

# Pelagic zone is an evolutionary catalyst, but an ecological dead end, for North American minnows

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## Abstract

The colonization of a novel geographic area is a classic source of ecological opportunity. Likewise, complex microhabitats are thought to promote biodiversity. We sought to reconcile these two predictions when they are naturally opposing outcomes. We assess the macroevolutionary consequences of an ancestral shift from benthic to pelagic microhabitat zones on rates of speciation and phenotypic evolution in North American minnows. Pelagic species have more similar phenotypes and slower rates of phenotypic evolution, but faster speciation rates, than benthic species. These are likely two independent, opposing responses to specialization along the benthic-pelagic axis, as rates of phenotypic evolution and speciation are not directly correlated. The pelagic zone is more structurally homogenous and offers less ecological opportunity, acting as an ecological dead end for minnows. In contrast, pelagic species may be more mobile and prone to dispersal and subsequent geographic isolation and, consequently, experience elevated instances of allopatric speciation. Microhabitat shifts can have decoupled effects on different dimensions of biodiversity, highlighting the need for nuance when interpreting the macroevolutionary consequences of ecological opportunity.

**Keywords:** continental radiation, ecological opportunity, macroevolution, morphological evolution, niche, speciation

## Introduction

The colonization of a novel environment, such as an island from the mainland, is held as a major catalyst of evolution (Simpson, 1953; Schluter, 2000). Geographic transitions are thought to provide release from competition and predation compared to the more saturated founding environment (Schluter, 1988; Losos & Ricklefs, 2009). In other words, species may be free to utilize resources that were previously inaccessible (Stroud & Losos, 2016). The rate of evolutionary change, either in terms of speciation or morphological evolution, is perhaps the key parameter used to assess the macroevolutionary signal of such ecological opportunity (Freckleton & Harvey, 2006; Glor, 2010; Harmon et al., 2010; Rabosky, 2014). For example, rates of phenotypic evolution are expected to be high as species adapt to novel niches (Schluter, 2000; Simpson, 1953). Structurally complex environments such as coral reefs are thought to promote phenotypic evolution as species adapt to an array of benthic niches (Corn et al., 2022; Evans et al., 2019; Price et al., 2011, 2013). By contrast, fewer niches may be viable in more simple environments such as the open ocean.

These two macroevolutionary predictions—that geographic transitions and habitat complexity promote evolution—are often viewed when they are cohesive or as separate phenomena. For example, the rise of modern coral reefs provided both novel and more complex habitat for fish lineages to exploit (Corn et al., 2022; Evans et al., 2019; Price et al., 2011, 2013). In other words, the directionality of the hypothesized effects of the two factors is cohesive. For terrestrial organisms such as anole lizards, colonizing an island from the mainland provides access to novel habitat (Stroud & Losos,

2016), but one of similar complexity, evidenced by the high degree of shared microhabitat specialists between island and mainland assemblages (Burress & Muñoz, 2022; Huie et al., 2021; Poe & Anderson, 2019).

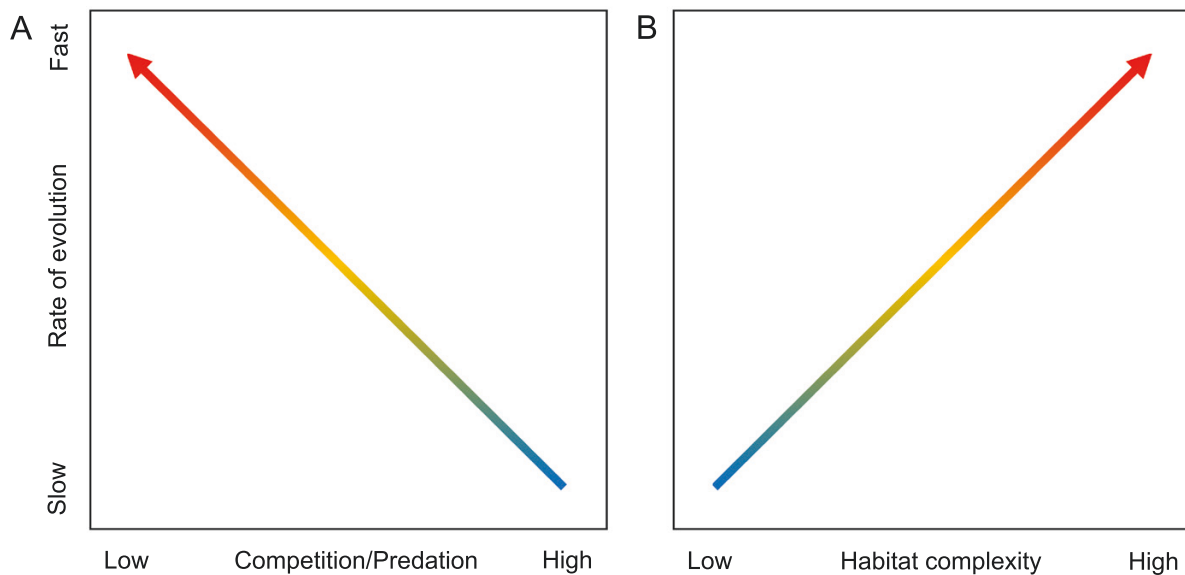
However, it remains uncertain how these macroevolutionary hypotheses resolve in scenarios in which they are opposing. For example, the continental radiation of North American minnows (Leuciscidae) exhibits an ancestral benthic-to-pelagic microhabitat shift (Burress et al., 2017; Hollingsworth et al., 2013). This evolutionary history presents a paradox: the pelagic lineages might experience ecological opportunity as they escape competition with a relatively saturated benthic assemblage (i.e., Schluter, 1998; Simpson, 1953) or they might encounter a narrowing of ecological opportunity as they adapt to a more homogenous environment (i.e., Friedman et al., 2020; Larouche et al., 2020). Furthermore, the benthic-to-pelagic habitat shift sparked a bout of lineage diversification (Burress et al., 2017; Hollingsworth et al., 2013), but its effect on the pace of phenotypic evolution is unknown, leaving it uncertain if there was a unified or decoupled macroevolutionary response to the benthic-to-pelagic shift across these different dimensions of biodiversity.

