



Trophic hierarchy of coastal marine fish communities viewed via compound-specific isotope analysis of amino acids

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ABSTRACT: Coastal marine ecosystems are very complex and composed of myriad organisms, including offshore, coastal, and migratory fish occupying diverse trophic positions (TPs) in food webs. The illustration of trophic hierarchy based on the TP and resource utilization of individual organisms remains challenging. In this study, we applied compound-specific isotope analysis of amino acids to estimate the TP and isotopic baseline (i.e. $\delta^{15}\text{N}$ values of primary resources at the base of food webs) for 13 fish and 1 squid species in a coastal area of Sagami Bay, Japan, where a large diversity in the isotopic baseline is caused by an admixture of ocean currents and artificial nitrogen inputs. Our results indicate that the TP of fish and squid varies between 2.9 and 3.9 (i.e. omnivorous, carnivorous, and tertiary consumers), with low variation within individual species. Moreover, the $\delta^{15}\text{N}$ values of phenylalanine revealed the diversity of isotopic baselines between and within species. Low values (7.8–10.3‰) and high values (18.6–19.2‰), with a small variation ($1\sigma < 1.0\%$), were found in 2 offshore species and 3 coastal species, respectively. In contrast, highly variable values (9.8–19.7‰), with large variation within species ($1\sigma > 1.0\%$), were found for the remaining 9 migratory species. These results represent evidence of differential trophic exploitation of habitats between offshore and coastal species, particularly among individuals of migratory species, that were all collected in a single area of Sagami Bay.

KEY WORDS: Trophic position · Habitat · Food web · Nutrient input · Compound-specific isotope analysis · Amino acids · Nitrogen isotope

1. INTRODUCTION

For the last several decades, marine ecosystems have been increasingly impacted by human activities, including eutrophication of coastal areas by nutrient inputs, ocean acidification by CO_2 emissions, in-

creased concentrations of pollutants by biomagnification, and the extinction of organisms by overfishing (Wren & Stephenson 1991, Jackson et al. 2001, Conley et al. 2009). These problems affect marine ecosystems in many ways, including alterations of the food webs (e.g. Ostrom et al. 2017, Morra et al. 2019).

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The characterization of the trophic positions (TPs) of organisms and their use of resources within food webs is useful in understanding changes in marine ecosystems (e.g. Wada et al. 1987, Vander Zanden & Rasmussen 1996, Post 2002, Fry 2006).

The TPs of organisms have long been estimated by stomach content analysis, based on the direct identification of diets that the organisms feed on. However, this method has several drawbacks, as it provides only a snapshot of feeding, is biased toward easily detectable prey species, and does not always allow clear identification of digested items. To solve these issues, stable isotope analysis of bulk nitrogen in protein-rich tissues of organisms has been used since the 1980s, based on the increase in nitrogen stable isotope ratios ($\delta^{15}\text{N}$) between food and consumers (e.g. DeNiro & Epstein 1981, Minagawa & Wada 1986). The use of stable isotopes allows us to see the integrated prey information at the turnover time of the tissue: days to weeks for zooplankton (Tiselius & Fransson 2016) and months to years for fish (Sweeting et al. 2005). However, this bulk isotope method is not always useful for the study of food webs, particularly for coastal marine ecosystems. In coastal marine ecosystems, the primary producers (mostly phytoplankton) can utilize multiple isotopically distinct inorganic nitrogen sources (e.g. NH_4^+ and NO_3^-) to synthesize the amino acids required to produce biomass protein, which is transferred to upper trophic levels by consumers such as zooplankton and fish. The large difference in the life span and integration time of the isotopes between phytoplankton and fish makes it difficult to compare the $\delta^{15}\text{N}$ values between primary producers and consumers, which is further complicated when the latter exploit different habitats (e.g. Cabana & Rasmussen 1996, Fourqurean et al. 1997, Vander Zanden et al. 1997, O'Reilly et al. 2002, Post 2002).

