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Spatial food-web structure in the eastern tropical Pacific Ocean based on compound-specific nitrogen isotope analysis of amino acids

Elizabeth D. Hetherington,[1,2,a]* Robert J. Olson,[3] Jeffrey C. Drazen,[4] Cleridy E. Lennert-Cody,[3] Lisa T. Ballance,[5] Ronald S. Kaufmann,[2] Brian N. Popp[6]

[1]Division of Biological Sciences, University of California, San Diego, La Jolla, California
[2]Department of Environmental and Ocean Sciences, University of San Diego, San Diego, California
[3]Inter-American Tropical Tuna Commission, La Jolla, California
[4]Department of Oceanography, University of Hawaii, Honolulu, Hawaii
[5]Marine Mammal and Turtle Division, Southwest Fisheries Science Center, NOAA Fisheries, La Jolla, California
[6]Department of Geology and Geophysics, University of Hawaii, Honolulu, Hawaii

Abstract

The effective evaluation of trophic interactions in pelagic food webs is essential for understanding food web ecology, conservation biology, and management. We tested the applicability of compound-specific isotope analysis of amino acids (CSIA-AA) for (1) characterizing trophic positions (TPs) of nine species from four trophic groups (tunas, squids, myctophids, and euphausiids) within a pelagic food web in the eastern tropical Pacific (ETP) Ocean, (2) evaluating trophic discrimination factors (TDFs) of each trophic group, and (3) detecting spatial changes in TPs and food chain length across a region with heterogeneous productivity. Although δ^{15}N values of bulk tissues generally increased from south-to-north, CSIA-AA revealed that trophic positions were uniform throughout our study area. These results suggest that variability in δ^{15}N values were largely driven by nitrogen cycling dynamics in the ETP, which highlights the importance of these processes for the interpretation of δ^{15}N values in food web studies. Absolute TP estimates were unrealistic for higher-level species, and TDFs (tunas: 4.0‰, squids: 4.6‰, myctophids: 5.0‰, and euphausiids: 7.0‰) were lower than a widely used ecosystem TDF. We used remotely sensed oceanographic data to evaluate the physical oceanography and biological productivity throughout our study area and found significant relationships between δ^{15}N values, nitrate concentrations, and SST across our study area. We did not find a gradient in phytoplankton cell size co-occurring with an expected productivity gradient across our sampling region, which substantiated our isotope results indicating non-significant spatial changes in TP and food chain length across the ETP.

Understanding and evaluating trophic structure and food web dynamics is a prerequisite for an ecosystem-based approach to fisheries and provides a means for assessing environmental and anthropogenic impacts on trophic ecology. Commercial fisheries can affect food webs by removing top predators and triggering top-down trophic cascades (Pauly et al. 1998; Worm and Myers 2003; Essington and Hansson 2004; Pauly and Palomares 2005). Simultaneously, environmental variability can affect nutrient availability and alter food webs through bottom-up processes at seasonal, inter-annual, and decadal scales (Watters et al. 2003; Fernández-Álamo and Färber-Lorda 2006; Pennington et al. 2006). In order to address questions about energy flow through ecosystems, we need effective methods for evaluating trophic interactions and food web structure in dynamic marine systems. Our aim here is to test the applicability of a proliferating technique, compound-specific isotope analysis of amino acids (CSIA-AA), on an entire pelagic food web to estimate trophic position (TP) and variability in trophic ecology of multiple species over a portion of the pelagic eastern tropical Pacific (ETP) Ocean.

