













RESEARCH ARTICLE

Looks Can Be Deceiving: Morphological Traits Are Weakly Associated With the Isotopic Niches of Freshwater Fishes

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Received: 13 June 2025 | **Revised:** 25 August 2025 | **Accepted:** 4 September 2025

Handling Editor: Fabien Leprieur

Funding: This research is a product of the Food-webs research group, funded by the Centre for the Synthesis and Analysis of Biodiversity (CESAB) of the Foundation for Research on Biodiversity (FRB). It is part of the FUNELAKES project (grant no. IRP 00020) from INEE CNRS (Centre National de la Recherche Scientifique). J.D.O. was supported by the Richard C. and Lois M. Worthington Endowed Professor in Fisheries Management from the School of Aquatic and Fishery Sciences, University of Washington. F.W.K. thanks the National Council for Scientific and Technological Development of Brazil (CNPq) for providing a postdoctoral scholarship (150773/2024-2).

Keywords: ecomorphology | functional traits | niche conservatism | stable isotopes | trophic niche

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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from 811 fish communities and ten species-level morphological traits with direct relevance for foraging behaviour (body length, body shape, and position of the mouth, eyes and fins) for 1282 species. Associations were assessed with Multiple Regression on Distance Matrices and Multivariate Generalised Linear Models (MGLM). Analyses were repeated at the individual level for five 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 $\delta^{13}\text{C}$ and 48% in $\delta^{15}\text{N}$, 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 $\delta^{15}\text{N}$, while no significant differences were observed for $\delta^{13}\text{C}$. At the individual level, morphology explained 0% to 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 recognise the limitations of assuming morphological traits are ‘functional’ in the sense that they can offer a reasonable proxy of a species’ trophic ecology at large scales.

1 | 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 behaviour of vertebrates worldwide (e.g., Brosse et al. 2021; Tobias et al. 2022; Osyrko 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 and 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 and Matthews 1992; Montaña and 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 and 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 size and 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, that is, many-to-many relationships between form and performance (Douglas and Matthews 1992; Burrell et al. 2016; Keppeler and 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 and 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 and McPhail 1996), and colonisation history (Leaver and Reimchen 2012). Given the strong context dependency of a

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 and 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 manoeuvrability 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 and 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 width, 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values, indicating benthic feeding, to have larger pectoral fins positioned higher in the body to enable manoeuvrability in structured environments (Table 1). For both lentic and lotic systems, we hypothesized that fishes with large gape lengths should have the highest $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values because these traits are associated with the pursuit of prey (Table 1).

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 behaviours 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Boulétreau et al. 2025). Briefly, $\delta^{13}\text{C}$ indicates the origin of the carbon in a consumer's diet, and $\delta^{15}\text{N}$ 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 and 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 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 rationalise the use of fish morphology as a faithful indicator of resource acquisition in broad-scale ecological investigations.

2 | Methods

2.1 | Species-Level Stable Isotope and Morphology Data

Trophic positions were estimated using species-level $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for 1282 fish species (123 families) from 811 fish communities in 65 countries across all continents except Antarctica. The data was curated from the global database—IsoFresh—a comprehensive database of species-level $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values within food webs was -25.58 (SD = 4.24) and the mean range of $\delta^{15}\text{N}$ 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 length, 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 1282 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 utilising a series of regression models based on the other variables in the dataset—from the R package *mice* (van Buuren and 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 8342 species in FISHMORPH and the species examined in this study (Figure S1).

2.2 | Individual-Level Stable Isotope and Morphology Data

We collated stable isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) 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.

