Integrative and Comparative Biology

Integrative and Comparative Biology, volume 62, number 2, pp. 406–423
https://doi.org/10.1093/icb/icac085

Society for Integrative and Comparative Biology

SYMPOSIUM

Patterns of Phenotypic Evolution Associated with Marine/Freshwater Transitions in Fishes

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From the symposium “The deep and shallow history of aquatic life’s passages between marine and freshwater habitats” presented at the annual meeting of the Society for Integrative and Comparative Biology virtual annual meeting, January 3–February 28, 2022.

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Synopsis Evolutionary transitions between marine and freshwater ecosystems have occurred repeatedly throughout the phylogenetic history of fishes. The theory of ecological opportunity predicts that lineages that colonize species-poor regions will have greater potential for phenotypic diversification than lineages invading species-rich regions. Thus, transitions between marine and freshwaters may promote phenotypic diversification in trans-marine/freshwater fish clades. We used phylogenetic comparative methods to analyze body size data in nine major fish clades that have crossed the marine/freshwater boundary. We explored how habitat transitions, ecological opportunity, and community interactions influenced patterns of phenotypic diversity. Our analyses indicated that transitions between marine and freshwater habitats did not drive body size evolution, and there are few differences in body size between marine and freshwater lineages. We found that body size disparity in freshwater lineages is not correlated with the number of independent transitions to freshwaters. We found a positive correlation between body size disparity and overall species richness of a given area, and a negative correlation between body size disparity and diversity of closely related species. Our results indicate that the diversity of incumbent freshwater species does not restrict phenotypic diversification, but the diversity of closely related taxa can limit body size diversification. Ecological opportunity arising from colonization of novel habitats does not seem to have a major effect in the trajectory of body size evolution in trans-marine/freshwater clades. Moreover, competition with closely related taxa in freshwaters has a greater effect than competition with distantly related incumbent species.

Advance Access publication June 8, 2022

Introduction

Evolutionary transitions between marine and freshwater ecosystems have occurred repeatedly throughout the fish tree of life (Bloom and Lovejoy 2011, 2017; Vega and Wiens 2012; Betancur-R et al. 2015; Corush 2019; Rabosky 2020). Transitions between marine and freshwaters represent an extreme ecological shift, exposing lineages to novel abiotic and biotic settings (Lee and Bell 1999; Vega and Wiens 2012; Seehausen and Wagner 2014; Davis et al. 2020). For example, marine/freshwater transitions may alter the selective landscape for traits associated with swimming efficiency, predator avoidance, and competition for resources (Lee and Bell 1999; Seehausen and Wagner 2014; Kolmann et al. 2020). While conceptually it is understood that novel biotic and abiotic factors can result in strong selective pressure on phenotypic traits, establishing empirical links between ecological factors driving evolutionary change over long time scales remains challenging (Benton 2009; Weber et al. 2017; Aristide and Morlon 2019; Harmon et al. 2019; Hembry and Weber 2020). A key, yet unresolved question is: How do transitions between marine and freshwaters influence patterns of phenotypic evolution across macroevolutionary scales? An integrative approach that combines comparative phylogenetics, species coexistence, and phenotypic trait data can advance our understanding of how colonization of new habitats and different community compositions influence evolution of species traits.

Biotic interactions play a crucial role in the successful invasion and subsequent phenotypic evolution in new habitats. The theory of ecological opportunity is a central concept used to predict macroevolutionary patterns based on ecological interactions (Schluter 2000; Glor 2010; Losos 2010; Yoder et al. 2010; Wagner et al. 2012; Wellborn and Langerhans 2015; Stroud and Losos 2016; Harmon et al. 2019; Martin and Richards 2019; McGee et al. 2020). Ecological opportunity can be defined as the availability of resources that can be evolutionarily exploited by ecologically capable taxa in a species-poor region or habitat where competition is low (Simpson 1953; Schluter 2000). This theory predicts that a lineage invading new areas, such as islands, depauperate habitats, or other competition-free spaces, could experience rapid speciation and morphological diversification (adaptative radiation) by occupying vacant niches (Simpson 1953; Schluter 2000; Glor 2010; Losos 2010; Yoder et al. 2010; Stroud and Losos 2016). Ecological opportunity can result from biogeographic processes (e.g., dispersal to a new habitat), ecological processes (e.g., extinction of an antagonist species reducing competition), and/or evolutionary processes (e.g., evolution of a key innovation allowing the access of new resources) (Simpson 1953; Yoder et al. 2010). Transitions between marine and freshwaters may also function as dispersal events, facilitating ecological opportunity in novel environments (Schluter 2000; Yoder et al. 2010).

Competition is often predicted to be greater in areas with higher species richness, which suggests that lineages that colonized species-poor regions may have experienced a greater opportunity for diversification than lineages that invaded species-rich regions (Betancur-R et al. 2012; Santini et al. 2013; Bloom and Lovejoy 2017). Previous macroevolutionary studies of fishes have found evidence for ecological opportunity resulting from habitat transitions into species poor regions. For example, Betancur-R et al. (2012) detected elevated rates of evolution in ariid catfish lineages that colonized freshwaters in otherwise species-poor regions, such as Australia and New Guinea. However, ariids that invaded hyperdiverse ecosystems, such as the Amazon River basin, experienced stymied diversification, suggesting the ecological context was a key factor in diversification following marine/freshwater transitions. In addition, numerous studies have investigated the impact of habitat transitions on lineage diversification, with higher speciation rates being reported in freshwater lineages than in marine fish lineages (e.g., Davis et al. 2012; Bloom et al. 2013; Guinot and Cavin 2015; Tedesco et al. 2017; Rabosky 2020; Miller 2021). This suggests that overall species richness alone may not be the only factor in generating ecological opportunity. If ecological opportunity is the primary mechanism influencing not only lineage diversification but also phenotypic evolution, we predict phenotypic variation will be negatively correlated with the overall diversity of freshwater species in the invading region.

While species richness can serve as a proxy to gauge the strength of competition, when lineages diversify in newly-invaded habitats the interaction among closely related species may have a stronger effect on phenotypic evolution than interactions with distantly related species (Weber and Strauss 2016). Initially proposed by Darwin (1859), the competition-relatedness hypothesis states that closely related species tend to have more similar traits, and thus can become stronger competitors than distantly related lineages (Cahill et al. 2008). Consequently, natural selection should favor the tendency of reduction in similarity between lineages, promoting niche separation, and preventing interaction and competition (Rabosky 2013; Anderson and Weir 2021). Thus, if lineages tend to diversify while avoiding competition-mediated extinction, we expect that phenotypic variation of fish lineages that invaded freshwaters should be higher in regions harboring a higher diversity of closely related species due to diversity-dependent factors. Alternatively, competitive exclusion might prevent closely related species with similar traits from establishment and subsequent diversification in sympatry; however, divergence in certain traits prior to the coexistence or subtle niche differences may overcome small competitive differences (Mayfield and Levine 2010; Germain et al. 2020). If interaction with closely related species has a greater impact on phenotypic evolution than the interaction with distantly related species, we predict that phenotypic variation will have a stronger correlation with the number of closely related species in a freshwater region than with the overall number of incumbent species.

In this study, we focus on the evolution of body size as a phenotypic trait. Body size co-varies with many attributes, including physiology, species interaction, life-history, migration, and biomechanical traits (Peters 1983; Bloom et al. 2018; Burns and Bloom 2020), and it is a strong predictor of trophic position in fishes (Romanuk et al. 2011). Fishes exhibit a remarkable diversity of sizes, making them well-suited for studies of body size diversity and evolution (Knouft and Page 2003; Rüber et al. 2007; Albert and Johnson 2012). Many phylogenetic comparative studies have used body size to explore patterns of phenotypic evolution using broad-scale datasets (Harmon et al. 2010; Uyeda et al. 2011; Rabosky et al. 2013; Cooney and Thomas 2021) because body size is directly comparable across wide phylogenetic scales. Previous studies indicated that the evolution of extreme body sizes can be associated with the colonization of freshwaters (Weitzman and Vari 1988; Steele and López-Fernández 2014; Bloom et al. 2020; Kolmann et al. 2020), but it remains unclear if general patterns emerge following transitions across the marine/freshwater boundary.

While most major fish lineages are restricted to either marine or freshwater environments, some clades, referred to trans-marine/freshwater clades (Bloom and Egan 2018), have crossed the marine/freshwater boundary over geological timeframes. Major trans-marine/freshwater clades include ariid catfishes (Family Ariidae) (Betancur-R 2010; Betancur-R. et al. 2012), clingfishes (Family Gobiesocidae) (Conway et al., 2017, 2020), gobies, and mudskippers (Families Gobiidae + Oxudercidae) (McCraney et al. 2020), pufferfishes (Family Tetraodontidae) (Santini et al. 2013), anchovies, herrings, and sardines (Order Clupeiformes) (Bloom and Egan 2018; Egan et al. 2018), sculpins (Superfamily Cottoidea) (Kinziger et al. 2005; Goto et al. 2015), silversides, rainbowfishes, and relatives (Order Atheriniformes) (Campanella et al. 2015), stingrays (Order Myliobatiformes) (Lim et al. 2015; Fontenelle et al. 2021a; Fontenelle et al. 2021b) and needlefishes (Family Belonidae) (Kolmann et al. 2020). These groups collectively have experienced dozens of transitions between marine and freshwaters on every continent except Antarctica (Lovejoy et al. 2006; Bloom and Lovejoy 2011, 2017). The numerous transitions to a wide variety of habitats and biogeographic settings, ranging from species-poor regions (e.g., Australia) to megadiverse realms (e.g., the Neotropics), and the collective diversity of body sizes allow us to study the role of habitat shifts and different levels of competition on phenotypic evolution. In addition, focusing on individual trans-marine/freshwater groups by taxonomic experts offers the opportunity of informed interpretation of patterns of phenotypic evolution that might otherwise be clouded in analyses at larger phylogenetic scales (Beaulieu and O'Meara 2018; Rabosky 2020; Clarke 2021).

In this study, we investigated the role of habitat transitions and the interaction with different community compositions in freshwater systems in the phenotypic evolution of trans-marine/freshwater fishes. We used phylogenetic comparative analyses to test (1) whether habitat transitions between marine and freshwaters drive consistent phenotypic changes across fish clades; (2) if transitions to freshwater habitats have increased body size disparity arising from ecological opportunity; and (3) whether there is a relationship between the richness of incumbent freshwater species or closely related species with body size disparity, indicating that competition reduces access to ecological opportunity.

Methods

Data acquisition and study systems

We used phylogenetic comparative methods to analyze the evolution of body size in 1446 trans-marine/freshwater species from nine individual major fish clades, including: Ariidae (ariid catfishes), Atheriniformes (silversides and rainbowfishes), Belonidae (needlefishes and halfbeaks), Clupeiformes (anchovies, allies, herrings, and sardines), Cottoidea (sculpins), Gobiesocidae (clingfishes), Gobiidae + Oxudercidae (gobies and mudskippers), Myliobatiformes (stingrays), and Tetraodontidae (pufferfishes).