Historically, the characterization of food web structure in marine ecosystems has been based on stomach contents analysis, which provides only a snapshot of a consumer's most recent meal, and can be biased by processes such as

*Correspondence: ehetheri@ucsd.edu

[a]Present address: Division of Biological Sciences, University of California, San Diego, La Jolla, California

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size-based gastric evacuation (e.g., Olson and Boggs 1986), regurgitation, and cod-end feeding. To circumvent such shortcomings, nitrogen isotope ratios have been used for elucidating trophic interactions and food web structure (DeNiro and Epstein 1981; Peterson and Fry 1987; Fry 1988). Nitrogen stable isotope values provide information about a consumer's diet because the ^{15}N value of a consumer's tissues is enriched relative to the ^{15}N of its dietary components (DeNiro and Epstein 1981; Hobson et al. 2002). Although the nitrogen stable isotopic composition of bulk tissue or whole animals can provide useful information, this approach also has limitations when the objective is to determine absolute TPs. Traditional TP estimates for predators require, in addition to $\delta^{15}N$ measurements of the predator's tissues, $\delta^{15}N$ measurements of the base of the food web (primary producers), or a proxy for baseline values (e.g., suspension-feeding scallops, Jennings and Warr 2003). Baseline $\delta^{15}N$ measurements are often difficult to obtain in open-ocean, pelagic ecosystems because primary producers typically have very short life spans, and isotopic compositions that can vary substantially (Bronk et al. 1994; Rolff 2000; Hannides et al. 2009). The $\delta^{15}N$ composition at the base of the food web is governed by the dominant N transformation processes in the region (i.e., N recycling, phytoplankton utilization of nitrate, N_2-fixation, or denitrification; Gruber and Sarmiento 1997; Altabet 2001; Voss et al. 2001). Without reliable measurements of baseline or baseline proxy $\delta^{15}N$ values, it is unclear whether isotopic variability in predator tissues is due to variability in trophic structure or due to nitrogen biogeochemistry that affects baseline isotopic values, which then propagate up the food web, or a combination of these two factors.

Time-integrated TPs of consumers and the nitrogen isotope composition at the base of the food web can be determined using results of CSIA-AA (McClelland and Montoya 2002; Popp et al. 2007; Chikaraishi et al. 2009). The $\delta^{15}N$ values of specific amino acids in a consumer's tissues provide information that overcomes limitations of bulk tissue isotope analysis. "Trophic" amino acids (e.g., glutamic acid, alanine) appear to be enriched in ^{15}N relative to "source" amino acids (e.g., phenylalanine, lysine) because of isotopic fractionation during transamination and deamination (Chikaraishi et al. 2007, 2009; Popp et al. 2007). Conversely, source amino acid metabolism does not cleave or form nitrogen bonds, so the isotope composition of these amino acids show little fractionation, and are conservative biomarkers that reflect the nitrogen isotope composition at the base of the food web (Chikaraishi et al. 2007, 2009; Popp et al. 2007). In essence, the fractional TP of a consumer is estimated as the difference between the $\delta^{15}N$ values of the trophic and source amino acids, while factoring in trophic discrimination values (McClelland and Montoya 2002; Popp et al. 2007; Chikaraishi et al. 2009).

The universality of the $\delta^{15}N$ CSIA-AA approach for measuring time-integrated trophic structure for multiple consumers spanning marine food webs, however, remains unclear. Previous CSIA-AA studies have provided reasonable TP estimates for zooplankton in culture (McClelland and Montoya 2002), gastropods in a marine coastal ecosystem (Chikaraishi et al. 2007), krill in the Antarctic (Schmidt et al. 2004; Schmidt et al. 2006), plankton in the Central Pacific (McCarthy et al. 2007), zooplankton near Hawaii (Hannides et al. 2009), leatherback turtles (*Dermochelys coriacea*) in the Pacific (Seminoff et al. 2012), lanternfishes across the globe (Choy et al. 2012), and yellowfin tuna in the eastern tropical Pacific Ocean (Popp et al. 2007; Olson et al. 2010). Recent studies, however, have shown conflicting results, i.e., underestimated TPs of mesozooplankton in the California Current ecosystem (Décima et al. 2013), elasmobranchs in coastal waters of Hawaii (Dale et al. 2011), penguins (Lorrain et al. 2009; McMahon et al. 2015a,b), teleosts (Bradley et al. 2015), and harbor seals in captivity (Germain et al. 2013). These underestimates were presumably due to interspecific differences between source and trophic amino acids in the trophic discrimination of ^{15}N. Studies on variability in trophic discrimination factors (TDFs) have also been widely discussed for bulk $\delta^{15}N$ values, with increasing evidence showing that a single ecosystem TDF value is unlikely and arguing for lower TDF values for higher trophic level organisms (Hussey et al. 2013; Reum et al. 2015). Despite a growing number of studies, applications of CSIA-AA have focused on isolated components of disparate ecosystems, and a systematic study encompassing an entire pelagic food web is lacking. Our approach was to sample the dominant functional groups occupying various TPs from a natural pelagic ecosystem to test the assumption of using a single ecosystem TDF value. Examining relationships within a single food web provides a holistic approach missing from previous ecological research based on $\delta^{15}N$ CSIA-AA.

