

The relationship between trophic level and body size in fishes depends on functional traits

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Abstract. Predators typically are larger than their prey, and consequently, trophic level should increase with body size. Whereas this relationship has helped in developing predictions about food web structure and dynamics in mesocosms and simple communities, a trophic-level–body-size relationship may not exist for all kinds of communities or taxa, especially those with many non-carnivorous species. Moreover, functional traits associated with trophic level generally have not been considered. Herein, we examine the correlation between trophic level and body size in fishes and how this relationship may vary in relation to functional traits (body dimensions, mouth size and orientation, tooth shape, gill rakers, and gut length) and trophic guilds (carnivorous vs. non-carnivorous). We analyzed data from morphological measurements and dietary analyses performed on thousands of specimens from freshwater and estuarine habitats across three zoogeographic regions (Nearctic, Neotropical, and Afrotropical). A positive relationship between trophic level and body size was only found for carnivorous fishes. No relationship was found when all species were analyzed together, rejecting the idea that trophic level is positively related with body size in fishes generally. This result was consistent even when using either body mass or standard length as the measure of body size, and trophic level for either species (average values) or individual specimens as the response variable. At the intraspecific level, trophic level varied consistently with size for one third of the species, among which only 40% had positive relationships. Body depth, tooth shape, and mouth width were all associated with the trophic-level–body-size relationship. Overall, predators with conical or triangular serrated teeth, large mouths, and elongated/and/or fusiform bodies tend to have positive trophic-level–body-size relationships, whereas primarily non-carnivorous species with unicuspid or multicuspid teeth, deep bodies and small to medium sized mouth gapes tended to have negative relationships. Given the diverse ecological strategies encompassed by fishes, trophic level and food web patterns and processes should not be inferred based solely on body size. Research that integrates multiple functional traits with trophic ecology will improve understanding and predictions about food web structure and dynamics.

Key words: body size; consumers; diet shift; food webs; functional traits; trophic level.

INTRODUCTION

Body size varies many orders of magnitudes in nature and has long been recognized as an important trait influencing not only fitness, but also species interactions and community dynamics (Elton 1927, Cohen et al. 1993). Animal body size affects many important aspects of physiology and ecological performance, including metabolism (Kleiber 1932), movement and home range (Reiss 1988), foraging and predation vulnerability (Peters 1983), fecundity (Roff 1992), and longevity (Speakman 2005). Body size distributions therefore can affect

population, community, and ecosystem dynamics (Brown et al. 2004, Caughlin et al. 2014, Ripple et al. 2017). Recent studies have suggested that overharvest (Pauly et al. 1998), deforestation (Ilha et al. 2018), global warming (Tseng et al. 2018), and other human impacts are reflected in population and community size structures. Given the many ways body size affects ecological processes, it should not be surprising that size relationships have been a major research focus in ecology (Cohen et al. 2003, Jonsson et al. 2005, Woodward et al. 2005, Petchey et al. 2008, McLaughlin et al. 2010, Gilljam et al. 2011).

A major body of research concludes that predators (here excluding parasites and parasitoids) are typically larger than their prey (Warren and Lawton 1987, Cohen et al. 1993, Jennings et al. 2002, Barnes et al. 2010, Nakazawa 2017). Small predators are limited to

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capturing and handling small prey, whereas large predators often feed on larger prey to meet their higher energetic demand (Werner and Hall 1974, Mittelbach 1981). Therefore, it is expected that trophic level is positively correlated with body size (Elton 1927). Evidence for a positive relationship between trophic level and body size in predators has been found for both aquatic (Jennings et al. 2007, Arim et al. 2010, Barnes et al. 2010, Gilljam et al. 2011, Riede et al. 2011, Reum and Marshall 2013) and terrestrial organisms (Riede et al. 2011), encompassing invertebrates and ectothermic and endothermic vertebrates. Furthermore, entire communities have been shown to be strongly size based (Jennings et al. 2002, Al-Habsi et al. 2008, Romero-Romero et al. 2016), with most studies to date conducted in the marine realm. At a global scale, fishes apparently show a positive relationship between trophic level and body size (Pauly et al. 1998, Romanuk et al. 2011). As a result, a growing number of food web models have been built under the assumption of a positive trophic-level–body-size relationship (McCann et al. 2005, Rooney et al. 2008, McCann 2011), and some have derived model parameters assuming certain size-based relationships among interacting species (Otto et al. 2007, Berlow et al. 2009, Schneider et al. 2016). These models have succeeded simulating the structure and dynamics of simple systems (Berlow et al. 2009, McCann 2011), but their predictive power seems to decrease as systems become more complex (Jonsson et al. 2018).

Despite numerous claims of a strong positive relationship between trophic level and body size, this pattern does not always hold. Potapov et al. (2019) found positive relationships between trophic level and body size in consumers of aquatic ecosystems, but not in terrestrial ones. Layman et al. (2005) found a flat relationship between trophic level and body size of carnivorous freshwater fishes from a tropical river. Similarly, no relationship was found between trophic level and body size in terrestrial and marine mammals (Tucker and Rogers 2014). The trophic-level–body-size relationship can even be negative, as exemplified in cyprinids, a diverse freshwater fish family (Burruss et al. 2016). Arim et al. (2007) hypothesized the existence of hump-shaped trophic-level–body-size relationships, such as the one found for animals from the coast of the southwestern Atlantic (Segura et al. 2015). This pattern arose, in part, because small organisms are restricted to feed at relatively high trophic levels due to morphological limitations (e.g., gape size), whereas adults are limited by the amount of energy available. The inconsistencies found in trophic-level–body-size relationships indicate that factors in addition to body size play significant roles in determining trophic level.

Body size is an important factor determining food web structure, but the predictive power of models has been shown to increase drastically with the inclusion of other functional traits (Eklöf et al. 2013, Brose et al. 2019). For instance, the largest animals on land (e.g.,

elephants, rhinos, giraffes, and hippos) and many large tropical freshwater fishes (e.g., frugivorous tambaqui, *Colossoma macropomum*, of the Amazon) are herbivores. These animals are usually not included in trophic-level–body-size analyses, often being dismissed as outliers. In these cases, characteristics of dentition and the gastrointestinal tracts are more indicative of trophic level than body size. Burruss et al. (2016) suggested that many herbivorous fishes evolved large body size to accommodate a long gastrointestinal tract required to process a cellulose-rich diet. Carnivores have protein-rich diets and tend to have relatively short gastrointestinal tracts, something that can be achieved at any body size (Wagner et al. 2009). Similar associations may be observed for other traits, such as tooth shape (e.g., in mammals, presence of canines and sharp molars in carnivores vs. absence of canines with flat molars in herbivores) and claws (e.g., raptorial talons in birds of prey vs. grasping claws in perching songbirds). Surprisingly, few studies have examined traits other than body size to predict trophic level and other features of food webs.

Several food web studies have emphasized how intraspecific variation in body size and trophic ecology affects dynamics of populations and communities (Werner and Gilliam 1984, Ingram et al. 2011, Klecka and Boukal 2013, Nakazawa et al. 2013, Rudolf et al. 2014). Ontogenetic shifts in trophic levels are common (Werner and Gilliam 1984). Anuran amphibians provide an extreme example, with most tadpoles feeding on algae and detritus, and most adult frogs and toads consuming terrestrial arthropods. Major ontogenetic dietary shifts are observed in many other vertebrates and invertebrates, including fishes, crocodylians, and spiders (Werner and Gilliam 1984, Nakazawa 2015, Sánchez-Hernández et al. 2019). Given that intraspecific variation is known to influence population dynamics and species interactions (Bruno and O'Connor 2005, Rudolf 2008), improved understanding of trophic-level–body-size relationship at the species level should facilitate development of more realistic food web models.

Here, we investigated the relationship between trophic level and body size at both interspecific and intraspecific levels by analyzing a large data set for freshwater and estuarine fishes. The relationship was assessed for both carnivorous and non-carnivorous fish guilds. We also explore how the trophic-level–body-size relationship at the species level varies according to seven morphological traits: body depth, body width, mouth width, mouth position, tooth shape, gill raker length and number, and gut length. Because these morphological attributes influence fish feeding performance (as well as fitness, indirectly, via effects on growth, survival, and reproduction; Villéger et al. 2017), we consider them to represent functional traits (Violle et al. 2007). Body depth and width influence maneuverability and swimming speed, and are strongly associated with habitat use (Keast and Webb 1966, Gatz 1979) and indirectly linked to foraging (Webb 1984a). Mouth gape limits the size of prey that can be

ingested whole (Nilsson and Brönmark 2000), and mouth position influences the efficiency of feeding at vertical positions within the water column (Helfman et al. 2009). Tooth shape affects food acquisition and processing (Winemiller 1991b), gill rakers affect processing and selection of particles within the orobranchial chamber (Helfman et al. 2009), and gut length affects digestion and nutrient absorption (Horn 1989, German and Horn 2006). We expected that trophic level is positively correlated with body size, especially for carnivorous fishes and that the strength and direction of the trophic-level–body-size relationship at the species level are mediated by other functional traits.

MATERIALS AND METHODS

Fish samples and trophic level estimation

We compiled new and previously published data for freshwater and estuarine fish diets based on examination of 30,341 specimens (excluding specimens with empty stomachs) and encompassing 367 species, 220 genera, 75 families, and 20 orders (Appendix S1). Dietary data originated from six field research projects conducted over the past 36 yr by the senior author and members of his lab in temperate and tropical freshwater systems, including two floodplain rivers (Tarim 2002, Robertson et al. 2008, Montaña and Winemiller 2013) and an estuary in Texas (USA) (Akin and Winemiller 2006), two coastal streams in Costa Rica (Winemiller 1990), four streams in Venezuela (Winemiller 1990, Peterson et al. 2017), and a floodplain river (Upper Zambezi River and Barotse Floodplain) in Zambia (Winemiller 1991a, Winemiller and Kelso-Winemiller 1994, 1996, 2003). In each of these systems, fishes were collected throughout one year using experimental gillnets, seines, cast nets, and dip nets, in order to acquire a good representation of the local fish assemblage and any seasonal variation in composition. Surveys were conducted during all seasons: Winter, Spring, Summer, and Fall seasons in temperate regions and Rainy-Flood and Dry-Low-water seasons in tropical regions. Thus, all samples contain broad ranges of species body sizes based on temporal patterns of reproduction, recruitment, and dispersal at the sites (Winemiller et al. 2014).