The family Ariidae is the most widespread group of the order Siluriformes, occupying mainly demersal habitats in warm-temperate and tropical regions, comprising 156 species, with about 56 of them occurring primarily in freshwaters (Nelson et al. 2016; Froese and Pauly 2021; Fricke et al. 2022). The origin of ariids is marine, with their fossil record dating back to the Late Cretaceous (∼70 ma) (Betancur-R 2009). They have invaded freshwaters in all the regions where they occur, including South and Central America, Africa, Madagascar, Southeast Asia (SE Asia), and Australia–New Guinea, and include species with diadromous life cycles (Betancur-R 2009, 2010). The greatest diversity of freshwater ariids (34 species) is concentrated in a clade endemic to the Australia–New Guinea region (Betancur-R et al. 2012).

Atheriniformes are distributed globally in tropical and temperate regions, comprising 385 species, with about 210 of them occurring primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022). Atheriniformes likely originated in marine waters during the Late Cretaceous (72.8 ma) (Campanella et al. 2015) and invaded freshwaters multiple times in the Americas, Africa, Southeast Asia, and Australia–New Guinea (Parenti 1996; Bloom et al., 2012, 2013; Unmack et al. 2013; Hughes et al. 2020). One of the clades with highest diversity of freshwater silversides (genus *Chirostoma*, 23 species) is found in lacustrine freshwater waters of central Mexico (Barbour 1973; Bloom et al. 2012).

Species of the family Belonidae are elongate pelagic fishes distributed worldwide in tropical and temperate waters, comprising 44 species, with 12 of them occurring primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022). Fossil evidence suggests that Belonidae has a minimum age of origin during the Lower Oligocene (33.9–28.4 ma) (De Sant'Anna et al. 2013). Belonids have invaded freshwater habitats six times, with no subsequent reversals to the marine habitats (Kolmann et al. 2020). The highest diversity of freshwater belonids (10 species) is found in South and Central American rivers.

The order Clupeiformes is distributed worldwide, comprising >400 mainly pelagic species, with about 79 of them occurring primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022) and about 30 species exhibiting diadromous life cycles (McDowall 2003). The origin of Clupeiformes dates to the Early Cretaceous (∼125 ma) (Malabarba and Di Dario 2017; Bloom and Egan 2018). Clupeiformes have invaded freshwaters across all continents but Antarctica (Whitehead et al. 1988), and among those, at least four independent transitions to South America generated their highest diversity of freshwater species in a region (Bloom and Lovejoy 2017).

Fishes of the superfamily Cottoidea inhabit mostly benthic habitats in temperate and boreal waters of the Northern Hemisphere, comprising 390 species, of those about 100 species occur primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022). Crown Cottoidea is dated to the Lower Oligocene (∼30 Ma) (Near et al. 2013). The great diversity of freshwater and diadromous sculpins distributed throughout North America, Europe, and Asia originated from a single transition from the marine habitat (Buser et al. 2019). Some freshwater lineages of this clade present remarkable adaptive radiation, including the diversification in the Lake Baikal, where they inhabit from pelagic to deep water benthic habitats (Goto et al. 2015).

The small-bodied fish of the family Gobiesocidae inhabit benthic habitats in intertidal zones of the Atlantic and Indo–Pacific Oceans, comprising 189 species, of those about 7 occur primarily in freshwaters (Briggs and Miller 1960; Conway et al. 2017; Fricke et al. 2022). The origin of clingfishes dates to the Mid to Late Eocene (50.8–32.0 ma), with a single habitat transition event from marine to freshwaters in their history (Conway et al. 2017, 2020). The freshwater species of Gobiesocidae inhabit tropical waters of fast flowing rivers and streams in South and Central America and Caribbean islands, draining into the Pacific Ocean and the Caribbean Sea (Briggs and Miller 1960; Conway et al. 2017).

The diverse families Gobiidae + Oxudercidae include benthic species distributed worldwide, comprising 1965 species, of which about 200 species occur primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022). This clade is estimated to have originated in the Early Eocene (∼54 ma) (Near et al. 2013), and their lineages have invaded freshwater habitats multiple times across all continents but Antarctica (Fricke et al. 2022). Fishes of Gobiidae + Oxudercidae display an incredible ecological diversification, including the shortest lifespans and some of the smallest body sizes recorded among vertebrates (Watson and Walker 2004; Depczynski and Bellwood 2005; Rittmeyer et al. 2012).

The stingrays of the order Myliobatiformes are distributed worldwide from tropical to temperate waters, comprising 385 species, with about 41 occurring primarily in freshwaters (Nelson et al. 2016; Fricke et al. 2022). Molecular evidence place the origin of this clade in the Late Jurassic (∼150 ma) (Aschliman et al. 2012). Myliobatiformes have invaded freshwater systems in Africa and Southeast Asia, but a single transition event to South America led to the origin of the greatest diversity of freshwater species in this clade (Fontenelle et al. 2021b).

Fishes of the family Tetraodontidae are distributed worldwide in tropical and subtropical waters, comprising 194 species, with about 30 species inhabiting primarily freshwaters (Nelson et al. 2016; Fricke et al. 2022). The origin of this clade is estimated by the age of the oldest fossil assigned to Tetraodontidae, which dates to Middle Eocene (∼50 Ma) (Santini et al. 2013). Lineages of this clade have transitioned to freshwaters multiple times, with the highest diversity occurring in Southeast Asia, but also occurring in Africa and South America (Yamanoue et al. 2011; Santini et al. 2013).

Each group was independently analyzed following an identical pipeline. We obtained the maximum body size data and habitat type for each species using data from measurements, primary literature, and FishBase (Froese and Pauly 2021) (Supplementary Table S1). We also leveraged our collective taxonomic expertise to provide quality control of these data from aggregated sources. Body size was recorded as disc width (DW) for stingrays and as standard length (SL) for all other groups, and $\log_{10}$ transformed for all statistical analyses. We classified fish species as freshwater, marine, or diadromous, and considered species that live primarily in brackish waters as marine. Our phylogenetic comparative analyses were conducted using the most recent time-calibrated phylogenic trees available for each trans-marine/freshwater group (Supplementary Table S1).

Body size evolution across habitats

We used boxplots to visualize differences in body size between marine, freshwater, and diadromous fishes. We tested for significant differences in body size of marine, freshwater, and diadromous species within each of the nine major trans-marine/freshwater clades using phylogenetic ANOVA (Garland et al. 1993) implemented in the R package *phytools* (Revell 2012). To analyze the variation of body size between marine, freshwater, and diadromous species we calculated the disparity of the species from each habitat using the function morphol.disparity in the R package *geomorph* (Adams and Otárola-Castillo 2013). All subsequent estimations of disparity were calculated using this function. Absolute differences in variances between groups were calculated in a permutation test with 9999 iterations to test for statistical differences in disparity between the species from each habitat.

To identify the number of habitat transitions and trends in body size evolution across trans-marine/freshwater groups, we estimated the ancestral habitat states and the ancestral values of body size across phylogenies using traitgrams (Revell 2013). Traitgrams visualize patterns of phenotypic convergence associated with habitat transitions by projecting the phylogenetic tree with the mapped ancestral habitats into a two-dimensional space, in which the *y*-axis represents the phenotype (body size) and the *x*-axis represents the time (Revell 2013). We reconstructed the ancestral habitat types across the phylogenies using stochastic character mapping (SIMMAP) (Nielsen 2002; Huelsenbeck et al. 2003). For each trans-marine/freshwater clade, we generated 1000 SIMMAP replicates and estimated the posterior probability of ancestral states for nodes by averaging habitat state frequencies across replicates. We then generated traitgrams with one random SIMMAP out of the 1000 to visualize how body size responded to shifts in habitat, and whether the direction of body size evolution was consistent for a given habitat. Both the SIMMAPs and traitgrams were created using the R package *phytools* (Revell 2012).

We also tested whether shifts in body size evolution were linked to habitat transitions by comparing estimated evolutionary shifts in trans-marine/freshwater groups without *a priori* assignment of ecological states as habitat transitions (Burns and Sidlauskas 2019) using the R package *PhylogeneticEM* (Bastide et al. 2018). We then visually inspected evolutionary shifts in body size and habitat shifts reconstructed from our SIMMAP analyses to determine if these occurred at the same nodes. If these co-occurrences were detected in a clade, we calculated the probability of these co-occurrences given the same number of morphological and ecological shifts randomly distributed across the phylogeny. The lower the probability of co-occurrence, the greater the indication that the evolutionary shifts in body size were driven by habitat transitions (Burns and Sidlauskas 2019).

Next, we used phylogenetically independent contrasts (PICs) to determine if there was a pattern of increase or decrease in body size at nodes associated with habitat transitions (Friedman et al. 2020). We obtained the independent contrasts and ancestral states of the nodes that immediately preceded habitat transitions in each SIMMAP and recorded the daughter branch in which the transition occurred, including the type of transition (e.g., marine to freshwater, freshwater to diadromous, etc.). We then calculated the average PIC values of each clade by type of transition, where negative values indicated decreases and positive values indicated increase in body size. We then used a *t*-test on the average PICs per habitat transition type in each clade. If an independent contrast differed significantly from zero, that indicated that the respective habitat transition was associated with directional morphological change (Friedman et al. 2020).

Habitat transitions and phenotypic disparity

We tested for a correlation between body size disparity and habitat transitions to infer whether transitions to freshwaters increased access to ecological opportunity, leading to subsequent increases in phenotypic disparity. We estimated disparity by calculating variance of body size of freshwater species from each trans-marine/freshwater clade. We then retrieved the average number of habitat transitions to freshwaters from the SIMMAPs of each clade. To remove the effect of clade size in the analysis, we divided the number of transitions by the total number of the nodes in each phylogeny, obtaining the relative number of transitions from marine/diadromy to freshwaters. We used linear regression to correlate body size disparity of the trans-marine/freshwater clades and their relative number of habitat transitions, and we tested for statistical significance using Spearman's rank correlation coefficient.

Next, we tested whether the observed body size disparity of freshwater lineages was higher or lower than expected by chance following the invasion of freshwaters. We simulated evolution of body size 1000 times under a Brownian motion model across the respective trees for each clade using the function sim.char in the R package *Geiger* (Pennell et al. 2014). We estimated body size disparity using the function morphol.disparity in the R package *geomorph* (Adams and Otárola-Castillo 2013) for each simulation, and extracted the values of body size disparity of freshwater lineages. To determine whether the observed body size disparities were higher or lower than the simulations, we counted the number of simulations that showed a body size disparity higher than the empirical disparity and compared the average value of disparity in each major trans-marine/freshwater clade with the observed value. The number of simulations with body size disparity higher than the observed in the empirical data determined the significance of the observed disparity difference.

