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Running-title: Microbial vs. metazoan trophic position

The microbial contribution to the trophic position of stomiiform fishes

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Abstract

The trophic position (TP) of fishes determines their importance in terms of energy flows within food webs. However, accurate estimations of TP are often prevented because of the difficulties in tracing all food sources. This is particularly challenging for omnivorous fishes, such as those from the Order Stomiiformes. In this study, we applied recent developments in stable isotope analysis of amino acids to untangle the contributions of microbial vs. metazoan food webs in 13 species of Stomiiformes. The inclusion of the microbial food web reduced the differences between TP estimates using stable isotopes and those derived from stomach content analysis. In addition, the new estimates allowed to quantify the relative contribution of the microbial food web to each species (6-21%), highlighting the importance of detritus consumption even in

piscivorous species (e.g. *Stomias boa*, *Chauliodus danae*). The comparison of TP estimates obtained with selected amino acids in fish muscle allowed for the detection of the microbial influence integrated at time scales relevant for net fish growth, even when trophic exchanges in the microbial food web occur at much shorter time scales. The assessment of TP considering the differential contribution of microbial and metazoan food webs challenges our current understanding of marine food webs; yet provides a new quantitative tool for the analysis of their structure and function.

Keywords: amino acids, metazoan food web, microbial food web, micronekton, stable isotopes

Introduction

The trophic position (TP) of a given species or individual summarizes its role in the food web by integrating all the food sources contributing to its biomass. Originated from the merely quantitative concept of discrete trophic levels, explaining the unidirectional flow of energy through an ecosystem (Lindeman, 1942), the definition of TP has evolved to a quantitative, fractional measure of trophic hierarchy, which takes into account the omnivory behaviors of most species, particularly in aquatic food webs (Vander Zanden and Rasmussen, 1996). Accurate estimations of TPs of fishes are critical to understand their role in the ecosystem and, ultimately, to improve our knowledge on energy fluxes and food web structure and resiliency. Multispecies and ecosystem management require robust predictions on the structure and dynamics of food webs (Grumbine, 1994; McCormack et al., 2019). In turn, regime shifts imply abrupt reorganizations of food-web and community structures that can be traced from changes in TPs (Möllman et al., 2015; Kröncke et al., 2019). Therefore, the large variability in TP values obtained through different methods must be taken into account when analysing food web dynamics, as outlined below.

Classical evaluations of TP for most fish species rely on the observations of identifiable prey remains in their stomachs (i.e. gut content analysis). This approach has been used to estimate TP values for individual species, such as those compiled in FishBase (Froese and Pauly, 2021), which have been key elements of biomass-based ecosystem models, including ECOPATH (Pauly and Christensen, 1995; Pauly et al., 1998). To overcome the uncertainties in identifying all prey items and in attributing a definite TP to each of them, alternative assessments of TP can be made using tracer molecules such as fatty

55 acids and stable isotopes (Post, 2002; Pethybridge et al., 2018). Fatty acids analysis is
56 commonly applied to identify the diet of consumers (Dalsgaard et al., 2003; Iverson et
57 al., 2004; Stowasser et al., 2009; Xu et al., 2020). However, lipids have faster turnover
58 rates than structural proteins, particularly in the white muscle (e.g. Lu et al., 2019), thus
59 integrating the diet over relatively short time scales (i.e. weeks to months). The analysis
60 of stable nitrogen isotopes is based on the progressive enrichment of the heavier
61 nitrogen isotopes (^{15}N) within organisms along the food web, and the quantification of
62 the TP of a given species is possible by measuring the accumulation of stable nitrogen
63 isotopes (^{14}N and ^{15}N) in its tissues (Post, 2002).

64 Despite a general agreement with diet-based TP estimates (e.g. Kline and Pauly, 1998),
65 stable isotope-based TP assessments using bulk tissues require a careful selection of
66 isotopic baselines and trophic discrimination factors, which is often challenging. The
67 baseline characterizes the locally-relevant nutrient sources (Jennings and van der
68 Molen, 2015) while the discrimination factor represents the isotopic enrichment
69 between adjacent trophic levels (Hussey et al., 2014; Bastos et al., 2017). In contrast,
70 the use of amino acid-specific stable isotopes has provided TP estimates increasingly
71 closer to those derived from dietary data (Choy et al., 2012; Bradley et al., 2015;
72 Nielsen et al., 2015). These values are based on the different isotopic fractionation rates
73 affecting the 'source amino acids' (i.e. those that barely change along the food web) and
74 the 'trophic amino acids' (i.e. those that undergo predictable isotopic enrichment
75 moving up the food web).

76 Recently it has been suggested that more realistic estimations of TP can be made by
77 taking into account trophic-level differences in isotopic enrichment (McMahon and
78 McCarthy, 2016) and markers of microbial consumers (Decima and Landry, 2020). In
79 contrast with previous applications based on the averaging of source and trophic amino
80 acids, the new TP estimates can be used to quantify the relative contribution of
81 microbial vs. metazoan food webs to the overall TP of a given species. Such
82 differentiation is possible because certain amino acids show isotopic enrichment in all
83 types of consumers, including protozoans in microbial food webs, while others are
84 enriched only for metazoans (Decima et al., 2017). In contrast to metazoans,
85 chemotrophic microbes exhibit a large plasticity for amino acid acquisition resulting in
86 more diverse isotopic enrichment patterns (McMahon and McCarthy, 2016; Ohkouchi
87 et al., 2017). External hydrolysis of seston by heterotrophic bacteria produces an even