Our study was conducted in the eastern tropical Pacific Ocean (ETP), a biogeochemically and oceanographically diverse region (Fiedler and Talley 2006; Pennington et al. 2006), for which an integrated representation of the food web has been provided (Olson and Watters 2003). The thermocline depth shoals from west-to-east across the ETP, and therefore nutrient availability and productivity varies spatially (Pennington et al. 2006). Additionally, oceanographic conditions vary on seasonal, interannual, and decadal time scales, which affect temporal nutrient availability and biological production in the ETP (Barber and Chavez 1986; Fiedler 2002).

Although several processes influence $\delta^{15}N$ values, previous work has demonstrated high denitrification rates in regions with pronounced oxygen minimum zones, like the ETP (Cline and Kaplan 1975; Voss et al. 2001; Somes et al. 2010; Lorrain et al. 2015). Since the ETP has one of the largest OMZs in the world, we expect high baseline $\delta^{15}N$ values

Table 1. Trophic groups, species names, and common names of the nine species utilized in our study. Trophic position (TP) estimates are from Olson and Watters (2003) and are used to compare with our TP estimates using $\delta^{15}N$ values from compound-specific isotope analysis of amino acids. Sample sizes (*N*) per species varied for bulk isotope analysis ($\delta^{15}N_{Bulk}$) and compound-specific isotope analysis of amino acids ($\delta^{15}N_{AA}$).

Trophic group	Species name	Common name	Trophic position	*N* ($\delta^{15}N_{Bulk}$)	*N* ($\delta^{15}N_{AA}$)
Micronektonivores	*Thunnus albacares*	Yellowfin tuna	4.57	14	6
	Thunnus obesus	Bigeye tuna	4.53	13	6
	Katsuwonus pelamis	Skipjack tuna	4.57	15	6
Cephalopods	*Dosidicus gigas*	Humboldt squid	4.40	8	3
	Sthenoteuthis oualaniensis	Purpleback flying squid		3	3
Mesopelagic micronekton	*Myctophum nitidulum*	Lanternfish	3.19	13	6
	Symbolophorus reversus			13	6
Macrozooplankton	*Euphausia distinguenda*	Krill	2.70	7	6
	Euphausia tenera			6	5

compared with areas dominated by nitrogen fixation. Nitrate concentrations (Somes et al. 2010) and isotopic fractionation due to nitrate utilization by phytoplankton (Waser et al. 1998; Altabet 2001) also influence $\delta^{15}N$ values, so we expect that $\delta^{15}N$ values will be related to local denitrification and nitrate concentrations.

