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Looks can be deceiving: Morphological traits are weakly associated with the isotopic niches of freshwater fishes

Abstract

Aim: Fueled by the emergence of global-scale databases, fish morphological traits are now routinely used as a proxy for trophic ecology when estimating functional diversity. Yet, a rigorous empirical validation of trophic-morphology relationships is lacking. This study offers a global test of whether species' morphology is associated with a known indicator of trophic ecology, stable isotope ratios of carbon and nitrogen.

Location: Worldwide.

Time period: 1992-2023.

Major taxa studied: Freshwater fishes (Actinopterygii).

Methods: Isotope-morphology relationships were examined using $\delta^{13}C$ and $\delta^{15}N$ values from 811 fish communities and ten species-level morphological traits with direct relevance for foraging behavior (body length, body shape, and position of the mouth, eyes, and fins) for 1,282 species. Associations were assessed with Multiple Regression on Distance Matrices and Multivariate Generalized Linear Models (MGLM). Analyses were repeated at the individual level for 5 fish species.

Results: Species morphological similarities explained, on average, 10% of the variation in stable isotope values for fish communities, with >6% of the communities exhibiting statistically significant associations. The MGLM explained 53% of the variation in $\delta^{13}C$ and 48% in $\delta^{15}N$, with approximately 97% and 77% of this variation, respectively, attributed to the site identity (random effect), not the morphological traits. Individual traits contributed minimally (each < 1% variation explained), except for oral gape length (8.3%) and maximum body length (4.7%). Herbivores had significantly weaker isotope-morphology correlations than omnivores and carnivores for $\delta^{15}N$, while no significant differences were observed for $\delta^{13}C$. At the individual level, morphology explained 0–16% of isotopic variation within populations, averaging 4%.

Main Conclusions: Weak isotope-morphology relationships for freshwater fishes may arise from the influence of a host of biological, environmental, or methodological factors, or may truly indicate a fundamental disconnection between morphological traits measured at the species level and the trophic niche of populations that are manifested in food webs. Ecologists should recognize the limitations of assuming morphology traits are "functional" in the sense that they can offer a reasonable proxy of a species' trophic ecology at large scales.

Introduction

Functional traits – measurable attributes of an organism that influence their performance or fitness – play a prominent role in ecology and biogeography (McGill *et al.*, 2006; Violle *et al.*, 2007). Decades of research illustrate the power of functional traits as an interpretive lens and investigative currency through which we can quantify patterns in functional diversity and understand processes shaping species responses to environmental change and anticipate effects on ecosystem processes (Mouillot *et al.*, 2013). Perhaps the greatest appeal of functional traits is the hope that they help move ecological and biogeographic knowledge beyond species-level patterns toward a more mechanistic understanding of ecosystem structure and function (Schleuning *et al.*, 2023).

Interspecific interactions, and their magnitude, are key drivers of community assemblages yet can be challenging to quantify. Morphological traits describing body size, body shape, and the structure and orientation of jaws, beaks, eyes, legs, fins, and wings offer key insights into the dietary niche and foraging behavior of vertebrates worldwide (e.g., Brosse *et al.*, 2021; Tobias *et al.*, 2022; Oskyrko *et al.*, 2024). Freshwater ecologists, like most others, continue to voice their aspirations that morphological traits offer new opportunities to describe the trophic functions of organisms that determine their response to, and ecosystem roles in, a changing environment (Villéger *et al.*, 2017; Luiz *et al.*, 2019). Morphological traits have been widely used as a proxy to reflect fish ecology, including habitat use and feeding ecology (e.g., Gatz, 1979; Hugueny & Pouilly, 1999; Villéger *et al.*, 2017; Kopf *et al.*, 2021). As morphological similarity is assumed to capture niche similarity, patterns of species separation in morphological trait space are often interpreted as indicators of trophic niche differentiation, shaped by the availability of, and competition for, resources (Winemiller, 1991; Douglas & Matthews, 1992; Montaña & Winemiller, 2013). Morphological traits are also routinely used to assess fish community assembly from local to regional scales and quantify patterns and drivers of fish functional diversity (Olden *et al.*, 2010).

The recent prominence of morphological traits in freshwater ecology was sparked by the release of a comprehensive compilation of morphological traits for almost half of the world's freshwater fish fauna (FISHMORPH, Brosse *et al.*, 2021). FISHMORPH has paved the way for numerous continental and global analyses by offering an extensive repository of trait information. Included among these are investigations related to the patterns and drivers of functional diversity (Toussaint *et al.*, 2016; Su *et al.*, 2022), effects of environmental disturbance and nonnative species on native functional diversity (Toussaint *et al.*, 2018; Chen *et al.*, 2023), functional similarities between native and nonnative fishes (Rocha *et al.*, 2025), morphological correlates of invasive species establishment (Su *et al.*, 2020; Bernery *et al.*, 2023) and trait-based estimates of native community invasibility (Xu *et al.*, 2022, 2025; Su *et al.*, 2023). This has led some to declare functional diversity, based solely on morphological traits, as a fundamental basis to guide large-scale freshwater fish diversity conservation and management (Wang *et al.*, 2025).

An enduring predicament facing ecologists is “which traits for which processes” (Westoby, 2025). Indeed, a persistent challenge is to identify and measure species traits that faithfully reflect, to the extent possible, the contributions of species to ecosystem function (McGill *et al.*, 2006; Streit & Bellwood, 2023). This is perhaps no more evident than for freshwater fishes, where attempts to reconcile form vs. function when selecting and interpreting morphological traits have long persisted (Villéger *et al.*, 2017). Past large-scale studies have made the fundamental assumption that morphological features of fish, such as body shape, fin structure, and the mouth and eye size and orientation, provide clues about their foraging habitat, feeding strategies and prey consumed, and therefore by extension, these traits provide a representation of species’ trophic niches within food webs (Villéger *et al.*, 2017). However, questions of the robustness of this assumption continue to linger in the literature. Local-scale investigations have reported highly variable diet-morphology associations in freshwater fishes. Some studies have shown moderate to high correlations ($R^2 > 0.30$) for various Brazilian and Bolivian rivers (Ibañez *et al.*, 2007; Prado *et al.*, 2016; Baldasso *et al.*, 2019), whereas others have demonstrated weaker relationships ($R^2 < 0.10$) where dietary variability exists among species with similar morphologies and diet convergence occurs between species with similar morphologies, i.e., many-to-many relationships between form and performance (Douglas & Matthews, 1992; Burress *et al.*, 2016; Keppeler & Winemiller, 2020; see Table S1). Similar weak associations between diet and morphology similarity have been highlighted in marine fishes (e.g., Albouy *et al.*, 2011).

Beyond functional constraints and feeding tactics inferred using fish morphological traits (Box 1), what an individual actually consumes is the most relevant indicator of the trophic niche (Elton, 1927). Many factors may weaken trophic-morphology relationships at both the individual and species level, including trophic variability expressed across ontogeny (Diallo & Olden, 2025) and invoked by local environmental conditions (Raffard *et al.*, 2020), prey type and availability (Osenberg *et al.*, 2004), presence of competitors and predators (Day & McPhail, 1996), and colonization history (Leaver & Reimchen, 2012). Given the strong context dependency of a species' trophic niche, therefore, it is not unreasonable to expect that morphological traits quantified at the species-level may poorly associate with dietary patterns captured at the population level. Yet, while such discussions are more common in studies of ecomorphology, past tests of this assumption have largely been limited in spatial scale (i.e., a single stream) and taxonomic scope (i.e., small number of species) and thus are not commensurate with the growing scientific application of morphological trait data to diverse species pools at continental and global scales.

Despite rarely being acknowledged in macroecological studies, we believe there is a need to explore whether fish morphological traits hold promise in explaining patterns in dietary behaviors and thus offer a meaningful surrogate for a species' trophic ecology. Here, we present the first global test of this question. Trophic ecology was estimated by assembling a global database of stable isotope ratios of δ^{13}C and δ^{15}N (Boulêtreau *et al.*, 2025). Briefly, δ^{13}C indicates the origin of the carbon in a consumer's diet, and δ^{15}N is reflective of the trophic position of the consumer within the food web (Fry, 2006). Stable isotope values offer insight into potential resource use of species and their relative trophic position within a community (Newsome *et al.*, 2007), while acknowledging the differences between isotopic niches and more traditional niche proxies that describe resource use through examination of stomach contents ("trophic niches") (Shipley & Matich, 2020; Matich *et al.*, 2021). As such, stable isotope patterns that represent horizontal and vertical dimensions of food webs should correspond to morphological differences among species that occupy different food-web positions (Box 1). The strength of isotope-morphology linkages was also compared between lotic (e.g., streams, rivers) and lentic (e.g., lakes, reservoirs) ecosystems, given potentially different ecological and evolutionary forces shaping the relationship between physical traits and food acquisition, and compared between trophic (feeding) guilds that are often used as a coarse-level classifier of a species' trophic niche. Next, a series of case studies was also used to evaluate the role of individual variation, specifically testing whether accounting for intraspecific variability increases the strength of isotope-morphology relationships. The implications of this research are significant. If morphological traits are not reasonably associated with the trophic ecology of fishes as estimated using stable isotope analysis, then additional consideration may be needed to rationalize the use of fish morphology as a faithful indicator of resource acquisition in broad-scale ecological investigations.

Methods

Species-level stable isotope and morphology data

Trophic positions were estimated using species-level δ^{13}C and δ^{15}N values for 1,282 fish species (123 families) from 811 fish communities in 65 countries across all continents except Antarctica (Figure 1). The data was curated from the global database – IsoFresh - a comprehensive database of species-level δ^{13}C and δ^{15}N values of freshwater food webs published in journal articles, university theses and reports between 1992 and 2023 (Boulêtreau *et al.*, 2025). Most important for the present study is the inclusion criterion that studies were only included when they stated in the original publication that fish communities were comprehensively sampled. Furthermore, studies with fewer than five species were excluded to ensure a sufficient gradient in fish morphology and isotopic variability. When more than one value was reported for each species (e.g., different life-stages or size-classes), we averaged the data to obtain one value per species using a weighted average procedure according to the sample sizes. The mean range of δ^{13}C values within food webs was -25.58 (SD=4.24) and the mean range of δ^{15}N values was 10.58 (SD=3.30).

We obtained ten morphological traits, quantified at the species-level, for ray-finned fishes (Actinopterygii) from FISHMORPH (Brosse *et al.*, 2021) – related to food detection and capture (Box 1) – describing body size, shape and position of the mouth, eyes, and fins with direct relevance to fish foraging and mobility (Table 1). All morphological traits are quantified as unitless ratios between comparable body parts to reduce the allometric (size-dependency) effect, except maximum body length (measured in mm). Of the 1,282 species retrieved from FISHMORPH, only 79 species had partially missing trait data (1.7% of total possible trait values). To retain all species in our analysis, we imputed the missing values using Multivariate Imputation by Chained Equations (MICE) – a statistical method for handling missing data by iteratively imputing each incomplete variable utilizing a series of regression models based on the other variables in the dataset – from the R package *mice* (van Buuren & Groothuis-Oudshoorn, 2011). MICE was selected over phylogenetic imputation to avoid circularity in subsequent analyses described below. Total variation in morphological traits of those species included in this study is broadly representative of the morphological diversity reported in FISHMORPH as revealed by a Principal Component Analysis of fish species according to the ten morphological traits comparing the 8,342 species in FISHMORPH and the species examined in this study (Figure S1).

Individual-level stable isotope and morphology data

We collated stable isotope ($\delta^{15}N$ and $\delta^{13}C$) and morphological trait data for individual fish to test whether accounting for intraspecific variability increases the strength of trophic-morphology relationships. Case study databases were selected to represent a range of fish feeding guilds that included: black bullhead (*Ameiurus melas*) from 9 artificial lakes in France (n=181, Jorigné, 2018); pumpkinseed sunfish (*Lepomis gibbosus*) from 6 artificial lakes in France (n=164, Evangelista *et al.*, 2015); largemouth bass (*Micropterus salmoides*) from a single pond in France (n=105, Zhao *et al.*, 2014); European minnow (*Phoxinus phoxinus*) from 15 populations in the Garonne River Basin, France (n=399, Raffard *et al.*, 2020); and European catfish (*Silurus glanis*) from Lake Bourget, France (n=61, Vagnon *et al.*, 2022). Standard stable isotope analyses based on muscle tissue were conducted, and the same external morphological traits used in the species-level analysis (Table 1) were estimated from direct measurements of each fish specimen when presented. This included: black bullhead (all traits except pectoral fin size and position), pumpkinseed sunfish (all traits), largemouth bass (all traits), European minnow (10 traits), and European catfish (all traits except caudal penuncle throttling). We refer the reader to the original publications for more details.

Stable isotope analyses

Species-level isotope values ($\delta^{13}C$ and $\delta^{15}N$) were standardized before subsequent analyses. To maximize sample size and account for spatial differences in baseline isotopic values, for each community, we standardized each fish species value (separately for $\delta^{13}C$ and $\delta^{15}N$) by subtracting the minimum value across all fish species in the community. This standardization process was also applied for the case studies, where we standardized each value (separately for $\delta^{13}C$ and $\delta^{15}N$) by subtracting the minimum value across all individuals in each population. Our use of standardized metrics for both species-level and individual-level analyses meant that each species in a given food web could have isotope values that are high or low relative to the remainder of the food web. In lakes, phytoplankton tend to be depleted in ^{13}C relative to both periphyton and terrestrial detritus, and so pelagic consumers will have more negative $\delta^{13}C$ values relative to benthic consumers. In streams and rivers, autochthonous (*in situ*) production can have both higher and lower $\delta^{13}C$ values versus allochthonous (*ex situ*) resources, and increased water velocity also decreases the $\delta^{13}C$ values of autochthonous prey. $\delta^{15}N$ is reflective of the trophic position of the consumer within the food web because of predictable enrichment in ^{15}N with each step in the food chain (Fry, 2006). *De novo* lipid synthesis is known to drive $\delta^{13}C$ in a negative direction in fatty tissues, which could influence the relative positioning of species within the food web that is independent of diet. But we chose not to correct $\delta^{13}C$ data for lipids because freshwater fish muscle tends to be low in lipids with only minimal expected influence on $\delta^{13}C$ values (Logan *et al.*, 2008).

