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RESEARCH ARTICLE

Geologic History Explains Freshwater Fish Species Richness Across the Conterminous USA

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ABSTRACT

Aim: Freshwater fishes comprise over 20% of vertebrate biodiversity despite occupying <1% of the Earth's surface. However, species richness differs substantially among river basins. Fundamentally, richness patterns can be explained by spatial variation in diversification rates, evolutionary time and habitat capacities, which are in turn shaped by landscape change over geologic timescales. To test how geologic disturbances have influenced the accumulation of freshwater fish biodiversity, we hypothesized species richness would be (1) ordered by regional geologic history, (2) associated with high or intermediate river capture rates, (3) higher in assemblages with older evolutionary origins and (4) positively associated with stream size.

Time Period: 2008–2019.

Location: Conterminous United States (USA).

Major Taxa: Freshwater fishes.

Methods: We analysed native species richness from a spatially representative survey of 5321 fish assemblages at 3609 sites. Geologic history was determined from surrogates of tectonic activity, glaciation, sea levels and river capture over the last 66 million years, which were paired with previously published evolutionary time estimates. Hypotheses were tested with spatial linear models.

Results: All hypotheses were at least partially supported. (1) Rank-order richness matched hypothesized effects of geologic disturbances on evolutionary time and diversification rates. (2) Richness peaked in lowlands with high putative river capture rates. (3) Richness increased with evolutionary time at broad scales, but this relationship was weak and influenced by non-teleost taxa. (4) Richness largely increased with stream size. Overall, the tectonically active western USA exhibited lower richness, weaker effects of stream size and a greater share of young lineages compared to the more geologically stable eastern USA, especially unglaciated lowlands within the Mississippi Basin.

Main Conclusions: We demonstrate that deep-time processes leave a persistent mark on fish species richness. Thus, accounting for geologic history can improve assessments of freshwater biodiversity and biological condition in the USA and beyond.

1 | Introduction

Fishes are exceptionally diverse, accounting for approximately half of all extant vertebrate biodiversity (Near et al. 2012). Roughly half of all known fish species live in freshwater environments, mostly in rivers and streams, likely because riverine habitats promote speciation through habitat fragmentation, niche diversity and reproductive isolation (Seehausen and Wagner 2014). Additionally, rivers globally support greater fish biodiversity than other freshwater systems, such as lakes because rivers are more stable over geologic timescales, thus providing more time for new species to accumulate (Miller 2021). Still, fish species richness varies by several orders of magnitude among river basins and exhibits a strong latitudinal gradient, with the highest diversity in large tropical lowland basins such as the Amazon, Congo and Mekong (Oberdorff et al. 2011). Moreover, riverine fishes are commonly affected by human activities such as dam construction, land use change and habitat alteration (Comte et al. 2021; Chen et al. 2023). These impacts are unevenly distributed and often most detrimental in highly developed temperate regions, although many tropical basins with the highest diversity still face substantial risks (Su et al. 2021). Given the threats to global freshwater biodiversity, there is a need to prioritise monitoring, assessment, and conservation of these crucial ecosystems (Albert et al. 2021).

Fundamentally, species richness patterns are determined by three macroevolutionary processes: net diversification rate, evolutionary time and habitat capacity (Rabosky 2010). These processes interact to shape how species accumulate over time and can be broken down into sub-processes that vary spatially (Rabosky et al. 2018), which is the primary interest of this study. Net diversification rates describe how rapidly new species are added to an area over a given timespan, which we define as the difference between in situ speciation and extinction rates. Local species accumulation can also be modified by dispersal within and among river basins, which can indirectly alter net diversification rates by changing the balance between speciation and extinction (Smith 1981; Val et al. 2022). Similarly, we define evolutionary time as the timespan available for species to accumulate in an area via in situ diversification (Stephens and Wiens 2003). Evolutionary time is often estimated from time-calibrated phylogenies as the time since a given lineage originated or colonised an area, which is influenced by deep-time continental drift and climate variability (Miller and Román-Palacios 2021). Lastly, we define habitat capacity (sometimes called 'ecological limits') as the maximum number of species that can be supported in an area, which could be related to habitat size or productivity (Oberdorff et al. 2011; McGarvey and Terra 2016). When habitat capacity is not limiting, richness is believed to accumulate at a constant rate, and spatial patterns are largely determined by variation in net diversification rates and evolutionary time. However, when habitat conditions impose an upper bound on richness, net diversification rates approach zero and richness accumulates asymptotically (Rabosky 2010). Taken together, richness should be highest in areas with a combination of rapid diversification, prolonged evolutionary time and high habitat capacities, which are characteristics shared by large tropical basins that harbour the most freshwater fish diversity (Miller and Román-Palacios 2021; Val et al. 2022; Melo et al. 2022).

Geologic history is hypothesized to be a key driver of spatial variation in aquatic species richness via its influence on diversification rates, evolutionary time and habitat capacity. These macroevolutionary processes can be altered by tectonic activity, glaciation, sea levels and river capture over millions of years (Figure 1). For example, tectonic activity may strongly reduce richness because mountain building causes drainage fragmentation and creates persistent barriers to dispersal among river basins (Val et al. 2022). Limits on dispersal can interact with long-term climatic fluctuations (e.g.,

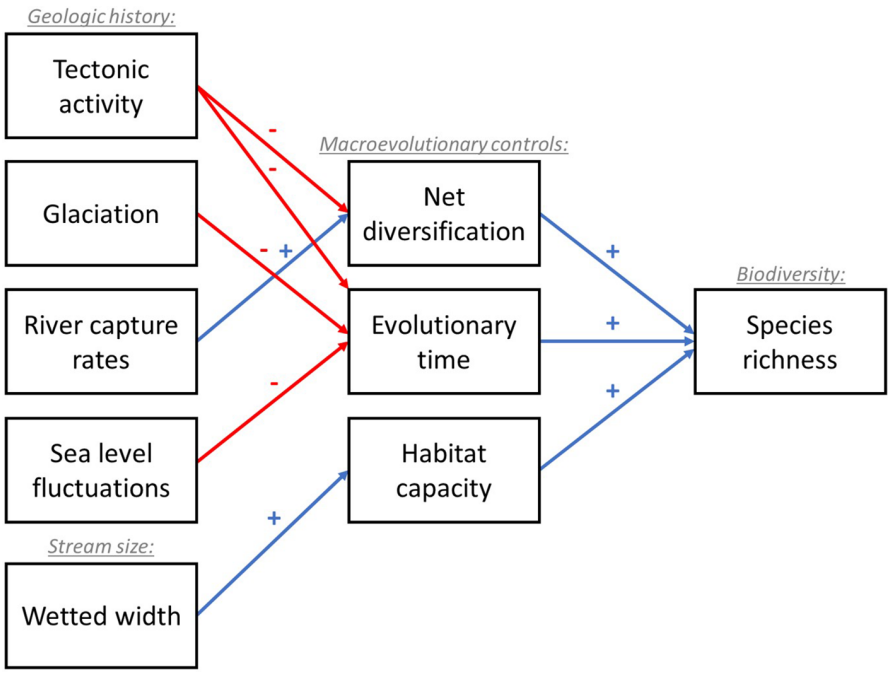

FIGURE 1 | Conceptual diagram showing expected influence of four geologic events and one stream size metric on three macroevolutionary processes, which in turn influence spatial variation in species richness. Negative effects are shown by red arrows and labelled with minus signs (–), while positive effects are shown by blue arrows and labelled with plus signs (+).

between pluvial and arid precipitation regimes; Ibarra et al. 2018; Cook et al. 2025) to destabilise habitats and increase extinction rates, resulting in shortened evolutionary time and slower net diversification rates in active landscapes (Smith et al. 2010). In contrast, river capture occurs when a drainage is diverted from its course and merged into a neighbouring watershed, which is thought to accelerate diversification by facilitating speciation and dispersal (Val et al. 2022). A recent study of river capture events since the mid-Eocene 45 mya suggested that the frequency and areal extent of river captures was highest in tectonically stable lowland basins (Albert et al. 2018), underlining that tectonic activity likely hinders species accumulation (Figure 1). Other geologic events effectively remove all species that previously existed in an area such as when freshwater systems get covered by glaciers or oceans. In these situations, species must avoid extinction by dispersing to nearby refugia and can only recolonize the area once freshwater systems reemerge, which reduces evolutionary time (Miller and Román-Palacios 2021; Figure 1). Interruptions in species accumulation may explain low freshwater richness in polar regions affected by Pleistocene glaciations (Cortés-Guzmán et al. 2024). Similarly, many coastal lowlands were flooded by high sea levels during warm periods 66–40 mya (Miller et al. 2020; Scotese 2021), which would have eliminated any freshwater fishes that were unable to disperse inland (Bellard et al. 2014; but see Noss et al. 2015). Finally, geologic history shapes basin area and riverine habitat size, which is longitudinally structured such that habitat capacity, and thus the number of species that can be supported, tends to increase with distance downstream (Figure 1). Indeed, high habitat volume, connectivity and complexity in large rivers may explain positive species-area and species-discharge relationships in freshwater fishes (McGarvey and Hughes 2008; Oberdorff et al. 2011; McGarvey and Terra 2016).