To avoid asynchronies in the life spans between primary producers and consumers, compound-specific isotope analysis of amino acids (CSIA-AA) has been proposed as an alternative tool for calculating the TPs of organisms (e.g. Chikaraishi et al. 2007, McCarthy et al. 2007, Popp et al. 2007). This method is based on predictable trophic increases in the $\delta^{15}\text{N}$ values between amino acids along food chains (Gaebler et al. 1966, McClelland & Montoya 2002, Steffan et al. 2015, McMahan & McCarthy 2016). Such increases (trophic discrimination factor, TDF) allow for estimating the TP from the $\delta^{15}\text{N}$ values of amino acids within a single organism, thus avoiding the problem with bulk isotope analysis caused by differences in the life span between primary producers

and consumers, and therefore illustrating the food web structure from TPs in marine ecosystems (e.g. Chikaraishi et al. 2014). However, CSIA-AA has not yet been applied to coastal areas where isotopically distinct multiple inorganic nitrogen forms are used as nitrogen sources for primary producers and incorporated into food webs. In such environments, the estimations of TP by the bulk method are less precise in assessing the dynamics of coastal ecosystems (e.g. Rolff 2000, Carscallen et al. 2012).

Sagami Bay (Japan) is a complex environment in which nutrient dynamics are largely affected by ocean currents, agricultural fertilization, and industrial nitrogen fixation, which translates into a large diversity in the $\delta^{15}\text{N}$ values of primary producers (Fujiki et al. 2004, Baek et al. 2009). This diversity has resulted in some serious underestimations of the TPs of starfish, bivalves, and gastropods, which were estimated to be autotrophs according to bulk isotope analysis (Won et al. 2007). Setting the wrong baseline also led to serious overestimation of the TP for the planktivorous Japanese anchovy *Engraulis japonicus* (e.g. James 1988), which was estimated to be predatory according to the bulk isotope analysis (Miyachi et al. 2015).

In this study, we applied CSIA-AA to a collection of fish and squid species from Sagami Bay to estimate their TPs and evaluate their dependence on isotopically distinct inorganic nitrogen inputs. Moreover, based on the TPs and the $\delta^{15}\text{N}$ values of amino acids of these organisms, the diversity of isotopic baselines (i.e. $\delta^{15}\text{N}$ value of primary resources at the base of food webs) for these species appears to represent differences in their preferential habitat at the level of species and individuals for this coastal area.

2. MATERIALS AND METHODS

2.1. Sample collection

In May 2010, we used a fixed shore net to collect 13 fish and 1 squid species (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m652p137_supp.pdf) from a coastal area in Sagami Bay, Japan ($35^\circ 37' \text{N}$, $139^\circ 25' \text{E}$) (Fig. 1). Sagami Bay is located in the southwest of Tokyo Bay, and is connected to the Pacific Ocean. Two major rivers (Sakawa and Hayakawa) near the study site deliver a large amount of agricultural nutrients from farms (including rice, vegetable, and fruit farms) to Sagami Bay. The squid *Todarodes pacificus*, the bluefin searobin *Chelidonichthys spinosus*, and the marbled flounder *Pseudo-*

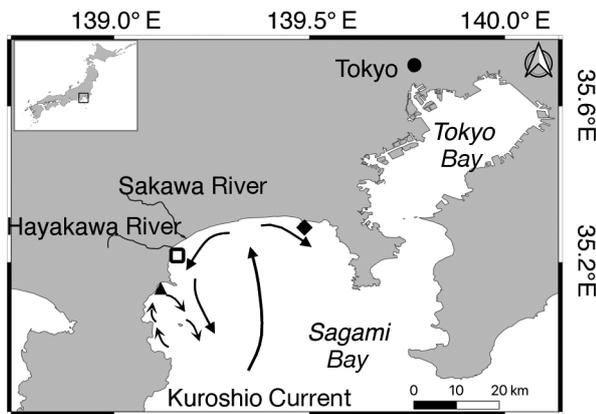


Fig. 1. Sagami Bay, Japan. Open square marks the sampling site for this study. Sites of previous studies marked as follows: filled triangle, Chikaraishi et al. (2014); filled diamond, Miyachi et al. (2015). Arrows represent the Kuroshio and its branched currents

pleuronectes yokohamae were collected as representatives of offshore–pelagic, offshore–benthic, and migrating–benthic species, respectively. The other fish were classified as coastal– or migrating–pelagic species based on body shapes (i.e. fusiform for 8 species and compressiform for 3 species, Table S1); fusiform fishes are characterized by a streamlined body shape for swimming long distances across offshore and coastal areas, whereas compressiform species are characterized by a laterally compressed body shape for producing quick bursts of speed for living in coastal areas (e.g. Roy et al. 2007). All studied fish and squid are ammonotelic, and we analyzed only adult fish and squid in the present study. The collected samples were cleaned with filtered seawater to remove surface contaminants and stored at -20°C before analysis.

