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A meta-analysis of amino acid δ^{15}N trophic enrichment factors in fishes relative to nutritional and ecological drivers

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Abstract. The use of amino acid (AA) nitrogen stable isotopes (δ^{15}N) from consumer tissues aims to provide precise estimates of trophic position (TP), but the drivers of AA isotope fractionation remain unclear. In particular, the main factors driving the variability in TEF$_{AA}$ among taxonomic groups and trophic levels remain largely unexplained, which challenges the application of universal values for TEFs. While the relationship between protein content and quality and TEFs has been examined, studies have yielded inconsistent results, and the role of protein and lipid nutritional requirements as well as feeding regime have not been considered. Likewise, drivers that influence physiological and nutritional processes have not been examined relative to TEF$_{AA}$ variation. We conducted a meta-analysis of controlled feeding experiments within a single group, teleosts fishes, to evaluate the relationship between five nutritional factors (protein and lipid content, protein and lipid content relative to nutritional requirements, and feeding regime) and three ecological drivers (diet type, life stage, and habitat type) on TEF$_{AA}$. We considered a broad range of protein levels (8–71%) in diets and found no relationship between source TEF$_{AA}$s and percent protein relative to nutritional requirements, whereas lipid content relative to nutrient requirements, feeding regime and habitat type partially explain the variability in TEFs of Lys, but not for Phe and Met TEFs. The variability for the latter was representative of robust source AAs. Among trophic AAs, Asp, Ile, Pro, and Leu TEFs were significantly higher in species from brackish than marine habitats possibly due to osmoregulation involvement. TEF$_{Glu}$ was sensitive to protein content and feeding regime within teleosts, but relatively constant when comparing TEFs among teleosts, non-teleosts, and all taxa. Our results indicate that TEF$_{AA}$ is less variable within a single taxon than among multiple taxa and that such variation is not negligible. Our results indicate that δ^{15}N$_{AA}$ values could provide better TP estimates if using taxon-specific values, and highlights the need to explain the mechanisms of AA fractionation and quantify the variability in TEFs used during error propagation for TP estimates.

Key words: amino acids; compound-specific isotope analysis (CSIA); fish nutrition; lipid levels; nitrogen stable isotopes; protein levels; trophic enrichment factors.

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INTRODUCTION

Nitrogen (N) stable isotope ratios from individual amino acids (AAs) have recently emerged as a potentially powerful technique in ecology for estimating trophic position (TP) estimates based on measurements of animal tissues (Chikaraishi et al. 2007, 2009, Popp et al. 2007). This

approach, widely known as compound-specific stable isotope analysis (CSIA) of AAs, is based on the premise that AA-specific $\delta^{15}N$ values quantified from consumer tissues provide information regarding both primary producers (the isotopic baseline) and tropic level (McClelland and Montoya 2002, Popp et al. 2007, Chikaraishi et al. 2009).

Amino acids have been classified as source and trophic AAs based on the degree of the isotopic discrimination per trophic step (known as trophic enrichment factors, or TEFs). The source AAs reflect the isotopic baseline because they show limited isotope discrimination with each trophic level and should thus reflect the isotopic composition at the base of the food web (e.g., TEF for phenylalanine or Phe ≤ 0.4‰), whereas trophic AAs reflect a consumers' trophic step due to their substantial enrichment in ^{15}N with each trophic level (e.g., TEF for glutamic acid or Glu = 8.0‰; Chikaraishi et al. 2009). Early in the application of CSIA-AA, source AAs included Phe, lysine (Lys), methionine (Met), glycine (Gly), serine (Ser), threonine (Thr), tyrosine (Tyr). Other AAs fractionate in a less predictable manner and are classified within a metabolic group despite the fact that all AAs follow metabolic pathways (Germain et al. 2013, O'Connell 2017). Trophic AAs included Glu, alanine (Ala), isoleucine (Ile), leucine (Leu), valine (Val), aspartic acid (Asp), proline (Pro) (Popp et al. 2007). Thr was subsequently re-classified as a metabolic AA because its $\delta^{15}N$ values are depleted in ^{15}N, especially for high trophic-level consumers (Germain et al. 2013). Ser and Gly have been also considered metabolic AAs due to a high level of variability in empirical TEF estimates, which questions its classification as source AA (McCarthy et al. 2007, McMahon and McCarthy 2016, Whiteman et al. 2019). Based on the relative consistency in TEF estimates across trophic levels and taxa, Phe and Glu have been considered the canonical source and trophic AA, respectively (Chikaraishi et al. 2009, O'Connell 2017), but other studies have advocated for the use of a suite of select or single AA for characterizing the baseline isotopic composition and animal trophic status (Nielsen et al. 2015, Ruiz-Cooley et al. 2017, Ishikawa et al. 2018).

Calculating TP requires accurate estimates of TEFs for trophic and source AAs following the relationship proposed by Chikaraishi et al. (2009):

$$TP_{x/y} = (\delta^{15}N_x - \delta^{15}N_y - \beta_{x/y})/(TEF_x - TEF_y) + 1 \tag{1}$$

where $\beta_{x/y}$ is the difference between the $\delta^{15}N$ values of trophic AA (x) and source AA (y) in primary producers. The trophic discrimination factor (TDF) is another parameter necessary to estimate the TP of a consumer, and it is based on the difference between the TEF_{AA} of a trophic and a source AA quantified from the same animal tissue (Popp et al. 2007, Chikaraishi et al. 2009).

Identifying the main factors driving N isotopic fractionation in AAs across tissues, species, and trophic levels is key for the use of CSIA-AA in food web studies. Estimation of TP depends on the precise and accurate estimation of β and TEF_{AA} values, which can vary substantially between consumer–prey relationships (Nielsen et al. 2015). The variability in $\delta^{15}N_{AA}$ and TEF_{AA} values in consumer tissues has been associated with specific metabolic pathways in AAs of primary producer (Hare et al. 1991) and consumer tissues (Hare et al. 1991, Chikaraishi et al. 2007, 2009, O'Connell 2017). The $\delta^{15}N_{AA}$ isotope fractionation in animal tissues can vary by habitat type (Dale et al. 2011), ontogenetic stage (Schwartz-Narbonne et al. 2015), wild vs. captive and taxa (McClelland and Montoya 2002, Chikaraishi et al. 2007, Nielsen et al. 2015, McMahon and McCarthy 2016), feeding habits (Bloomfield et al. 2011, Hoen et al. 2014), mode of N excretion (Dale et al. 2011, Germain et al. 2013, Nielsen et al. 2015), dietary protein quantity (Nuche-Pascual et al. 2018), and protein quality (Nakashita et al. 2011, McMahon et al. 2015, Nuche-Pascual et al. 2018). Several studies have concluded that the TP of marine consumers, especially top predators, are often underestimated using CSIA-AA (Dale et al. 2011, Nielsen et al. 2015).

Recent studies reported high variability (rather than stability) in empirical estimates of TEFs of specific source and trophic AAs of a consumer, indicating that no universal TEF values would adequately reflect isotope enrichment for all terrestrial and aquatic taxa as initially assumed. Nielsen et al. (2015) conducted the first meta-

analysis that compared TP estimates derived from $\delta^{15}N_{AA}$ and stomach contents for captive and wild marine organisms, and found that TEF_{AA} differed between carnivorous, omnivorous, and herbivorous consumers, and also differed by mode of nitrogen excretion (urea vs. ammonia). Another meta-analysis highlighted the variability in TEF_{AA} across aquatic and terrestrial taxa, but indicated that the level of variability for Phe and Glu was relatively low, $-0.1‰ \pm 1.6‰$ and $6.4‰ \pm 2.5‰$ (mean $\pm$ standard deviation [SD]), respectively, in comparison with other AAs (McMahon and McCarthy 2016). To date, it is well recognized that the underlying factors controlling the variability in AA isotopic fractionation are strongly linked to mode of N excretion, nutrient sources, and direct routing, but more experimental and theoretical studies are needed to advance the application of CSIA-AA (Whiteman et al. 2019). Focusing on specific taxonomic group can reduce the effect of variation due to nitrogen excretion (Nielsen et al. 2015) and thermal metabolism (poikilothermic vs. endotherms; Thomas and Crowther 2014). We focus on teleosts because of their widespread presence in aquatic ecosystems, their well-studied nutritional requirements derived from aquaculture research, and the pressing need for furthering the understanding of the trophic role of commercially and ecologically important species. Fishes have complex life cycles and undergo distinct physiological and development processes at early life stages (larvae, and juveniles) that are linked to bioenergetic requirements and metabolic processes (Kamler 1991, Finn et al. 2002). Because N isotope discrimination is linked to AA synthesis and catabolic pathways (O'Connell 2017), variability in TEF_{AA} values could be highly linked to life stages and its bioenergetic requirements.

Recent studies on fish have yielded inconsistent relationships between TEF_{AA} of source and trophic AAs and protein content. In juvenile mummichog (*Fundulus heteroclitus*) fed with varying diets of varying protein quantity and quality, muscle TEFs of Asp, Glu, Ile, Leu, Val, Ala were significantly higher in individuals fed a very low-protein vegetable-based diet in comparison with those fed with higher quality animal feeds (McMahon et al. 2015). Barreto-Curiel et al. (2018) found that juvenile totoaba (*Totoaba*

macdonaldi) Met, Gly, Glu, and Pro TEFs were higher in fish fed a lower protein content diet. In contrast, Phe showed mean higher TEFs with higher protein content, and Val TEF was higher in fish fed the lowest protein content. Phe and Lys exhibited different levels of isotope discrimination in response to protein quantity in juvenile Pacific yellowtail, possibly due to the tissue-specific function of AAs relative to dietary availability and requirements (Nuche-Pascual et al. 2018). Their results indicate that protein availability plays a role in AA isotope discrimination, even for source AAs, which has long been suggested for bulk tissue isotopic analysis (see review by Martínez del Río and Wolf 2005).

Proteins and lipids are key substrates for fish growth and meeting bioenergetic requirements, while carbohydrates are not required per se and their role as an energy source is limited (Tocher 2003, NRC 2011). Specifically, protein and lipid requirements, defined as the minimum amount of protein or lipids needed to maximize growth, are determined by species-specific essential AA and fatty acid requirements, respectively (Dacosta-Calheiros et al. 2003, NRC 2011). Fish require higher protein levels in feeds than terrestrial homeotherms to achieve maximum growth rate and have lower energy requirements (Kaushik and Seiliez 2010). Therefore, fish fed with limiting or excess amount of protein or lipid would trigger AA catabolic and anabolic pathways to modulate protein accretion and meet species-specific nutrient and energetic requirements (NRC 2011). Because AA catabolism and anabolism control nitrogen metabolism and N isotope discrimination (O'Connell 2017) and consequently the excretion of lighter nitrogen and enrichment in ^{15}N in AA in consumer tissues, experimental designs should consider species nutritional requirements to evaluate variability in $TEFs_{AA}$.

Empirical studies on fish fed with a fixed amount of food or to satiation (a physiological process that results in the termination of food ingestion) can determine the quantity of macronutrients, and the quantity of protein assimilated, catabolized, and channeled to growth (Ritter 2004, Saravanan et al. 2012). The type of feeding regime can alter the catabolic activity of AAs and consequently influence isotopic discrimination. Fish- and invertebrate-based diets can be

considered high protein quality diets in terms of their AA profile relative to fish nutritional requirements and higher protein digestibility. In contrast, plant-based diets tend to have low-protein content and digestibility (NRC 2011). If fish are fed with plant-based diets, nutritional stress and macronutrient catabolism are triggered to support energy demands and growth, and fish often sustain only limited growth.