This study assessed isotope-morphology relationships within each community, not across communities, which further reduced the effects of varying isotopic baselines. However, the validity of this decision was evaluated by examining a reduced number of communities (n=330) where the isotope values of primary consumers were also reported in the study, including zooplankton and macroinvertebrates. For these communities, we used the following baseline standardizations according to (Olsson *et al.*, 2009):

$$\delta^{13}\mathrm{C}_{fish-corr} = \frac{\delta^{13}\mathrm{C}_{fish} - \mathrm{mean}\,(\delta^{13}\mathrm{C}_{baseline})}{\mathrm{abs}\,(\mathrm{range}\,(\delta^{13}\mathrm{C}_{baseline}))} \qquad [1]$$

$$\delta^{15}\mathrm{N}_{fish-corr} = \delta^{15}\mathrm{N}_{fish} - \mathrm{mean}(\delta^{15}\mathrm{N}_{baseline}) \qquad [2]$$

The choice of isotopic baseline was determined according to the original published study, under the reasonable assumption that this reflected a well-informed decision by the authors (Boulêtreau *et al.*, 2025).

Statistical analyses

We used two complementary approaches to quantify the strength of associations between the trophic ecology (inferred from $\delta^{13}C$ and $\delta^{15}N$) and morphological traits at both the species and individual (case studies) level. We performed Multiple Regression on Distance Matrices (MRM), implemented in the R package ecodist (Goslee & Urban, 2007), to test the relationship between the isotope similarity matrix (response) and the trait similarity matrix (predictor), both based on standardized Euclidean distances, separately for each fish community. Statistical significance was assessed using a permutation approach. MRM is an extension of the Mantel test that tests for associations between distance matrices. Next, we evaluated the spatial autocorrelation in the data using Moran's I index (Gittleman & Kot, 1990). The index varies from -1 (closely related sites have opposite values) to 1 (closely related sites have similar values). The strength of isotope-morphology relationships (R^2) was compared between lotic (stream and rivers) and lentic (lakes and reservoirs) ecosystems using a two-sample Student's t-test. We also report Mantel test statistics (based on standardized Euclidean distances) to allow for comparison to past studies. Finally, MRMs were conducted for each case study population according to individual-level stable isotope and morphology data.

We used a Bayesian Multivariate Generalized Linear Model (MGLM) implemented in the R package Hmsc (Tikhonov *et al.*, 2020) to relate individual traits (predictors) to stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$ values) at the species level. Site ID was included as a random effect, as our goal was to broadly isolate the explanatory power of morphological traits versus other unaccounted for sources of variation that are predictive of $\delta^{13}C$ and $\delta^{15}N$ values. The model assumed a Gaussian distribution with non-informative priors following (Ovaskainen & Abrego, 2020). A trait was considered significant when its respective parameters' 95% credible intervals did not encompass zero. Variance partitioning was done using the method proposed by Ovaskainen & Abrego (2020) to identify which variables were more relevant to explain $\delta^{13}C$ and $\delta^{15}N$ variation. We set the model with five chains of 50,000 iterations each to ensure convergence, discarding 50% as burn-in, and then retained 250 samples from the remaining iterations using a thinning interval of 100.

The MGLM residuals were used to assess whether the stable isotope ratios of certain fish feeding guilds (herbivore, omnivore, carnivore) or fish families were better explained by their morphology than others. First, mean absolute residuals for each species were calculated across all communities in which the species was present. Second, species were assigned to feeding guilds using trophic position estimates obtained from FishBase (http://www.fishbase.org, accessed 05/01/25). In FishBase, trophic position is calculated by adding 1 to the mean trophic position, weighted by relative abundance, of all food items consumed by a species reported in the literature (Froese & Pauly, 2000). Species were classified as herbivore (TP<2.2), omnivore (TP=2.2-2.8), or carnivore (TP>2.8). MGLM absolute residuals were compared between fish feeding guilds (herbivore, omnivore, carnivore) using an Analysis of Variance and pairwise Tukey HSD tests. Third, the Blomberg's K metric, implemented in the phytools R package, was used to test for a phylogenetic signal in the MGLM absolute residuals. Due to the unresolved nature of fish phylogenies, we constructed a phylogenetic tree based on taxonomic similarity. Taxonomic information was sourced from the Integrated Taxonomic Information System (ITIS; National Museum of Natural History, Smithsonian Institution, 2024) using the taxize R package (Chamberlain & Szöcs, 2013). All analyses were performed in R ver. 4.3.1 (R Development Core Team).

Results

Isotope-morphology relationships for freshwater fish communities were universally weak, but notably highly variable across fish communities. Overall, associations between species similarities based on morphological traits and stable isotope ratios were low according to the MRMs, with an average R² of 0.100 (SD = 0.143) (Figure 1). Statistically significant associations ($P<0.05$) were identified in 48 fish communities (~5.9% of the 810 sites examined), and only 26 fish communities (~3.2%) displayed R² values that exceeded 0.50. No clear geographic patterns were evident in the strength of the trophic-morphology correlations (Figure 1), despite some evidence for the detection of spatial autocorrelation ($I_{observed} = 0.063$, $I_{expected} = -0.002$, SD = 0.020, $p < 0.001$). Isotope-morphology correlations differed slightly between lotic (mean = 0.114) and lentic ecosystems (mean = 0.090) ($t_{1,809}=2.56$, $p=0.01$; Figure 2). Associations between morphological traits and stable isotope ratios were also low according to Mantel tests, with an average R² of 0.05 (SD = 0.31). Weak associations were also evident when using baseline-corrected stable isotope values, with an average R² of 0.102 (SD = 0.141).

Predominantly weak associations between individual traits and each stable isotope ratio were similarly evident. Overall, 53% of the variation in $\delta^{13}C$ and 48% in $\delta^{15}N$ were explained by the MGLM models, but the majority of this variation, 97% and 77%, respectively, was attributed to the identity of the studied site (random effect) and not the explanatory power of the traits (Table 2). Of the minimal variation explained in stable isotope ratios, significant relative importances (95% credible intervals not encompassing zero) were revealed for eye size (positive: $\delta^{15}N$), eye position (positive: $\delta^{15}N$), body length (positive: $\delta^{13}C$, $\delta^{15}N$), body elongation (negative: $\delta^{15}N$) and lateral shape (positive: $\delta^{15}N$), pectoral fin size (negative: $\delta^{15}N$), pectoral fin position (positive: $\delta^{13}C$), and oral gape length (positive: $\delta^{13}C$, $\delta^{15}N$) (Table 2). However, the relative importance of all these traits was exceedingly minor, except $\delta^{15}N$ - oral gape length and $\delta^{15}N$ - body length, which were 0.109 (8.3% of the total variation) and 0.062 (4.7% of the total variation), respectively (Table 2). The strength of isotope-morphology relationships separated according to lotic and lentic ecosystems also demonstrated similarly low associations between individual traits and each stable isotope ratio (Table S2). The directional influence of traits on each stable isotope value was the same for lotic and lentic ecosystems, except for eye position (lotic: negative; lentic: positive), caudal peduncle throttling (lotic: positive; lentic: negative), oral gape position (lotic: positive; lentic: negative) and oral gape length (lotic: positive; lentic: negative) concerning $\delta^{13}C$ (Table S2). No differences between ecosystems in the directional influence of traits were observed for $\delta^{15}N$. Relationships between baseline-corrected stable isotope values and species traits were similarly weak and demonstrated the same directional relationships reported above (Table S3).

Variability in isotope-morphology correlations, inferred from the absolute MGLM residuals, differed according to species feeding guilds. Only slight differences were evident for δ¹³C (ANOVA, $F_{2,1279}$=3.098, P=0.046, Figure 3A). In contrast, larger differences were apparent for δ¹⁵N (ANOVA, $F_{2,1279}$=7.086, P<0.001), where herbivorous species demonstrated significantly higher δ¹⁵N residuals than omnivores and carnivores according to Tukey HSD tests (Figure 3B).

The phylogenetic signal of the MGLM residuals was significant for δ¹³C (K = 0.26, P = 0.018) and δ¹⁵N (K = 0.25, P = 0.019) at the species level (Figure 4). Species within families such as Lepisosteidae (gars), Salangidae (noodlefishes), and Comephoridae (golomyankas) exhibited lower morphology-δ¹⁵N correlations, reflected in higher residuals, compared to species in Cyprinodontidae (pupfish), Umbridae (pike, pickerel, and mudminnows), and Psilorhynchidae (torrent minnows). Conversely, species in Hypophthalmidae (maparás) and Pristigasteridae (longfin herrings) had lower morphology-δ¹³C correlation than others, such as Cyprinodontidae and Alestiidae (African tetras).

The strength of isotope-morphology relationships evaluated at the intraspecific (individual) level largely mirrored the interspecific (species) level findings. Individual differences in stable isotope ratios explained by variability in morphological traits (R^2) ranged from 0.00 to 0.16 across populations, averaging just 0.04 across all populations of the five species included in the case studies (Table 3). Largemouth bass exhibited the strongest isotope-morphology associations.

Discussion

Ecologists routinely posit that functional trait analyses, founded either solely or in part on morphological traits, provide opportunities to guide global fish biodiversity conservation, management, and sustainability (Toussaint *et al.*, 2016; Toussaint *et al.*, 2018; Su *et al.*, 2022; Xu *et al.*, 2022; Chen *et al.*, 2023; Bernery *et al.*, 2023; Su *et al.*, 2023; Rocha *et al.*, 2025; Wang *et al.*, 2025). Support of such claims depends on the fundamental, albeit often unspoken, assumption that morphological characteristics of fish, such as body shape, fin configuration, and the size and orientation of the mouth and eyes, serve as reliable indicators of their feeding strategies and prey preferences. By extension, these external physical attributes – often easily measurable on individuals in the field or estimated from photographs – are regarded as robust predictors of their trophic ecology within freshwater food webs. In a global test of this assumption, we show that morphological traits show weak, but variable, associations with carbon and nitrogen stable isotope values of freshwater fishes, thus questioning the functional underpinnings for the use of such traits to infer the trophic ecology of species in broad-scale investigations.

We found that species morphological similarities explained, on average, just 10% of the stable isotope variation in freshwater fish communities, and that isotope-morphology relationships were statistically significant in just over one of every twenty communities. These weak associations, revealed at the global scale, are supported by past local-scale studies examining stomach contents that also reported poor reliability of diet-morphology relationships (Table S1). In the Roanoke River, USA, researchers were among the first to report weak associations for fish assemblages (Douglas & Matthews, 1992). They noted that after accounting for taxonomy, the relationship between body shape and trophic ecology was small and nonsignificant. Similarly, Ibañez *et al.* (2007) revealed little association between diet and morphology for 30 fish species from tropical forest streams of the Bolivian Amazon, and Manna *et al.* (2019) reported that morphology–dietary relationships were weak at both individual and species levels for two Brazilian streams. In summary, our results offer a global perspective on existing evidence pointing to the considerable variability in the strength of trophic-morphology relationships shown at local scales (see Table S1 for more examples).

Body size and mouth gape size are considered to oppose fundamental constraints on fish hunting ability and the size of prey that can be swallowed whole (Wainwright & Richard, 1995; Blake, 2004). We report that maximum body length and oral gape length were the only morphological traits that explained variation in δ^{15}N values; albeit just a total 13% at the species level. This finding provides some support of past studies that have incorporated body length as well as gape size—or a common proxy, gape (maxillary jaw) length—into trait-based analyses of fish communities, based on the assumption that a larger body sizes gape allows access to a wider variety of prey sizes and types (Gatz, 1979; Villéger *et al.*, 2017; Kopf *et al.*, 2021). For example, maximum gape width correlates with the largest prey a fish can consume (Keppeler *et al.*, 2020), and body size and jaw length are linked to higher trophic positions (Romanuk *et al.*, 2011; Kopf *et al.*, 2021). Measurements of gape size from physical specimens and gape length from photographs may offer some utility in functional trait analyses.

Biogeographical studies of trophic niches have primarily focused on interspecific differences among species, often overlooking the role of intraspecific variability caused by individual trophic specialization (Luiz *et al.*, 2019). In populations of black bullhead, pumpkinseed sunfish, largemouth bass, European minnow, and European catfish, we found that individual variation in morphological traits showed generally weak correlations with trophic niches inferred from carbon and nitrogen stable isotopes. Therefore, accounting for intraspecific variability did not appear to significantly improve diet-morphology correlations (also see Zhao *et al.*, 2014; Manna *et al.*, 2019), with the notable exception of largemouth bass, an omnivorous species that transitions to piscivory later in age. These results support the notion that fish with similar morphologies may feed on different resources (Ross, 1986), whereas fish with different morphologies may display trophic convergence (Norton & Brainerd, 1993). Past research suggests that resource-related trait variation may not always be captured by general morphology. Despite this, individual body sizes have been shown to be related to stable isotope-based estimates of trophic positions (e.g., Keppeler *et al.*, 2020). Our results for largemouth bass support the findings of Kopf *et al.*, (2025) who reported positive relationships between individual body mass and trophic position (estimated by δ^{15}N values) for fishes in the Murray-Darling Basin, Australia. Similarly, Kopf *et al.*, (2025) noted that this relationship was not evident for algivores-detritivores, mirroring our finding of weak isotope-morphology relationships for herbivorous fishes.