2.2. Isotope analysis

Muscle tissues were taken from these samples and prepared for isotopic analysis. The nitrogen isotopic composition of amino acids was determined following the procedure described by Chikaraishi et al. (2009). In brief, the muscle tissues were hydrolyzed with 12 N HCl at 110°C overnight (>12 h). The hydrolysate was washed with *n*-hexane/dichloromethane (3/2, v/v) to remove hydrophobic constituents. Derivatizations were then performed sequentially with thionyl chloride/2-propanol (1/4) and pivaloyl chloride/dichloromethane (1/4). The *N*-pivaloyl/isopropyl (Pv/iPr) esters of amino acids were extracted with *n*-hexane/dichloromethane (3/2, v/v). The nitro-

gen isotopic composition of amino acids was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) (Chikaraishi et al. 2014). To assess the reproducibility of the isotope measurement and to obtain the amino acid isotopic composition, reference mixtures of 9 amino acids (alanine, glycine, leucine, norleucine, aspartic acid, methionine, glutamic acid, phenylalanine, and hydroxyproline) with known $\delta^{15}\text{N}$ values (-26.6 to 45.7 ‰, Indiana University, Shoko Science) were derivatized to Pv/iPr esters and analyzed after every 4 to 6 sample runs, and 3 pulses of reference N_2 gas were discharged into the IRMS instrument at the beginning and end of each chromatography run for both reference mixtures and samples. The isotopic composition of amino acids in samples was expressed relative to atmospheric nitrogen (air) on scales normalized to known $\delta^{15}\text{N}$ values of the reference amino acids. The accuracy and precision for the reference mixtures were always 0.0 ‰ (mean of Δ) and 0.4 – 0.7 ‰ (mean of 1σ), respectively, for sample sizes of ≥ 1.0 nmol N. We note that glutamine was converted into glutamic acid during the acid hydrolysis; as a result, the $\delta^{15}\text{N}$ value of glutamic acid was the combined value of glutamic acid itself and the α -amino group of glutamine (Chikaraishi et al. 2009). In this study, we obtained the $\delta^{15}\text{N}$ value of 6 amino acids (glycine, valine, leucine, isoleucine, glutamic acid, and phenylalanine) for all species and 3 amino acids (alanine, proline, and serine) for several species based on the peak intensity and separation on the GC/IRMS chromatogram.

2.3. Calculation of TP and isotopic baseline

The TP of samples was calculated based on the comparison of large (3 – 8 ‰) and small (0 – 1 ‰) TDFs between trophic (e.g. glutamic acid and alanine) and source amino acids (e.g. phenylalanine and methionine), respectively (e.g. Chikaraishi et al. 2007, McCarthy et al. 2007, Popp et al. 2007). Indeed, the distinct TDF between glutamic acid and phenylalanine has been frequently used to calculate the TP of diverse organisms including fish (Chikaraishi et al. 2014, Steffan et al. 2015), based on the following equation:

$$\text{TP} = (\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} - \beta) / \text{TDF}_{\text{Glu-Phe}} + 1 \quad (1)$$

where $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ represent the $\delta^{15}\text{N}$ values of glutamic acid and phenylalanine, respectively, in a studied organism, β represents a constant offset between $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ values in primary producers (e.g. 3.4 ‰, Chikaraishi et al. 2009), and $\text{TDF}_{\text{Glu-Phe}}$ represents the difference in the TDF

between glutamic acid and phenylalanine in consumers (e.g. 7.6‰, Chikaraishi et al. 2009).

Moreover, the $\delta^{15}\text{N}$ values of source amino acids (AAs) in high TP consumers represent the mean $\delta^{15}\text{N}$ values of primary producers (i.e. $\delta^{15}\text{N}_{\text{Baseline}}$, which is approximately equal to the mean $\delta^{15}\text{N}$ value of inorganic nitrogen in tropical and temperate areas, e.g. Minagawa & Wada 1986, Altabet & Francois 1994) (Fig. 2). Because there is a small TDF in phenylalanine (e.g. 0.4‰ for each trophic level, Chikaraishi et al. 2009), Eq. (2) was used to minimize this effect, and the $\delta^{15}\text{N}_{\text{Baseline, Phe}}$ values were obtained to compare the resource utilization among samples:

$$\delta^{15}\text{N}_{\text{Baseline, Phe}} = \delta^{15}\text{N}_{\text{Phe}} - 0.4 (\text{TP} - 1) \quad (2)$$