We conducted a meta-analysis that compiled teleost TEF_{AA} estimates from studies on captive teleosts subjected to controlled feeding experiments to evaluate the role of lipids and protein, diet type, feeding regime, life stages, and habitat type on AA $\delta^{15}N$ TEF variation for source and trophic AAs. We quantified the level of variation in AA-specific TEFs for teleosts in comparison with TEFs derived from multiple taxa to illustrate the potential error for estimating TP of natural animal populations using a universal TEF. Specifically, we assessed the relationship between TEF_{AA} and dietary protein (DP) and dietary lipid (DL) content, and DP and DL relative to taxon-specific estimates of protein and lipid requirement. We also evaluated whether TEF_{AA} varied as a function of diet type (fish, invertebrates, plant-based feeds), feeding regime (fixed vs. satiation), life stages (larvae, early juvenile, subadult, and adults), and aquatic habitat type (marine vs. brackish vs. freshwater). We hypothesized that TEFs of source AAs would not differ as a function of nutritional and ecological parameters since isotope discrimination is limited because transamination does not involve cleavage of a C–N bond (Chikaraishi et al. 2007, 2009; but note that deamination can lead to isotope discrimination; O'Connell 2017). For trophic AAs, TEFs should increase with high protein levels relative to protein requirements, because fish should catabolize excess dietary protein resulting in higher excretion of ^{15}N-depleted nitrogen (Martínez del Río and Wolf 2005). Since type of feeding regime and dietary protein sources influence the amount of food consumption and protein metabolic pathways, we hypothesized that satiation feeding regime and plant-based feeds would lead to higher AA isotopic fractionation and therefore higher $TEFs_{AA}$. In addition, TEF_{AA} should be lower for early life stages since larvae and early juveniles fed animal protein that meets AA nutritional requirements will assimilate protein efficiently that will be routed for growth (especially in muscle tissue), while AA catabolism and AA isotope discrimination should be reduced.

MATERIALS AND METHODS

We compiled literature of controlled laboratory feeding experiments reporting $\delta^{15}N$-AA values for fish muscle tissue and diets, from which TEFs were reported or could be calculated using published data (Appendix S1). Since TEF_{AA} varies between tissues (Nuche-Pascual et al. 2018), our compilation was limited to muscle, which is the most common tissue type analyzed for CSIA-AA. When TEFs were not reported, we calculated TEF_{AA} values as follows:

$$TEF_{AA} = \delta^{15}N\text{-}AA_{tissue} - \delta^{15}N\text{-}AA_{diet} \quad (2)$$

where $\delta^{15}N$-AA_{tissue} and $\delta^{15}N$-AA_{diet} represent the nitrogen isotopic composition of each AA in a consumer's muscle tissue and the diet, respectively (Popp et al. 2007).

In our meta-analysis, each TEF_{AA} value-obtained from an individual feeding experiment within a study was considered as an individual data point (similar to individual participant data that includes raw data from an individual specific for meta-analysis; Riley et al. 2010, Tierney et al. 2015), regardless of whether multiple TEFs were reported within a single study that conducted different dietary treatments. Hereafter, an experiment refers to a single feeding experiment with specific conditions from which a TEF_{AA} value is estimated. As in other meta-analysis of bulk and CSIA AA TEFs (McCutchan et al. 2003, Vanderklift and Ponsard 2003, McMahon and McCarthy 2016), TEFs reported within a single paper are not strictly independent in the sense that experimental conditions are more similar within rather than across studies. This is an inherent and common feature of meta-analyses. However, within a single controlled laboratory study, feeding experiments are considered statistically independent because fish are raised in different tanks and samples from each treatment are measured. When possible, we strengthen our inferences based on the comparison of the results of our meta-analysis with those of specific studies.

We selected experiments in which fish tissues reached isotopic equilibrium, which was evaluated based on author analysis or by estimating

the relative weight gain (WR) achieved during each feeding experiment. A minimum threefold increase in weight was considered as indicative of equilibrium (Herzka 2005, Nuche-Pascual et al. 2018). We included all the AAs reported in at least two studies, except for Thr, because this AA exhibits very depleted δ^{15}N values in contrast to the other AAs (Hare et al. 1991), and is not considered an adequate tracer for baseline or TP (Germain et al. 2013, McMahon et al. 2015). In addition, although recent studies on rats suggest Thr may be a good indicator of protein deficiency (Fuller and Petzke 2017), only six feeding experiments met our criteria for inclusion in the meta-analysis, which was insufficient for the meta-analysis.

Gly and Ser were originally classified as source AA (Popp et al. 2007), but no longer fit into this classification due to the large variability reported across multiple consumers: Gly (3.9‰ ± 4.9‰) and Ser (2.9‰ ± 4.6‰; McMahon and McCarthy 2016). Hence, we considered Gly and Ser metabolic AA. We place particular emphasis on Phe and Glu due to their prevalent use for estimating TP.

Nutritional characteristics included the dietary protein and lipid content reported in each study, and same content relative to species-specific protein and lipid requirements. We evaluated the relationship between protein and lipid content or TEFs AA by using regression analysis and compared our results with other feeding experiments on fish species that explicitly reported protein and lipid content (Appendix S3). Because some lipid extractions methods can change the natural abundance of N isotopic values in bulk tissues possibly due to the removal of lipoproteins (Sotiropoulos et al. 2004, Logan et al. 2008, Ruiz-Cooley et al. 2011), we report whether lipid extraction methods were used. However, we do not evaluate TEFs with and without lipid extraction because that is outside the scope of this study and requires a specific and controlled experimental design.

To examine the role of protein and lipid content relative to dietary requirements, each study dietary treatment was classified into one of the three categories: low, optimum, or high protein or lipid level relative to the species' requirements. A diet was considered to contain an optimum dietary protein level if it was within ±5% of the protein requirement reported in the literature (hence an optimum protein level reflects a 10% range in protein content). A 10% difference in dietary protein content results in a strong influence on growth performance in most fish nutrition studies (Catacutan et al. 2001, Nuche-Pascual et al. 2018). Diets classified as containing a high protein level had >5% protein content than a specific species' requirement and those with low-protein level had <5% or less protein content than the requirement. A diet was considered to contain an optimum lipid level when it contained within 3% of the lipid requirement reported in the literature, whereas a high and low lipid content had greater than and less than 3% of lipid requirement, respectively (Watanabe et al. 2001). Species-specific protein and lipid requirements were obtained from the literature when available. If unavailable, published genus or family-specific protein and lipid requirements were used (Appendix S3).

The feeding regime used in each experiment was classified as either fixed feeding rate, when a pre-established quantity of feed was provided or as satiation feeding. Food types used during feeding experiments were classified into three categories based on the predominant protein source: plant, invertebrate, or fish meal or tissue. Ecological factors included life stage and aquatic habitat. The life stage of the fish during feeding experiments was classified into four categories: larvae, early juvenile, subadult, and adult stages. Each species was classified as marine, brackish, or freshwater, based on their predominant habitat.

We evaluated differences between teleost TEF_{AA} values with those calculated based on reviews of multiple taxonomic groups (mold, bacteria, fungus, insects, crustaceans, mollusks, amphibians, reptiles, teleosts, elasmobranchs, and mammals) and derived exclusively from feeding experiments as reported by McMahon and McCarthy (2016; $n = 73$). Values for multiple taxonomic groups are referred to in our study as global TEF_{AA}. Our analysis included a total of 32 teleost–prey discrete feeding experiments (some from the same study) that encompass 23 additional feeding experiments (and hence TEFs) than those considered by McMahon and McCarthy's (2016). In addition, we calculated average non-teleost TEF_{AA} from McMahon and

McCarthy (2016)'s data set for comparison with our estimates for teleost.

Statistical analyses

For comparative purposes, both the mean and median TEF for each AA were estimated for each source and trophic AA (Fig. 1). The mean and median are both central tendency indicators (Miller 1991), and although the mean and SD are the more commonly used indicators, they are sensitive to outliers. Errors were calculated for the mean (SD) and the median value (median absolute deviation; MAD). The calculation of SD assumes a normal distribution and values are influenced by sample size. In contrast, the median is not influenced by outliers, and the MAD does not assume a normal distribution and it is not influenced by the sample size (Leys et al. 2013). Given that the sample size TEFs for some AAs, such as Met and Ser, was limited, the median was considered a robust central tendency

indicator for comparison with mean values. Median absolute deviation is defined as the median of the absolute deviations from the overall median (Huber 1981) and was estimated following Leys et al. (2013):

$$MAD = b \times M_i(|x_i - M_j(x_j)|) \quad (3)$$

where x_i refers to each of the original observations (i.e., the TEF_{AA} values from each feeding experiment), x_j refers to the number of observations (i.e., the number of TEFs included in the estimate), and M_i is the median, M_j is defined as the absolute value of $(x_i - M_i)$. The constant $b = 1.4826$ is applied when the data have an underlying normal distribution (Rousseeuw and Croux 1993), or $b = 1/Q$ (0.75) in cases when the data are non-normal (normality was previously evaluated for each TEF_{AA} in separate tests) and where $Q(0.75)$ represents the value of the third quartile (Huber 1981). Only TEFs of Phe, Gly, Asp, and Leu were not normal. Mean and
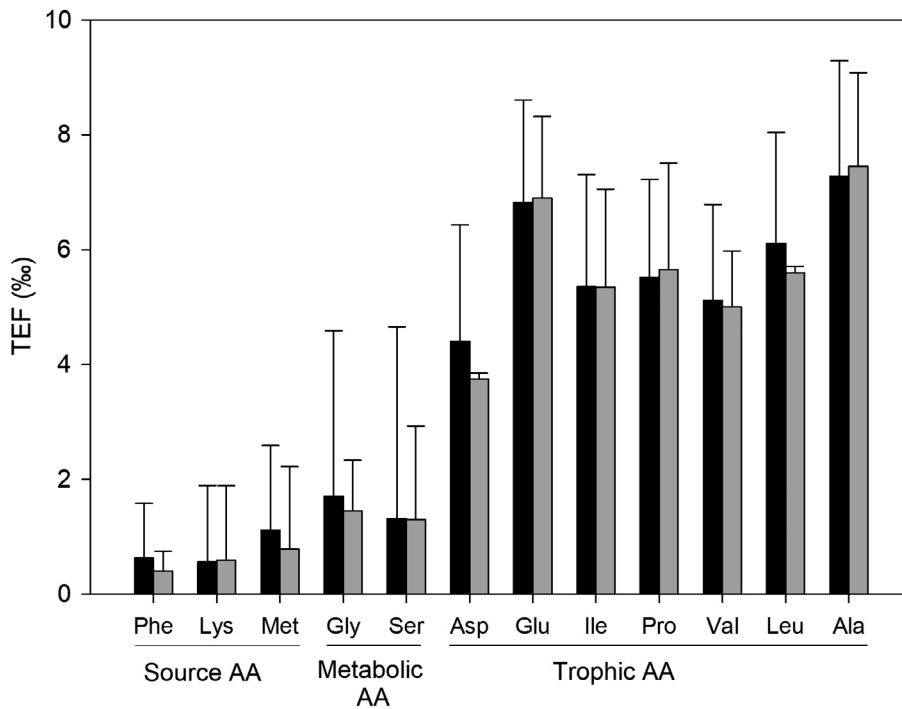

Fig. 1. Mean (black bars) and median (gray bars) trophic enrichment factors (TEFs) for amino acids (AAs) measured in fish muscle tissue of all studies included in the meta-analysis. Raw data are reported in Appendix S1, S2. Errors are represented as standard deviation and median absolute deviation for mean and median values, respectively. Phe, phenylalanine; Lys, lysine; Met, methionine; Gly, glycine; Ser, serine; Asp, aspartic acid; Glu, glutamic acid; Ile, isoleucine; Pro, proline; Val, valine; Leu, leucine; Ala, alanine.

median values, as well as SD and MAD, were similar for most TEFs of source and trophic AAs, indicating that there were few extreme values influencing mean TEFs. Hence, to report our results we refer solely to the mean values.