2.3 | Stable Isotope Analyses

Species-level isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were standardised before subsequent analyses. To maximise sample size and account for spatial differences in baseline isotopic values, for each community, we standardised each fish species value (separately for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) by subtracting the minimum value across all fish species in the community. This standardisation process was also applied for the case studies, where we standardised each value (separately for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) by subtracting the minimum value across all individuals in each population. Our use of standardised 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 $\delta^{13}\text{C}$ relative to both periphyton and terrestrial detritus, and so pelagic consumers will have more negative $\delta^{13}\text{C}$ values relative to benthic consumers. In streams and rivers, autochthonous (in situ) production can have both higher and lower $\delta^{13}\text{C}$ values versus allochthonous (ex situ) resources, and increased water velocity also decreases the $\delta^{13}\text{C}$ values of autochthonous prey. $\delta^{15}\text{N}$ is reflective of the trophic position of the consumer within the food web because of predictable enrichment in $\delta^{15}\text{N}$ with each step in the food chain (Fry 2006). De novo lipid synthesis is known to drive $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ data for lipids because freshwater fish muscle tends to be low in lipids with only minimal expected influence on $\delta^{13}\text{C}$ values (Logan et al. 2008).

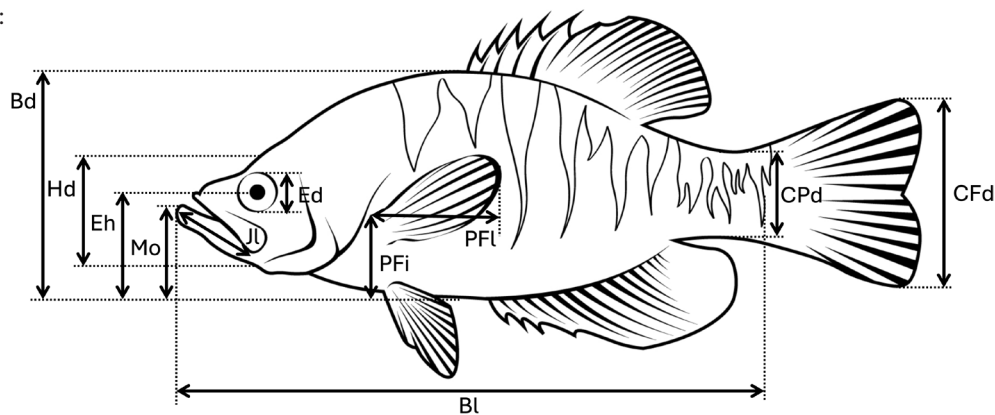
This study assessed isotope-morphology relationships within each community, not across communities, which further reduced the effects of varying isotopic baselines. However, the validity of this decision was evaluated by examining a reduced number of communities ($n = 330$) where the isotope values of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

$$\delta^{13}\text{C}_{\text{fish-corr}} = \frac{\delta^{13}\text{C}_{\text{fish}} - \text{mean}(\delta^{13}\text{C}_{\text{baseline}})}{\text{abs}(\text{range}(\delta^{13}\text{C}_{\text{baseline}}))} \quad (1)$$

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 ^{4,5,6}
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 (PFI) to standard body length (Bl)	PFI/Bl	Manoeuvrability when foraging in structured environments ^{6,11}
	Pectoral fin position: ratio of pectoral fin distance from upper insertion to bottom of body (PFI) to maximum body depth (Bd)	PFI/Bd	Swimming duration ^{4,6} and manoeuvrability 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 ^{3,6,9,10,11,12,13,14}

Fish measurements:



¹Wikramanayake (1990).

²Piet (1998).

³Dumay et al. (2004).

⁴Mahon (1984).

⁵Watson and Balon (1984).

⁶Hugueny and Pouilly (1999).

⁷Webb (1984).

⁸Blake (2004).

⁹Gatz (1979).

¹⁰Sibbing and Nagelkerke (2000).

¹¹Pouilly et al. (2003).

¹²Gerking (1994).

¹³Nikolski (1933).

¹⁴Kopf et al. (2021).

$$\delta^{15}\text{N}_{\text{fish-corr}} = \delta^{15}\text{N}_{\text{fish}} - \text{mean}(\delta^{15}\text{N}_{\text{baseline}}) \quad (2)$$

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 (Boulétreau et al. 2025).