In this study, we test two alternative hypotheses (Figure 1). First, macroevolutionary-scale habitat shifts may result in a competitive release from a more saturated ancestral assemblage and/or community (e.g., Schluter, 1988). In this scenario, we expect rates of phenotypic evolution and speciation to be elevated in pelagic species as they escape competition with benthic species (Figure 1A). Second, the pelagic zone is rather homogeneous when compared to the benthos, and thus may offer less opportunity to interact with the environment

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**Figure 1.** Hypothesized macroevolutionary responses to competition and/or predation (A) (i.e., Schluter, 1988) and habitat complexity (B) (i.e., Friedman et al., 2020; Larouche et al., 2020). Arrows and color gradient depict the direction and magnitude of change in evolutionary rate.

and ultimately fewer available niches (e.g., Friedman et al., 2020). In this scenario, we expect rates of phenotypic evolution and speciation to be reduced in pelagic species (Figure 1B). We then integrate these results with existing literature and discuss how the benthic-to-pelagic axis influences speciation and phenotypic evolution and ultimately shapes the evolutionary history of a major continental radiation of fishes.

## Material and methods

### Study group

Leuciscid fishes—commonly known as “minnows”—include chubs, dace, and shiners (Stout et al. 2022; Tan & Armbruster, 2018). Most leuciscids in eastern North America are united as a clade by the osteological character of a small opening at the base of the skull (open posterior myodome: OPM). The OPM clade contains about 250 species (Hollingsworth et al., 2013; Stout et al., 2022) and is particularly diverse in rivers throughout the southeastern United States where they often co-occur in assemblages of up to 15 species (Baker & Ross, 1981; Burruss et al., 2016a; Gorman, 1988; Page & Burr, 2011). Much of this diversity arose following an ancestral benthic-to-pelagic microhabitat shift (Burruss et al., 2017; Hollingsworth et al., 2013).

### Morphological traits

We measured the body shape of 684 individuals representing 145 species (one to five individuals per species) accessioned at the Auburn University Museum of Natural History (approximately 58% of the described species). We measured 12 linear dimensions that characterize body shape variation in fishes (Price et al., 2019; Winemiller et al., 1995) and are known to have ecological implications in leuciscid fishes (Burruss et al., 2016, 2017). These measurements included: head length, depth, and width, snout length, lower jaw length, eye diameter, body depth and width, caudal peduncle (CP) length, depth, and width, and standard fish length. Head length was measured as the linear distance from the posterior edge of the operculum to the anterior tip of the snout. Head depth

was measured as the vertical distance through the center of the eye. Head width was measured as the distance at the center of the eyes. Snout length was measured as the horizontal distance from the center of the eye to the anterior tip of the snout. Lower jaw length was measured as the distance from the jaw joint to the anterior tip of the mandible. Eye diameter was measured as the horizontal distance between the anterior and posterior margins of the eye. Body depth was measured as the vertical distance at the anterior insertion point of the pelvic fins. Body width was measured as the maximum distance at the anterior insertion point of the pelvic fins. Caudal peduncle length (CPL) was measured as the horizontal distance from the posterior insertion of the anal fin to the posterior tip of the hypural plate. Caudal peduncle depth was measured as the vertical distance at the midpoint of the CP (i.e., the midpoint of the CPL measurement). Caudal peduncle width (CPW) was measured as the width at the CP midpoint. The standard fish length was measured as the distance from the anterior tip of the snout to the posterior tip of the hypural plate. All measurements were taken by the authors using digital calipers to a precision of 0.01 cm. To account for body size, we calculated phylogenetic residuals by regressing ln-transformed shape variables against ln-transformed standard length using the `phyl.resid` function in PHYTOOLS (Revell, 2012), a common method employed in macroevolutionary studies when traits scale strongly with size (Arbour & López-Fernández, 2016; Burruss & Muñoz, 2023; Friedman et al., 2020; see Price et al., 2019) for a detailed treatment of alternative options and their effects). This method fits a clade-wide slope between trait values and body size, which we confirmed by assessing slopes among four subclasses of similar species richness: subclasses comprised of *Campostoma*, *Nocomis*, *Exoglossum*, and *Rhinichthys*; *Phenacobius*, *Erimystax*, and *Dionda*; *Cyprinella*; as well as *Notropis* (in part), *Alburnops*, and *Hybognathus* (results not shown). During this procedure and all subsequent phylogenetic comparative methods, we used an existing multi-locus phylogeny of North American minnows (Hollingsworth et al., 2013), which included 223 species (approximately 90% of the described species). Since