The longstanding field of fish ecomorphology offers considerable insight into how associations between organismal morphology and ecological performance, such as prey detection, capture success and choice, can depend on the biological and environmental context (e.g., Gatz, 1979; Page & Swofford, 1984; Winemiller, 1991). The influence of these external factors, the quantification of which was not the objective of this study, is reflected in the study site (random effect in our models), accounting for almost all the variation in fish δ^{13}C and δ^{15}N values as opposed to the morphological traits. Trophic polymorphisms are not uncommon in freshwater fishes. For example, pumpkinseed sunfish have specialized pharyngeal structures and feeding behaviors that enable them to crush and eat snails, typically comprising 70–90% of their diet. In lakes with few snails, their diet shifts to soft-bodied invertebrates, and their pharyngeal muscles and bones become reduced due to lack of use (Wainwright *et al.*, 1991). Indeed, site-specific resource availability may mask true form-function relationships, such as what is captured by Liem's Paradox, where species that have specialized feeding traits may forage as generalists when resources are abundant (Liem, 1980). Predation risk can also cause some prey species to alter their food preferences and foraging habitats in ways that may decouple diet from morphology. For example, largemouth bass are widely known to reduce foraging, shift habitat use and alter food choice of their prey (Werner & Hall, 1988). Predators can also cause morphological changes in fish prey as they increase their defense. Fathead minnows develop deeper heads and bodies, longer dorsal fin bases, shorter caudal peduncles and fins, and a larger relative eye size when exposed to conspecific alarm cues triggered by predators

(Meuthen *et al.*, 2019). Furthermore, the diet of freshwater fishes can vary according to environmental characteristics, such as temperature, hydrology, habitat complexity, and light levels. Using eye morphology as just one example, eye sizes of Eurasian perch (*Perca fluviatilis*) were negatively related to increasing water clarity in lakes, suggesting that more turbid and darker waters select for larger eye size that promote the navigation of environments to find food and avoid predators (Andersson *et al.*, 2024). In summary, phenotypic traits of organisms can respond to varying environmental and ecological conditions and weaken associations between morphological and diet variability of freshwater fishes.

This study leveraged a comprehensive database of stable isotope values for fish communities worldwide (Boulêtreau *et al.*, 2025), thus choosing to focus on the temporally integrative isotope ratios (from weeks to months) rather than instantaneous stomach contents to estimate the diet of species. While this time-integrated approach was an advantage, it does introduce additional considerations that are worth noting. Differences in stable isotope turnover rates between species (Vander Zanden *et al.*, 2015), lipid contents in the samples that differ between tissues (Post *et al.*, 2007), and uncertainties in trophic fractionation among species (Vander Zanden & Rasmussen, 2001) are factors that can limit the ability of stable isotope analyses to reflect the exact trophic position of organisms (Fry, 2006; Hette-Tronquart, 2019). Furthermore, we point to the confounding effects of variation in carbon isotope ratios of basal sources and that nitrogen isotope ratios of basal sources often differ within and among food webs (Hoeinghaus & Zeug, 2008). In many cases, different prey, resources and habitats may also have similar isotope ratios and limit the ability of stable isotope analyses to provide high taxonomic or habitat resolution (Alp & Cucherousset, 2022). For this reason, the value of stable isotope data to infer species' trophic niche is enhanced when used in conjunction with direct observations of trophic relationships from stomach contents analyses. Nevertheless, global investigations using stable isotope data in freshwater fishes have provided important information on food chain length patterns (Vander Zanden & Fetzer, 2007), the structure of fish communities (Pool *et al.*, 2016) and their response to fish invasions (Sagouis *et al.*, 2015), and testing isotopic niche conservatism of nonnative species (Comte *et al.*, 2017), to name just a few examples. Therefore, by quantifying the realized trophic positions of organisms in their environment, isotopes can provide insight into trophic relations within communities.

The widespread availability of publicly accessible trait data has dramatically expanded the possibilities for ecological research. However, while large trait datasets can be generated through broad aggregation, such traits may not necessarily be well-suited to specific research questions. Limitations in taxonomic and geographic coverage and data resolution are significant challenges for future-proofing the use of traits in ecology (Keller *et al.*, 2023), and here we demonstrate that ecological relevance may further constrain their utility in large-scale freshwater investigations. The abundance of morphological trait data offered by FISHMORPH (Brosse *et al.*, 2021) has appeared to unintentionally shift the emphasis from hypothesis-driven research to data-driven exploration, thereby increasing the risk of spurious correlations or post hoc interpretations. Of the many hypothesized relationships between morphology and diet in fish (Box 1), we found that only body and gape length were positively associated with $\delta^{15}N$. We urge ecologists to guide their trait selection by clearly defining hypotheses rather than leveraging the convenience of existing datasets where the prevailing trend is to 'functionalize' existing traits (Streit & Bellwood, 2023).

We support efforts to close the significant gaps in morphological trait data for freshwater fishes, especially given that more than half of the world's freshwater fish species still lack any such measurements (Brosse *et al.*, 2021). However, if the primary motivation for expanding morphological datasets is to advance studies of biodiversity and biogeography, we encourage fish ecologists to broaden their focus beyond external morphology. Specifically, we advocate for including internal anatomical traits related to prey capture and digestion (e.g., gill rakers, jaw structure, dentition, intestinal tract length), which may provide more ecologically relevant insights into feeding strategies and trophic ecology (Keppeler *et al.*, 2020). Emerging bioimaging techniques using micro-computed tomography with contrast enhancement for soft tissues offer new opportunities to analyze the internal structure of fish, including anatomical measurements conducted at scale (Gignac *et al.*, 2016). Therefore, combining biodiversity metrics with trophic morphology and stable isotopes may be an approach to testing this hypothesis and others. Combining morphological traits with complementary insights offered by recent global diet (Ridgway & Wesner, 2025) and stable isotope databases (Boulêtreau *et al.*, 2025) now offers new avenues to more robustly depict the trophic ecology of freshwater fishes by accounting for the uncertainty inherent to each data source.

Conclusion

The growing enthusiasm for traits-based ecology, particularly approaches that are grounded solely in morphological traits, is increasingly shaping freshwater fish biogeography. However, we caution that this enthusiasm should not overlook the possible limitations of morphological traits as a meaningful lens or currency for functional inference. While we do not dispute the limitations of using stable isotopes to infer trophic dynamics and continue to believe that morphological traits hold value in depicting habitat use (Villéger *et al.*, 2017), representing ecological diversity (Côte *et al.*, 2022) and offering insight into fish movement ability (Comte & Olden, 2018), our results do raise the question of whether such traits are "functional" in the sense that they can offer a reasonable proxy of a species' trophic ecology and hence their role in ecosystem functioning. If morphological traits are indeed just traits and not functional traits, then their exclusive use in exploring patterns and drivers of functional diversity at broad spatial scales deserves careful consideration.

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Data Availability Statement: IsoFresh (Boulêtreau *et al.*, 2025) - a database of δ^{13}C and δ^{15}N values of freshwater food webs – is available at https://doi.org/10.5281/zenodo.14982323), and FISHMORPH (Brosse *et al.*, 2021) – a database of morphological traits of freshwater fish species – is available at: https://doi.org/10.6084/m9.figshare.14891412. Data and R code to conduct the analysis and reproduce all figures and tables is available at: https://doi.org/10.5281/zenodo.16945396.

References

Albouy, C., Guilhaumon, F., Villéger, S., Mouchet, M., Mercier, L., Culioli, J., Tomasini, J., Le Loc'h, F. & Mouillot, D. (2011) Predicting trophic guild and diet overlap from functional traits: statistics, opportunities and limitations for marine ecology. *Marine Ecology Progress Series*, **436**, 17–28.

Alp, M. & Cucherousset, J. (2022) Food webs speak of human impact: Using stable isotope-based tools to measure ecological consequences of environmental change. *Food Webs*, **30**, e00218.

Andersson, M.L., Scharnweber, K. & Eklöv, P. (2024) Environmental and ecological drivers of eye size variation in a freshwater predator: A trade-off between foraging and predation risk. *Functional Ecology*, **38**, 2470–2477.

Baldasso, M.C., Wolff, L.L., Neves, M.P. & Delariva, R.L. (2019) Ecomorphological variations and food supply drive trophic relationships in the fish fauna of a pristine neotropical stream. *Environmental Biology of Fishes*, **102**, 783–800.

Bernery, C., Marino, C. & Bellard, C. (2023) Relative importance of exotic species traits in determining invasiveness across levels of establishment: Example of freshwater fish. *Functional Ecology*, **37**, 2358–2370.

Blake, R.W. (2004) Fish functional design and swimming performance. *Journal of Fish Biology*, **65**, 1193–1222.

Boulêtreau, S., Vagnon, C., Comte, L., Sagouis, A., Pool, T., Stiling, R.R., Harrod, C., South, J., McIntosh, A.R., Perga, M.-E., Sánchez-Hernández, J., Roussel, J.-M., Tunney, T.D., Jackson, M., Olden, J.D. & Cucherousset, J. (2025) IsoFresh: A global stable isotope database of freshwater food webs. *Knowledge & Management of Aquatic Ecosystems*, 15.

Brosse, S., Charpin, N., Su, G., Toussaint, A., Herrera-R, G.A., Tedesco, P.A. & Villéger, S. (2021) FISHMORPH: A global database on morphological traits of freshwater fishes. *Global Ecology and Biogeography*, **30**, 2330–2336.

Burress, E.D., Holcomb, J.M. & Armbruster, J.W. (2016) Ecological clustering within a diverse minnow assemblage according to morphological, dietary and isotopic data. *Freshwater Biology*, **61**, 328–339.

van Buuren, S. & Groothuis-Oudshoorn, K. (2011) **mice** : Multivariate Imputation by Chained Equations in *R*. *Journal of Statistical Software*, **45**.

Caves, E.M., Sutton, T.T. & Johnsen, S. (2017) Visual acuity in ray-finned fishes correlates with eye size and habitat. *Journal of Experimental Biology*, jeb.151183.

Chamberlain, S.A. & Szöcs, E. (2013) taxize: taxonomic search and retrieval in R. *F1000Research*, **2**, 191.

Chen, M., Liang, Y., Cheng, X., Wang, J., Ding, L., Huang, M., Wang, G., Tao, J. & Ding, C. (2023) How do fish functional traits respond to dams at the global scale? *Hydrobiologia*.

Comte, L., Cucherousset, J. & Olden, J.D. (2017) Global test of Eltonian niche conservatism of nonnative freshwater fish species between their native and introduced ranges. *Ecography*, **40**, 384–392.

Comte, L. & Olden, J.D. (2018) Evidence for dispersal syndromes in freshwater fishes. *Proceedings of the Royal Society B: Biological Sciences*, **285**, 20172214.

Côte, J., Kuczynski, L. & Grenouillet, G. (2022) Morphology reflects differently the various facets of species traits in stream fish. *Freshwater Biology*, **67**, 1203–1213.

Day, T. & McPhail, J.D. (1996) The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (Gasterosteus sp.). *Oecologia*, **108**, 380–388.

Diallo, J. & Olden, J. (2025) Invasive species invoke a lifetime of trophic change in native stream fishes. *Ecosphere*, **16**, e70304.

Douglas, M.E. & Matthews, W.J. (1992) Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. *Oikos*, **65**, 213.

Dumay, O., Tari, P.S., Tomasini, J.A. & Mouillot, D. (2004) Functional groups of lagoon fish species in Languedoc Roussillon, southern France. *Journal of Fish Biology*, **64**, 970–983.

Elton, C.S. (1927) *Animal Ecology*, Sidgewick and Jackson, London, U.K.

Evangelista, C., Britton, R.J. & Cucherousset, J. (2015) Impacts of invasive fish removal through angling on population characteristics and juvenile growth rate. *Ecology and Evolution*, **5**, 2193–2202.

Froese, R. & Pauly, D. (2000) FishBase.

Fry, B. (2006) *Stable Isotope Ecology*, Springer New York, New York, NY.

Gatz, J. (1979) Ecological morphology of freshwater stream fishes. *Tulane Studies in Zoology and Botany*, **21**, 91–124.

Gerking, S. (1994) *Fedding Ecology of Fish*, Academic Press, San Diego, CA.

Gignac, P.M., Kley, N.J., Clarke, J.A., Colbert, M.W., Morhardt, A.C., Cerio, D., Cost, I.N., Cox, P.G., Daza, J.D., Early, C.M., Echols, M.S., Henkelman, R.M., Herdina, A.N., Holliday, C.M., Li, Z., Mahlow, K., Merchant, S., Müller, J., Orsbon, C.P., Paluh, D.J., Thies, M.L., Tsai, H.P. & Witmer, L.M. (2016) Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *Journal of Anatomy*, **228**, 889–909.

Gittleman, J.L. & Kot, M. (1990) Adaptation: Statistics and a null model for estimating phylogenetic effects. *Systematic Zoology*, **39**, 227.

Goslee, S.C. & Urban, D.L. (2007) The ecodist Package for Dissimilarity-based Analysis of Ecological Data. *Journal of Statistical Software*, **22**.

Hette-Tronquart, N. (2019) Isotopic niche is not equal to trophic niche. *Ecology Letters*, **22**, 1987–1989.

Hoeinghaus, D.J. & Zeug, S.C. (2008) Can stable isotope rations provide for community-wide measures of trophic structure? *Ecology*, **89**, 2353–2357.