enrichment of all amino acids (Hannides et al., 2013), while amino acids synthesized from inorganic nitrogen by chemoautotrophic bacteria show an enrichment pattern similar to that of algae, and those obtained from metabolic processing and salvage of amino acid-rich dissolved substrates by heterotrophic bacteria are enriched following a pattern similar to that of animals (Ohkouchi et al., 2017). However, dominance of chemoautotrophy and heterotrophy on amino acid-rich substrates are generally restricted to specific ecosystems or to experimental settings and the isotopic enrichment of microbial amino acids of most oceanic ecosystems is expected to be a combination of the different patterns (McMahon and McCarthy, 2016). For instance, selective resynthesis of some amino acids (as alanine or glycine) and direct uptake of others (as glutamic acid) have been invoked to explain the large variability observed in the enrichment of individual amino acids after bacterial degradation of dissolved organic matter (Calleja et al., 2013). Both processes were observed in experimental food webs including protists (Gutierrez-Rodriguez et al., 2014; Decima et al., 2017). The selective enrichment cannot be traced using bulk isotopic determinations, thus leading to an underestimation of the contribution of the microbial food web to the TP of metazoan consumers (Gutiérrez-Rodríguez et al., 2014). Examples of TP taking into account the microbial food web were recently provided for zooplankton (Decima and Landry, 2020) and several micronekton species (Bode et al., 2021a).

In this study, we aimed to provide new insights to reconcile isotopic TP estimates with diet-based TP estimates in 13 fish species of the Order Stomiiformes. To do so, we compared results derived from the natural abundance of stable nitrogen isotopes (bulk and amino acid compound-specific) with those based on diet and reported in the literature. The objective was to produce accurate estimations of TP, which take into account the contribution of organisms from the microbial food web, and that may be applied to the comparison of fish species within and across ecosystems. The selected fish species were representative of different migration and feeding habits, and were distributed over different water depths. This allowed for the examination of potential differences in TP caused by generalist feeding expected in migrant and omnivorous fishes (Choy et al., 2012; Carmo et al., 2015). The new estimations will contribute to quantify the close links between microbial and metazoan food webs in oceanic ecosystems.

Material and Methods

Sampling

Samples of 13 species of stomiiforms were collected within different water column layers during the MAFIA cruise in the subtropical N Atlantic using a 35 m^2 midwater trawl fitted with a Multisampler (Olivar et al., 2017, 2019). These species were representative of different daily migratory and dietary habits, as well as depth distributions (Supplementary Table S1). Fishes were sorted, identified on board, and kept frozen (–20 °C) for up to 12 months. In the laboratory, the standard length (SL) of each fish was measured before freeze-drying. In this study, one individual per species was processed for stable isotope analysis. However for some species) up to three individuals of the same size were combined to obtain sufficient mass for analysis (Supplementary Table S1). Details of the fish samples and raw data can be found in Bode et al. (2021b). Samples of calanoid copepods (*Calanoides* spp.) were collected from the same sampling stations where fishes were caught using a MOCNESS-1m^2 net (200 µm mesh) between the surface and 800 m depth to provide a baseline reference for TP estimations (Bode and Hernández-León, 2018a, b). Calanoid copepods were sorted in the laboratory and dried (50°C, 48 h) prior to stable isotope analysis.

Stable isotope analysis

Determinations of stable nitrogen isotope ratios were made in bulk for copepod and fish tissue samples and in derivatized amino acids for fish samples only. Nitrogen isotopic ratios were reported as $\delta^{15}N$ values (‰) with respect to air (Coplen et al., 2011). Between 6 and 16 Copepod samples, each containing between 5 and 15 individuals, were analysed for each station. Final copepod $\delta^{15}N$ values were pooled by station. Portions of the dorsal musculature of fish were selected, except for very small specimens (i.e. < 35 mm) that were analysed as whole after removal of the gut and gonads. All samples were ground to a fine and homogeneous powder with a mixer mill (Retsch Mixer Mill MM-200). The quantification of bulk samples was made using an elemental analyser coupled to an isotope-ratio mass spectrometer. Isotope standards of caffeine IAEA-600 (International Atomic Energy Agency), IA-R041-15N/13C L-alanine, (Iso-Analytical Limited) and urea IVA33802174 (IVA Analysentechnik e.K.) were analysed with the samples along with internal acetanilide and sample standards (cyanobacteria culture of known isotope composition used as an internal control). Precision of triplicate determinations of standards or samples was <0.4‰.

For the quantification of amino acid specific $\delta^{15}N$ ratios we followed the procedure detailed in McCarthy et al. (2013) and Mompeán et al. (2016). Briefly, 10 mg sample aliquots were hydrolysed with 6N HCl (20 h, 110 °C), filtered through 0.20 μm hydrophilic filters, evaporated to dryness under an N_2 stream, and then treated with 2.5 ml of 1:5 acetyl chloride:2-propanol, flushed with N_2 and heated (110°C, 60 min). Subsequently, the solvents were evaporated under N_2 and the extracts treated with 0.9 ml of 3:1 diclomethane:trifluoracetic anhydride (DCM:TFAA) and heated (110 °C, 15 min). The resulting derivatized amino acids were purified by solvent extraction in 1:2 chloroform:phosphate buffer and centrifugation (Loick-Wilde et al., 2019), evaporated at room temperature under N_2, and stored at −20 °C in 3:1 DCM:TFAA until further analysis.

The individual amino acids were separated using a gas chromatograph equipped with a TraceGOLD TG-5MS chromatographic column (60 m, 0.32 mm ID, 1.0 μm film), and were subsequently injected into a mass spectrometer using a continuous flow interface and a combustion module. The $\delta^{15}N$ of each amino acid in the sample was calibrated with the values obtained for isolated standards (Shoko Science) analysed by combustion as described for bulk analysis. Additional corrections were made using an internal L-norleucine standard (SIGMA) added to each sample. The molar fraction of individual amino acids (%molar) was also determined in the same analytical run by calibration of the area of the Mass 28 from the spectrometer with amino acid standards (McCarthy et al., 2013). Mean precision of triplicate samples (two injections per sample) was <0.3‰ per individual amino acid. All isotopic determinations were made at the Servicio de Análisis Instrumental of the Universidade da Coruña (Spain).