minnows with larger mouth angles consume more terrestrial insects and less benthic prey such as aquatic insects and algae (Burruss et al., 2017), we measured mouth angle as a proxy for specialization along the benthic-pelagic axis. We defined mouth angle as the angle formed by the linear plane that passes through the lower jaw joint and the tip of the lower jaw relative to the linear plane that passes through the center of the eye and the midpoint of the hypural plate (i.e., the horizontal axis of the fish; adapted from Burruss et al., 2017). Mouth angle was measured from photographs using the angle tool in tpsDIG2 ver. 2.31 (Rohlf, 2017).

### Phylogenetic comparative methods

First, we estimated the evolutionary history of mouth angle using maximum likelihood via the `contMap()` function implemented in PHYTOOLS (Revell, 2012). We visualized the body shape diversity of the sampled species using principal component analysis using the `prcomp()` function in base R (version 4.1.2). To assess body shape disparity across the benthic-pelagic axis, we partitioned species into quartiles based on mouth angles. We then calculated body shape disparity for each quartile using the `morphol.disparity()` function in the GEOMORPH package (Baken et al., 2021) using 999 iterations. During this procedure, disparity is measured as the variance.

To assess rates of body shape evolution along the benthic-pelagic axis, we employed a state-dependent, multivariate, relaxed model of Brownian motion (May & Moore, 2020) implemented in RevBayes (Höhna et al., 2016). As continuous characters, we used the phylogenetic residuals for the 11 body shape variables described above. As a discrete character, we used the quartiles delimited using mouth angle as a proxy for specialization along the benthic-pelagic axis. The model jointly estimates the evolutionary histories of the discrete and continuous characters, accounts for correlated evolution of the continuous characters, and accounts for background rate variation, thereby reducing type I error (i.e., false positives; Burruss & Muñoz, 2022; May & Moore, 2020). The Markov chain Monte Carlo (MCMC) was run for 500,000 generations with 10% burnin. To evaluate sensitivity to priors, replicates of the MCMC were run with different priors on the number of rate shifts (25, 50, 75, and 100 shifts). To evaluate sensitivity to the cut-off points during discretization of mouth angle, we repeated the analyses with two and three states.

To assess the relative effects of the benthic-pelagic axis on rates of body shape evolution and speciation, we calculated tip rates (i.e., species-specific rates; Title & Rabosky, 2019). First, to calculate phenotypic tip rates, we estimated branch-specific rates of evolution using a multivariate, relaxed model of Brownian motion (May & Moore, 2020), implemented in RevBayes (Höhna et al., 2016), following Burruss et al. (2020). The MCMC was run for 500,000 generations with 10% burnin and a prior of 50 rate shifts. Second, to calculate speciation tip rates, we estimated branch-specific speciation rates using a birth-death-shift (BDS) process (Martínez-Gómez et al., 2023), implemented in RevBayes (Höhna et al., 2016), following Burruss and Muñoz (2022). The MCMC was run for 500,000 generations with 10% burnin and four rate categories (i.e.,  $K = 4$ ). Due to inherent limitations while estimating extinction (Rabosky, 2010) and by extension species diversification (Louca & Pennell, 2020), the BDS model was only employed to estimate speciation. Missing taxa non-randomly

distributed across the phylogeny could bias the estimation of tip rates (e.g., if missing taxa were concentrated in a specific subclade). Since the birth-death-shift model cannot account for incomplete taxon sampling, we assume unsampled taxa are random. Lastly, since tip rates are not phylogenetically independent, we used phylogenetic generalized least squares (Revell, 2010) to assess relationships between specialization along the benthic-pelagic axis (i.e., mouth angle) and both types of tip rates as well as between phenotypic and speciation tip rates.