Hugueny, B. & Pouilly, M. (1999) Morphological correlates of diet in an assemblage of West African freshwater fishes. *Journal of Fish Biology*, **54**, 1310–1325.

Ibañez, C., Tedesco, P.A., Bigorne, R., Hugueny, B., Pouilly, M., Zepita, C., Zubieta, J. & Oberdorff, T. (2007) Dietary-morphological relationships in fish assemblages of small forested streams in the Bolivian Amazon. *Aquatic Living Resources*, **20**, 131–142.

Jorigné, B. (2018) Pratiques de gestion et conséquences écologiques sur des espèces exotiques envahissantes dans des milieux aquatiques artificiels.

Keller, A., Ankenbrand, M.J., Bruelheide, H., Dekeyzer, S., Enquist, B.J., Erfanian, M.B., Falster, D.S., Gallagher, R.V., Hammock, J., Kattge, J., Leonhardt, S.D., Madin, J.S., Maitner, B., Neyret, M., Onstein, R.E., Pearse, W.D., Poelen, J.H., Salguero-Gomez, R., Schneider, F.D., Tóth, A.B. & Penone, C. (2023) Ten (mostly) simple rules to future-proof trait data in ecological and evolutionary sciences. *Methods in Ecology and Evolution*, **14**, 444–458.

Keppeler, F.W., Montaña, C.G. & Winemiller, K.O. (2020) The relationship between trophic level and body size in fishes depends on functional traits. *Ecological Monographs*, **90**, e01415.

Keppeler, F.W. & Winemiller, K.O. (2020) Can ancestry and morphology be used as surrogates for species niche relationships? *Ecology and Evolution*, **10**, 6562–6578.

Kopf, R.K., McPhan, L., McInerney, P.J., Zampatti, B., Thiem, J., Koster, W., Butler, G.L., Bond, N. & Thompson, R.M. (2025) Intraspecific body size determines isotopic trophic structure of a large river fish community. *Journal of Animal Ecology*, **94**, 1435–1448.

Kopf, R.K., Yen, J.D.L., Nimmo, D.G., Brosse, S. & Villéger, S. (2021) Global patterns and predictors of trophic position, body size and jaw size in fishes. *Global Ecology and Biogeography*, **30**, 414–428.

Leaver, S.D. & Reimchen, T.E. (2012) Abrupt changes in defence and trophic morphology of the giant threespine stickleback (*Gasterosteus* sp.) following colonization of a vacant habitat: Abrupt morphological shifts in stickleback. *Biological Journal of the Linnean Society*, **107**, 494–509.

Liem, K.F. (1980) Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. *American Zoologist*, **20**, 295–314.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A. & Lutcavage, M.E. (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, **77**, 838–846.

López-Fernández, H., Winemiller, K.O., Montaña, C. & Honeycutt, R.L. (2012) Diet-morphology correlations in the radiation of South American Geophagine Cichlids (Perciformes: Cichlidae: Cichlinae). *PLoS ONE*, **7**, e33997.

Luiz, O.J., Olden, J.D., Kennard, M.J., Crook, D.A., Douglas, M.M., Saunders, T.M. & King, A.J. (2019) Trait-based ecology of fishes: A quantitative assessment of literature trends and knowledge gaps using topic modelling. *Fish and Fisheries*, **20**, 1100–1110.

Mahon, R. (1984) Divergent Structure in Fish Taxocenes of North Temperate Streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 330–350.

Manna, L.R., Villéger, S., Rezende, C.F. & Mazzoni, R. (2019) High intraspecific variability in morphology and diet in tropical stream fish communities. *Ecology of Freshwater Fish*, **28**, 41–52.

Matich, P., Bizzarro, J.J. & Shipley, O.N. (2021) Are stable isotope ratios suitable for describing niche partitioning and individual specialization? *Ecological Applications*, **31**, e02392.

McGill, B., Enquist, B., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178–185.

Meuthen, D., Ferrari, M.C.O., Lane, T. & Chivers, D.P. (2019) Predation risk induces age- and sex-specific morphological plastic responses in the fathead minnow Pimephales promelas. *Scientific Reports*, **9**, 15378.

Montaña, C.G. & Winemiller, K.O. (2013) Evolutionary convergence in Neotropical cichlids and Nearctic centrarchids: evidence from morphology, diet, and stable isotope analysis. *Biological Journal of the Linnean Society*, **109**, 146–164.

Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H. & Bellwood, D.R. (2013) A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, **28**, 167–177.

Newsome, S.D., Martinez Del Rio, C., Bearhop, S. & Phillips, D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429–436.

Nikolski, G.V. (1933) On the Influence of the Rate of Flow on the Fish Fauna of the Rivers of Central Asia. *The Journal of Animal Ecology*, **2**, 266.

Norton, S.F. & Brainerd, E.L. (1993) Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology*, **176**, 11–29.

Olden, J.D., Kennard, M.J., Leprieur, F., Tedesco, P.A., Winemiller, K.O. & García-Berthou, E. (2010) Conservation biogeography of freshwater fishes: recent progress and future challenges. *Diversity and Distributions*, **16**, 496–513.

Olsson, K., Stenroth, P., Nyström, P. & Granéli, W. (2009) Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology*, **54**, 1731–1740.

Osenberg, C.W., Huckins, C.J.F., Kaltenberg, A. & Martinez, A. (2004) Resolving within- and between-population variation in feeding ecology with a biomechanical model. *Oecologia*, **141**, 57–65.

Oskyrko, O., Mi, C., Meiri, S. & Du, W. (2024) ReptTraits: a comprehensive dataset of ecological traits in reptiles. *Scientific Data*, **11**, 243.

Ovaskainen, O. & Abrego, N. (2020) *Joint Species Distribution Modelling: With Applications in R*, 1st edn. Cambridge University Press.

Page, L.M. & Swofford, D.L. (1984) Morphological correlates of ecological specialization in darters. *Environmental Biology of Fishes*, **11**, 139–159.

Piet, G.J. (1998) Impact of environmental perturbation on a tropical fish community. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1842–1853.

Pool, T.K., Cucherousset, J., Boulêtreau, S., Villéger, S., Strecker, A.L. & Grenouillet, G. (2016) Increased taxonomic and functional similarity does not increase the trophic similarity of communities. *Global Ecology and Biogeography*, **25**, 46–54.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J. & Montaña, C.G. (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, **152**, 179–189.

Pouilly, M., Lino, F., Bretenoux, J. -G. & Rosales, C. (2003) Dietary–morphological relationships in a fish assemblage of the Bolivian Amazonian floodplain. *Journal of Fish Biology*, **62**, 1137–1158.

Prado, A.V.R., Goulart, E. & Pagotto, J.P.A. (2016) Ecomorphology and use of food resources: inter- and intraspecific relationships of fish fauna associated with macrophyte stands. *Neotropical Ichthyology*, **14**.

Raffard, A., Santoul, F., Blanchet, S. & Cucherousset, J. (2020) Linking intraspecific variability in trophic and functional niches along an environmental gradient. *Freshwater Biology*, **65**, 1401–1411.

Ribble, D.O. & Smith, M.H. (1983) Relative intestine length and feeding ecology of freshwater fishes. *Growth*, **47**, 292–300.

Ridgway, J. & Wesner, J. (2025) A global dataset of freshwater fish trophic interactions. *Scientific Data*, **12**, 160.

Rocha, B.S., Rodrigues, A.C. & Granzotti, R.V. (2025) Widespread freshwater non-native fishes exhibit synchronized population dynamics with functionally similar natives. *Scientific Reports*, **15**, 19753.

Romanuk, T.N., Hayward, A. & Hutchings, J.A. (2011) Trophic level scales positively with body size in fishes: Trophic level and body size in fishes. *Global Ecology and Biogeography*, **20**, 231–240.

Ross, S.T. (1986) Resource partitioning in fish assemblages: A review of field studies. *Copeia*, **1986**, 352.

Sagouis, A., Cucherousset, J., Villéger, S., Santoul, F. & Boulêtreau, S. (2015) Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, **38**, 979–985.

Schleuning, M., García, D. & Tobias, J.A. (2023) Animal functional traits: Towards a trait-based ecology for whole ecosystems. *Functional Ecology*, **37**, 4–12.

Shipley, O.N. & Matich, P. (2020) Studying animal niches using bulk stable isotope ratios: an updated synthesis. *Oecologia*, **193**, 27–51.

Sibbing, F.A. & Nagelkerke, L.A.J. (2000) [No title found]. *Reviews in Fish Biology and Fisheries*, **10**, 393–437.

Streit, R.P. & Bellwood, D.R. (2023) To harness traits for ecology, let's abandon 'functionality.' *Trends in Ecology & Evolution*, **38**, 402–411.

Su, G., Mertel, A., Brosse, S. & Calabrese, J.M. (2023) Species invasiveness and community invasibility of North American freshwater fish fauna revealed via trait-based analysis. *Nature Communications*, **14**, 2332.

Su, G., Tedesco, P.A., Toussaint, A., Villéger, S. & Brosse, S. (2022) Contemporary environment and historical legacy explain functional diversity of freshwater fishes in the world rivers. *Global Ecology and Biogeography*, **31**, 700–713.

Su, G., Villéger, S. & Brosse, S. (2020) Morphological sorting of introduced freshwater fish species within and between donor realms. *Global Ecology and Biogeography*, **29**, 803–813.

Tikhonov, G., Opedal, Ø.H., Abrego, N., Lehikoinen, A., De Jonge, M.M.J., Oksanen, J. & Ovaskainen, O. (2020) Joint species distribution modelling with the R -package H MSC. *Methods in Ecology and Evolution*, **11**, 442–447.

Tobias, J.A., Sheard, C., Pigot, A.L., Devenish, A.J.M., Yang, J., Sayol, F., Neate-Clegg, M.H.C., Alioravainen, N., Weeks, T.L., Barber, R.A., Walkden, P.A., MacGregor, H.E.A., Jones, S.E.I., Vincent, C., Phillips, A.G., Marples, N.M., Montaño-Centellas, F.A., Leandro-Silva, V., Claramunt, S., Darski, B., Freeman, B.G., Bregman, T.P., Cooney, C.R., Hughes, E.C., Capp, E.J.R., Varley, Z.K., Friedman, N.R., Korntheuer, H., Corrales-Vargas, A., Trisos, C.H., Weeks, B.C., Hanz, D.M., Töpfer, T., Bravo, G.A., Remeš, V., Nowak, L., Carneiro, L.S., Moncada R., A.J., Matysioková, B., Baldassarre, D.T., Martínez-Salinas, A., Wolfe, J.D., Chapman, P.M., Daly, B.G., Sorensen, M.C., Neu, A., Ford, M.A., Mayhew, R.J., Fabio Silveira, L., Kelly, D.J., Annorbah, N.N.D., Pollock, H.S., Grabowska-Zhang, A.M., McEntee, J.P., Carlos T. Gonzalez, J., Meneses, C.G., Muñoz, M.C., Powell, L.L., Jamie, G.A., Matthews, T.J., Johnson, O., Brito, G.R.R., Zyskowski, K., Crates, R., Harvey, M.G., Jurado Zevallos, M., Hosner, P.A., Bradfer-Lawrence, T., Maley, J.M., Stiles, F.G., Lima, H.S., Provost, K.L., Chibesa, M., Mashao, M., Howard, J.T., Mlamba, E., Chua, M.A.H., Li, B., Gómez, M.I., García, N.C., Päckert, M., Fuchs, J., Ali, J.R., Derryberry, E.P., Carlson, M.L., Urriza, R.C., Brzeski, K.E., Prawiradilaga, D.M., Rayner, M.J., Miller, E.T., Bowie, R.C.K., Lafontaine, R., Scofield, R.P., Lou, Y., Somarathna, L., Lepage, D., Illif, M., Neuschulz, E.L., Templin, M., Dehling, D.M., Cooper, J.C., Pauwels, O.S.G., Analuddin, K., Fjeldså, J., Seddon, N., Sweet, P.R., DeClerck, F.A.J., Naka, L.N., Brawn, J.D., Aleixo, A., Böhning-Gaese, K., Rahbek, C., Fritz, S.A., Thomas, G.H. & Schleuning, M. (2022) AVONET: morphological, ecological and geographical data for all birds. *Ecology Letters*, **25**, 581–597.

Toussaint, A., Charpin, N., Beauchard, O., Grenouillet, G., Oberdorff, T., Tedesco, P.A., Brosse, S. & Villéger, S. (2018) Non-native species led to marked shifts in functional diversity of the world freshwater fish faunas. *Ecology Letters*, **21**, 1649–1659.

Toussaint, A., Charpin, N., Brosse, S. & Villéger, S. (2016) Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread. *Scientific Reports*, **6**, 22125.

Vagnon, C., Bazin, S., Cattanéo, F., Goulon, C., Guillard, J. & Frossard, V. (2022) The opportunistic trophic behaviour of the European catfish (*Silurus glanis*) in a recently colonised large peri-alpine lake. *Ecology of Freshwater Fish*, **31**, 650–661.

Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T. & Weidel, B.C. (2015) Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis. *PLOS ONE*, **10**, e0116182.

Vander Zanden, M.J. & Fetzer, W.W. (2007) Global patterns of aquatic food chain length. *Oikos*, **116**, 1378–1388.

Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in δ^{15} N and δ^{13} C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.

Villéger, S., Brosse, S., Mouchet, M., Mouillot, D. & Vanni, M.J. (2017) Functional ecology of fish: current approaches and future challenges. *Aquatic Sciences*, **79**, 783–801.

Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007) Let the concept of trait be functional! *Oikos*, **116**, 882–892.