For all specimens, dietary analysis followed a protocol described by Winemiller (1990). Prey categories were assigned within trophic levels according to information reported in literature sources (Appendix S2). The trophic level of each fish specimen (herein referred as TL) was calculated using the formula proposed by Adams et al. (1983)

$$TL_i = 1.0 + \sum_{j=0}^n (T_j \times p_{ij}),$$

where T_j is the trophic level of a prey taxon j and p_{ij} is the fraction of prey taxon j ingested by predator i . The

mean trophic level (herein referred as MTL) of each fish species was calculated as the arithmetic mean of the weighted mean trophic levels (TL) of every analyzed specimen of a species, and based on MTL, we classified each fish species in one of two groups: carnivorous or non-carnivorous. Carnivorous species were those that included more animal than non-animal material in their diets, resulting in $MTL > 2.5$ ($N = 223$), whereas non-carnivorous species were omnivores, herbivores and detritivores that included minor fractions of animal material in their diets with $MTL < 2.5$ ($N = 52$).

Functional traits

Standard length was used as the main descriptor for body size. Standard length (mm) was measured for all specimens examined for dietary analysis. Given that standard length is an imperfect index for fish body mass because of diverse body shapes (ranging from anguilliform to gibbose to compressiform), we converted standard length to mass (g) using the allometric formula (Keys 1928)

$$W_i = aL_i^b$$

where W_i is the predicted weight of individual i , L_i is the length of individual i , and $\log(a)$ and b are the intercept and slope, respectively, of the logarithmic form of the length–weight relationship of individual i 's population. The values of the parameters a and b for each species were estimated by posterior modes (i.e., kernel density estimation) generated by the Bayesian hierarchical approach proposed by Froese et al. (2014). This Bayesian method generates posteriori distributions for parameters a and b for a target species using priors based on body shape classifications and length–mass data (i.e., a and b estimates from other studies) available for the species of interest and/or closely related species in FishBase (Froese and Pauly 2019). A full list of the priors and data used to predict a and b values, and the length–mass relationship predicted for each species used in this study are available in Appendix S3 and S4.

Morphological measurements, including maximum body depth (maximum distance from ventrum to dorsum; mm), maximum body width (maximum horizontal distance from side to side; mm), gut length (mm), tooth shape (absent, unicuspid, multicuspid, conical, triangular serrated), gill raker shape (absent, short/blunt/tooth-like, intermediate/long and sparse, long, and comb-like), mouth orientation (superior, terminal, subterminal, inferior) and mouth width (mm), were made on adult specimens (i.e., body length exceeding the minimum size of maturation reported for the species or most closely related species for which data are reported) based on the protocol described by Winemiller (1991b). For most species, we measured three individuals, although in a few cases, this number was higher or lower depending on availability of preserved specimens from the field studies

(Appendix S5). Three specimens per species were deemed sufficient for reliable mean values for body-size-standardized measures (Winemiller 1991b) and facilitated detection of outlier values caused by measurement error. In all cases, measurements were made on formalin-fixed and alcohol-preserved (70% EtOH) specimens, including specimens deposited in ichthyology collections at Texas A&M University (Biodiversity Research and Teaching Collections, BRTC) and The Texas University at Austin (Texas Natural History Collections, THNC). In few cases, specimens from the original field studies were unavailable, and we measured specimens collected from nearby locations.

Maximum body depth, maximum body width, gut length, and mouth width were converted to body proportions in order to create standardized measurements that are independent of body size. Following Winemiller (1991b), we used standard length as the denominator for ratios involving body depth, body width, and gut length, and maximum body width was used as the denominator for the ratio of mouth width. The measurements were then averaged for each species. Although intraspecific variation in body shape and other morphological traits caused by sexual dimorphism, ontogeny, and polymorphism are common (Bolnick et al. 2011), we only estimated species averages of morphological traits ratios based on adult specimens with the objective of analyzing how interspecific variation may influence MTL and the TL–body-size relationships. Standardization based on proportions can introduce allometric biases in morphometric analyses (Albrecht et al. 1993), however, this source of potential bias should have little influence for broad interspecific comparisons (Winemiller 1991b). Moreover, body size ratios have straightforward ecological and functional interpretations (Winemiller 1991b, Montaña and Winemiller 2013, Villéger et al. 2017) and have been widely used in functional ecology studies (Toussaint et al. 2018, Su et al. 2019). Standard length and body mass (both indicators of body size), and relative gut length for all species were log transformed prior analyses.

Data analysis

We used two approaches to test the relationship between trophic level and body size and its association with other functional traits. One approach analyzed trophic level and body size averages per species (using MTL, herein called *averaged data*) while the second approach analyzed data for individual specimens and encompassed within-species variation (using TL, herein called *raw data*). The analyses were conducted using a Bayesian framework that has advantages over traditional frequentist approaches, including a capability to generate exact confidence intervals for the parameters and to account for uncertainty at multiple levels of the model, independent of sample size (Kéry 2010, Reum and Marshall 2013). No evidence of nonlinearities

(Segura et al. 2015) was detected in our data; consequently, statistical analyses were based on linear models. The lack of independence among species due to shared ancestry was considered by adding phylogenetic components in the analyses (see details below). To do that, we used a hundred different phylogenetic hypotheses generated by a recent study that analyzed ~30,000 fish species (Rabosky et al. 2018). The backbone of these super trees is based on molecular data of ~15,000 fish species and time-calibrated with fossil records. Rabosky et al. (2018) placed the remaining unsampled species (i.e., those lacking molecular data) in the backbone tree using stochastic polytomy resolution in order to generate consistent taxonomic resolution through a conservative constant-rate birth–death process.

Averaged data.—We used average values per species to examine patterns of interspecific variation. Species with fewer than five specimens having gut contents were removed from our data set to reduce the potential for outliers to skew dietary data. The remaining species ($n = 275$) in the data set were analyzed using Bayesian phylogenetic linear mixed models in which MTL was the response variable and the arithmetic mean of body size (standard length or body mass) was the main factor. In addition to analysis of the full fish data set, the relationship of MTL with body size was examined separately for carnivorous and non-carnivorous fishes. Consistency of the MTL–body-size relationships was assessed using three models: (1) NULL model, which was run without any explanatory variable, (2) SUB model, which used the main factor alone (body size), and (3) FULL model, which used the main factor (body size) along with seven co-variables (body depth, body width, mouth orientation, mouth width, tooth shape, gill raker shape, gut length). To account for non-independence, species phylogeny was included in the models as a random term (forming what is called an animal model; Hadfield 2010). To account for uncertainty from shared ancestry (i.e., multiple phylogenetic hypotheses), we performed analyses on all phylogenetic trees published by Rabosky et al. (2018) ($N = 100$) and calculated a combined posterior distribution of the model parameters using the R package *mulTree* (Guillerme and Healy 2018). We used non-informative priors (variance = 0.5, belief parameter = 0.002) for both fixed and random effects (Hadfield 2010) and conducted three chains for 240,000 interactions with a thinning value of 100 and burning of 40,000. The strength of the phylogenetic component to explain the trait variance was accessed using Lynch's phylogenetic heritability index (H^2 ; Lynch 1991), which is comparable to Pagel's lambda (Pagel 1999) and varies from 0, when the trait is evolving independently of the phylogeny, to 1, when the trait is evolving according to Brownian motion (Hansen and Orzack 2005). Convergence of the model chains was verified using the Gelman-Rubin statistic (Gelman and Rubin 1992). Potential scale reduction values were lower than 1.1, and

autocorrelations of posterior probabilities were lower than 0.1. The effective sample sizes of the models were all greater than 1,000. Q-Q plots of the posterior density of parameter estimates indicated that Gaussian was an adequate probability distribution for the response variable. Multicollinearity was tested prior to Bayesian analyses using the variance inflation factor (VIF), but no evidence of autocorrelation among the traits was found ($VIF < 2$). We considered a variable significant when the 95% credible intervals did not encompass zero. We also compared the NULL, SUB, and FULL models using the deviance information criterion (DIC), which is a hierarchical generalization of Akaike's information criterion (AIC) for Bayesian models (Spiegelhalter et al. 2002). Marginal R^2 and conditional R^2 , which represent the variance explained by the fixed factors and by both fixed and random factors, respectively, were calculated according to Nakagawa and Schielzeth (2013).

Raw data.—The second approach used data for individual specimens to test for intraspecific variation in TL–body-size relationships and employed two steps. First, we conducted Bayesian semi-parametric generalized mixed models on a Dirichlet Process Mixtures (DPM, family = Gaussian, nit = 240,000, thin = 100, burning = 40,000, chains = 3) using non-informative priors ($\alpha = 1$, $\tau_{11} = 0.01$, $\tau_{22} = 0.01$, $\nu_0 = 4.01$, $\tau_{inv} = 10$, $\nu_{ub} = 4.01$, $\tau_{binv} = 10$, $m_b = 0$, $S_b = 1,000$; see Jara et al. 2011 for more details), with TL used as the response variable, body size (standard length or body mass) as the main factor, and fish species as a random variable (random slope and intercept). For this model, we used data from all dissected specimens for those species for which at least 30 specimens contained food items in the gut, which reduced the number of observations to 28,710 and the number of species to 179. The threshold of 30 specimens was necessary to allow the mixed models to estimate the relationship between body size and TL for individual species. In contrast to other studies (Arim et al. 2010), we did not split body size into size categories. The use of size categories is usually justified on the basis of reducing uncertainty in TL estimation, but exploratory analyses indicated that splitting body size into size categories did not consistently reduce uncertainty nor changed the parameter estimates (see Appendix S6). We used a semi-parametric mixed model because of its modeling flexibility and robustness to deviance from parametric assumptions, including multivariate normal distribution of random effects (Escobar and West 1995, Jara et al. 2011, Müller et al. 2018).