Correlation between body size disparity and freshwater diversity

We used linear regressions to determine the relationship between the richness of freshwater species and phenotypic disparity of invading lineages. To infer the body size disparity of the trans-marine/freshwater clades in each freshwater region, we added the available body size data of freshwater taxa that were not present in the phylogenies to the original dataset (Supplementary Table S1). The body size data of the additional freshwater species not present in the phylogenies and the geographical distribution of all taxa were obtained from FishBase (Froese and Pauly 2021) using the R package *rfishbase* (Boettiger et al. 2012). First, we assessed the correlation between body size disparity of trans-marine/freshwater species that colonized freshwaters against the diversity of primary freshwater fish per region. To explore whether the overall trend in body size disparity is consistent across all clades, we regressed body size disparity of trans-marine/freshwater fishes against the diversity of closely related species for each clade separately. Second, we regressed body size disparity of trans-marine/freshwater species that colonized freshwaters against the diversity of closely related species per region. For the diversity of closely related taxa, we considered the total number of freshwater species of each trans-marine/freshwater clade in each biogeographical region (e.g., all the freshwater clupeiforms in the Neotropics). To explore whether the overall trend in body size disparity is consistent across all regions, we regressed body size disparity of trans-marine/freshwater fish against the diversity of closely related species in each region separately. The additional list of closely related taxa in each freshwater region not present in the phylogenies was obtained from FishBase (Froese and Pauly 2021) using the R package *rfishbase* (Boettiger et al. 2012) (Supplementary Table S1). We classified freshwater systems into six major biogeographical regions: Australian, Ethiopian, Nearctic, Neotropical, Oriental, and Palearctic. For each of the nine trans-marine/freshwater clades, we calculated the disparity of body size of each region by obtaining the variance of body size of freshwater species in that region. We obtained the number of primary freshwater fish species in each biogeographical region from Leroy et al. (2019). We used the current diversity of fish species as a proxy for past competition during the evolution of the trans-marine/freshwater lineages in each freshwater invasion event (Betancur-R. et al. 2012).

Results

Body size evolution across habitats

Our results show that there are few differences in body size between marine and freshwater species of trans-marine/freshwater fishes (Fig. 1). Our phylogenetic ANOVAs showed that size differences are not statistically significant for most trans-marine/freshwater clades: Ariidae (*P*-value = 0.792), Atheriniformes (*P-value* = 0.098), Belonidae (*P*-value = 0.009), Clupeiformes (*P*-value = 0.005), Cottoidea (*P-value* = 0.453), Gobiesocidae (*P-value* = 0.184), Gobiidae + Oxudercidae (*P*-value = 0.023), Myliobatiformes (*P*-value = 0.89), Tetraodontidae (*P*-value = 0.378). Only Belonidae showed significant differences in body size between marine and freshwater habitats. In Clupeiformes and Gobiidae + Oxudercidae, the pairwise difference between marine and freshwater species is not significant (Supplementary Table S2), but our ANOVA detected a significant difference among habitats due to the larger body size of diadromous taxa in both groups, respectively.

Our analysis of the variation in body size between habitats showed that the disparity of body size is higher in marine than freshwater habitats in Atheriniformes and Cottoidea; Belonidae and Tetraodontidae revealed the opposite pattern, with higher disparity of body size in freshwater than marine habitats. Ariidae, Clupeiformes, Gobiesocidae, Gobiidae + Oxudercidae, and Myliobatiformes had higher disparity of body size in diadromous species (Table 1). Cottoidea and Myliobatiformes had statistically significant higher disparity in marine habitats than in freshwaters. Clupeiformes,

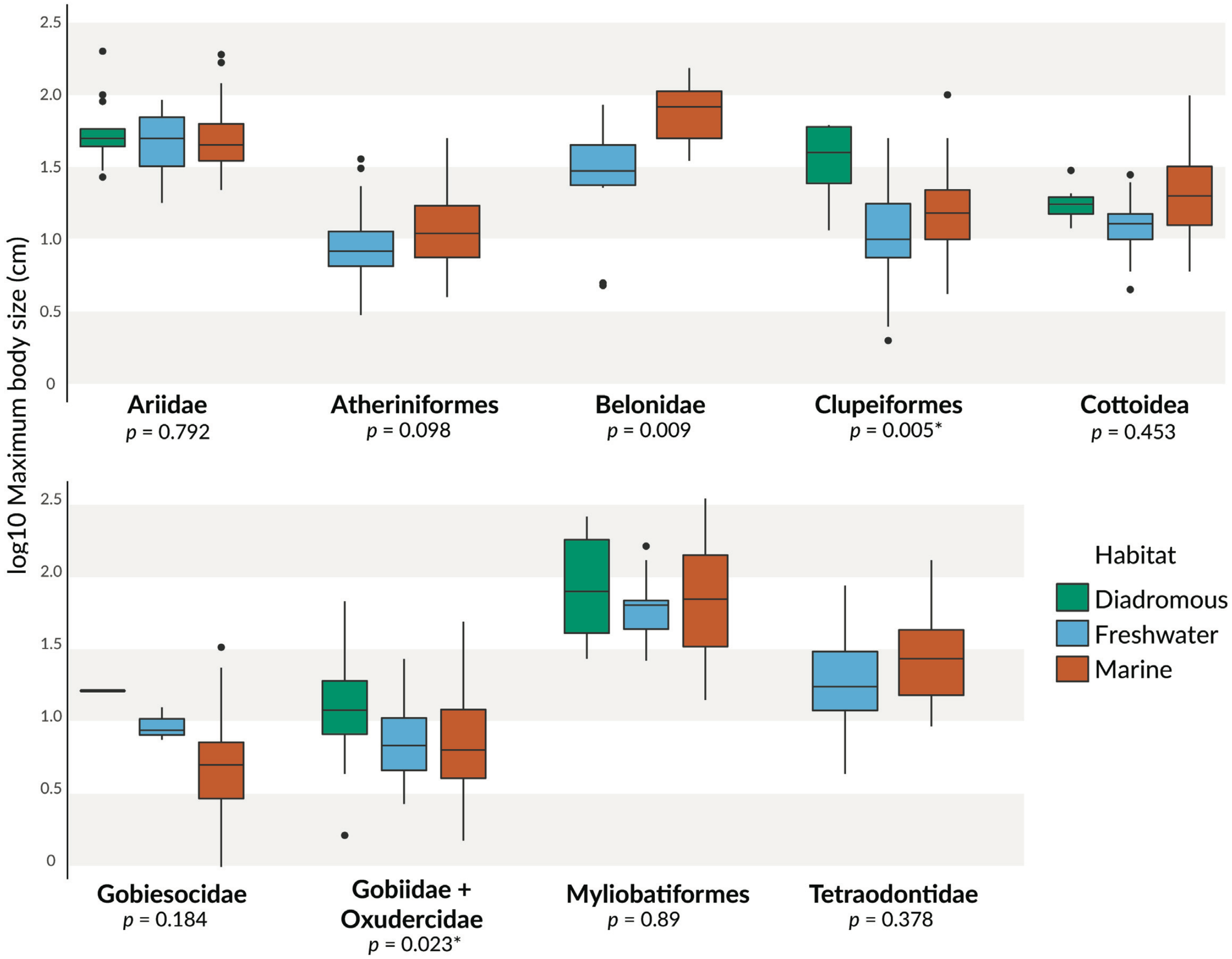

Figure 1. Boxplots of the $\log_{10}$ transformed body sizes of diadromous (green), freshwater (blue), and marine (orange) species for nine trans-marine/freshwater clades. The *P*-values reported for each clade are from phylogenetic ANOVAs. Asterisks (∗) indicate non-significant pairwise differences between marine and freshwater species.

Table 1. Variance (disparity) of body size in diadromous, freshwater, and marine species from nine trans-marine/freshwater clades

Clade	Diadromous	Freshwater	Marine
Ariidae	0.0532	0.0380	0.0373
Atheriniformes	–	0.0604	0.0797
Belonidae	–	0.2313	0.0713
Clupeiformes	0.1863	0.1322	0.0582
Cottoidea	0.0124	0.0428	0.0816
Gobiesocidae	0.2585	0.0796	0.0831
Gobiidae + Oxudercidae	0.1261	0.0638	0.1021
Myliobatiformes	0.1499	0.0354	0.1381
Tetraodontidae	–	0.1074	0.0848

showed the opposite pattern, with statistically significant higher disparity in freshwater than in marine habitats. Clupeiformes also had statistically significant higher disparity in diadromous species than in marine species. In contrast, Cottoidea had statistically significant higher disparity in marine than in diadromous species. Gobiidae + Oxudercidae and Myliobatiformes had statistically significant higher disparity in diadromous species than in freshwater species. There was a variation in body size disparity among other clades, but the differences were not statistically significant (Table 2).

The traitgrams show that transition to a new habitat was often associated with a divergence in body size (Fig. 2), but the directionality was not consistent. How-

Table 2. Pairwise comparison of the body size variance (disparity) between marine, freshwater, and diadromous species from trans-marine/freshwater clades

Clade	Marine–freshwater		Marine–diadromous		Freshwater–diadromous	
	Difference	*P*-value	Difference	*P*-value	Difference	*P*-value
Ariidae	0.0007	0.9621	0.0159	0.3858	0.0152	0.4564
Atheriniformes	0.0193	0.3452	–	–	–	–
Belonidae	0.1600	0.092	–	–	–	–
Clupeiformes	0.0740	**$8e^{-04}$**	0.1281	**$7e^{-04}$**	0.0541	0.1180
Cottoidea	0.0388	**0.0046**	0.0692	**0.0300**	0.0304	0.3483
Gobiesocidae	0.0035	0.9660	0.1754	0.0854	0.1789	0.1436
Gobiidae + Oxudercidae	0.0383	0.0780	0.0240	0.1264	0.0623	**0.0157**
Myliobatiformes	0.1027	**0.0004**	0.0118	0.8279	0.1145	**0.0405**
Tetraodontidae	0.0226	0.3688	–	–	–	–

Values in the columns labeled "Difference" are the observed pairwise absolute differences between the body size variance in each habitat. Values in the subsequent columns are the *P*-values associated with the pairwise differences. Significant *P*-values are in bold.

ever, we found that habitat transitions may be associated with an increase in body size variation. Belonidae is the only group with a clear pattern of body size evolution, with a consistent size reduction in freshwater lineages. Our traitgrams indicated there is no common optimum body size associated with habitat shifts.

Our analysis of evolutionary shifts without *a priori* assignment of habitat states estimated 23 evolutionary shifts in body size among the trans-marine/freshwater clades. Of those, we identified only three that were linked with habitat shifts on the SIMMAPs (one in each of Ariidae, Belonidae, and Tetraodontidae; see Supplementary Fig. S1). The probability of obtaining the same number of co-occurrences by randomizing the same number of evolutionary shifts in body size and habitat transitions on the phylogenies is high (59% in Ariidae; 15% in Belonidae; 34% in Tetraodontidae), indicating no significant association between shifts in body size and shifts in habitat.

Our analysis of the phylogenetically independent contrasts (PICs) revealed no consistent patterns of increase or decrease in body size across the different types of habitat transitions in trans-marine/freshwater fish groups (Table 3; Supplementary Table S3). All types of habitat transitions presented both positive and negative PIC values across the different groups, indicating no consistent pattern of body size increase of decrease. A *t*-test revealed that 38 of the 41 average PICs were not statistically different than zero, indicating no association between most of the habitat transitions and directional morphological change. The three significant average PICs indicate increase in body size in transitions from marine habitat to diadromy in Ariidae and Gobiesocidae and a decrease in body size moving from freshwaters to marine habitats in Gobiidae + Oxudercidae. These results indicate that the direction of body size variation (increase or decrease) of trans-marine/freshwater lineages that colonize a new habitat is not predictable on the type of habitat transitions.