Amino acids were classified as either source or trophic (McClelland and Montoya, 2002; McCarthy et al., 2013; McMahon and McCarthy, 2016). Source amino acids included glycine (Gly), threonine (Thr), serine (Ser), methionine (Met), phenylalanine (Phe), and lysine (Lys). Trophic amino acids included alanine (Ala), leucine (Leu), isoleucine (Ile), proline (Pro), valine (Val), and the mixtures of glutamine and glutamic acid (Glx), and of aspartamine and aspartic acid (Asx). The variability of nitrogen sources among samples was investigated using both the canonical source amino acid Phe and the molar-weighted average $\delta^{15}N$ of all source amino acids. Trophic position estimates were made using the $\delta^{15}N$ values of the canonical trophic amino acids Glx (Chikaraishi et al., 2009) and Ala (Decima and Landry, 2020). Values of TP computed

from Glx represented only the metazoan food web while those computed from Ala represented both the microbial and metazoan food webs. (Gutiérrez-Rodriguez et al., 2014; Decima et al., 2017; Decima and Landry, 2020).

Trophic position estimations

The TP of each species was obtained from stable isotope measurements by using four models (Table 1). In the first two models, bulk measurements of fish $\delta^{15}N$ were combined with baseline reference values of calanoid copepods by assuming either constant (TP_{bulk1}) or scaled values (TP_{bulk2}) of the trophic discrimination factor (TDF). In both cases the baseline values (either constant or scaled) were considered to be TP = 2, as generally assumed in similar studies (e.g. Kline & Pauly, 1998; Hussey et al., 2014; Valls et al., 2014). In the third and fourth models, amino acid $\delta^{15}N$ values were used to estimate TP taking into account metazoan-only (TP_{Glx}, Chikaraishi et al., 2009) and microbial + metazoan trophic steps (TP_{Ala}, Decima and Landry, 2020), respectively. In both cases, the amino acid TP estimates were obtained using different TDF values for the trophic steps in plankton and in fish (McMahon and McCarthy, 2016). The propagated error (sd) in the mean values of TP for each species was calculated using first-order Taylor series expansions of the corresponding equations in Table 1 by considering the analytical errors in the individual determinations $\delta^{15}N$ for bulk, trophic and source amino acids, as well as the variability in the coefficients employed in each model (Bradley et al., 2015; Ohkouchi et al., 2017). Values of TP derived from stable isotopes were compared with those reported in the global fish species database FishBase (Froese & Pauly, 2021).

Statistical analysis

Non-parametric ANOVA (Kruskal-Wallis) was used to test differences in isotopic composition and TP by three different factors (i.e. habitat depth layer, migration habit, and feeding type) that were analysed one at a time because not all species occurred in each combination of factors. Habitat depths were provided by FishBase and data from our samples (Olivar et al., 2017), and defined as mesopelagic (in this case considering species distributed between the surface and1000 m depth) and bathypelagic layers (for species reaching depths below 1000 m depth). Migrants (i.e. species performing large diel vertical movements to layers near the surface) and partial-migrants (i.e. species with limited diel vertical migrations and not reaching the upper 100 m layer) were

grouped together and compared with non-migrant species (those always living below 200 m depth). Finally, diet diversity as reported in FishBase and in additional references (Supplementary Table S1) was summarized in three categories: plankton (mainly copepods), nekton (small fish and non-copepod crustaceans including large amphipods, euphausiids and decapods), and mixed (plankton and nekton) diets. Comparisons between the different TP estimates were made using ANOVA and *post-hoc* Bonferroni tests. Statistical analyses were made using SPSS 17.0 (SPSS Inc.) and graphics using Past 4.0 (Hammer et al., 2001).

Results

The different TP estimates ranged from high values (4.02 for TP_{Ala}) for those derived from amino acids to unrealistically low values (<1.5) for those derived from bulk $\delta^{15}N$ (Figure 1). Mean values of TP_{Ala} and TP_{Glx} were not significantly different from TP values reported in FishBase ($p>0.05$) while those from other estimates were significantly lower ($p<0.01$). Isotope-based TP estimates did not vary significantly when the species were grouped by migration habit, habitat depth layer, or feeding type (Supplementary Figure S1, $p>0.05$). In addition, there were no significant differences in the nitrogen baselines by habitat depth layer, either estimated by $\delta^{15}N$ in phenylalanine or by the mean value in source amino acids (Supplementary Figure S2, $p>0.05$).

Some of the differences in TP values were also evident when considering individual species, with the lowest values and largest variation observed for bulk estimates (Figure 2). *Borostomias elucens*, *Malacosteus niger*, and *Stomias boa* were the species with the highest TP values (ca. 4) when estimated from amino acids. Interestingly, not all species considered as piscivores or with a mixed plankton and nekton diets had always high TP. For instance, mean TP_{Ala} for *Chauliodus danae* was 3.46, almost equivalent to the values for planktivorous species as *Cyclothone acclinidens*, *Argyropelecus sladeni*, *C. pseudopallida* or mixed diet species as *A. hemigymnus*. Conversely, planktivorous species as *Polyipnus polli* and *Vincigueria nimbaria* had mean TP_{Ala} values equivalent to those of species with a mixed diet (e.g. *Sternoptyx diaphana* or *Sigmops elongatus*). The difference between mean TP_{FB} and TP values estimated from stable isotopes for individual species were larger for those based on bulk isotopes (mean ± sd = 0.70 ± 0.94, and 0.91 ± 0.53, for TP_{bulk1} and TP_{bulk2}, respectively) than for those based on amino acids (0.38 ± 0.40, and -0.12 ± 0.29 for TP_{Glx} and TP_{Ala},respectively). These

differences did not vary significantly when considering migration habits, depth layers, or diet types ($p>0.05$), except for the difference between TP_{FB} and TP_{Glx} in species with a dominant nektonic diet ($p<0.05$) that were on average ca. 1 TP lower for the latter (Figure 3).