2.4 | Statistical Analyses

We used two complementary approaches to quantify the strength of associations between the trophic ecology (inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 and Urban 2007), to test the relationship between the isotope similarity matrix (response) and the trait similarity matrix (predictor), both based on standardised 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 and 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 standardised 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 Generalised Linear Model (MGLM) implemented in the R package *Hmsc* (Tikhonov et al. 2020) to relate individual traits (predictors) to stable isotope

composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The model assumed a Gaussian distribution with non-informative priors following (Ovaskainen and 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 and Abrego (2020) to identify which variables were more relevant to explain $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 and Pauly 2025). 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 R package *phytools*, 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

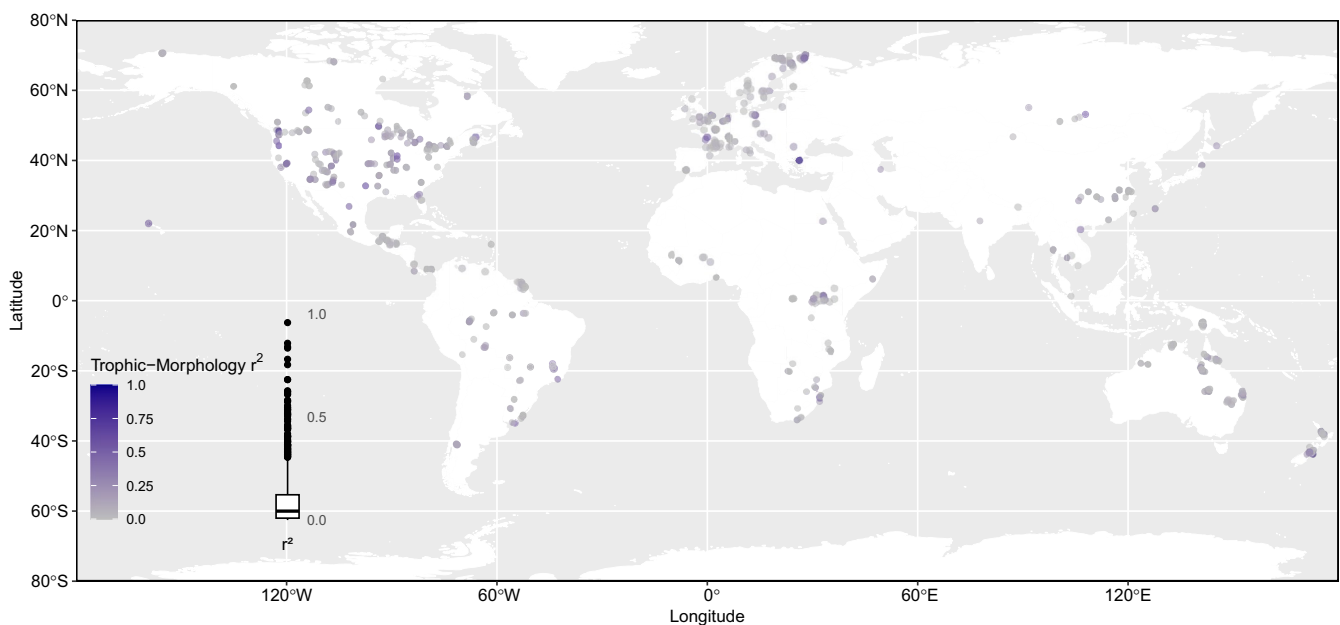


FIGURE 1 | Magnitude (r^2) and direction (coefficient) of the trophic-morphology relationships between species similarities according to stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and species traits based on multiple regression analyses on distance matrices.

Museum of Natural History, Smithsonian Institution 2025) using the R package *taxize* (Chamberlain and Szöcs 2013). All analyses were performed in R ver. 4.3.1 (R Development Core Team).

