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Divergent processes drive parallel evolution in marine and freshwater fishes

Friedman, S.T.[1], Collyer, M.L.[2], Price, S.A.[3], and Wainwright, P. C.[1]

[1]Department of Evolution and Ecology, University of California Davis, Davis, CA 95616, USA

[2]Department of Science, Chatham University, Pittsburgh, Pennsylvania 15232, USA

[3]Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA

Corresponding Author: S. T. Friedman, Department of Evolution and Ecology, University of California Davis, Davis, CA 95616, USA. Email: sarahtfried@gmail.com.

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Abstract

Evolutionary comparisons between major environmental divides, such as between marine and freshwater systems, can reveal the fundamental processes governing diversification dynamics. Although processes may differ due to the different scales of their biogeographic barriers, freshwater and marine environments nevertheless offer similar opportunities for diversification in benthic, demersal, and pelagic habitats. Here, we compare the evolutionary patterns and processes shaping teleost diversity both in each of these three habitats and between marine and freshwater systems. Using specimens from the National Museum of Natural History, we developed a dataset of linear measurements capturing body shape in 2,266 freshwater and 3,344 marine teleost species. With a novel comparative approach, we contrast the primary axis of morphological diversification in each habitat with the major axis defined by phylogenetic signal. By comparing angles between these axes, we find that fish in corresponding habitats have more similar primary axes of morphological diversity than would be expected by chance, but that different historical processes underlie these parallel patterns in freshwater and marine environments. Marine diversification is more strongly aligned with phylogenetic signal and shows a trend toward lineages occupying separate regions of morphospace. In contrast, ecological signal appears to be a strong driver of diversification in freshwater lineages through repeated morphological evolution in densely packed regions of morphospace. In spite of these divergent histories, our findings reveal that habitat has driven convergent patterns of evolutionary diversification on a global scale.

Keywords: body shape, benthic-pelagic axis, morphological diversification, phylogenetic signal, convergent evolution

Introduction

One of the largest and most dramatic environmental divides on Earth is between freshwater and marine systems. These two ecosystems have attracted much interest among evolutionary biologists, particularly with respect to fishes, which have successfully transitioned between the two systems numerous times (Betancur-R. 2010; Yamanoue et al. 2011; Davis et al. 2012; Davis and Betancur-R. 2017; Buser et al. 2019). Though marine environments dwarf freshwater environments in both area and volume, paradoxically, they contain roughly equivalent numbers of fish species, potentially hinting at different processes governing evolutionary dynamics (Dawson 2012; Rabosky 2020).

There are many reasons to expect differences in evolutionary patterns and processes between the two realms. The different physical and environmental properties of the two ecosystems can have substantial effects on macroevolutionary diversification patterns. Freshwater habitats tend to be strongly fragmented and isolated even on small spatial scales, providing ample opportunity for speciation (Puebla 2009; Seehausen and Wagner 2014). In contrast, marine habitats are more open and interconnected. Though the marine realm is not without geographic barriers, these barriers, such as the open expanses of the Atlantic Ocean, are often permeable and tend to operate on much larger spatial scales (Rocha et al. 2007; Rocha and Bowen 2008). The relative lack of physical barriers coupled with higher dispersal potential means that marine organisms tend to exhibit greater gene flow and less genetic structuring (Ward et al. 1994; Puebla 2009; Bloom et al. 2013). Habitat stability also differs between marine and freshwater systems. Large freshwater lakes and rivers, for example, are much younger than marine habitats and many have undergone dramatic water-level fluctuations, sometimes to the

point of complete desiccation (Lowe-McConnell 1969). These ephemeral events can lead to population fragmentation and local adaptation, with potential consequences for evolutionary trends even when water levels resume (Matschiner et al. 2010).

In spite of these differences, marine and freshwater realms offer some similar ecological opportunities for diversification along the depth gradient. Transitions along the benthic-pelagic axis have been one of the most widespread and consistently reported drivers of intra- and interspecific diversification in the literature. There are numerous examples of both freshwater and marine lineages transitioning to benthic (in physical contact with the substrate), demersal (close proximity to the substrate), and pelagic (limnetic/open water) habitats with well-recognized ecomorphological trends (Walker 1997; Cooper et al. 2010; Willacker et al. 2010; Cooper et al. 2017; Muschick et al. 2012; Hollingsworth et al. 2013; Hulsey et al. 2013; Kusche et al. 2014; Burress et al. 2017; Ribeiro et al. 2018; Tavera et al. 2018). For example, pelagic species often evolve a more streamlined body shape, thought to be adaptive for efficient, steady locomotion (Hatfield and Schluter 1999; Cooper et al. 2010, but see Lujan and Conway 2015), while many demersal lineages are characterized by deeper bodies which enhance maneuverability (Webb 1984a; Robinson and Wilson 1994; Tavera et al. 2018). Although many studies have focused on the trait changes associated with transitions along the depth gradient, we lack an understanding of how the primary axis of phenotypic diversification proceeds in each habitat and how uniform these axes are across habitats. Yet, consistent ecomorphological trends associated with habitat transitions have been shown on a macroevolutionary scale (Friedman et al. 2020; Larouche et al. 2020), suggesting that the selective pressures and opportunities