Habitat transitions and phenotypic disparity

Our analysis of the correlation between body size disparity of freshwater species from trans-marine/freshwater clades and habitat transitions to freshwater shows a trend of increase in body size disparity as the relative number of habitat transitions to freshwaters increases ($r^2 = 0.25$), but the relationship is statistically non-significant (*P*-value = 0.52) (Fig. 3). Eight of the nine trans-marine/freshwater clades had average simulated body size disparities in freshwater lineages higher than the observed disparities. Ariidae had an average simulated body size disparity in freshwater lineages of 0.1121; an average of 0.6301 in Atheriniformes; 0.2067 in Clupeiformes; 0.3688 in Cottoidea; 0.1550 in Gobiesocidae; 0.3810 in Gobiidae + Oxudercidae; 3.4790 in Myliobatiformes; and 1.8329 in Tetraodontidae. Belonidae had an average simulated body size disparity in freshwater lineages of 0.1175, the only clade to exhibit a value lower than the observed disparity. In Atheriniformes, Cottoidea, Gobiidae + Oxudercidae, Myliobatiformes, and Tetraodontidae, all the simulated body size disparities were higher than the observed disparities. In Ariidae, 0.8% of the simulated disparities were lower than the observed disparities; in Clupeiformes, 28.2%; and in Gobiesocidae, 34.2%. In Belonidae, 5.9% of the simulated body size disparities were higher than the observed disparities.

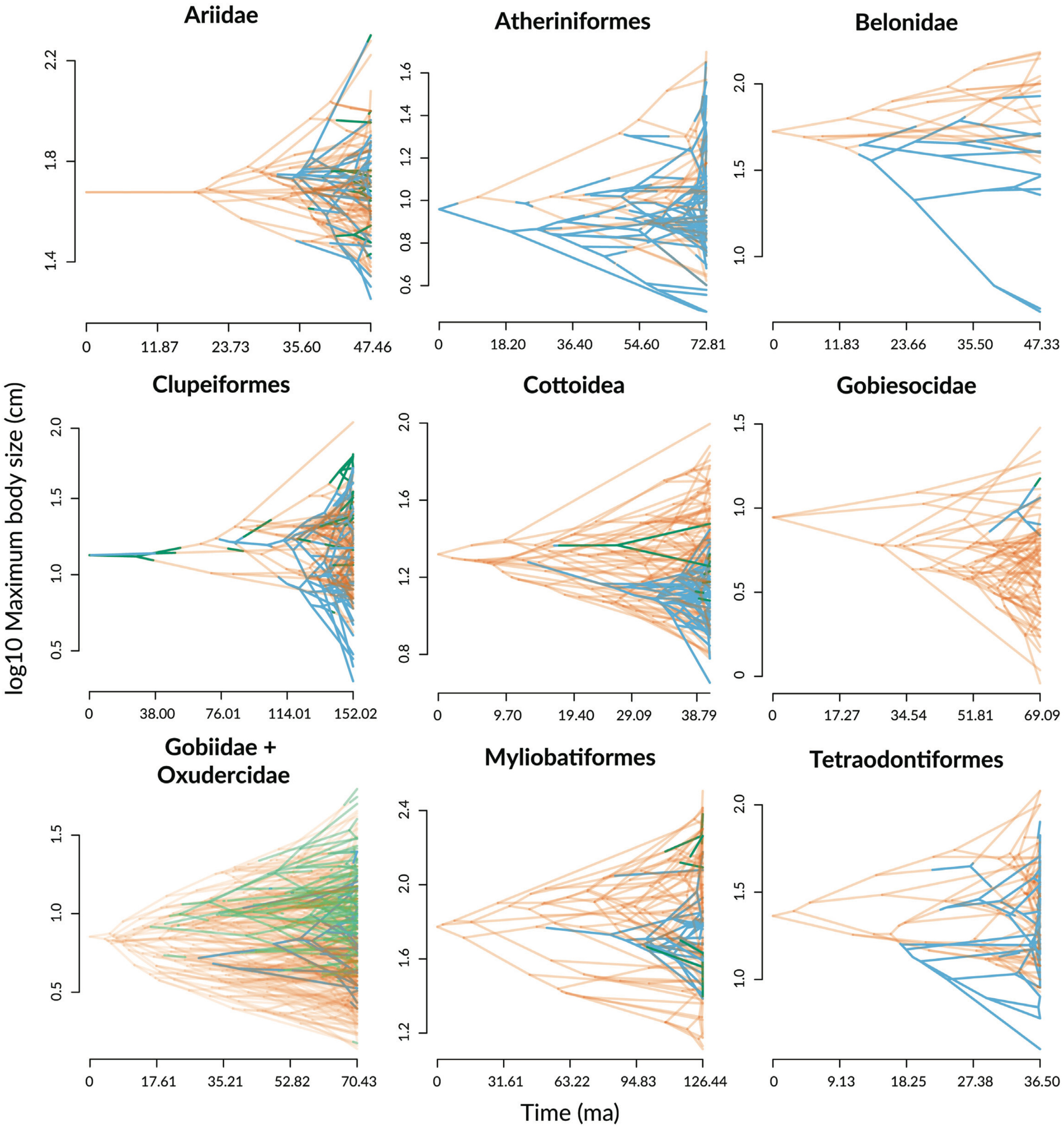

Figure 2. Traitgram projections of the $\log_{10}$ transformed body size for trans-marine/freshwater groups using their respective phylogenies. The *y*-axis represents the log transformed body size and the *x*-axis represents the time in millions of years (ma). Marine taxa are orange, freshwater taxa are blue, and diadromous are green.

Correlation between body size and community data

Our analysis of the correlation between the disparity of body size of trans-marine/freshwater fishes and the richness of incumbent and closely related freshwater species revealed a positive correlation between the disparity of body size and the diversity of primary freshwater fish species in each zoogeographical region ($r^2 = 0.28$, P-value $= 1.2\mathrm{e}^{-13}$) (Fig. 4 left), and a negative correlation between the disparity of body size and the diversity of closely related species (r^2 = -0.3, P-value $< 2.2\mathrm{e}^{-16}$) (Fig. 4 right). The plots show that the greater the diversity of primary freshwater fish species in a region, the greater the disparity of body size among invading species, but conversely that the greater the diversity of a trans-marine/freshwater clade in a re-

Table 3. Average PIC values on nodes that immediately preceded habitat transitions

Group	Marine to freshwater	Freshwater to marine	Marine to diadromous	Diadromous to marine	Freshwater to diadromous	Diadromous to freshwater
Ariidae	0.004574395	0.002191148	**0.012073512**	0.005481187	0.003305798	– 0.004053804
Atheriniformes	– 0.002757934	0.002290687	–	–	–	–
Belonidae	– 0.000134211	0.000320001	–	–	–	–
Clupeiformes	0.000309326	– 0.001252346	0.002151545	0.000311829	0.001563676	0.000599415
Cottoidea	– 0.000217989	0.042609876	– 0.007294902	0.025480435	– 0.001070917	– 0.009175285
Gobiesocidae	0.01236303	0.01918168	**0.05037158**	–	0.09703473	– 0.038373
Gobiidae + Oxudercidae	– 0.00161966	**– 0.006775675**	– 0.000560568	– 0.003332464	0.001376711	– 0.0038905 1
Myliobatiformes	0.001375258	– 0.009185546	– 0.01427276	0.065984325	0.0081808	– 0.012857319
Tetraodontidae	0.008886041	– 0.015950729	–	–	–	–

The values significantly different from zero are highlighted in bold.

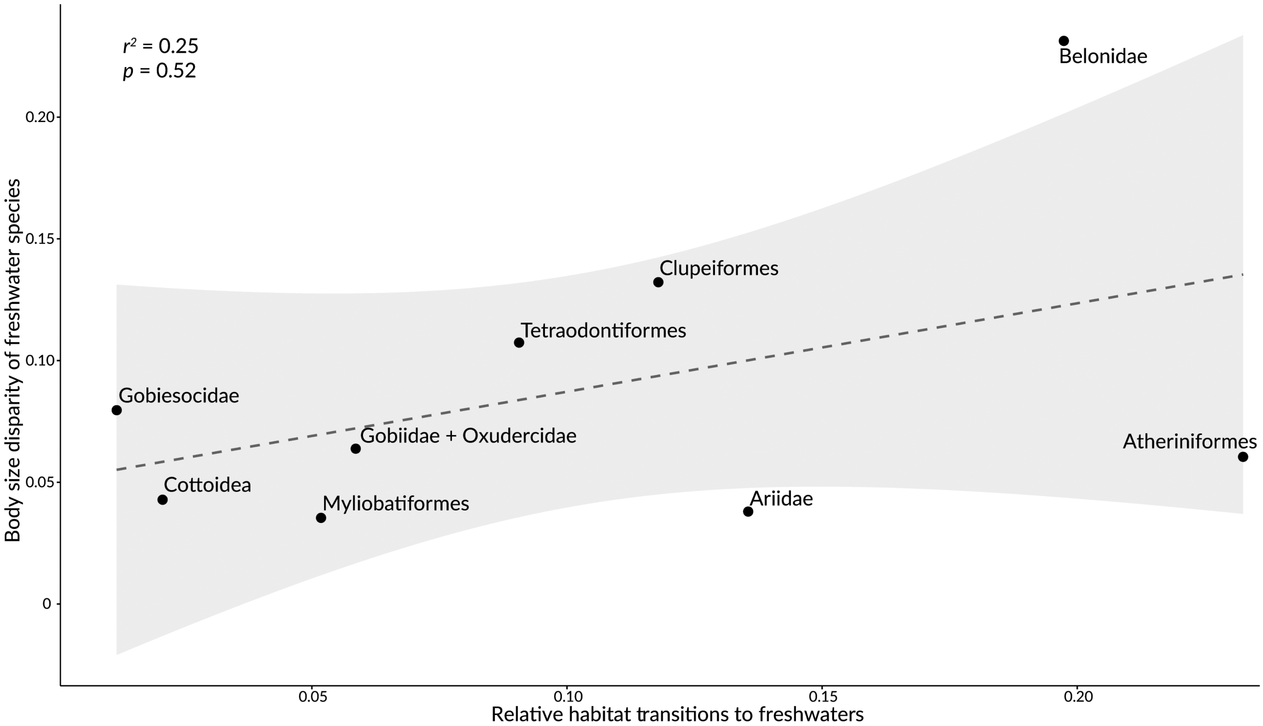

Figure 3. Scatterplot of the body size disparity and the relative number of habitat transitions to freshwaters for each trans-marine/freshwater group. The shaded region in light gray around the regression line represents the 95% confidence interval.

gion, the less diverse in body size their species tend to be.

Our analysis of body size variation in each clade (Fig. 4 left) revealed a positive correlation between the disparity of body size of trans-marine/freshwater fishes and the diversity of primary freshwater fish species in each zoogeographical region, congruent with the overall trend, in Ariidae ($r^2 = 0.49$, P-value = 2.1e^{-4}), Atheriniformes ($r^2 = 0.58$, P-value < 2.2e^{-16}), Myliobatiformes ($r^2 = 0.41$, P-value = 0.012), and Tetraodontidae ($r^2 = 0.56$, P-value = 8.2e^{-05}). The trend in body size variation was negative in Clupeiformes (r^2 = -0.84, P-value < 2.2e^{-16}), Cottoidea (r^2 = -1, P-value < 2.2e^{-16}), Gobiesocidae (r^2 = -1, P-value < 2.2e^{-16}), and Gobiidae + Oxudercidae (r^2 = -0.59, P-value < 2.2e^{-16}). Belonidae only has more than two freshwater species in the Neotropical region, preventing analysis of body size disparity.