Discussion

The general agreement between TP estimates using the $\delta^{15}N$ values of the trophic amino acid Ala, instead of the commonly used Glx, and TP values reported in FishBase points to a new way to compare TP estimates based on stable isotopes analysis with those based on diet information. While the gut content data was generally considered an oversimplification of the food web, particularly at low TPs, the inclusion of microbial trophic steps (i.e. those involving consumption of bacteria, flagellates, and protozoa) along with metazoan trophic steps (e.g. consumption of copepods) in TP_{Ala} supports the general validity of FishBase estimates intended for modelling purposes, at least for mid trophic levels as the stomiiform fish species considered in this study. Computation of TP from diet data requires a good understanding of the trophic pathways involved and the collection of sufficient data at large spatial and temporal scales, which is particularly challenging in the case of opportunistic feeders such as the pelagic fishes (Jennings and van der Molen, 2015). However, diet-based TP provide conservative values for comparison with TP computed by other methods (Pethybridge et al., 2018). FishBase estimates were based on ECOPATH models made by assuming that the species TP were the weighted average of the TP of all the food items reported in the literature for each species (Pauly and Christensen, 1995; Pauly et al., 1998), following the convention of attributing TP = 1 for primary producers, detritus, and the associated bacteria (Mathews, 1993). This procedure implies the propagation of uncertainties as the TP of the different prey are combined, but it is assumed that for a given species there would be a compensation of errors with opposite signs. Previous comparisons in different ecosystems revealed a general correlation between ECOPATH and TP values computed from $\delta^{15}N$ in bulk tissues (Kline and Pauly, 1998) but more detailed studies concluded that the former were lower (Milessi et al, 2010; Navarro et al., 2011; Lasalle et al., 2014; Du et al., 2015) or higher than the isotopic-based TP (Du et al., 2020). Such differences may have resulted from the use of inappropriate values for the reference baseline, as most studies assumed TP = 2 but employed different organisms as representative primary consumers (from copepods to filter-feeding molluscs). The

copepods (*Calanoides* spp.) used in our study are considered a filter-feeding herbivore (e.g. McGinty et al., 2018), but related species of Family Calanidae were reported to have TP values between 2 and 2.5 (Decima & Landry, 2020). However, even if we assumed a mean TP = 2.5 for our baseline, TP_{bulk1} and TP_{bulk2} values would be still lower than those of TP_{FB}.

The use of $\delta^{15}N$ averaged by trophic and source amino acids in TP estimations reduced the difference with those derived from gut contents at species and group level by levelling the isotopic signatures of individual amino acids (Choy et al., 2012; Bradley et al., 2015). However, while pooling various amino acids improves the precision of TP estimates (Nielsen et al., 2015), this procedure prevents the separation of the contribution of the microbial vs. the metazoan trophic steps. The results obtained in this study showed that models based on bulk $\delta^{15}N$ underestimated by ca. 1 the TP reported in FishBase, and had larger errors than those derived from amino acids, as showed in previous studies (e.g. Bradley et al., 2015). The amino acid-based TP values were comparable to those reported for the same species but using averaged trophic and source amino acids in other studies. For instance, our TP_{Ala} estimate for *M. niger* (3.91 ± 0.51) was equivalent to the value reported by Bradley et al. (2015) in the North Atlantic (3.87 ± 0.56), and those for *S. elongatus* and *A. hemigymnus* (3.34 ± 0.40 and 3.40 ± 0.51, respectively) were within the values reported in Richards et al. (2020) for these species in the Gulf of Mexico (3.44 ± 0.29 and 3.38 ± 0.36). Our analysis also revealed that, despite a general relationship between TP values and the diet reported for each species, the literature assigned values, including FishBase and additional references (Supplementary Table S1) may not be applicable to all populations of each species, likely due to the opportunistic feeding behaviour of most micronektonic fishes (e.g. Bernal et al., 2015). This may be the case for *A. hemigymnus* whose TP reported showed high variability even when obtained from the same methodology (Valls et al., 2014; Bradley et al., 2015; this study). Some species categorized as piscivores, including *S. elongatus* and *C. danae*, had mean TP values of ca. 3.5, suggesting a substantial dependence on plankton prey. In turn, planktivorous species (e.g. *P. polli*, *V. nimbaria*) showed TP values overlapping those of species with mixed plankton and fish diets (e.g. *S. diaphana*, *A. hemigymnus*). Indeed, *C. livida*, a species with no reported dietary information, showed TP values close to those of piscivorous species, while it

would be considered to have a planktivorous diet, as reported for other species of the same genus (Supplementary Table S1).

The distinction between TP contributions by the metazoan only vs. the metazoan + microbial food webs (Decima et al., 2017) allows the assessment of the importance of the microbial trophic steps in different types of consumers. Specifically, this is possible by analysing the difference between TP_{Ala} and TP_{Glx} values (Decima and Landry, 2020). In this study, this difference did not vary among species grouped by migration habits, habitat depth layers, or feeding types. Similar results were found in a previous study conducted on micronekton fishesof various taxonomic orders, including Stomiiformes (Bode et al., 2021a). The lack of a clear pattern of this difference suggests that the microbial contribution to the TP of meso- and bathypelagic fishes is not primarily controlled by a single factor but rather by a combination of depth, migration, and diet, including feeding on detritus.