92 presented by each habitat may also shape subsequent patterns of morphological evolution across
93 the shared adaptive landscape.
94
95 Given the different physical and environmental characteristics of marine and freshwater
96 realms combined with similar ecological opportunities (in terms of the benthic-pelagic axis), this
97 system offers a particularly interesting evolutionary juxtaposition. Though studies have shown
98 that speciation dynamics likely differ between these major ecosystems (Puebla 2009; Bloom et
99 al. 2013; Rabosky 2020), none have compared the patterns and processes of morphological
100 diversification in these realms across teleost fishes. Here, we ask three questions about
101 macroevolutionary dynamics in teleost fish body shapes: (1) Is the primary axis of
102 morphological diversification in benthic, demersal and pelagic habitats shared across marine and
103 freshwater systems? (2) What evolutionary signals are associated with the major dimensions of
104 morphological evolution in each habitat? (3) Have the primary patterns of diversification within
105 each habitat been produced by similar processes, or has the history of body shape evolution
106 proceeded differently in these landscapes?
107
108 **Methods**
109 *Data Collection and Preparation*
110 We collected linear measurements on body shape from three adult specimens from each
111 of 5,610 species, including 2,266 freshwater and 3,344 marine teleost species. Using specimens
112 from the Smithsonian National Museum of Natural History, we measured eight ecologically and
113 functionally relevant features of body shape and averaged these values across specimens for
114 species means. The measurements included standard length, maximum body depth, maximum

body width, minimum caudal peduncle depth, minimum caudal peduncle width, lower jaw length, mouth width, and head depth. For further details on data collection, measurements, and collation methods see Price *et al.* (2019).

Species were categorized into three habitats: benthic, demersal, and pelagic based on their adult habitat preferences and behavior. For more details on this habitat classification system see Friedman *et al.* (2020). Briefly, benthic fishes generally spend the majority of the time with their body in contact with the substrate and may lack a swim bladder, while pelagic fishes live in the water column or at the air/water interface, rarely or never coming into contact with the benthos. Demersal fishes have some degree of interaction with the substrate, but spend little time with their body physically in contact with the benthos. We further separated species into freshwater and marine ecosystems for a total of six discrete habitat groups (freshwater benthic, marine benthic, freshwater demersal, etc.). Due to the differences in the amount of vertical partitioning between freshwater and marine ecosystems, we note the potential for uncertainty in the habitat categorization between ecosystems, however, given the scale of the study, any ambiguity is unlikely to substantially influence our findings. Exclusively brackish fishes or species that can be found in both marine and freshwater systems were removed from the dataset. Euryhaline species that live in brackish habitats as well as either marine or freshwater environments were grouped with their primary (freshwater/marine) environment to maintain a binary coding system. All salinity information was collected from fishbase.org (Froese and Pauly 2019). Species habitats were determined using both fishbase and primary literature when habitat information was unavailable (Table S1; Friedman *et al.* 2020).

For comparative analyses we used a previously-published time-calibrated phylogeny of ray-finned fishes pruned to our species list (Rabosky et al. 2018). We first ln-transformed all linear traits and, for each of the six habitat groups, used 'phytools' (Revell 2012) to size-correct by taking the residuals of a phylogenetic regression on body size. We used the geometric mean (cube-root of the product of species averages for standard length, maximum body width, and maximum body depth) as a composite metric for size that incorporates the three major dimensions of body shape (Mosimann 1970; Klingenberg 2016). All analyses for this study were implemented in the R statistical computing environment version 3.6.2 (R Core Team 2017).

Comparing Axes of Diversity Across Habitats

To determine the primary axis of morphological diversity in each of the six habitat groupings, we performed six independent principal components analyses (PCA) with the correlation matrix on all eight morphological traits, extracting the first principal component (PC1) from each. PC1 represents the linear combination of morphological traits with the maximum within-habitat variance. This vector is of interest because it is the primary axis of multivariate diversity – capturing both variation between species expected from the phylogeny as well as non-phylogenetic diversification. To determine if the primary axes of trait divergence are shared across habitats, we calculated pairwise angles (θ) between each of the PC1s using the formula: $\theta_{i,j} = \cos^{-1}\left(\frac{(PC1_i)'}{D_{PC1_i}} \frac{PC1_j}{D_{PC1_j}}\right)$ (equation from appendix of Adams and Collyer 2009). In other words, the angle is calculated as the inner product between the two PC1s, standardized by the Euclidean distances of the vectors (D). If $\theta_{i,j}$ was greater than 90°, then (180 - $\theta_{i,j}$) was used instead to account for arbitrary differences in PC vector direction (Adams and Cerney 2007). Thus, pairwise angles range from 0° (equivalent trajectories) to 90° (completely uncorrelated

trajectories), with smaller angles indicating the trajectories are closely aligned in multidimensional space (Adams and Collyer 2009).

To evaluate whether the observed pairwise angles differed from the null expectation, we iteratively simulated an eight-trait morphological dataset under Brownian motion using the default parameters of the *mvSIM* function in 'mvMORPH'. We then recalculated the pairwise angle estimates between habitat groups in 1,000 simulated datasets, comparing the empirical angle to the null distribution of angles with a one-tailed test statistic.

As multiple axes of morphological diversification can also be shared between habitats, we also implemented a multivariate approach to compare similarity among habitats beyond just PC1. The Krzanowski correlation metric is commonly used to compare covariance matrices in quantitative genetics studies (Aguirre et al. 2014; McGlothlin et al. 2018). It is calculated as mean of the squared vector correlations between all pairs of eigenvectors included (Krzanowski 1979). Using the KrzCor function in the R package 'evolqg' (Melo et al. 2016), we compared covariance matrices between each habitat, retaining the first three eigenvectors (half of the total number of eigenvectors minus 1, as recommended by Krzanowski (1979)). Correlation values range from 0 (no common variation within subspaces) to 1 (identical variation within subspaces).

To more directly evaluate the directions through morphospace that were most privileged by evolution for each habitat (sensu Schluter 1996), we also compared rate matrices between regimes. Due to computational constraints on a dataset of this size (pers. comm. Julien Clavel), we first averaged the trait data by family (n = 380) and designated the habitat and ecosystem by

the most common regimes found in the family. We then pruned the phylogeny to family-level and generated 100 stochastic character maps (simmaps) under an all-rates-different model using the make.simmap function implemented in phytools (Revell 2012). Using the mvBM function in mvMORPH (Clavel et al. 2015), we fit a separate rate matrix to each of the six regimes across 100 simmaps. These rate matrices were then averaged across simmaps and compared using the Krzanowski correlation procedure detailed above.