3 | 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 R^2 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 R^2 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 ($I_{\text{observed}} = 0.063$, $I_{\text{expected}} = -0.002$, 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 R^2 of 0.05 (SD = 0.31). Weak associations were also evident when using baseline-corrected stable isotope values, with an average R^2 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 $\delta^{13}\text{C}$ and 48% in $\delta^{15}\text{N}$ were explained by the MGLMs, 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 importance (95% credible intervals not encompassing zero) were revealed for eye size (positive: $\delta^{15}\text{N}$), eye position (positive: $\delta^{15}\text{N}$), body length (positive: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$), body elongation (negative: $\delta^{15}\text{N}$) and lateral shape (positive: $\delta^{15}\text{N}$), pectoral fin size (negative: $\delta^{15}\text{N}$), pectoral fin position (positive: $\delta^{13}\text{C}$), and oral gape length (positive: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) (Table 2). However, the relative importance of all these traits was exceedingly minor, except $\delta^{15}\text{N}$ —oral gape length and $\delta^{15}\text{N}$ —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 $\delta^{13}\text{C}$ (Table S2). No differences between ecosystems in the directional influence of traits were observed for $\delta^{15}\text{N}$. Relationships between baseline-corrected stable isotope values and species traits were similarly weak and demonstrated the same directional relationships reported above (Table S3).

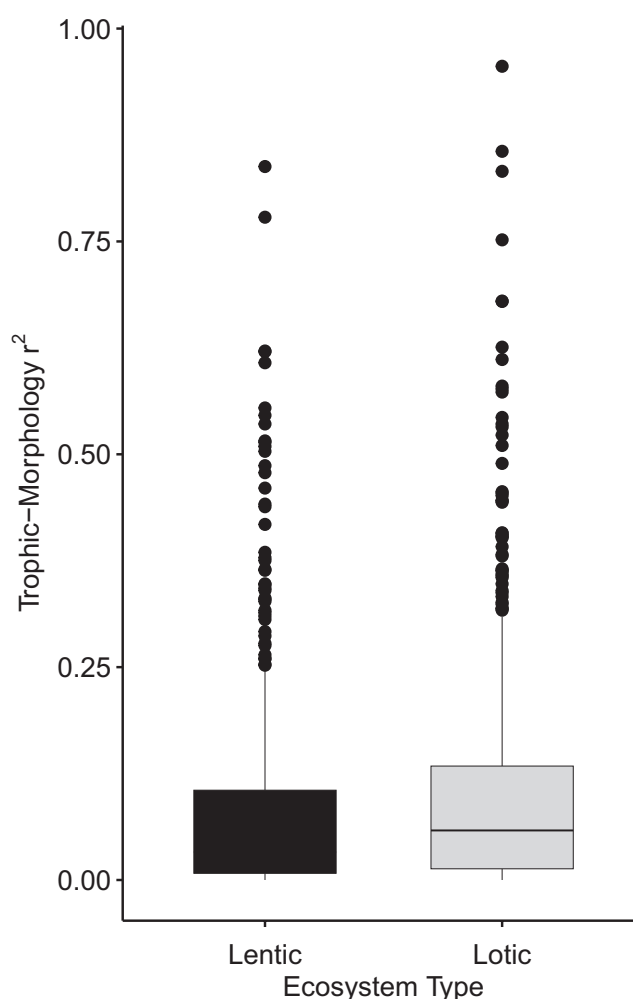


FIGURE 2 | Magnitude (r^2) of the trophic-morphology relationships between species similarities according to stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (trophic) and species morphological traits for lentic and lotic communities based on analyses of multiple regression on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

Variability in isotope-morphology correlations, inferred from the absolute MGLM residuals, differed according to species feeding guilds. Only slight differences were evident for $\delta^{13}\text{C}$ (ANOVA, $F_{2,1279} = 3.098$, $p = 0.046$, Figure 3A). By contrast, larger differences were apparent for $\delta^{15}\text{N}$ (ANOVA, $F_{2,1279} = 7.086$, $p < 0.001$), where herbivorous species demonstrated significantly higher $\delta^{15}\text{N}$ residuals than omnivores and carnivores according to Tukey's HSD tests (Figure 3B).