Second, we extracted the random slopes generated for species from the Bayesian semi-parametric generalized mixed model in step 1. These slopes describe the relationship between body size (standard length or body mass) and TL for each species and allowed us to explore how this relationship may vary according to species functional traits. Thus, we conducted another series of Bayesian phylogenetic linear mixed models (nit = 240,000,

thin = 100, burning = 40,000, chains = 3) using: (1) the random slopes as a response variable, (2) mean body size (standard length or body mass), body depth, body width, mouth orientation, mouth width, tooth shape, gill raker shape, and gut length as main factors, (3) phylogenetic trees ($N = 100$) as random components, and (4) non-informative priors (variance = 0.5 and belief parameter = 0.002). Similar to the first approach, the posterior distribution of each variable parameter generated here accounts for the uncertainty associated with species shared ancestry. We compared the FULL model (i.e., with all explanatory variables) with the NULL model (i.e., a model without any explanatory variables) using the DIC to test the importance of these functional traits in explaining TL–body-size relationships. All diagnostic techniques applied to approach 1 were repeated for approach 2. Furthermore, generalized linear models (GLM) based on the binomial distribution were performed to explore whether species with consistent relationships between TL and body size (i.e., species with a random slope CI not encompassing zero) were those that had larger sample sizes and wider body size ranges. Body size range was estimated by dividing the body size range observed in our samples by the total body size range, which is the distance between minimum and maximum body size of a given species. Maximum body size of each species was obtained from FishBase (Froese and Pauly 2019), and minimum body size was set to 1 mm for standard length and 0.001 g for body mass; although arbitrary, these minimum values were necessary because newly hatched or live born fishes are always larger than 0 mm and heavier than 0 g. Given the lack of maximum body size information reported as standard length for many species, the effect of body size range on the consistency of the TL–body-size relationship was tested using only 87 species.

A Bayesian phylogenetic linear mixed models with non-informative priors was also performed to explore the possibility that the TL–body-size relationship becomes positive at positions higher in the food web. The random slopes generated by the Bayesian semi-parametric generalized mixed models served as the response variable, MTL was used as the main factor, and the phylogenetic trees constructed by Rabosky et al. (2018) were used as the random components. We did not introduce co-variables for this analysis (e.g., relative gut length) because of high levels of multicollinearity ($VIF > 10$). These models (herein named SUB-MTL models) also were compared with the FULL and NULL models using the DIC.

Hypothesis 1 (trophic level is positively correlated with body size) was rejected if the relationship between trophic level and body size was not consistently positive (credible intervals of slopes encompassing 0) according to approach 1 (species averages data) and the first step of approach 2 (individual specimen data). Hypothesis 2 (strength of the TL–body size relationship is mediated by functional traits) was supported if any of the seven

functional traits affected the slope of the TL–body-size relationship, and this was assessed based on results from step 2 of approach 2.

Bayesian phylogenetic linear mixed models were performed in R (R Core Team 2019) using the packages MCMCglmm (Hadfield 2010). Bayesian semi-parametric generalized mixed models were performed in DPPackage (Jara et al. 2011), and model comparisons and VIF were done using the packages MuMIn (Barton 2019) and fmsb (Nakazawa 2018), respectively. Length–mass data were obtained from the FishBase database using the R package rfishbase (Boettiger et al. 2012).

RESULTS

Averaged data

Mean standard length was not a consistent predictor of MTL when all fishes were analyzed together (FULL, slope of the MTL–standard-length relationship = 0.06 [lower CI = -0.01 , upper CI = 0.13]; SUB, slope of the MTL–standard-length relationship = 0.04 [lower CI = -0.05 , upper CI = 0.13]; Figs. 1a, 2a). Models with only standard length (SUB model) reached a maximum conditional R^2 lower than 1% and had lower performance on average when compared to models with other functional traits (FULL model) and without any explanatory variable (NULL model; Table 1). Both large and small fishes fed at low and high trophic levels, but at intermediate MTL (~ 2.5) there was a tendency for fishes to have small body sizes (Fig. 1a, Appendix S7). The fixed variables in the FULL model explained 35% of MTL variation (Table 1). Body depth and gut length were negatively correlated with MTL (Fig. 2a, e respectively), whereas mouth width had a positive association (Fig. 2c, Table 2). MTL varied according to tooth shape, whereby species without teeth had the lowest MTL, fishes with unicuspid and multicuspid teeth had intermediate values, fishes with conical teeth had high values, and final species with triangular serrated teeth had highest MTL (Fig. 3a, d). MTL also varied among fishes with different mouth orientations. Fishes with superior mouths tend to have higher MTL compared to fishes with inferior, subterminal and terminal mouths (Fig. 3c, e). MTL was not associated with the shape of gill rakers (Table 2, Appendix S7). Similarly, no correlations were observed between relative maximum width and MTL (Table 2, Appendix S7). In all models (FULL, SUB, and NULL), the H^2 and the conditional R^2 were high ($H^2 = 0.96\text{--}0.98$, conditional $R^2 = 0.77\text{--}0.84$; Table 1), indicating that MTL has a strong phylogenetic component, independent of the phylogenetic tree used.

These results did not appear to change when mean standard length was replaced with mean body mass (Table 2, Fig. 1b). Despite having slightly higher DIC than the NULL model, mean body mass alone was not strongly associated with MTL (FULL, slope of the MTL–body-mass relationship = 0.02 [lower CI = 0.00,

upper CI = 0.05]; SUB, slope of the MTL–body-mass relationship = 0.01 [lower CI = -0.02 , upper CI = 0.04]; Figs. 1b, 2b), and mean body mass explained less than 1% of the variation in MTL (Table 1). The addition of other functional traits in the model (FULL) improved the model predicting variation in MTL (Table 1; Appendix S7). Again, body depth, mouth orientation, mouth width, tooth shape, and gut length were consistent predictors of MTL, and their effects were similar to the models based on standard length as the measure of body size (Appendix S7). H^2 and the conditional R^2 values were high in all models ($H^2 = 0.96$ to 0.98 , conditional $R = 0.77\text{--}0.84$; Table 1), indicating a role for phylogenetic constraint and/or niche conservatism in explaining interspecific variation in MTL.

Both mean standard length and mean body mass were positively associated with MTL only when carnivorous fishes were analyzed separately (Table 2; Appendix S8). The FULL models were still the most relevant as gut length, tooth shape, and mouth gape influenced MTL (Tables 1, 2; Appendix S8). The SUB models had lower DIC values than NULL models, which indicate that even being a consistent predictor, body size is redundant given its strong phylogenetic component. On the other hand, mean body size (either standard length or body mass) was not a good predictor of MTL when only non-carnivorous fishes were analyzed (Table 2). The only functional trait that significantly influenced MTL was gut length (Table 1, 2; Appendix S8). Overall, the division of MTL into carnivorous and non-carnivorous groups reduced the R^2 of the models (Table 1). H^2 values were also reduced, but remained high, especially when compared to models of the TL–body-size relationship (Table 1; see Raw data section).

Raw data

The Bayesian semi-parametric generalized mixed model using TL for individual specimens rather than species MTL indicated that standard length alone was not a good predictor of TL (slope of the TL–standard-length relationship = -0.04 [lower CI = -0.13 , upper CI = 0.04]). However, TL varied consistently with standard length for 56 out of 179 species (31%) as indicated by the values of the random slopes of the model (Fig. 4, Appendix S9). From those species with consistent relationships, 34 (61%) had negative relationship between standard length and TL, whereas 22 (39%) had positive relationships. The correlation between individual fish body mass and TL also was weak and inconsistent (slope of the TL–body-mass relationship = -0.02 [lower CI = -0.08 , upper CI = 0.04]). Random slope estimates generated by the model using body mass were convergent with the estimates generated using standard length (Fig. 4). In eight cases (4.5%), the models did not converge regarding the direction of the relationship (negative vs. positive, or vice versa; Fig. 4). However, the credible intervals of the slopes encompassed zero in all eight cases, indicating that the TL–body-size (either

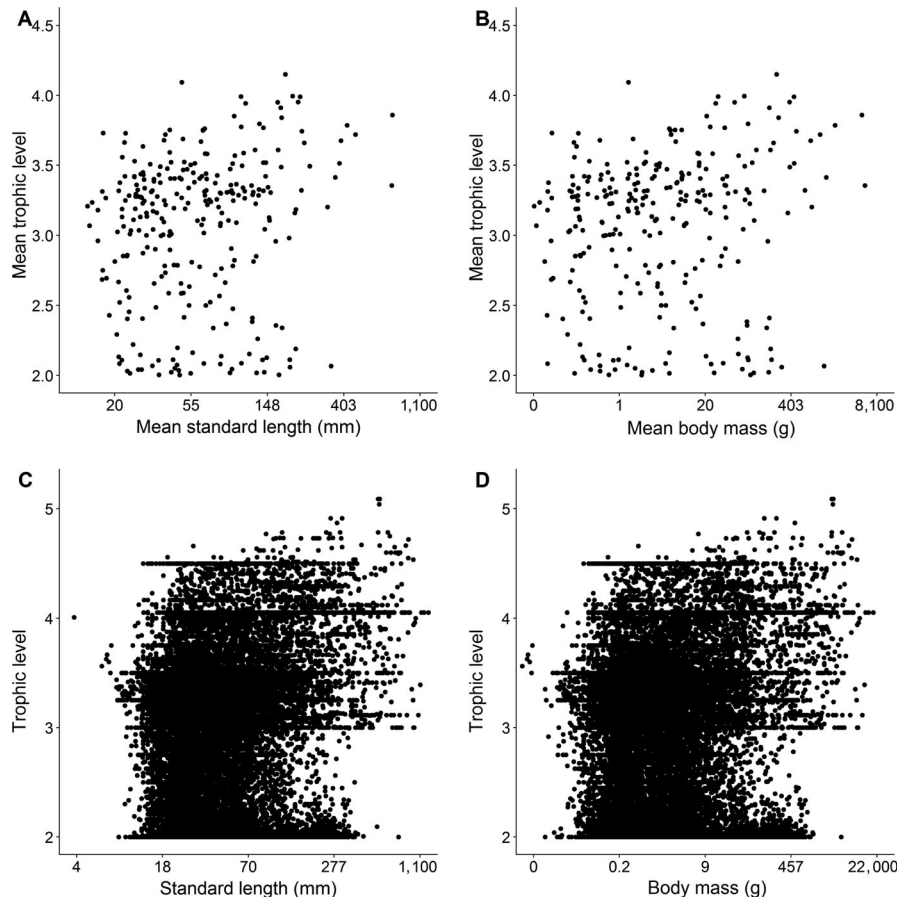


FIG. 1. Relationship between body size and trophic level (TL or MTL) for freshwater and estuarine fishes from Texas (USA), Costa Rica, Venezuela, and Zambia: (A) mean trophic level (MTL) and mean standard length and (B) MTL and mean body mass are presented for fish species with more than five specimens dissected for dietary analysis. Data for individual trophic level (TL) and (C) standard length and TL and (D) body mass are shown for species with at least 30 specimens. Parameter estimation and credible intervals for each one of these relationships can be found in Tables 2 and 3.