Environmental and oceanographic variability affect trophic ecology and $\delta^{15}N$ values by altering the nutrient supply to the base of the food web, and ultimately determining the amount of energy that reaches higher-level consumers (Barnes et al. 2010; Polovina and Woodworth 2012; Rousseaux and Gregg 2012; Young et al. 2015). For example, in oligotrophic areas, small picophytoplankton (i.e., cyanobacteria) are capable of taking up nutrients in low concentrations and therefore are typically the dominant phytoplankton functional group. Phytoplankton communities in oligotrophic areas are typically dominated by small phytoplankton, which can result in an increase, of up to two trophic levels, in the number of steps in the food web, compared with food webs in eutrophic areas dominated by larger phytoplankton (i.e., diatoms) because many zooplankton cannot directly consume picophytoplankton (Seki and Polovina 2001; Barnes et al. 2010; Young et al. 2015). Therefore, variability in productivity throughout the ETP may be reflected by differences in phytoplankton cell size and subsequent food web structure. We utilized remotely sensed oceanographic data to evaluate spatial variability in estimated phytoplankton cell size in our study area.

The goal of this study is to evaluate the applicability of CSIA-AA for providing insight into the trophic structure of a pelagic ecosystem in the ETP. Our first objective was to estimate TPs of nine species in four trophic groups in the ETP, including krill, lanternfishes, squids, and tunas using CSIA-AA, and empirically evaluate variability in trophic discrimination factors (TDFs) for these components. We hypothesized that the previously published whole-ecosystem TDF value of 7.6‰ (Chikaraishi et al. 2009) would not be appropriate for our higher TP species (i.e., tunas) given findings of recent studies (Bradley et al. 2015; McMahon et al. 2015a,b). Our second objective was to evaluate spatial differences in TPs of the aforementioned trophic groups. Due to a previously described east-west productivity gradient in the ETP, we hypothesized that there would be significant differences in food web structure across our study area, specifically in food chain length and TPs, as a result of differences in phytoplankton size. The final objective was to evaluate relationships between remotely sensed oceanographic data and nitrogen isotopic values. We hypothesized that nutrient concentrations would provide a mechanism to help explain variability in $\delta^{15}N$ values, as nutrient dynamics, specifically nitrate concentrations, affect biogeochemical cycling and $\delta^{15}N$ values (Deutsch et al. 2001; Voss et al. 2001; Somes et al. 2010). This study is the first to utilize nitrogen CSIA-AA to evaluate the major components of a complete open-ocean, pelagic food web, and contributes to the development of CSIA-AA as a tool for measuring time-integrated trophic structure for multiple marine taxa that occupy a range of TPs.

Methods

Sample collection and processing

We analyzed tissue samples from nine species that represent four distinct trophic groups across the food web in the pelagic ETP: macrozooplankton (euphausiid crustaceans), micronekton (myctophid fishes), cephalopods (squids), and micronektonivores (tunas; Table 1). Zooplankton, small mesopelagic fishes, and squids were collected from 28 July 2006 to 08 December 2006 during the National Oceanic and Atmospheric Administration's (NOAA's) *Stenella* Abundance Research (STAR) surveys (Gerrodette et al. 2008). We defined our study area to include a subset of sample locations (Fig. 1, Supporting Information 1) from the STAR surveys based on the presence of both east-west and north-south productivity gradients across the region, with greater surface chlorophyll