Most research to date has studied fish species richness at the scale of entire river basins (e.g., Tedesco et al. 2017; Miller and Román-Palacios 2021; Val et al. 2022). However, biodiversity, geologic history and hydrologic connectivity can differ considerably within basins and over time (e.g., Cassemiro et al. 2023; Makki et al. 2023). For example, some habitats within a river basin may be older and more stable over geologic timescales than others (Graham 2011). Thus, leveraging fine-scale data and diverse data types could provide new insights into macroevolutionary processes that shaped freshwater biodiversity.

The United States of America (USA) contains some of the most diverse temperate freshwater fish assemblages on Earth (Oberdorff et al. 1997, 2011), which are structured by regional differences in geologic history (Smith et al. 2010; Figure 2). Riverine fishes in the USA exhibit a latitudinal gradient in species richness (Figure S1), but latitude alone is a poor proxy for deep-time processes that build richness. Indeed, the latitudinal gradient is weaker in the western USA and is largely driven by exceptionally high diversity within the central-eastern Mississippi basin (Jenkins et al. 2015; Qian et al. 2020). Richness is highest around the Ohio and Tennessee drainages, which have mostly been tectonically stable, unglaciated, and part of an extensive system of interconnected freshwater lowlands for tens of millions of years (Bentley Sr et al. 2016; Russell et al. 2021). Geologic stability in this region may have thus promoted species accumulation by increasing diversification rates, evolutionary time and habitat capacity, especially compared to the tectonically active western USA (Smith 1981). The apparent association between geologic stability and fish biodiversity in the USA mirrors global patterns, where richness is highest in stable lowland basins formed by multiple river captures and drainage network reorganisations such as the Amazon and Congo (Albert et al. 2018; Val et al. 2022). Additionally, freshwater fish assemblages are extensively monitored in the USA (e.g., Herlihy et al. 2006; Hughes et al. 2023), providing opportunities to relate biodiversity patterns to geologic history at finer spatial scales than previous studies (e.g., Smith et al. 2010). For example, site-level data could help explain spatial variation in richness within the Mississippi basin, which was partially glaciated and reorganised by river capture after the Pleistocene (Strange 1999). Overall, fine-scale data coupled with more detailed proxies of geologic history can improve biological assessments, establish biodiversity baselines and ultimately support better inferences about the causes of biodiversity loss (Strange 1999; Hilburn et al. 2024).

In this study, we analysed native fish species richness patterns from the National Rivers and Streams Assessment, a spatially representative survey of rivers and streams across the conterminous USA (USEPA 2020). Our objective was to explain site-level richness with novel descriptors of regional tectonic activity, glaciation, river capture, sea level fluctuations and stream size (see Section 2), which may be useful proxies for macroevolutionary controls that govern spatial patterns in richness (Rabosky 2010; Figures 1 and 2). To our knowledge, our study is the first to assess how these five aspects of geologic history jointly influenced modern freshwater fish diversity. Additionally, we tested four hypotheses inspired by previous research (Table 1).

1.1 | (H1) Geologic Stability Hypothesis

Rank-order richness patterns will follow expected effects of geologic history on diversification rates and evolutionary time (after Smith et al. 2010; Miller and Román-Palacios 2021; Val et al. 2022). Specifically, we expected high richness in geologically stable regions that have not been tectonically active, glaciated, or flooded by high sea levels in the last 66 million years. We expected intermediate richness in tectonically stable regions where high sea levels or subsequent glaciation reduced evolutionary time. We expected the lowest richness in tectonically active regions that exhibited high extinction rates and reduced evolutionary time (Figure 2b, Table 1).

1.2 | (H2) River Capture Hypothesis

Richness will be greater in lowlands than highlands (Figure 2b), and greatest in areas with putatively high or intermediate river capture rates within each region. River capture rates are largely unknown (Albert et al. 2018), but we assumed high topographic relief in uplifted areas is associated with harder and more variable rock types, which limit river capture rates compared to low-relief areas dominated by soft alluvial rocks (Val et al. 2022). Therefore, we used associations between richness and topographic relief in lowland regions to assess two competing predictions (after Val et al. 2022). Specifically, if richness is maximised in lowlands with high river capture rates (i.e., in low-relief landscapes), we expected to observe negative associations with relief. Alternatively, if richness is maximised in lowlands with

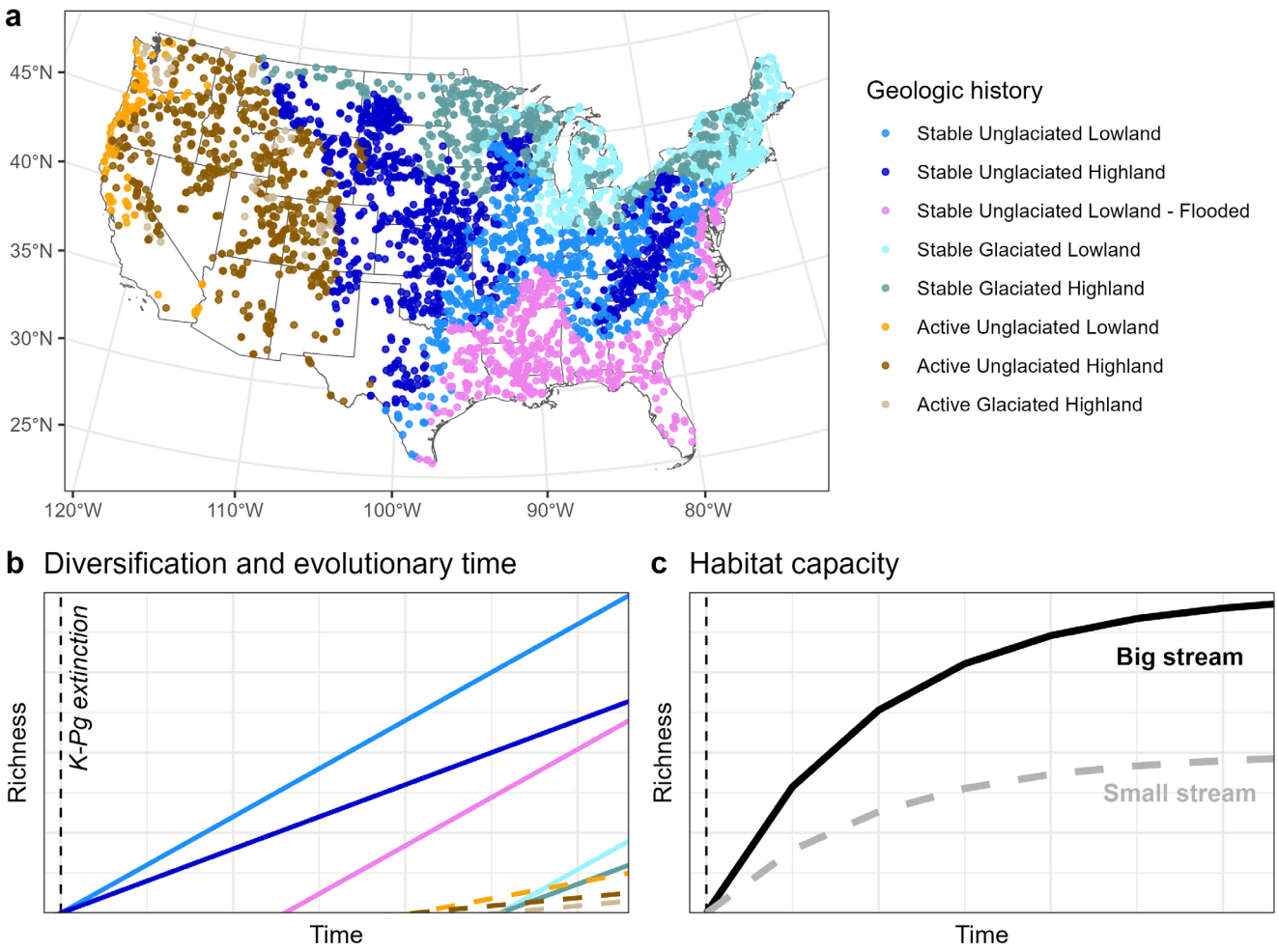

FIGURE 2 | Geologic history categories and hypothesized impacts on freshwater fish species richness across the conterminous United States. Geologic history categories in the legend are ordered by their hypothesized rank-order species richness (Table 1). Line colours in (b) correspond to point colours for geologic history categories in (a), and tectonically active regions (orange, brown, and tan) are shown as dashed lines to improve visibility. Thin dashed vertical lines in (b, c) signify the timing of the K-Pg extinction 66 mya.

intermediate river capture rates, we expected positive associations (Table 1).

1.3 | (H3) Evolutionary Time Hypothesis

Richness will be positively associated with evolutionary time, such that assemblages with higher richness will contain lineages with older evolutionary origins on average (after Miller and Román-Palacios 2021). Furthermore, the distribution of evolutionary time within species pools and assemblages should reflect differences in the relative timing of regional geologic disturbances (Figure 2b; Qian et al. 2020).