Regression analysis was used to examine the relationship between TEF_{AA} and dietary percent protein and lipids for each AA. Levene's test was used to test the homogeneity of variance between source AA and trophic AA (separate tests). Since the assumption of homogeneity of variance was met, one-way ANOVAs were used to test for differences in mean TEF_{AA} for each factor. Statistical analyses were carried out using STATISTICA V 7, TIBCO Software, Palo Alto, California, USA. Categories with only one TEF_{AA} value (i.e., $n = 1$) were included in graphs for comparative purposes only but excluded from statistical analyses.

Levene's test was used to test for homogeneity of variances between global TEF, non-teleost TEFs, and our teleosts TEFs for each AA. A Student t test was applied to test for differences in mean TEFs when the variances were homogeneous, and a non-parametric statistical test was used when variances were not homogeneous. Standard deviations were calculated and compared among taxonomic groups.

RESULTS

We found nine studies published between 2009 and 2018 that included 11 teleosts consumer species and 32 individual consumer–diet feeding experiments reporting a total of 236 AA-specific consumer–diet relationships (Appendix S2, S3). We compiled TEFs for the five AAs initially classified as source AAs (Phe, Lys, Met, Gly, and Ser) and seven trophic AAs (Asp, Glu, Ile, Pro, Val, Leu, and Ala; Appendix S2). The number (n) of TEF_{AA} estimates in published studies varied (Phe = 31, Lys = 18, Met = 11, Gly = 20, Ser = 9, Asp = 17, Glu = 32, Ile = 18, Pro = 20, Val = 20, Leu = 20, Ala = 20). Box plots and individual TEFs for each AA are included as Appendix S2. Only the TEFs for Glu were reported in all studies and experiments.

Dietary protein and lipid content

The dietary protein content for all feeding experiments ranged from 8% to 71% of the diet (Appendix S3). A single feeding experiment included a very low-protein content (8%) treatment; the other experiments had 40% or higher protein in the diets. The mean TEFs for each of the seven trophic AAs were highest for fish fed with the lowest (8%) protein treatment than those fed with ≥40% protein diets. However, regression analyses between protein percent and each source and metabolic AA TEFs were not significant (Fig. 2a). In contrast, regression analysis between dietary protein content and TEF values for Glu and Ala was significantly and negatively related ($P = 0.011$ and $P = 0.027$, respectively; Fig. 2b), and regressions for the other five trophic AAs were not significant ($P > 0.05$). Percent of dietary lipid used in the feeding experiments ranged between 2% and 24% of the diet, which represents a broad range relative to fish nutritional requirements (Miller et al. 2005). We found no significant relationships between lipid content and either source, trophic, or metabolic AA TEFs (Fig. 3).

On a species-specific level and focusing solely on studies that fed fish with diets varying in protein content, significant negative relationships between TEFs and dietary protein were found for Lys ($R^2 = 0.95$, $P = 0.025$, $TEF_{Lys} = 3.12 - 0.02 \times \%protein$), Glu ($R^2 = 0.95$, $P = 0.024$, $TEF_{Glu} = 11.37 - 0.07 \times \%protein$), Ile ($R^2 = 0.94$, $P = 0.029$, $TEF_{Ile} = 9.81 - 0.06 \times \%protein$), Pro ($R^2 = 0.91$, $P = 0.044$, $TEF_{Pro} = 7.42 - 0.01 \times \%protein$), and Leu ($R^2 = 0.99$, $P = 0.003$, $TEF_{Pro} = 10.53 - 0.07 \times \%protein$) using data from mummichog (McMahon et al. 2015), but significant relationships were not found for Pacific yellowtail (Nuche-Pascual et al. 2018) nor totoaba (Barreto-Curiel et al. 2018). With regard to dietary lipid content, on a species-specific level significant relationships were only found between percent lipid and TEFs of Met ($R^2 = 0.66$, $P = 0.049$, $TEF_{Met} = -2.20 + 0.24 \times \%lipid$) for yellowtail (Nuche-Pascual et al. 2018). There were no relationships between dietary lipid content and TEFs in the experiments conducted on mummichog.

Dietary protein and lipid content relative to nutritional requirements

The $\delta^{15}N$ TEF_{AA} values were relatively variable among categories of AA and nutritional requirements (high, optimum, or low; Fig. 4). The mean of TEF_{Ser} exhibited the highest value (3‰) for

Fig. 2. Nitrogen trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids (AAs) in muscle of fish fed different levels of dietary protein. TEF$_{AA}$ values are represented individually for each consumer–diet combination. Only significant regressions are plotted.
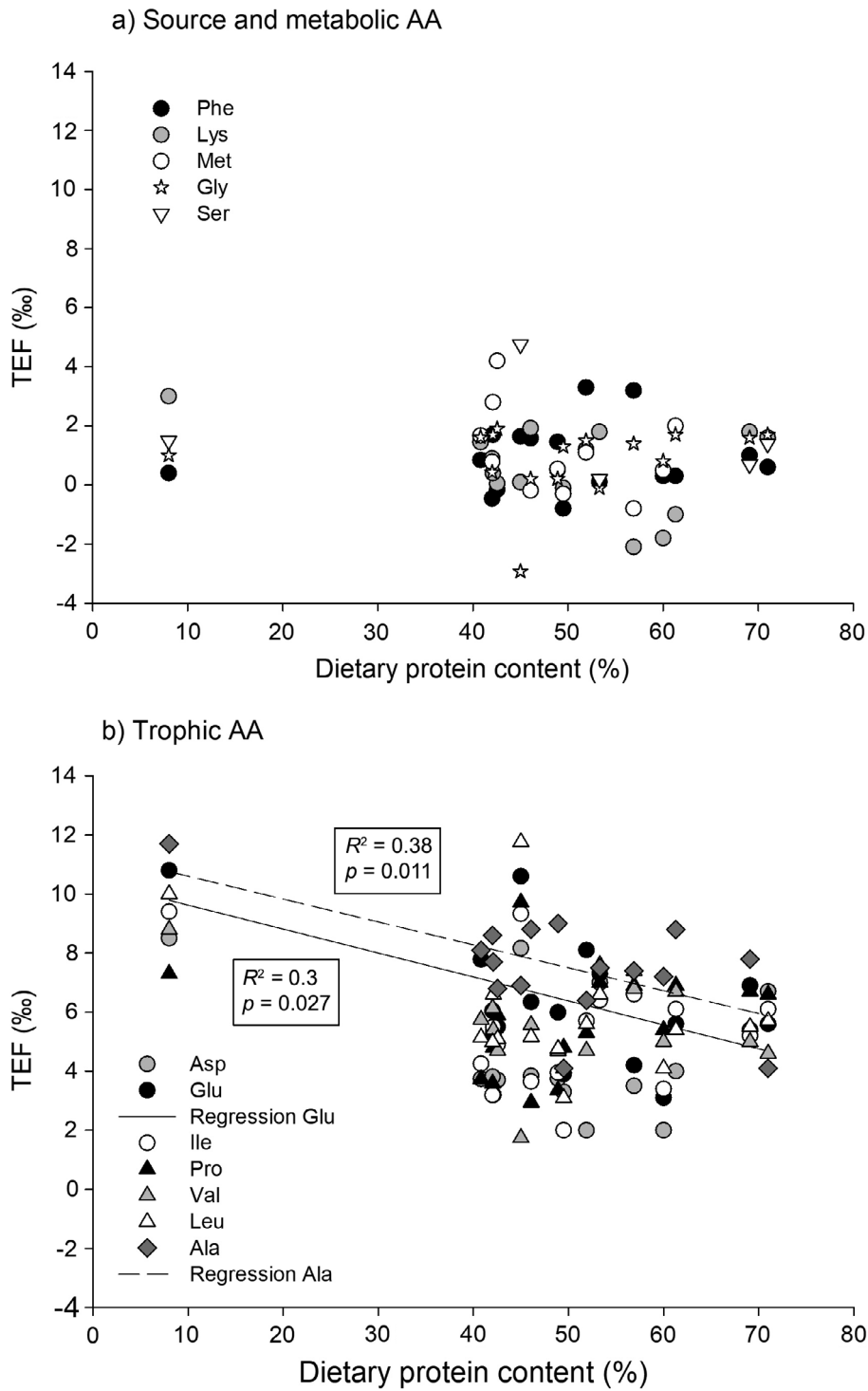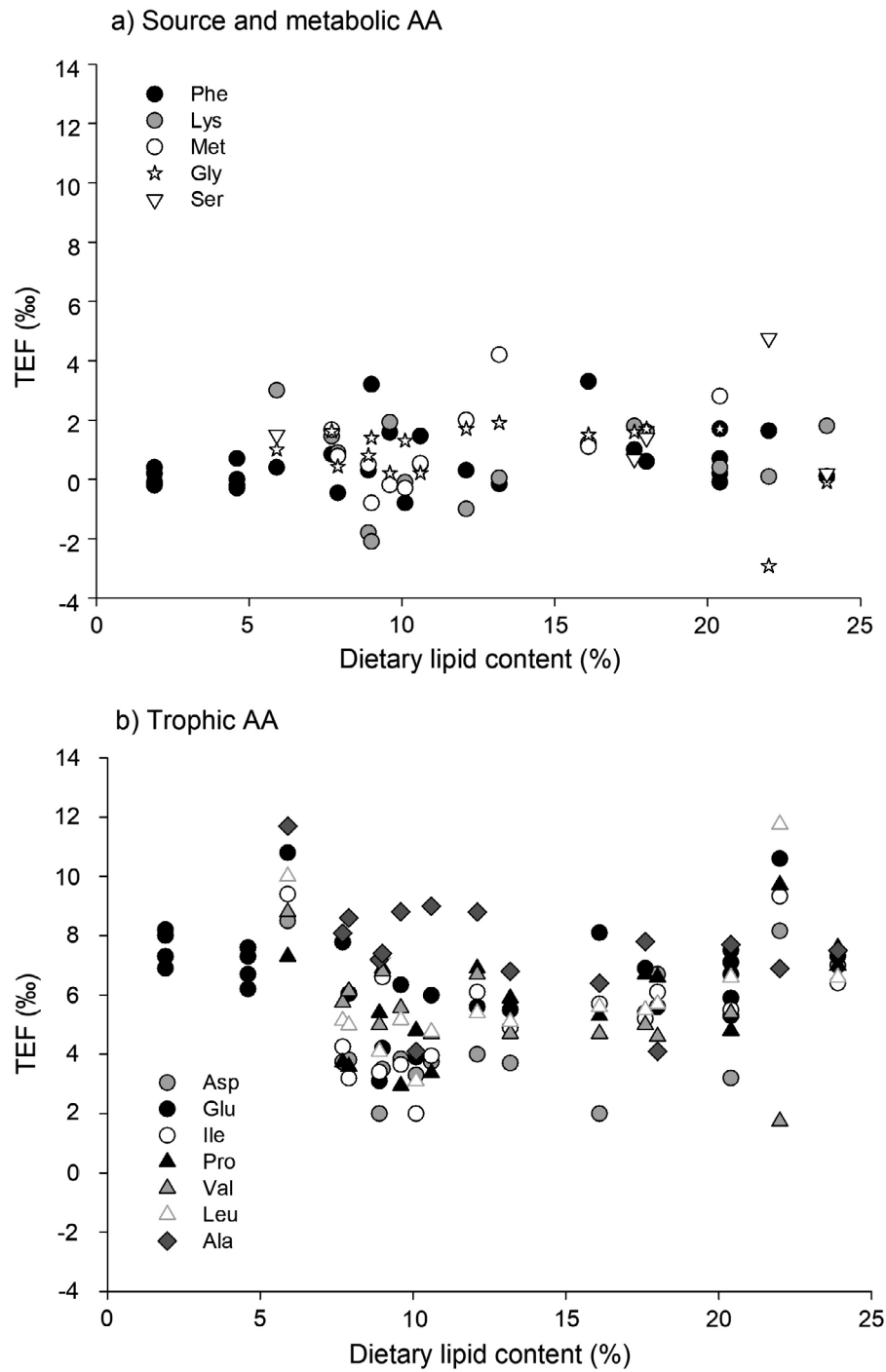
Fig. 3. Nitrogen trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids (AAs) in muscle of fish fed different levels of dietary lipid content. TEF_{AA} values are represented individually for each consumer–diet combination. Regression analysis yielded no significant relationships between percent dietary lipids and AA-specific TEFs.