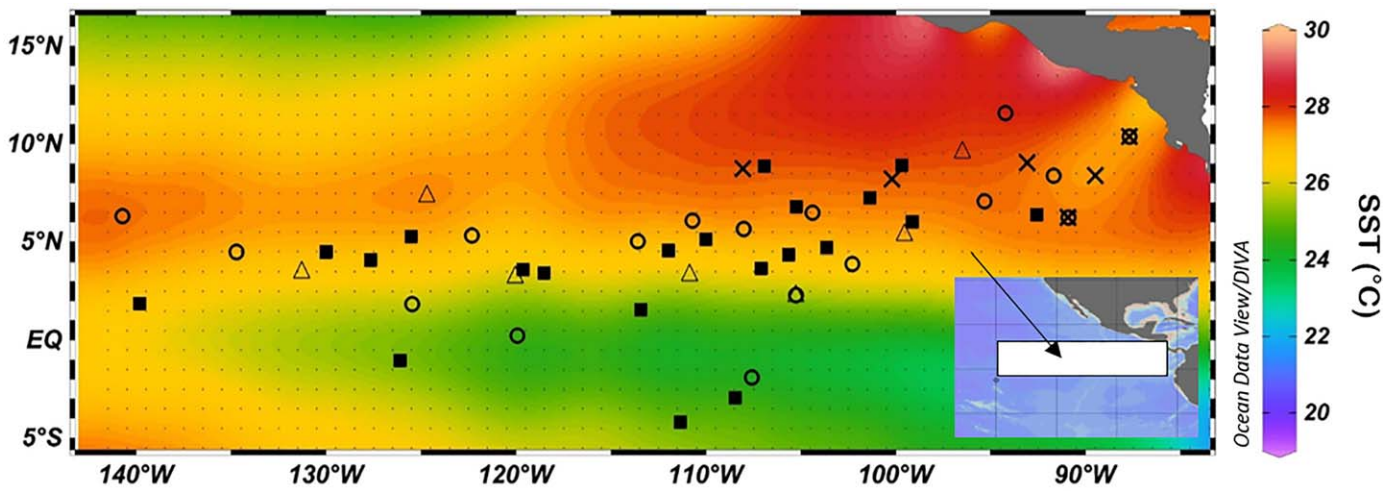

Fig. 1. Map of sea-surface temperature and sampling locations in the eastern tropical Pacific, with an inset showing the larger geographic context of our study area. Nine species are clustered into four trophic groups: 1) small micronektonivores (■): *Thunnus albacares*, *Katsuwonus pelamis*, and *T. obesus*, 2) cephalopods (X): *Dosidicus gigas* and *Sthenoteuthis oualaniensis*, 3) mesopelagic micronekton (○): *Myctophum nitidulum* and *Symbolophorus reversus*, and 4) macrozooplankton (▲): *Euphausia distinguenda* and *E. tenera*. Sea-surface temperature (SST) data are 2005 annual means from NODC's World Ocean Atlas (https://www.nodc.noaa.gov/). Refer to Supporting Information 1 for locations of replicate samples.

a (Chl *a*) concentrations at the eastern end of the study area and along the equator, according to published oceanographic data. Zooplankton were sampled with a cylindrical-conical bongo net (333 μm mesh), towed to 200 m approximately 2 h after sunset, and specimens were processed within 1 h of collection. Specimens of mesopelagic myctophid fishes *Myctophum nitidulum* (M.n.) and *Symbolophorus reversus* (S.r.) were collected by dipnet at night. Specimens of the squids *Dosidicus gigas* (D.g.) and *Sthenoteuthis oualaniensis* (S.o.) also were collected at night, using handlines and jigs. (See Olson et al. 2010; Philbrick et al. 2001 for detailed methods).

Three species of tuna, yellowfin (T.a.; *Thunnus albacares*), skipjack (K.p.; *Katsuwonus pelamis*), and bigeye (T.o.; *Thunnus obesus*) tunas, were sampled year-round during 2003–2005 by observers of the Inter-American Tropical Tuna Commission onboard purse-seine fishing vessels. Samples of dorsal white muscle were taken from each fish adjacent to the second dorsal fin. Fish of uniform size were used for analysis: skipjack tuna 450–550 mm, yellowfin tuna 500–700 mm, and bigeye tuna 450–550 mm. All samples were stored frozen until further processing in the laboratory.

Zooplankton samples were thawed slowly, and euphausiids were identified to species, and immediately refrozen in seawater. Two species, *Euphausia distinguenda* (E.d.) and *Euphausia tenera* (E.t.), were present in all bongo samples in sufficient numbers for isotope determinations, and were therefore chosen for the analysis. Only fully intact individuals were analyzed, and due to small body size, multiple individuals of each species from each sample were pooled for the analysis. Myctophid samples consisted of muscle and skin from individual fish, whereas the heads, fins, stomachs, and vertebral columns were removed. Skin was removed from the tuna white muscle samples and squid mantle samples before analysis. Each sample was lyophilized for 24 h and homogenized to a fine powder using a ceramic mortar and pestle.