3. RESULTS

The $\delta^{15}\text{N}$ values varied from 5.5–22.0‰ for phenylalanine ($14.0 \pm 4.3\%$, mean \pm 1 SD) and from 25.0–43.2‰ for glutamic acid ($34.4 \pm 5.1\%$) in the studied species (Table S1). Among these species, the chicken grunt *Parapristipoma trilineatum* and the seabass *Lateolabrax japonicus* had the lowest and highest $\delta^{15}\text{N}$ values of glutamic acid ($\delta^{15}\text{N}_{\text{Glu}}$), re-

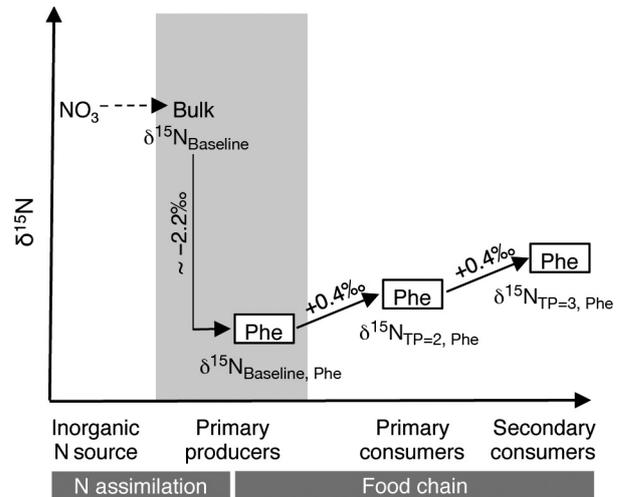


Fig. 2. Relationship of $\delta^{15}\text{N}_{\text{Phe}}$, $\delta^{15}\text{N}_{\text{Baseline}}$, and $\delta^{15}\text{N}_{\text{Baseline, Phe}}$. Phe: phenylalanine, TP: trophic position. Shaded bar: isotopic fractionation associated with phenylalanine synthesis in primary producers

spectively, whereas *P. trilineatum* and whitetongue jack *Uraspis helvola* had the lowest and highest $\delta^{15}\text{N}$ values of phenylalanine ($\delta^{15}\text{N}_{\text{Phe}}$), respectively.

The mean $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ values for each of the 13 fish and 1 squid species (Fig. 3) are plotted along the trophoclines that were defined from Eq. (1) as