Our analysis of body size variation in each region (Fig. 4 right) revealed a negative correlation between the disparity of body size of trans-marine/freshwater fishes and the richness of closely related freshwater species in each zoogeographical region, congruent with the overall trend, in the re-

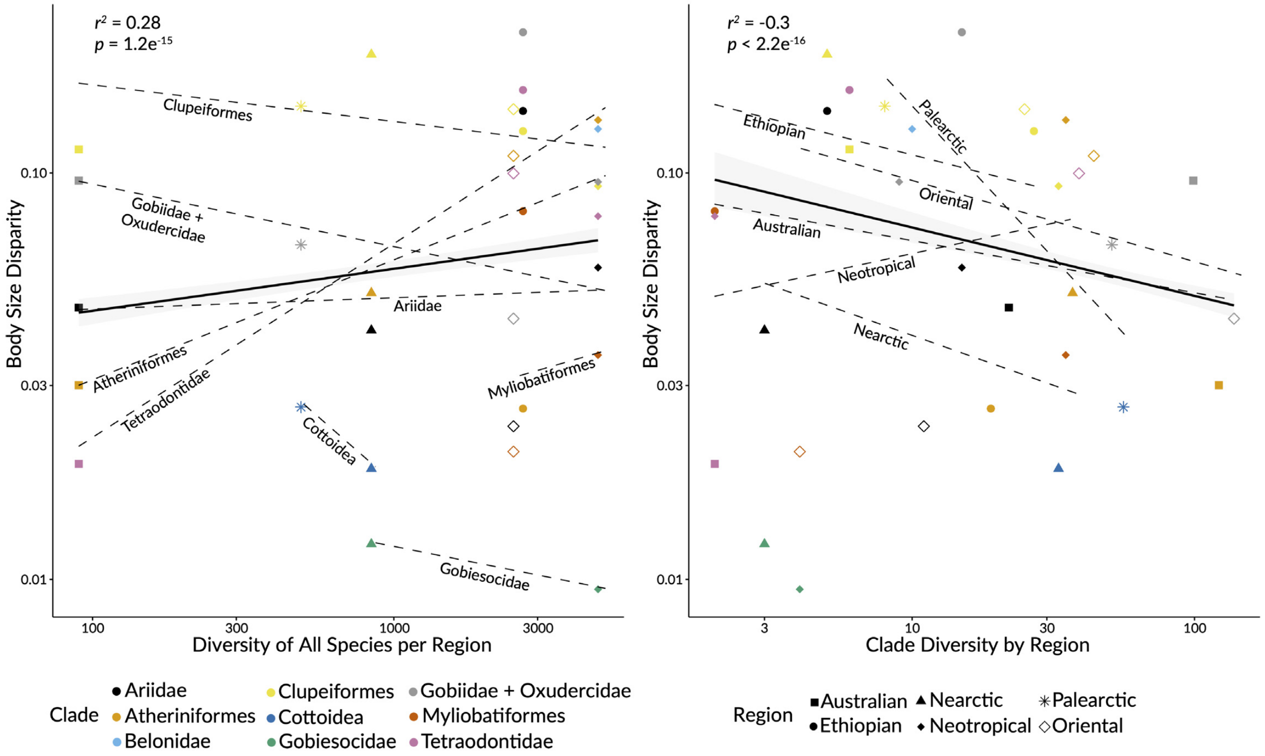

Figure 4. Correlation of body size disparity in trans-marine/freshwater species and the richness of primary freshwater fish species in each zoogeographical region (left) and the number of closely related fish species in each zoogeographical region (right). The overall regression line is represented by the solid black line and the covaries are represented by the dashed lines. The shaded region around the regression line represents the 95% *CI*.

gions Australian (r^2 = -0.82, *P*-value $< 2.2e^{-16}$), Ethiopian (r^2 = -0.42, *P*-value $= 2.1e^{-5}$), Neotropical (r^2 = -0.064, *P*-value = 0.49), Palearctic (r^2 = -1, *P*-value $< 2.2e^{-16}$), and Oriental (r^2 = -5, *P*-value $= 2.1e^{-14}$). The trend in body size variation was positive in the region Nearctic (r^2 = 0.46, *P*-value $= 1.4e^{-5}$).

Discussion

Unpredictable trajectories in body size evolution following habitat transitions

Evolutionary transitions between marine and freshwaters represent an extreme ecological shift and expose lineages to novel abiotic and biotic settings (Lee and Bell 1999; Vega and Wiens 2012; Seehausen and Wagner 2014; Davis et al. 2020). These transitions have been hypothesized to drive shifts in evolution of phenotypic traits (Lee and Bell 1999; Betancur-R et al. 2012; Seehausen and Wagner 2014; Bloom et al. 2020; Davis et al. 2020; Kolmann et al. 2020). However, our analyses showed that evolutionary transitions between marine and freshwater environments generally do not result in predictable patterns of body size evolution (Fig. 2). Our phylogenetic ANOVA (Fig. 1) confirmed there is no significant difference in body size between marine and freshwater taxa, and our PIC (Table 3) showed that the invasion of marine or freshwater systems is not directly correlated to changes in body size in trans-marine/freshwater fishes. Additionally, only three of nine clades had statistically significant differences in body size disparity between marine and freshwaters (Tables 2 and 3), and the difference in disparity is not predictable by habitat or directionality of habitat transition. Our results show that habitat transitions between marine and freshwaters are not the main drivers of body size evolution in fishes and only the adoption of diadromy seems to have a predictable effect on increasing the body size of trans-marine/freshwater clades.

Our results suggest that there is not a predictable shift in body size associated with the invasion of marine or freshwater habitats in fishes. These results contrast with previous studies that showed that fishes that transition between marine and freshwater habitats are prone to convergence, such as diet and life history traits (Egan et al. 2018; Davis et al. 2020). For instance, the transition to freshwaters could offer access to novel prey resources (e.g., aquatic larval insects and adult terrestrial insects) compared to the marine environment (Egan et al. 2018; Kolmann et al. 2020, 2022). If those novel prey resources are smaller than ancestral marine prey items, such as the evolution of terrestrial invertivory by freshwater clupeiforms (Egan et al. 2018) and stingrays (Kolmann et al. 2022), this dietary shift could lead to reduction in body size, as prey size can be positively correlated with predator body size in fishes (Romanuk et al. 2011; Egan et al. 2017). Conversely, our results suggest other factors, such as biogeography, ecological aspects, and intrinsic clade differences may impose stronger selective pressures, resulting in contingency in body size evolution. Moreover, similar ecological settings may present themselves in both marine and freshwater systems, offering opportunity for convergent phenotypic patterns. For instance, Weitzman and Vari (1988) suggested extremely small body sizes may facilitate the exploration of complex freshwater microhabitats, but complex microhabitats and extreme body size reduction can occur in both marine and freshwater environments. In trans-marine/freshwater fishes, miniature marine gobies and clingfishes have cryptic life cycles associated with reefs and complex marine habitats (Herler et al. 2011; Conway et al. 2019; Fujiwara et al. 2021), and extreme body size reduction is also reported in freshwater species of atheriniforms, clupeiforms, and belonids that inhabit freshwaters in tropical forests (Parenti 1996; Bloom et al. 2020; Kolmann et al. 2020). The reduction of body size in freshwater Amazonian belonids (needlefishes and halfbeaks) may be associated with greater maneuverability in structurally complex environments, such as smaller rivers, streams, and wetlands (Kolmann et al. 2020). Similar to their marine relatives, freshwater belonids occupy epipelagic or limnetic habitats, typically cruising just below the water's surface (Goulding and Carvalho 1983). However, only freshwater species that occupy open-water habitats in medium to large rivers (e.g., *Pseudotylosurus*) maintain a highly piscivorous trophic niche and retain a body plan similar to marine taxa (Kolmann et al. 2020). The similarity in body size among some marine and freshwater taxa indicates a degree of phenotypic conservatism despite clades undergoing repeated habitat transitions.

Although marine/freshwater transitions are not strong drivers of body size evolution in fishes, the adoption of migratory life cycles may have generated strong pressure to increase size in comparison to non-migratory relatives. The pattern of larger body size of diadromous fishes in comparison to their non-migratory relatives detected in our study is consistent with previous studies (Griffiths 2012; Bloom et al. 2018; Burns and Bloom 2020). The evolution of larger body size in diadromous fishes is linked to the decrease in the proximate costs of migration (Hendry and Stearns 2003). Larger body size allows for greater swimming efficiency and lower energy costs, required to survive long migration distances and bypass barriers to movement (Roff 1991; Hendry and Stearns 2003). Macroevolutionary studies have linked these proximate causes to higher rates of morphological evolution in migratory lineages than non-migratory lineages, demonstrating this is a broad pattern that spans wide phylogenetic scales (Burns and Bloom 2020). Our results further demonstrate that these differences in diadromous fishes are likely driven by factors other than differences between marine and freshwater environments.

Habitat transitions and body size disparity

We did not detect an increase in body size disparity associated with transitions to freshwater habitats (Fig. 3). Although our analysis returned a positive correlation between habitat transitions and body size disparity, these differences were not statistically significant. Additionally, our comparison between the observed body size disparity and simulated body size disparity under Brownian motion in freshwater lineages indicates that in most clades, the transition to freshwaters constrained the diversification of body size in fishes, leading to a lower diversity than expected by stochastic processes. These results suggest that transitions to freshwaters are not consistently presenting ecological opportunity and facilitating adaptative radiations. Instead, there are isolated instances where transitions to freshwaters lead to increased morphological disparity. For example, marine sticklebacks have colonized multiple freshwater streams and lakes independently after the retreat of Pleistocene glaciers, and they have experienced adaptive radiation in these novel habitats, varying the number of lateral plates and body size according to local selective pressures (Hagen and Gilbertson 1973; Bell and Foster 1994; Colosimo et al. 2005; Berner et al. 2009; Deagle et al. 2012; Jones et al. 2012; Kaeuffer et al. 2012; Lucek et al., 2013, 2014). New world anchovies possibly have experienced ecological diversification following the invasion of freshwater habitats, given the early and substantial diversification of large- and small-bodied lineages (Bloom and Lovejoy 2012). Freshwater ariid catfishes have higher rates of phenotypic evolution than their marine relatives (Betancur-R et al. 2012), but this pattern is mostly driven by ecological opportunity experienced by lineages that colonized Australian and New Guinean inland waters, regions with depauperate diversity of incumbent freshwater species. Ecological diversification in freshwaters could also affect the evolution of traits other than body size, such as body shape (Bloom et al. 2020; Kolmann et al. 2020; Friedman et al. 2021), trophic niches (Davis et al. 2012; Egan et al. 2018), reproduction (Davis et al. 2020), and behavior (Fuller et al. 2007).

Conversely, the reduced body size disparity in freshwater lineages compared to that expected under a stochastic process could stem from constraining selective pressures resulting from marine/freshwater transitions. Habitat transitions may act as strong environmental filters, constraining the phenotypes able to undergo these extreme events (Kraft et al. 2015). It is widely held that generalist species are best suited to colonize novel environments, which suggests that phenotypic extremes might be selected against during and following habitat transitions (Bamber and Henderson 1988). Few major clades have successfully colonized both marine and freshwaters across the evolutionary history of fishes (Vega and Wiens 2012), suggesting there are strong barriers between these habitats. The stark difference in abiotic conditions between marine and freshwaters may increase the strength of environmental filtering, leading to reduced trait diversity as environmental stress and stabilizing selection increase (Weiher and Keddy 1995).