1.4 | (H4) Habitat Capacity Hypothesis

Richness will be positively associated with stream size, reflecting higher habitat capacity in large rivers and streams (Figure 2c). We expect this relationship will be observed regardless of regional geologic history, reflecting strong species–area and species–discharge relationships observed across diverse basin types within the USA (McGarvey and Hughes 2008) and globally (Val et al. 2022).

2 | Methods

Analysis was conducted in *R* version 4.4.0 (R Core Team 2024). Data handling and visualisation were done within the *tidyverse* package (Wickham et al. 2019), and basemaps were obtained from the *tigris* package (Walker 2024). Our workflow can be reproduced using a single R script, and the code is freely available as a Quarto document (see File S1).

2.1 | Native Species Richness Data

We used previously collected fish assemblage data from the National Rivers and Streams Assessment (NRSA) by the United States Environmental Protection Agency over three survey periods (2008–2009, 2013–2014, 2018–2019), which were accessed using the *finsyncR* package (Mahon et al. 2024). NRSA sites were selected using a randomised probability-based design, which was spatially balanced across states, ecoregions and stream orders to provide a representative sample across the conterminous USA (Olsen and Peck 2008). Fish were collected via backpack or boat electrofishing, with standardised protocols based on stream wetted width and whether the site was

TABLE 1 | Geologic history categories, number of observations (*N*), and a priori hypotheses for rank-order species richness (H1; geologic stability hypothesis), slopes with topographic relief assuming that richness is maximised at low relief or intermediate relief (H2; river capture hypothesis), slopes with mean assemblage evolutionary time (H3; evolutionary time hypothesis), and slopes with stream width (H4; habitat capacity hypothesis).

Geologic history	*N*	Richness rank (H1)	Justification of richness rank (H1)	Low relief slope (H2)	Int. relief slope (H2)	Evo. time slope (H3)	Width slope (H4)
Stable unglaciated lowland	974	1	Highest evolutionary time, high river capture rates	Negative	Positive	Positive	Positive
Stable unglaciated highland	1291	2	Highest evolutionary time, reduced river capture rates	Negative	Negative	Positive	Positive
Stable unglaciated lowland—flooded	764	3	Reduced evolutionary time, high river capture rates	Negative	Positive	Positive	Positive
Stable glaciated lowland	774	4	Lowest evolutionary time, high river capture rates	Negative	Positive	Positive	Positive
Stable glaciated highland	661	5	Lowest evolutionary time, reduced river capture rates	Negative	Negative	Positive	Positive
Active unglaciated lowland	112	6	Reduced evolutionary time, high river capture rates, highest extinction rates	Negative	Positive	Positive	Positive
Active unglaciated highland	674	7	Reduced evolutionary time and river capture rates, highest extinction rates	Negative	Negative	Positive	Positive
Active glaciated highland	72	8	Lowest evolutionary time, reduced river capture rates, highest extinction rates	Negative	Negative	Positive	Positive

Note: Justification for hypothesized species richness patterns for H1 is also provided and can be visualised in Figure 2b. Note that two separate models were built to test hypotheses (see Section 2).

wadeable or boatable (USEPA 2017a, 2017b; Hughes et al. 2023). Wetted width measures the distance between the water's edge from bank to bank, which is a consistent indicator of stream size (Hughes et al. 2011; Figure S2).

As part of NRSA, fish sampling occurred in 1-day visits during low-flow conditions, mostly from June to September (94% of observations). All individuals with total length > 25 mm were identified, counted, then released. Species names were based on Nelson et al. (2004) and Page et al. (2013), and a small number of individuals visually identified as hybrids were excluded from all analyses. After removing sites where fish were absent or where wetted width measurements were missing, we obtained 5321 samples from 3609 sites. Species at each site were assigned as native or non-native using the SEINeD tool from the nonindigenous aquatic species database (USGS 2024), which aligned georeferenced observations with species-specific range maps based on 8-digit hydrologic unit codes (HUC8s). The USGS database successfully classified 98.8% of observations, but was missing range maps for 84 species, most of which were rare. For 61 missing species, we identified native occurrences based on NatureServe HUC8-scale range maps (NatureServe 2010). The remaining 23 missing species were manually classified by mapping all occurrences (1–31 observations) and visually comparing them to range maps obtained from various online sources. This process identified 466 native fish species, which were used to calculate native richness in each NRSA sample (Figure 3).

2.2 | Geologic History

We divided the conterminous USA into eight regional geologic histories based on proxies of tectonic activity, glaciation, river capture and sea level fluctuations over the last 66 million years since the Cretaceous–Paleogene (K-Pg) mass extinction (Figure 2a). We focused on this period because most extant freshwater fishes in North America evolved during this time (Miller and Román-Palacios 2021). Listed in order of expected

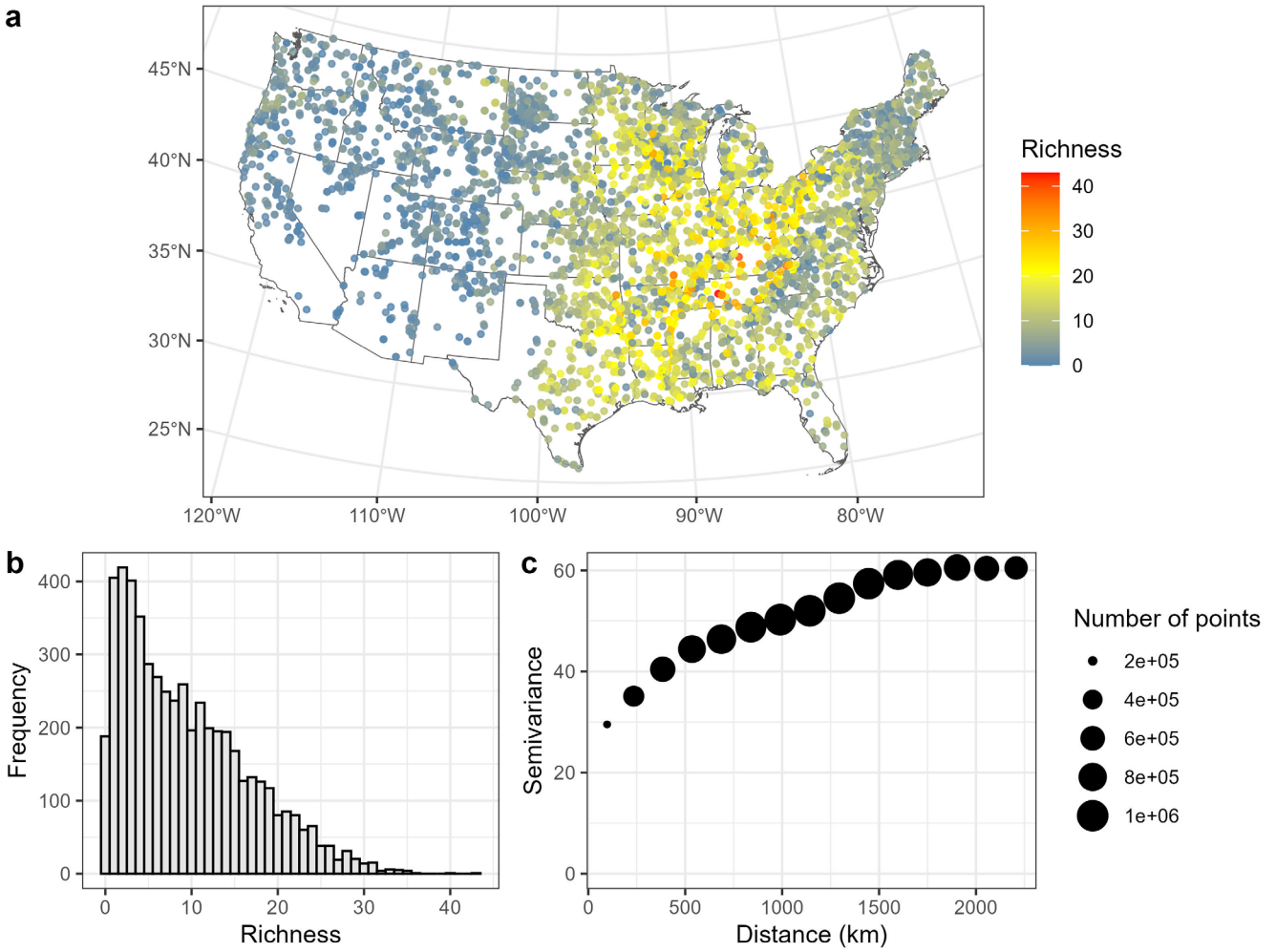

FIGURE 3 | Species richness patterns for native freshwater fish assemblages across the conterminous United States. The map (a) and histogram (b) show native species richness observed by the EPA National Rivers and Streams Assessment during six sampling years (2008–2009, 2013–2014, 2018–2019; see Section 2). The empirical semivariogram (c) suggests strong spatial autocorrelation, as pairwise differences in species richness between sites increase with distance (i.e., mean semivariance increases across 15 distance bins).

richness, the regions were *Stable Unglaciated Lowlands*, *Stable Unglaciated Highlands*, *Stable Unglaciated Lowlands—Flooded*, *Stable Glaciated Lowlands*, *Stable Glaciated Highlands*, *Active Unglaciated Lowlands*, *Active Unglaciated Highlands* and *Active Glaciated Highlands* (Table 1; written in italics hereafter). Six sites were not initially classified into these classes, so we instead combined them with existing categories (File S1).