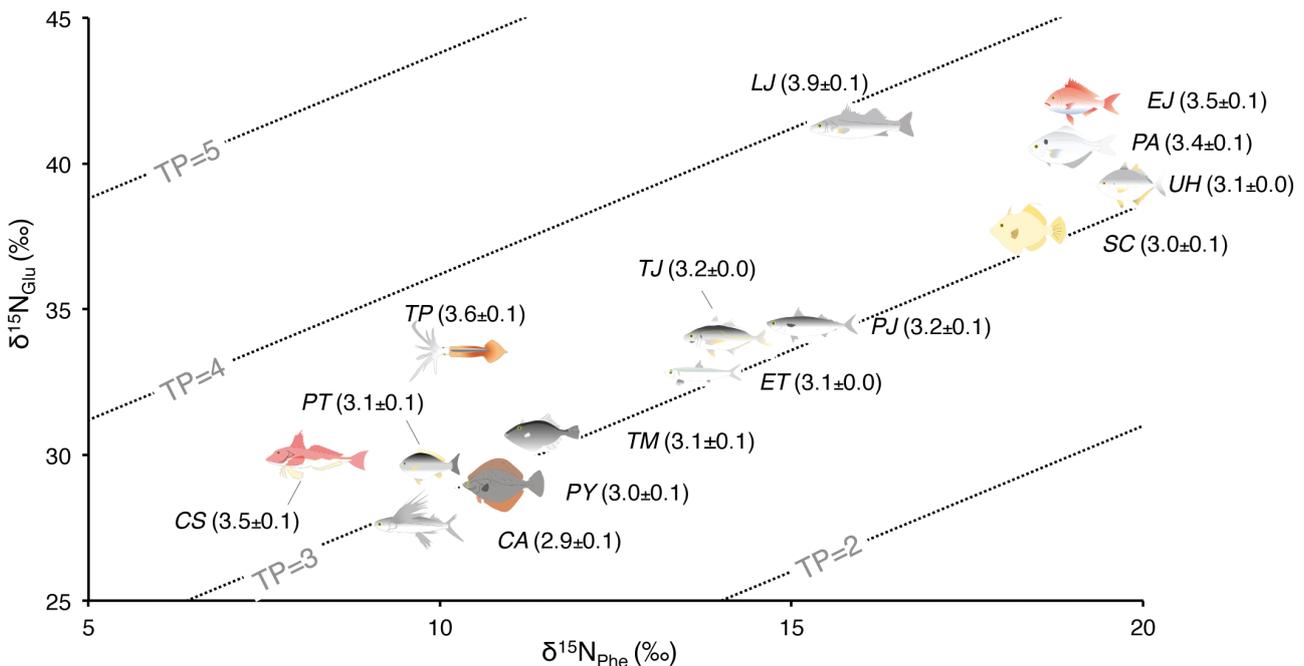


Fig. 3. Cross-plot of the $\delta^{15}\text{N}$ values of glutamic acid (Glu) and phenylalanine (Phe). Lines represent integer trophic positions (TPs, i.e. 2, 3, 4, and 5) estimated with Eq. (1). Numbers in brackets show the mean \pm SD of TP for the species (CA: *Cypselurus agoo*, CS: *Chelidonichthys spinosus*, EJ: *Evynnis japonica*, ET: *Etrumeus teres*, LJ: *Lateolabrax japonicus*, PA: *Psenopsis anomala*, PJ: *Pneumatophorus japonicus*, PT: *Parapristipoma trilineatum*, PY: *Pseudopleuronectes yokohamae*, SC: *Stephanolepis cirrifer*, TJ: *Trachurus japonicus*, TM: *Thamnaconus modestus*, TP: *Todarodes pacificus*, UH: *Uraspis helvola*)

having a slope of 1.0 for each TP and an interval of 7.6‰ for each trophic transfer in food webs (e.g. Steffan et al. 2013, Chikaraishi et al. 2014, Bowes & Thorp 2015, Nielsen et al. 2015). Notwithstanding a large variation in the $\delta^{15}\text{N}_{\text{Phe}}$ value, the TP values varied only between 2.9 (e.g. flyingfish *Cypselurus agoo*) and 3.9 (*L. japonicus*) (Table S1, Fig. 3), and did not correlate with the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values ($R^2 = 0.02$, $p = 0.4$). The TP values significantly but weakly correlated with the $\delta^{15}\text{N}$ values of glutamic acid ($R^2 = 0.30$, $p = 0.0002$, Fig. S1A) but did not correlate with those of phenylalanine ($R^2 = 0.02$, $p = 0.318$, Fig. S1B). For example, *C. spinosus* and the crimson seabream *Evynnis japonica* had the same TP (both 3.5 ± 0.1), although there were large differences in their $\delta^{15}\text{N}_{\text{Glu}}$ (29.8 ± 1.2 and 41.5 ± 0.5 ‰, respectively) and $\delta^{15}\text{N}_{\text{Phe}}$ values (7.8 ± 0.7 and 18.9 ± 0.3 ‰, respectively) between these 2 species (Table S1).

The $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values (13.1 ± 4.3 ‰) followed the $\delta^{15}\text{N}_{\text{Phe}}$ values, ranging from 4.6‰ (*P. trilineatum*) to 21.1‰ (*U. helvola*) among studied samples (Table S1). The variability in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value was species-dependent, as exemplified by the smallest ($1\sigma = 0.6$ ‰) and largest ($1\sigma = 8.0$ ‰) variation for *E. japonica* and *P. trilineatum*, respectively. The TP did not correlate with the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values ($R^2 = 0.02$, $p = 0.4$).

4. DISCUSSION

4.1. Food web structure

The trophoclines defined by the $\delta^{15}\text{N}$ values of trophic and source amino acids have been used to understand food web structure, not only in terms of TP but also in the simultaneous interpretation of isotopic baselines (e.g. Chikaraishi et al. 2014, Bowes & Thorp 2015, Nielsen et al. 2015, Steffan et al. 2015). In 2-dimensional trophocline space, all samples are expected to fall within a relatively vertical line when there is a single food chain starting from isotopically uniform primary producers in the studied area. Alternatively, the samples are expected to distribute along the trophoclines, according to their proximities to the mean of their diet resources, when there are multiple food chains that have multiple, isotopically distinct primary producers (Chikaraishi et al. 2014). For instance, the $\delta^{15}\text{N}$ values of phenylalanine can be directly used as isotopic baseline for illustrating the trophocline, because of its small TDF (0.4‰ for each trophic level) (e.g. Chikaraishi et al. 2014, Ohkouchi et al. 2017).