measured as standard length or body mass) relationship was not consistent. The number of species with consistent relationships between body size (standard length or body mass) and TL could have been underestimated given that sample size had a small influence on the likelihood of finding consistent relationships (standard length, $z = 4.60$, $P < 0.001$, $R^2 = 0.13$; body mass, $z = 3.71$, $P < 0.001$, $R^2 = 0.07$; Appendix S9). Overall, there was no evidence that the range of body size affected the chance of finding consistent relationships between body size and TL (standard length, $z = 0.63$, $P = 0.53$, $R^2 < 0.01$; body mass: $z = -0.71$, $P = 0.48$, $R^2 < 0.01$).

FULL models performed better than NULL models (Table 1), with the former explaining an average of 31.5% of total variation of the TL–body-size relationships (CI marginal $R^2 = 0.30$ to 0.32). The relationship between standard length and TL was mainly influenced by three functional traits: body depth, mouth width, and tooth shape (Table 3, Fig. 4). Fishes with laterally compressed bodies had more negative relationships between standard length and TL than fishes with elongate and

fusiform bodies (Fig. 2b). Positive relationships were more likely to occur in fishes with relatively large mouths (Fig. 2d). Fishes with unicuspid or multicuspid teeth tended to have negative slopes, fishes without teeth tended to have flat slopes, and those with conical and triangular serrated teeth had positive slopes (Fig. 3b, d). Inconsistent patterns were found for body width, mouth orientation, gill raker shape, and gut length (Table 3; Appendix S10). H^2 values and conditional R^2 were lower on average for analyses with TL–standard-length as response variable compared to values generated from analyses using species MTL ($H^2 = 0.55$ –0.72, conditional $R^2 = 0.10$ –0.39; Table 1), indicating only a moderate influence of phylogenetic relationships on TL–standard-length relationships.

FULL models performed better than NULL models in explaining the variation of TL–body-mass relationship (Table 1). Body depth, mouth width, and tooth shape consistently influenced TL–body-mass relationships, and their effects were similar to those observed with models using standard length (Table 3; Appendix S10). Body

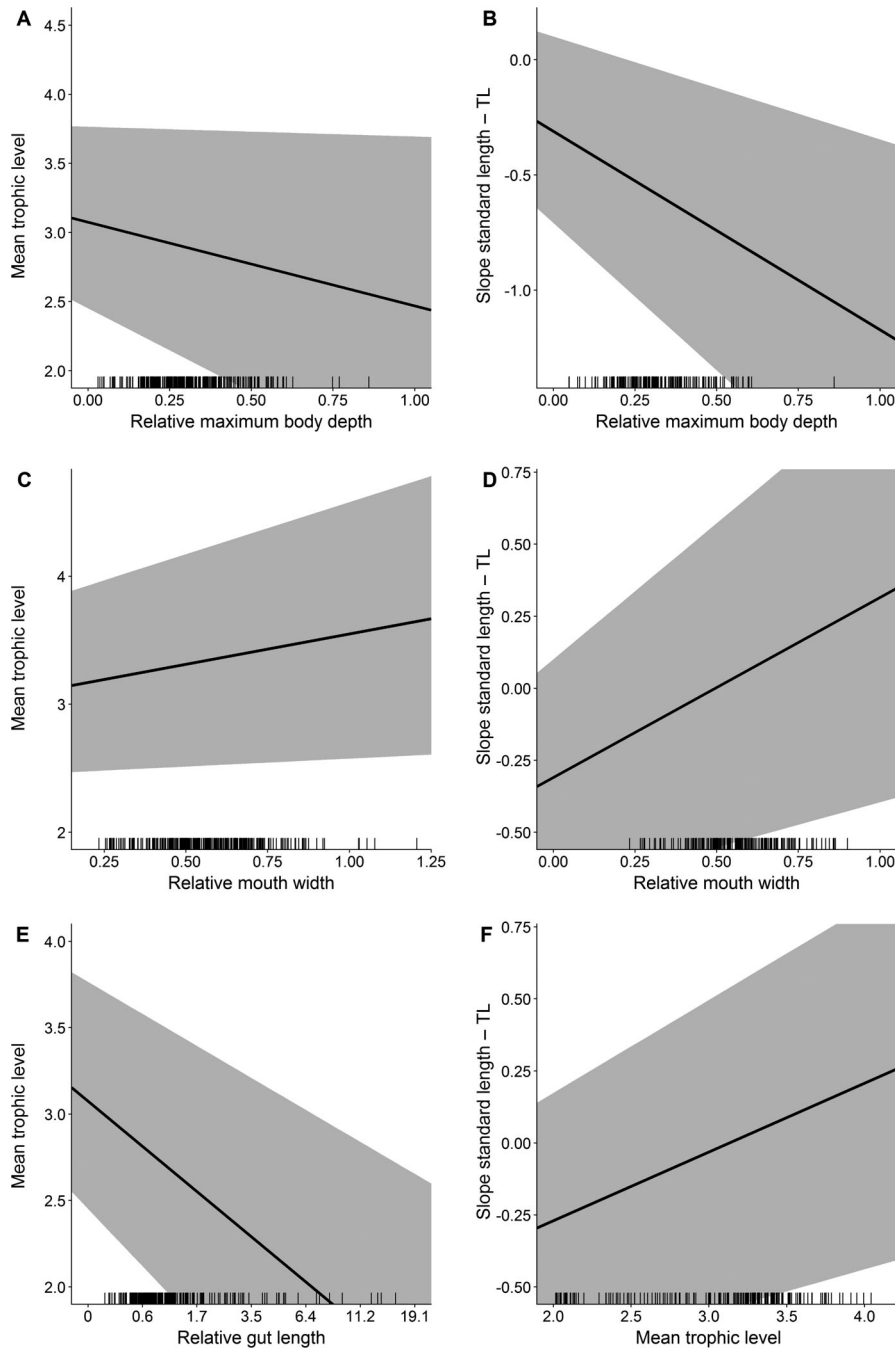


FIG. 2. Marginal effects of (A, B) relative maximum body depth, (C, D) relative mouth width, (E) relative gut length, and (F) mean trophic level (MTL) on MTL and/or the slope of standard length–trophic level (TL) relationship according to Bayesian phylogenetic linear mixed models performed on 100 different trees. Both carnivorous and non-carnivorous species are included in these analyses. Tick marks represent the position of the species according to the x variable. The 95% credible intervals of slopes are shown as gray ribbons. Statistically nonsignificant results (i.e., credible intervals encompassing 0) and results based on body mass rather than standard length can be found in Appendix S7 and S10.

width, mouth orientation, gill rakers, and gut length did not influence TL–body-mass relationships (Table 3; Appendix S10). Marginal and conditional R^2 and H^2 values were virtually the same as those obtained from models using standard length (Table 1).

Despite the fact that the SUB-MTL models (containing only MTL as explanatory variable) performed poorly when compared to FULL models, MTL influenced both the TL–standard-length and TL–body-mass relationships, explaining 14% and 9% of their variation,

TABLE 1. Comparisons between Bayesian phylogenetic linear mixed models aiming to explain mean trophic level (MTL) and the slope of the trophic level (TL)–body size relationship with mean body size and seven other traits (FULL models), with only mean body size (SUB models) and without any explanatory variables (NULL).