Tectonically active sites were identified using NRSA Western Mountain and Xeric ecoregions (Omernik 1987), which mostly contained land west of the Rocky Mountain Front (Figure S3a). While only some of this region remains active today, extensive mountain building occurred from the late Cretaceous to the Eocene 80–50 mya (Bentley Sr et al. 2016) and earthquake faults from the Pleistocene 2.5–15 kya exist throughout the area (USGS 2020, https://www.usgs.gov/programs/earthquake-hazards/faults). Glaciated sites were identified using the *sf* package (Pebesma 2018) based on a shape file of global maximum glacial extent during the Pleistocene (Figure S3b), which included continental ice sheets and mountain glaciers (Ehlers and Gibbard 2003, https://booksite.elsevier.com/9780444534477/). Topographic data were used to indirectly infer landscape stability and river capture rates, which are thought to be greatest in lowlands with low topographic relief (Albert et al. 2018; Val et al. 2022). Specifically, we used the *elevatr* (Hollister et al. 2017) and *terra* packages (Hijmans 2024) to extract elevations from the USGS Elevation Point Query Service, which were used to designate sites as lowland (≤250 m) or highland (>250 m; Figure S3c). Topographic relief was calculated in a scale-independent manner, with a consistent grain size and spatial extent that was unaffected by basin area. We added a 1 km buffer around each site, joined buffers with elevation raster data from Amazon Web Service (25–35 m resolution), extracted minimum and maximum elevation, then calculated the difference as a measure of relief. Finally, sites flooded by past sea levels were identified using the NRSA Coastal Plain ecoregion (Omernik 1987; Figure S3d). The Coastal Plain is a common biogeographic break for freshwater fishes, and much of this area was intermittently submerged by high sea levels 66 mya (100–200 m higher) until the Pliocene 3 mya (22 m higher; Russell et al. 2021).

2.3 | Evolutionary Time

We used published evolutionary time estimates for Nearctic freshwater fishes from Miller and Román-Palacios (2021). They used historical biogeographic approaches to estimate the timing of colonisation by parent lineages within continental regions based on the mega-phylogeny by Rabosky et al. (2018), which was attached to descendant species as a measure of evolutionary time (time allowed for in situ diversification). For example, darters (Percidae) were inferred to have colonised North America ~62 million years ago; this estimate was attached to all living darter species descended from this event. Data were missing for 44 out of 466 native fish species (9.6%), which were filled in using two methods. First, we identified 29 species with estimates available from other members of their genus, then imputed missing data using a stochastic single imputation method, drawing a random number from a normal distribution centered on the genus mean with a standard deviation that incorporated both observation-level and mean-level uncertainty. This imputation method is preferred to simply replacing missing values with their mean, which can cause resulting models to mischaracterize natural variability (Little and Rubin 2019; Van Buuren and Van Buuren 2012). Lastly, we used values from the literature for the remaining 15 species missing from Miller and Román-Palacios (2021). Three coastal species (*Brevoortia tyranus*, *Conger oceanicus* and *Leiostomus xanthurus*) had values taken from phylogenetic studies (Wang et al. 2022; Santini et al. 2013; Lo et al. 2015, respectively), whereas 12 lamprey species from four genera (*Petromyzon*, *Ichthyomyzon*, *Entosphenus*, *Lampetra*) had values taken from a time-calibrated phylogeny by Brownstein and Near (2023).

After filling in missing data, we calculated mean assemblage evolutionary time in NRSA samples with one or more native species, which was used as a covariate (see below). We also assessed the distribution of evolutionary time across regional geologic histories for species pools (i.e., all unique species observed in a region) and assemblages. During this process, we confirmed that a minority of species originated before the K-Pg extinction (23.3%), whereas only 24 species (~5%) originated more than 105 mya, 10 of which were non-teleost taxa (i.e., bowfin, gars, paddlefish, sturgeons). To account for the very different ages of teleost and non-teleost lineages in North America, richness and evolutionary time in teleosts and non-teleosts were explored by subsetting data according to taxonomic class using the *rfishbase* package (Boettiger et al. 2012).

The underlying phylogenetic tree by Rabosky et al. (2018) may be skewed towards relatively old clade ages (see Miller et al. 2022), but we considered Miller and Román-Palacios (2021) to be the best available dataset for this study due to its broad taxonomic coverage. Moreover, the relative age of clades (i.e., distinguishing older vs. younger lineages) is most important for testing the evolutionary time hypothesis, and Miller and Román-Palacios (2021) found that rank-order clade ages were very similar across two different phylogenies. Nonetheless, phylogenetic models are sensitive to taxonomic sampling, genetic markers used, availability of fossil calibrations, and other factors (Near et al. 2011; Dornburg et al. 2014; Miller et al. 2025). For example, phylogenies calibrated with mitochondrial data can estimate divergence times that are consistently older than those based on nuclear genetic data (Dornburg et al. 2014), potentially altering relationships between clade age and species richness (Title and Rabosky 2017). To explore phylogenetic uncertainty in more detail, we used lampreys as an example to assess the effect of using different trees to estimate evolutionary time. Specifically, we recalculated assemblage evolutionary time using values from an alternative phylogeny for North American lampreys by Hughes et al. (2025), which suggested more recent origination times compared to Brownstein and Near (2023).

2.4 | Statistical Analysis

We tested our hypotheses by using the *spmodel* package (Dumelle et al. 2023) to fit two spatial linear models, which are similar to conventional linear models but account for spatial autocorrelation in the response variable. Spatial models often yield more accurate estimates of fixed effects and their uncertainty, thereby improving model performance and the validity of hypothesis tests (Cressie 1993). We used native species richness as the response variable in both models, but we re-ran analyses with total richness (including non-natives) and native teleost richness to ensure results were robust. Both metrics were strongly correlated with native species richness ($r = 0.95–0.99$). Since richness is considered count data, we also explored negative binomial generalised linear models, but spatial linear models were preferred because they yielded similar results and were easier to fit, interpret and reproduce.

The first model was stratified by geologic history (eight levels; Figure 2a) and included topographic relief and $\log_{10}$-transformed wetted width ($\log_{10}$(width) hereafter) as additional covariates, with the fixed effect structure:

$$Richness_{ij} = \beta_{0,i} + \beta_{1,i}\left(Relief_{ij}\right) + \beta_{2,i}\left(\log_{10}\left(Width_{ij}\right)\right) + \beta_{3,i}\left(\log_{10}\left(Width_{ij}\right) \times Relief_{ij}\right)$$

where richness at site j within geologic history i was a function of intercepts (β_0) and slopes (β_1, β_2, β_3) estimated for each geologic history i and covariate values for site j (32 fixed effects). To test the geologic stability hypothesis, we used the *emmeans* package (Lenth 2024) to estimate marginal means for each level of geologic history, assuming median width and relief. We then compared ranked point estimates to expected rank-order richness (Table 1) using a Spearman correlation test. To test the river capture hypothesis, we used the *emtrends()* function to estimate slopes with relief (β_1) assuming median width, while slopes in lowlands were used to assess our two competing expectations (negative or positive associations if richness peaks at low or intermediate river capture rates, respectively; Val et al. 2022). Similarly, slopes with $\log_{10}$(width) (β_2) at median values of relief were used to test the habitat capacity hypothesis.

The second model had a similar fixed effect structure but used mean assemblage evolutionary time (Evo) at site j as a covariate (32 fixed effects), with the formula:

$$Richness_{ij} = \beta_{0,i} + \beta_{1,i}\left(Evo_{ij}\right) + \beta_{2,i}\left(\log_{10}\left(Width_{ij}\right)\right) + \beta_{3,i}\left(\log_{10}\left(Width_{ij}\right) \times Evo_{ij}\right)$$

To test the evolutionary time hypothesis, we used *emtrends()* to estimate whether slopes with evolutionary time (β_1) were positive for each geologic history, assuming median width. Like the previous model, slopes with $\log_{10}$(width) (β_2) at median values of relief were also used to test the habitat capacity hypothesis. We did not report *p*-values from *emmeans* results but instead assessed whether 95% confidence intervals overlapped with zero.

For both models, the formulations above were supported over two simpler alternatives ($\Delta AIC > 23$), and the influence of interaction terms (β_3) was visualised using the *emmip()* function. Model performance was assessed by inspecting residuals and conducting variance partitioning within *spmodel*, which estimated the variance attributable to fixed effects, random effects, spatial covariance and residual error (Dumelle et al. 2023). Additionally, we assessed out-of-sample performance via leave-one-out cross-validation, which dropped each observation, refitted the model, then used the model to predict the missing value. To account for spatial autocorrelation in richness (Figure 3c), we compared three spatial covariance functions to a non-spatial model and found the exponential function was best supported ($\Delta AIC > 291$). We were also interested in covariance directionality because river basins with a largely north–south orientation are thought to facilitate dispersal and postglacial recolonization more than those oriented east–west (Oberdorff et al. 1997; Smith et al. 2010). Thus, both models included exponential spatial covariance terms, anisotropic terms modelling covariance directionality, and spatial random effects allowing intercepts to vary by hydrologic unit (Figure S4; but see Omernik et al. 2017) and by site. These features significantly improved performance in both models. Specifically, including spatial covariance and random effects increased leave-one-out cross-validation R^2 by ~50% and decreased mean-squared-prediction errors by ~50% compared to nonspatial models without random effects.