The mean contribution of microbial trophic steps, measured as the difference between TP_{Ala} and TP_{Glx} relative to TP_{Ala}, varied between 6% for *A. sladeni* and 21% for *B. elucens*. These values were within those observed for omnivorous plankton (Decima and Landry, 2020) and other micronekton fish species (Bode et al., 2021a), and suggest a major importance of detritus consumption along with the associated microbial food web). Indeed, this is not unexpected because unidentified detrital remains were reported in the stomachs of some *Cyclothone* species, as *C. acclinidens* (DeWitt and Cailliet 1972) or *C. braueri* (Palma, 1990; Bernal et al., 2015), and are also likely present in most species considered as planktivores or mixed feeders. Detrital aggregates, or marine snow, constitutes a nutritious and relatively abundant trophic resource in deep ocean waters and can support zooplankton (Fanelli et al., 2011; Kiorboe, 2011) but also small fish and larvae (Miller et al., 2013; Tsukamoto and Miller, 2020). Marine snow aggregates are micro ecosystems containing organic matter remains of phytoplankton (e.g. dead cells, exopolymers), zooplankton (e.g. crustacean, carcasses, appendicularian houses), and all other kind of detrital remains and minerals, as well as bacteria and their protozoan predators (Alldredge and Silver, 1988; Passow, 2002). These aggregates, which can attain sizes of several centimetres (Burd and Jackson, 2009; Guidi et al., 2009), offer a concentrated food source for consumers that would not be able to reach otherwise.

The inclusion of biomass recycling processes through microbes and detritus has challenged the application of food web models based on stable isotopes (Gutierrez-Rodriguez et al., 2014; Flynn et al., 2018). However, the identification of the appropriate markers for microbial trophic steps (Decima et al., 2017) allows for the quantification of these processes and their influence in the overall TP of consumers at ecologically relevant time scales (months, in this case). While trophic processes are typically fast in the microbial food web, with the propagation of changes in the source baseline at the scale of days (e.g. Gutierrez-Rodriguez et al., 2014), the effect for metazoan consumers can only be detected at longer time scales related to the turnover time of isotopes in their tissues. For instance, the stable isotope turnover rate in animals varies inversely with individual body mass, and equations have been provided for estimating turnover rates (expressed as half-life) in different tissues and organisms (Vander Zanden et al., 2015). Using the equation for ectotherms and the individual weight of the specimens analysed in this study, we estimated that half-lives of nitrogen stable isotopes in the species analysed here varied between 15 days, for the small-sized *C. acclinidens*, and 44 days for the much larger *C. danae*. Since almost 95% isotopic renovation is roughly equivalent to ca. 5 half-lives (Hobson and Clark, 1992), we can estimate that the TP determined with both bulk or amino acid-specific stable isotopes correspond to the diet integrated between ca. 3 and 7 months.

The results of this study align with those of previous reports indicating that TP estimations of micronekton including the contribution of the microbial food web can be achieved using $\delta^{15}N$ values of selected trophic and source amino acids (Bode et al., 2021a). The new TP values are equivalent to values derived from models based on simplified assumptions on the food web and literature diet data as provided by FishBase. However, in contrast to previous models (e.g. Nielsen et al., 2015), the separation between microbial vs. metazoan trophic step contributions provides a new quantitative tool for the analysis of food web structure and function. These estimates are particularly needed in the case of oceanic food webs dominated by omnivore species that also feed on detritus (Libralato, 2013; Heymans et al., 2014).

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Author contributions

S.H.L. designed the project. M.P.O and C.L.-P., obtained the samples. A.B. and M.P.O. conceived this specific research, and analysed the data. A.B. wrote the manuscript with contributions from all co-authors.

Data availability statement

The original data on sample location, individual fish characteristics and stable isotope composition, including amino acids, can be accessed through the PANGAEA repository (Bode et al., 2021b). Similarly, the original data for copepods can be found in Bode et al. (2018b).

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Table 1. Equations employed for the estimation of trophic position (TP) used in this study. $\delta^{15}N_s$: natural abundance of bulk stable nitrogen isotopes in stomiiform fishes; $\delta^{15}N_p$: natural abundance of bulk stable nitrogen isotopes in calanoid copepods; $\delta^{15}N_{Ala}$, $\delta^{15}N_{Glx}$, $\delta^{15}N_{Phe}$: natural abundance of stable nitrogen isotopes of alanine, glutamine + glutamic acid, and phenylalanine, respectively. TEF: trophic enrichment factor. CSIA: compound-specific stable isotope analysis

Type	Equation	Parameters	References
Additive (bulk)	$TP_{bulk1} = \frac{(\delta^{15}N_s - \delta^{15}N_p)}{TEF_{bulk}} + 2$	TEF_{bulk} = 3.4 ± 1.0‰	Post (2002)
Scaled (bulk)	$TP_{bulk2} = \frac{[\log(\delta^{15}N_{lim} - \delta^{15}N_p) - \log(\delta^{15}N_{lim} - \delta^{15}N_s)]}{k} + 2$	$\delta^{15}N_{lim}$ = 2.93 ± 0.71‰ k = 0.14 ± 0.49	Hussey et al. (2014)
Total (CSIA)	$TP_{Ala} = \frac{(\delta^{15}N_{Ala} - \delta^{15}N_{Phe} - TEF_p - \beta)}{TEF_s} + 2$	TEF_p = 4.5 ± 2.1‰† TEF_s = 6.1 ± 0.3‰‡ β = 3.2 ± 1.2‰†	McMahon and McCarthy (2016) Decima and Landry (2020)
Metazoan (CSIA)	$TP_{Glx} = \frac{(\delta^{15}N_{Glx} - \delta^{15}N_{Phe} - TEF_p - \beta)}{TEF_s} + 2$	TEF_p = 7.6 ± 1.2‰† TEF_s = 5.7 ± 0.3‰‡ β = 3.6 ± 0.5‰‡	McMahon and McCarthy (2016) Bradley et al. (2015)

† Chikaraishi et al. (2009)

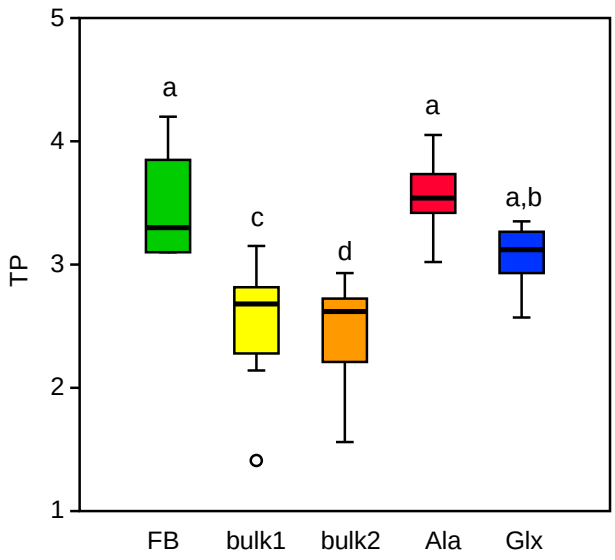
‡ Bradley et al. (2015)