As family averages will only capture trends deeper within the tree, we ran an additional analysis to capture more tipward trends by splitting the phylogeny into subtrees that were largely fixed within a single habitat regime. We first identified candidate nodes that did not change state, nor had any descendent nodes that changed state across 95/100 stochastic character maps and led to at least 10 tips. We then split the phylogeny into subtrees at each of these nodes, removing any species that did not match the primary habitat regime, resulting in a total of 3624 species across 120 subtrees. For each subtree, we calculated independent contrasts (Felsenstein 1985) across the 8 morphological traits and then combined these datasets into regime-specific variance-covariance matrices, which were compared using the same Krzanowski correlation procedure as described earlier. To test for consistency between this and the previous (family-level) correlation matrix, we performed a singular value decomposition on the cross-product between correlation matrices (sensu Rohlf and Corti 2000). Consistency would be revealed by a tendency for the first singular value to be large compared to others, and for pairs of "left" and "right" vectors to be correlated.

Comparing Axes of Phylogenetic Signal in Habitats

206 To further tease apart major patterns of morphological evolution associated with habitat,
207 we implemented a newly developed method, phylogenetically-aligned PCA (PACA), which we
208 used to evaluate how closely aligned PC1 was with phylogenetic signal in each habitat (Collyer
209 and Adams 2020). Phylogenetic signal, in this case, refers to the tendency for closely-related
210 species to exhibit phenotypic similarity (Revell et al. 2008; Goolsby 2015). In PACA, the first
211 component is represented by the combination of traits that aligns most closely with phylogenetic
212 signal in the dataset, with each subsequent component orthogonal to all others and successively
213 describing the maximum portion of remaining phylogenetic signal (Collyer and Adams 2020).
214 As some traits may evolve in tandem with the phylogeny, while others are more labile and may
215 strongly reflect the organism's ecology or other life history characteristics, PACA can be used to
216 distinguish differing amounts of phylogenetic signal partitioned in different morphological traits.
217 Unlike traditional phylogenetic PCA (phylo-PCA), which reveals the major axes of divergence
218 between lineages or clades (Revell 2009; Uyeda et al. 2015), PACA finds the traits that are most
219 similar among closely related species, making the two methods somewhat antagonistic to one
220 another, although neither method removes phylogenetic signal from the dataset.
221
222 We performed a PACA on each of the six habitat groups with the correlation matrix
223 using the *gm.prcomp* function in 'geomorph' (Adams et al. 2019; Collyer and Adams 2020),
224 again extracting the first component. Using the same equation as before, we calculated the angle
225 between PC1 and PAC1 *within* each habitat. This allowed us to determine how closely aligned
226 the primary axis of diversity was with the major axis describing phylogenetic signal in a given
227 habitat. If PC1 and PAC1 are similar, this implies that phylogenetic signal tends to align with the
228 main axis of morphological variation in the dataset, while dissimilarity between the vectors

indicates that some other signal (e.g., ecology, development, allometry, etc.) may be shaping diversification. We also ran a phylo-PCA on each habitat using 'geomorph' and estimated angles between phylo-PC1, PAC1, and PC1 within each habitat. Phylo-PCA is a natural complement to PACA that can reveal how other signals contribute to morphological variation. Finally, to confirm that allometric variation did not significantly alter the major axis of diversification within habitats, we re-ran all analyses using a log-shape ratio (Mosimann 1970; Claude 2013) size correction, which preserves allometric variation.

To ensure that our results were not affected by the unequal numbers of species in each habitat, we randomly resampled 477 species (the smallest group) from each habitat and recalculated the angle between PC1 and PAC1 for 1,000 iterations. Finally, we estimated a multivariate Blomberg's K (Blomberg et al. 2003), a commonly used statistic for quantifying phylogenetic signal, across all traits for each of the six groups. This analysis was conducted using the K_{mult} statistic implemented in geomorph (Adams et al. 2019) and iterated 1,000 times to assess significance.

Patterns of Morphospace Occupation

Morphological disparity was estimated for the six habitat groups across all eight trait dimensions using the morphol.disparity function in 'geomorph' (1000 iterations). To evaluate if the evolutionary processes in each ecosystem deviates from Brownian motion, we constructed disparity-through-time (DTT) plots for the freshwater and marine habitats (Foote 1997; Harmon et al. 2003; Slater et al. 2010). These plots were created from freshwater and marine datasets that were size corrected separately prior to analysis. This allowed us to compare trends in average

252 subclade morphological disparity through time with the expectation under a constant rate
253 Brownian motion process by simulating evolution 1,000 times across the phylogeny. To
254 determine the significance of the DTT plots, we implemented a rank envelope test (Murrell
255 2018), which avoids multiple testing issues while retaining the power to detect deviations from
256 Brownian motion trait evolution.
257
258 To visualize the rate of habitat transitions through time in each environment, and thus
259 another aspect of the evolutionary process, we first split the phylogeny into marine and
260 freshwater trees. We then reconstructed habitat occupation across 100 simmaps for each
261 phylogeny under an all-rates-different model, in which transition rates differ between habitat
262 states. We determined this was the best-fit model by comparing log-likelihoods of the Q-matrices
263 from models that allowed for equal, symmetric, and asymmetric rate transitions between habitat
264 states. Using the 'ctt' function in phytools (Revell 2012), we calculated the average rate of
265 habitat transitions through time for each ecosystem. With the 'sim.multiCtt' function, we then
266 compared these estimates to a null distribution of 100 changes-through-time (CTT) plots
267 simulated under the Q matrix estimated from the simmap procedure.
268
269 To qualitatively assess the ecological and morphological diversification of species
270 through time, we first conducted a phylogenetic size correction followed by a non-phylogenetic
271 PCA with the correlation matrix on the entire dataset. We then used the function 'anc.BM' in the
272 geomorph package to determine the ancestral state at each node across all eight dimensions of
273 the PCA for each ecosystem. By creating 100 stochastic character maps for each ecosystem
274 under an all-rates-different transition model using 'make.simmap' in phytools, we were able to