Wainwright, P.C., Osenberg, C.W. & Mittelbach, G.G. (1991) Trophic polymorphism in the pumpkinseed sunfish (Lepomis gibbosus Linnaeus): Effects of environment on ontogeny. *Functional Ecology*, **5**, 40.

Wainwright, P.C. & Richard, B.A. (1995) Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes*, **44**, 97–113.

Wang, J., Tao, J., Heino, J., Huang, M. & Ding, C. (2025) Geodiversity as a surrogate to inform freshwater fish diversity conservation and management. *Journal of Applied Ecology*, 1365-2664.70057.

Watson, D.J. & Balon, E.K. (1984) Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *Journal of Fish Biology*, **25**, 371–384.

Webb, P.W. (1984) Body Form, Locomotion and Foraging in Aquatic Vertebrates. *American Zoologist*, **24**, 107–120.

Werner, E.E. & Hall, D.J. (1988) Ontogenetic habitat shifts in bluegill: The foraging rate-predation risk trade-off. *Ecology*, **69**, 1352–1366.

Westoby, M. (2025) Trait-based ecology, trait-free ecology, and in between. *New Phytologist*, **245**, 33–39.

Wikramanayake, E.D. (1990) Ecomorphology and Biogeography of a Tropical Stream Fish Assemblage: Evolution of Assemblage Structure. *Ecology*, **71**, 1756–1764.

Winemiller, K.O. (1991) Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecological Monographs*, **61**, 343–365.

Xu, M., Fang, M., Usoof, A.M., Mandrak, N.E., Chu, C., Qian, H., Cao, Y., Liu, C., Cuthbert, R.N., Li, S., Su, G., Tedesco, P.A., Dick, J.T.A., Wei, H., Yu, F., Shu, L., Wang, X., Mu, X. & Gu, D. (2025) Ecological similarity governs non-native fish establishment while human pressure and native diversity shape invasion richness. *Science Advances*, **11**, eadw4347.

Xu, M., Li, S., Dick, J.T.A., Gu, D., Fang, M., Yang, Y., Hu, Y. & Mu, X. (2022) Exotic fishes that are phylogenetically close but functionally distant to native fishes are more likely to establish. *Global Change Biology*, **28**, 5683–5694.

Zhao, T., Villéger, S., Lek, S. & Cucherousset, J. (2014) High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. *Ecology and Evolution*, **4**, 4649–4657.

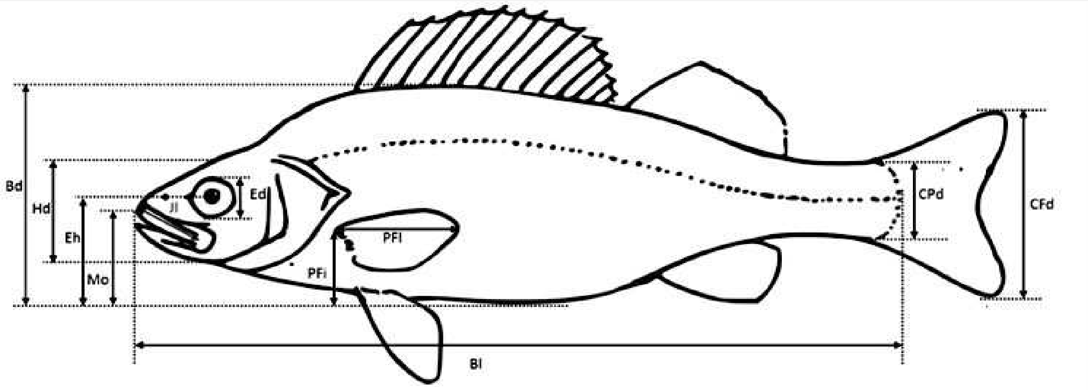
Box 1. Morphological (external) and anatomical (internal) traits of freshwater fishes seek to describe the relative size, shape and/or position of the body parts involved in each step of the food acquisition process (e.g., detection, capture and digestion). Prey detection typically occurs through visual stimuli, with eye size relative to head dimensions serving as a measure of visual acuity and light sensitivity. Fish with disproportionately large eyes are often nocturnal predators, relying on enhanced vision in low-light environments to detect prey. By contrast, species with small eyes may depend more on other senses like touch or smell for foraging, especially in murky water or sediment-filled environments (Caves *et al.*, 2017). Eye position also indicates feeding strategies, with top-eyed fish often being ambush predators or surface feeders, and fish with ventrally-located eyes having a general proclivity for benthic feeding (Hugueny & Pouilly, 1999).

Prey capture in fish is influenced by body size and shape, describing swimming ability and the biomechanical properties of the mouth and gills that allow ingestion through biting, suction or filtering. Strong swimming ability increases opportunities for individuals to expand their home range and compete for food (Winemiller, 1991). Larger-bodied species with highly streamlined bodies are typically fast swimmers (Webb, 1984; Blake, 2004) and are often predatory (Kopf *et al.*, 2021). By contrast, laterally compressed fish usually live in complex benthic environments where maneuverability is crucial for foraging among structures. Elongated species can be associated with burrowing or hiding in crevices and tend to be ambush predators (Gatz, 1979). Orientation of the oral gape influences prey capture modes, with ventral mouths suited for bottom feeding on benthic resources, terminal mouths for feeding from the water column, and dorsal mouths for surface feeding, which will mediate the importance of particular prey items associated with different habitats (López-Fernández *et al.*, 2012). Fishes with superior mouths and longer jaws tend to have higher trophic positions (Keppeler *et al.*, 2020; Kopf *et al.*, 2021). Dentition can also indicate a fish's diet and feeding strategies. For example, sharp, pointed teeth suggest a carnivorous diet, while flat, crushing teeth indicate feeding on hard-shelled prey or plant matter. Pharyngeal (throat) teeth also play a role in processing food, particularly in species that feed on shelled invertebrates and plant material. Additionally, species with long and thin gill rakers are generally associated with the consumption of plankton.

Prey digestion is facilitated by longer intestines that offer greater digestive processing time due to the lower nutrient content and greater assimilation of difficult to digest food resources such as plant tissues containing cellulose. As a result, an inverse relationship between intestine length and trophic level has been well documented, where longer lengths are commonly positively correlated with detritivory and herbivory (Ribble & Smith, 1983). Global databases like FISHMORPH are limited to external morphological traits that are measurable from 2-D photos or drawings of specimens and thus do not include gape size, dentition, gill rakers, or internal anatomical traits related to prey digestion.

Morphological differences among species are expected to be related to isotope patterns that represent different food-web positions, along both horizontal and vertical dimensions, of food webs. In lakes, for example, species with lower $\delta^{13}C$ values, typically associated with pelagic feeding (Fry, 2006), were hypothesized to have eyes and mouths positioned higher on the head (Table 1). Conversely, we expected species with higher $\delta^{13}C$ values, indicating benthic feeding, to have larger pectoral fins positioned higher in the body to enable maneuverability in structured environments (Table 1). For both lentic and lotic systems, we hypothesized that fishes with large gape lengths should have the highest $\delta^{15}N$ values, indicative of positions higher on the food chain (Kopf *et al.*, 2021). Species with longer bodies and greater caudal fin-to-peduncle ratios were also expected to have higher $\delta^{15}N$ values because these traits are associated with the pursuit of prey (Table 1).

Table 1. List of morphological traits used in the analysis and sourced from FISHMORPH (Brosse et al. 2021). Trait abbreviations follow FISHMORPH. All morphological traits are quantified as unitless ratios.

Trophic axis	Morphological trait	Measure	Ecological relevance
Prey detection	Eye size Ratio of eye diameter (Ed) to head depth (Hd) at the vertical of the eye	Ed/Hd	Visual acuity that influences the size of food items detected [1-3]
	Eye position Ratio of eye position (Eh) measured between center of the eye to the bottom to the body to maximum body depth (Bd)	Eh/Bd	Feeding position in the water column [4-6]
Prey capture	Body length Maximum adult standard body length	Bl	Swimming speed, duration, and endurance when pursuing prey [7,8]
	Body elongation Ratio of standard body length (Bl) to maximum body depth (Bd)	Bl/Bd	Hydrodynamics [9,10], turning ability [11], and hiding ability when pursuing or ambushing prey [9]
	Body lateral shape Ratio of head depth at the vertical of the eye (Hd) to maximum body depth (Bd)	Hd/Bd	Hydrodynamics [6,9] and turning ability [10] when foraging.
	Caudal peduncle throttling Ratio of maximum caudal fin depth (CFd) to minimum caudal peduncle depth (CPd)	CFd/CPd	Swimming speed, propulsion and endurance when pursuing prey [4,9]
	Pectoral fin size Ratio of pectoral fin length at longest ray (PFl) to standard body length (Bl)	PFl/Bl	Maneuverability when foraging in structured environments [6,11]
	Pectoral fin position Ratio of pectoral fin distance from upper insertion to bottom of body (PFl) to maximum body depth (Bd)	PFl/Bd	Swimming duration [4,6] and maneuverability when foraging in structured environments [9,11]
	Oral gape position Ratio of mouth height (Mo) to body depth (Bd)	Mo/Bd	Feeding position in the water column and method of food capture [3,10-12]
	Oral gape length Ratio of gape length from snout to corner of mouth (Jl) to head depth (Hd) at the vertical of the eye	Jl/Hd	Maximum prey size that can be captured [3,6,9-14]
Fish measurements 			

[1] Wikramanayake, 1990, [2] Piet, 1998, [3] Dumay *et al.*, 2004, [4] Mahon, 1984, [5] Watson & Balon, 1984, [6] Hugueny & Pouilly, 1999, [7] Webb, 1984, [8] Blake, 2004, [9] Gatz, 1979, [10] Sibbing & Nagelkerke, 2000, [11] Pouilly *et al.*, 2003, [12] Gerking, 1994, [13] Nikolski, 1933, [14] Kopf *et al.*, 2021

Table 2. Relationships between stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and individual species traits according to the Bayesian Multivariate Generalized Linear Models. For each isotope, the relative amount of variation explained by each trait, the slope of the isotope-trait relationship, and the percentage of variance explained are reported. A relationship was considered significant when the 95% credibility interval did not encompass zero. Site ID was included as a random effect in the analysis.

Morphological Trait	**$\delta^{13}C$**		**$\delta^{15}N$**	
	Relative importance	**Slope (lower, upper)**	**Relative importance**	**Slope (lower, upper)**
Eye size	0.001	-0.03 (-0.09, 0.03)	0.003	0.05 (0.01, 0.09)
Eye position	0.002	-0.08 (-0.16, 0.00)	0.024	0.15 (0.10, 0.20)
Body length	0.012	0.19 (0.12, 0.27)	0.062	0.24 (0.2, 0.29)
Body elongation	0.003	-0.09 (-0.17, 0.00)	0.013	-0.11 (-0.17, -0.06)
Body lateral shape	0.001	0.06 (-0.02, 0.13)	0.005	0.07 (0.02, 0.11)
Caudal peduncle throttling	0.000	-0.01 (-0.07, 0.05)	0.001	0.03 (-0.01, 0.07)
Pectoral fin size	0.001	0.05 (-0.03, 0.13)	0.015	-0.12 (-0.17, -0.06)
Pectoral fin position	0.004	0.11 (0.04, 0.18)	0.001	0.02 (-0.03, 0.06)
Oral gape position	0.001	0.06 (-0.03, 0.13)	0.001	-0.02 (-0.07, 0.02)
Oral gape length	0.002	0.07 (0.00, 0.14)	0.109	0.32 (0.28, 0.36)
Random effect (Site ID)	0.974		0.766	
Variation explained	53.1%		47.9%	

Table 3. Intraspecific relationships between species similarities according to stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and species traits based on analyses of multiple regression on distance matrices. The mean (and range) in the correlation and statistical significance across populations are reported. The following traits were available: black bullhead (all traits except pectoral fin size and position), pumpkinseed sunfish (all traits), largemouth bass (all traits), European minnow (10 traits), and European catfish (all traits except caudal peduncle throttling).

Species common name (*Scientific name*)	**# populations**	**r2**	**p**
black bullhead (*Ameiurus melas*)	9	0.018 (0.000,0.080)	0.519 (0.021,0.896)
pumpkinseed sunfish (*Lepomis gibbosus*)	6	0.060 (0.002,0.160)	0.195 (0.001,0.732)
largemouth bass (*Micropterus salmoides*)	1	0.100	0.001
European minnow (*Phoxinus phoxinus*)	15	0.013 (0.000,0.055)	0.453 (0.093,0.909)
European catfish (*Silurus glanis*)	1	0.002	0.648

Figure 1. Magnitude (R^2) and direction (coefficient) of the trophic-morphology relationships between species similarities according to stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and species traits based on multiple regression analyses on distance matrices.