Fig. 4. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish fed different dietary protein content relative to taxon-specific protein requirements. Error bars represent the standard deviation. The number of TEF estimates included in each mean is presented above the error bars. The optimum level is ±5% species-specific protein requirement, the low level is ≤5% species-specific protein requirement, and the high level is ≥5% species-specific protein requirement.

the low-protein content category and the lowest TEF_{Ser} (0.2‰) for the optimum protein content category. TEF_{Lys} was the only source AA that showed a negative TEF among source AAs. Specifically, TEF_{Lys} was −0.3‰ in fish-fed diets with a high protein level and showed positive values for the low and optimum protein levels (1.0‰ and 1.1‰, respectively). However, due to the high variability in TEFs within each category of nutrient requirements, there were no significant differences in mean TEF_{AA} values among high, optimum, or low-protein content relative to requirement, including lysine ($P > 0.05$). The mean TEF of Phe remained consistent regardless of protein level (0.7‰, 1.1‰, and 1.1‰ for the low, optimum, and high protein levels, respectively; Fig. 4a).

Among trophic AAs, Asp showed the lowest mean TEF for each of the three dietary protein level categories (5.2‰, 4.0‰, and 4.3‰ for low, optimum, and high protein diets, respectively). Only TEF_{Glu} decreased as dietary protein level increased (from 7.7‰ to 5.1‰; Fig. 4b), although differences were not statistically significant (ANOVA, $F = 166.9$, df $= 13.0$, $P = 0.130$).

The relationships between AA TEFs values and lipid content relative to nutritional requirements showed clear patterns of variation for some source and trophic AAs. Among the source AAs, only TEF_{Lys} varied significantly in fish fed with diets of low to high dietary lipid content categories (ANOVA, $F = 3.9$, df $= 13.0$, $P = 0.47$); there was a 1.8‰ difference between lipid content categories for the mean TEF Lys (Fig. 5a). Among trophic AAs, no significant differences were observed in TEFs among categories.

Feeding regime

Among all the studies considered in our meta-analysis, very few (only 2.5%) used a fixed feeding regime and 97.5% used a satiation feeding regime (Appendix S2). For source AA, TEF_{Lys} differed significantly between those two feeding regimes. The TEF_{Lys} of fish fed a fixed feeding regime was significantly lower than under satiation ($F = 11.6$, $P = 0.004$; Fig. 6a). The metabolic AA Gly and Ser did not exhibit significant differences. Among trophic AAs, the TEFs of Asp and Glu exhibited significant differences between fixed and satiation feeding regimes. In both cases, the TEFs values from feeding experiments using a fixed feeding protocol were significantly lower ($F = 8.33$, $P = 0.012$ for TEF_{Asp}, and $F = 10.46$, $P = 0.003$ for TEF_{Glu}; Fig. 6b).

Diet type

Most feeding experiments (177 out of 236) used fish protein as the main component of the teleost diets, while 48 used invertebrates and only 11 (one per AA) incorporated plants as the main protein source (Appendix S3). Comparisons with TEFs for plant-based diets are therefore purely qualitative.

Mean TEF_{Phe} was similar between diet types (0.7‰ and 0.4‰ for fish fed fish and invertebrate-based diets, respectively) and did not differ significantly. There were no TEF_{Met} reports for invertebrate-based feeds. TEF_{Gly} showed significant differences between experiments conducted with fish or invertebrate-based diets ($F = 7.87$, $P = 0.012$); the mean TEF with the fish-based diets was 4‰ lower (Fig. 7a). The TEFs of trophic AAs did not show significant differences between fish- and invertebrate-based diets, but the mean TEFs of Asp, Glu, Ile, and Pro ranged from 3.9‰ to 8.5‰, from 6.4‰ to 10.8‰, from 5.0‰ to 9.4‰, and from 5.2‰ to 7.3‰, respectively, when comparing fish-based diet to a plant-based diet (Fig. 7b).

Life stage

Of the feeding experiments, 79% were conducted on early-juvenile fishes. Among source AAs, only TEF_{Gly} showed significant differences between the larval and early-juvenile life stages ($F = 24.6$, $P = 0.0003$); the TEF_{Gly} for larvae was significantly higher (8.1‰) than for early juvenile (0.9‰; Fig. 8). TEF_{Ser} exhibited mean negative values for the subadult (−4.2‰) and adult (−1.3‰) stages, although these data were not included in the statistical analysis because they were single values. For trophic AAs, there were no significant differences in TEF_{AA} between larvae and juveniles, and mean values for specific AA varied by less than 3.2‰.

Aquatic habitat

Most of the feeding experiments (157 out of 236) were conducted on fish species from marine habitats, and 24 feeding experiments were on freshwater fishes. Differences in the TEF of Phe and Ser were not significant. TEFs of Phe were
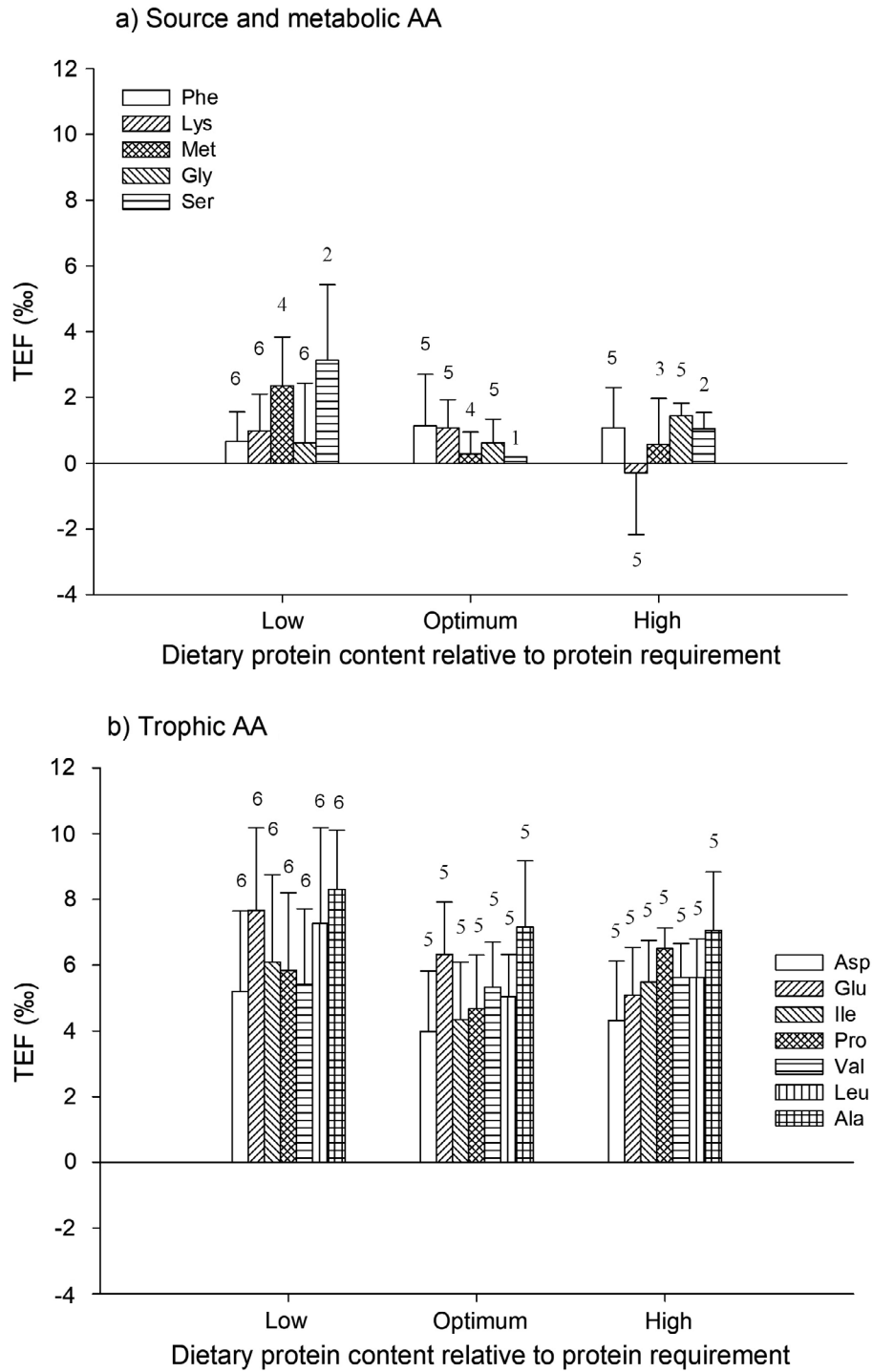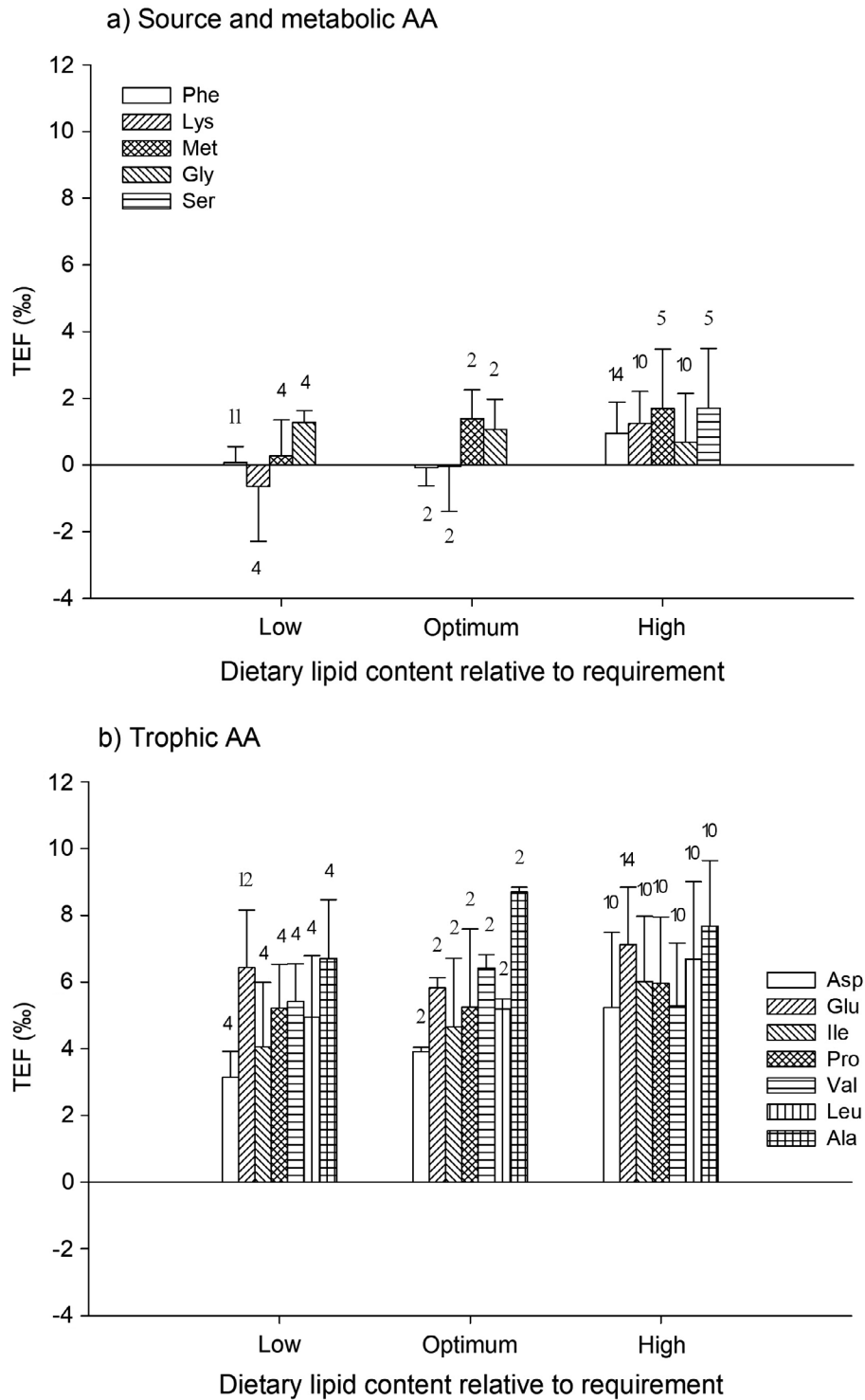
Fig. 5. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish fed different dietary lipid content relative to taxon-specific requirement. Error bars represent the standard deviation. The number of TEF estimates included in each mean is presented above the error bars. The optimum level is ±3% species-specific lipid requirement, the low level is ≤3% species-specific lipid requirement, and the high level is ≥3% species-specific lipid requirement.