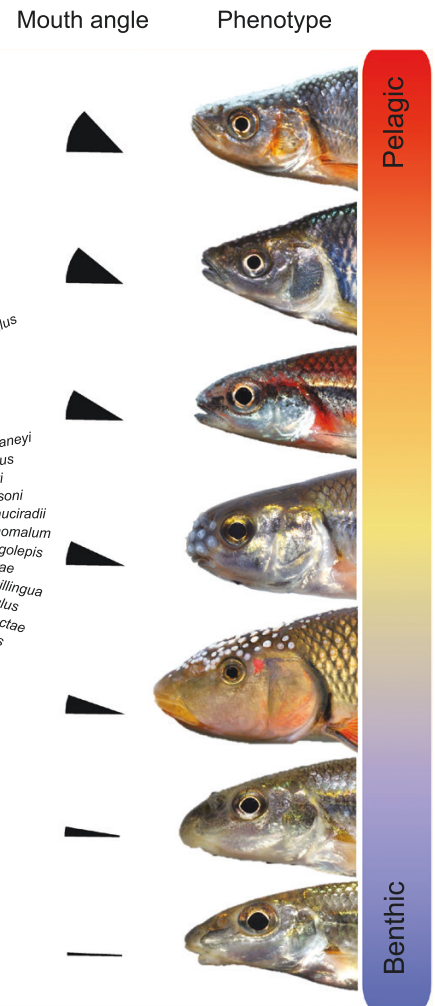
### Results

Mouth angles varied from 1° to 53°, reflecting the continuum of specialization along the benthic-pelagic axis (Figure 2). The transition from inferior to terminal and superior-oriented mouth angles corresponded with the ancestral benthic-to-pelagic habitat shift reported by Hollingsworth et al. (2013; Figure 2). Benthic species tended to have wider and elongated bodies, longer heads, shorter jaws, and smaller eyes than pelagic species (Figure 3A). Mouth angle was approximately normally distributed, with a slight bias toward more species with larger mouth angles (Figure 3B). Body shape disparity declined across quartiles, with the lower quartile (i.e., most benthic) having twofold to 2.5-fold higher morphological disparity than the other quartiles (Figure 3C), indicating that pelagic species are more similar in body shape.

Rates of phenotypic evolution were state-dependent (posterior probability [PP] = 1.0), with the highest quartile (i.e., most pelagic) having the slowest rates (Figure 4). This result was consistent across models with different priors (all PP = 1.0) and different numbers of discrete character states (all PP > 0.966). Phenotypic tip rates were negatively correlated with mouth angle ( $r^2 = 0.469$ ;  $t = -4.909$ ;  $p < .0001$ ; Figure 5A and B), whereas speciation tip rates were positively correlated with mouth angle ( $r^2 = 0.296$ ;  $t = 2.560$ ;  $p = .012$ ; Figure 5A and C). This result likely represents two independent, opposite responses to specialization along the benthic-pelagic axis, as phenotypic and speciation tip rates were not correlated ( $r^2 = 0.032$ ;  $t = 0.637$ ;  $p = .525$ ; Figure 5D).

### Discussion

The benthic-pelagic axis is a major dimension along which fish lineages specialize (Burruss, 2015; Friedman et al., 2020; Ghezelayagh et al., 2022; Ribeiro et al., 2018; Seehausen & Wagner, 2014). Its importance spans vast temporal and geographic scales, ranging from incipient species pairs in isolated glacial lakes (Bernatchez et al., 2010; Østbye et al., 2006) to species-rich endemic species flocks in large lakes (Cooper et al., 2010; Hulsey et al., 2013), to geographically dispersed continental and marine radiations (Burruss et al., 2017; Friedman et al., 2020; Hollingsworth et al., 2013; López-Fernández et al., 2013). In Earth's vast oceans, two fundamental environmental axes—the depth gradient and the benthic-pelagic axis—can become decoupled and each may have profound effects on the evolution of fish diversity. The deep sea contains relatively few species compared to the photic zone, especially considering its immense volume (Miller et al., 2022), yet is a hotspot for phenotypic diversity, characterized by unique phenotypes and rapid phenotypic evolution (Martínez et al., 2021). Importantly, some lineages, such as Lophiiformes, include benthic species that occupy shallow coral reefs (i.e.,



**Figure 2.** Evolutionary history of mouth angle among leuciscid fishes. Phylogeny from [Hollingsworth et al. \(2013\)](#). Taxonomy follows [Stout et al. \(2022\)](#). Asterisks indicate the location of the ancestral benthic-to-pelagic shift as estimated by [Hollingsworth et al. \(2013\)](#) and [Burress et al. \(2017\)](#). Images depict the continuum of mouth angles in North American minnows from benthic specialists with inferior mouths (bottom) to pelagic specialists with superior mouths (top).

frogfishes) as well as pelagic species that occupy the abyss (i.e., anglerfishes; [Hart et al., 2022](#)), highlighting the potential for depth and the benthic-pelagic axis to become decoupled. Like depth, the benthic-pelagic axis also influences the evolution of fish diversity, as benthic fishes are more phenotypically diverse and exhibit rapid rates of phenotypic evolution ([Friedman et al., 2020](#)).