In this study, the trophocline space defined by the $\delta^{15}\text{N}$ of glutamic acid and phenylalanine illustrates a food web structure with TP varying from 2.9 to 3.9 among species. Nine species (e.g. *Pseudopleuronectes yokohamae*) are secondary consumers (TP = 3.0 ± 0.1), 1 species (i.e. *L. japonicus*) is a tertiary consumer (TP = 3.9 ± 0.1), and the other 4 species occupy intermediate positions (TP = 3.5 ± 0.1). These results are consistent with the expected TP of the studied fish and squid species, as reported in the literature; for instance, the sardine *Etrumeus teres* and the sardine *L. japonicus* were characterized as zooplanktivorous and piscivorous fish, respectively (Nip et al. 2003, Falautano et al. 2006); also, the estimated TP of *P. trilineatum* (TP = 3.1 ± 0.1) in the studied area is equivalent to the value reported by Chikaraishi et al. (2014) for this species collected from a different area in Sagami Bay (TP = 2.9 ± 0.1).

Based on the $\delta^{15}\text{N}$ values of phenylalanine (i.e. isotopic baseline), we infer the existence of multiple distinct food chains for the fish and squid species investigated. The offshore–pelagic and offshore–benthic species (e.g. *P. yokohamae*) belong to food chains characterized by low baseline values (6.8–9.3‰), the compressiform species (e.g. *E. japonica*) belong to food chains with high baseline values (17.8–18.3‰), and fusiform species (e.g. *L. japonicus*) belong to food chains with intermediate baseline values (9.6–18.9‰). These results are consistent with our expectation of a large variation in the $\delta^{15}\text{N}$ baseline values in Sagami Bay (Won et al. 2007, Miyachi et al. 2015).

4.2. Effect of $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values on the TP

A large variation in the $\delta^{15}\text{N}$ values at the base of food webs theoretically represents diversity in the $\delta^{15}\text{N}$ value of nutrient sources. Alterations in these sources pose potential risks for the preservation of ecological conditions, primary production, and ultimately food web structures (e.g. Cabana & Rasmussen 1996, Baeta et al. 2017, Hicks et al. 2017). In this study, variability in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value between individuals of a single species is species dependent (Table S1). Indeed, a small variation in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value was found for all offshore–pelagic and offshore–benthic species, as well as for the 3 compressiform species (Fig. 4a,b, respectively), whereas the 8 fusiform species (Fig. 4c) had a much larger variability. In contrast, for all species investigated in this study, the variation in TP within species was small ($1\sigma = 0.1$) (Fig. 3). For instance, we found no substantial difference in the TP among 3 indi-

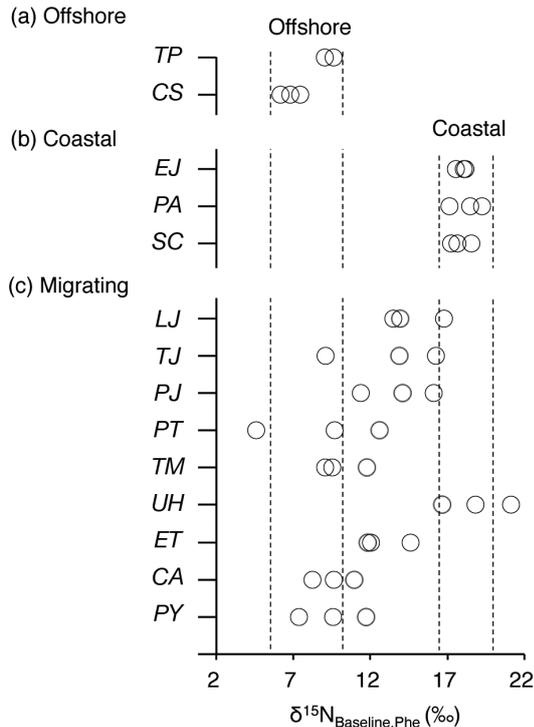


Fig. 4. Diversity in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value of fish and squid with respect to their habitats (i.e. offshore vs. coastal, and pelagic vs. benthic). Vertical dashed lines demarcate the coastal and offshore regions. See Fig. 3 for fish abbreviations

viduals of *P. trilineatum* ($3.1 \pm 0.1\text{‰}$), despite their variable $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values ($9.0 \pm 4.1\text{‰}$). However, *E. japonica* had a low variability in both TP (3.5 ± 0.1) and $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value ($17.9 \pm 0.3\text{‰}$) among the 3 specimens investigated. In addition to the lack of correlation between TP and $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values when all species were pooled, these results suggest that the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values do not affect the TPs of organisms in Sagami Bay. Although the investigation of the underlying cause is outside of the scope of this study, our findings imply that the inclusion of different sources of inorganic nitrogen does not affect the size of food chains in the study area.