The phylogenetic signal of the MGLM residuals was significant for $\delta^{13}\text{C}$ ($K = 0.26$, $p = 0.018$) and $\delta^{15}\text{N}$ ($K = 0.25$, $p = 0.019$) at the species level (Figure 4). Species within families such as Lepisosteidae (gars), Salangidae (noodlefishes) and Comephoridae (golomyankas) exhibited lower morphology- $\delta^{15}\text{N}$ 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- $\delta^{13}\text{C}$ correlation than others, such as Cyprinodontidae and Alestiidae (African tetras).

TABLE 2 | Relationships between stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and individual species traits according to the Bayesian Multivariate Generalised 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. Site ID was included as a random effect in the analysis.

Morphological trait	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	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%	

The strength of isotope-morphology relationships evaluated at the intraspecific (individual) level largely mirrored the inter-specific (species) level findings. Individual differences in stable isotope ratios explained by variability in morphological traits (R^2) ranged from 0.00–0.16 across populations based on MRMs, 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.

4 | 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, 2018; Su et al. 2022, 2023; Xu et al. 2022; Chen et al. 2023; Bernery 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 size and 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 and 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 impose fundamental constraints on fish hunting ability and the size of prey that can be swallowed whole (Wainwright and Richard 1995; Blake 2004). We report that maximum body length and oral gape length were the only morphological traits that explained variation in $\delta^{15}\text{N}$ values; albeit just a total of 8% at the species level (17.1% of a total 47.9% explained by the MGLM). This finding provides some support for 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 size's 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