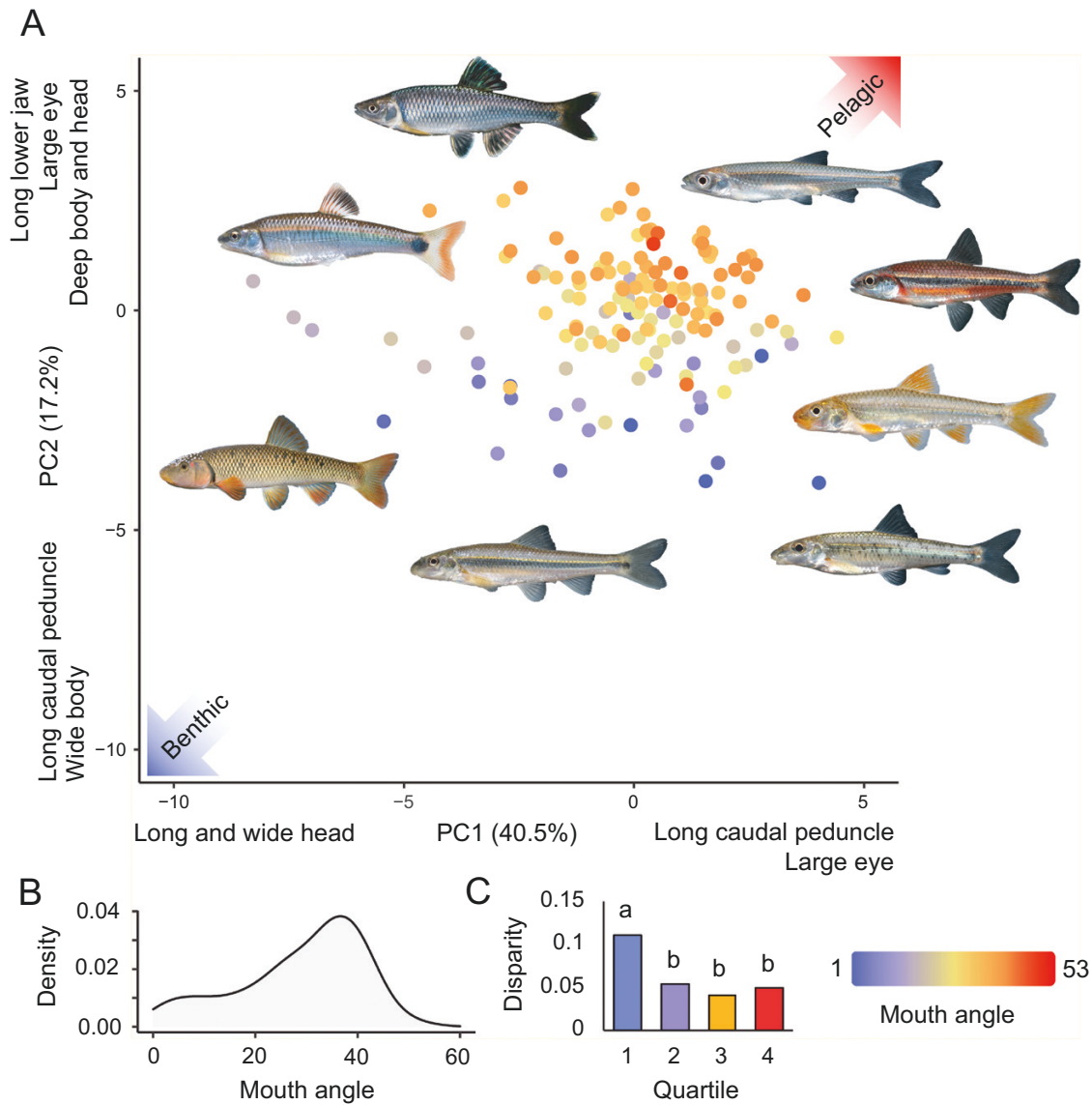
The magnitude of interactions between habitat transitions and the benthic-pelagic axis varies across ecosystems. Transitions from marine to freshwater ecosystems can also alter the evolutionary trajectory of lineages, including morphological and ecological expansion ([Kolmann et al., 2022](#)). Within freshwaters, the importance of the benthic-pelagic axis is best known in insular lake ecosystems, perhaps most notably in stickleback and cichlids in which specialization along the benthic-pelagic axis has been a fundamental component of their diversity ([Cooper et al., 2010](#); [Hulsey et al., 2013](#); [Schluter, 1993](#)). Continental radiations are more geographically dispersed, usually diversifying across river drainages, their tributaries, and in the face of a more spatially constrained vertical habitat dimension that is also spatially integrated with the littoral zone (i.e., along stream margins). Of the many megadiverse continental radiations of fishes,

including Neotropical cichlids, characiforms, and loricariids, there has been considerable variation in the extent of diversification along the benthic-pelagic axis ([Burns & Sidlauskas, 2019](#); [López-Fernández et al., 2013](#); [Melo et al., 2022](#); [Burress et al., 2022](#); [Silva et al., 2016](#)).