275 determine the most commonly reconstructed habitat state (benthic, demersal, pelagic) at each
276 node in the phylogeny. We then plotted the position of all nodes in each ecosystem on PC1 over
277 time, colored by habitat. Combined, this allowed us to visually contrast the temporal and spatial
278 diversification of species in morphospace with respect to habitat and ecosystem
279 (marine/freshwater) occupation.
280
281 Another method to evaluate major patterns of morphological diversification is to quantify
282 clade overlap in morphospace (Price et al. 2015). Using the same PCA on the entire dataset, we
283 separated species into marine and freshwater and removed families with fewer than 20 species in
284 either ecosystem. Then, we estimated 95% data hypervolumes across the first four principal
285 components (89.8% variance explained) for each family in marine and freshwater habitats, as our
286 analyses revealed we lack the data to accurately estimate family hypervolumes beyond the first
287 four dimensions. We opted for 95% data hypervolumes as opposed to minimum area convex
288 polygons (Price et al. 2015) to avoid potential outlier species biasing morphospace area estimates
289 for a given family. We then estimated the volume of the data hypervolumes for each family, as
290 well as the pairwise overlap between all families in the ecosystem using the 'hypervolume'
291 package in R (Blonder et al. 2014). Finally, we determined the median volume and overlap of
292 families in each ecosystem.
293
294 **Results**
295 *Comparing Axes of Diversity Across Habitats*
296 Corresponding demersal and pelagic habitats across freshwater/marine realms had much
297 smaller PC1 angles than any other pairwise comparisons, while benthic habitats had a slightly

larger angle between them (29.8°) (Fig. 1A, Table S2). Our simulations also show that PC1 angles between corresponding habitats are much smaller than would be expected under Brownian motion (Fig. S1), suggesting that species in corresponding habitats diversify along very similar morphological axes. Interestingly, we also find that some of the pairwise comparisons between non-corresponding habitats are statistically significant as well, though the angles are nearly always larger than those between corresponding habitats. This may be due to universal developmental processes governing the body shape axis with the most potential for morphological change in fishes (Ward and Brainerd 2007; Ward and Mehta 2010), particularly in demersal and pelagic habitats where the angles are smallest. For example, body elongation is commonly found to be a primary axis of diversification across large swaths of fish diversity (Ward and Mehta 2010; Maxwell and Wilson 2013; Claverie and Wainwright 2014; Collar et al. 2016). Consistent with these other studies, we find that two traits — maximum body depth and standard length — load particularly high and in opposite directions on nearly all of the PC1 vectors regardless of habitat (Fig. 1A, Table S3, Table S4). Combined, these traits capture variation in elongation of the body, creating what amounts to a prevailing morphological axis across teleosts with deep-bodied fishes at one end and elongate, eel-like fishes at the other (Fig. 2).

Comparing Axes of Phylogenetic Signal in Habitats

Angles between PC1 and PAC1 in a given habitat varied substantially across the six habitat groups. The largest discrepancy between corresponding habitats varied between 15.1° in marine benthic fishes to 64.4° in freshwater benthic fishes (Fig. 1B). With an average angle of 19.4°, we found greater concordance between PC1 and PAC1 in the marine habitats than in the

three freshwater habitats, where the average angle was 58.7°. This implies that body shape diversification in marine realms generally proceeds along the trajectory aligned with phylogenetic signal. In contrast, freshwater fishes occupy primary axes of diversity that differ substantially from the axes defined by phylogenetic signal. As expected, comparisons with the first component of a phylogenetic PCA yielded large angles in all habitats (Table S5). Traits associated with feeding, mouth width and jaw length, load strongly on phylo-PC1 in most habitats (Table S3). All patterns are also consistent even when allometric signal remains in the dataset (Table S6) and there is no evidence to suggest that this finding is influenced by the difference in sample size between our habitat groups (Fig. S2). Results from the multivariate matrix comparisons are also consistent with our findings, with the highest correlations between corresponding habitats (range: 0.86-0.90), though many of the non-corresponding habitat comparisons are also quite high (range: 0.61-0.77) (Table S7). The multivariate rate matrix comparisons of families roughly mirror these findings, with the highest correlations between corresponding demersal and benthic habitats, while corresponding pelagic habitats appear to be slightly less correlated (Table S8). This marginal difference may be attributable to the family-level resampling procedure, which was essential for this analysis due to the size of our dataset. The rate matrix comparisons from the subtree approach also yielded fairly similar results, although benthic habitats are less correlated and some non-corresponding habitats are relatively highly correlated (Table S9). These differences are likely due to unequal retention of species within each regime after the subtree pruning procedure. For example, 63% of freshwater pelagic species were removed from the dataset, while only 19% of marine benthic species were dropped. These results may also imply that transitions between habitat regimes and deeper phylogenetic nodes are contributing to the evolutionary signal. However, the correlations (between Tables S8

and S9) were fairly consistent (99.9% representation of squared singular value sum associated with principal vectors, and a vector correlation of 0.998 for the first vector pair). Although we find a tendency for correlations to increase with subsampling, the results are qualitatively similar.

Estimates of Blomberg's K across all eight trait dimensions indicate that phylogenetic signal is universally higher in marine groups than in freshwater groups (Table 1). With all marine groups possessing higher K values ($K_{mult} > 0.5$), we find statistically significant ($p < 0.05$) evidence for relatively strong phylogenetic signal in the marine realm. Meanwhile, freshwater groups all have lower K values, implying weaker, but still significant phylogenetic signal across all eight trait dimensions.