Figure legends

Figure 1. Box plot of mean TP values estimated through the different methods (see Table 1). FB: FishBase, bulk1: additive model, bulk2: scaled model, Ala: microbial + metazoan food web, Glx: metazoan food web. Circle: outlier. Different letters indicate significant means (Bonferroni post-hoc test, $P<0.05$). Each box encompasses the 25 and 75% quartiles, whereas the whiskers indicate 1.5 times the interquartile range, the horizontal line indicates the median, and circles indicate outliers (>1.5 times the interquartile range).

Figure 2. Mean (± propagated sd) trophic positions (TP) of the 13 stomiiform fish species analysed estimated using bulk (a) or amino acid-specific (b) stable nitrogen isotope ratios. Values compiled in FishBase (FB) are included for comparison.The equations used to obtain the different estimates are provided in Table 1.

Figure 3. Box plot of mean differences in the trophic position (TP) estimates of individual species (see Table 1) across migration habits (migrants and partial migrants vs. non-migrants), habitat depth layers (mesopelagic, bathypelagic), and feeding types (plankton, nekton, mixed). Each box encompasses the 25 and 75% quartiles, whereas the whiskers indicate 1.5 times the interquartile range, the horizontal line indicates the median, and circles indicate outliers (>1.5 times the interquartile range). The red arrow indicates significant differences (Bonferroni post-hoc test, $p<0.05$).



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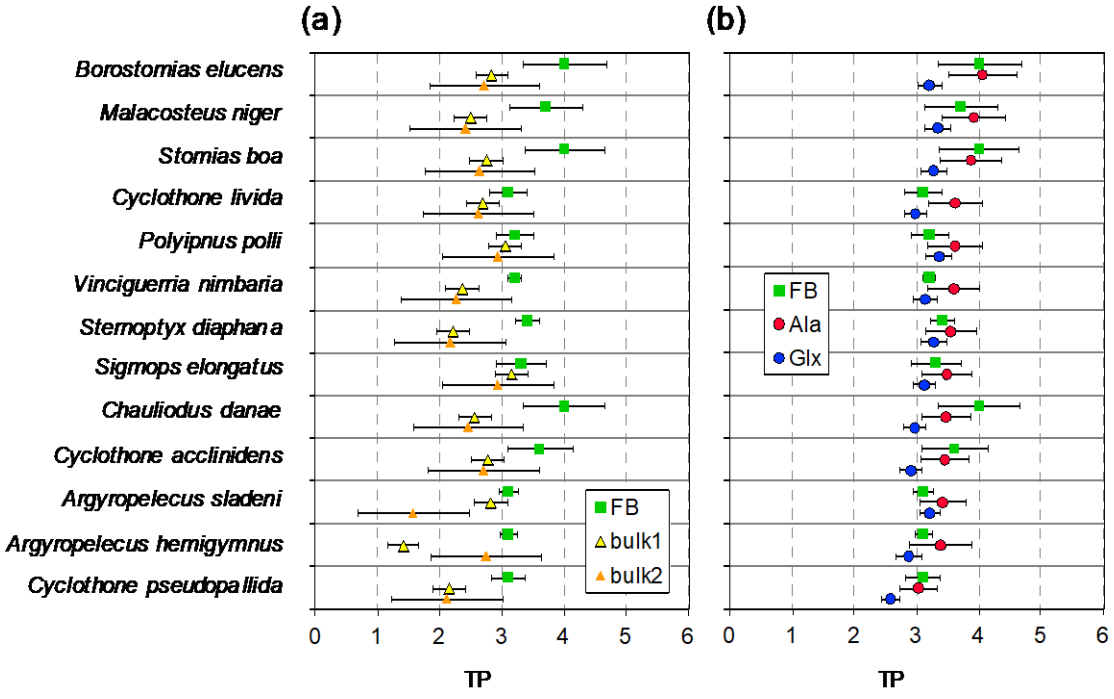