Correlation between body size disparity and freshwater diversity

Our analysis of the relationship between richness and phenotypic disparity revealed an overall positive correlation between body size disparity and diversity of incumbent freshwater species, and an overall negative correlation between body size disparity and diversity of closely related species (Fig. 4). These results indicate that competition with a broad community of incumbent freshwater species does not limit body size evolution following transitions to freshwater habitats. Previous works suggest that interaction between trans-marine/freshwater clades with incumbent freshwater taxa has a great impact on the phenotypic evolution of those fishes (Betancur-R et al. 2012; Bloom and Lovejoy 2012; Santini et al. 2013a; but see Kolmann et al. 2022). Regions with diverse incumbent fauna are thought to limit the diversification of new lineages due to the lack of niche availability (Patterson and Givnish 2002). Our results indicate that competition with a diverse community of incumbent freshwater species may have led to increase in the body size disparity of trans-marine/freshwater groups. The theory of ecological opportunity posits that diversification could be facilitated by little or no competition (Simpson 1953; Schluter 2000). However, species interaction could act as a strong selective pressure to drive adaptive changes in phenotype (e.g., character displacement theory) (Brown and Wilson 1956; Grant 1972; Pfennig and Pfennig 2009). The effect of incumbency and competition in diverse communities could be driving trans-marine/freshwater lineages to occupy open niches unexplored by incumbent freshwater fishes, instead of restricting their diversification, leading to the evolution of diverse body sizes, such as the extreme size reduction in Belonidae (Kolmann et al. 2020) and Clupeiformes (Bloom et al. 2020). In other fish groups, such as the haplochromine cichlids in Lake Victoria, release from competition alone does not explain their ecological radiation, as other lineages of fishes occupied the same environment during the formation of the lake (Muschick et al. 2018). In muroid rodents distributed across the globe, studies suggest that decelerating rates of phenotypic evolution in lineages of secondary colonists may not result from interaction with incumbent species (Schenk et al. 2013; Rowsey et al. 2019). In these rodents, it is also thought that incumbency may act as a biotic filter, competitively excluding potential ecologically similar invaders, leading secondary colonists to occupy distinct areas of trait space from incumbent clades (Rowsey et al. 2019). Thus, the diversity of incumbent freshwater taxa might have acted as filters, possibly limiting invaders that were too ecologically similar from colonizing new regions outright (i.e., the priority effect; MacArthur 1972; Chase 2007), but also redirecting phenotypic diversification of trans-marine/freshwater fishes that were able to overcome the initial filter and successfully establish themselves.

We found a significant relationship between the reduction in body size disparity in freshwater regions and an increase in diversity of closely related species (Fig. 4), which supports our prediction that body size variation has a stronger correlation with the richness of closely related taxa than the overall richness of incumbent species. This trend in decreasing body size disparity in freshwaters indicates that competition with closely related species did not drive character displacement in trans-marine/freshwater fishes, but instead may have limited phenotypic diversification. Darwin's competition-relatedness hypothesis states that closely related species tend to be stronger competitors than distantly related species due to higher trait similarity (Cahill et al. 2008). This hypothesis predicts that natural selection may lead to an increase in phenotypic disparity, favoring the tendency of reduction in similarity between lineages to avoid costly interactions (Rabosky 2013; Anderson and Weir 2021). Conversely, our results suggest that body size diversification is restricted by the presence of more closely related lineages in the same region. Competition by close relatives stymieing diversification has been demonstrated in laboratory microbial experiments, in which the ecological diversification of the bacterium *Pseudomonas fluorescens* was progressively more restricted in the presence of ecologically similar populations of the same species (Brockhurst et al. 2007). High species richness of closely related species may inhibit, rather than promote phenotypic diversification, and thus regulate the scope of diversification attainable by ecologically similar lineages.

The limited body size diversification in the presence of higher diversity of closely related species may also be explained by instances of non-adaptive radiation (Gittenberger 1991; Kozak and Wiens 2006; Rundell and Price 2009). Non-adaptive radiation occurs when increased speciation precedes significant ecological differentiation and is not coupled with increased phenotypic disparity (Rundell and Price 2009; Martin and Richards 2019). A signature of non-adaptive diversification is the retention of ancestral niches over prolonged evolutionary time, and in some cases associated with elevated rates of speciation (Kozak and Wiens 2006). For example, in eastern North American woodland salamanders, phylogenetic niche conservatism contributes to rapid lineage diversification by promoting vicariant isolation and speciation across spatially dynamic environments (Kozak and Wiens 2006). In the flannelmouth characin families Curimatidae and Prochilodontidae, many species occupy a small cluster of related niches varying within a small range of morphologies (Sidlauskas 2008). These fishes have a series of morphological adaptations to detritivorous feeding habits, which allows them to exploit an ecological niche unexplored by other South American characins (Bowen 1983; Flecker 1996). The successful ecological exploitation has likely promoted widespread distribution and increased the likelihood of allopatric speciation in these clades. A similar process may explain phenotypic similarity of lineages that colonized and diversified across continental freshwaters.

In trans-marine/freshwater fishes, lineages that invaded freshwaters are also subjected to dynamic habitat fragmentation and limited dispersal ability (Bloom et al. 2013; Tedesco et al. 2017; Albert et al. 2020). The rapid cladogenesis arriving from this biogeographical pattern can be decoupled from morphological diversification (Simões et al. 2016), which may not generate body size diversification. Additionally, species that transition between marine and freshwaters are subjected to environmental filtering (Emerson and Gillespie 2008; Cavender-Bares et al. 2009). The ecological constraints imposed by the transitions between marine and freshwater habitats could result in stabilizing selection on traits that allowed the successful establishment of fishes in those habitats. This pattern of community assembly mediated by abiotic factors would select for species with similar niches as a consequence of their phylogenetic relatedness (Mayfield and Levine 2010). Furthermore, closely related species with small niche differences can coexist even in the presence of only slight differences in competitive ability (Mayfield and Levine 2010; Kraft et al. 2015; Cadotte and Tucker 2017). For marine fishes which invaded freshwaters, lower body size disparity in regions with higher richness of closely related species might be overcome by low differences in competitive ability among invaders and their relatives. We argue that transitions between marine and freshwater habitats alone are not the best predictors of morphological diversification generated by ecological opportunity. The theory of ecological opportunity posits that transitions to new habitats can lead to ecological release, generating rapid morphological diversification in turn (Schluter 2000; Yoder et al. 2010). However, the lack of direct association between changes in body size evolution and habitat transitions indicate that transitions between marine and freshwaters are often not linked to ecological opportunity and subsequent phenotypic diversification. Indeed, while there are examples of trans-marine/freshwater groups where the invasion of a depauperate freshwater habitat led to instances of adaptive radiation, this pattern is far from ubiquitous. We argue that abiotic factors, biogeographic patterns, biotic interactions, and intrinsic constraints are important to understand patterns of phenotypic evolution in fishes that cross the marine/freshwater boundary.

Acknowledgments

We thank Kathryn M Docherty, Sharon A Gill, and Hernán López-Fernández for constructive feedback during the early stages of this project. We also thank Joshua P Egan, Lindsey M Dehaan, Darby L Finnegan, and the other members of the Bloom lab for helpful discussions about fish evolution across the marine/freshwater boundary.

Funding

Funding for attendance and participation in the associated symposium was provided by divisions of the Society for Integrative and Comparative Biology; the American Microscopical Society; The Crustacean Society; the National Science Foundation [grant numbers EAR-1659006 and IOS-2135085]; and the Company of Biologists. This work was supported by the National Science Foundation [grant number DEB-1754627 to D.D.]; the Society for the Study of Evolution [R. C. Lewontin Early Award to V. de B.]; and the American Society of Ichthyologists & Herpetologists [Edward C. Raney Fund Award to V. de B.].

Supplementary data

Supplementary data available at *ICB* online.

References

Adams DC, Otárola-Castillo E. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol Evol 4: 393–9.

Albert JS, Johnson DM. 2012. Diversity and evolution of body size in fishes. Evol Biol 39: 324–40.

Albert JS, Tagliacollo VA, Dagosta F. 2020. Diversification of Neotropical freshwater fishes. Annu Rev Ecol Evol Syst 51: 27–53.

Anderson SAS, Weir JT. 2021. Character displacement drives trait divergence in a continental fauna. Proc Natl Acad Sci 118.

Aristide L, Morlon H. 2019. Understanding the effect of competition during evolutionary radiations: an integrated model of phenotypic and species diversification. Ecol Lett 22: 2006–17.

Aschliman NC, Nishida M, Miya M, Inoue JG, Rosana KM, Naylor GJP. 2012. Body plan convergence in the evolution of skates and rays (Chondrichthyes: Batoidea). Mol Phylogenet Evol 63: 28–42.

Bamber RN, Henderson PA. 1988. Pre-adaptive plasticity in atherinids and the estuarine seat of teleost evolution. J Fish Biol 33: 17–23.

Barbour CD. 1973. A biogeographical history of Chirostoma (Pisces: Atherinidae): a species flock fromtheMexican Plateau. Copeia 1973: 533–56.

Bastide P, Ané C, Robin S, Mariadassou M. 2018. Inference of adaptive shifts for multivariate correlated traits. Syst Biol 67: 662–80.

Beaulieu JM, O'Meara BC. 2018. Can we build it? Yes we can, but should we use it? Assessing the quality and value of a very large phylogeny of campanulid angiosperms. Am J Bot 105: 417–32.

Bell MA, Foster SA. 1994. Introduction to the evolutionary biology of the threespine stickleback. In: Bell MA, Foster SA, editors. The evolutionary biology of the three spine sticklebacks. Oxford: Oxford University Press. p. 1–27.

Benton MJ. 2009. The red queen and the court jester: species diversity and the role of biotic and abiotic factors through time. Science 323: 728–32.

Berner D, Grandchamp A-C, Hendry AP. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution 63: 1740–53.

Betancur-R. R, Ortí G, Stein AM, Marceniuk AP, Alexander Pyron R. 2012. Apparent signal of competition limiting diversification after ecological transitions from marine to freshwater habitats. Ecol Lett 15: 822–30.

Betancur-R R. 2009. Molecular phylogenetics and evolutionary history of ariid catfishes revisited: a comprehensive sampling. BMC Evol Biol 9: 1–18.

Betancur-R R. 2010. Molecular phylogenetics supports multiple evolutionary transitions from marine to freshwater habitats in ariid catfishes. Mol Phylogenet Evol 55: 249–58.

Betancur-R R, Ortí G, Pyron RA. 2015. Fossil-based comparative analyses reveal ancient marine ancestry erased by extinction in ray-finned fishes. Ecol Lett 18: 441–50.

Bloom DD, Burns MD, Schriever TA. 2018. Evolution of body size and trophic position in migratory fishes: a phylogenetic comparative analysis of Clupeiformes (anchovies, herring, shad, and allies). Biol J Linn Soc 125: 302–14.