Bulk isotope analysis

Isotopic analysis of bulk muscle tissue or whole animals was performed at the University of Hawaii's Isotope Biogeochemistry Laboratory. All isotopic results from this study are available through BCO-DMO (http://www.bco-dmo.org/project/491309). Stable isotope values of nitrogen were determined using an on-line carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer (FinniganConFlo II/Delta-Plus). Isotope values are reported in δ notation relative to air (DeNiro and Epstein 1981). Mean accuracy of all stable isotopic analyses was $< \pm$ 0.1‰ (1 SD) based on triplicate analysis of in-house reference materials (glycine standard and tuna muscle) with known $\delta^{15}N$ values.

The number of samples used for bulk isotope analysis per location for each species was typically one (Table 1), although there was a subset of locations at which we analyzed more than one individual per species (Supporting Information 1). To avoid weighting certain locations more than others in the spatial analyses (because of the imbalance in samples per location by species), we averaged the individual bulk isotope values by species at each location where multiple samples per species were taken. Isotope values from tuna samples collected at the same location were averaged, as specimens that were caught in the same set on a single tuna school were not independent. Myctophids vertically

migrate in groups, and specimens caught together at the same location were also considered to not be independent. We analyzed bulk $\delta^{15}N$ of tuna white muscle tissue from three individuals per species at two locations in our study area, and at other stations we analyzed single samples per species. We analyzed two *Symbolophorus reversus* and *Myctophum nitidulum* individuals values at three and two locations, respectively, and averaged those values. Since the squid species we utilized are non-schooling species, we considered sample from those species independent, and did not average squid isotope values. Fewer euphausiid samples were available compared with other taxa, so we were unable to average euphausiid isotope values by location.

CSIA-AA

Due to analytical costs, we conducted CSIA-AA on a subset of 48 of the samples. The basis for sample selection was to represent the range of variability in bulk $\delta^{15}N$ values and the range of sample locations along the transect. Samples were prepared for CSIA-AA following the protocol of Popp et al. (2007), Hannides et al. (2009), and Hannides et al. (2013). To isolate amino acids, we hydrolyzed samples (6N HCl, 150°C for 70 min; Cowie and Hedges 1992), esterified (4 : 1 isopropanol : acetyl chloride), and derivatized (3 : 1 methylene chloride : trifluoroacetyl anhydride). The derivatization method can affect the yield of amino acids. We analyzed trifluoroacetyl and isopropyl ester (TFA) derivatives (Hannides et al. 2013). Derivatives were analyzed with ISODAT software by a Trace GC gas chromatograph and a Thermo Delta XP mass spectrometer through a GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap. We injected samples (split/splitless, 5 : 1 split ratio) with a 180°C injector temperature and a constant helium flow rate of 2 mL min^{-1} onto a 0.32 i.d., 50-m HP column with 0.52 μm thickness. More details of CSIA-AA methodology can be found in Popp et al. (2007) and Dale et al. (2011).

To maximize analytical precision and accuracy during sample analysis, we performed multiple assays (i.e., machine runs), until standard deviations for the $\delta^{15}N$ values of each amino acid (AA) were $< 1‰$. Analytical errors for amino acid $\delta^{15}N$ were generally under 1‰, but ranged from 0.08‰ to 1.65‰, and averaged 0.43‰. The number of assays required was typically three, but on occasion, an additional assay was required. Amino acid values were corrected to the $\delta^{15}N$ values of norleucine and aminoadipic acid internal reference standards with known nitrogen isotopic compositions; these were co-injected with each sample. Quality control was confirmed by analyzing a suite of several AAs with known $\delta^{15}N$ values every 4–5 assays. $\delta^{15}N$ values of 18 amino acids were analyzed, although some amino acids were not detected on the chromatographs. Amino acids were grouped into two categories, trophic amino acids: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), leucine (Leu), and proline (Pro); and source amino acids: glycine (Gly), lysine (Lys), phenlyalanine (Phe), serine (Ser), and threonine (Thr). Since Thr values are extremely negative relative to other source AAs, we recognize that including Thr as a source AA may not be appropriate and recent studies are classifying Thr as a "metabolic" amino acid (Germain et al. 2013; Bradley et al. 2015).