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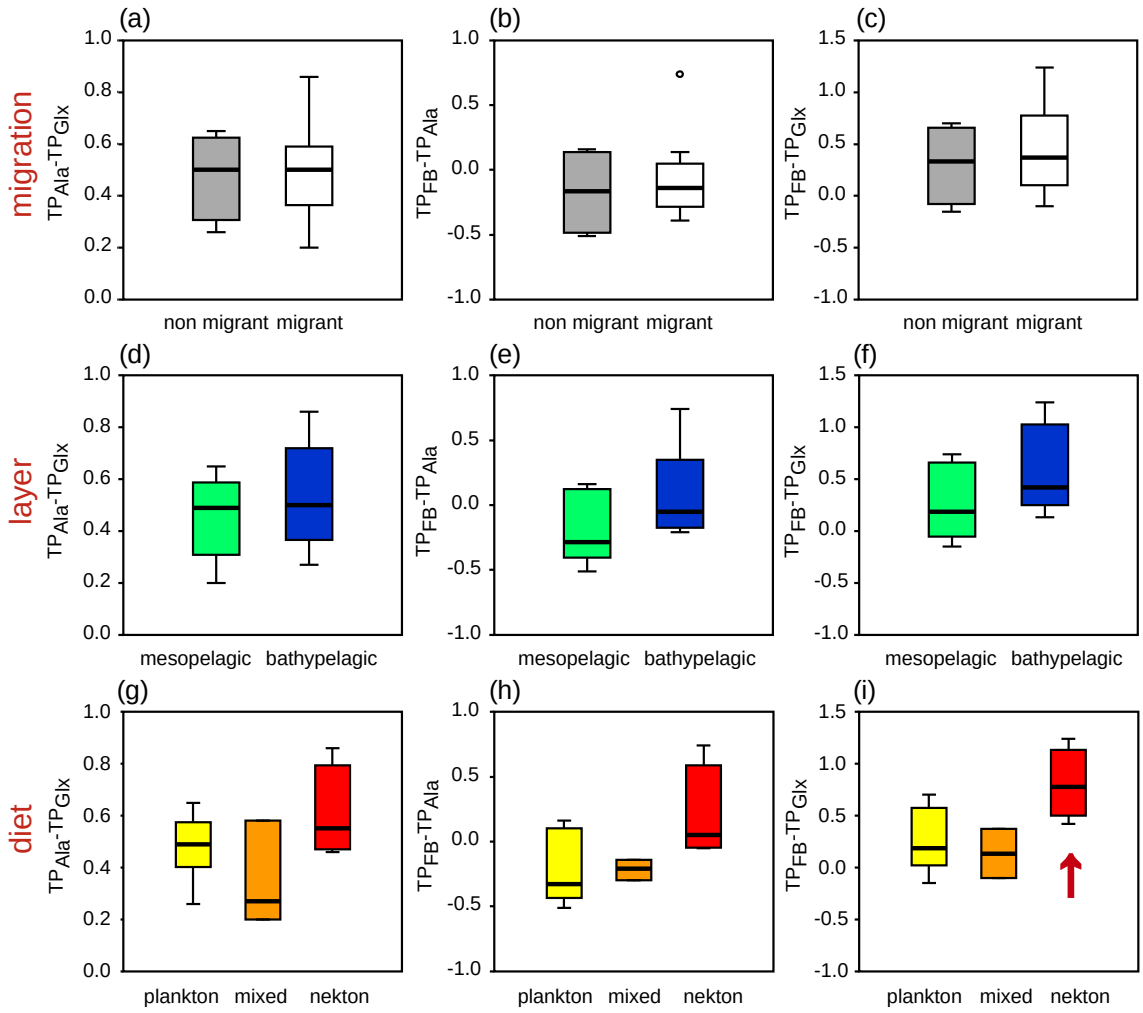

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Supplementary Table S1. Details of the stomiiform fishes analysed in this study. Longitude (lon) and latitude (lat) of collection, and standard length (SL, mm) of specimens are provided. One individual was analysed for most species, except for *S. boa* (pool of 3 individuals), *P. polli* and *C. pseudopallida* (pools of 2 individuals each). Species vertical depth range (layer, m) and migration habits (M: migrant; NM: non-migrant; PM: partial migrant) were obtained from Olivar et al. (2017), own unpublished data, and FishBase. Main diet according to FishBase and additional references.

Species	Family	lon	lat	depth layer	SL	migration habit	diet†	diet
Cyclothone acclinidens	Gonostomatidae	-21.3721	14.3818	300-800	31	NM	plankton[1-3]	zooplankton (mainly copepods and debris)
Cyclothone livida	Gonostomatidae	-21.3721	14.3818	400-800	31	NM	no data	zooplankton ?
Cyclothone pseudopallida	Gonostomatidae	-22.6762	10.8212	300-800	31	NM	plankton[4,5]	zooplankton (mainly copepods)
Sigmops elongatus	Gonostomatidae	-17.3951	25.3535	100-1200	111	PM	nekton[5-8]	midwater fish and crustaceans
Vinciguerria nimbaria	Phosichthyidae	-23.9500	7.2500	0-800	48	M	plankton[6,8-10]	zooplankton (mainly copepods)
Argyropelecus hemigymnus	Sternoptychidae	-21.3722	14.5105	0-1000	30	PM	mixed[2,4,5,8,11-13]	zooplankton (also midwater fish)
Argyropelecus sladeni	Sternoptychidae	-22.6762	10.8212	100-700	31	PM	plankton[14,15]	crustaceans
Polyipnus polli	Sternoptychidae	-20.1641	18.1283	200-600	35	NM	plankton[16]	zooplankton (mixed crustaceans)
Sternoptyx diaphana	Sternoptychidae	-20.2150	18.0719	100-1200	27	PM	mixed[2,8,13,15,17]	zooplankton (also midwater fish)
Borostomias elucens	Stomiidae	-20.1641	18.1283	0-1500	98	M	nekton[18]	midwater fish and crustaceans
Chauliodus danae	Stomiidae	-20.2150	18.0719	0-1800	152	M	nekton[16,19-21]	midwater fish and crustaceans
Malacosteus niger	Stomiidae	-22.6462	10.9406	0-1800	71	M	mixed[8,16,18,22,23]	zooplankton (also crustaceans and fish)
Stomias boa	Stomiidae	-20.1641	18.1283	0-700	91	M	nekton[11,24]	midwater fish and crustaceans