Bloom DD, Egan JP. 2018. Systematics of clupeiformes and testing for ecological limits on species richness in a transmarine/freshwater clade. Neotrop Ichthyol 16: 1–14.

Bloom DD, Kolmann M, Foster K, Watrous H. 2020. Mode of miniaturisation influences body shape evolution in new world anchovies (Engraulidae). J Fish Biol 96: 194–201.

Bloom DD, Lovejoy NR. 2011. The biogeography of marine incursions in South America. In: Albert JS, Reis RE, editors. Historical biogeograhpy of neotropical freshwater fishes. Berkely and Los Angeles: University of California Press. p. 137–44.

Bloom DD, Lovejoy NR. 2012. Molecular phylogenetics reveals a pattern of biome conservatism in New World anchovies (family Engraulidae). J Evol Biol 25: 701–15.

Bloom DD, Lovejoy NR. 2017. On the origins of marine-derived freshwater fishes in South America. J Biogeogr 44: 1927–38.

Bloom DD, Unmack PJ, Gosztonyi AE, Piller KR, Lovejoy NR. 2012. It's a family matter: molecular phylogenetics of Atheriniformes and the polyphyly of the surf silversides (Family: Notocheiridae). Mol Phylogenet Evol 62: 1025–30.

Bloom DD, Weir JT, Piller KR, Lovejoy NR. 2013. Do freshwater fishes diversify faster than marine fishes? a test using state-dependent diversification analyses and molecular phylogenetics of new world silversides (Atherinopsidae). Evolution 67: 2040–57.

Boettiger C, Lang DT Wainwright PC. 2012. rfishbase: exploring, manipulating and visualizing FishBase data from R. J Fish Biol 81: 2030–39.

Bowen SH. 1983. Detritivory in Neotropical fish communities. Environ Biol Fishes 9: 137–44.

Briggs JC, Miller RR. 1960. Two new freshwater clingfishes of the genus *Gobiesox* from southern Mexico. Occas Pap Univ Michigan 616: 1–15.

Brockhurst MA, Colegrave N, Hodgson DJ, Buckling A. 2007. Niche occupation limits adaptive radiation in experimental microcosms. PLoS One 2: e193.

Brown WL, Wilson EO. 1956. Character displacement. Syst ZoolSyst Zool 5: 49–64.

Burns MD, Bloom DD. 2020. Migratory lineages rapidly evolve larger body sizes than non-migratory relatives in ray-finned fishes. Proc R Soc B Biol Sci 287: 20192615.

Burns MD, Sidlauskas BL. 2019. Ancient and contingent body shape diversification in a hyperdiverse continental fish radiation. Evolution 73: 569–87.

Buser TJ, Finnegan DL, Summers AP, Kolmann MA. 2019. Have niche, will travel. new means of linking diet and ecomorphology reveals niche conservatism in freshwater cottoid fishes. Integr Org Biol 1: obz023.

Cadotte MW, Tucker CM. 2017. Should environmental filtering be abandoned? Trends Ecol Evol 32: 429–37.

Cahill JF, Kembel SW, Lamb EG, Keddy PA. 2008. Does phylogenetic relatedness influence the strength of competition among vascular plants? Perspect Plant Ecol Evol Syst 10: 41–50.

Campanella D, Hughes LC, Unmack PJ, Bloom DD, Piller KR, Ortí G. 2015. Multi-locus fossil-calibrated phylogeny of Atheriniformes (Teleostei, Ovalentaria). Mol Phylogenet Evol 86: 8–23.

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW. 2009. The merging of community ecology and phylogenetic biology. Ecol Lett 12: 693–715.

Chase JM. 2007. Drought mediates the importance of stochastic community assembly. Proc Natl Acad Sci 104: 17430–4.

Clarke JT. 2021. Evidence for general size-by-habitat rules in actinopterygian fishes across nine scales of observation. Ecol Lett 24: 1569–81.

Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G, Dickson H, Grimwood J, Schmutz J, Myers RM, Schluter D, Kingsley DM. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307: 1928–33.

Conway KW, Kim D, Rüber L, Espinosa Pérez HS, Hastings PA. 2017. Molecular systematics of the New World clingfish genus *Gobiesox* (Teleostei: Gobiesocidae) and the origin of a freshwater clade. Mol Phylogenet Evol 112: 138–47.

Conway KW, King CD, Summers AP, Kim D, Hastings PA, Moore GI, Iglésias SP, Erdmann M V., Baldwin CC, Short G et al. 2020. Molecular phylogenetics of the clingfishes (Teleostei: Gobiesocidae)—implications for classification. Copeia 108: 886–906.

Conway KW, Moore GI, Summers AP. 2019. A new genus and two new species of miniature clingfishes from temperate southern Australia (Teleostei, Gobiesocidae). Zookeys 864: 35–65.

Cooney CR, Thomas GH. 2021. Heterogeneous relationships between rates of speciation and body size evolution across vertebrate clades. Nat Ecol Evol 5: 101–10.

Corush JB. 2019. Evolutionary patterns of diadromy in fishes: more than a transitional state between marine and freshwater. BMC Evol Biol 19: 1–13.

Darwin C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life . Br Foreign Med Chir Rev 25: 367–404.

Davis AM, Pusey BJ, Betancur-R R. 2020. Effects of adoption of freshwater residency on life-history ecology of terapontid grunters. Freshw Biol 65: 1139–52.

Davis AM, Unmack PJ, Pusey BJ, Johnson JB, Pearson RG. 2012. Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). J Evol Biol 25: 1163–79.

De Sant'Anna VB, Collette BB, Godfrey SJ. 2013. †*Belone countermani*, a new Miocene needlefish (Belonidae) from the St. Marys formation of Calvert Cliffs, Maryland. Proc Biol Soc Washingt 126: 137–50.

Deagle BE, Jones FC, Chan YF, Absher DM, Kingsley DM, Reimchen TE. 2012. Population genomics of parallel phenotypic evolution in stickleback across stream–lake ecological transitions. Proc R Soc B Biol Sci 279: 1277–86.

Depczynski M, Bellwood DR. 2005. Shortest recorded vertebrate lifespan found in a coral reef fish. Curr Biol 15: R288–9.

Egan JP, Bloom DD, Kuo CH, Hammer MP, Tongnunui P, Iglésias SP, Sheaves M, Grudpan C, Simons AM. 2018. Phylogenetic analysis of trophic niche evolution reveals a latitudinal herbivory gradient in Clupeoidei (herrings, anchovies, and allies). Mol Phylogenet Evol 124: 151–61.

Egan JP, Chew US, Kuo CH, Villarroel-Diaz V, Hundt PJ, Iwinski NG, Hammer MP, Simons AM. 2017. Diets and trophic guilds of small fishes from coastal marine habitats in western Taiwan. J Fish Biol 91: 331–45.

Emerson BC, Gillespie RG. 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends Ecol Evol 23: 619–30.

Flecker AS. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77: 1845–54.

Fontenelle JP, Lovejoy NR, Kolmann MA, Marques FPL. 2021a. Molecular phylogeny for the Neotropical freshwater stingrays (Myliobatiformes: Potamotrygoninae) reveals limitations of traditional taxonomy. Biol J Linn Soc 134: 381–401.

Fontenelle JP, Marques FPL, Kolmann MA, Lovejoy NR. 2021b. Biogeography of the Neotropical freshwater stingrays (Myliobatiformes: Potamotrygoninae) reveals effects of continent-scale paleogeographic change and drainage evolution. J Biogeogr 48: 1406–19.

Fricke R, Eschmeyer WN, Van der Laan R. 2022. Eschmeyer's catalog of fishes: genera, species, references. San Francisco (CA): California Academy of Sciences.

Friedman ST, Collyer ML, Price SA, Wainwright PC. 2021. Divergent processes drive parallel evolution in marine and freshwater fishes. Syst Biol 0: 1–12.

Friedman ST, Price SA, Corn KA, Larouche O, Martinez CM, Wainwright PC. 2020. Body shape diversification along the benthic–pelagic axis in marine fishes. Proc R Soc B Biol Sci 287: 20201053.

Froese R, Pauly D. 2021. FishBase. (www.fishbase.org) last accessed February 18, 2022.

Fujiwara K, Conway KW, Motomura H. 2021. Description of a new genus and two new species of Indo–Pacific clingfishes (Gobiesocidae: Diademichthyinae) with redescription and reassignment of two species previously assigned to Lepadichthys Waite, 1904. Ichthyol Herpetol 109: 753–84.

Fuller RC, Mcghee KE, Schrader M. 2007. Speciation in killifish and the role of salt tolerance. J Evol Biol 20: 1962–75.

Garland T, Dickerman AW, Janis CM, Jones JA. 1993. Phylogenetic analysis of covariance by computer simulation. Syst Biol 42: 265.

Germain RM, Hart SP, Turcotte MM, Otto SP, Sakarchi J, Rolland J, Usui T, Angert AL, Schluter D, Bassar RD et al. 2021. On the origin of coexisting species. Trends Ecol Evol 36: 284–93.

Gittenberger E. 1991. What about non-adaptive radiation? Biol J Linn Soc 43: 263–72.

Glor RE. 2010. Phylogenetic insights on adaptive radiation. Annu Rev Ecol Evol Syst 41: 251–70.

Goto A, Yokoyama R, Sideleva VG. 2015. Evolutionary diversification in freshwater sculpins (Cottoidea): a review of two major adaptive radiations. Environ Biol Fishes 98: 307–35.

Goulding M, Carvalho ML. 1983. Ecology of Amazonian needlefishes (Belonidae). 2: 99–111.

Grant PR. 1972. Convergent and divergent character displacement. Biol J Linn Soc 4: 39–68.

Griffiths D. 2012. Body size distributions in North American freshwater fish: large-scale factors. Global Ecol Biogeogr 21: 383–92.

Guinot G, Cavin L. 2015. Contrasting "fish" diversity dynamics between marine and freshwater environments. Curr Biol 25: 2314–8.

Hagen DW, Gilbertson LG. 1973. Selective predation and the intensity of selection acting upon the lateral plates of threespine sticklebacks. Heredity 30: 273–87.

Harmon LJ, Andreazzi CS, Débarre F, Drury J, Goldberg EE, Martins AB, Melián CJ, Narwani A, Nuismer SL, Pennell MW et al. 2019. Detecting the macroevolutionary signal of species interactions. J Evol Biol 32: 769–82.

Harmon LJ, Losos JB, Jonathan Davies T, Gillespie RG, Gittleman JL, Bryan Jennings W, Kozak KH, McPeek MA, Moreno-

Roark F, Near TJ et al. 2010. Early bursts of body size and shape evolution are rare in comparative data. Evolution 64: 2385–96.

Hembry DH, Weber MG. 2020. Ecological interactions and macroevolution: a new field with old roots. Annu Rev Ecol Evol Syst 51: 215–43.

Hendry AP, Stearns SC. 2003. To sea or not to sea? Anadromy vs. non-anadromy in salmonids. In: Hendry AP, Stearns SC, editors. Evolution illuminated: Salmon and their relatives. Oxford: Oxford University Press. p. 1–69.

Herler J, Munday P, Hernaman V. 2011. Gobies on coral reefs. In: Patzner RA, Tassell JL Van, Kovačić M, Kapoor BG, editors. The Biology of Gobies. Boca Raton (FL): Science Publishers CRC Press. p. 493–529.