| Model | DIC | Delta | Weight | Marginal R^2 | Conditional R^2 | H^2 |
|---------------------------------------|-------------------------|----------------------|----------------|--------------------|-------------------|-------------------|
| MTL (overall)/standard length | | | | | | |
| FULL | 201.9 (176.7, 217.7) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.35 (0.31, 0.38) | 0.77 (0.73, 0.82) | 0.96 (0.94, 0.97) |
| NULL | 288.5 (246.9, 321.0) | 86.7 (56.0, 127.6) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.84 (0.81, 0.86) | 0.98 (0.97, 0.98) |
| SUB | 290.4 (247.6, 323.3) | 89.5 (54.4, 130.9) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.78 (0.84, 0.88) | 0.98 (0.97, 0.98) |
| MTL (overall)/body mass | | | | | | |
| FULL | 203.8 (177.6, 219.8) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.35 (0.31, 0.39) | 0.77 (0.72, 0.82) | 0.96 (0.94, 0.97) |
| SUB | 285.5 (243.8, 319.6) | 81.9 (48.7, 124.9) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.84 (0.81, 0.86) | 0.98 (0.97, 0.98) |
| NULL | 288.5 (246.9, 321.0) | 84.3 (54.7, 126.5) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.84 (0.81, 0.86) | 0.98 (0.97, 0.98) |
| MTL (carnivorous)/standard length | | | | | | |
| FULL | 93.3 (70.1, 100.4) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.23 (0.19, 0.26) | 0.52 (0.43, 0.68) | 0.92 (0.88, 0.95) |
| NULL | 120.9 (96.7, 137.3) | 28.0 (17.7, 38.7) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.61 (0.54, 0.68) | 0.94 (0.92, 0.96) |
| SUB | 122.4 (103.3, 125.8) | 28.9 (24.3, 38.6) | 0.0 (0.0, 0.0) | 0.11 (0.09, 0.12) | 0.32 (0.26, 0.47) | 0.87 (0.73, 0.93) |
| MTL (carnivorous)/body mass | | | | | | |
| FULL | 93.6 (67.5, 100.4) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.23 (0.18, 0.26) | 0.51 (0.42, 0.69) | 0.92 (0.86, 0.95) |
| NULL | 120.9 (96.7, 137.3) | 28.3 (18.9, 39.8) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.61 (0.54, 0.68) | 0.94 (0.92, 0.96) |
| SUB | 121.4 (96.3, 126.6) | 28.6 (22.7, 36.2) | 0.0 (0.0, 0.0) | 0.10 (0.08, 0.11) | 0.35 (0.27, 0.51) | 0.89 (0.76, 0.94) |
| MTL (non-carnivorous)/standard length | | | | | | |
| FULL | −75.5 (−100.6, −50.9) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.18 (0.16, 0.20) | 0.80 (0.66, 0.87) | 0.96 (0.94, 0.96) |
| NULL | −45.2 (−50.6, −43.1) | 30.2 (7.2, 53.3) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.36 (0.32, 0.46) | 0.86 (0.78, 0.93) |
| SUB | −43.6 (−49.0, −41.6) | 31.8 (8.8, 55.2) | 0.0 (0.0, 0.0) | 0.01 (0.00, 0.01) | 0.36 (0.32, 0.44) | 0.85 (0.77, 0.93) |
| MTL (non-carnivorous)/body mass | | | | | | |
| FULL | −75.2 (−101.6, −50.1) | 0.0 (0.0, 0.0) | 1.0 (0.9, 1.0) | 0.18 (−0.16, 0.20) | 0.79 (0.65, 0.87) | 0.96 (0.94, 0.97) |
| NULL | −45.2 (−50.6, −43.1) | 29.3 (6.8, 51.9) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.36 (0.32, 0.46) | 0.85 (0.78, 0.93) |
| SUB | −43.7 (−48.9, −41.7) | 31.0 (8.1, 53.8) | 0.0 (0.0, 0.0) | 0.01 (0.01, 0.01) | 0.36 (0.33, 0.45) | 0.84 (0.76, 0.93) |
| Slope/standard length | | | | | | |
| FULL | 56.1 (53.5, 56.7) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.31 (0.30, 0.32) | 0.37 (0.36, 0.42) | 0.60 (0.39, 0.83) |
| SUB-MTL | 73.8 (68.9, 74.3) | 17.6 (13.6, 19.5) | 0.0 (0.0, 0.0) | 0.14 (0.14, 0.15) | 0.22 (0.20, 0.27) | 0.55 (0.35, 0.84) |
| NULL | 100.6 (96.9, 101.2) | 44.4 (41.9, 46.0) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.10 (0.07, 0.15) | 0.59 (0.43, 0.83) |
| Slope/body mass | | | | | | |
| FULL | −246.0 (−250.5, −244.5) | 0.0 (0.0, 0.0) | 1.0 (1.0, 1.0) | 0.28 (0.26, 0.29) | 0.39 (0.36, 0.47) | 0.72 (0.49, 0.88) |
| SUB-MTL | −224.2 (−231.7, −223.1) | 21.5 (17.1, 24.0) | 0.0 (0.0, 0.0) | 0.09 (0.08, 0.09) | 0.21 (0.19, 0.27) | 0.63 (0.45, 0.88) |
| NULL | 100.6 (96.9, 101.2) | 346.3 (344.5, 350.4) | 0.0 (0.0, 0.0) | 0.00 (0.00, 0.00) | 0.10 (0.07, 0.15) | 0.59 (0.43, 0.83) |

Notes: MTL was analyzed in three different ways: all fishes combined (overall), carnivorous fishes only (MTL > 2.5), and non-carnivorous fishes only (MTL < 2.5). MTL was also used alone as a predictor of the slope of TL–body size variation (SUB-MTL models). Standard length and body mass were used as two different estimates of body size. Numbers in parentheses are 2.5% and 97.5% quantiles based on the variation associated with phylogeny uncertainty (100 trees, see more details in Rabosky et al. 2018). DIC (deviance information criterion) is a hierarchical generalization of Akaike’s information criterion (AIC) for Bayesian models. Delta AIC (Delta) is an index that represents the comparison of the AIC values of each model to the best model (values < 2 are usually interpreted as good evidence in support of the model). Akaike weight (Weight) constitutes the ratio of delta AIC values relative to the entire set of candidate models and measures the strength of evidence for each model (1, total evidence; 0, no evidence). Marginal R^2 represents the variance explained only by the fixed factors, and Conditional R^2 represents the variance explained by both fixed and random factors. Marginal R^2 is zero for NULL models due to the absence of fixed factors. For SUB-models, marginal R^2 were lower than 0.01 in some cases (e.g., MTL [overall]) and therefore were rounded to zero. H^2 indicates Lynch’s phylogenetic heritability index and varies from 0, when the trait is evolving independently of the phylogeny, to 1, when the trait is evolving according to Brownian motion.

respectively (Table 1, Fig. 2f; Appendix S10). In both cases, the slopes of the TL–body-size relationships tended to be positive for species with higher MTL (Fig. 2f).

DISCUSSION

Our analysis of freshwater and estuarine fishes revealed a wasp-waist distribution between trophic level (either TL or MTL) and body size, which is not

consistent with the general positive linear relationship for fishes reported by Romanuk et al. (2011) and others. However, MTL increased with mean body size when carnivorous fishes were analyzed separately, a finding in agreement with several previous studies (Riede et al. 2011). Similar results were obtained using either body mass or standard length as the estimate of body size. The TL–body-size relationship varied considerably among species, and patterns were associated with certain

TABLE 2. Coefficient estimates generated by Bayesian phylogenetic linear mixed models performed on 100 different trees aiming to explain the variation in the mean trophic level (MTL) of freshwater and estuarine fishes.

| Coefficients | Overall | | | Carnivorous | | | Non-carnivorous | | |
|-----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------|--|
| | Model with standard length | Model with body mass | Model with standard length | Model with standard length | Model with body mass | Model with standard length | Model with standard length | Model with body mass | |
| Intercept | 3.11 (2.46/3.78) | 3.38 (2.82/3.94) | 2.79 (2.34/3.29) | 3.34 (2.97/3.71) | 3.34 (2.97/3.71) | 2.41 (1.80/3.01) | 2.42 (1.87/2.95) | | |
| Size | 0.06 (-0.01/0.13) | 0.02 (0.00/0.05) | 0.12 (0.05/0.18) | 0.04 (0.02/0.06) | 0.04 (0.02/0.06) | 0.00 (-0.06/0.07) | 0.00 (-0.02/0.02) | | |
| Maximum body depth | -0.63 (-1.21/-0.07) | -0.62 (-1.19/-0.06) | -0.23 (-0.78/0.29) | -0.27 (-0.80/0.22) | -0.27 (-0.80/0.22) | 0.08 (-0.41/0.62) | 0.07 (-0.43/0.61) | | |
| Maximum body width | 0.17 (-1.25/1.57) | 0.10 (-1.30/1.50) | 0.15 (-1.00/1.27) | -0.06 (-1.20/1.07) | -0.06 (-1.20/1.07) | -0.33 (-2.03/1.35) | -0.32 (-2.02/1.37) | | |
| Gut length | -0.52 (-0.66/-0.37) | -0.53 (-0.68/-0.38) | -0.31 (-0.54/-0.08) | -0.32 (-0.55/-0.09) | -0.32 (-0.55/-0.09) | -0.13 (-0.25/-0.01) | -0.13 (-0.25/-0.02) | | |
| Mouth width | 0.47 (0.12/0.81) | 0.43 (0.08/0.77) | 0.37 (0.08/0.66) | 0.31 (0.02/0.61) | 0.31 (0.02/0.61) | 0.01 (-0.42/0.44) | 0.02 (-0.41/0.44) | | |
| Tooth shape (unicuspid) | 0.15 (-0.08/0.37) | 0.14 (-0.08/0.37) | 0.14 (-0.07/0.36) | 0.12 (-0.10/0.33) | 0.12 (-0.10/0.33) | 0.08 (-0.12/0.28) | 0.08 (-0.11/0.28) | | |
| Tooth shape (multicuspid) | 0.16 (-0.05/0.38) | 0.16 (-0.06/0.38) | 0.08 (-0.12/0.29) | 0.06 (-0.14/0.26) | 0.06 (-0.14/0.26) | -0.02 (-0.26/0.22) | -0.01 (-0.25/0.22) | | |
| Tooth shape (conical) | 0.29 (0.08/0.51) | 0.30 (0.08/0.51) | 0.15 (-0.06/0.35) | 0.13 (-0.07/0.33) | 0.13 (-0.07/0.33) | 0.00 (-0.20/0.19) | 0.00 (-0.19/0.19) | | |
| Tooth shape (triangular serrated) | 0.63 (0.27/0.99) | 0.60 (0.24/0.97) | 0.54 (0.19/0.92) | 0.49 (0.14/0.87) | 0.49 (0.14/0.87) | 0.02 (-0.28/0.30) | 0.02 (-0.27/0.30) | | |
| Gill raker (short) | -0.02 (-0.20/0.17) | -0.04 (-0.23/0.15) | -0.05 (-0.23/0.12) | -0.08 (-0.26/0.09) | -0.08 (-0.26/0.09) | 0.00 (-0.18/0.17) | 0.00 (-0.18/0.18) | | |
| Gill raker (intermediate) | -0.07 (-0.27/0.12) | -0.10 (-0.29/0.10) | -0.01 (-0.20/0.17) | -0.04 (-0.23/0.14) | -0.04 (-0.23/0.14) | -0.06 (-0.22/0.10) | -0.06 (-0.23/0.10) | | |
| Gill raker (comb-like) | -0.12 (-0.34/0.10) | -0.15 (-0.36/0.07) | -0.07 (-0.29/0.15) | -0.10 (-0.32/0.12) | -0.10 (-0.32/0.12) | 0.00 (-0.17/0.16) | 0.00 (-0.17/0.16) | | |
| Mouth orientation (terminal) | -0.21 (-0.33/-0.09) | -0.20 (-0.32/-0.08) | -0.12 (-0.23/-0.02) | -0.12 (-0.22/-0.02) | -0.12 (-0.22/-0.02) | -0.10 (-0.27/0.07) | -0.10 (-0.27/0.07) | | |
| Mouth orientation (subterminal) | -0.23 (-0.39/-0.06) | -0.23 (-0.39/-0.06) | -0.12 (-0.26/0.02) | -0.13 (-0.27/0.01) | -0.13 (-0.27/0.01) | 0.03 (-0.18/0.23) | 0.03 (-0.18/0.23) | | |
| Mouth orientation (inferior) | -0.30 (-0.63/0.03) | -0.29 (-0.62/0.03) | -0.25 (-0.55/0.04) | -0.27 (-0.57/0.02) | -0.27 (-0.57/0.02) | -0.02 (-0.32/0.29) | -0.03 (-0.33/0.28) | | |
| Phylogenetic variance | 0.18 (0.05/0.36) | 0.17 (0.04/0.36) | 0.05 (0.00/0.16) | 0.05 (0.00/0.17) | 0.05 (0.00/0.17) | 0.04 (0.00/0.13) | 0.04 (0.00/0.13) | | |
| Residual variance | 0.09 (0.06/0.13) | 0.09 (0.06/0.13) | 0.07 (0.05/0.10) | 0.07 (0.05/0.10) | 0.07 (0.05/0.10) | 0.01 (0.00/0.03) | 0.01 (0.00/0.03) | | |