Fig. 6. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish fed different feeding regimes. Error bars represent the standard deviation. The number of TEF estimates included in each mean is presented above the error bars.

Fig. 7. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish feed different diet types. Error bars represent the standard deviation. The number of TEF estimates included in each mean is presented above the error bars.
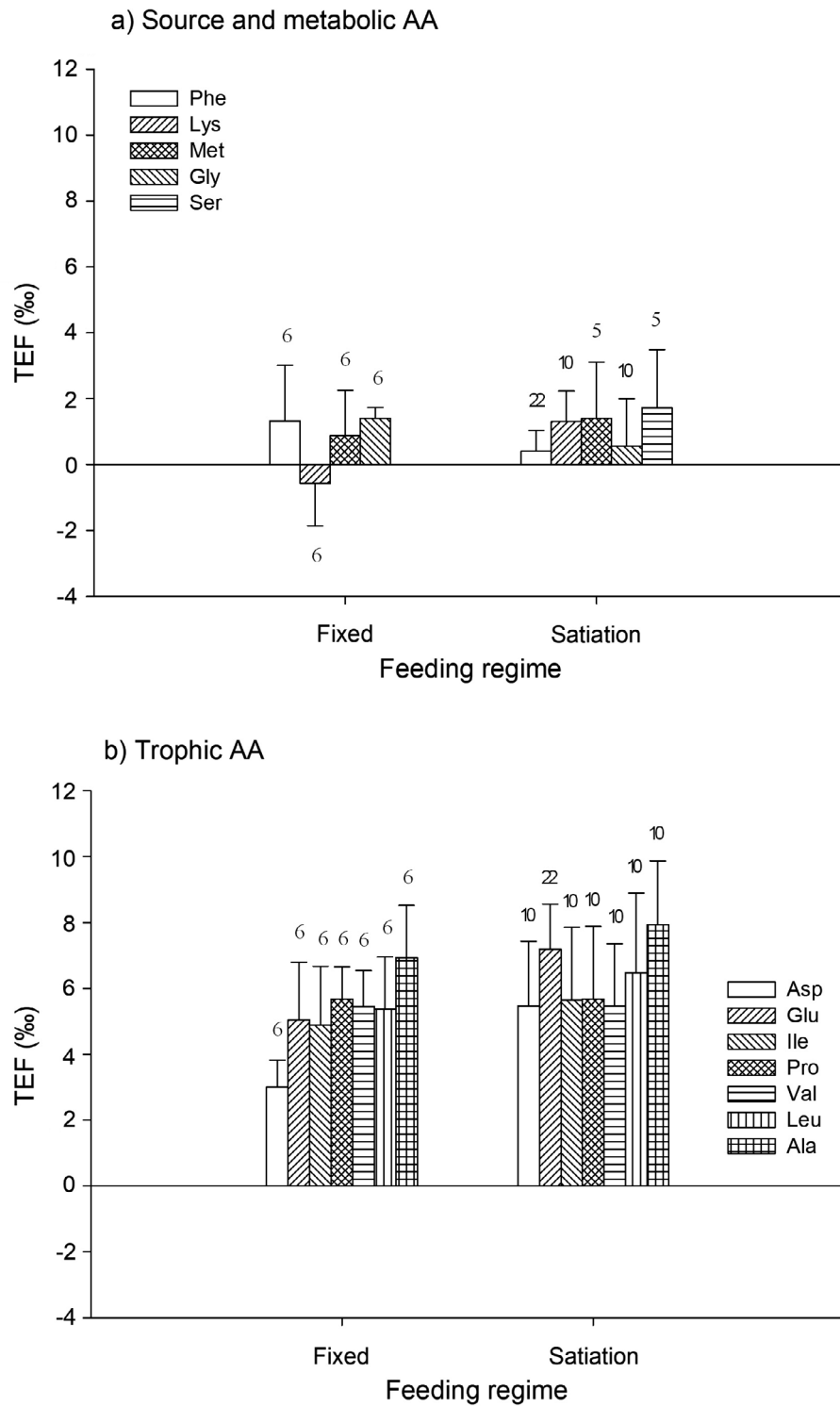
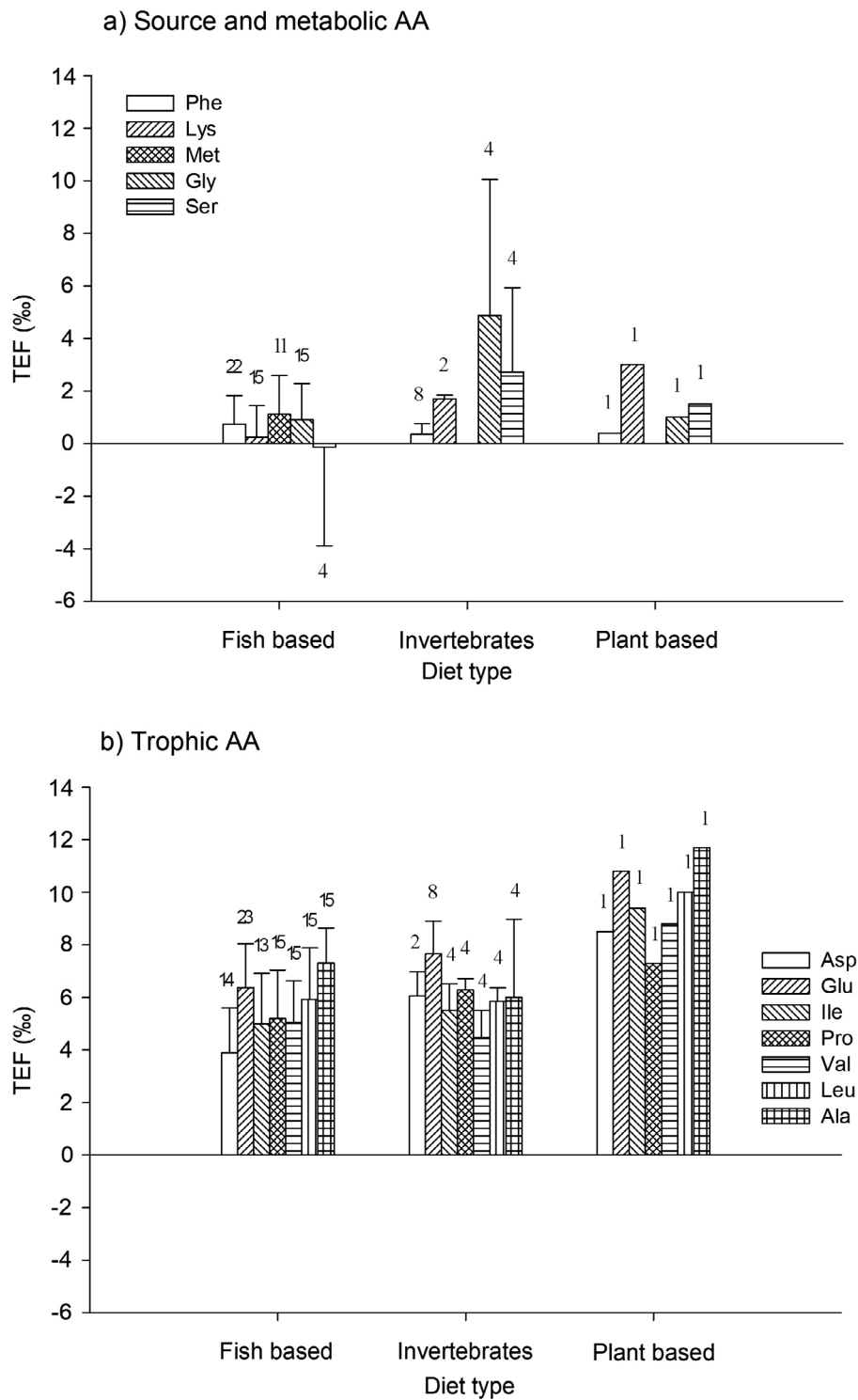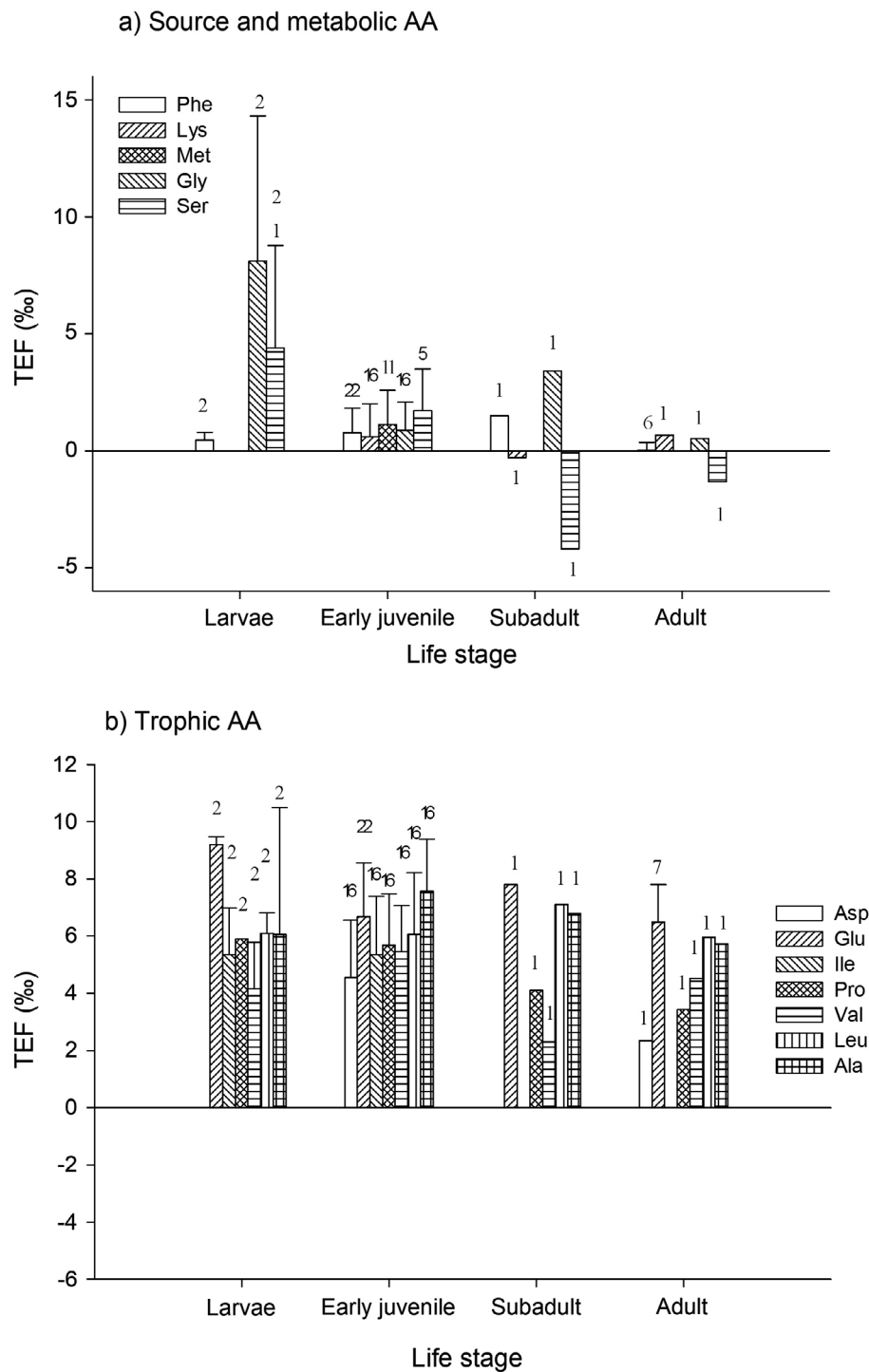Fig. 8. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish of different life stages. Error bars represent the standard deviation of TEF_{AA} values. The number of TEF estimates included in each mean is presented above the error bars.