### Pelagic zone as an ecological dead-end

Minnows often form species-rich assemblages (up to 15 sympatric species) that vertically partition the water column ([Baker & Ross, 1981](#); [Gorman, 1988](#); [Page & Burr, 2011](#)). Since the pelagic zone is sparsely inhabited by other small-bodied lineages ([Page & Burr, 2011](#)), it likely acted as a novel adaptive zone for minnows by providing competitive release from more saturated benthic microhabitats (i.e., [Schluter, 2000](#); [Simpson, 1953](#)).

Phenotypically and trophically, minnow assemblages are often distributed along the benthic-pelagic axis, as species specialize in terms of functional morphology ([Burress et al., 2016a, b](#)). Minnows have rarely transitioned directly between herbivory and allochthonous prey ([Pos et al., 2019](#)), suggesting that specialization along the benthic-pelagic axis can constrain the evolution of the trophic niche. Indeed, we found that pelagic minnows have more similar phenotypes and reduced



**Figure 3.** Phenotypic diversity in North American minnows (A). Each point represents the mean of a species. Points are color-coded according to their mouth angle. Traits listed along the axes denote traits that have larger values in that direction. Distribution of mouth angles among sampled minnows (B). Phenotypic shape disparity across quartiles delimited based on mouth angle (C). Letters above bars denote significant comparisons (different letters indicate significant differences between groups). Images depict a species (and phenotype) that represents an adjacent data point.

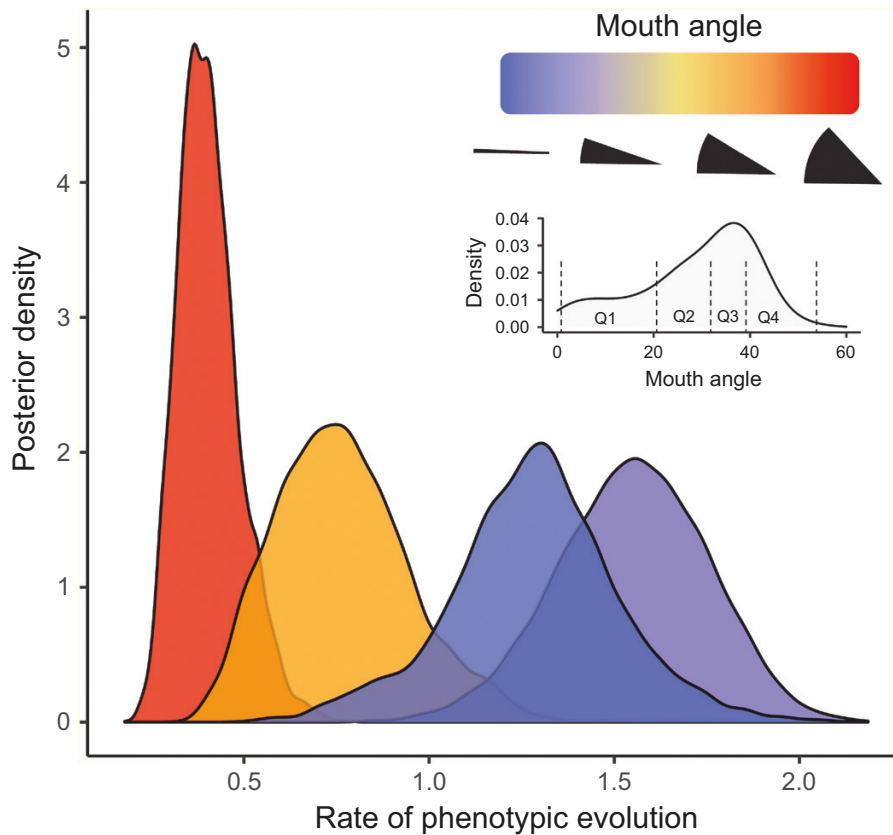
rates of phenotypic evolution than their benthic counterparts (Figures 3 and 4). Pelagic minnows occupy a higher trophic position than benthic species (Burress et al., 2016b), likely in response to consuming less algae and more terrestrial insects (Burress et al., 2017). Taken together, these patterns suggest that ecological opportunity is not evenly distributed across the benthic-pelagic environmental axis, as the pelagic zone funnels species into specialized ecologies associated with a limited range of phenotypes.