Patterns of Morphospace Occupation

From the DTT plots we find an MDI of 0.155 in freshwater environments and an MDI of -0.038 in marine species, both of which are statistically significant ($p < 0.05$) in the rank envelope test. These findings imply that evolution proceeds differently in marine and freshwater habitats, but that evolution in both ecosystems significantly deviates from pure Brownian motion (Fig. 2). Subclades of marine fishes tend to diverge in morphospace, exhibiting a subtle pattern that is generally associated with adaptive radiation or an Early Burst model (Harmon et al. 2003), while subclades of freshwater fishes tend to overlap extensively in morphospace.

We find that the rate of habitat transitions is generally stable or slightly increases through time in freshwater fishes, while the rate of habitat transitions decreases in marine fishes (Fig. S3,

Fig. S4). Consistent with our other analyses, evolution across both marine and freshwater fishes appears to deviate from constant-rate evolution in the CTT plots.

All of the pairwise comparisons of morphological disparity between marine and freshwater habitats were statistically significant ($p<0.05$), with higher estimates of morphological disparity across all trait dimensions for marine fishes in the three habitats. While we were able to recover significant disparity differences among marine habitat comparisons, none of the freshwater habitat comparisons significantly differed, implying that habitat has more of an effect on the variance of shapes in marine environments. Visualizing the diversification of species along PC1 through time, it is clear that marine species show a pattern of expansion through morphospace that is somewhat partitioned by habitat (Fig. 2). For example, marine benthic fishes tend to occupy regions of morphospace corresponding with higher values along PC1, reflecting the presence of elongate (eel-like) bodies, while marine demersal fishes are most dense at the low end of PC1, indicating a predominance of deeper body shapes. Freshwater fishes from the three habitats overlap more in morphospace and tend to occupy a more densely localized region near the middle of PC1, corresponding to an intermediate body shape (Fig. 2, Table S3). These observed morphospace trends also appear to align well with our analysis of clade occupation patterns. Across the first four PCs, we find that the median morphospace volume that freshwater families occupy is 15.8 (n = 25 families), compared to just 10.2 in marine families (n = 46). Median pairwise overlap of these family-level hypervolumes is also greater among freshwater families (freshwater: 0.914, marine: 0.0), further demonstrating that freshwater families tend to explore similar regions of morphospace (Fig. S5). Thus, with respect to habitat, marine species

389 are qualitatively and quantitatively more morphologically diverse than freshwater fishes and
390 exhibit more clade-specific expansion in morphospace.
391
392 **Discussion**
393 We find substantial evidence for parallel diversification along habitat-specific PC1s
394 spanning the marine-freshwater divide, indicating that habitat plays a dominant role in fish body
395 shape evolution on a global scale. However, the underlying evolutionary processes driving these
396 patterns of body shape diversification differ substantially between ecosystems. Marine fishes
397 consistently exhibit both high morphological disparity as well as strong phylogenetic signal that
398 is aligned with the primary axis of morphological diversity, while freshwater lineages show
399 weaker phylogenetic signal that is poorly aligned with their primary axis of morphological
400 diversity. We also find evidence that the rate of habitat transitions through time is stable in
401 freshwater fishes, whereas it decreases in marine fishes. Taken together, our findings imply that
402 fishes in the two realms have evolved similar primary axes of diversity via different processes.
403
404 *Parallel Evolution in Corresponding Habitats*
405 We find that major axes of morphological diversity are roughly parallel in corresponding
406 habitats, indicating that habitat plays a large role in organizing patterns of diversification. There
407 are two non-exclusive explanations for such a pattern: either the ecological opportunities or the
408 intrinsic constraints on body shape evolution are shared between corresponding habitats in
409 freshwater and marine systems (Collar et al. 2016). Habitat can select on functionally relevant
410 morphological traits, creating a primary axis of variation that reflects the habitat-specific
411 evolutionary responses. Shared intrinsic constraints (i.e. genetics, development, etc.) may limit

412 the inhabitable regions of morphospace (Raup 1966) as well as bias the direction of phenotypic
413 variation, such that morphological diversification primarily proceeds along the axis of available
414 genetic variation (Schluter 1996). We suggest that both factors play a strong role in this system.
415 Given that we find high correlations between non-corresponding habitats, endogenous
416 constraints may shape shared patterns of morphological diversification across fishes. However,
417 we also find that major axes of body shape diversity are similar among corresponding habitats,
418 presumably reflecting habitat-specific adaptive landscapes which can also drive differences in
419 the primary axis of diversification across habitats.
420
421 There are clear differences in the functional demands of benthic, demersal, and pelagic
422 habitats which have the potential to drive patterns of morphological diversification. For example,
423 a pelagic lifestyle often places a premium on efficient steady locomotion, reflected in the
424 streamlined body shapes commonly seen in many open water fishes, whereas demersal fishes
425 tend to be deep-bodied, thought to enhance maneuverability around the structurally complex
426 habitats on which they live (Webb 1984a, 1984b). As such, we find that standard length and
427 body depth both load particularly high on PC1 for demersal and pelagic fishes, resulting in
428 widespread variation along the elongation axis for species in these habitats. However, we do
429 recover a slightly lower correlation between corresponding pelagic rate matrices, perhaps due to
430 differences in vertical structuring between freshwater and marine pelagic habitats. Relaxation of
431 the selective pressures imposed by locomotion may have allowed sedentary benthic fishes to
432 explore novel regions of morphospace, such as wide, dorsoventrally depressed forms
433 (particularly in marine benthic fishes), which are conducive to station-holding in high flow
434 environments (Carlson and Lauder 2010; Friedman et al. 2020), as well as eel-like forms which

move effectively in physically cramped spaces. We find that standard length loads high on PC1 for both freshwater and marine benthic fishes, but, while body depth contributes strongly to variation in marine freshwater species, it is not an important component of PC1 for marine benthic fishes. Flow regimes not only differ between the three habitats, but also between freshwater and marine ecosystems and can have broad consequences for morphological evolution (Langerhans 2008). This may be one explanation as to why PC1s of freshwater and marine benthic habitats are the least aligned of the three corresponding habitat comparisons, as flow regimes can be more pronounced in freshwater benthic environments (Lujan and Conway 2015). Not only do locomotion demands differ between these three habitats, but other ecological correlates such as diet, can also impose consistent selective pressures on morphological features related to prey capture and processing (Cooper et al. 2010, 2017; Collar et al. 2016; Tavera et al. 2018). Indeed, our findings indicate that different suites of functional demands in each habitat have established predictable axes of diversification through morphospace.