Huelsenbeck JP, Nielsen R, Bollback JP. 2003. Stochastic mapping of morphological characters. Syst Biol 52: 131–58.

Hughes LC, Cardoso YP, Sommer JA, Cifuentes R, Cuello M, Somoza GM, González-Castro M, Malabarba LR, Cussac V, Habit EM et al. 2020. Biogeography, habitat transitions and hybridization in a radiation of South American silverside fishes revealed by mitochondrial and genomic RAD data. Mol Ecol 29: 738–51.

Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, Johnson J, Swofford R, Pirun M, Zody MC, White S et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484: 55–61.

Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66: 402–18.

Kinziger AP, Wood RM, Neely DA. 2005. Molecular systematics of the genus *Cottus* (Scorpaeniformes: Cottidae). Copeia 2: 303–11.

Knouft JH, Page LM. 2003. The evolution of body size in extant groups of North American freshwater fishes: speciation, size distributions, and Cope's rule. Am Nat 161: 413–21.

Kolmann MA, Burns MD, Ng JYK, Lovejoy NR, Bloom DD. 2020. Habitat transitions alter the adaptive landscape and shape phenotypic evolution in needlefishes (Belonidae). Ecol Evol 10: 3769–83.

Kolmann MA, Marques FPL, Weaver JC, Dean MN, Fontenelle JP, Lovejoy NR. 2022. Ecological and phenotypic diversification after a continental invasion in Neotropical freshwater stingrays. Integr Comp Biol icac019.

Kozak KH, Wiens JJ. 2006. Does niche conservatism promote speciation? A case study in North American Salamanders. Evolution 60: 2604.

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM. 2015. Community assembly, coexistence and the environmental filtering metaphor. Funct Ecol 29: 592–9.

Lee CE, Bell MA. 1999. Causes and consequences of recent freshwater invasions by saltwater animals. Trends Ecol Evol 14: 284–8.

Leroy B, Dias MS, Giraud E, Hugueny B, Jézéquel C, Leprieur F, Oberdorff T, Tedesco PA. 2019. Global biogeographical regions of freshwater fish species. J Biogeogr 46: 2407–19.

Lim KC, Lim P-E, Chong VC, Loh K-H. 2015. Molecular and morphological analyses reveal phylogenetic relationships of stingrays focusing on the family dasyatidae (Myliobatiformes). PLoS One 10: e0120518.

Losos JB. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. Am Nat 175: 623–39.

Lovejoy NR, Albert JS, Crampton WGR. 2006. Miocene marine incursions and marine/freshwater transitions: evidence from Neotropical fishes. J South Amer Earth Sci 21: 5–13.

Lucek K, Sivasundar A, Kristjánsson BK, Skúlason S, Seehausen O. 2014. Quick divergence but slow convergence during ecotype formation in lake and stream stickleback pairs of variable age. J Evol Biol 27: 1878–92.

Lucek K, Sivasundar A, Roy D, Seehausen O. 2013. Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake–stream divergence in parapatric Swiss stickleback. J Evol Biol 26: 2691–709.

MacArthur RH. 1972. Geographical ecology: patterns in the distribution of species. New York (NY): Harper & Row.

McCraney WT, Thacker CE, Alfaro ME. 2020. Supermatrix phylogeny resolves goby lineages and reveals unstable root of Gobiaria. Mol Phylogenet Evol 151: 106862.

McDowall RM. 2003. Shads and diadromy: implications for ecology, evolution, and biogeography. Am Fish Soc Symp 2003: 11–23.

McGee MD, Borstein SR, Meier JI, Marques DA, Mwaiko S, Taabu A, Kishe MA, O'Meara B, Bruggmann R, Excoffier L et al. 2020. The ecological and genomic basis of explosive adaptive radiation. Nature 586: 75–9.

Malabarba MC, Di Dario F. 2017. A new predatory herring-like fish (Teleostei: Clupeiformes) from the early cretaceous of Brazil, and implications for relationships in the Clupeoidei. Zool J Linn Soc 180: 175–94.

Martin CH, Richards EJ. 2019. The paradox behind the pattern of rapid adaptive radiation: how can the speciation process sustain itself through an early burst? Annu Rev Ecol Evol Syst 50: 569–93.

Mayfield MM, Levine JM. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecol Lett 13: 1085–93.

Miller EC. 2021. Comparing diversification rates in lakes, rivers, and the sea. Evolution 75: 2055–73.

Muschick M, Russell JM, Jemmi E, Walker J, Stewart KM, Murray AM, Dubois N, Stager JC, Johnson TC, Seehausen O. 2018. Arrival order and release from competition does not explain why haplochromine cichlids radiated in Lake Victoria. Proc R Soc B Biol Sci 285: 20180462.

Near TJ, Dornburg A, Eytan RI, Keck BP, Smith WL, Kuhn KL, Moore JA, Price SA, Burbrink FT, Friedman M et al. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc Natl Acad Sci 110: 12738–43.

Nelson JS, Grande TC, Wilson MVH. 2016. Fishes of the world. Hoboken (NJ): John Wiley & Sons, Inc.

Nielsen R. 2002. Mapping mutations on phylogenies. Syst Biol 51: 729–39.

Parenti LR. 1996. Phylogenetic systematics and biogeography of Phallostethid fishes (Atherinomorpha, Phallostethidae) of northwestern Borneo, with description of a new species. Copeia 1996: 703–12.

Patterson TB, Givnish TJ. 2002. Phylogeny, concerted convergence, and phylogenetic niche conservatism in the core liliales: insights from rbcL and ndhF sequence data. Evolution 56: 233–52.

Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, Fitzjohn RG, Alfaro ME, Harmon LJ. 2014. Geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. Bioinformatics 30: 2216–8.

Peters RH. 1983. The ecological implications of body size, the ecological implications of body size. Shaftesbury Rd (CA): Cambridge University Press.

Pfennig KS, Pfennig DW. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. Q Rev Biol 84: 253–76.

Rabosky DL. 2013. Diversity-dependence, ecological speciation, and the role of competition in macroevolution. Annu Rev Ecol Evol Syst 44: 481–502.

Rabosky DL. 2020. Speciation rate and the diversity of fishes in freshwaters and the oceans. J Biogeogr 47: 1207–17.

Rabosky DL, Santini F, Eastman J, Smith SA, Sidlauskas B, Chang J, Alfaro ME. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat Commun 4: 1958.

Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3: 217–23.

Revell LJ. 2013. Two new graphical methods for mapping trait evolution on phylogenies. Methods Ecol Evol 4: 754–9.

Rittmeyer EN, Allison A, Gründler MC, Thompson DK, Austin CC. 2012. Ecological guild evolution and the discovery of the world's smallest vertebrate. PLoS One 7: e29797.

Roff DA. 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. Integr Comp Biol 31: 205–16.

Romanuk TN, Hayward A, Hutchings JA. 2011. Trophic level scales positively with body size in fishes. Global Ecol Biogeogr 20: 231–40.

Rowsey DM, Heaney LR, Jansa SA. 2019. Tempo and mode of mandibular shape and size evolution reveal mixed support for incumbency effects in two clades of island-endemic rodents (Muridae: Murinae). Evolution 73: 1411–27.

Rüber L, Kottelat M, Tan HH, Ng PKL, Britz R. 2007. Evolution of miniaturization and the phylogenetic position of *Paedocypris*, comprising the world's smallest vertebrate. BMC Evol Biol 7: 1–10.

Rundell RJ, Price TD. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. Trends Ecol Evol 24: 394–9.

Santini F, Nguyen MTT, Sorenson L, Waltzek TB, Lynch Alfaro JW, Eastman JM, Alfaro ME. 2013. Do habitat shifts drive diversification in teleost fishes? An example from the pufferfishes (Tetraodontidae). J Evol Biol 26: 1003–18.

Schenk JJ, Rowe KC, Steppan SJ. 2013. Ecological opportunity and incumbency in the diversification of repeated continental colonizations by muroid rodents. Syst Biol 62: 837–64.

Schluter D. 2000. The ecology of adaptive radiation. Oxford: Oxford University Press.

Seehausen O, Wagner CE. 2014. Speciation in freshwater fishes. Annu Rev Ecol Evol Syst 45: 621–51.

Sidlauskas B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. Evolution 62: 3135–56.

Simões M, Breitkreuz L, Alvarado M, Baca S, Cooper JC, Heins L, Herzog K, Lieberman BS. 2016. The evolving theory of evolutionary radiations. Trends Ecol Evol 31: 27–34.

Simpson GG. 1953. The major features of evolution. New York (NY): Columbia University Press.

Steele SE, López-Fernández H. 2014. Body size diversity and frequency distributions of Neotropical Cichlid fishes (Cichliformes: Cichlidae: Cichlinae). PLoS One 9: e106336.

Stroud JT, Losos JB. 2016. Ecological opportunity and adaptive radiation. Annu Rev Ecol Evol Syst 47: 507–32.

Tedesco PA, Paradis E, Lévêque C, Hugueny B. 2017. Explaining global-scale diversification patterns in actinopterygian fishes. J Biogeogr 44: 773–83.

Unmack PJ, Allen GR, Johnson JB. 2013. Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. Mol Phylogenet Evol 67: 15–27.

Uyeda JC, Hansen TF, SJ Arnold, Pienaar J. 2011. The million-year wait for macroevolutionary bursts. Proc Natl Acad Sci 108: 15908–13.

Vega GC, Wiens JJ. 2012. Why are there so few fish in the sea? Proc R Soc B Biol Sci 279: 2323–9.

Wagner CE, Harmon LJ, Seehausen O. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature 487: 366–9.

Watson W, Walker HJ. 2004. The world's smallest vertebrate, *Schindleria brevipinguis*, a new paedomorphic species in the family Schindleriidae (Perciformes: Gobioidei). Rec Aust Museum 56: 139–42.

Weber MG, Strauss SY. 2016. Coexistence in close relatives: beyond competition and reproductive isolation in Sister Taxa. Annu Rev Ecol Evol Syst 47: 359–81.

Weber MG, Wagner CE, Best RJ, Harmon LJ, Matthews B. 2017. Evolution in a community context: on integrating ecological interactions and macroevolution. Trends Ecol Evol 32: 291–304.

Weiher E, Keddy PA. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74: 159.

Weitzman SH, Vari RP. 1988. Miniaturization in South American freshwater fishes: an overview and discussion. Proc Biol Soc Washingt 101: 444–65.

Wellborn GA, Langerhans RB. 2015. Ecological opportunity and the adaptive diversification of lineages. Ecol Evol 5: 176–95.

Whitehead PJP, Nelson GJ, Wongratana T. 1988. Clupeoid Fishes of the World (Suborder Clupeoidei), FAO Species Catalogue, Vol. 7 Rome: UNDP FAO.

Yamanoue Y, Miya M, Doi H, Mabuchi K, Sakai H, Nishida M. 2011. Multiple invasions into freshwater by pufferfishes (Teleostei: Tetraodontidae): a mitogenomic perspective. PLoS One 6: e17410.

Yoder JB, Clancey E, Des Roches S, Eastman JM, Gentry L, Godsoe W, Hagey TJ, Jochimsen D, Oswald BP, Robertson J et al. 2010. Ecological opportunity and the origin of adaptive radiations. J Evol Biol 23: 1581–96.