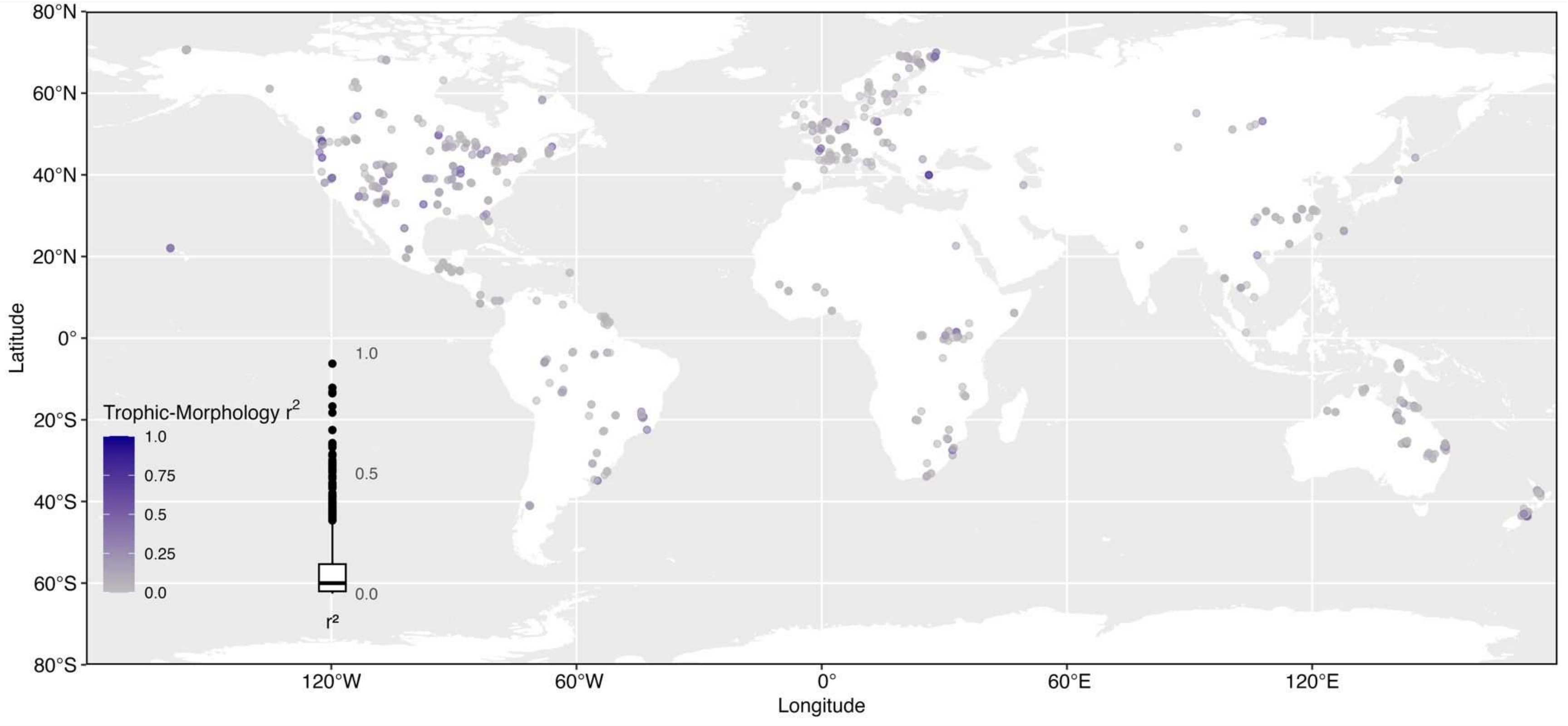


Figure 2. Magnitude (r^2) of the trophic-morphology relationships between species similarities according to stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) (trophic) and species morphological traits for lentic and lotic communities based on analyses of multiple regression on distance matrices. Boxplots report median (center line), interquartile range (box), 95% CIs (whiskers), and outliers (points).

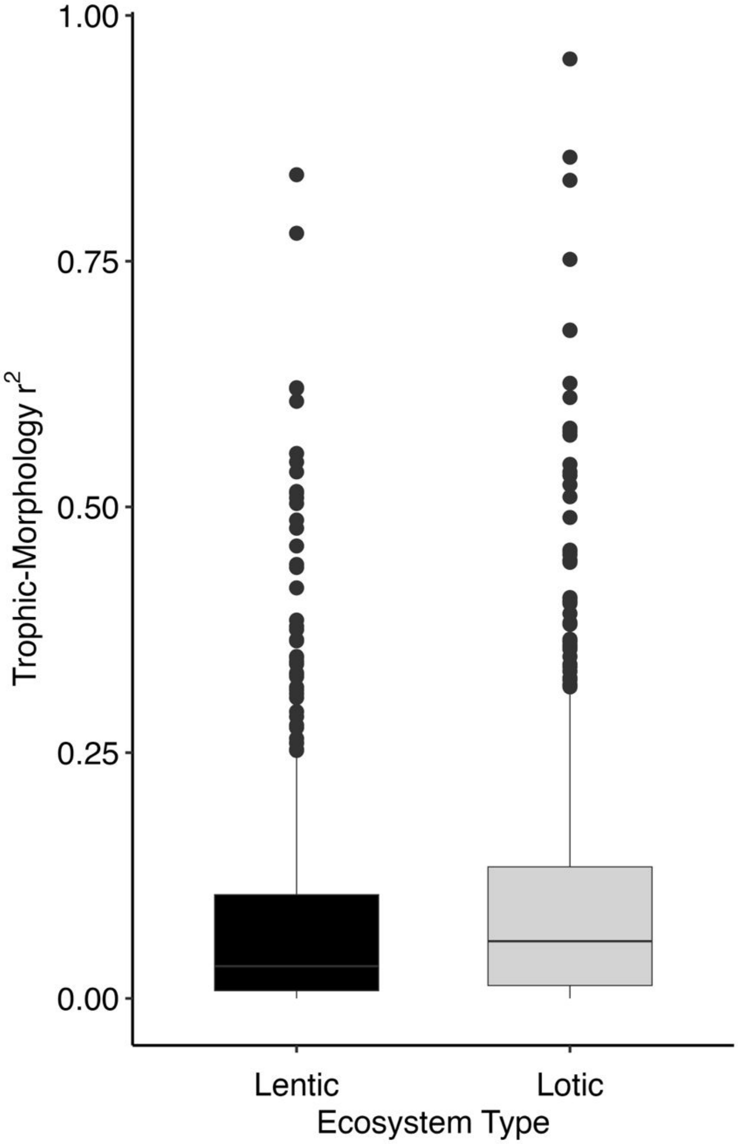


Figure 3. Species residuals of isotope-morphology associations from the multivariate generalized linear models, reported for (A) $\delta^{13}C$ and (B) $\delta^{15}N$. Mean absolute residuals for each species were calculated across all communities in which the species was present, where larger residuals represent weaker isotope-trait associations. Boxplots report median (center line), interquartile ranges (boxes), 95% CIs (whiskers), and outliers (points). Horizontal lines and p-values represent pairwise comparisons according to Tukey's HSD tests.

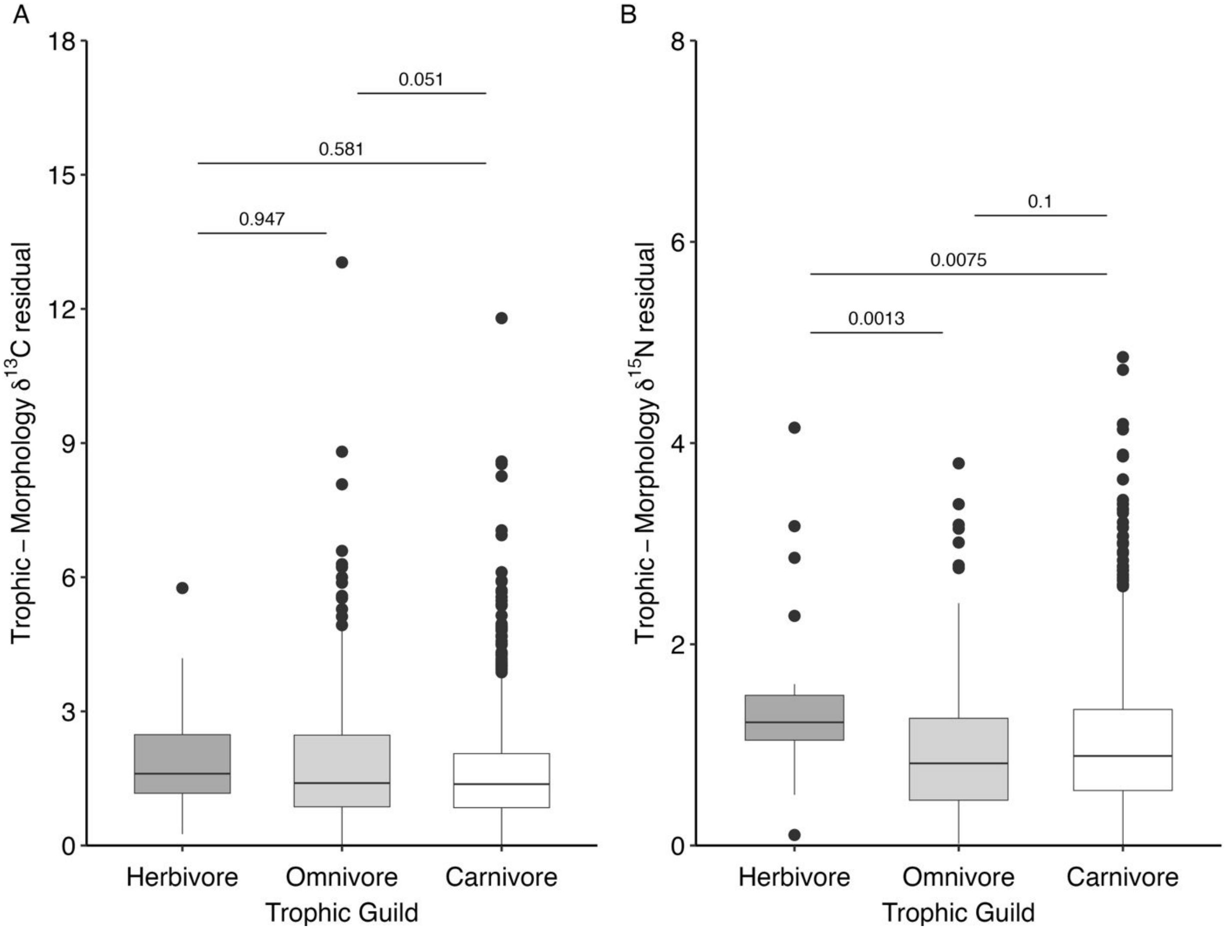
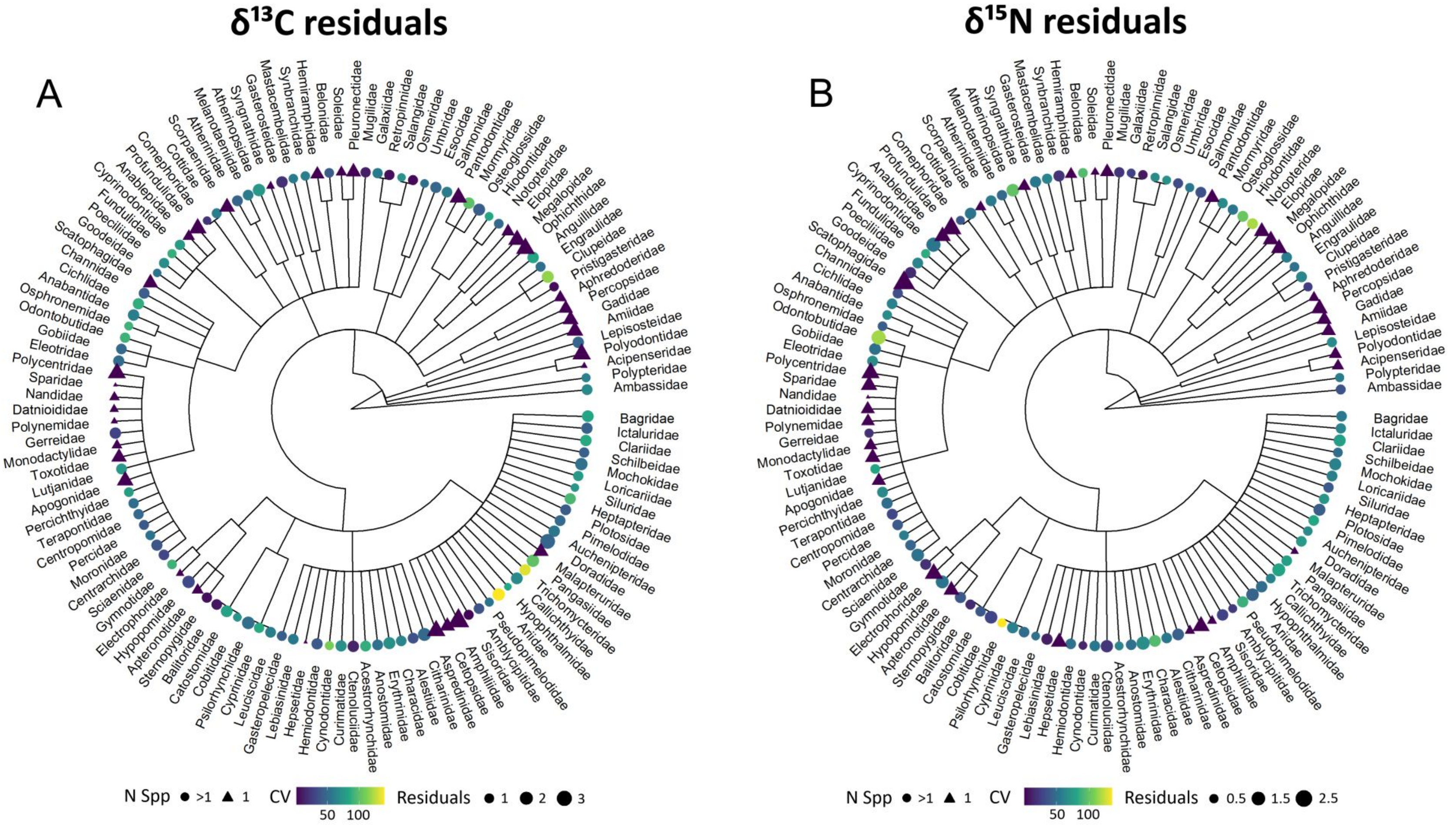


Figure 4. Species residuals of isotope-morphology associations from the multivariate generalized linear models, reported for families and displayed according to phylogeny. Reported are the family-wide mean (resid) and variability (CV) in absolute residuals across all species, where larger residuals (symbol size) represent weaker isotope-trait associations. N. Spp represents the number of species in each family.