1.0‰, 0.7‰, and 0.2‰ for fish from marine, brackish, and freshwater habitats, respectively. The mean TEF of Lys was significantly different (by 1.6‰) between fish from marine vs. brackish habitats ($F = 6.14$, $P = 0.025$); marine fishes exhibited less isotope discrimination (Fig. 9a). The mean TEF of the canonical trophic AA Glu differed between fish from marine (6.1‰), brackish (8.2‰), and freshwater (7.1‰) habitats, but did not differ significantly. The mean TEF of Asp of marine fishes (3.3‰) was significantly lower ($F = 66.3$, $P = 0.0002$) than that of fishes inhabiting brackish habitats (7.2‰). The TEFs of Ile ($F = 10.6$, $P = 0.006$), Pro ($F = 15.13$, $P = 0.0012$), and Leu ($F = 7.9$, $P = 0.0117$) also differed significantly between marine and brackish habitats; marine TEFs had lower values for all trophic AAs (4.6‰, 4.9‰ and 5.5‰ for Ile, Pro, and Leu vs. 7.3, 7.5, and 7.9 for Ile, Pro, and Leu, respectively; Fig. 9b).

Comparison of teleost, global, and non-teleost TEFs

The mean TEF_{Phe} (0.6‰) for teleost was higher than the mean global TEF_{Phe} (0.0‰) (Fig. 10a; Appendix S4), but did not differ significantly. The mean TEF_{Lys} (mean ± SD, 0.6‰ ± 1.3‰) for teleosts was significantly lower than the global mean (0.9‰ ± 1.9‰, $Z = 3.064$, $P = 0.002$). The means and SDs of TEF_{Met} did not differ significantly when comparing teleosts (1.1‰ ± 1.5‰), global (1.2‰ ± 2.1‰), and non-teleost (1.2‰ ± 2.1‰). Statistical differences were found between teleost mean TEF_{Lys} (0.6‰ ± 1.3‰) and the global mean (TEF = 0.9‰ ± 1.9‰, $Z = 2.6$, $P = 0.009$) as well as between the mean TEF_{Lys} for teleosts and non-teleosts (TEF = 0.5‰ ± 1.6‰, $Z = 3.064$, $P = 0.002$). The mean and SD of TEF_{Gly} were statistically different among the three categories ($Z = 2.5$, $P = 0.014$).

Among trophic AAs, only the mean TEFs of Asp, Pro and Leu were significantly different between teleosts and non-teleosts ($Z = 2.9$, $P = 0.004$, $Z = 2.3$, $P = 0.020$, and $Z = 2.5$, $P = 0.014$, respectively); means were higher for teleosts.

The SD of the mean for Phe, Lys, and Met was lower when considering only teleosts, and higher but relatively similar when comparing global and non-teleost values that average over various taxonomic groups (Fig. 10b). Gly and Ser, the two AA

classified as metabolic, had the highest means and SD, and the largest differences between mean TEFs for teleost, global, or non-teleosts (>2.0‰; Fig. 10a), but means did not differ significantly. The SD for teleost TEFs of trophic AA was generally 1.5–2.0‰ lower when considering teleosts vs. global values. Asp had similar SD when comparing teleosts and non-teleosts (~2.0‰), while the SD for Glu, Ile, Pro, Val, Leu, and Ala showed lower variability when considering only teleosts compared with non-teleosts.

DISCUSSION

Influence of macronutrients and feeding regime

The lack of significant linear relationships between source and metabolic TEF_{AA} and percent protein suggests that source AA TEFs are not dependent on dietary protein content, despite the broad range of protein levels (8–71%) evaluated. TEFs of source and metabolic AAs also did not differ significantly between diets classified as containing low, optimum, or high protein content. This is consistent with previous studies that found no differences in source AA TEFs with protein content, possibly due to similarity in deamination processes between terrestrial and aquatic consumers from different trophic levels (Popp et al. 2007, Chikaraishi et al. 2009, 2015, McMahon and McCarthy 2016, O'Connell 2017). However, species-specific studies on teleosts have yielded differences in source AAs TEFs vs. protein level (see Appendix S3; McMahon et al. 2015, Barreto-Curiel et al. 2018, Nuche-Pascual et al. 2018). This suggests that other factors contribute to the TEFs variability and that these relationships are likely AA-specific. In addition, the variability inherent to a meta-analysis may mask the proposed relationship between protein content and isotope discrimination, and co-variation of protein quantity and quality in experimental dietary studies can obscure the effect of specific macronutrients on TEF_{AAs}. Importantly, our analysis does show a broad dispersion in TEF $\delta^{15}N$ values relative to percent protein (Fig. 2), which suggests that other factors contribute to the variability.

