Title: Comparing diversification rates in lakes, rivers, and the sea

Short title: Diversification in aquatic habitats

Elizabeth Christina Miller[1]

[1]School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington 98105, U.S.A.

Correspondence to: emille3@uw.edu

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TITLE:

Comparing diversification rates in lakes, rivers, and the sea

ABSTRACT:

The diversity of species inhabiting freshwater relative to marine habitats is striking, given that freshwater habitats encompass <1% of Earth's water. The most commonly proposed explanation for this pattern is that freshwater habitats are more fragmented than marine habitats, allowing more opportunities for allopatric speciation and thus increased diversification rates in freshwater. However, speciation may be generally faster in sympatry than in allopatry, as illustrated by lacustrine radiations such as African cichlids. Differences between rivers and lakes may be important to consider when comparing diversification broadly among freshwater and marine lineages. Here I compared diversification rates of teleost fishes in marine, riverine and lacustrine habitats. I found that lakes had faster speciation and net diversification rates than other aquatic habitats. However, most freshwater diversity arose in rivers. Surprisingly, riverine and marine habitats had similar rates of net diversification. Biogeographic models suggest that lacustrine habitats are evolutionary unstable, explaining the dearth of lacustrine species in spite of their rapid diversification. Collectively, these results suggest that diversification rate differences are unlikely to explain the freshwater paradox. Instead, this pattern may be attributable to the comparable amount of time spent in riverine and marine habitats over the 200-million-year history of teleosts.

KEY WORDS:

Freshwater paradox, rivers, lakes, marine fishes, freshwater fishes, diversification rates

INTRODUCTION:

The great biodiversity of freshwater habitats is striking. Habitable freshwater makes up 0.009% of the Earth's water volume but contain at least 40% of ray-finned fish species (Cohen 1970; Horn 1972), a pattern called the "freshwater paradox" (Tedesco et al. 2017a). The diversity of freshwater relative to marine fishes is thought to be due to the greater fragmentation and isolation of freshwater habitats, creating more opportunities for geographic population subdivision than in the ocean (Cohen 1970; Horn 1972; May 1994; Grosberg et al. 2012). For this reason, speciation and net diversification rates are expected to be higher in freshwater than in marine lineages (e.g., Tedesco et al. 2017a).

Two innovations have allowed researchers to rigorously test longstanding ideas about the origin of biodiversity: the expansion in size and taxonomic scope of molecular phylogenies (Rabosky et al. 2013; Rabosky et al. 2018), and the development of increasingly sophisticated approaches to estimate diversification rates (Maddison et al. 2007; Rabosky 2014). The hypothesis that freshwater fishes have faster diversification rates than marine fishes has been tested in at least 10 studies over the past decade, with mixed results (Table 1). Most studies focusing on a single clade of fishes spanning both marine and freshwater biomes have found little difference in net diversification rates (speciation minus extinction rates; see references in Table 1). This could perhaps be due to low power to detect diversification rate differences within small clades (Davis et al. 2013), or because speciation and extinction rates are both higher in freshwater leading to small differences in net diversification (Bloom et al. 2013). Note that higher turnover in freshwater compared to marine habitats cannot explain the freshwater paradox, because species richness will not change when speciation and extinction rates are about equal (see also Meseguer and Condamine 2020). Studies including all ray-finned fishes have

47 found no difference in diversification rates (Carrete Vega and Wiens 2012), faster rates in marine
48 habitats (Betancur-R et al. 2015), faster rates in freshwater (Tedesco et al. 2017a), or faster rates
49 in only some freshwater clades (Rabosky 2020). The only study to my knowledge that
50 exclusively used the fossil record to test this hypothesis found that the accumulation of family
51 diversity has slowed over time in marine but not freshwater fishes (Guinot and Cavin 2015).
52 However, familial diversity is lower overall in freshwater, so it is unknown if freshwater
53 diversity will also plateau if it ever reaches a similar magnitude as the ocean (Figure 1 of Guinot
54 and Cavin 2015). Studies of organisms other than ray-finned fishes are also collectively unclear
55 as to whether marine, freshwater or terrestrial habitats have the fastest diversification rates
56 (Table 1). In sum, whether freshwater or marine fishes have faster diversification rates could be
57 influenced by choice of methods, focal clade, or the breadth of clades included in the study. This
58 sensitivity could exist because the difference in rates between biomes is actually narrower than
59 expected (and therefore hard to detect), or because the habitat with the faster rate differs among
60 clades.

61 Both 'freshwater' and 'marine' are broad categories that encompass diverse habitats and
62 lineages. Diversification rates are known to vary among marine fishes: for example,
63 diversification is faster on coral reefs than in other marine habitats (Santini et al. 2013; Tedesco
64 et al. 2017a). I am not aware of similar studies in fishes comparing diversification rates among
65 different freshwater habitats. An examination of diversification-rate variation among freshwater
66 fish clades (Rabosky et al. 2013; Seehausen and Wagner 2014) shows that some species-rich
67 clades do not have fast rates (e.g. cyprinids), while some groups with fast rates are not species-
68 rich (e.g. sticklebacks). To what extent can these patterns be explained by differences in habitat?

69 The most commonly proposed mechanism for faster freshwater diversification rates, that
70 freshwater habitats give more opportunities for population subdivision than marine habitats,
71 implies that allopatric speciation has generated much of the world's freshwater diversity (Lynch
72 1989). This may be the case, as major river systems such as the Amazon are among the most
73 diverse freshwater habitats (Lévêque et al. 2008; Albert et al. 2020; Miller and Román-Palacios
74 2021). The fragmentation of rivers has been positively related to endemism and cladogenesis in
75 fishes (Dias et al. 2013). However, lakes are also known for their diverse faunas (Lévêque et al.
76 2008). Ancient lakes such as the African rift lakes and Lake Baikal contain species flocks, or
77 monophyletic groups that underwent rapid speciation within a single lake (Cohen 1995;
78 Cristescu et al. 2010; Wagner et al. 2012; Seehausen 2015). While allopatric speciation is also
79 common within and among lakes (Parenti 1984; Seehausen and van Alphen 1999; Rico and
80 Turner 2002; Hrbek et al. 2002; Martin et al. 2015), intralacustrine radiations represent some of
81 the best-supported cases of sympatric speciation across the Tree of Life (Schluter and McPhail
82 1993; Bolnick and Fitzpatrick 2007; Seehausen and Wagner 2014; Kautt et al. 2020). The
83 relative contribution of speciation in rivers versus lakes to freshwater diversity overall is an open
84 question (Seehausen and Wagner 2014).
85 A related question is the pace of speciation inherent to allopatric versus sympatric
86 speciation modes. Speciation is thought to proceed slowly in allopatry because genetic
87 differences between isolated populations accumulate passively (genetic drift) or through gradual
88 local adaptation. Reproductive isolation may be achieved faster in sympatry than in allopatry
89 because disruptive selection may actively select against hybridization (Bush 1975; McCune and
90 Lovejoy 1998; Crow et al. 2010; Germain et al. 2020). McCune and Lovejoy (1998) showed that
91 sympatric lacustrine sister-species had fewer genetic differences between them than allopatric

92 riverine sister-species, suggesting that reproductive isolation was achieved faster between
93 sympatric sister-species. However, later studies in other fishes found that speciation can also be
94 rapid in allopatry in both lacustrine and riverine habitats (Hrbek et al. 2002; Near and Bernard
95 2004). It is unclear what generalities can be drawn from these studies limited to a few well-
96 studied fish clades.

97 There are at least two reasons why we may expect differences in net diversification rates
98 in fishes found in riverine versus lacustrine habitats. First, these habitats may be differently
99 conducive to alternative speciation modes which differ in their time to completion. Allopatric
100 speciation seems to be the dominant mode in rivers because rivers become fragmented and
101 change course through time. Evidence for this statement comes from the allopatric distribution of
102 closely related species in river systems around the world (Near and Bernard 2004; Dias et al.
103 2013; Albert et al. 2020; Sholihah et al. 2021). A few cases of sympatric or parapatric speciation
104 within rivers are well-supported (mormyrid fishes, Sullivan et al. 2002; African *Teleogramma*
105 cichlids, Alter et al. 2017; Neotropical *Crenicichla* cichlids, Burress et al. 2018), but in general
106 sympatric speciation appears to be less common in rivers than in lakes (Seehausen and Wagner
107 2014; Seehausen 2015). If speciation rates are faster in sympatry than in allopatry, then we may
108 expect lacustrine fishes to have faster speciation and net diversification rates than riverine fishes
109 (McCune and Lovejoy 1998).

110 Second, rivers and lakes may differ in ecological opportunity (Yoder et al. 2010). Early
111 literature posited that lakes should have faster speciation rates for ecological and structural
112 reasons. Briggs (1966) and Lowe-McConnell (1969) thought that rates of speciation should
113 generally be faster in peripheral habitats than in centers of species diversity, with both authors
114 comparing rivers and lakes. Lowe-McConnell (1969) and later Seehausen (2015) also argued

115 that there may be fewer available niches in rivers: since rivers are locally unstable, riverine fishes
116 are more likely to be opportunistic feeders. The more stable conditions of lakes allow for trophic
117 specialization. In addition, since lakes tend to be wider and deeper than most rivers, they offer
118 three dimensions to partition habitats: onshore-offshore, shallow-deep, and along the shoreline
119 (Seehausen 2015). This dimensionality may encourage divergent selection, promote species
120 coexistence and allow for faster speciation (Germain et al. 2020).

121 Differences in diversification rates among freshwater habitats may be a crucial missing
122 piece to interpreting comparisons between freshwater and marine diversification. Specifically, an
123 alternative hypothesis to explain the freshwater paradox is that freshwater diversity generally
124 accumulated over long periods of time rather than at a very fast rate (Wiens 2012; Carrete Vega
125 and Wiens 2012; Miller and Román-Palacios 2021). This could still be true if most freshwater
126 species arose through fine-scale allopatric speciation as predicted (e.g., Grosberg et al. 2012). If
127 so, the higher diversification rates sometimes observed in freshwater relative to marine fishes
128 (Table 1) may actually be driven by rapid speciation within lakes, not allopatric speciation in
129 rivers. This would call into question the most commonly proposed mechanism for why
130 diversification rates seem to differ between biomes.