Supplemental Materials

Table S1. Representative examples of studies testing for the association between freshwater fish morphology and diet. Traits refer to broad categories of traits that were examined (e.g., size, position, structure) according to the body shape (B), dentition (D), eye (E), fins (F), gill rakers (G), mouth (M) or intestinal tract (I). Studies using morphometric analysis based on measured landmarks were marked as B, E, F, and M. Studies that included body length as a separate variable in the analysis (i.e., not associated with the standardization of other traits) are noted (BL). Diet refers to whether fish trophic behavior was quantified using stomach contents or stable isotopes. Evidence refers to the reported morphology-diet correlation (and method), and "partial" is noted in all instances where the influence of taxonomy/phylogeny was accounted for. Statistical tests are indicated as Canonical Correspondence Analysis (CCA), Co-inertia analysis, Constrained Principal Analysis on Coordinates (CAP), G-test, Mantel test, Partial Least Squares (PLS), Redundancy Analysis (RDA), Procrustean superimposition approach (Procrustes)

Location	**N. sp**	**N. sites**	**Traits**	**Diet**	**Evidence**	**Reference**
Iguaçu River, Brazil	1	12	B, E, F, M	Stomach contents	r2 = 0.35 (p = 0.0012) (Mantel)	Baldasso et al. (2019)
Alto Jacuí River, Brazil	10	11	D, M	Stomach contents	% var = 15.1 (P < 0.05) (CAP)	Bonato et al. (2017)
New River, USA	1	15	B, E, F, M	Stomach contents	r2 = 0.02 (0.109) (Mantel)	Burress et al. (2016)
	1	15	B, E, F, M	Stable isotopes	r2 = 0.12 (p = 0.016) (Mantel)	Burress et al. (2016)
Floodplain lakes, Brazil	2	8	B, E, F, M	Stomach contents	r2 = 0.001 (p = 0.572) (partial-Mantel)	Cardoso et al. (2019)
Comte River, French Guiana	4	42	B, F, E, M, D, G, I	Stomach contents	% var = 0.90 (CCA)	de Mérona et al. (2008)
Upper Parana River, Brazil	1	6	M, I, D, G	Stomach contents	r2 = 0.25 (p < 0.05) (Mantel)	Delariva and Agostinho (2001)

Upper Uruguay River, Brazil	14	5	B, E, F, M, D, G, I	Stomach contents	rPLS = 0.382 (p < 0.05) (PLS)	Delariva and Neves (2020)
Roanoke River, USA	11	17	B, E, F, M	Stomach contents	r2 = 0.04 (p = 0.001) (partial-Mantel)	Douglas and Matthews (1992)
Magadi Lake, Kenya; Natron Lake, Tanzania	2	6	B, E, F, M	Stable isotopes	cor = 0.444 (p < 0.0001) - δ13C, cor = 0.199 (p = 0.2814) - δ15N (PLS)	Ford et al. (2016)
Kogon, Fatala, Konkour and Kolente rivers, Guinea (museum specimens)	4	18	B, E, M, I, (BL)	Stomach contents	r2 = 0.20 (p = 0.014) (partial-Mantel)	Hugueny and Pouilly (1999)
Rio Chipiriri, Brazil	27	30	B, E, M, I, (BL)	Stomach contents	r2 = 0.30 (p = 0.001) (RDA)	Ibañez et al. (2007)
Caño Maraca, Venezuela; Caño Agua Fría Viejo, Costa Rica	2	65	B, D, E, F, G, M, I, (BL)	Stomach contents	r2 = 0.11 (p = 0.001) (partial-Mantel)	Keppeler and Winemiller (2020)
	2	65	B, D, E, F, G, M, I, (BL)	Stable isotopes	r2 = 0.01 (p = 0.030) (partial-Mantel)	Keppeler and Winemiller (2020)
Neotropics (museum specimens)	NA	23	B, M, G, (BL)	Stomach contents	m12 = 0.801 (p = 0.03) (uncorrected matrices); m12 = 0.9572 (p = 0.94) (independent contrasts) (Procrustes)	López-Fernández et al. (2012)
Lake Titicaca, Peru and Bolivia	1	4	B, E, M	Stomach contents	RV = 0.14 (p < 0.001) (Co-inertia analysis)	Maldonado et al. 2009
Brazil	2	28	B, E, F, M	Stomach contents	r2 = 0.11 (p = 0.023), r2 = 0.42 (p = 0.002) (Mantel)	Manna et al. (2019)
Sinnamary River, French Guiana	6	66	B	Stomach contents	G-value = 46.131 (p < 0.001) (G-test)	Mérigoux and Ponton (1998)
Cinaruco River, Venezuela; Tambopata River, Peru; Neches and Brazos rivers, USA	4	37	B, M, E, I, G, (BL)	Stomach contents	r2 = 0.08 (p < 0.001) (Mantel), r2 = 0.02 (p < 0.001) (partial-Mantel)	Montaña and Winemiller (2013)
Iguaçu River, Brazil	1	7	B, F, M, E	Stomach contents	r2 = 0.22 (p = 0.02) (partial-Mantel)	Neves et al. (2015)
Cuiaba River, Brazil	6	4	B, M, G	Stomach contents	r2 = 0.18 (p = 0.20) (Mantel)	Novakowski (2016)

Grijalva-Usumacinta Basin, Mexico	1	8	B, E, F, G, M, I, (BL)	Stomach contents	% var = 0.808 ($p < 0.01$) (CCA)	Pease et al. (2018)
Tissawewa, Sri Lanka	1	10	B, E, M, I, (BL)	Stomach contents	% var = 0.77 ($p < 0.01$) (CCA)	Piet (1998)
Sundarbans, India	7	37	B, E, M, G	Stomach contents	% var = 0.899 (PLS)	Podder et al. (2021a)
Eastern Himalayan, India	23	45	B, F, E, M, G	Stomach contents	% var = 0.95 (PLS)	Podder et al. (2021b)
Mamoré River, Bolivia	8	48	B, D, E, G, M, I, (BL)	Stomach contents	r2 = 0.07 ($p = 0.001$) (Mantel)	Pouilly et al. (2003)
Upper Paraná River, Brazil	10	6	B, F, M, E	Stomach contents	r2 = 0.86 ($p = 0.002$) (Mantel)	Prado et al. (2016)
Sanaga River, Cameroon	15	9	B, E, F, M	Stable isotopes	Procrust. coordinates vs d13C (RV=0.144, $p < 0.001$), PC1 Morpho vs d13C (RV=0.118, $p < 0.001$), Procrust. coordinates vs d15N (RV=0.024, $p = 0.503$), PC1 Morpho vs d15N (RV<0.001, $p = 0.884$), (PLS)	Sommer et al. (2024)
Tzendales River, Mexico	1	14	B, E, M, G, D, I	Stomach contents	% var = 0.622 ($p = 0.001$) (CCA)	Soria-Barreto et al. (2019)
Vermelho River, Brazil	3	18	B, F, I, M, E	Stomach contents	% var = 0.783 ($p < 0.05$) (RDA)	Wolff et al. (2022)
Liangzi Lake, China	1	9	B, M, I, R, (BL)	Stomach contents	% var = 0.85 ($p < 0.05$) (CCA)	Xie et al. (2001)

References

Baldasso, M.C., Wolff, L.L., Neves, M.P. & Delariva, R.L. (2019) Ecomorphological variations and food supply drive trophic relationships in the fish fauna of a pristine neotropical stream. *Environmental Biology of Fishes*, **102**, 783–800.

Bonato, K.O., Burress, E.D. & Fialho, C.B. (2017) Dietary differentiation in relation to mouth and tooth morphology of a neotropical characid fish community. *Zoologischer Anzeiger*, **267**, 31–40.

Burress, E.D., Holcomb, J.M. & Armbruster, J.W. (2016) Ecological clustering within a diverse minnow assemblage according to morphological, dietary and isotopic data. *Freshwater Biology*, **61**, 328–339.

Cardoso, D.C., deHart, P., Freitas, C.E.D.C. & Siqueira-Souza, F.K. (2019) Diet and ecomorphology of predator fish species of the Amazonian floodplain lakes. *Biota Neotropica*, **19**, e20180678.

De Mérona, B., Hugueny, B., Tejerina-Garro, F.L. & Gautheret, E. (2008) Diet-morphology relationship in a fish assemblage from a medium-sized river of French Guiana: the effect of species taxonomic proximity. *Aquatic Living Resources*, **21**, 171–184.

Delariva, R.L. & Agostinho, A.A. (2001) Relationship between morphology and diets of six neotropical loricariids. *Journal of Fish Biology*, **58**, 832–847.

Delariva, R.L. & Neves, M.P. (2020) Morphological traits correlated with resource partitioning among small characin fish species coexisting in a Neotropical river. *Ecology of Freshwater Fish*, **29**, 640–653.

Douglas, M.E. & Matthews, W.J. (1992) Does Morphology Predict Ecology? Hypothesis Testing within a Freshwater Stream Fish Assemblage. *Oikos*, **65**, 213.

Ford, A.G.P., Rüber, L., Newton, J., Dasmahapatra, K.K., Balarin, J.D., Bruun, K. & Day, J.J. (2016) Niche divergence facilitated by fine-scale ecological partitioning in a recent cichlid fish adaptive radiation. *Evolution*, **70**, 2718–2735.

Hugueny, B. & Pouilly, M. (1999) Morphological correlates of diet in an assemblage of West African freshwater fishes. *Journal of Fish Biology*, **54**, 1310–1325.

Ibañez, C., Tedesco, P.A., Bigorne, R., Hugueny, B., Pouilly, M., Zepita, C., Zubieta, J. & Oberdorff, T. (2007) Dietary-morphological relationships in fish assemblages of small forested streams in the Bolivian Amazon. *Aquatic Living Resources*, **20**, 131–142.

Keppeler, F.W. & Winemiller, K.O. (2020) Can ancestry and morphology be used as surrogates for species niche relationships? *Ecology and Evolution*, **10**, 6562–6578.

López-Fernández, H., Winemiller, K.O., Montaña, C. & Honeycutt, R.L. (2012) Diet-Morphology Correlations in the Radiation of South American Geophagine Cichlids (Perciformes: Cichlidae: Cichlinae). *PLoS ONE*, **7**, e33997.

Maldonado, E., Hubert, N., Sagnes, P. & De MÉrona, B. (2009) Morphology–diet relationships in four killifishes (Teleostei, Cyprinodontidae, *Orestias*) from Lake Titicaca. *Journal of Fish Biology*, **74**, 502–520.

Manna, L.R., Villéger, S., Rezende, C.F. & Mazzoni, R. (2019) High intraspecific variability in morphology and diet in tropical stream fish communities. *Ecology of Freshwater Fish*, **28**, 41–52.

Merigoux, S. & Ponton, D. Body shape, diet and ontogenetic diet shifts in young fish of the Sinnamary River, French Guiana, South America.

Montaña, C.G. & Winemiller, K.O. (2013) Evolutionary convergence in Neotropical cichlids and Nearctic centrarchids: evidence from morphology, diet, and stable isotope analysis: Evolutionary Convergence in Perciform Fishes. *Biological Journal of the Linnean Society*, **109**, 146–164.

Neves, M.P., Delariva, R.L. & Wolff, L.L. (2015) Diet and ecomorphological relationships of an endemic, species-poor fish assemblage in a stream in the Iguaçu National Park. *Neotropical Ichthyology*, **13**, 245–254.

Novakowski, G.C., Cassemiro, F.A.S. & Hahn, N.S. (2016) Diet and ecomorphological relationships of four cichlid species from the Cuiabá River basin. *Neotropical Ichthyology*, **14**.

Pease, A.A., Mendoza-Carranza, M. & Winemiller, K.O. (2018) Feeding ecology and ecomorphology of cichlid assemblages in a large Mesoamerican river delta. *Environmental Biology of Fishes*, **101**, 867–879.

Piet, G.J. (1998) Ecomorphology of a size-structured tropical freshwater fish community. *Environmental Biology of Fishes*, **51**, 67–86.

Podder, A., Panja, S., Chaudhuri, A., Roy, A., Biswas, M. & Homechaudhuri, S. (2021a) Patterns of morphological traits shaping the feeding guilds in the intertidal mudflat fishes of the Indian Sundarbans. *Journal of Fish Biology*, **99**, 1010–1031.

Podder, A., Panja, S., Chaudhuri, A., Roy, A., Biswas, M. & Homechaudhuri, S. (2021b) Role of morphology in shaping the feeding niche of fishes inhabiting Eastern Himalayan torrential rivers of West Bengal, India. *Ecology of Freshwater Fish*, **30**, 444–465.

Pouilly, M., Lino, F., Bretenoux, J. -G. & Rosales, C. (2003) Dietary–morphological relationships in a fish assemblage of the Bolivian Amazonian floodplain. *Journal of Fish Biology*, **62**, 1137–1158.

Prado, A.V.R., Goulart, E., Pagotto, J.P.A. (2016) Ecomorphology and use of food resources: inter- and intraspecific relationships of fish fauna associated with macrophyte stands. *Neotropical Ichthyology*, **14**.

Sommer, G.M., Njom, S.D., Indermaur, A., Nyom, A.R.B., Jandová, K., Kukla, J., Petrtýl, M., Horká, P. & Musilova, Z. (2024) Trophic ecology of the African riverine elephant fishes (Mormyridae). *Ecology and Evolution*, **14**, e70173.