Notes: MTL was analyzed in three different ways: all fishes combined (overall), carnivorous fishes only (MTL > 2.5), and non-carnivorous fishes only (MTL < 2.5). Coefficient estimation was based on FULL models containing the explanatory variables: mean body size (either standard length or body mass), body depth, body width, mouth width, mouth position, tooth shape, gill raker length and number, and gut length. Numbers in boldface type indicate that the coefficient estimate was consistent (i.e., credible intervals not encompassing zero). The coefficients associated with levels of categorical variables (presented in parenthesis) represent their difference to a fixed baseline level (tooth shape: absence of teeth, gill raker: absence of gill rakers, mouth orientation: superior mouth). For pairwise comparisons between all levels of categorical variables, see Fig. 3 and Appendix S7 and S8.

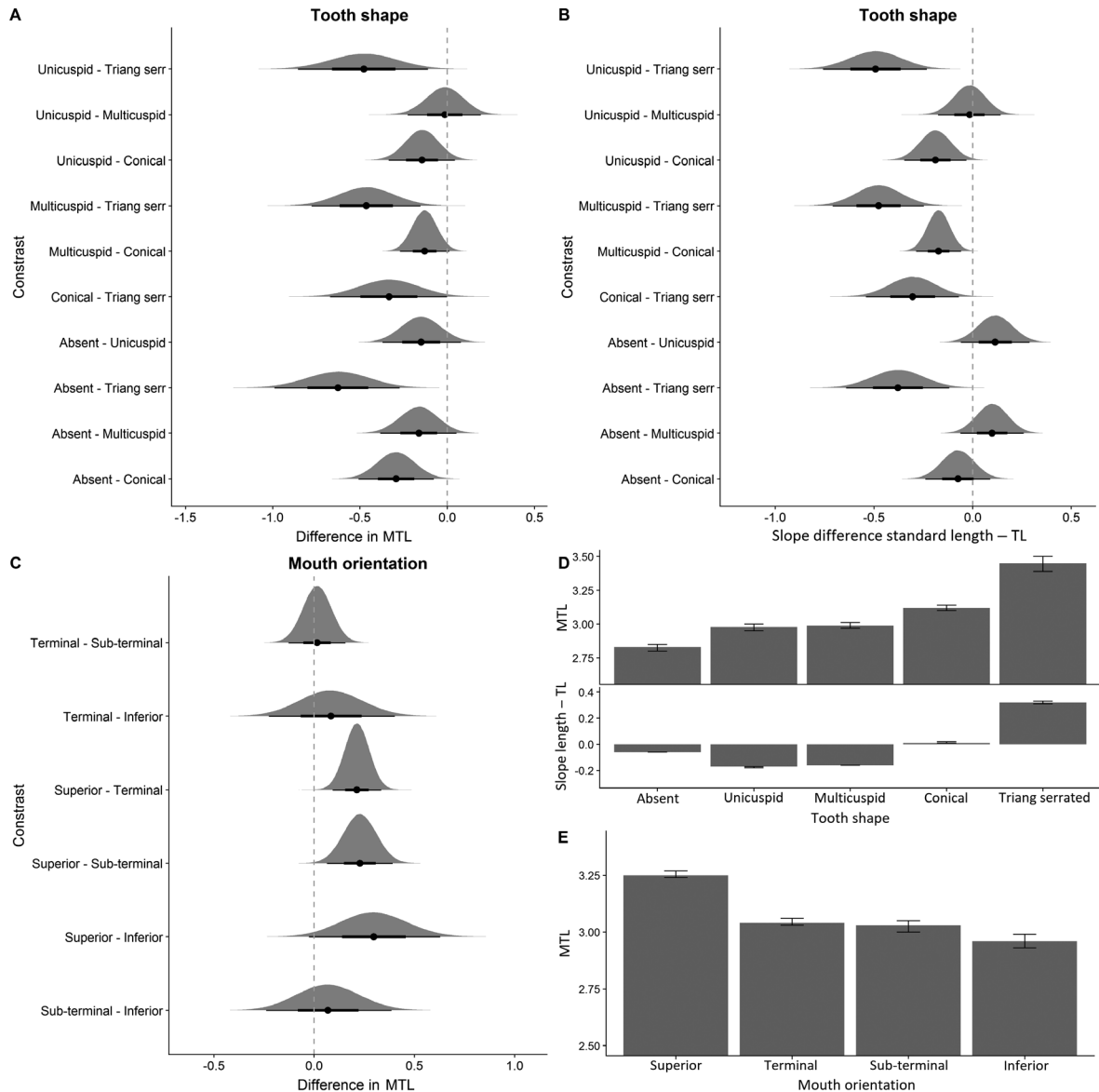


FIG. 3. Posterior distributions of estimated marginal mean differences of (A) mean trophic level (MTL) and/or (B) the slope of the standard length–trophic level (TL) relationship among different types of tooth shape (triang serr, triangular serrated) and (C) mouth orientation (just for MTL) according to Bayesian phylogenetic linear mixed models performed on 100 different trees. Estimated marginal means of MTL are presented for different types of (D) tooth shape and (E) mouth orientation. Estimated marginal means of the standard length–TL slopes are shown for different types of tooth shape (D). In panels A–C, the black dot shows mean values, the heavy black line shows 66% interval, and the thin line shows 95% interval. In panels D and E, values are mean and error bars are 95% intervals. Both carnivorous and non-carnivorous species are included in these analyses. Statistically nonsignificant results (i.e., credible intervals encompassing 0) and results based on body mass rather than standard length can be found in Appendix S7 and S10.

functional traits, especially body depth, mouth width, and tooth shape. This finding supports our prediction that functional traits associated with feeding and food processing mediate the relationship between TL and body size.

The lack of evidence for a general positive relationship between trophic level (either TL or MTL) and body size contrast with other studies conducted in both aquatic (Jennings et al. 2001, 2002, 2007, Al-Habsi et al. 2008,

Rooney et al. 2008, Arim et al. 2010, Barnes et al. 2010, Gilljam et al. 2011, Riede et al. 2011, Romanuk et al. 2011, Reum and Marshall 2013, Romero-Romero et al. 2016, Potapov et al. 2019) and terrestrial ecosystems (Rooney et al. 2008, Riede et al. 2011). Most of these studies involved communities that are strongly size structured (Jennings et al. 2001, 2002, Al-Habsi et al. 2008, Romero-Romero et al. 2016) and/or were strongly focused on carnivores (Jennings et al. 2007, Arim et al.