3 | Results

3.1 | Model Performance

The first model using geologic history, topographic relief and $\log_{10}$(width) as covariates performed similarly to the second model, which used geologic history, assemblage evolutionary time and $\log_{10}$(width) as covariates. Variance components indicated that fixed effects explained 29.5%–29.9% of the variance based on pseudo-R^2 values, while 21.1%–22.9% of variance was attributed to spatial covariance, and 31.3%–31.5% was attributed to random effects. Out-of-sample performance was good, as leave-one-out R^2 was 0.75–0.76 with low bias and prediction errors. Spatial covariance was ~1.35 times stronger in the north–south direction than the east–west direction in both models (File S1).

Model performance and hypothesis test results were mostly similar when using total richness and native teleost richness as response variables (Tables S2 and S3), but any notable differences are listed below.

3.2 | Geologic Stability Hypothesis

The first model supported the geologic stability hypothesis, as rank-order richness across geologic histories was strongly correlated with expected ranks (Spearman $r = 0.81$, $p = 0.02$). However, marginal mean richness was higher than expected in the Coastal Plain (*Stable Unglaciated Lowlands—Flooded*) and in *Stable Glaciated* regions within the eastern USA, whereas richness was lower than expected in *Stable Unglaciated Highlands* (Figure 4c).

3.3 | River Capture Hypothesis

The first model generally supported the river capture hypothesis but suggested high uncertainty. Relief was highest in the tectonically *Active* western USA and low in *Stable Unglaciated Lowlands* throughout the Mississippi Basin and Coastal Plain (Figure 4a). Areas with low topographic relief tended to have higher richness (Figure 4b). Associations with relief were negative in 7/8 regions, but only *Stable Unglaciated Highlands* had a 95% confidence interval that did not contain zero (Figure 4d, Table S1). Associations were similar when using native teleost richness (Table S2) as the response, whereas all slope estimates were more consistently negative when total richness was used (8/8 regions; Table S3).

Notably, *Lowlands* consistently had higher predicted richness than comparable *Highland* regions (Figure 4d). Interactions between relief and stream width did not strongly influence hypothesis tests (Figure S5). Overall, richness was highest in *Stable Lowlands* with low topographic relief, which likely had high river capture rates. There was little support for the alternative expectation that high richness was associated with intermediate river capture rates.

3.4 | Evolutionary Time Hypothesis

The second model provided some, albeit inconsistent, support for the evolutionary time hypothesis. Based on our measure of evolutionary time, assemblages were often younger (< 50 mya) in the tectonically *Active* western USA and older (> 100 mya) in species-rich *Stable Unglaciated Lowlands* around the Mississippi River mainstem and Coastal Plain (Figure 5a). However, richness showed a complex dome-shaped relationship with assemblage evolutionary time (Figure 5b), which largely reflected colonisation times of regional species pools (Figure 5c). An exception to this was the *Flooded* Coastal Plain, where assemblages were skewed towards older lineages than the underlying species pool, likely due to the prevalence of ancient non-teleost taxa (see below). Associations with evolutionary time were positive in 5/8 regions, but of these only *Stable Unglaciated Lowlands* had a 95% confidence interval that did not contain zero (Table S1). Moreover, the strength of associations was influenced by interactions between evolutionary time and $\log_{10}$(width) in the tectonically *Stable* eastern USA, such that effects of evolutionary time on richness tended to be positive in small streams but negative in large streams (Figure 5d).

Support for the evolutionary time hypothesis was weaker when using native teleost richness as the response. Assemblages in the *Flooded* Coastal Plain were no longer skewed towards older lineages (Figure S6), and the positive slope in *Stable Unglaciated Lowlands* had a confidence interval that contained

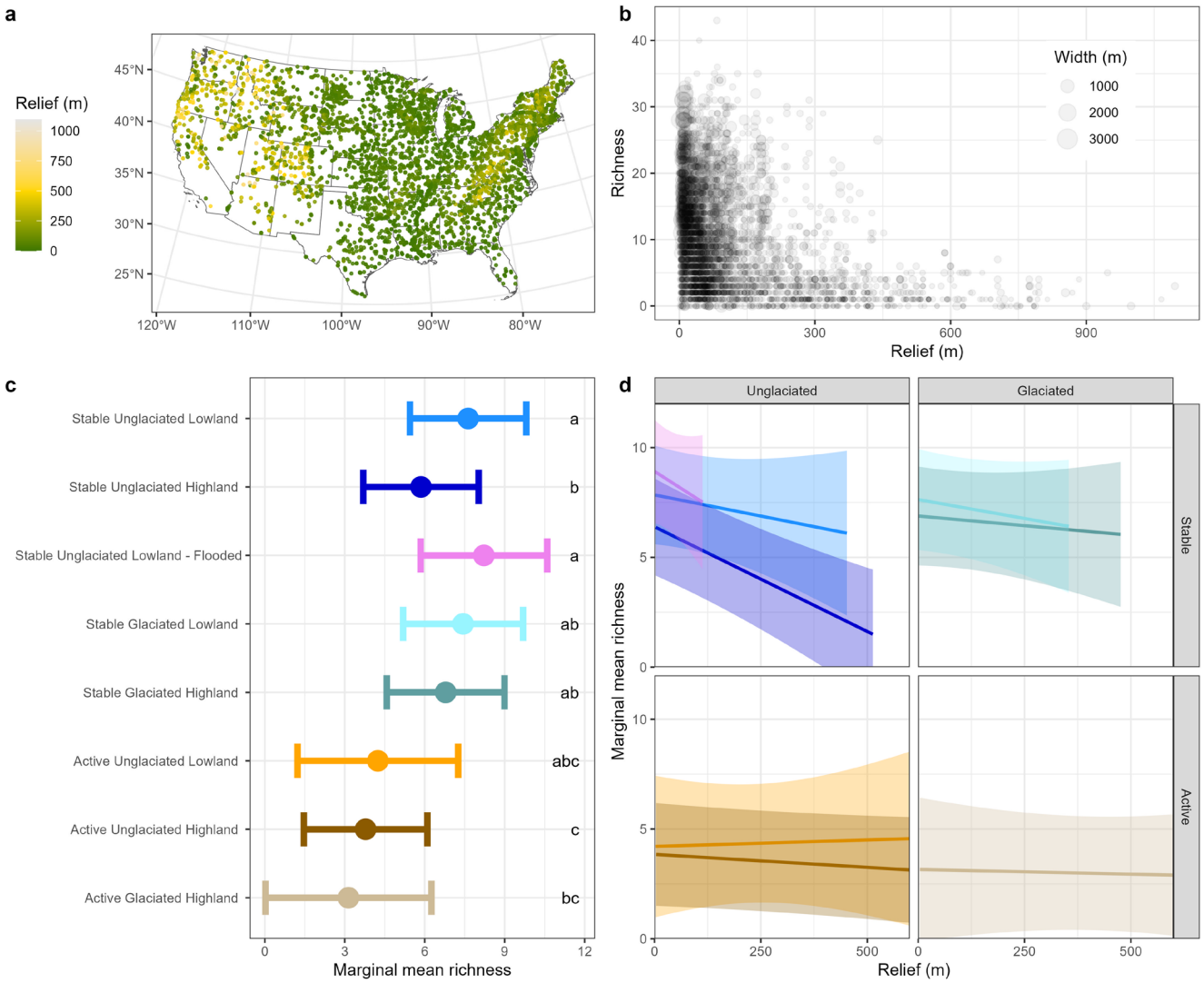

FIGURE 4 | Test results for the geologic stability hypothesis and river capture hypothesis. Map of topographic relief (a) and its relationship with richness (b). The partial effect plot (c) shows marginal mean richness (with 95% confidence intervals) within each geologic history category, assuming median values for stream width (11.85 m) and topographic relief (56 m). Linear partial effect plots (d) show marginal mean species richness (with 95% confidence intervals) across the observed range of topographic relief up to 600 m within each geologic history category, assuming median stream width. Geologic history categories in (c) are ordered by their hypothesized rank-order species richness (Table 1), and letters summarize results of Tukey pairwise contrasts.

zero (Table S2). In contrast, associations with evolutionary time were very similar when total richness was used as the response variable (Table S3).

Exploratory analysis of North American lampreys suggested that phylogenetic uncertainty likely influenced our results, as estimates of assemblage evolutionary time differed depending on the phylogeny used (range: 1.4–47.5 mya). Notably, differences were typically negligible in the *Stable* eastern USA but were larger in the *Active* west where richness was lower (Figure S7).

3.5 | Habitat Capacity Hypothesis

Both models largely supported the habitat capacity hypothesis, as $\log_{10}$(width) had a positive effect on richness across all geologic histories. However, associations were much stronger in the tectonically *Stable* eastern USA, where average richness increased by 4–6 species for every 10-fold increase in stream width (Table S1). In contrast, all slopes in the *Active* western USA had 95% confidence intervals containing zero.