Given the many differences between freshwater and marine systems, it is striking that habitat is sufficiently powerful to drive similar major axes of body shape diversification. Though consistent shape changes accompanying transitions along the depth gradient have been extensively reported in fishes (Walker 1997; Cooper et al. 2010; Willacker et al. 2010; Cooper et al. 2017; Muschick et al. 2012; Hollingsworth et al. 2013; Hulsey et al. 2013; Kusche et al. 2014; Burress et al. 2017; Ribeiro et al. 2018; Tavera et al. 2018; Friedman et al. 2020), this is the first study to demonstrate that fish evolution is also shaped by the predictable patterns of diversification *within* habitats. With nearly 6,000 species, this dataset encompasses broad ecological, behavioral, and morphological diversity, and yet our finding of significant, parallel

458 axes of diversification between general habitat categories points to a widespread effect of habitat
459 in organizing teleost body shape diversification.
460
461 *Patterns of Morphospace Occupation*
462 While the primary axes of morphological diversification in corresponding habitats are
463 similar, the arrangement of closely related species in morphospace differs substantially between
464 marine and freshwater fishes. The similar PCA and PACA vectors found in marine fishes imply
465 that the primary dimension of variation (PC1) is *across* clades in the marine realm, while PC1 is
466 more descriptive of variation *within* clades in freshwater fishes. Combined with results from the
467 disparity-through-time plots and the reduced morphospace overlap in marine families, it appears
468 that marine lineages diversify in relatively secluded regions of morphospace. Though similar
469 morphospace occupation patterns have been documented for families of coral reef fishes (Price
470 et al. 2015), this is the first study to demonstrate the role of a strong phylogenetic signal
471 underlying the greater diversity of marine fish shapes, as compared with freshwater fishes. This
472 pattern suggests that much of the body shape diversity in marine fishes is a consequence of deep,
473 conserved divergences between major lineages. Though it is unclear what initiated this
474 exploration in morphospace, a relaxation of constraints in marine fishes may have served to
475 expand the available trait space for these lineages. To the extent that morphology reflects
476 ecology, it is possible that they also exhibit a pattern of phylogenetic niche conservatism,
477 however we lack the detailed ecological information to empirically evaluate this possibility.
478 Such a pattern would be consistent with our findings, resulting in the large amounts of
479 morphological disparity we find in each of the three habitats as well as a decreasing rate of
480 habitat transitions through time.

481

482 In contrast, freshwater clades exhibit a different history, repeatedly radiating in some of
483 the most densely packed regions of morphospace with reduced total disparity compared to
484 marine fishes. The primary axis of diversification in freshwater fishes is considerably less
485 aligned with phylogeny than in marine fishes (Fig. 1B; Table S2) and morphological variation is
486 primarily distributed within, rather than across, freshwater clades (Fig. 2). While fish in both
487 marine and freshwater show similar primary axes of body shape variation in corresponding
488 habitats, the history of morphological evolution in freshwater involves repeatedly evolving a
489 smaller range of forms. Given the substantial influence of habitat on body shape, it may be an
490 ecological signal that overwhelms the phylogenetic signal in freshwater fishes. Likewise, the
491 limited morphospace occupation of freshwater fishes may also indicate that intrinsic constraints
492 play a larger role in the freshwater realm, driving differences in the evolutionary patterns and
493 processes between ecosystems. Repeated diversification within a constrained region of
494 morphospace could also serve to erode the phylogenetic signal within this system. In all
495 likelihood these two mechanisms—adaptation and constraint—strike a balance to generate the
496 evolutionary patterns and processes of marine and freshwater ecosystems. Efforts to tease apart
497 the individual contributions of these factors in driving macroevolutionary patterns may present a
498 fruitful avenue for future research.

499

500 Implementing the three separate sets of components analyses has also lent exceptional
501 resolution into the ecological and phylogenetic trends within each of the habitat groups and
502 across ecosystems. Trends along PAC1 within each habitat have been largely shaped by just a
503 few monophyletic assemblages, whose key morphological traits drive variation along this axis.

For example, we find the strongest phylogenetic signal associated with elongation (standard length and body depth) in marine benthic fishes, which is primarily driven by elongate clades like eels (Anguilliformes) and pipefishes (Syngnathidae). Meanwhile, phylogenetic trends in freshwater benthic species appear to be dominated by catfishes (Siluriformes), with mouth width loading quite high on PAC1 in this group. Demersal species all exhibit the highest phylogenetic signal in standard length and depth measurements, prompted by cichlids (Cichlidae) and characins (Characidae) in freshwater fishes and the contrast between rattails (Macrouridae) and filefishes (Monacanthidae) in marine fishes. Caudal peduncle width and jaw length load high on PAC1 for marine pelagic species, driven by the contrast between cutlassfishes (Trichiuridae) with tapered tails and small-mouthed damselfishes (Pomacentridae). Conversely, freshwater pelagic species are more variable in mouth width, largely due to needlefishes (Belonidae) and small-mouthed pupfishes (Cyprinodontidae). Phylogenetic signal is nearly always concentrated within a few select traits, rather than diffusely spread across all data dimensions. However, the traits that load highest on PAC1 are quite variable across habitats, implying that no single combination of traits harbors greater phylogenetic signal across fishes. We also find that traits associated with either the mouth or caudal peduncle contribute most to strongly variation along phylo-PC1 in every habitat. As this method reveals the orientation of data space that minimizes phylogenetic signal, the primary axis can be correlated with a suite of non-phylogenetic signals (i.e., developmental, ecological, allometric, etc.) (Collyer and Adams 2020). Here, it appears to have an ecological basis, as traits related to either feeding or locomotion load highest using this ordination method, suggesting that these features may be some of the more labile morphological traits (of those measured here) across fishes. Thus, ecological factors that co-vary with habitat may also be significant motors for morphological diversification in fishes (Corn et al. 2021;