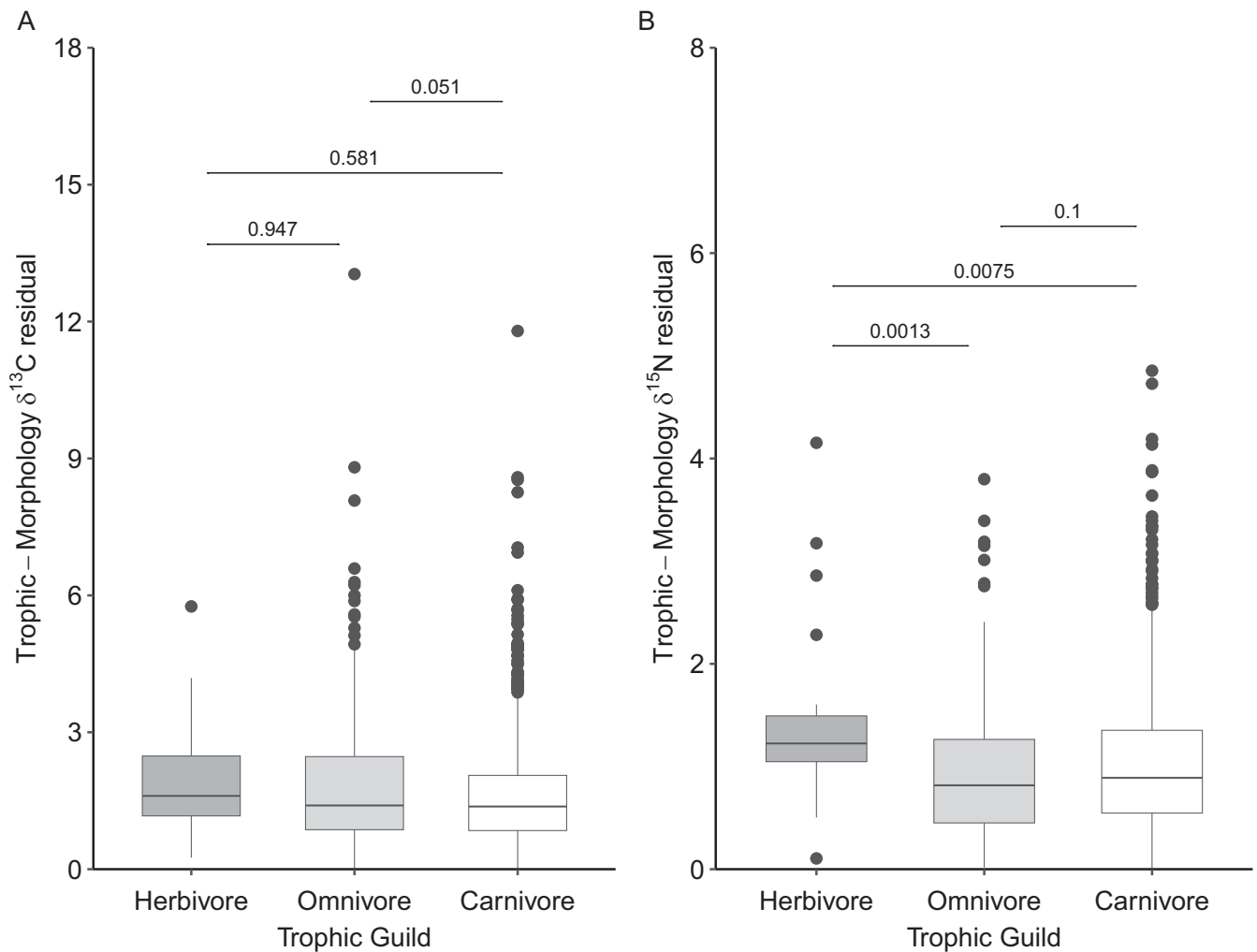


FIGURE 3 | Species residuals of isotope-morphology associations from the multivariate generalised linear models, reported for (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$. Mean absolute residuals for each species were calculated across all communities in which the species was present, where 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.

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 specialisation (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 (4% of the variation, on average). 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 and 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 $\delta^{15}\text{N}$ 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 and 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as opposed to the morphological traits. Trophic polymorphisms are