4 | Discussion

We developed novel proxies of geologic history and stream size that strongly influenced native freshwater fish species richness at sites across the conterminous USA. Our proxy variables allowed us to indirectly infer mechanisms shaping deep-time spatial variation in net diversification rates, evolutionary time and habitat capacities (Figures 1 and 2), in accordance with previous global studies (Miller and Román-Palacios 2021; Val et al. 2022). We found strong support for the geologic stability hypothesis and consistent support for the river capture hypothesis, albeit

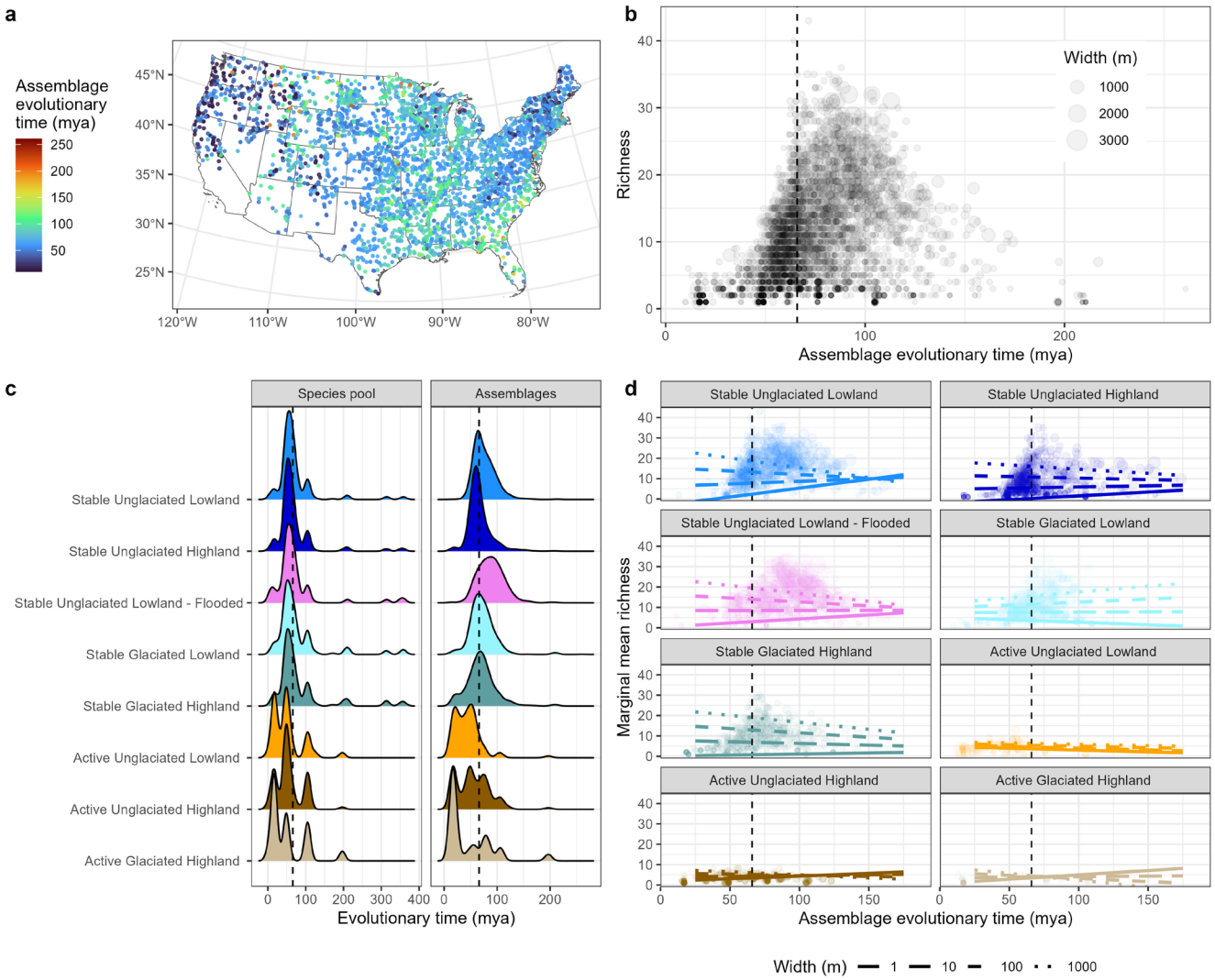

FIGURE 5 | Test results for the evolutionary time hypothesis. Map of mean assemblage evolutionary time (a) and its relationship with richness (b). Ridgeline plots (c) show the distribution of evolutionary time for species pools and assemblages within each geologic history category, which are ordered by their hypothesized rank-order species richness (see Table 1). Scatter plots (d) show relationships between mean assemblage evolutionary time and species richness across geologic history categories, which are fitted with linear marginal means at stream width values of 1 m (solid lines), 10 m (long dashed), 100 m (dashed), and 1000 m (dotted). Points (b, d) are sized according to stream width; dashed vertical lines (b–d) denote the approximate timing of the K-Pg extinction 66 mya.

with greater uncertainty (i.e., most slopes had confidence intervals containing zero). Support for the evolutionary time hypothesis was more equivocal, as regions dominated by older lineages often had higher richness, but this relationship alone did not explain differences among sites within most regions. Finally, we uncovered consistent support for the Habitat Capacity Hypothesis, which was strongest in the tectonically *Stable* eastern USA. Spatial models were powerful tools for testing hypotheses and analysing site-level biodiversity data from spatially balanced designs, achieving out-of-sample R^2 values of ~0.75.

Overall, richness was highest in the geologically stable eastern USA, especially in *Stable Unglaciated Lowlands* around the eastern Mississippi basin and surrounding *Flooded* Coastal Plain, which had signatures of prolonged evolutionary time and putatively high river capture rates (Albert et al. 2018). In contrast, the tectonically *Active* western USA had consistently low richness, low putative river capture rates and assemblages dominated by lineages with more recent colonisation times, which is consistent with high extinction rates and low connectivity inferred from the fossil record (Smith et al. 2002, 2010). Beyond the strong divergence based on tectonic activity, quantitative differences in richness among regions were difficult to predict a priori, likely owing to variable diversification rates and the influence of dispersal. Finally, our research integrated multiple open-source datasets within a reproducible framework, which can be adapted for future research and improve assessment models used to monitor freshwater biodiversity (Strange 1999; Hilburn et al. 2024).

4.1 | Geologic Stability Promotes Fish Biodiversity

As expected based on the geologic stability hypothesis, richness was highest in *Stable Unglaciated Lowlands* within the Tennessee and Ohio River drainages of the Mississippi Basin, like

previous studies (Jenkins et al. 2015; Qian et al. 2020). Although some areas within the Coastal Plain were *Flooded* by fluctuating sea levels (Russell et al. 2021) and river captures occurred (Albert et al. 2018), this area has been relatively stable since 66 mya. Indeed, many species-rich sites are within the Paleo-Tennessee drainage, which drained the southern Appalachian highlands from the late Cretaceous until it was captured by the Mississippi 30–23 mya (Blum et al. 2017) and underwent subsequent drainage rearrangements (Galloway et al. 2011; Odom and Granger 2022). Thus, drainage evolution in this region is well resolved and suggests that riverine habitats connecting highlands and lowlands have existed for over 100 million years, while the surrounding climate has also been warm, wet, and relatively stable for much of this time (Galloway et al. 2011). Together, these conditions have likely allowed lineages to diversify over long periods (Miller and Román-Palacios 2021; Val et al. 2022).

The availability of stable habitat within *Stable Unglaciated Lowlands* may be especially relevant for teleost lineages such as darters (Percidae) and minnows (Leuciscidae) that dominate richness in the eastern USA (Figure S6). For example, both lineages often occupy isolated habitat patches in headwater streams or springs that are spatially fragmented and thus promote allopatric speciation (Fluker et al. 2014; Burress et al. 2017). In minnows, ecological opportunity within persistent habitat patches may have encouraged microhabitat partitioning along the benthic-pelagic axis and facilitated sympatric speciation (Martin and Bonett 2015; Burress et al. 2017). Through these mechanisms, the geologic stability of the southeastern USA may have allowed species to accumulate steadily and/or in bursts over millions of years. However, these two key teleost lineages largely lack a fossil record and thus have greater uncertainty when estimating the timing and mode of diversification (Near et al. 2011), which may obscure the influence of habitat stability and evolutionary time.

Long-term habitat stability in the southeastern USA may have also resulted in relatively low extinction rates, as evidenced by the persistence of non-teleost lineages such as Holostei (bowfin and gars) for over 300 million years. Holostei were mostly observed in large rivers within *Stable Unglaciated Lowlands* (Figure S8), contributing to relationships between richness and evolutionary time (Figure 5d). The slow-moving river, swamp and backwater habitats favoured by Holostei have probably existed for over 100 mya (Graham 2011) and may have shielded freshwater fishes from the K-Pg extinction (Brownstein and Lyson 2022). While this pattern was less evident in teleosts (Figure S6b), the oldest extant teleost lineages (mooneyes, pikes and mudminnows) are only found in the eastern USA and, like Holostei, prefer large river or backwater habitats (Robison 1986). In contrast, richness may have been unexpectedly low in *Stable Unglaciated Highlands* because many endemic highland species prefer small, clear, high-gradient streams that formed more recently (Hoagstrom et al. 2014) and likely have low habitat capacity.