The mean TEF of Phe was consistent among protein content categories (~1‰), but variability of up to 2‰ was detected. Phe can follow different pathways that may or may not break N
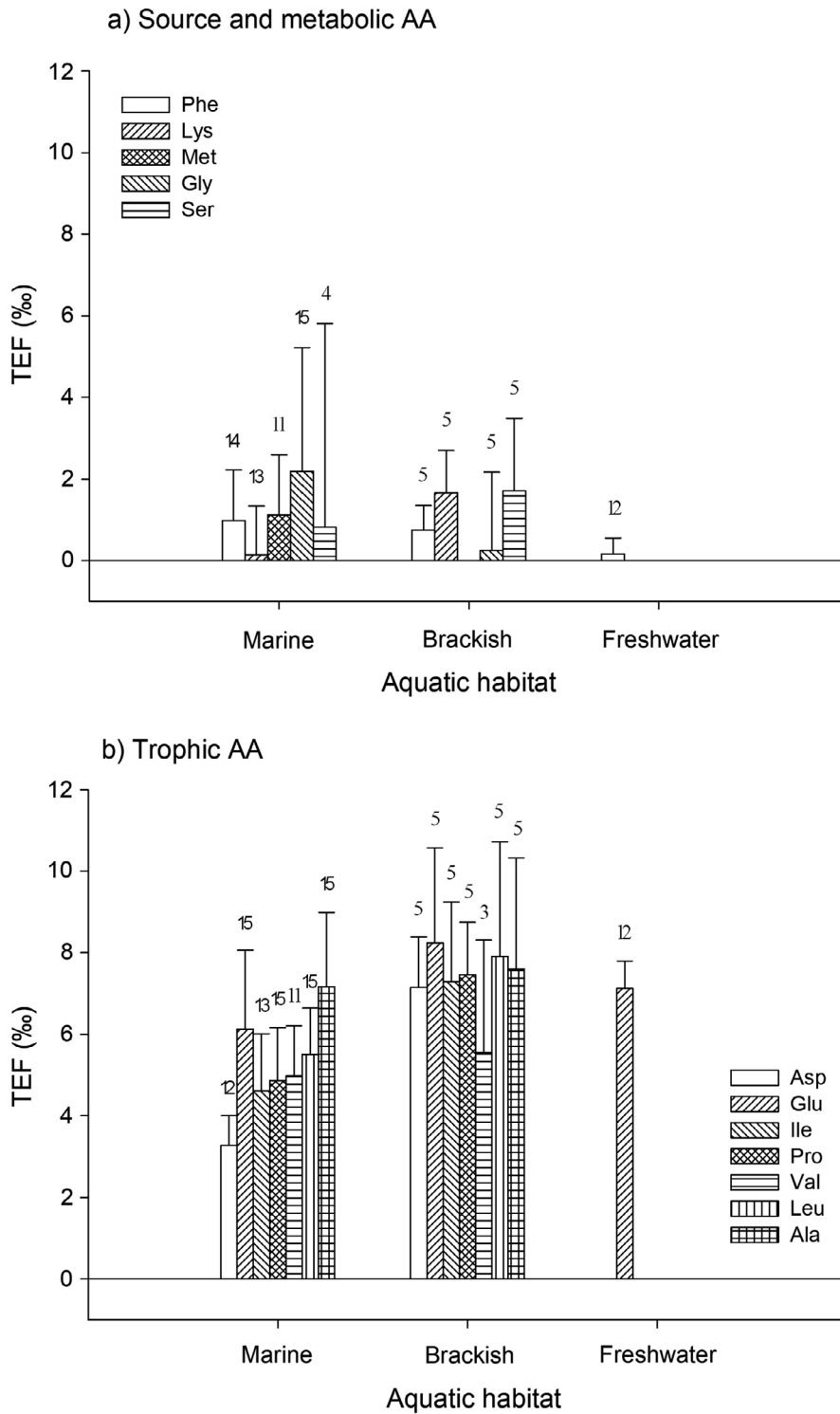
Fig. 9. Mean trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids for fish that differ in their dominant habitat. Error bars represent the standard deviation. The number of TEF estimates included in each mean is presented above the error bars.
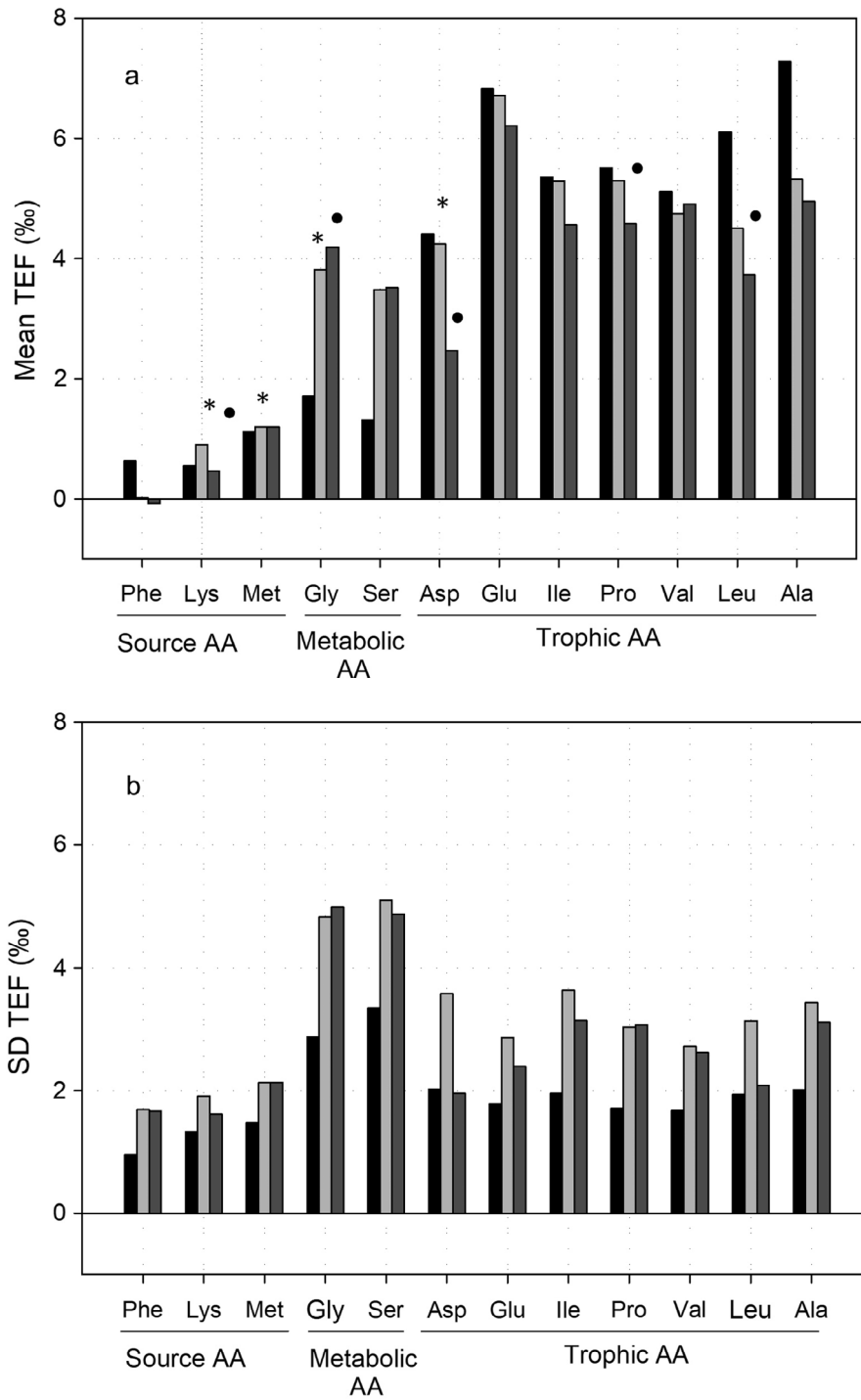
Fig. 10. Mean (a) and SD (b) trophic enrichment factors (TEFs) of source, metabolic, and trophic amino acids (AAs) derived from teleosts (black bars), global values means estimated from McMahon and McCarthy (2016; light gray bars) and non-teleosts means calculated from McMahon and McCarthy (2016; dark gray). The asterisks and the solid circles in (a) represent significant differences between mean teleosts vs. mean global values and mean teleosts vs. mean non-teleosts values, respectively.

bonds (O'Connell 2017). In addition, the nutritional requirements of the aromatic AA Phe and Tyr are closely coupled in fishes; Tyr requirements can be met by conversion of Phe to Tyr (Wilson and Halver 1986) and hence the N in Phe can be transferred to Tyr, which could lead to differences in δ^{15}N-Phe and hence TEFs. Whatever the biochemical mechanism underlying the limited but important differences in TEF_{Phe}, there are clearly inconsistent relationships between TEF_{Phe} and protein levels relative to nutritional requirements at the meta-analysis and species-specific levels. The specific nutritional conditions leading to the limited (but detectable) variation in isotope discrimination of Phe remain unclear and warrant further experimental work.

The negative relationship between the TEF of Glu and Ala and percent protein we observed was largely driven by the high TEFs of the low-protein (8%) diet in McMahon et al. (2015); the estimated protein requirement for mummichog is 52% (Prinslow et al. 1974). McMahon et al. (2015) also reported negative relationships between the TEFs of Glu, Ala, Ile, Pro, and Leu and percent protein. Other studies have not found a significant relationship between the TEFs of Ala, Ile, Pro, and Leu and protein content (Barreto-Curiel et al. 2018, Nuche-Pascual et al. 2018); these studies considered a narrow range of protein content for carnivorous fishes based on their species-specific nutritional requirements. The discrepancy may be related to nutritional and metabolic stress induced when fish are fed with exceedingly low and insufficient dietary protein content diets (Schreck et al. 2001), which may have triggered high transamination and deamination of both endogenous and exogenous AAs to meet fish energetic requirements (Goto et al. 2018), or to compensate for AA imbalances (Li et al. 2009, McMahon et al. 2015) resulting in high TEFs of trophic AAs.

It is important to note that in the wild, it would be very unlikely that an omnivorous fish would feed and survive solely on an exceedingly low-protein diet that does not meet protein requirements. For example, mummichog feed on a wide variety of prey, including benthic diatoms, plant detritus, and invertebrates such as amphipods, copepods, insects, ostracods, and chironomids (McMahon et al. 2005, James-Pirri et al. 2011). Hence, the exceptionally high TEFs

reported by McMahon et al. (2015) for fish fed a very low-protein diet should not be applied to omnivorous or carnivorous species. Glu TEFs were significantly higher in the study that fed mummichog a plant-based, low-protein category diet compared with higher protein diets (McMahon et al. 2015). This was not observed in other species-specific studies with a more limited range of protein levels (Barreto-Curiel et al. 2018, Nuche-Pascual et al. 2018, Appendix S3). Because Glu is central to AA metabolism and is involved in the transamination of many AAs (Cammarata and Cohen 1950, O'Connell 2017), we suggest that higher mean TEF_{Glu} at lower protein contents may result from fish catabolizing higher amounts of endogenous Glu (possibly from muscle tissue) to meet energy and growth requirements (Goto et al. 2018), which would lead to higher isotope discrimination.

No significant correlations were found between TEF_{AA} of Phe and lipid content and categories relative to requirements. Our results agree with those of Blanke et al. (2017) and Barreto-Curiel et al. (2018). Dietary lipid content has a direct effect on protein metabolism because its availability (relative to requirements) is directly related to protein metabolism; fish fed a diet limited in lipids will use protein as an energy source (see review by Kaushik and Seiliez 2010, NRC 2011) leading to deamination and consequently N isotope discrimination. However, Phe TEFs do not appear to be sensitive to dietary lipid content, at least within the range of lipid levels examined.

For Lys, in contrast, TEFs were smaller at the lower lipid category, which was also reported in Nuche-Pascual et al.'s (2018) species-specific study (all of McMahon et al.'s 2015 treatments fell into the high lipid category). Because Lys is involved in the synthesis of carnitine, which transports long-chain fatty acids from the cytosol into the mitochondria in mammalian and fish tissues (Vaz and Wanders 2002, Li et al. 2009), higher dietary lipid content may require more carnitine triggering the catabolism of Lys, increasing excretion of light nitrogen and resulting in a higher TEF of consumer tissues.

The δ^{15}N values from TEF_{Glu} and the other trophic AAs did not vary significantly among low, optimum, and high lipid content relative to requirements. In contrast, Nuche-Pascual et al.

(2018) and Barreto-Curiel et al. (2018) did find significantly higher TEF_{Glu} for lower lipid content diets. Hence, although our meta-analysis suggests lipid content has little effect on trophic AAs fractionation, specific studies suggest otherwise. Future research should evaluate the role of protein to energy ratios and NFE and TEF_{AA}.

We anticipated that feeding regimes would affect trophic AAs. Our meta-analysis indicates that Lys TEFs varied in response to feeding regime; mean TEF_{Lys} was significantly higher in fish fed to satiation, suggesting a greater degree of metabolic activity under higher feed consumption. Among trophic AAs, only Asp and Glu differed significantly. Although this meta-analysis necessarily includes data from different species and experimental conditions, it appears that at least Lys, Glu, and Asp are sensitive to feed intake. Because fish fed a fixed feeding regime cannot increase protein consumption to compensate for essential AA or energy deficiencies, dietary AAs would likely be routed to active metabolic tissues like muscle to sustain protein accretion (particularly in fast-growing juveniles; NRC 2011). Efficient dietary AA routing would limit catabolism and N isotope discrimination (O'Connell 2017). For natural populations, this could imply that the level of isotope discrimination of some AA may be sensitive to ingestion rates, although this remains to be evaluated.

TEFs and ecological factors

We found similar mean TEF_{Phe} among the three diet types. Within a category, TEF_{Phe} varied by less than 2‰ (mean SD ~1‰), despite the expected underlying differences in feed AA profiles. Fish- and invertebrate-based diets had a larger sample size and hence the results of the meta-analysis are more robust for these two diet types. The results support the use of Phe as proxy for food web baseline values due to its limited isotopic fractionation despite drastic differences in protein sources and the consequent changes in AA profiles and digestibility (i.e., food quality).