†Numbers indicate references listed below

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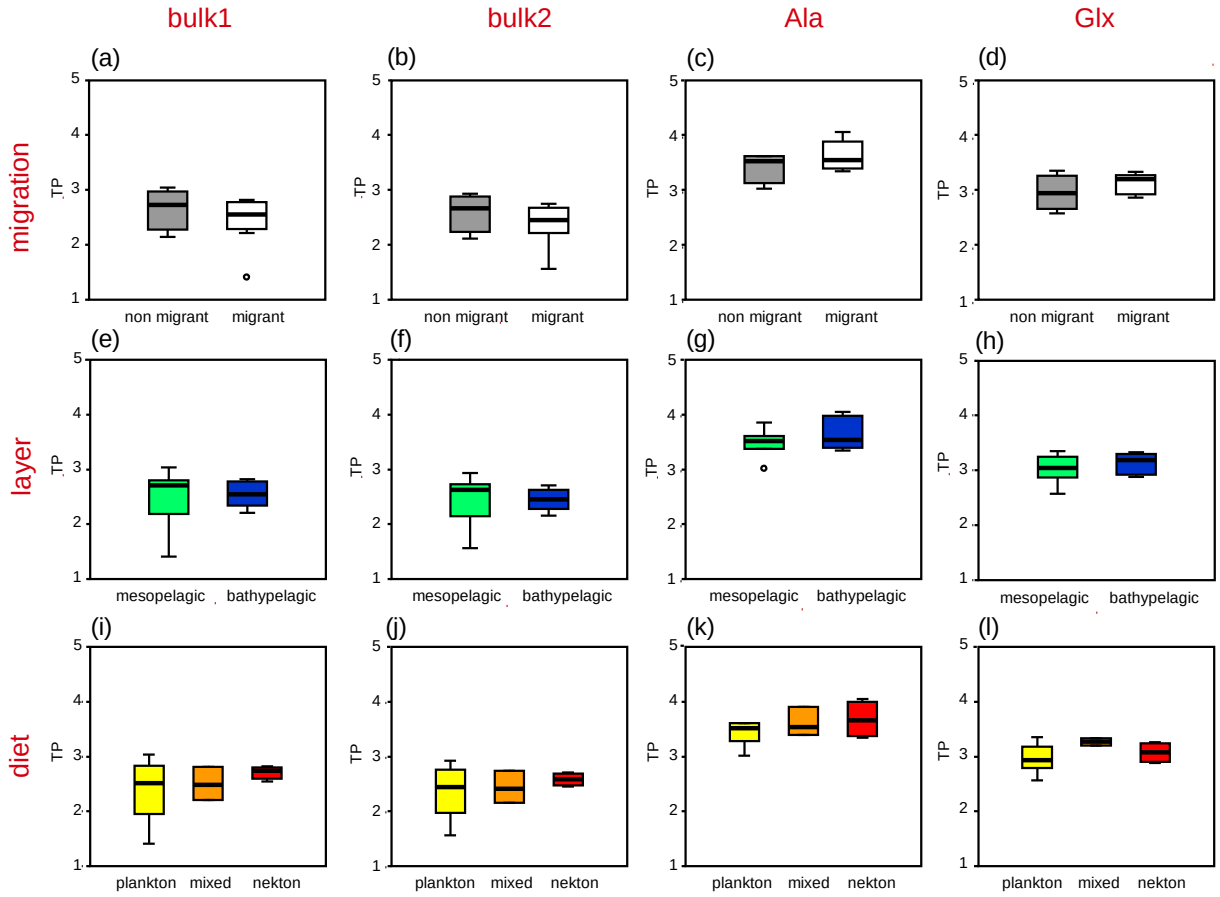
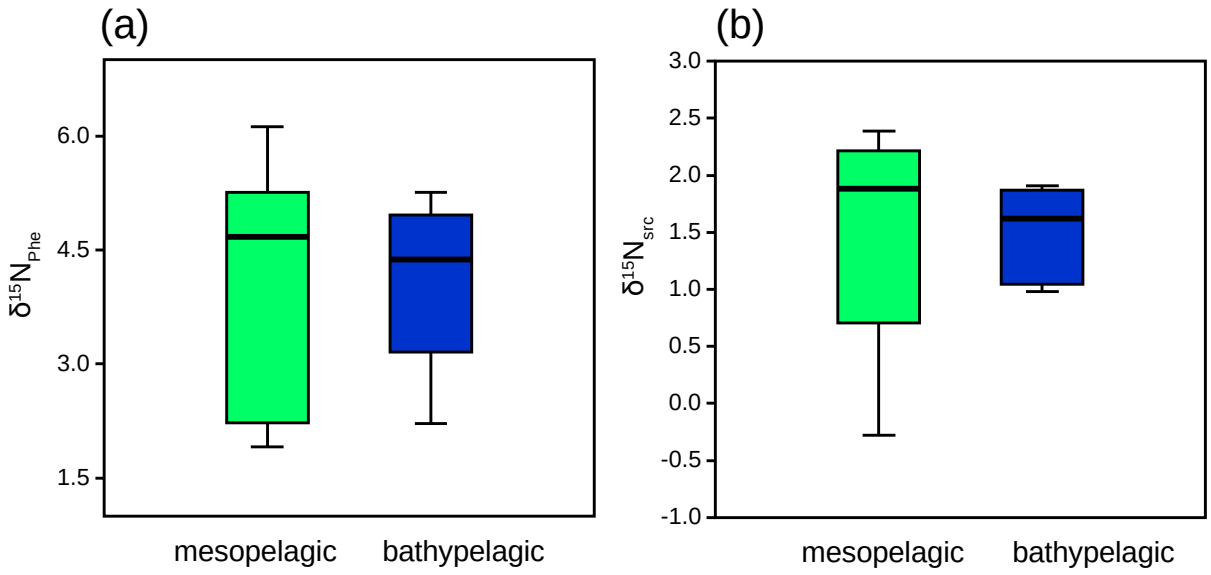


Supplementary Figure S1. Box plots of trophic position (TP) estimates (see Table 1) of the stomiiform fishes analysed in this study grouped by migration habits (migrants and partial migrants vs. non-migrants), habitat depth layers (mesopelagic, bathypelagic), and feeding types (plankton, mixed, nekton). Each box encompasses the 25 and 75% quartiles, whereas the whiskers indicate 1.5 times the interquartile range, the horizontal line indicates the median, and circles indicate outliers (>1.5 times the interquartile range).



Supplementary Figure S2. Box plot of $\delta^{15}N$ baseline in (a) phenylalanine ($\delta^{15}N_{Phe}$) or (b) averaged source amino acids ($\delta^{15}N_{src}$) in the stomiiform fishes analysed in this study grouped by habitat depth layers. The box encompasses the 25 and 75% quartiles, the whiskers indicate 1.5 times the interquartile range, and the horizontal line indicates the median.