527 Friedman et al. 2021), particularly in freshwater ecosystems where phylogenetic signal is
528 reduced.
529
530 *Hypothesized Evolutionary Mechanisms*
531 We speculate that discordance in patterns of body shape evolution between freshwater
532 and marine realms reflects large differences in spatial organization of these systems. Oceans are
533 highly interconnected ecosystems which allow greater population connectivity and dispersal
534 potential in marine organisms (Palumbi 1994). These systems are also characterized by vast,
535 largely uninhabited open space dotted with densely populated islands of habitat, a prime example
536 being coral reefs (Grosberg et al. 2012). We hypothesize that evolution of marine fishes is
537 characterized by continual dispersal to new locations as well as early invasion of unexploited
538 regions of morphospace, allowing clades to establish in already-flourishing communities while
539 avoiding competition. These features are consistent with Simpson's ecospace model, which
540 postulates that adaptive radiations are a consequence of early rapid expansion in morphospace
541 followed by the extinction of intermediate forms (Simpson 1944; Benton 2015). Such a process
542 could manifest in the patterns we find for marine fishes: widespread morphological
543 conservatism, strong phylogenetic signal, a pattern of adaptive radiation on the DTT plot, and a
544 decreasing rate of habitat transitions through time.
545
546 Meanwhile, freshwater habitats are highly fragmented even on small spatial scales,
547 creating high levels of physical isolation and resulting in increased opportunities for local
548 adaptation and allopatric speciation in freshwater relative to marine fishes (Puebla 2009;
549 Seehausen and Wagner 2014). Because freshwater river systems are inherently structured as

dendritic networks, there is the potential for iterative diversification along environmental gradients as lineages invade new, unoccupied branches of river systems. This could lead to an overall pattern of extensive overlap in morphospace and would overwhelm an expected phylogenetic signal, consistent with our findings in this study. Unfortunately, it is exceedingly difficult to rigorously test our hypothesized mechanisms on macroevolutionary scales (but see Barraclough and Vogler 2000; Cavender-Bares et al. 2009). Our habitat categorization system provides only a coarse look at the diversity of ecologies present in this dataset. We recommend that future studies incorporate biogeographic data or more detailed ecological information to empirically test for differences in the extent of iterative ecological diversification in marine and freshwater fishes.

Many studies have found evidence that factors related to the physical fragmentation of habitats have played an important role in the diversification processes of teleost fishes (Puebla 2009; Carrete Vega and Wiens 2012; Bloom et al. 2013; Tedesco et al. 2017). Higher rates of speciation and diversification appear to characterize freshwater fishes compared to marine fishes (Bloom et al. 2013; Dias et al. 2013; Tedesco et al. 2017; Manel et al. 2020), though this may reflect the influence of a few dominant clades (Rabosky 2020). Freshwater fish species have also been shown to possess nearly double the amount of nucleotide diversity compared to marine fishes, attributable to the island-like structure of the freshwater realm (Manel et al. 2020). At a smaller scale, freshwater clades such as cichlids, livebearers, and sticklebacks are renowned for their tendency to iteratively ecologically radiate after invading new habitats (Tobler et al. 2011; Hulsey et al. 2013; Schluter 2016). We hypothesize that these patterns scale up to the point

where the divergent speciation processes between freshwater and marine ecosystems may have implications for morphological and ecological diversification across teleost fish evolution.

Conclusions

In conclusion, teleost fishes exhibit striking parallel morphological evolution in the major habitats shared between the two realms. This result is remarkably consistent, despite different histories of body shape evolution in freshwater and marine ecosystems. Phylogenetic signal is tightly aligned with morphological diversification in marine fishes, while freshwater fish diversification more strongly reflects ecological transitions. We hypothesize that different scales of biogeographic barriers in the two ecosystems alter speciation dynamics, producing a history of iterative morphological radiations in freshwater fishes and a relative lack of phenotypic variation within established marine lineages. While differences in habitat fragmentation and physical isolation may have resulted in different temporal patterns of body shape evolution, it appears that the ecological opportunities present in the major habitat types have nevertheless molded very similar major axes of body shape variation in both freshwater and marine realms.