131 In this study, I compared rates of speciation, extinction, and net diversification between
132 marine and freshwater teleost fishes. However, unlike past studies, I compared these rates in
133 riverine and lacustrine habitats separately rather than treating freshwater as a single category. I
134 found that diversification rates in riverine and marine fishes are actually similar on average.
135 However, diversification rates in lakes are much faster than in other aquatic habitats. These
136 results show that more nuance is needed when comparing marine and freshwater diversification
137 patterns.

MATERIAL AND METHODS:

Data acquisition:

In this study I used the largest available time-calibrated phylogeny of ray-finned fishes for all analyses (Rabosky et al. 2018). This maximum likelihood phylogeny was constructed by aggregating genetic data from many past studies, and contains 11,638 species (36.9% of described actinopterygian fishes). I limited the present study to teleost fishes, a group containing 99.8% of ray-finned fishes, because living non-teleost clades are much older with many extinct members (Betancur-R et al. 2015) and it may not be appropriate to compare their diversification rates with teleosts. I removed 51 tips that were duplicate or unresolved species and 48 tips belonging to the four living non-teleost orders (Polypteriformes, Acipenseriformes, Lepisosteiformes, and Amiiformes), leaving phylogenetic data for 11,539 species.

To assign each species in the phylogeny to aquatic habitat categories I used descriptions of geographic range and habitat in FishBase (Froese and Pauley 2019), the IUCN Red List version 6.2 (2020), and a recent compilation of habitat in fishes (Corush 2019). I identified species that were marine, freshwater, diadromous, or estuarine/brackish. Freshwater species were further categorized as riverine, lacustrine, or occurring in both lakes and rivers. Some species were found in additional freshwater habitats including swamps, ponds, temporary pools, caves, and springs. I found that diversification rates in these habitats were similar to those in rivers (details in Extended Text 1; Figure S1), perhaps because these habitats tend to be created or maintained by fluvial action. Species found in these other habitats were simultaneously found in rivers much more often than in lakes (Table S1). For these reasons, I used the species' distribution in rivers and/or lakes as the sole basis for its habitat categorization. This approach

161 was not applicable to the (very few) species that were endemic to these other habitats (Table S1).
162 Rather than exclude these species or include many additional habitat categories in my analyses, I
163 coded these species as riverine.

164 Of the 11,539 species in the phylogeny, I identified 4,984 as exclusively marine (44.4%),
165 631 as brackish, estuarine or euryhaline (5.6%), 263 as diadromous (2.3%), 3,985 as riverine
166 (35.5%), 510 as lacustrine (4.5%), and 854 in both rivers and lakes (7.6%). I removed the
167 remaining 312 species with unclear habitat affinities from analyses, leaving 11,227 species.
168

169 ***Comparing diversification rates among habitats:***

170 I compared diversification rates among habitat types using tip-associated rates (Title and
171 Rabosky 2019) calculated for each species by Rabosky et al. (2018). Rates associated with each
172 species were downloaded using the R package fishtree version 0.2.0 (Chang et al. 2019). I
173 compared seven alternative rate types: (1) the DR statistic (Jetz et al. 2012) which was calculated
174 from the phylogeny of ray-finned fishes with all missing species imputed on the phylogeny, (2)
175 speciation calculated by Bayesian Analysis of Macroevolutionary Mixtures (BAMM; Rabosky
176 2014) under a time-varying model on the phylogeny with genetic data only, (3) speciation
177 calculated by BAMM under a time-constant model, (4) extinction calculated by BAMM under a
178 time-varying model, (5) extinction calculated under a time-constant model, (6) BAMM net
179 diversification rates (speciation minus extinction rates) under a time-variable model, and (7)
180 BAMM net diversification rates under a time-constant model. Note that tip rates were not
181 available for 172 species (Chang et al. 2019). Analysis settings used to obtain these rates are
182 detailed in the original study (Rabosky et al. 2018). The DR statistic for any given species is
183 estimated from the number of branches (i.e. splitting events) between the species and the root of

184 the tree and the length of those branches (Jetz et al. 2012). This type of rate estimate resembles
185 speciation rates more closely than net diversification rates (Title and Rabosky 2019). BAMM
186 uses a model of diversification to simulate a posterior distribution of rate shift configurations, in
187 which taxa in the same rate shift cohort have identical tip rates. While -SSE models can also be
188 used to compare diversification rates among habitats (Table 1), I chose to use rates calculated
189 using methods agnostic to traits (DR, BAMM). This is because it is straightforward to compare
190 rates among several habitat categories at once using these trait-agnostic rates, while -SSE models
191 become very computationally intensive when more than two traits are considered especially at
192 this broad phylogenetic scale.

193 I statistically compared the mean tip rates of habitats using phylogenetic ANOVA
194 (Garland et al. 1993) implemented in phytools version 0.6–44 (Revell 2012). The *phylANOVA*
195 function in phytools implements a simulation-based post hoc test to compare pairwise
196 differences in means, and also uses a Holm-Bonferroni correction for multiple comparisons. I
197 made a total of four habitat comparisons with and without cichlids, using each of the seven rate
198 types (see above), for a total of 56 comparisons. First, I compared tip-associated diversification
199 rates between species in two habitats, as classically done (Table 1): exclusively marine and
200 exclusively freshwater (excluding diadromous or estuarine species). Second, I compared rates
201 among six habitat categories: riverine, lacustrine, both riverine and lacustrine, marine, estuarine,
202 and diadromous. Third, I compared four habitats: exclusively marine, riverine, lacustrine, and
203 both riverine and lacustrine. I excluded diadromous and euryhaline species from this comparison
204 because the habitat where speciation occurred may be unclear when species interact with both
205 freshwater and marine habitats. Fourth, I compared only freshwater habitat types (three
206 categories: riverine, both riverine and lacustrine, and lacustrine). Finally, I repeated these four

habitat comparisons after removing the family Cichlidae. This was to test if cichlids were driving any differences in rates observed among habitats. Lacustrine cichlids possess the fastest diversification rates among fishes (Burress and Tan 2017; McGee et al. 2020) and so may have an overwhelming influence on the results.

Alternative divergence times:

The age of clades can potentially affect diversification rate estimates by shortening or lengthening branch lengths within the clade. Several new divergence time estimates are available for fishes using phylogenomic techniques. However, the dense sampling of the Rabosky et al. (2018) phylogeny is needed to estimate tip-associated diversification rates. To test whether alternative divergence estimates have cascading effects on diversification rate differences among habitats, I used the "congruification" approach of Eastman et al. (2013). This approach uses a reference phylogeny, which is a tree containing few exemplar tips representing higher taxa, to time-calibrate a target phylogeny with shared higher taxa but denser species-level sampling. Thus, the congruification approach attempts to join the efforts of systematists using alternative approaches to build and date trees (few exemplar taxa with many base pairs per species versus broad sampling with less data per species).

I used the genomic phylogenies of Hughes et al. (2018) for teleosts and Alfaro et al. (2018) for Acanthomorpha (spiny-rayed fishes) as alternative target phylogenies. Notably, Alfaro et al. (2018) found that most major clades within acanthomorphs diverged near the K-Pg boundary (66 mya), more recently than in Hughes et al. (2018) or Rabosky et al. (2018). I downloaded the undated maximum likelihood phylogeny of fishes (branch lengths scaled by molecular substitutions) from the Fish Tree of Life website

230 (https://fishtreeoflife.org/downloads/). I implemented the congruification using the function
231 *congruify.phylo* in the R package geiger version 2.0.7 (Pennell et al. 2014) which uses TreePL
232 (Smith & O'Meara 2012) to time-calibrate the tree. This procedure resulted in two rescaled trees:
233 a phylogeny of teleosts with identical sampling as Rabosky et al. (2018) but dates similar to
234 Hughes et al. (2018), and a phylogeny of acanthomorphs with identical sampling as Rabosky et
235 al. (2018) but dates similar to Alfaro et al. (2018).
236 I calculated the DR statistic using each rescaled tree and compared diversification rates
237 among habitat categories as detailed above. This set of analyses added 16 comparisons (4 habitat
238 combinations, with and without cichlids, using each re-scaled phylogeny) to the 56 performed
239 using the original phylogeny for a total of 72 comparisons among three phylogenies.
240
241 ***Biogeographic model fitting:***
242 I found that global freshwater biodiversity was mostly found in rivers, with much lower
243 richness distributed among lakes (Fig. 1). Species richness among habitats can be explained by
244 three processes: in-situ speciation, extinction, and dispersal (transitions) among habitats
245 (Ricklefs 1987; Wiens 2012). To understand why there are much fewer species in lakes than in
246 rivers, I used biogeographic models to trace the history in each habitat along the phylogeny
247 (Rabosky et al. 2018). I fit alternative biogeographic models implemented in the program
248 BioGeoBEARS version 1.1.2 (Matzke 2014). I summarize my approach to model fitting here and
249 give more details in Extended Text 2. Note that these analyses are similar to those in Miller and
250 Román-Palacios (2021). The goal of that study was to compare colonization patterns and
251 diversification rates among the freshwater faunas of biogeographic regions, but did not
252 distinguish between lakes and rivers within these regions.