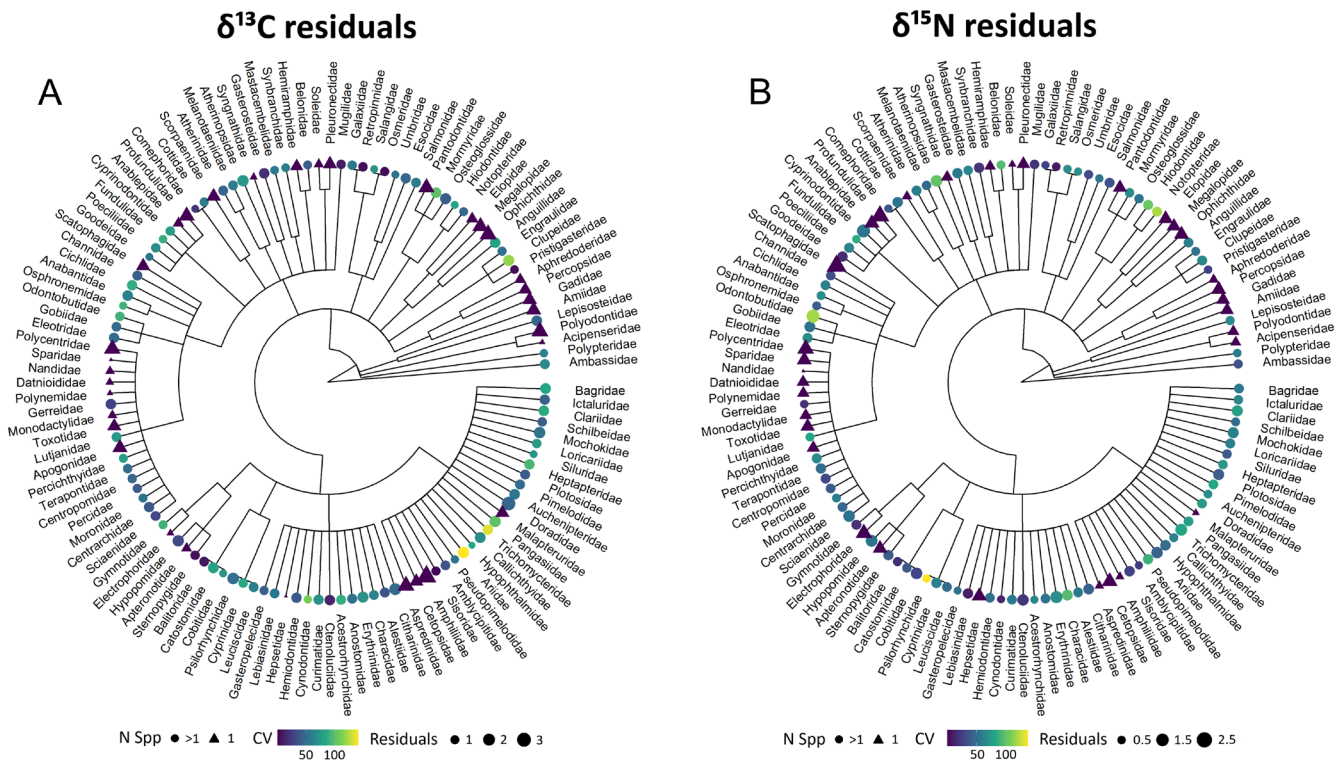


FIGURE 4 | Species residuals of isotope-morphology associations from the multivariate generalised linear models, reported for families and displayed according to phylogeny. Reported are the family-wide mean (resid) and variability (CV) in absolute residuals across all species, where larger residuals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

TABLE 3 | Intraspecific relationships between species similarities according to stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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.

Species common name (scientific name)	No. of populations	r^2	p
Black bullhead (<i>Ameiurus melas</i>)	9	0.018 (0.000, 0.080)	0.519 (0.021, 0.896)
Pumpkinseed sunfish (<i>Lepomis gibbosus</i>)	6	0.060 (0.002, 0.160)	0.195 (0.001, 0.732)
Largemouth bass (<i>Micropterus salmoides</i>)	1	0.100	0.001
European minnow (<i>Phoxinus phoxinus</i>)	15	0.013 (0.000, 0.055)	0.453 (0.093, 0.909)
European catfish (<i>Silurus glanis</i>)	1	0.002	0.648

not uncommon in freshwater fishes. For example, pumpkinseed sunfish have specialised pharyngeal structures and feeding behaviours that enable them to crush and eat snails, typically comprising 70% to 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 specialised 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 and Hall 1988). Predators can also cause morphological changes in fish prey as they increase their defence. 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 sizes 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 (Boulétreau 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 and 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 and 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 and 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, dietary trophic position estimates correspond closely with $\delta^{15}\text{N}$ estimates for freshwater fishes (Vander Zanden et al. 1997), and global investigations using stable isotope data in freshwater fishes have provided important information on food chain length patterns (Vander Zanden and 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 non-native species (Comte et al. 2017), to name just a few examples. Therefore, by quantifying the realised 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 hypothesised relationships between morphology and diet in fish (Box 1), we found that only body and gape length were positively associated with $\delta^{15}\text{N}$, albeit very weakly. 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 and 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 analyse the internal structure of fish, including anatomical measurements conducted at scale (Gignac et al. 2016). Combining morphological traits with complementary insights offered by recent global diet (Ridgway and 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.

5 | 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 and 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.

Acknowledgements

This research is a product of the Food-webs research group, funded by the Centre for the Synthesis and Analysis of Biodiversity (CESAB) of the Foundation for Research on Biodiversity (FRB). It is part of the FUNELAKES project (grant no. IRP 00020) from INEE CNRS (Centre National de la Recherche Scientifique). J.D.O. was supported by the Richard C. and Lois M. Worthington Endowed Professor in Fisheries Management from the School of Aquatic and Fishery Sciences, University of Washington. F.W.K. thanks the National Council for Scientific and Technological Development of Brazil (CNPq) for providing a postdoctoral scholarship (150773/2024-2). We thank David Hoeinghaus for early conversations, and Javier Sánchez-Hernández and Tyler Tunney for their contributions to Food-webs and comments on the manuscript. Two anonymous reviewers offered constructive feedback that improved the final paper.

Disclosure

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

IsoFresh (Boulêtreau et al. 2025)—a database of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** geb70124-sup-0001-DataS1.docx.