TABLE 3. Coefficient estimates generated by Bayesian phylogenetic linear mixed models performed on 100 different trees aiming to explain the variation in the relationship between trophic level (TL) and body size (either standard length or body mass) of freshwater and estuarine fishes.

| Coefficients | Model with standard length | Model with body mass |
|-----------------------------------|----------------------------|----------------------------|
| Intercept | -0.30 (-0.71/0.10) | -0.13 (-0.27/0.01) |
| Size | 0.04 (-0.10/0.18) | 0.01 (-0.01/0.03) |
| Maximum body depth | -0.87 (-1.28/-0.45) | -0.36 (-0.54/-0.18) |
| Maximum body width | 0.11 (-0.93/1.16) | 0.10 (-0.35/0.56) |
| Gut length | 0.01 (-0.09/0.12) | 0.02 (-0.03/0.06) |
| Mouth width | 0.63 (0.31/0.94) | 0.25 (0.12/0.39) |
| Tooth shape (unicuspid) | -0.11 (-0.29/0.06) | -0.03 (-0.11/0.04) |
| Tooth shape (multicuspid) | -0.10 (-0.26/0.06) | -0.03 (-0.10/0.04) |
| Tooth shape (conical) | 0.07 (-0.09/0.24) | 0.05 (-0.03/0.12) |
| Tooth shape (triangular serrated) | 0.38 (0.12/0.64) | 0.15 (0.04/0.27) |
| Gill raker (short) | 0.12 (-0.03/0.28) | 0.05 (-0.01/0.12) |
| Gill raker (intermediate) | 0.08 (-0.09/0.24) | 0.02 (-0.05/0.10) |
| Gill raker (comb-like) | 0.04 (-0.15/0.23) | 0.01 (-0.07/0.09) |
| Mouth orientation (terminal) | 0.01 (-0.10/0.12) | 0.01 (-0.04/0.06) |
| Mouth orientation (subterminal) | -0.01 (-0.15/0.13) | 0.01 (-0.05/0.07) |
| Mouth orientation (inferior) | 0.01 (-0.23/0.24) | 0.01 (-0.10/0.11) |
| Phylogenetic variance | 0.01 (0.00/0.04) | 0.00 (0.00/0.01) |
| Residual variance | 0.07 (0.06/0.09) | 0.01 (0.01/0.02) |

Notes: Coefficient estimation was based on FULL models containing the explanatory variables: mean body size (either standard length or body mass), body depth, body width, mouth width, mouth position, tooth shape, gill raker length and number, and gut length. Numbers in boldface type indicate that the coefficient estimate was consistent (i.e., credible intervals not encompassing zero). The coefficients associated with levels of categorical variables (presented in parenthesis) represent their difference to a fixed baseline level (tooth shape, absence of teeth; gill raker, absence of gill rakers; mouth orientation, superior mouth). For pairwise comparison between all levels of categorical variables, see Fig. 3, and Appendix S10.

2010, Barnes et al. 2010, Riede et al. 2011, Reum and Marshall 2013). However, exclusion of species at lower trophic levels neglects an important part of community trophic diversity, especially for highly diverse taxa, such as teleost fishes and faunas of species-rich tropical regions. In our study, carnivorous fishes that have higher MTL tended to have positive relationships between TL and body size. Furthermore, the relationship between MTL and mean body size was significantly positive only

when the analysis was restricted to carnivorous fishes (MTL > 2.5). Therefore, it is possible that studies that failed to include omnivorous, herbivorous and detritivorous species in their analysis (Romanuk et al. 2011) may have overestimated the strength and slope of the MTL–body-size relationship. Consequently, studies that analyze regional faunas or phylogenetic lineages that mostly or exclusively include carnivorous fishes should discuss potential bias when using resultant MTL–body-size relationships for food web modeling.

The lack of a general relationship between MTL and body size likely reflects differences in the morphology, physiology, and behavior of carnivorous and non-carnivorous fishes. In our study, MTL of carnivorous species increased with mean body size, which corroborates findings from several studies that analyzed only predatory fishes (Jennings et al. 2007, Arim et al. 2010, Gilljam et al. 2011). In order to meet energetic requirements, large predators may feed preferentially on the largest and most profitable prey that can be successfully subdued (Werner and Hall 1974, Mittelbach 1981). Interestingly, a study conducted in a Neotropical floodplain river found that the MTL of carnivorous fishes did not increase with mean body size (Layman et al. 2005). A possible explanation is that low availability of prey fish at higher trophic positions during certain periods of the annual hydrologic cycle forces large piscivores to feed at lower trophic levels to meet metabolic demands (Arim et al. 2007), especially at tropical regions where the temperatures tend to be high (Dantas et al. 2019). Research that explores the relationship between MTL and mean body size across multiple species of non-carnivorous fishes appears to be lacking. Our results indicated that the relationship is flat, but given our relatively small sample size for this guild (52 species), inferences should be made with caution.

Shallow freshwater and estuarine habitats are in some respects more similar to terrestrial habitats than the marine pelagic habitats for which a strong trophic level (both TL and MTL)–body-size relationships are believed to influence food web dynamics (Jennings et al. 2007, Al-Habsi et al. 2008, Riede et al. 2011, Reum and Marshall 2013, Romero-Romero et al. 2016, Potapov et al. 2019). Marine pelagic food webs are largely supported by unicellular phytoplankton that are relatively nutrient rich and easy to digest (Tucker and Rogers 2014). Consequently, these food webs are characterized by efficient transfer of energy and biomass and long food chains (McGarvey et al. 2016). Under these circumstances, it is difficult for large animals to exploit food resources at or near the bottom of food chains due to the difficulty of ingesting and handling small particles. The exception is large filter feeders, such as basking sharks and whale sharks that strain large volumes of water through comb-like gill rakers, and baleen whales that strain particles using the comb-like baleen. However, the great majority of marine phytoplankton feeders are zooplankton, and most zooplankton are consumed

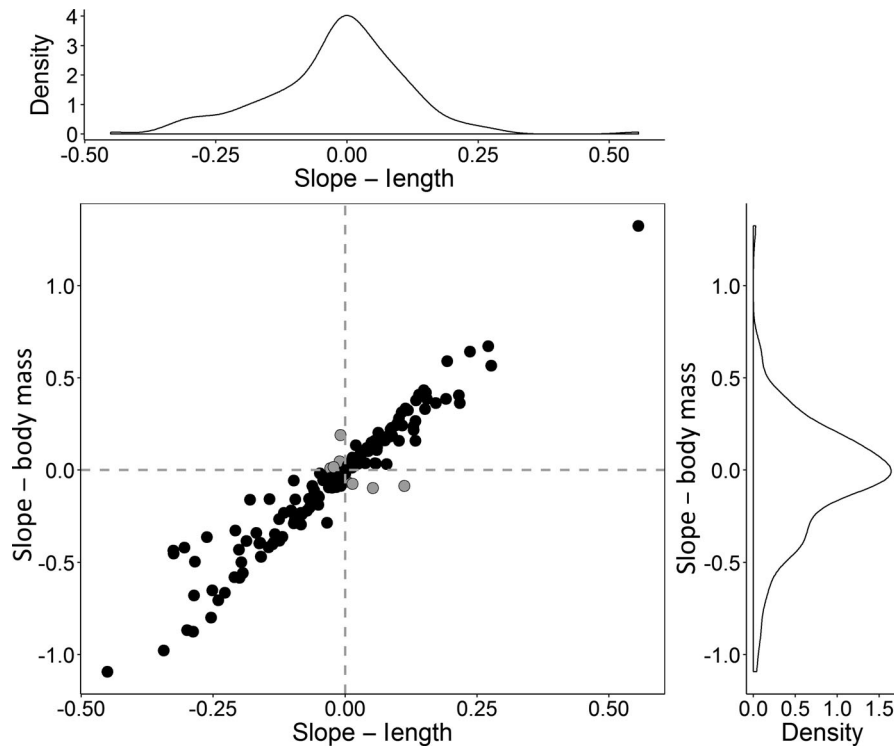


FIG. 4. Comparison of the slopes of the trophic level (TL)–standard length relationship (x -axis) and the slopes of the TL–body mass relationship (y -axis) for 179 freshwater and estuarine fish species. Slopes were generated using Bayesian semiparametric generalized mixed models, where species were treated as a random variable (random intercept and slope). All species analyzed contained at least 30 specimens dissected for dietary analysis. Gray dots represent cases ($N = 8$) where the models did not converge regarding the direction of the relationship (negative vs. positive or vice versa). Kernel density estimation plots show the distribution of the slopes generated using both standard length and body mass.

by larger zooplankton and small fishes. Shallow freshwater and estuarine systems, on the other hand, have food webs supported by combinations of unicellular and multicellular autotrophs, including phytoplankton, periphyton, aquatic macrophytes, and allochthonous plant material (Correll 1978, Vannote et al. 1980, Junk et al. 1989, Winemiller 1990, Roach et al. 2014). Detritus, both of autochthonous and allochthonous origin, also is an important food resource that is directly exploited by fishes in shallow freshwater and estuarine ecosystems (Darnell 1967, Mann 1988, Winemiller 1990, Zeug and Winemiller 2008). Many medium- and large-sized fishes are well adapted to exploit food resources at the base of aquatic food webs, e.g., frugivorous pacus of the Amazon (e.g., *Colossoma macropomum*, *Piaractus brachipomus*) and omnivorous and herbivorous carps of Asia (e.g., *Catlocarpio siamensis*, *Ctenopharyngodon idella*). Basal production sources in freshwater ecosystems are exploited by other kinds of large vertebrates, including manatees (*Trichechus* spp.), beavers (*Castor* spp.), turtles (e.g., *Peltecephalus dumerilianus*), geese (e.g., *Anser* spp., *Branta* spp., and *Chen* spp.), and ducks (e.g., *Anas* spp.). Conversely, many small freshwater and estuarine fishes that feed on aquatic and terrestrial invertebrates are positioned at high trophic levels. These small invertebrates include many species of tetras (Alestidae,

Characidae, Lebiasinidae), minnows (Cyprinidae), killifishes (Fundulidae, Rivulidae), ricefishes (Adrianichthyidae), and mosquitofishes (Poeciliidae). Carnivorous arthropods that are prey of these small fishes also feed at high trophic levels: examples include spiders (*Argyroneta aquatica*), beetles (e.g., Hydrophilidae), water bugs (e.g., Belostomatidae, Nepidae), and water mites (e.g., *Hydracarina* spp.). On land, the high diversity of plants provides a vast range of options for herbivores (e.g., granivores, frugivores, browsers, grazers) spanning a wide range of body sizes, including some of Earth's largest terrestrial animals (e.g., elephants, giraffes, hippos, rhinos). Half of all insect species, the most diverse group of animals on earth, are estimated to feed primarily on plants (Grimaldi and Engel 2005, Nakadai 2017), which implies that consumers smaller than their food resource should be the rule rather than the exception in terrestrial food webs. Therefore, positive TL–body-size relationships should not be expected to be the rule for the majority of consumers in many communities, and the relationship between MTL and body size across all consumers in terrestrial communities as well as freshwater and estuarine ecosystems, should be very weak or absent, a position argued previously by others (Layman et al. 2005, Tucker and Rogers 2014, Ou et al. 2017, Potapov et al. 2019).