253 I modeled dispersal among 13 habitat-region combinations. I included six biogeographic
254 regions following Leroy et al. (2019), who used clustering algorithms to identify a
255 bioregionalization scheme based on shared freshwater fish diversity (region names also follow
256 this study). The six regions were: (1) Nearctic, (2) Neotropical, (3) Palearctic, (4) Ethiopian, (5)
257 Sino-Oriental, and (6) Australia. Note that Miller and Román-Palacios (2021) used the earlier
258 regionalization of Tedesco et al. (2017b) which has minor differences in boundaries. Species in
259 these six regions were further assigned to riverine and/or lacustrine habitats, giving 12 region-
260 habitat combinations (e.g. Neotropical rivers and Neotropical lakes). Marine and estuarine
261 species were coded as belonging to a 13th "marine" region. I restricted the root of the teleost
262 phylogeny to the marine state in accordance with the fossil record of fishes (Betancur-R et al.
263 2015). Following prior biogeographic studies in freshwater systems (Toussaint et al. 2017; Miller
264 and Román-Palacios 2021), I restricted dispersal probabilities in accordance with changing
265 regional connectivity through time (Table S11). These dispersal constraints were common to all
266 models.
267 I also compared the fit of six model variants with modified transition matrices in order to
268 test hypotheses about the role of lakes in dispersal. In variant 1, dispersal among biogeographic
269 regions was unrelated to habitat. For example, dispersal from the Neotropical to the Nearctic
270 region could occur as easily within the riverine as the lacustrine state, and marine-to-lacustrine
271 transitions could occur as easily as marine-to-riverine transitions. In variant 2, the probability of
272 transition between marine and lacustrine habitats was reduced (multiplied by a scalar value of
273 0.05). This model would be preferred if most transitions from marine to freshwater habitats
274 occurred via rivers. In variant 3, the probability of dispersal among biogeographic regions was
275 much lower via lakes than via rivers (i.e., all regional dispersal probabilities within the lacustrine

276 state were multiplied by 0.05). For example, dispersal from Neotropical to Nearctic rivers was
277 more likely than dispersal from Neotropical to Nearctic lakes. This model would be preferred if
278 most biogeographic dispersal occurred within rivers. In variant 4, restrictions on lacustrine
279 dispersal were relaxed such that lacustrine dispersal occurred at the same probability as riverine
280 dispersal across adjacent regions or short marine barriers, but lacustrine dispersal was
281 downweighed across large overland or overseas barriers (multiplied by 0.05). For example,
282 dispersal from Neotropical to Nearctic rivers was equally likely as from Neotropical to Nearctic
283 lakes; however, dispersal from the Neotropical rivers to Ethiopian rivers was more likely than
284 the equivalent through lakes. Variant 5 combined marine-lake constraints with strict constraints
285 on biogeographic dispersal through lakes. Variant 6 combined marine-lake constraints with
286 relaxed constraints on dispersal through lakes.

287 In total, I fit 36 alternative biogeographic models (Table 2). These were six models with
288 varying dispersal matrices for each of six base model types (DEC, DEC+J, DIVALIKE,
289 DIVALIKE+J, BAYAREALIKE, and BAYAREALIKE+J). Models with +J denote the addition
290 of a jump parameter allowing cladogenetic dispersal; otherwise, an assumption of models is that
291 all dispersal occurs anagenetically (further differences among models are described in Matzke
292 2014). The best-fit model was chosen by comparing Akaike weights (Burnham and Anderson
293 2002).

294

295 ***Biogeographic patterns:***

296 In order to identify independent dispersal events and visualize uncertainty in the ancestral
297 reconstruction, I simulated 100 biogeographic stochastic maps (Dupin et al. 2017) using the best-
298 fit model. Each individual simulation is a realized history that is possible given the model and

299 data, including the time and location on the branches for biogeographic events. Averaging over
300 all of these simulations will approximate the ancestral state probabilities calculated by the model.
301 To understand why there are fewer species in lakes than rivers, I used biogeographic
302 stochastic maps to answer the following questions. First, what is the rate of transition into and
303 out of lakes relative to other habitats? To obtain habitat transition rates, I counted the number of
304 transitions to marine, riverine only, lacustrine only, and riverine+lacustrine states (combining
305 these counts among regions for each habitat type). The count of events alone can be a misleading
306 measure of the transition rate. As an example, if most freshwater lineages occur in rivers then
307 there may be more transitions from rivers to lakes simply by chance. To convert these counts
308 into a transition rate, for each transition type I divided the number of transitions from the source
309 habitat to the receiving habitat by the sum of branch lengths reconstructed in the source habitat.
310 For example, to calculate the transition rate from marine to riverine I divided the count of these
311 transitions by the sum of branch lengths reconstructed in the marine state. These counts vary
312 slightly among stochastic maps, so I did this for all 100 simulations individually and then took
313 the mean of the 100 rate estimates.
314 Second, what is the contribution of individual transition events to overall freshwater
315 diversity, and are there replicated patterns shared by habitats across biogeographic regions?
316 More specifically, for each lineage representing an independent transition to a habitat I counted
317 the number of descendants of that transition (species richness; note that this count only included
318 species remaining in that habitat by the present), the mean tip-associated diversification rate
319 among the descendants (DR), and the age of the lineage (time of the transition). Then, I took the
320 mean of these values among lineages found in each habitat and biogeographic region. Again, the
321 estimated location of habitat transitions on the phylogeny varies slightly among stochastic maps,

322 so I did this for all 100 simulations. Note that the richness of independent habitat transitions was
323 inferred by counting tips in the phylogeny descended from these transitions, because I could not
324 assign unsampled species in the phylogeny to individual biogeographic events. I assessed the
325 potential sensitivity of these results to biased sampling in Extended Text 3.

326 Finally, I visualized the accumulation of diversity through time in each freshwater habitat
327 and region using the lineage-through-space-and-time approach of Skeels (2019) implemented
328 using the R package ltstR version 0.1.0. This approach is analogous to a lineage-through-time
329 plot but uses the output of biogeographic stochastic mapping to separate temporal patterns of
330 species accumulation by region.

331

332 **RESULTS**

333 ***Diversification rates in aquatic habitats:***

334 The results of 56 rate-habitat comparisons using the phylogeny of Rabosky et al. (2018)
335 are summarized in Table 3 and given in full in Tables S2–S8. When comparing only two habitat
336 categories, net diversification rates in marine and freshwater teleosts were broadly overlapping
337 but slightly higher in freshwater (mean net diversification rate=0.10 species/million years in
338 marine and 0.17 in freshwater under a BAMM time-varying model; Fig. 1). This difference was
339 not significant using phylogenetic ANOVA no matter whether speciation, extinction or net
340 diversification rates were used, the method to calculate these rates, or whether the family
341 Cichlidae was included (Table 3).

342 When comparing six habitat categories, lacustrine fishes had much faster speciation,
343 extinction and net diversification rates than other aquatic habitats (mean net diversification
344 rate=0.63 under a BAMM time-varying model; Fig. 1). Surprisingly, rates in marine and riverine

habitats were similar (mean net rate=0.10 in marine and 0.12 in riverine habitats). Species found in both rivers and lakes diversified at rates more similar to rivers than to lakes (mean net rate=0.15). Estuarine and diadromous species had slightly lower net diversification rates than other habitats (mean net rate=0.07 in estuaries and 0.08 in diadromous species).

Comparisons of net diversification rates among six habitat categories using phylogenetic ANOVA were significant when including all teleosts (BAMM time-varying model: P=0.047; BAMM time-constant model: P=0.041). Differences in speciation and extinction rates were not significant, nor were net diversification rates after excluding Cichlidae (Table 3). Still, lakes were significantly different from the other five habitats in speciation and net diversification rates in all pairwise post hoc tests, even when the ANOVAs were not globally significant. This was true using all methods to calculate rates and whether or not cichlids were included (Table 3). Comparing four habitat categories (excluding estuarine and diadromous species) resulted in the same pattern as comparing six: phylogenetic ANOVAs were not globally significant, but lakes were significantly different in speciation and net diversification rates from the other three habitats in pairwise post hoc tests. When comparing just the three freshwater habitats (riverine, lacustrine, and both riverine and lacustrine), phylogenetic ANOVAs became globally significant in speciation, extinction and net diversification rates. This was driven by faster rates in lakes compared to rivers or rivers+lakes, no matter the rate method or exclusion of cichlids. Among all 56 comparisons, pairwise tests were never significant between other habitat categories except for one other case (diadromous species had faster extinction rates than estuarine or marine species; Tables S3, S6).

Taken together, these results suggest that: (1) lakes have faster speciation and net diversification rates than other freshwater habitats (and potentially faster extinction, though this

368 was not significant), (2) rapid lacustrine diversification rates are not limited to cichlids, and (3)
369 marine rates are similar to riverine rates. As a consequence of the latter result, comparing just
370 two categories (freshwater and marine) can mask the signal of rapid lacustrine diversification
371 since riverine species are much more common than lacustrine species (Fig. 1). The similarity
372 between marine and riverine diversification rates also explains why phylogenetic ANOVAs were
373 not significant when comparing four categories but were significant when comparing the three
374 freshwater categories (Table 3).

375 These patterns held when using phylogenies re-dated in reference to phylogenomic
376 hypotheses (Alfaro et al. 2018; Hughes et al. 2018). The results of the 16 additional comparisons
377 using re-dated trees are summarized in Fig. 2 and shown in full in Tables S9–S10.
378 Unsurprisingly, re-scaling the phylogeny of Acanthomorpha using the Alfaro et al. (2018)
379 hypothesis resulted in slightly faster rates for all habitats because the younger divergence time
380 estimates shortened branch lengths across the entire phylogeny. Still, diversification rates (DR
381 statistic) were generally similar before and after time-calibrating the Rabosky et al. (2018) tree
382 based on either genomic phylogeny (Fig. 2; Fig. S2). Lacustrine species still had the fastest
383 diversification rates among aquatic habitats as shown by pairwise post hoc tests.

384

385 ***Habitat transitions and the formation of freshwater diversity:***

386 The best-fitting biogeographic model (BAYAREA+J with dispersal matrix variant 6;
387 Table 2) constrained marine-lacustrine transitions to be less probable than marine-riverine
388 transitions. This model also applied some constraints on regional dispersal while in the lacustrine
389 state. Rates of cladogenetic ("jump") dispersal were six times higher than rates of anagenetic

390 dispersal (*j*=0.0078 events/million years versus *d*=0.0013; Table 2), suggesting that founder
391 events are an important means of dispersal and speciation among freshwater habitats and regions.
392 In general, marine and riverine habitats were reconstructed in deep ancestral nodes, while
393 the lacustrine state was concentrated towards more recent nodes (Fig. 3A). The rate of transition
394 from marine to riverine habitats was seven times higher than the rate from marine to lacustrine
395 (0.0007 versus 0.0001 events per million years; Fig. 3B). The transition rate from the joint
396 river+lake state into lakes alone was over twice as high as the rate of direct dispersal from rivers
397 to lakes (0.0031 versus 0.0013). Thus, the most typical means of becoming a lacustrine fish was
398 to first transition from marine to riverine habitats, then from rivers alone to both rivers and lakes,
399 then finally becoming endemic to lakes.
400 Transition rates out of lakes were higher than transition rates into lakes (Fig. 3B). The
401 rate of transition from lakes alone back to rivers+lakes was about seven times higher than the
402 reverse (0.0223 events/million years versus 0.0031). The direct dispersal rate from lakes to rivers
403 was over three times as high as the reverse (0.0048 versus 0.0013). This suggests that of the few
404 lineages that become endemic to lakes, many will eventually enter rivers again.
405 I compared four metrics characterizing lineages associated with habitat transitions: the
406 number of independent habitat transitions, mean richness per habitat transition, mean timing of
407 habitat transitions, and mean diversification rate (DR). Differences in these metrics among
408 rivers, lakes, and rivers+lakes were generally shared across the six biogeographic regions (Fig.
409 3D). Transitions into the rivers+lakes state were much more common than transitions into rivers
410 or lakes alone for four of six regions (all but Neotropical and Australian regions). Transitions
411 into lakes were the least common in five of six regions (all but Sino-Oriental).