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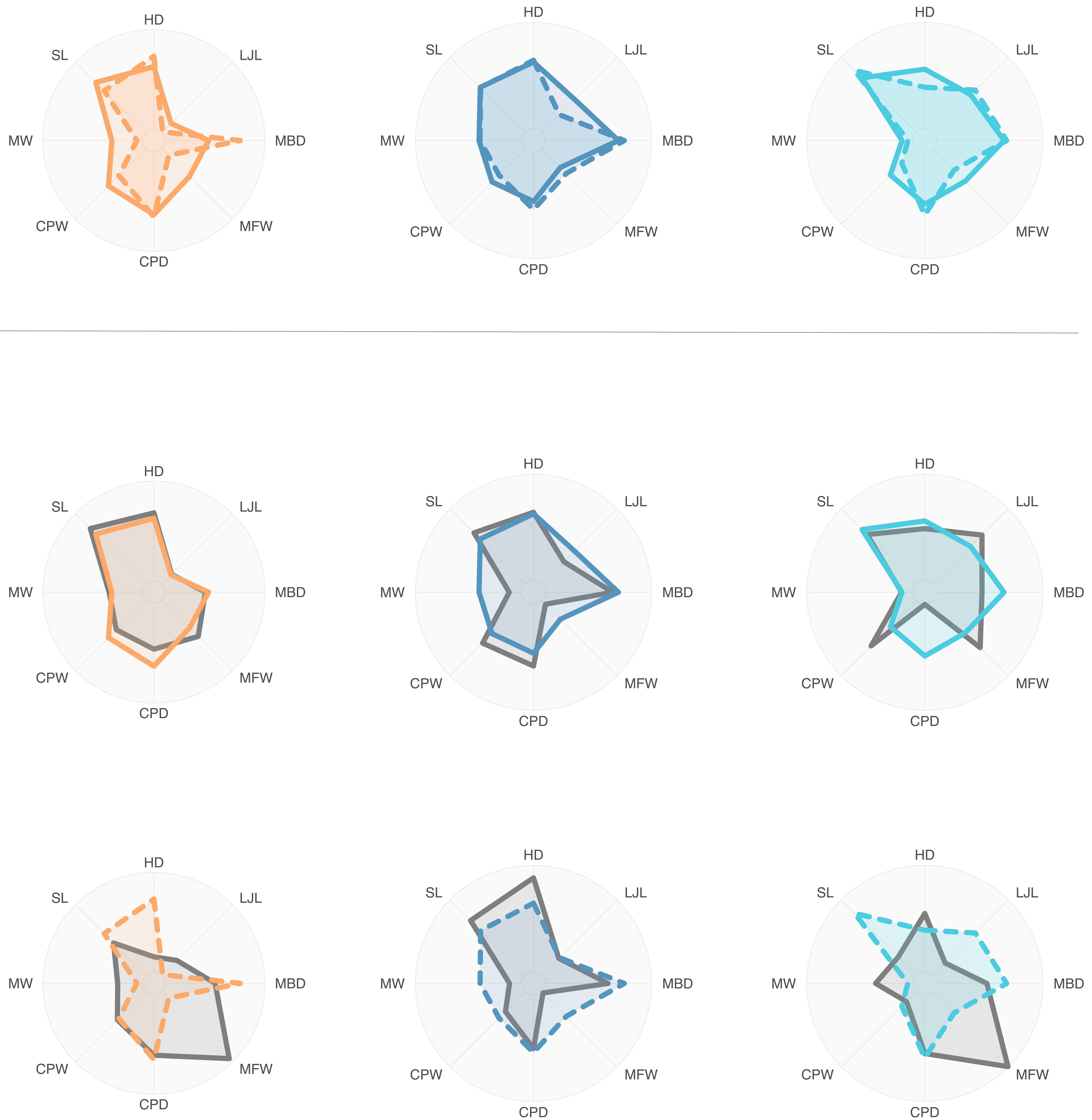
795
796
797 **Figure 1.** Radar plots of the trait loadings on the first principal component for normal PCA
798 (colored lines) and phylogenetically-aligned PCA (grey lines). PCA loadings are colored by
799 habitat (benthic: orange; demersal: dark blue; pelagic: light blue). Line type corresponds with
800 freshwater (dashed lines) or marine (solid lines) realms. Panel A compares the loadings on PC1
801 between corresponding habitats, while panel B compares the first component from each PCA

802 method within a single habitat. The angle above each radar plot is the angle in multidimensional
803 space between the two vectors. Abbreviations for traits are as follows, clockwise from top: HD:
804 head depth; LJL: lower jaw length; MBD: maximum body depth; MFW: maximum fish width;
805 CPD: minimum caudal peduncle depth; CPW: minimum caudal peduncle width; MW: mouth
806 width; SL: standard length.
807
808 **Figure 2.** Disparity through time plots for freshwater and marine realms (top plots). Grey lines
809 and the shaded regions designate the expectation under Brownian motion and the associated 95%
810 confidence interval, respectively. The bottom plots show the diversification of species along PC1
811 through time. Fish outlines designate the maximum (*Nemichthys curvirostris*) and minimum
812 (*Symphysodon discus*) shapes along PC1 for extant species. Each point is a node in the
813 phylogeny colored by the reconstructed habitat (benthic: orange, demersal: dark blue, pelagic:
814 light blue). Labelled nodes designate the most recent common ancestor of select major clades.
815 Vertical lines in right margin show where 90% of extant species fall along PC1 for each habitat.
816
817 **Table 1.** Estimates of morphological disparity and Blomberg's K (with associated p-values and z
818 score) across all eight trait dimensions for each of the six habitat groups. The amount of
819 phylogenetic signal contained in just PC1 for each group is reported in the final column.



Freshwater

Marine

Average subclade disparity

0.0

0.3

0.6

0.9

PC1 (39% variance explained)

5

0

Ostariophysi

Percomorpha

Ovalentaria

Anguilliformes

Ovalentaria

Squamipinnes

150

100

50

0

Time (MYA)

Ecosystem	Habitat	Disparity	K_{mult}	p-value	Z score	PC1 K_{mult}
freshwater	benthic	0.942	0.284	0.001	16.366	0.486
	demersal	0.760	0.241	0.001	14.311	0.781
	pelagic	1.043	0.486	0.001	6.337	0.751
marine	benthic	1.925	0.525	0.001	21.432	1.155
	demersal	1.301	0.606	0.001	20.669	2.157
	pelagic	1.482	0.530	0.001	14.482	1.017

Table 1. Estimates of morphological disparity and Blomberg's K (with associated p-values and z score) across all eight trait dimensions for each of the six habitat groups. The amount of phylogenetic signal contained in just PC1 for each group is reported in the final column.