The geologic stability hypothesis was also supported by low richness in the western USA, which has been tectonically *Active* for much of the last 66 mya, resulting in mountainous terrain, harsh climate and many small, highly fragmented drainages (Minckley et al. 1986; Cook et al. 2025). Fishes in the western USA display higher endemism and speciation rates than eastern lineages (Griffiths 2025), likely because of their physical isolation (Smith 1981). However, long-term climate fluctuations also played a critical role as many western species diversified in large lakes created during periods of cool, wet climate conditions (Ibarra et al. 2018), then went extinct when arid conditions caused lakes to shrink or disappear (Smith 1981; Smith et al. 2002). Thus, by creating topographic barriers to dispersal and unpredictable climate conditions, tectonic activity often trapped fishes in unstable habitats, ultimately causing high extinction rates and low net diversification in the western USA (Smith et al. 2002, 2010). Unstable habitats may have also increased vacant niche space and altered selection pressures, as evidenced by the evolution of larger body sizes in western minnow species compared to their eastern counterparts (Martin and Bonett 2015). Additionally, geologic and climatic disturbances in the west may have limited richness by constraining basin area and stream width as expected under the Habitat Capacity Hypothesis (Figure S2). Like Smith et al. (2010), we found that geologic history broadly shaped freshwater fish biodiversity in North America, and richness was reduced most consistently in tectonically *Active* regions.

Geologic stability likely contributed to interactions between net diversification rates, evolutionary time and habitat capacities, which influenced other hypothesis tests. For example, within the relatively stable eastern USA, richness was highest in large streams with low topographic relief (Figure 4, Table S1), as expected based on the River Capture and Habitat Capacity Hypotheses. However, the frequency and areal extent of river captures are likely higher in flat, tectonically stable settings, producing larger rivers with higher habitat capacity like the Mississippi (Albert et al. 2018; Val et al. 2022). Indeed, more frequent river captures likely created more permeable dispersal barriers between fish faunas in the more diverse eastern USA, whereas these barriers are stronger and more persistent in the species-poor western USA, providing further indirect support for the river capture hypothesis (Matamoros et al. 2016). Similarly, the evolutionary time hypothesis was partly supported by younger origins of taxa in the unstable western USA (Figure 5), where aquatic habitat instability caused high extinction rates and subsequent diversification of lineages that could tolerate these conditions (Minckley et al. 1986; Qian et al. 2020).

Overall, the weak effects of topographic relief, stream width and evolutionary time on richness within the western USA (Table S1) suggest that long-term tectonic stability may be 'necessary but not sufficient' to build sizable fish biodiversity in rivers, similar to the conclusions of Val et al. (2022). Further research is needed to understand how richness is shaped by interactions between diversification rate, evolutionary time and habitat capacity, which are likely not mutually exclusive processes in freshwater systems.

4.2 | Importance of Dispersal

Historical connectivity and dispersal may explain why richness in some regions was higher than we hypothesized based on macroevolutionary processes (Figure 2b). Specifically, three regions within the eastern USA had higher rank-order richness than

expected: the Coastal Plain categorised as *Stable Unglaciated Lowlands—Flooded*, and northern areas categorised as *Stable Glaciated Lowlands* or *Stable Glaciated Highlands* (Table 1, Figure 4c).

The *Flooded* Coastal Plain contains much of the lower Mississippi Basin up to the confluence of the species-rich Ohio, Cumberland and Tennessee Rivers, as well as smaller coastal watersheds with high richness and endemism such as the Mobile Basin (Jenkins et al. 2015). We initially hypothesized that high sea levels reduced evolutionary time in many freshwater habitats in this region, but this is likely an oversimplification. For example, connectivity with the ocean may have also facilitated immigration of marine and estuarine lineages from the tropics (Hernández-Ávila et al. 2024; Massip-Veloso et al. 2024) and enabled marine-freshwater transitions over time (Miller 2021). However, even when considering strictly freshwater fishes, the central Coastal Plain is a distinct faunal province in North America (Matamoros et al. 2016). Local refugia likely allowed endemic freshwater fishes and other organisms to persist in this area during times of high sea levels, then recolonize habitats once sea levels were lowered (Noss et al. 2015). Similarly, upstream dispersal could have provided refugia within most river basins except those completely flooded by oceans (Swift et al. 1986). Dispersal barriers may also be lower and drainage rearrangements more frequent in the Coastal Plain due to its low topographic relief, soft alluvial sediments and periodic watershed fragmentation driven by high sea levels or avulsions (Phillips 2013; Albert et al. 2018). Indeed, the cyclical rise and fall of sea levels within the *Flooded* Coastal Plain over the last 66 million years may have created a speciation pump by repeatedly isolating and reconnecting drainages (Phillips 2013), thereby increasing diversification rates (Swift et al. 1986; Hernández-Ávila et al. 2024). Together, these factors may explain why our *Stable Unglaciated Lowlands—Flooded* region displayed higher species richness than expected and why it has been highlighted as a biodiversity hotspot for a wide range of organisms (Noss et al. 2015).

Similarly, we expected the two *Stable Glaciated* regions to have the lowest evolutionary time because they were recently covered by ice sheets, which should have limited richness. However, richness at *Glaciated* sites connected to the species-rich Mississippian refugium in the central USA was above the national average (11 vs. 9.4), and significantly higher than northeastern sites connected to the species-poor Atlantic refugium ($t = 13.6$, $p > 0.001$; Figure S9). The extensive connectivity and north–south orientation of the Mississippi Basin may have facilitated higher postglacial colonisation than Atlantic coastal basins that are oriented east–west (Smith et al. 2010), a pattern supported by stronger north–south spatial autocorrelation in our models (File S1). Indeed, Mississippian taxa dominate postglacial species pools from the Great Lakes to the Mackenzie Basin in northern Canada (Rempel and Smith 1998), while immigration from distinct glacial refugia contributes to northern fish biodiversity patterns in North America (April et al. 2013) and Europe (Cortés-Guzmán et al. 2024).

Overall, areas with positive net dispersal rates (i.e., immigration > emigration) after geologic disturbance had higher richness than expected, especially if connected to diverse source fauna (Smith et al. 2010). Net dispersal rates appeared to far exceed net diversification in *Stable Glaciated* regions, since richness increased from zero to > 20 species in ~12,000 years at many sites connected to the Mississippian refugium (Figure S9). This implies postglacial dispersal can add species to an area several orders of magnitude faster than the average net diversification rate for riverine fishes (Miller 2021). Indeed, most species that recolonized glaciated sites originated well before the Pleistocene, as evidenced by low endemism in the northern USA (Jenkins et al. 2015) and similar evolutionary time distributions in *Stable Glaciated* and *Stable Unglaciated* regions (Figure 5c).

Increases in richness via dispersal are obviously limited by habitat capacity, while species traits and habitat characteristics modify dispersal ability (Griffiths 2025; Cortés-Guzmán et al. 2024). For example, dispersal did not increase richness as much in *Active Glaciated* regions of the western USA, presumably due to small stream size and steep channel gradients limiting species accumulation in high-elevation postglacial streams (Smith et al. 2010). Throughout the *Active* western USA, species accumulation was slower and more staggered due to steep topographic barriers that limited net dispersal over millions of years, resulting in high extinction and low colonisation (Smith 1981; Smith et al. 2002). More research is needed to quantify the influence of net dispersal rates on richness and determine how it modifies richness patterns independent of, or in combination with, net diversification and other macroevolutionary processes.

4.3 | Future Research Directions

This study supports prior assertions that deep-time geologic history is a key determinant of native species richness in North American freshwater fishes (Smith et al. 2010), which should inform future research, management and conservation. First, our approach can be extended to assess turnover, composition and endemism across multiple spatial scales while incorporating habitat data, which could improve biodiversity assessments and management actions. When linking fish biodiversity to habitat characteristics (e.g., Moi et al. 2024) across large heterogeneous landscapes such as the conterminous USA, assessment models could be stratified by geologic history categories like the ones we developed (Figure 2). Watershed or ecoregion boundaries (e.g., Abell et al. 2008; Tedesco et al. 2017) could also be incorporated and contextualised with information on watershed formation and historical connectivity (e.g., Galloway et al. 2011; Blum et al. 2017). Stratifying models in this way should help minimise confounds and identify causal mechanisms to support decision-making. For example, NRSA rates the condition of fish assemblages and stream habitats as good, fair, or poor relative to a baseline, but using unstratified habitat condition to explain fish condition across the USA might lead to erroneous conclusions because geologic history strongly influences both factors (Smith et al. 2010; Kaufmann et al. 2022). Similarly, fish phylogenetic diversity in western and eastern North America shows divergent responses to temperature and precipitation gradients, suggesting that tectonic activity alters climatic controls on assemblages (Qian et al. 2020). Because NRSA uses a spatially balanced design (Olsen and Peck 2008), baseline levels for multiple biodiversity metrics could potentially be estimated across all streams in the conterminous USA (e.g., Hill et al. 2017). Future

models could predict baseline biodiversity by supplementing our hypothesis-driven covariates with measures of streamflow and stream temperature, while controlling for indices of terrestrial and aquatic human impacts (e.g., Sanderson et al. 2002; McManamay et al. 2022).