412 Mean species richness per habitat transition was highest for the riverine state in five of
413 six regions (Fig. 3D). The mean richness of transitions into lakes was low in all but the Ethiopian
414 region, where richness-per-transition was similar between rivers and lakes. Note that these
415 richness differences cannot be attributed to sampling biases by habitat: lacustrine species were
416 better sampled in the phylogeny than riverine species (Extended Text 3). In all six regions,
417 lineages transitioning into the rivers+lakes state were usually limited to single species,
418 suggesting that this habitat is a transitional state between rivers and lakes.

419 In all six regions, riverine transitions were older on average than transitions into the
420 rivers+lakes or lacustrine states (Fig. 3D).

421 The mean diversification rate (DR) of lacustrine lineages was higher than that of the other
422 habitat states in all regions. The difference between lacustrine and riverine diversification rates
423 was large in the Ethiopian, Australian, Nearctic and Palearctic regions, and smaller in the
424 Neotropical and Sino-Oriental regions (Fig. 3D).

425 The temporal patterns of lineage accumulation in rivers and lakes worldwide reflect these
426 differences in transition rates, transition timing, and diversification rates with habitat (Fig. 3C).
427 All six regions have much greater diversity contained in rivers than lakes, even though average
428 diversification rates were always higher in lakes (Fig. 3C). Freshwater diversity has been highest
429 overall in Neotropical and Sino-Oriental rivers for the past 75 million years, reinforcing the role
430 of time for explaining these mega-diverse faunas (Miller and Román-Palacios 2021). Only the
431 Ethiopian region has lacustrine diversity rivalling total riverine diversity in some other regions,
432 which is due to the exceptionally fast diversification rates in African lakes (Fig. 3D). Note that
433 time and diversification-rate patterns characterizing biogeographic regions themselves were
434 consistent with those found by Miller and Román-Palacios (2021).

435 Taken together, these biogeographic results (Table 2; Figure 3) suggest that most global
436 freshwater diversity is riverine in spite of faster lacustrine diversification rates for the following
437 reasons. First, most lineages transitioning to freshwater from marine biomes enter rivers first.
438 Second, transitions from rivers to lakes are relatively infrequent. Third, lineages leave lakes more
439 often than they enter lakes. For these reasons, riverine lineages have had greater opportunity to
440 build diversity over long periods of time than lacustrine lineages.
441
442 **DISCUSSION**
443 Why do freshwater habitats have higher species richness than expected given their total
444 volume on Earth, especially compared to the ocean (Cohen 1970; Horn 1972)? Many past studies
445 compared speciation and/or net diversification rates between freshwater and marine organisms in
446 order to answer this question, but collectively have found mixed results (Table 1). In this study, I
447 compared diversification rates in marine versus freshwater teleost fishes while separating
448 freshwater species by riverine and lacustrine habitats. Contrary to expectations, marine and
449 riverine fishes have similar net diversification rates on average. Lacustrine fishes have much
450 faster diversification rates than all other aquatic habitats yet make up a small fraction of overall
451 freshwater diversity. Therefore, I suggest that diversification rate differences are not sufficient to
452 explain the freshwater paradox, and a more nuanced explanation is needed.
453 It is generally expected that freshwater fishes should have faster speciation and net
454 diversification rates than marine fishes because freshwater habitats are more fragmented, so there
455 are presumably more opportunities for speciation in freshwater than in the ocean (Grosberg et al.
456 2012; Tedesco et al. 2017a). This explanation implies that most freshwater diversity arose
457 through allopatric speciation, an implication supported by the geographic distributions of closely

458 related freshwater fish species (Lynch 1989; Hrbek et al. 2002; Near and Benard 2004; Dias et
459 al. 2013; Albert et al. 2020; Sholihah et al. 2021). One potential problem with this explanation is
460 that allopatric speciation is thought to proceed more slowly than sympatric speciation (McCune
461 and Lovejoy 1998; Crow et al. 2010). Most freshwater diversity arose in rivers (Figs. 1, 3),
462 which suggests that most freshwater species are indeed the product of allopatric speciation
463 (assuming that this is the most common speciation mode in rivers). However, speciation and net
464 diversification rates of riverine fishes were not much faster than those of marine fishes on
465 average (Fig. 1). This finding seems to contradict the leading hypothesis for why freshwater
466 fishes should have faster speciation rates than marine fishes. Still, this hypothesis is supported in
467 part: allopatric speciation *sensu lato* appears to be important for explaining high freshwater
468 species richness, but not necessarily fast rates of allopatric speciation. The fine-scale
469 fragmentation of rivers might allow allopatric populations to complete the speciation process
470 without being outcompeted by congeners or reabsorbed through hybridization (Germain et al.
471 2020), thus allowing the accumulation of many species over a relatively small spatial scale
472 compared to other habitats. The density of speciation events in space can be decoupled from the
473 frequency of events over time (Boucher et al. 2020).

474 How then can we explain the freshwater paradox, if not by faster diversification rates in
475 freshwater versus the ocean? The results of biogeographic reconstructions (Fig. 3) suggest that
476 explanations invoking geologic time could be more appropriate (Stephens and Wiens 2003;
477 Wiens 2012). Diversity has been accumulating for comparably long periods of time in modern
478 marine and riverine lineages of teleosts (over 100 million years; Fig. 3A). Given this amount of
479 time, faster speciation is not necessary to explain high richness in freshwater. In fact, it seems
480 that speciation is about equally as frequent in freshwater and the ocean (Fig. 1). This similarity in

481 time-for-speciation, as well as the similarity in average net diversification rates, is consistent
482 with the roughly similar species richness in riverine and marine habitats today (Fig. 1). Put
483 another way, high freshwater diversity might be explained by the early colonization of
484 freshwater in the history of teleosts, regular opportunities for speciation because of the
485 continuous fragmentation of rivers, and protection from extinction allowing diversity to
486 accumulate over long time scales. This time-based explanation for the freshwater paradox has
487 also been suggested by past studies (Carrete Vega and Wiens 2012, Seehausen and Wagner
488 2014, Betancur-R et al. 2015; see also Miller and Wiens 2017, Miller and Román-Palacios
489 2021), but seems to have been given less attention from researchers than diversification rates
490 (Table 1).

491 How do we reconcile the observation that speciation rates do not differ between riverine
492 and marine habitats with evidence to the contrary from past studies? Manel et al. (2020) found
493 that freshwater fishes have higher intraspecific genetic diversity than marine fishes. This
494 presumably reflects the greater fragmentation of freshwater habitats, which could in principle
495 lead to faster speciation rates. However, the authors found that genetic diversity was weakly
496 related to local species richness (Correlation coefficient=0.20 in marine and 0.36 in freshwater,
497 Manel et al. 2020). Population subdivision may not have a straightforward relationship with
498 speciation rates since there may be distinct (and sometimes competing) factors driving
499 population isolation, reproductive isolation, persistence and coexistence of incipient species
500 (Harvey et al. 2019; Germain et al. 2020). For example, allopatric speciation creates daughter
501 species with smaller population sizes than the parent species, which leads to greater risk of
502 stochastic extinction (Moen and Morlon 2014). Further, species richness is itself only partially
503 related to diversification rates and also depends on historical factors (Ricklefs 1987; Wiens

504 2012). Spatial patterns of species richness are more closely related to dispersal frequency and
505 timing than to diversification rates in both the ocean (Miller et al. 2018) and freshwater (Miller
506 and Román-Palacios 2021). Therefore, the links between population subdivision, speciation, and
507 species richness can break down or be influenced by confounding factors.

508 Some macroevolutionary studies did find faster speciation and/or net diversification rates
509 in freshwater (e.g., Bloom et al. 2013, Tedesco et al. 2017a, Miller et al. 2018; see Table 1). In
510 light of the present study, it is possible that faster freshwater rates inferred by past studies were
511 actually driven by lacustrine species, and not reflective of a general difference between marine
512 and freshwater biomes. Indeed, Rabosky (2020) found that apparently faster average freshwater
513 rates of speciation in actinopterygians were driven by select clades and were not broadly
514 characteristic of marine-to-freshwater transitions. Lacustrine cichlids in particular have the
515 fastest speciation rates among all fishes (Burress and Tan 2017; McGee et al. 2020). Future
516 studies should aim to explain the variation in diversification rates seen within freshwater fishes,
517 which are diverse in age and ecology.

518 The other side of the coin is that diversification rates in marine fishes are faster than
519 expected given the apparent lack of barriers to dispersal in the ocean. Some marine lineages are
520 known to have exceptionally fast speciation rates (Rüber and Zardoya 2005; Rabosky et al. 2018;
521 Siqueira et al. 2020; see also Miller and Wiens 2017 for marine amniotes). Marine fishes are not
522 a monolithic group, and a general expectation for low speciation rates in the ocean seems overly
523 simplistic. The mechanisms for speciation in the marine environment appear to be as varied as
524 those found in continental biomes, and speciation with gene flow may be common (Crow et al.
525 2010; Bowen et al. 2013). Furthermore, while there is a 7,500-fold difference in water volume
526 between the ocean and freshwater (Horn 1972), most marine biodiversity is found along

527 coastlines, not in the open ocean. In particular, coral reefs contain ~40% of marine fishes within
528 only 0.1% of the Earth's surface area (Cowman et al. 2017). The similarity in species richness
529 between marine and freshwater fishes (Fig. 1) does not seem as puzzling when considering the
530 distribution of species within the ocean.