A conceptually analogous habitat shift may be surface-to-cave transitions in which the lineage likely experienced competitive release from saturated surface communities, yet faced a resource-poor environment, and by extension, limited ecological opportunity. For example, in amblyopsid fishes, there have been multiple subterranean invasions from surrounding spring and swamp habitats (Hart et al., 2020). These surface-to-cave transitions led to an increase in species richness and the evolution of different phenotypes but had no effect on

rates of phenotypic evolution (Armbruster et al., 2016; Hart et al., 2020). In this case, amblyopsids may have been funneled into few viable niches in cave environments but were more prone to geographic isolation and subsequent speciation (Hart et al., 2023; Niemiller et al., 2012).

### Decoupled macroevolutionary responses to microhabitat shifts

Rates of speciation and phenotypic evolution are generally correlated in fishes (Rabosky et al., 2013), yet the degree and direction of this relationship can vary among vertebrates (Cooney & Thomas, 2021). Indeed, we found that microhabitat transitions may have decoupled effects on speciation and phenotypic evolution. We corroborated previous work showing that pelagic minnows exhibit rapid speciation (Burress et al., 2017; Hollingsworth et al., 2013). We expand our understanding of the dynamics between speciation and phenotypic evolution by demonstrating that these two macroevolutionary



**Figure 4.** State-dependent rates of phenotypic evolution in North American minnows. Cooler colors depict smaller mouth angles (i.e., more benthic species), whereas warmer colors depict larger mouth angles (i.e., more pelagic species). Inset distribution indicates the cut-offs for the bins. See [Supplementary Figure S1](#) for an alternative model using evenly spaced bins rather than percentiles.

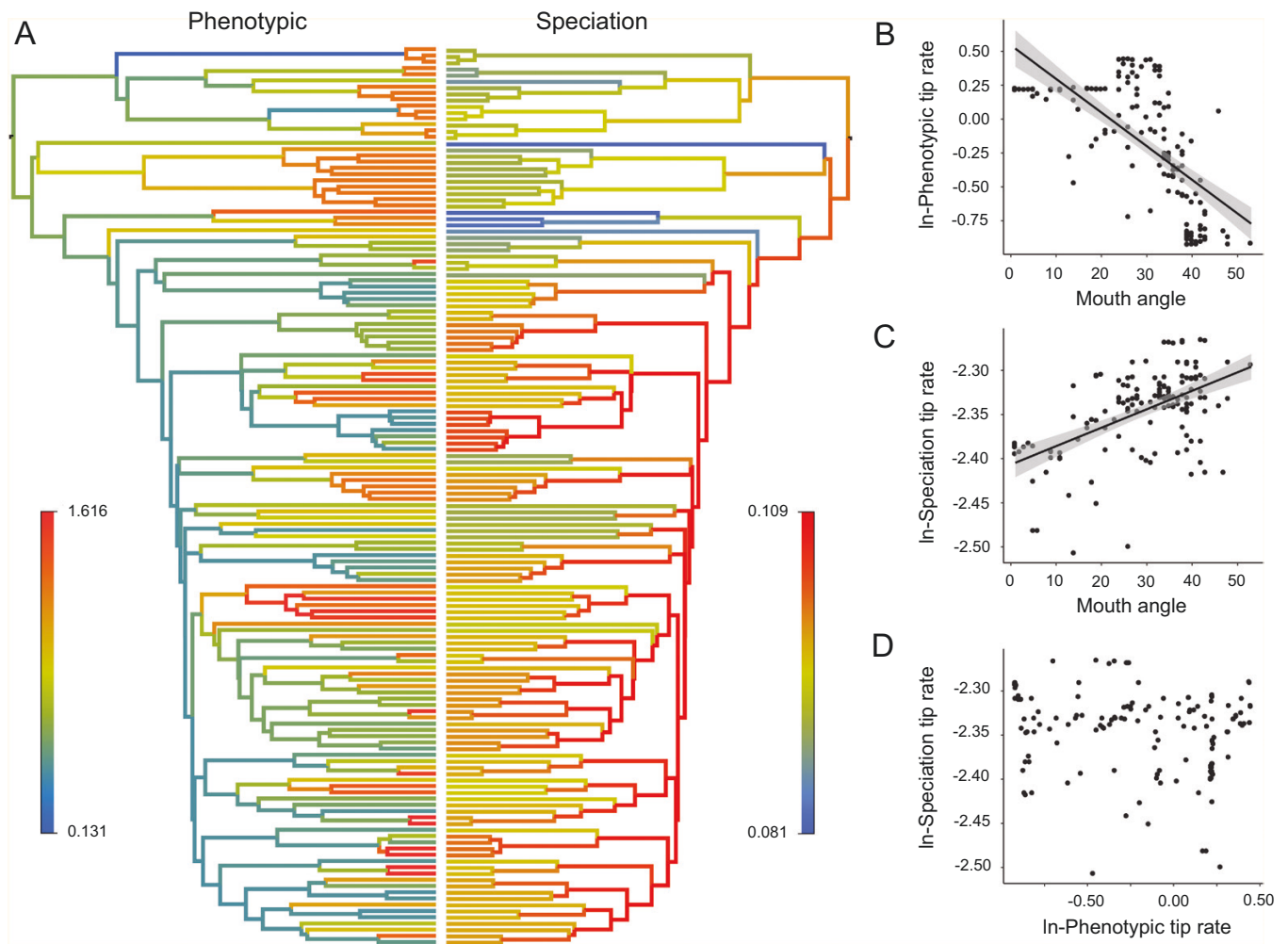
parameters were decoupled in response to benthic-to-pelagic transitions ([Figure 5](#)).