Mean body size was a weak predictor of MTL compared to other functional traits (e.g., gut length, tooth shape, mouth orientation, mouth width, and body depth) when the relationship was assessed across all consumers. These other functional variables directly influence foraging success, yet surprisingly, they have received little attention in empirical and theoretical food web studies. Tooth shape influences food acquisition and processing by both herbivorous and predatory fishes (McCollum and Sharpe 2001). Fishes without teeth or having unicuspid or multicuspid teeth tended to have low MTL. Some of these fishes (e.g., Neotropical Curimatidae, Loricariidae, and Prochilodontidae, African Citharinidae and Distichodontidae, many Asian carps, and the North American gizzard shad, *Dorosoma cepedianum*) feed on fine particulate organic matter (FPOM). Unicuspid teeth are common in fishes that scrape periphyton adhered to surfaces (e.g., loricariids), whereas many fishes with multicuspid teeth (e.g., alestids, characids, some serrasalmids) use them to crush fruits and seeds (Winemiller 1991b). Conical and triangular serrated teeth are common among predators that use them to pierce or tear flesh (Winemiller 1991b). Fish mouth orientation is a strong indicator of the position for feeding within the water column (Keast and Webb 1966, Winemiller 1991b). In our study, fishes with a superior mouth orientation had higher MTLs than much larger fishes with terminal, subterminal, and inferior mouth positions. Most fishes with upturned mouths, such as the Neotropical characiforms *Thoracocharax stellatus* and *Triporthes* spp., feed on small terrestrial arthropods that fall onto the water surface (Cushing and Allan 2001). Fishes with terminal and subterminal mouths were commonly observed among midwater-dwelling and epibenthic fishes, many of which were omnivorous with broad diets. Fishes with an inferior mouth orientation usually fed on substrates, either scraping periphyton from rocks and logs or using suction to ingest small aquatic invertebrates or FPOM. Mouth gape, here indicated by relative mouth width, sets an upper limit on the size of food items that can be consumed by animals that ingest food items whole, and therefore influences predator–prey size ratios and trophic level (Montaña et al. 2011, Mihalitsis and Bellwood 2017). Carnivorous species, especially piscivores that ingest prey whole (e.g., largemouth bass [*Micropterus salmoides*], peacock bass [*Cichla* spp.], and many catfishes [Siluriformes]) tended to have large mouths when compared to omnivorous, algivorous, and detritivorous fishes. This difference would be expected given that predator–prey body size ratios tend to decrease with trophic level (Riede et al. 2011), when mouth gape is the major factor limiting the size of prey that can be ingested. Gut length was associated with a diet gradient ranging from herbivory/detritivory (long gut) to carnivory (short gut). Because gut length affects digestion and nutrient absorption, it may be the single trait that best predicts trophic level in fishes (Kapoor et al. 1975,

Horn 1989, German and Horn 2006, Wagner et al. 2009), as well as reptiles and amphibians (Stevens and Hume 1995, O’Grady et al. 2005), birds (Ricklefs 1996, Battley and Piersma 2005), and mammals (Schieck and Millar 1985). Body shape is highly variable among teleosts and can affect both swimming performance and susceptibility to gape-limited predators. The negative relationship between body depth and MTL indicated that carnivorous fishes tend to have fusiform or elongated bodies that enhance pursuit speed (Webb 1984a,b). However, some piscivorous fishes, such as snappers (*Lutjanus* spp.) and certain cichlids (*Serranochromis* spp.), have relatively deep bodies and use ambush as a foraging strategy within structurally complex habitats (Webb 1984a,b). Among fishes that inhabit structurally complex habitats, a relatively deep body also may facilitate agile movements to avoid capture (Webb 1984a,b, Wood and Bain 1995). Functional traits other than body size also significantly influenced statistical models predicting the MTL of both carnivorous and non-carnivorous fishes. This suggests that more than just disentangling carnivorous from non-carnivorous fishes, functional traits can help deepen our understanding of how MTL varies within more restricted compartments of food webs.

From our intraspecific analyses, negative TL–body-size correlations were more common than positive correlations, a finding that contrasts with those from some earlier studies (Jennings et al. 2002, 2007, Reum and Marshall 2013). Negative TL–body-size relationships were observed for many species with low values for MTL, and those with deep bodies, small mouths, and unicuspid or multicuspid teeth. These are characteristic of herbivorous fishes, many of which undergo diet shifts during early ontogeny. Their early life stages generally feed on heterotrophic microfauna, such as protozoa and rotifers, and zooplankton, such as copepods and cladocerans, and later shift to consuming algae or macrophyte tissues that are less nutritious but often abundant in their environments (Horn 1989, German and Horn 2006). Less is documented about diets of early life stages of detritivorous fishes, especially those from tropical freshwaters. Most detritivores in our study had TL–body-size relationships with slightly negative or flat slopes (e.g., *Ancistrus triradiatus*, *Loricariichthys brunneus*, *Cyphocharax spilurus*, *Prochilodus mariae*; Appendix S9), suggesting a less abrupt diet change than seen in herbivorous fishes. In our study, detritivores had the longest relative gut lengths, which may have contributed to the lack of a significant linear relationship between gut length and the TL–body size relationship. In contrast, herbivores and some omnivorous species had shorter guts and negative TL–body-size relationships. Detritus varies in quality and generally contains bacteria, fungi, and other microorganisms that may supply nutrition sufficient for growth of early life stages of detritivorous fishes (Bowen 1980, 1983, Mann 1988). Species with high MTL, elongated or fusiform bodies, conical or triangular serrated teeth, and large mouths

tended to have positive TL-body size relationships, which likely was influenced by the fact that larger predators can ingest larger prey without necessarily eliminating small prey from their diets (Woodward and Hildrew 2002, Costa 2009). This leads to an increase in maximum TL of most predatory species while minimum TL stays flat, resulting in an increase in both the mean and variance of TL (Dalponti et al. 2018).

Potential sources of bias are always a concern for empirical studies. Body size distributions and sample sizes varied among species. Exploratory analysis with a portion of our data set indicated that TL–body-size relationships were not sensitive to the range of body sizes in species data sets. However, sample size influenced the statistical significance of results (i.e., parameter estimation either encompassing or non-encompassing zero), with small sample sizes sometimes producing nonsignificant relationships. We therefore conclude that the number of species with significant relationships may be underestimated. However, because we analyzed parameter estimates rather than their credible intervals, our major inferences should be largely unaffected by sample size. Trophic levels of our non-fish prey categories were estimated based on literature information, a potential source of error. The same issue confronts studies that estimate trophic level based on isotopic analysis (e.g., assumptions about trophic fractionation values, assimilation of material from basal resources, tissue turnover, sample size, body size, and habitat; Hoetinghaus and Zeug 2008, Layman et al. 2012). The use of mean trait values based on measurements of adult specimens used in Approach 1 and step 2 of Approach 2 discount the potential for ontogenetic allometry (German and Horn 2006). To minimize this issue, we measured adult specimens with lengths near the mode of the species distribution. Functional traits were measured primarily on adult specimens, whereas our diet data were obtained from a broader size range that included some immature size classes. This source of variation could have weakened relationships between functional traits (including body size) and TL, especially if intraspecific dietary and morphological variation increases with size (Keppeler et al. 2015). In a separate analysis (unpublished), we found that restricting the diet data to only adults vs. including a broader range of sizes had minimal effect on correlations between functional traits and food web descriptors.

Body size is acknowledged as one of the most important traits affecting ecological performance (Woodward et al. 2005), but sometimes, in the interest of simplifying complex systems, it has been emphasized to the exclusion of other traits that are equally or more influential. An increasing number of models and theories assume that food web structure and dynamics are strongly size based (Cohen et al. 1990, Otto et al. 2007, Petchey et al. 2008, Berlow et al. 2009, Arim et al. 2010, McCann 2011, Schneider et al. 2016). Furthermore, it has been proposed that additional traits should correlate with body size and trophic level, such as traits affecting

locomotion (McCann et al. 2005, Rooney et al. 2008), brain size (as a surrogate for cognitive ability and behavioral complexity; McCann et al. 2005, Rooney et al. 2008), and mouth gape size (Arim et al. 2010), although exceptions are found in all these examples (Chittka and Niven 2009, De Bie et al. 2012, Dunic and Baum 2017). Thus, while the importance of body size has been overemphasized in food web studies, we consider that the importance of other influential traits has been overlooked. Recent advances in ecological modeling and computation power allow the creation of more complex and realistic food web models that incorporate multiple traits of individual organisms (individual-based models) or life stages classes (age or stage-structured models; DeAngelis and Grimm 2014, Fujiwara 2016).

CONCLUSIONS

General rules that explain complex natural systems have been a major goal in ecology, and the use of functional traits has increasingly been promoted as an avenue for advancement (Winemiller et al. 2015, Funk et al. 2017). Body size has been seen as an important determinant of predator-prey interactions and, consequently, food web structure and dynamics (Woodward et al. 2005, Brose et al. 2006). Here, we found that the MTL was positively related with body size in fishes only when non-carnivorous species were excluded from the analysis. We did not find a general positive association between trophic level and body size in fishes as widely reported (e.g., Romanuk et al. 2011), and this was true for both interspecific and intraspecific comparisons. We speculate that similar results may be found in other systems wherein higher plants and detritus are important food resources for animals. TL–body-size relationships varied considerably among fish species and also were influenced by several functional traits. This was expected given the high trophic and morphological diversity observed among teleost fishes. Body size can have a relationship with TL, but mainly for fish that are strict carnivores, and therefore may influence the structure of food webs dominated by predatory fishes (e.g., pelagic marine systems; Jennings et al. 2001, 2002, Al-Habsi et al. 2008). However, this relationship was not strong for any trophic group in our data set for freshwater and estuarine fishes, and it was very weak for these fishes overall. Further exploration of relationships among key functional traits and trophic ecology likely will lead to improved predictions about food web patterns and dynamics of both carnivorous and non-carnivorous species.

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