531 The most famous examples of lacustrine radiation are cichlids in the African rift lakes
532 (Wagner et al. 2012; Seehausen 2015; Burress and Tan 2017; McGee et al. 2020). However,
533 rapid diversification in lakes was not limited to cichlids (Fig. 2) nor African lakes (Fig. 3).
534 Diversification rates were faster in lakes than in rivers within all six continental regions of the
535 world (Fig. 3D). Lacustrine radiations with elevated diversification rates other than cichlids
536 include: Lake Titicaca *Orestias* (mean DR=0.6 species/million years), *Coregonus* of Nearctic
537 (DR=2.67) and Palearctic (2.09) postglacial lakes, Lake Baikal sculpins (0.71), Lake Tana
538 *Labeobarbus* (4.08), and Sulawesi telmatherinid rainbowfishes (0.31). Additional examples are
539 summarized in Table 4.

540 If diversification rates are so fast in lakes, then why do lakes contain a small proportion
541 of freshwater diversity (Figs. 1, 3C)? Biogeographic models suggested that this contradictory
542 pattern can be explained by transition rates among habitats (Fig. 3B). In the best-fitting model,
543 direct transitions from the ocean into lakes were constrained to be rarer than marine-riverine
544 transitions (Table 2). This is intuitive, as lakes are fed by rivers and do not usually interface
545 directly with the ocean (but see Schluter and McPhail 1992 and Hughes et al. 2020 for examples
546 of marine-lacustrine connections). The most common path to entering lakes is by first entering
547 rivers, then occurring in both rivers and lakes, then finally becoming endemic to lakes (Fig. 3B).
548 Since there are several evolutionary steps to becoming lacustrine, lacustrine radiations were
549 usually nested within riverine lineages (Fig. 3A). Therefore, lacustrine radiations had less time-

550 for-speciation than riverine counterparts (Fig. 3D; Stephens and Wiens 2003). In addition,
551 transition rates out of lakes were higher than those into lakes (Fig. 3B). This suggests that being
552 endemic to lakes is an evolutionarily unstable state.

553 These biogeographic results can be framed in light of the age of rivers versus lakes. On a
554 local scale, conditions in rivers change frequently and seasonally, while conditions in lakes are
555 more stable. However, lakes are generally younger than rivers over geologic timescales because
556 lakes dry out and can disappear entirely (Lowe-McConnell 1969; McCune 1987; Joyce et al.
557 2005; Cristescu et al. 2010). Indeed, the fossil record documents past lacustrine radiations that
558 went extinct and were replaced as lakes dried out and refilled (McCune 1987). Lacustrine fishes
559 may avoid extinction by entering rivers (Joyce et al. 2005; Seehausen and Wagner 2014),
560 explaining the high transition rates out of lakes. Another consideration is that rapid
561 diversification rates are probably short lived, and the richness of lacustrine radiations may
562 ultimately be constrained by the features of individual lakes (Wagner et al. 2012, 2014). It is
563 noteworthy that many intralacustrine radiations are limited to populations within a single species,
564 or pairs of species (McPhail and Schluter 1992, 1993; Hudson et al. 2007). In contrast, some
565 large and ancient lakes host a greater diversity of species (Cohen 1995; Wagner et al. 2014) but
566 these lake systems may be atypical.

567 It is somewhat unexpected that rivers have lower speciation rates than lakes, since the
568 fragmentation of rivers promotes population subdivision and endemism (Dias et al. 2013). Note
569 however that a speciation rate represents the number of species generated per unit of time, and
570 not simply the number of species generated; therefore, the occurrence of many speciation events
571 might not translate to a fast speciation rate if these events occurred over several million years.
572 McCune and Lovejoy (1998) suggested that the reason for faster speciation rates in lakes was

573 that sympatric speciation completes faster than allopatric speciation. My results across fishes are
574 consistent with McCune and Lovejoy's findings, assuming that sympatric speciation is more
575 common in lakes than in rivers (Bolnick and Fitzpatrick 2007; Seehausen and Wagner 2014).
576 Still, I did not directly identify geographic modes of speciation in this study. At the time of
577 writing, phylogenetic sampling across fishes is still generally below that needed to accurately
578 infer the speciation mode from present-day geographic ranges (Skeels and Cardillo 2019). In
579 addition, allopatric speciation occurring over short distances within a lake can be difficult to
580 distinguish from sympatric speciation without fine-scale spatial and ecological data (Rico and
581 Turner 2002; Seehausen and van Alphen 1999). An unanswered question is whether allopatric
582 speciation within and among lakes is also generally faster than allopatric speciation in rivers.
583 Some lacustrine radiations for which allopatry appears to be the dominant mode also have rapid
584 diversification rates (Table 4).

585 Besides speciation mode, there are ecological differences between rivers and lakes that
586 could explain faster lacustrine diversification rates. Compared to rivers, lakes may allow for
587 more ecological specialization because of their more stable conditions and greater structural
588 diversity (Seehausen 2015). Stronger competition and predation pressure in rivers may also
589 inhibit speciation (Briggs 1966; Lowe-McConnell 1969; Seehausen 2015), such that fishes
590 experience ecological release when they colonize lakes (Yoder et al. 2010; Burress and Tan
591 2017). The observation that the present-day richness of many lakes appears to be below the
592 lake's carrying capacity (Barbour and Brown 1974; Doenz et al. 2018; Hauffe et al. 2020)
593 supports the idea of high ecological opportunity in lakes.

594 In conclusion, I found that lacustrine fishes have faster speciation and net diversification
595 rates than other aquatic habitats. Still, lakes represent a small fraction of freshwater diversity

596 compared to rivers. Surprisingly, riverine and marine fishes had similar rates of diversification,
597 despite past literature predicting that these habitats had opposite effects on diversification (Horn
598 1972; May 1994; Grosberg et al. 2012; Tedesco et al. 2017a). While allopatric speciation is
599 indeed important for generating freshwater diversity, this may not be the mechanism driving
600 faster speciation rates between freshwater and marine habitats observed in some past studies.
601 Furthermore, these results support an alternative explanation for the freshwater paradox.
602 Speciation is roughly as frequent in freshwater and marine habitats on average, and teleosts have
603 occupied these habitats for comparable amounts of time over their 200-million-year history.
604 Future research should aim to identify ecological and evolutionary mechanisms for how riverine
605 habitats support long-term diversification and lineage persistence while lacustrine habitats
606 promote rapid diversification.
607
608

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889 **Table 1.** Studies comparing diversification rates between (A) freshwater and marine ray-finned fishes, and (B) freshwater, marine,

890 and/or terrestrial habitats using other organisms. Here I note if the study supports the hypothesis that net diversification rates are faster

891 in freshwater than other habitats. See footnotes for method references.

892

Study	Clade	Method	Result	Supports hypothesis?
A: Ray-finned fishes:				
Davis et al. 2012	Terapontidae (grunters)	MEDUSA[1]	Higher freshwater net diversification rates	Yes
Carrete Vega and Wiens 2012	Ray-finned fishes	Method-of-moments[2]	No difference in net diversification rates	No
Betancur-R et al. 2012	Ariidae (sea catfishes)	GeoSSE[3]	No differences in rates	No
Bloom et al. 2013	Atherinopsidae (New World silversides)	BiSSE[4]	Higher freshwater speciation and extinction rates; little difference in net diversification rates	No
Santini et al. 2013	Tetraodontidae (puffers)	MuSSE[5]	Higher marine speciation rates	No
Betancur-R et al. 2015	Ray-finned fishes	GeoSSE	Higher marine net diversification rates	No
Guinot and Cavin 2015	Ray-finned fishes	Fossil family diversity through time	Accumulation of families has slowed through time in marine but not freshwater fishes	Unclear (see text)

Tedesco et al. 2017a	Ray-finned fishes	Method-of-moments	Higher freshwater net diversification rates	Yes
Miller et al. 2018	Percomorpha (e.g. cichlids, guppies, reef fishes)	HiSSE[6]	Slightly higher freshwater speciation, extinction and net diversification rates	Yes
Rabosky 2020	Ray-finned fishes	STRAPP[7]	Higher freshwater speciation rates driven by a few clades, but not a general pattern among fishes	Potentially
B: Other organisms:				
Wiens 2015a	All animal phyla	Method-of-moments	Higher non-marine net diversification rates	Unclear-freshwater and terrestrial combined
Wiens 2015b	Vertebrates	Method-of-moments	Higher terrestrial net diversification rates compared to aquatic habitats, but no difference between non-marine and marine habitats	Unclear-freshwater combined with other habitats
Poulin 2016	Fish parasites	Species-to-genus ratios	Higher parasite species-to-genus ratios in freshwater versus marine fishes	Yes
Miller and Wiens 2017	Amniotes	HiSSE	No consistent difference between marine and non-marine rates among clades	Unclear-freshwater and terrestrial combined

Kirchhoff et al. 2017	Myliobatiformes (stingrays)	ClaSSE[8]	No difference in rates between freshwater and marine habitats; brackish rates were lowest	No
Nakov et al. 2019	Diatoms	HiSSE	Higher freshwater speciation, extinction, and net diversification rates	Yes

1: Alfaro et al. 2009; 2: Magallón and Sanderson 2001; 3: Goldberg et al. 2011; 4: Maddison et al. 2007; 5: Fitzjohn 2012; 6: Beaulieu and O'Meara 2016; 7: Rabosky and Huang 2016; 8: Goldberg and Igić 2012

898 **Table 2.** Comparing the fit of alternative biogeographic models fit using BioGeoBEARS (Matzke 2014). Models differed in the
899 constraints set on dispersal in order to test hypotheses about biogeographic connections among lakes. Constraints were set by
900 multiplying the probability of dispersal by 0.05 (more details in Extended Text 2 and Table S11). The best-fitting model (in bold) was
901 used in downstream analyses (Figure 3).
902