A pelagic lifestyle involves enhanced mobility that may facilitate dispersal, subsequently promoting speciation as populations were more prone to becoming physically (and genetically) isolated from one another ([Wiley & Mayden, 1985](#)). Yet, the pelagic zone provides little ecological opportunity. Marine fishes have frequently invaded the pelagic zone, a transition sometimes accompanied by morphological expansion ([Ribeiro et al., 2018](#)), yet rates of phenotypic evolution have generally been higher among benthic fishes ([Friedman et al., 2020](#)). For minnows, the transition into the pelagic zone is associated with an increased reliance on terrestrial subsidies. For example, many species feed upon terrestrial insects directly from or near the water surface. The ephemeral and unpredictable nature of terrestrial subsidies may facilitate co-existence among species-rich minnow assemblages (i.e., [Cody, 1974](#); [Jepsen & Winemiller, 2002](#)), perhaps eroding competition that would otherwise lead to local exclusion ([Connell, 1980](#)). If benthic resources—algae, vegetation, insect larvae, and nymphs—were more predictable than terrestrial subsidies, it may explain why pelagic minnows may experience competitive release from a saturated benthic community in the face of an ecologically homogenous pelagic zone. Alternatively, since pelagic species tend to have smaller body sizes than their benthic counterparts ([Burruss et al., 2016b](#)), their elevated speciation rates may instead be due to faster generation times ([Martin & Palumbi, 1993](#)). However, since rates of speciation and phenotypic evolution were not directly correlated ([Figure 5](#)), we favor the hypothesis that

the two parameters exhibit independent, opposing responses to benthic-to-pelagic transitions.

The widespread notion that coral reefs are catalysts of rapid evolution is often attributed to their structural complexity and, subsequently, the abundance of ecological opportunity afforded by the viability of many available niches ([Corn et al., 2022](#); [Price et al., 2011, 2013](#)). North American minnows provide a stark contrast to this paradigm. On one hand, the pelagic zone served as a catalyst for much of the groups' species diversity ([Hollingsworth et al., 2013](#)). Yet, minnows' ecological diversity may have been stifled in the face of an ecologically homogenous pelagic zone ([Figures 3–5](#)). In this sense, minnows provide a clear example of the need for nuance when interpreting macroevolutionary signatures of ecological opportunity across different dimensions of biodiversity.

Continental radiations often exhibit macroevolutionary patterns consistent with adaptive radiation, as either early bursts of speciation or phenotypic evolution ([Burns & Sidlauskas, 2019](#); [DerryBerry et al., 2011](#); [López-Fernández et al., 2013](#)). While both are often interpreted the same, as evidence of lineages rapidly expanding to fill available niches ([Freckleton & Harvey, 2006](#); [Glor, 2010](#); [Schluter, 2000](#); [Simpson, 1953](#); [Yoder et al., 2010](#))—our study casts doubt on this interpretation. For example, minnows' transition from benthic to pelagic habitat coincident with a bout of speciation has been interpreted as a possible signature of adaptive radiation ([Burruss et al., 2017](#); [Hollingsworth et al., 2013](#)). However, we further our understanding of this microhabitat transition by showing that it resulted in reduced phenotypic



**Figure 5.** Branch-specific rates of phenotypic evolution and speciation among North American minnows (A). Relationships between mouth angle and phenotypic tip rates (B) and speciation tip rates (C). Relationship between phenotypic and speciation tip rates (D). Each point represents a species. Best-fit lines indicate significant correlations ( $p < .05$ ) based on phylogenetic generalized least squares (Revell, 2010). See text for detailed statistics for each model.

disparity and evolutionary rates (Figures 3–5). These patterns of phenotypic evolution are not consistent with adaptive radiation, instead pointing to pelagic minnows occupying a singular adaptive peak (i.e., Collar et al., 2009). In this regard, minnows are like some terrestrial radiations in which a high rate of speciation is not paired with exceptional morphological evolution, including Neotropical rodents (Maestri et al., 2017) and Neotropical birds (DerryBerry et al., 2011).

**Supplementary material**

Supplementary material is available online at *Evolution*.

**Data availability**

Data for this study have been archived in the Dryad data repository (<https://doi.org/10.5061/dryad.w3r228108>).

**Author contributions**

E.D.B. designed the study. E.D.B. and P.B.H. collected data. E.D.B. analyzed data. E.D.B. and P.B.H. wrote the article.

*Conflict of interest:* The authors declare no conflict of interest.

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