In our meta-analysis, Lys TEFs did not differ significantly between fish and invertebrate diets due to high isotopic variability within each group. However, in McMahon et al. (2015), the plant-based diet yielded a higher TEF_{Lys} (3.0‰) than for fish or invertebrate-based diets (~1.6–

1.8‰). Since vegetable-based diets tend to be limited in Lys, the higher TEF_{Lys} likely reflect a nutritional deficiency and higher catabolism of endogenous protein, which would lead higher isotope discrimination due to the excretion of light nitrogen.

Mean TEF_{Gly} was significantly higher (4.9‰) in fish fed with invertebrate-based diets (mainly driven by results from Chikaraishi et al. 2009 for fish larvae) compared with fish-based diets (0.9‰). High mean TEF_{Gly} values and variability (SD; 3.9‰ ± 4.9‰) were also reported by McMahon and McCarthy (2016) in their meta-analysis. Gly was formerly classified as source AA but its high variability in isotopic fractionation has led to questions regarding its potential as a robust tracer of the isotopic baseline in food webs (Nielsen et al. 2015, McMahon and McCarthy 2016). Our results also support Gly unsuitability as a source AA.

Glu TEFs were slightly higher for fish fed an invertebrate-based (7.7‰) vs. fish-based diets (6.4‰), although differences were not significant. The incorporation and isotopic routing of Glu are likely more efficient in carnivorous and omnivorous fish fed with high-quality digestible animal proteins such as those found in fish and invertebrates, leading to similar levels of isotope discrimination due to more limited AA reworking (McMahon and McCarthy 2016). Considering all trophic AAs, higher TEFs were observed for Asp, Glu, Ile, Pro, Val, Leu, and Ala when an omnivorous fish was fed plant-based diet, although this comparison is purely qualitative due to a low *n*. Aquaculture studies indicate that carnivorous and omnivorous fish fed a low-protein plant-based diet with lower digestibility provides insufficient AA to meet energy and nutrient requirements (NRC 2011, Saravanan et al. 2012). Carnivorous and omnivorous fish also have a limited ability to digest plant protein; when fed a low-protein diet, fish boost feed intake (Beltrán et al. 2009). This would increase AA metabolism and lead to the excretion of light nitrogen and higher isotope discrimination (McMahon and McCarthy 2016, O'Connell 2017).

The studies that met the criteria for our meta-analysis did not include herbivorous fishes, which have a distinct digestive morphology and physiology compared to that of omnivores and carnivores (see review in Farrell 2011).

Herbivores have higher nitrogen TEFs for bulk tissues (Vander Zanden and Rasmussen 2001, Mill et al. 2007, but see Wyatt et al. 2010) as well as for trophic AA (Nielsen et al. 2015). Higher TEFs in herbivorous fishes may be due to higher ingestion rates (that leads to higher metabolic reworking), lower assimilation efficiencies, and higher N excretion rates (Mill et al. 2007), as well as AA production by the gut microflora (Newsome et al. 2011). Hence, TEF selection for estimating the TP in fishes must consider a species' feeding ecology (Nielsen et al. 2015).

Our analysis included feeding experiments conducted mainly on juvenile fishes (79.6% of the experiments) and larvae, while subadults and adults were poorly represented in the literature. Among source AAs, we found similar TEFs for Phe, Lys, Ser, and Met among larvae and early juveniles, whereas Ser and Lys were highly variable between life stages. Only TEF_{Gly} showed statistically significant differences between larvae and early juveniles, with values for larvae as high as 8.8‰ reported by Chikaraishi et al. (2009). High isotope discrimination in Gly during the larval stage suggests high catabolism of this AA, which is because this AA is a biochemically simple NEAA that can be easily catabolized (Li and Wu 2018). Fish require high protein consumption to sustain fast protein accretion during the early life stages (NRC 2011), and Gly and Ser have an important function in protein synthesis, gluconeogenesis, and energy acquisition for growth (Walton and Cowey 1982). In addition, there is evidence to suggest that Gly is preferentially catabolized as an energy substrate in fish larvae compared with other AA (Conceição et al. 2002). Hence, the abnormally high TEF Gly reported by Chikaraishi et al. (2009) may reflect stage-specific AA metabolism of Gly, which should be confirmed through additional larval feeding studies.

Mean $\delta^{15}N$ values of trophic AA TEFs did not vary significantly between larvae and early juveniles (although sample size differed substantially). Noticeably, the mean TEF Glu did not differ between these early two life stages and adults, suggesting stability in isotopic fractionation during fish growth. This low variability in TEF Glu supports its use for estimating TP in fish.

Marine, brackish, and freshwater habitats differ in salinity, with important consequences for fish physiology and likely AA isotopic fractionation (Vanderklift and Ponsard 2003). Fish regulate their cell and body osmotic pressure depending on the ambient salinity concentration and must allocate energy to osmoregulate in order to maintain ionic balance and osmotic homeostasis (Edwards and Marshall 2013, Marshall 2013). Therefore, the metabolic pathways (and hence isotope discrimination) of AAs involved in osmoregulation, such as Gly (Li et al. 2009), could vary among brackish, marine, and freshwater, as our analysis seems to indicate although the sample size was limited.

We found that mean Phe did not yield significant differences between aquatic habitat types. The mean TEF_{Lys} for fish of brackish habitats (1.7‰) was significantly higher than for marine fish (0.1‰), which may be linked to Lys involvement for maintaining osmotic pressure and acid–base balance in the body fluids (Chiu et al. 1988). More experiments are needed to investigate the effect of aquatic habitat type on AA isotopic fractionation.

Glu TEFs did not differ significantly between habitat types, although there are no empirical TEFs for freshwater species. We found that Asp, Ile, Pro, and Leu had significant higher TEFs (~7–8‰) in fish from brackish habitat compared with marine species (3–5‰). The catabolism of Asp, Ile, Pro, and Leu provides additional energy during osmoregulation in brackish, estuarine, and marine habitats (Walton and Cowey 1977, Bystriansky et al. 2007), leading to higher isotope discrimination as light nitrogen is excreted during metabolic processes. This pattern is supported by results from Vanderklift and Ponsard (2003) on bulk tissue; they found lower mean TEF_{bulk} values in marine species than freshwater taxa; this meta-analysis included vertebrates and invertebrates. The differences in trophic AA TEFs in relation to habitat type may be particularly relevant for migratory species that feed and move between aquatic habitats, like salmonids.

Comparison of teleost, global, and non-teleost TEFs

Including multiple taxa in the calculation of global TEFs can lead to high variability due to differences in physiological processes such as mode of nitrogen excretion, protein requirements, and digestive physiology. For source

AAs, teleosts, non-teleosts, and global mean TEFs had comparable values for Phe, Lys, and Met, with differences ≤1‰. Although these differences are relatively limited, means for non-teleosts were negative due to the inclusion of a single arthropod group (29% of the TEF values for non-teleosts were for insects and were negative; McMahon and McCarthy 2016). Mean TEF Met showed remarkable stability among teleost, non-teleost, and global estimates, supporting its role as a robust source AA (Ishikawa et al. 2018). Mean $\delta^{15}N$ TEFs for the three groups were similar for Glu, which supports the concept of Glu as canonical trophic AA for estimating TP across taxa. The mean TEFs of Asp, Pro, and Leu were significantly higher in teleosts than in non-teleosts, possibly reflecting taxon-specific metabolic processes.

Gly and Ser, the so-called metabolic AAs, exhibited the highest variability among all AAs, highlighting their high sensitivity to dietary changes in response to intra- and inter-species specific metabolic processes, which excludes these AAs from a strict classification as source or trophic AAs. In contrast, lower variation was observed for Phe, Lys, Met, Gly, Ser, Asp, Glu, Ile, Pro, Val, Leu, and Ala within a taxonomic group (teleosts) than between multiple taxonomic groups. For non-teleosts and global estimates, the SD of the TEFs of trophic AAs was higher than ~2‰, suggesting the presence of intra-specific differences in AA metabolism that warrant detailed study. Among trophic AAs, Ala, and Leu were particularly variable, having SD between 2.0‰ and 3.4‰ and 1.9‰ and 3.1‰, respectively.

The overall lower variation (SD) in our teleosts TEFs$_{AA}$ compared to the global or non-teleosts TEFs indicates that taxon-specific values would yield more precise estimates of TP. Our results nevertheless show high variability in the TEFs of Phe (SD = 1.0‰) and Glu (SD = 1.8‰) within teleosts. For example, a SD TEF$_{Glu}$ of 1.8‰ accounts for about a fifth of one trophic level if a TDF of 7.6‰ is assumed (Chikaraishi et al. 2009) or a third of a trophic level using Nielsen et al. (2015)'s mean TEF of 6.6‰. The mean TEFs of Phe and Glu (0.6‰ and 6.8‰, respectively) yield a TDF for teleost's of 6.2‰ ± 1.9‰ (where 1.9‰ is the propagated error). The variability in TEFs as well as analytical variability should be considered in the calculation and interpretation of TP by including error propagation efforts (Choy et al. 2012, Bradley et al. 2015).

CONCLUSIONS

Mode of excretion and diet quality are the leading hypotheses for explaining high isotopic fractionation of trophic AA across multiple taxa (McMahon and McCarthy 2016). We conducted this review by selecting controlled feeding experiments on ammonotelic teleosts. The results of our study validate the use of Phe as the canonical source AA. However, its variability as a function of protein content, diet, life stage, and aquatic habitat is not negligible, and should be accounted for in TP calculations, and included in error propagation efforts. Lys showed limited isotope discrimination but was highly influenced by lipid content relative to fish nutritional requirements, feeding regime, diet type, and aquatic habitat. While our results indicate Lys is not a robust indicator of baseline values, it may serve as powerful indicator of dietary quality and quantity if the main catabolic pathways relative to the degree of isotopic fractionation are understood. Gly and Ser showed large variability as a function of diet type and life stage, which supports excluding them as source and trophic AAs. Mean values of TEF$_{Met}$ were relatively stable between factors. However, it is a difficult AA to measure (Ohkouchi et al. 2017) and is present in low concentrations in top predator tissues (Reid et al. 2005).

Among trophic AAs, TEF of Asp, Ile, Pro, and Leu varied mainly with lipid content relative to nutritional requirements, feeding regime, diet type, and aquatic habitat. TEF$_{Val}$ varied largely with diet type. TEF$_{Ala}$ varied with protein and lipid content and diet type. TEF$_{Glu}$ was more influenced by protein content, protein content relative to requirements, feeding regime, and diet type; however, only protein content and feeding regime were significant. These results are consistent with the key metabolic role of Glu. As the canonical trophic AA, complete insensitivity of TEF$_{Glu}$ to dietary characteristics and ecological factors would be highly desirable, but our results together with those reported in species-specific studies on teleosts indicated that is not strictly the case. Its application as a trophic AA for

estimating trophic level must therefore consider the level of variability within a single taxonomic group.

We highly recommend focusing future research on understanding the main mechanisms driving AA isotopic fractionation within a single infraclass or taxonomic group. Despite the long experimental periods required for obtaining empirical TEF estimates in subadult and adult fish due to slower isotope rates (Herzka 2005), there is a pressing need for empirical studies focused on these two late life stages that are characterized by different growth rates, reproductive activity, and senescence. In addition, feeding regimes must be considered in experimental designs seeking to evaluate AA isotopic fractionation as a function of dietary nutritional characteristics.

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