Marine-lake transitions	**Regional dispersal via lakes**	**Base model**	**LnL**	**No. params**	***d***	***e***	***j***	**AIC**	**Akaike weight**
No constraints	No constraints	BAYAREA	-8746.8	2	0.0015	0.0035	-	17497.6	<0.01
		BAYAREA+J	-7317.8	3	0.0010	0.0004	0.0061	14641.7	<0.01
		DEC	-7580.9	2	0.0019	0.0001	-	15165.9	<0.01
		DEC+J	-7468.5	3	0.0016	<0.0001	0.0024	14943.0	<0.01
		DIVA	-7993.1	2	0.0020	<0.0001	-	15990.1	<0.01
		DIVA+J	-7870.4	3	0.0017	<0.0001	0.0017	15746.8	<0.01
Constrained	No constraints	BAYAREA	-8526.4	2	0.0020	0.0033	-	17056.8	<0.01
		BAYAREA+J	-7084.7	3	0.0013	0.0003	0.0076	14175.3	<0.01
		DEC	-7295.1	2	0.0024	0.0001	-	14594.3	<0.01
		DEC+J	-7186.0	3	0.0021	<0.0001	0.0032	14377.9	<0.01
		DIVA	-8740.3	2	0.0031	0.0063	-	17484.5	<0.01
		DIVA+J	-7555.9	3	0.0022	<0.0001	0.0035	15117.7	<0.01

No constraints	Always constrained	BAYAREA	-8767.7	2	0.0015	0.0035	-	17539.5	<0.01
		BAYAREA+J	-7324.8	3	0.0010	0.0004	0.0063	14655.7	<0.01
		DEC	-7599.6	2	0.0019	0.0001	-	15203.2	<0.01
		DEC+J	-7485.6	3	0.0017	<0.0001	0.0020	14977.3	<0.01
		DIVA	-8007.6	2	0.0021	<0.0001	-	16019.1	<0.01
		DIVA+J	-7868.5	3	0.0017	<0.0001	0.0030	15743.1	<0.01
No constraints	Some types constrained	BAYAREA	-8746.6	2	0.0015	0.0035	-	17497.3	<0.01
		BAYAREA+J	-7312.5	3	0.0010	0.0004	0.0062	14631.0	<0.01
		DEC	-7580.0	2	0.0019	0.0001	-	15163.9	<0.01
		DEC+J	-7466.5	3	0.0016	<0.0001	0.0024	14939.0	<0.01
		DIVA	-7991.6	2	0.0020	<0.0001	-	15987.2	<0.01
		DIVA+J	-7853.1	3	0.0017	<0.0001	0.0027	15712.2	<0.01
Constrained	Always constrained	BAYAREA	-8546.5	2	0.0020	0.0033	-	17096.9	<0.01
		BAYAREA+J	-7086.3	3	0.0014	0.0003	0.0080	14178.6	<0.01
		DEC	-7318.8	2	0.0027	<0.0001	-	14641.6	<0.01
		DEC+J	-7198.6	3	0.0022	<0.0001	0.0027	14403.1	<0.01
		DIVA	-8741.0	2	0.0031	0.0062	-	17486.1	<0.01
		DIVA+J	-7564.1	3	0.0023	<0.0001	0.0037	15134.2	<0.01
Constrained	**Some types constrained**	BAYAREA	-8526.1	2	0.0020	0.0033	-	17056.2	<0.01
		BAYAREA+J	**-7077.1**	**3**	**0.0013**	**0.0003**	**0.0078**	**14160.2**	**1.00**

DEC	-7293.0	2	0.0024	0.0001	-	14590.0	<0.01
DEC+J	-7183.9	3	0.0022	<0.0001	0.0029	14373.9	<0.01
DIVA	-8732.9	2	0.0031	0.0063	-	17469.8	<0.01
DIVA+J	-7550.6	3	0.0022	<0.0001	0.0036	15107.2	<0.01

906 **Table 3:** Summary of 56 phylogenetic ANOVA and post hoc tests comparing diversification rates (seven types) among habitat
907 categories (four habitat combinations with and without cichlids). Rates were calculated by Rabosky et al. (2018; see also Chang et al.
908 2019). Full results including post hoc tests are reported in Tables S2–S8. Six habitats=marine, estuarine, diadromous, riverine,
909 lacustrine, both riverine and lacustrine. Four habitats=marine, riverine, lacustrine, both riverine and lacustrine. Three
910 habitats=riverine, lacustrine, both riverine and lacustrine. Two habitats=marine and freshwater. Boldface=ANOVA was significant.
911 *=Lakes were significantly different from all other habitats in pairwise post hoc tests, even if the ANOVA was not globally
912 significant.
913

	All teleosts, P-value				Teleosts excluding Cichlidae, P-value			
Rate type	Six habitats	Four habitats	Three habitats	Two habitats	Six habitats	Four habitats	Three habitats	Two habitats
DR	0.093*	0.121*	**0.001***	0.653	0.464*	0.468*	**0.001***	0.789
BAMM, speciation, time-varying	0.062*	0.071*	**0.001***	0.625	0.428*	0.448*	**0.001***	0.789
BAMM, speciation, time-constant	0.059*	0.071*	**0.001***	0.537	0.418*	0.417*	**0.001***	0.747

BAMM, extinction, time-varying	0.298	0.344	0.061	0.727	0.355	0.487*	**0.001***	0.832
BAMM, extinction, time-constant	0.270	0.266	**0.038**	0.635	0.379	0.460*	**0.001***	0.748
BAMM, net diversification, time-varying	**0.047***	0.071*	**0.001***	0.624	0.555*	0.546*	**0.001***	0.811
BAMM, net diversification, time-constant	**0.041***	0.056*	**0.001***	0.597	0.562*	0.561*	**0.001***	0.793

917 **Table 4.** Examples of lacustrine radiations other than cichlids. Note that this list is not exhaustive. These radiations represent
918 independent transitions to the lacustrine state based on biogeographic reconstructions (Fig. 3). Radiations are ranked by diversification
919 rate in descending order. The mean DR represents the mean tip-associated diversification rate (species/million years) of members of
920 the lacustrine lineage identified by BioGeoBEARS (Fig. 3D). Taxonomy follows Rabosky et al. (2018). Here I note whether
921 monophyletic clade(s) are found within individual lakes (intralacustrine radiations) based on prior phylogenetic studies.

922
923

Radiation	**Family**	**Region**	**Lake system**	**Mean DR**	**Intralacustrine radiation(s)?**	**References**
Labeobarbus	Cyprinidae	Ethiopian	Lake Tana, Ethiopia	4.08 ± 2.39	yes	Nagelkerke and Sibbing 2000
Coregonus	Salmonidae	Nearctic	North American postglacial lakes	2.67 ± 1.16	no	Hudson et al. 2007
Coregonus	Salmonidae	Palearctic	Eurasian postglacial lakes	2.09 ± 2.4	some members	Hudson et al. 2007; Doenz et al. 2018
Salvelinus and *Salvethymus*	Salmonidae	Palearctic	Lake El'gygytgyn and surrounding basins, Russia	1.06 ± 0.73	no	Osinov et al. 2015
Bathyclarias	Clariidae	Ethiopian	Lake Malawi, East Africa	0.96 ± 0.04	yes	Anseaume and Teugels 2005
Lake Baikal sculpins	Cottidae	Palearctic	Lake Baikal, Russia	0.71 ± 0.4	yes	Cohen 1995; Sherbakov 1999

Orestias	Cyprinodontidae	Neotropics	Lake Titicaca and surrounding basins, Peru/Bolivia	0.6 ± 0.42	no	Parenti 1984
Synodontis	Mochokidae	Ethiopian	Lake Tanganyika, East Africa	0.54 ± 0.23	yes	Day et al. 2009
Poblana	Atherinopsidae	Neotropics	Central Mexican Plateau	0.38 ± 0.12	some members	Bloom et al. 2013
Sulawesi telmatherinid rainbowfishes	Telmatherinidae	Sino-Oriental	Malili Lake system, Sulawesi	0.31 ± 0.16	some members	Herder et al. 2006
Prosopium	Salmonidae	Nearctic	Bear Lake, Idaho/Utah, U.S.A.	0.22 ± 0.07	yes	Vuorinen et al. 1998; Hudson et al. 2007
Mastacembelus	Mastacembelidae	Ethiopian	Lake Tanganyika, East Africa	0.19 ± 0.07	yes	Brown et al. 2010
Sarcocheilichthys	Cyprinidae	Sino-Oriental	Lake Biwa, Japan	0.15 ± 0.01	yes	Hauffe et al. 2020
Oryzias	Adrianichthyidae	Sino-Oriental	Malili Lake system, Sulawesi	0.12 ± 0.08	no	Parenti 2008
Paragalaxias	Galaxiidae	Australian	Central Plateau of Tasmania	0.10 ± 0.03	no	McDowall 1998