Soria-Barreto, M., Rodiles-Hernández, R. & Winemiller, K.O. (2019) Trophic ecomorphology of cichlid fishes of Selva Lacandona, Usumacinta, Mexico. *Environmental Biology of Fishes*, **102**, 985–996.

Wolff, L.L., De Oliveira, E.F. & Lobón-Cerviá, J. (2023) Linking fish ecomorphotypes to food categories and local-scale habitat uses along a Brazilian coastal stream. *Ecology of Freshwater Fish*, **32**, 195–208.

Xie, S., Cui, Y. & Li, Z. (2001) Dietary-morphological relationships of fishes in Liangzi Lake, China. *Journal of Fish Biology*, **58**, 1714–1729.

Table S2. Relationships between stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and species traits for lotic and lentic food webs according to the Bayesian Multivariate Generalized Linear Models. For each isotope, the relative amount of variation explained by each trait, the slope of the isotope-trait relationship, and the percentage of variance explained are reported. A relationship was considered significant when the 95% credibility interval did not encompass zero. Site ID was included as a random effect in the analysis.

Morphological Trait	**$\delta^{13}C$**		**$\delta^{15}N$**	
	Relative importance	**Slope (lower, upper)**	**Relative importance**	**Slope (lower, upper)**
Lotic food webs (n=396)				
Eye size	0.001	-0.03 (-0.13, 0.05)	0.002	0.04 (-0.02, 0.10)
Eye position	0.008	-0.17 (-0.27, -0.06)	0.01	0.10 (0.04, 0.17)
Body length	0.023	0.28 (0.19, 0.38)	0.045	0.22 (0.15, 0.28)
Body elongation	0.001	-0.01 (-0.12, 0.12)	0.016	-0.13 (-0.20, -0.05)
Body lateral shape	0.001	0.01 (-0.09, 0.12)	0.007	0.08 (0.01, 0.14)
Caudal peduncle throttling	0.005	0.13 (0.04, 0.22)	0.001	0.01 (-0.05, 0.06)
Pectoral fin size	0.001	0.02 (-0.08, 0.12)	0.011	-0.10 (-0.17, -0.04)
Pectoral fin position	0.001	0.02 (-0.07, 0.12)	0.001	0.03 (-0.03, 0.08)
Oral gape position	0.022	0.27 (0.16, 0.38)	0.003	-0.05 (-0.12, 0.02)
Oral gape length	0.006	0.13 (0.05, 0.22)	0.058	0.25 (0.20, 0.30)
Random effect (Site ID)	0.932		0.846	
Variation explained	64.6%		48.1%	
Lentic food webs (n=417)				
Eye size	0.004	-0.08 (-0.18, 0.03)	0.004	0.05 (-0.01, 0.11)
Eye position	0.004	0.09 (-0.04, 0.20)	0.027	0.16 (0.08, 0.24)
Body length	0.009	0.14 (0.02, 0.26)	0.049	0.22 (0.16, 0.28)
Body elongation	0.012	-0.17 (-0.30, -0.05)	0.006	-0.07 (-0.15, 0.01)
Body lateral shape	0.009	0.14 (0.03, 0.25)	0.002	0.03 (-0.04, 0.09)
Caudal peduncle throttling	0.005	-0.11 (-0.19, -0.02)	0.002	0.04 (-0.01, 0.10)
Pectoral fin size	0.002	0.03 (-0.10, 0.16)	0.011	-0.10 (-0.18, -0.02)
Pectoral fin position	0.010	0.16 (0.05, 0.25)	0.001	0.02 (-0.04, 0.08)
Oral gape position	0.011	-0.16 (-0.27, -0.04)	0.002	-0.02 (-0.09, 0.05)
Oral gape length	0.008	-0.12 (-0.22, -0.02)	0.203	0.45 (0.39, 0.51)
Random effect (Site ID)	0.924		0.693	
Variation explained	39.1%		48.7%	

Table S3. Relationships between baseline-corrected stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and species traits according to the Bayesian Multivariate Generalized Linear Models. For each isotope, the relative amount of variation explained by each trait, the slope of the isotope-trait relationship, and the percentage of variance explained is reported. A relationship is considered significant when the 95% credibility interval does not encompass zero. Site ID was included as a random effect in the analysis.

Morphological Trait	$\delta^{13}C$		$\delta^{15}N$	
	Relative importance	Slope (lower, upper)	Relative importance	Slope (lower, upper)
Eye size	0	-0.01 (-0.27, 0.26)	0.001	0.05 (-0.02, 0.12)
Eye position	0	-0.03 (-0.34, 0.26)	0.009	0.15 (0.07, 0.24)
Body length	0	0.31 (0.04, 0.59)	0.022	0.25 (0.17, 0.32)
Body elongation	0	0.06 (-0.27, 0.39)	0.009	-0.16 (-0.24, -0.07)
Body lateral shape	0	0.06 (-0.23, 0.34)	0.005	0.12 (0.04, 0.19)
Caudal peduncle throttling	0	-0.02 (-0.26, 0.21)	0.001	-0.03 (-0.10, 0.03)
Pectoral fin size	0	0.13 (-0.19, 0.44)	0.014	-0.20 (-0.28, -0.12)
Pectoral fin position	0	-0.03 (-0.30, 0.23)	0.001	-0.03 (-0.10, 0.04)
Oral gape position	0	0.20 (-0.10, 0.48)	0.001	-0.02 (-0.10, 0.05)
Oral gape length	0	-0.03 (-0.29, 0.23)	0.056	0.39 (0.33, 0.45)
Random effect (Site ID)	0.999		0.880	
Variation explained	87.4%		66.8%	

Figure S1. Principal Component Analysis of fish species according to the ten morphological traits (Table 1) labelled according to 8,342 species in FISHMORPH and 1,284 species examined in this study. Strong overlap in ordination space indicates that the fish species in this study are representative of the global freshwater fish diversity.

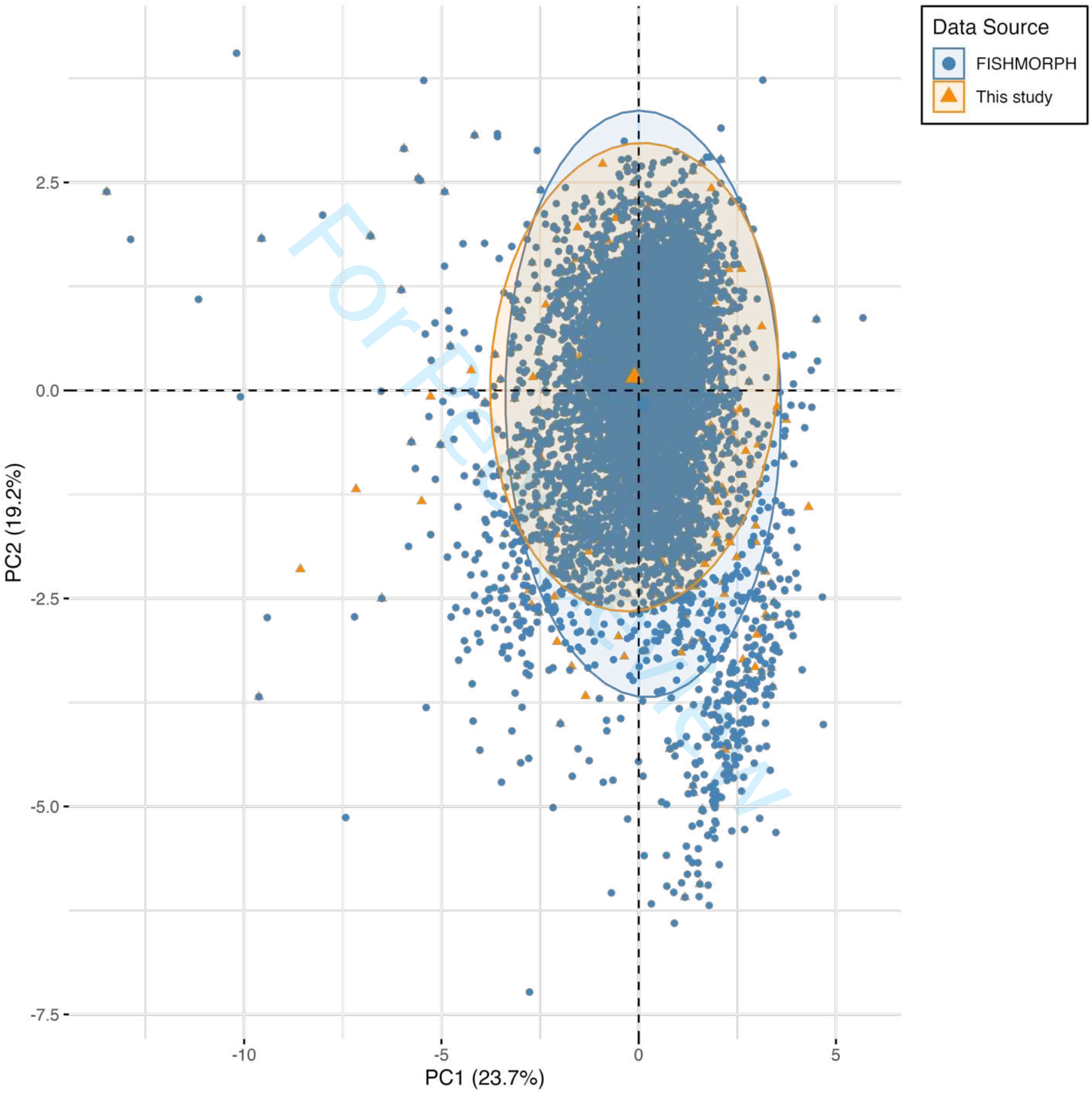


Figure S2. Pairwise associations between species' morphology traits and standardized (z-scores) δ¹³C values across study sites. Lines are fitted using local polynomial regression.

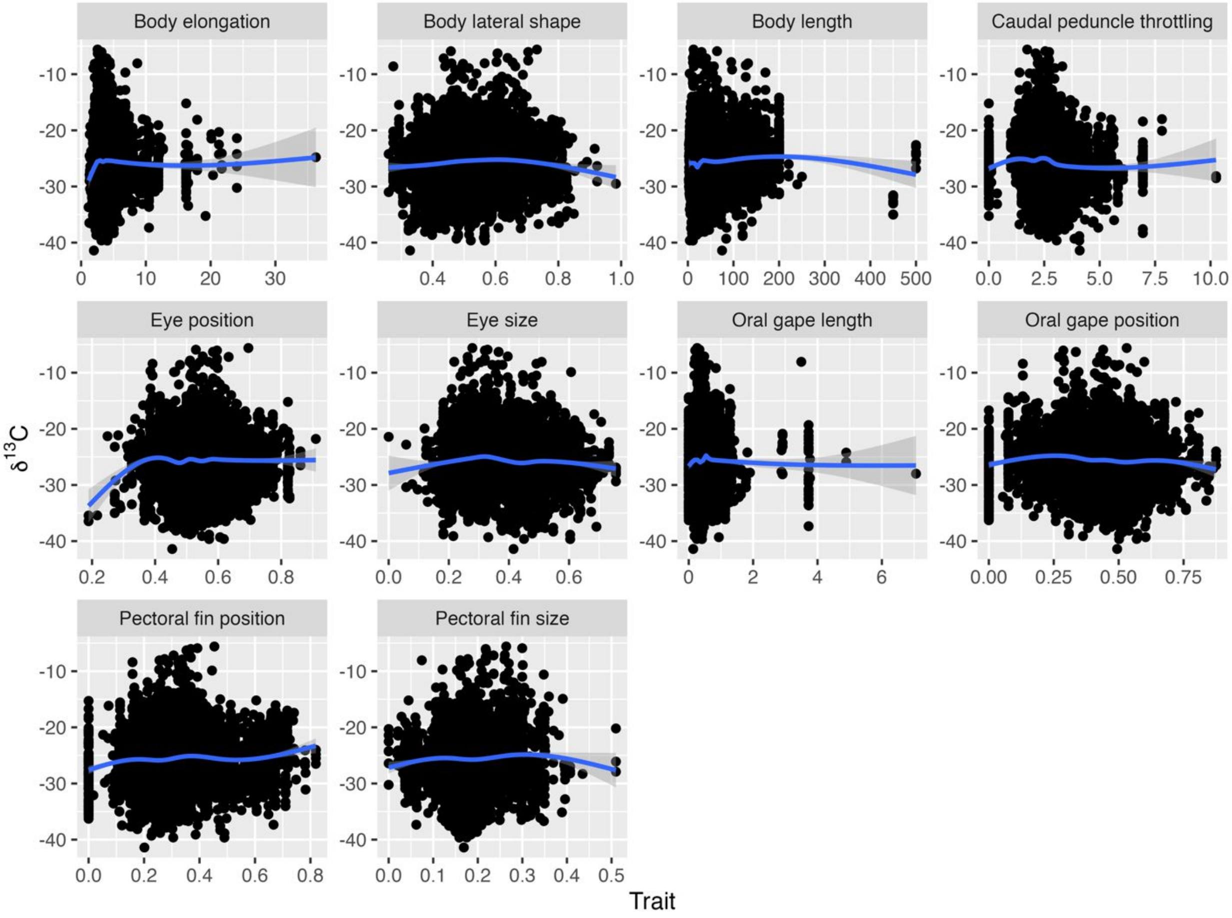


Figure S3. Pairwise associations between species' morphology traits and standardized (z-scores $\delta^{15}N$ values across study sites. Lines are fitted using local polynomial regression.