4.3. Habitats

Agricultural runoff (e.g. from rice, vegetable, and fruit farms), as well as artificial dams and wastewater treatment plants frequently cause anoxic conditions. In these conditions, denitrification produces N_2 gas, enriched in ^{14}N and leaving behind a pool of ^{15}N -enriched nitrate (e.g. Kellman & Hillaire-Marcel 2003, Sebilo et al. 2006). The abundance of nutrients with high $\delta^{15}\text{N}$ is temporally dependent on agricul-

tural cycles and precipitation, and they are incorporated into the Bay and mixed with the coastal water by ocean currents. The spatial dependence of such inputs likely explains the difference between the results of this study, with high and variable $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ ($13.0 \pm 4.1\text{‰}$), and the low and homogeneous values ($4.6 \pm 1.1\text{‰}$) found at the previous study site (Chikaraishi et al. 2014) within Sagami Bay. This is consistent with our expectation of isotopically distinct multiple inorganic nitrogen forms in the food web of our study site, but not at the previous site. We conclude that our study site has been considerably affected by agricultural and artificially derived nutrient inputs, while the latter has been affected by natural sources of nutrients, with negligible inputs of agricultural and artificially derived nutrients (Chikaraishi et al. 2014).

Low $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values for *T. pacificus* and *Chelidonichthys spinosus* (Fig. 4) can be attributed to their preferential use of offshore–pelagic and offshore–benthic habitats, respectively (Watanabe et al. 1996, Byun et al. 2013), where a lower contribution from agricultural and artificial nutrient inputs relative to the coastal area can be expected. In contrast, high $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values of 3 compressiform fish species (i.e. *E. japonica*, the Pacific rudderfish *Psenopsi anomala*, and the threadsail filefish *Stephanolepis cirrifer*) inhabiting the coastal area (Yamaoka et al. 2003, Wang et al. 2004), can be attributed to a large contribution from agricultural and artificial nutrient inputs. The other 9 fish are migrating fusiform and benthic species, as they are found in a variety of habitats extending from offshore to coastal areas (Wada et al. 2007, Fuji et al. 2011). The use of a wide range of habitats is consistent with highly variable $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values overlapping those of specialized offshore and coastal species. Interestingly, there was a large individual variability in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value within the migrating fusiform species. In the case of *T. japonicus*, the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ values of 3 individuals were 9.1, 13.9, and 16.3‰. Applying a 2-end member mixing model, with reference baseline values of 7.8 and 18.0‰ for offshore and coastal habitats, respectively, we can estimate the offshore:coastal habitat ratio of these specimens as 9:1, 2:3, and 1:4, respectively. These results imply that each individual used both habitats differently, maybe because they belonged to independent groups or subpopulations of this species. Based on these results, the large difference in the $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ value among the species in this study reveals diversity in the use of habitats (i.e. offshore vs. coastal) at the level of species and of individuals, respectively, even though they were collected from a single study site.

4. CONCLUSIONS

The CSIA-AA method provides greater resolution to TP estimation (vs. traditional bulk methods, per Chikaraishi et al. 2009), and has been used to elucidate the trophic structure of food webs characterized by 2 isotopically distinct primary producers (algae vs. seagrass: Choi et al. 2017; algae vs. terrestrial plants: Ishikawa et al. 2018). In the present study, we illustrated the trophic tendencies of fish and squid species with a wide range of isotopically distinct primary producers (i.e. $\delta^{15}\text{N}_{\text{Baseline,Phe}}$ ranging from 4.6–21.2‰), and show a large diversity in the habitat use among and within species. These findings further support the applicability of the CSIA-AA method to food web studies in which there is large variation among $\delta^{15}\text{N}_{\text{Baseline}}$ values, which can cause large errors in TP estimation when using the traditional bulk isotope method.

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