Our research synthesised information from a variety of disciplines and thus demonstrates the value of publicly sharing data, which has become more common in the last decade (e.g., Miller and Román-Palacios 2021; Mahon et al. 2024; USGS 2024). Most notably, we believe that future research would benefit from using empirical species richness data to complement phylogenetic, paleoclimatic and morphological approaches that are often used to study the formation of fish biodiversity (e.g., Brownstein and Near 2023; Hughes et al. 2025; Miller et al. 2025). This line of inquiry could be pursued further by linking data from biodiversity surveys to speciation rates, species distributions, physical habitat and other site-level information (Kaufmann et al. 2022; Moi et al. 2024; Griffiths 2025). Alternatively, phylogenetic models could explain richness patterns by assessing whether temporal changes in speciation, extinction and immigration rates match the timing of geological events that formed the Mississippi Basin (Blum et al. 2017; Russell et al. 2021), like recent studies of Amazonian fishes (Melo et al. 2022; Cassemiro et al. 2023). Phylogenetic uncertainty remains an important consideration, as differences in fossil sampling and dating methodologies can produce vastly different clade ages (e.g., Mongiardino Koch et al. 2021; Brocklehurst and Field 2024), thereby influencing tests of the evolutionary time hypothesis. Indeed, our example with North American lampreys demonstrates that estimates of assemblage evolutionary time can differ by as much as 20–40 million years depending on the phylogeny used, which could potentially influence regional comparisons (Figure S7). Future research can reduce phylogenetic uncertainty in fishes by leveraging whole-genome approaches that analyse tens of thousands of loci across taxa, which has been done in other vertebrate groups (Foley et al. 2023; Stiller et al. 2024), and could be improved further by accounting for recombination (Burbrink et al. 2025).

Biodiversity data from contemporary surveys should also benefit efforts to understand the evolution of life across different geologic settings, which is a key focus in transdisciplinary fields such as geogenomics (Baker et al. 2014; Dolby et al. 2022). Specifically, future research could test whether geologic history influences the prevalence of different speciation mechanisms. For example, erosion of heterogenous rock types in headwater streams of the Appalachian Mountains can progressively isolate habitat patches and promote allopatric speciation in darters (Stokes et al. 2023), but this process may require *Stable Unglaciated* settings to proceed with minimal interruption. Conversely, hybridization among recently diverged taxa could contribute to sympatric speciation in tectonically *Active* (e.g., cichlids in African rift lakes; Meier et al. 2023) and *Stable* regions (e.g., minnows in the eastern USA; Zbinden et al. 2023), but the broader effects of landscape history remain poorly understood.

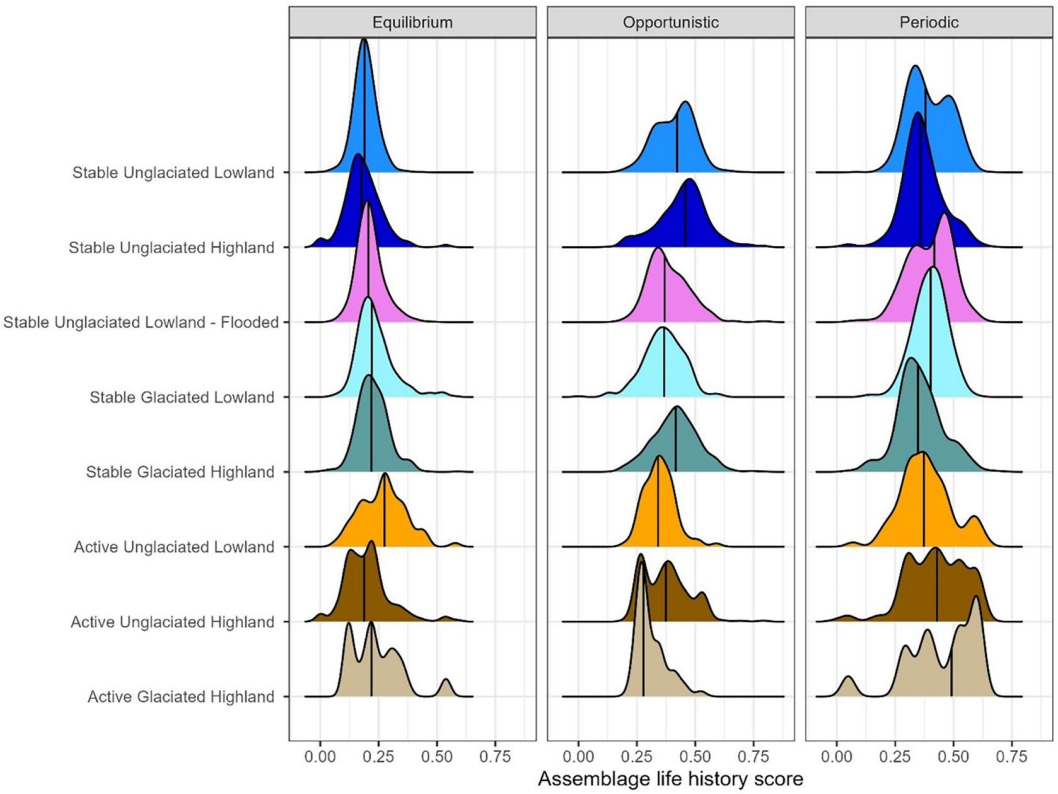

FIGURE 6 | Ridgeline plots visualising assemblage life history traits across geologic histories. We followed the methodology of Thorson et al. (2023) to estimate native species life history scores from a matrix of 33 imputed traits, which describe how well each species fits into periodic, opportunistic, or equilibrium archetypes (sensu Winemiller and Rose 1992). Species scores were then averaged within assemblages, and medians within each geologic history are displayed as solid vertical lines. Note that imputed trait data were missing for 29 native species (6.2% of taxa sampled by NRSA), and all assemblages containing these species were excluded. Code to reproduce this figure is available in File S1.

Lastly, we believe that the formation of North American freshwater fish biodiversity can be better understood by combining spatially representative surveys from NRSA with other comprehensive databases recently assembled by fish biologists. As an example, we followed the methodology of Thorson et al. (2023) to estimate life history scores describing how each species fits into three fish life history archetypes (opportunistic, periodic, equilibrium) devised by Winemiller and Rose (1992). We then linked these estimates to our NRSA fish assemblage database, calculated average scores within each sampled assemblage and plotted them by geologic history (Figure 6; see code in File S1). This exploratory analysis revealed that assemblages in the *Active* western USA are often dominated by species with periodic life histories that tend to be larger, longer-lived and more migratory (Figure 6; Winemiller and Rose 1992). This pattern is consistent with previous research (Mims et al. 2010) and may suggest that these traits are an adaptation to pluvial climate cycles and unstable habitat conditions in the western USA (Moyle and Herbold 1987; Minckley et al. 1986). Future studies could potentially leverage these rich empirical datasets to test previously untestable biogeographic and macroevolutionary hypotheses in fishes.

4.4 | Implications for Biodiversity Assessment

Our work attempted to answer previous calls to improve biodiversity assessments by incorporating macroevolutionary and biogeographic perspectives (Strange 1999; Hilburn et al. 2024). This approach can help identify and control for sources of natural variability, which should allow better inferences to be made about the most pressing threats to freshwater biodiversity (Albert et al. 2021). For example, recent research suggests streams in the tectonically *Active* western USA may be more susceptible to fish biodiversity loss induced by warming stream temperatures (Rumschlag et al. 2025) and non-native species (Qian et al. 2023). If true, this raises an intriguing possibility that deep-time processes can shape assemblages in ways that fundamentally alter their vulnerability to human activities in the 21st century. Indeed, geologic history could conceivably influence persistence by modifying species population structure (Leitão et al. 2021), niche space (Walker and Valentine 1984) and traits (Thorson et al. 2023; Figure 6). This line of research merits more attention and could help explain the considerable variation in observed responses of aquatic biodiversity to environmental change around the world (Comte et al. 2021; Su et al. 2021). By integrating deep-time processes with contemporary data that describe how environments are changing, we can better understand how freshwater biodiversity was generated, how it is threatened, and how it can be conserved.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are published and publicly available. All data and R code necessary to reproduce our analysis are provided in the Supporting Information and archived on Dryad (https://doi.org/10.5061/dryad.sqv9s4nh3). Raw data come from the US EPA National Rivers and Streams Assessment (https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys), and metadata for all other publicly available data sources used in this work can be found on EPA ScienceHub (https://doi.org/10.23719/1532249).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** geb70139-sup-0001-AppendixS1.pdf. **File S1:** geb70139-sup-0002-FileS1.html. **Data S1:** geb70139-sup-0003-DataS1.csv. **Data S2:** geb70139-sup-0004-DataS2.csv.

Biography

Brian K. Gallagher is a postdoctoral researcher who studies the effects of human activities on fish ecology, evolution and habitat. He is fascinated by the deep-time origins of fish biodiversity in North America and wants to understand how this diversity can be conserved in the 21st Century.