927 **FIGURE LEGENDS**

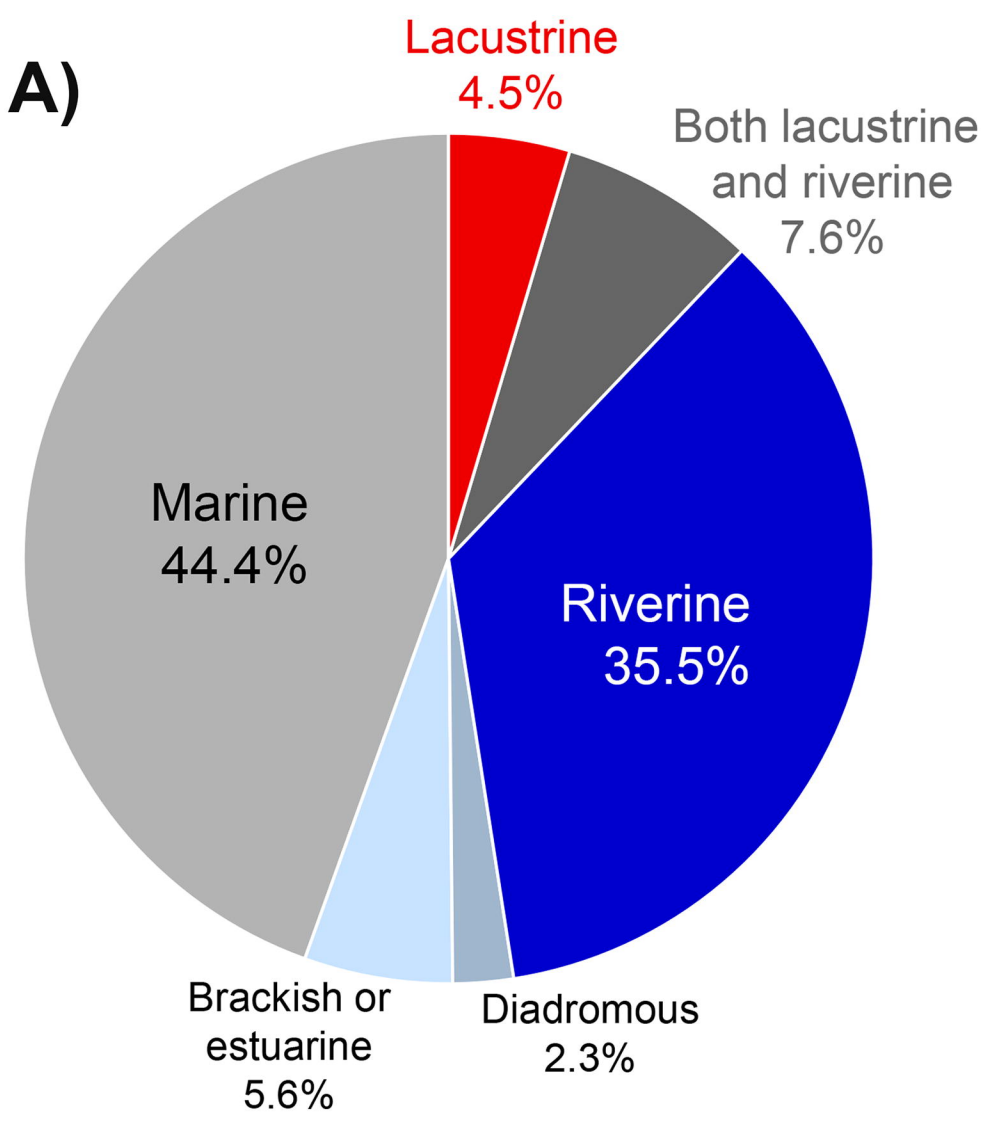
928

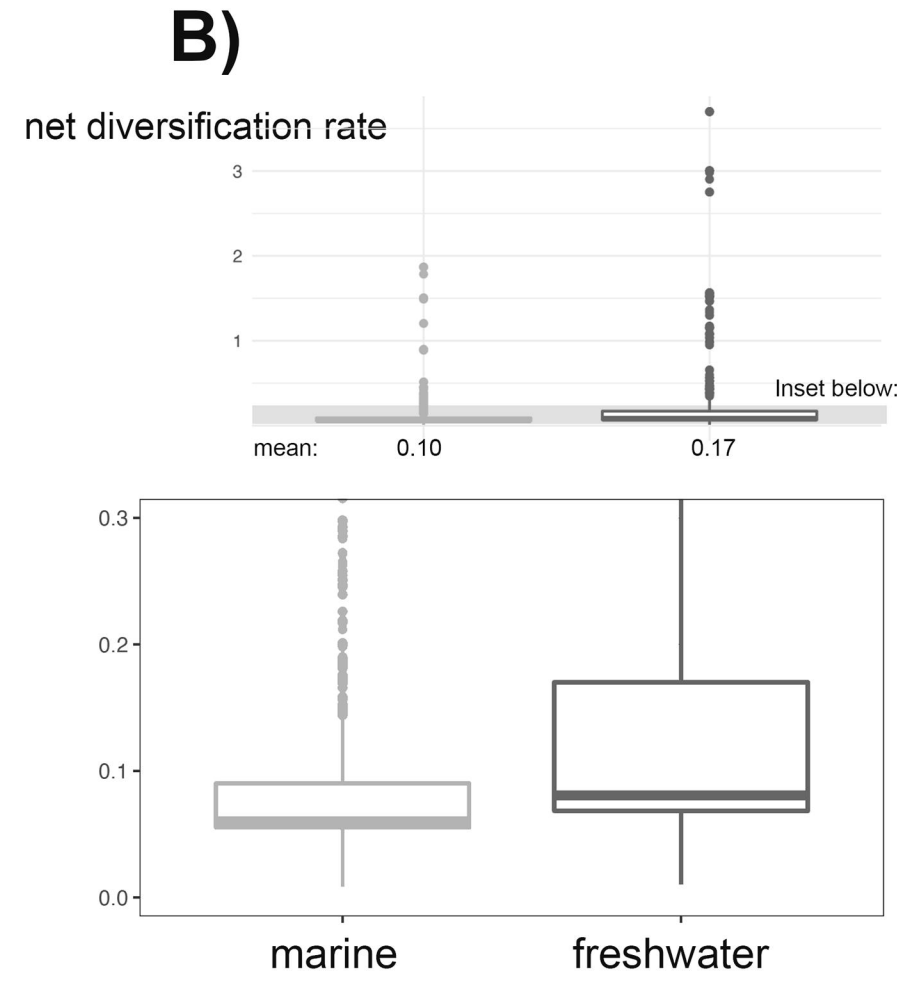
929 **Figure 1.** Diversity and diversification rates in aquatic habitats. (A) Proportion of teleost species richness in each habitat (n=11,227
930 species). (B) Tip-associated net diversification rates in species found exclusively in marine or freshwater habitats (n=10,177 species).
931 (C) Tip-associated speciation, extinction, and net diversification rates among six aquatic habitats. Boxplots summarize rates for
932 individual species, including medians, first and third quartiles, and outliers (outside the 95% confidence interval). Mean rates are
933 printed below each habitat. All rates shown here were calculated using BAMM under a time-varying model of diversification rates
934 (Rabosky et al. 2018; Chang et al. 2019). Results were similar using other methods of calculating diversification rates (Table 3; Table
935 S2–S8).

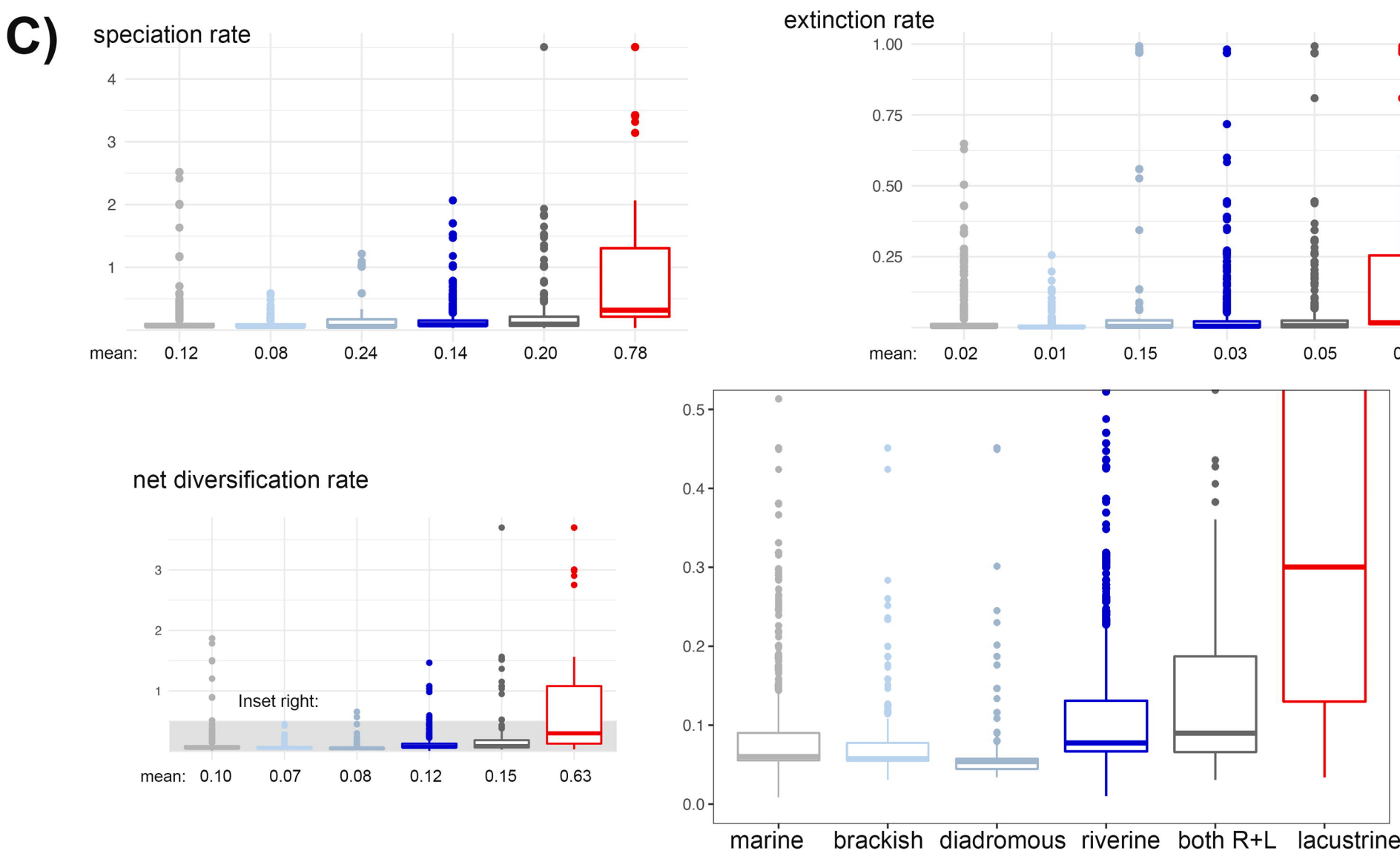
936

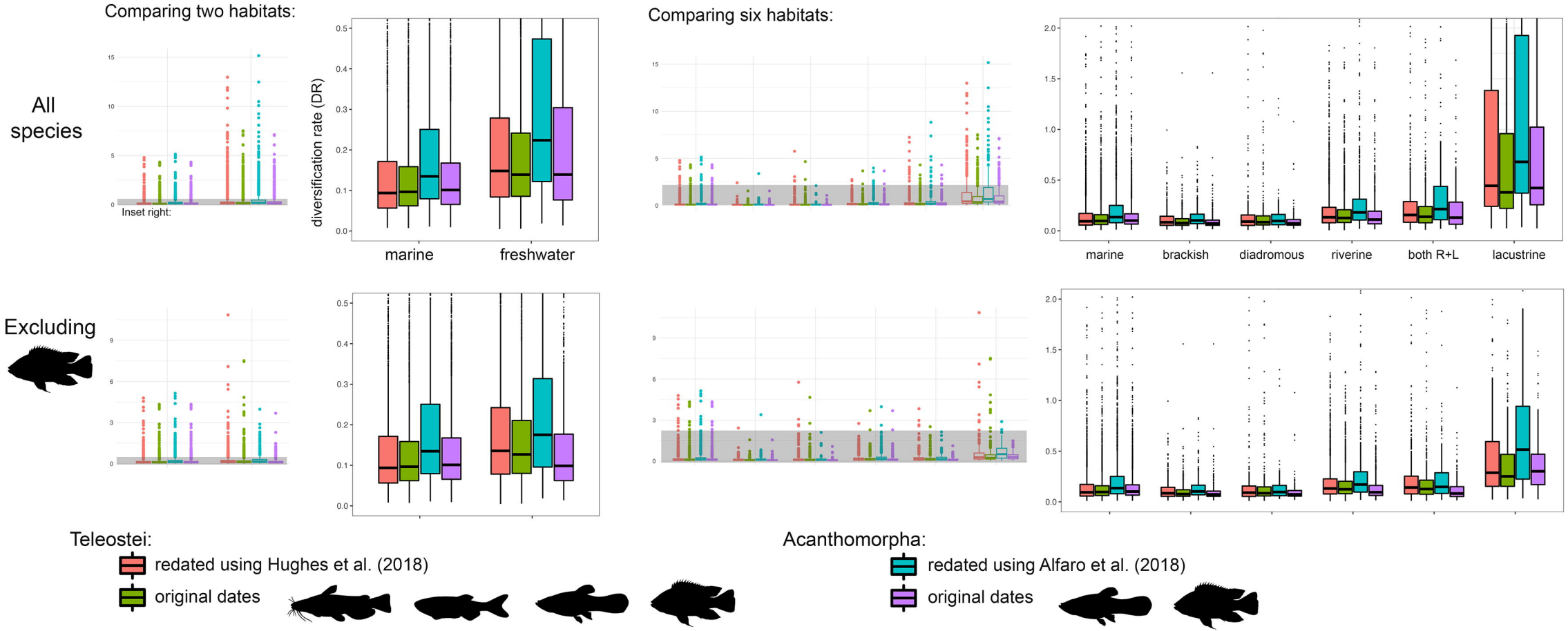
937 **Figure 2.** Diversification rate variation by habitat using alternative divergence time estimates and clade selection. All rates shown
938 were calculated using the DR statistic (full results in Tables S2–S10). Boxplots summarize rates for individual species, including
939 medians, first and third quartiles, and outliers (outside the 95% confidence interval). Left panels compare rates in species exclusive to
940 marine and freshwater habitats; right panels compare rates among six aquatic habitats. Top panels compare rates using all members of
941 the clade; right panels compare rates after excluding the family Cichlidae. For teleosts, red bars represent rates calculated from the
942 Rabosky et al. (2018) phylogeny redated using Hughes et al. (2018) as a reference; green bars represent rates using the original tree.
943 For acanthomorphs (spiny-rayed fishes), blue bars represent the Rabosky et al. (2018) phylogeny redated using Alfaro et al. (2018) as

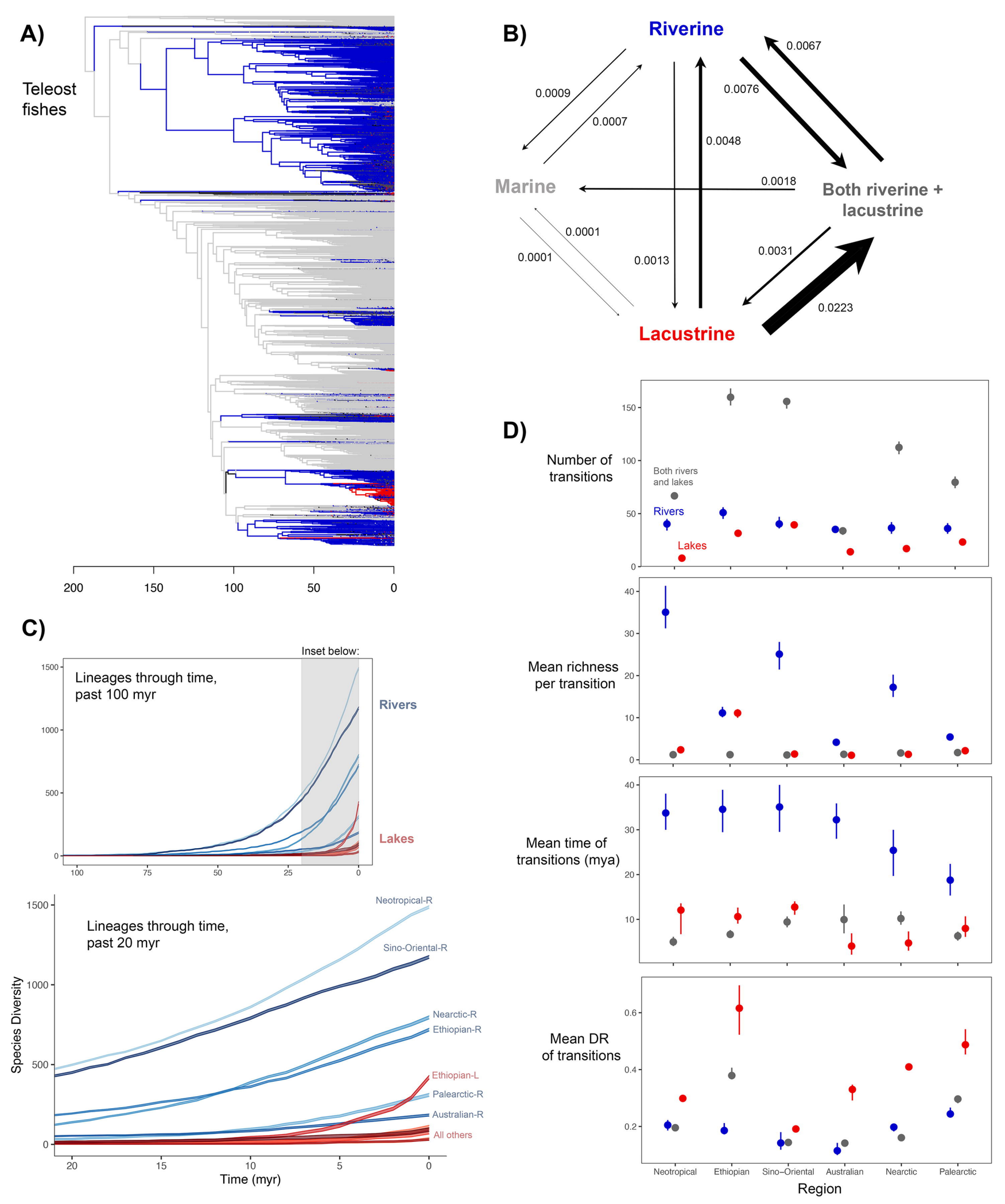
944 a reference; purple bars represent rates using the original tree (Acanthomorpha alone). Fish icons from PhyloPic (http://phylopic.org/)
945 with credit to Lily Hughes, Tyler McCraney, and Milton Tan.
946
947 **Figure 3.** Results of biogeographic and habitat modelling. In panels A and B, biogeographic regions were combined to illustrate
948 habitat transitions overall. In C and D, habitats are shown separately for each region. (A) Ancestral habitats based on best-fit model
949 (Table 2). Here, light grey=marine, blue=riverine, red=lacustrine, dark grey=both riverine and lacustrine, and black=uncertain (no
950 habitat with probability >80%). (B) Transition rates among habitats. Black arrows are sized to scale of transition rates. Number is the
951 mean transition rate calculated from 100 biogeographic stochastic maps. (C) Lineages-through-space-and-time plot (Skeels 2019) of
952 total riverine and lacustrine diversity within six biogeographic regions. The width of lines represents the range of values observed
953 among 100 stochastic maps. Bioregionalization follows Leroy et al. (2019). (D) Properties of lineages representing independent
954 habitat transitions by region. From top to bottom: number of independent transitions into that habitat, mean number of species
955 descending from transitions, mean time of transitions, and mean tip-associated diversification rate (DR) of descendants. Points show
956 the mean and range of mean values among 100 stochastic maps.


A)
Lacustrine
4.5%
Both lacustrine
and riverine
7.6%
Marine
44.4%
Riverine
35.5%
Brackish or
estuarine
5.6%
Diadromous
2.3%


B)
net diversification rate
3
2
1
Inset below:
mean:
0.10
0.17
0.3
0.2
0.1
0.0
marine
freshwater


C)
speciation rate
4
3
2
1
mean:
0.12
0.08
0.24
0.14
0.20
0.78
extinction rate
1.00
0.75
0.50
0.25
mean:
0.02
0.01
0.15
0.03
0.05
0.15
net diversification rate
3
2
1
Inset right:
mean:
0.10
0.07
0.08
0.12
0.15
0.63
0.5
0.4
0.3
0.2
0.1
0.0
marine
brackish
diadromous
riverine
both R+L
lacustrine


Comparing two habitats:
Comparing six habitats:
All species
Excluding
Inset right:
diversification rate (DR)
marine
freshwater
marine
brackish
diadromous
riverine
both R+L
lacustrine
Teleostei:
redated using Hughes et al. (2018)
original dates
Acanthomorpha:
redated using Alfaro et al. (2018)
original dates


A)
Teleost fishes
200
150
100
50
0
B)
Riverine
Marine
Both riverine + lacustrine
Lacustrine
0.0009
0.0007
0.0067
0.0076
0.0048
0.0018
0.0001
0.0001
0.0013
0.0031
0.0223
C)
Inset below:
Lineages through time, past 100 myr
Rivers
Lakes
Lineages through time, past 20 myr
Neotropical-R
Sino-Oriental-R
Nearctic-R
Ethiopian-R
Ethiopian-L
Palearctic-R
Australian-R
All others
Species Diversity
Time (myr)
D)
Number of transitions
Both rivers and lakes
Rivers
Lakes
Mean richness per transition
Mean time of transitions (mya)
Mean DR of transitions
Neotropical
Ethiopian
Sino–Oriental
Australian
Nearctic
Palearctic
Region