We calculated fractional TPs using glutamic acid, a trophic amino acid, and phenylalanine, a source amino acid (Chikaraishi et al. 2009; Hannides et al. 2009; Choy et al. 2012). Nitrogen isotope values of phenylalanine show minimal isotopic fractionation, which indicates that it is an appropriate source amino acids to use for estimating TPs (Chikaraishi et al. 2009), and has been used to evaluate spatial patterns in $\delta^{15}N$ at the base of the food web (Popp et al. 2007; Chikaraishi et al. 2009; Sherwood et al. 2011; Seminoff et al. 2012; Décima et al. 2013). We used glutamic acid as our trophic amino acid, since Chikaraishi et al. (2009) found the greatest $\delta^{15}N$ enrichment and smallest standard errors associated with this trophic AA compared to others. We used the following equation to estimate TP:

$$TP_{Glu-Phe} = \frac{\left(\delta^{15}N_{Glu} - \delta^{15}N_{Phe}\right) - \beta}{TDF} + 1 \quad (1)$$

where $TP_{Glu\text{-}Phe}$ is trophic position based on glutamic acid (Glu) and phenylalanine (Phe), TDF is the trophic discrimination factor (the ^{15}N enrichment of $\delta^{15}N_{Glu}$ with respect to $\delta^{15}N_{Phe}$ per trophic step, reported as 7.6‰ by Chikaraishi et al. 2009), and β represents $\delta^{15}N_{Glu}$-$\delta^{15}N_{Phe}$ in primary producers ($3.4 \pm 0.9‰$; Chikaraishi et al. 2009; McCarthy et al. 2013). We used standard error-propagation formulas to calculate errors associate with TP estimates (see Blum et al. 2013; Bradley et al. 2015).

Oceanographic data

We analyzed pre-processed oceanographic data to evaluate relationships between oceanographic conditions and bulk nitrogen stable isotope values for nine species. The variables we chose were: nitrate and phosphate concentrations ($\mu g\ L^{-1}$), a denitrification index (N^*), sea-surface temperature [SST (°C)], sea-surface height [SSH (cm)], Chl *a* [Chl *a* (mg/m^3)], thermocline depth [TD (m)], and phytoplankton cell diameter (μm). Preliminary analyses indicated that there were poor relationships between isotope data and Chl *a*, TD, and SSH. Additionally, several of the variables were correlated with each other (see Supporting Information 2), so we limited our emphasis mostly to nitrate concentrations, phytoplankton cell diameter, and N^*. See Supporting Information for more detailed information on the remainder of the oceanographic variables.

Ideally, the oceanographic variables should be averaged over time periods equivalent to $\delta^{15}N$ turnover rates in the tissues of the various taxa. However, estimates of tissue turnover rates were unavailable for most species. As a

compromise, we averaged oceanographic variables annually, as estimated tissue turnover rates for yellowfin tuna are within a 12-month time interval (94% turnover; Graham 2008). Nutrient concentration data were acquired from the 2005 World Ocean Atlas (WOA2005) through NOAA's National Oceanographic Data Center (www.nodc.noaa.gov) for one-degree, objectively analyzed mean annual fields, representing annual averages of surface concentrations. N^* was derived using nitrate and phosphate concentrations (Gruber and Sarmiento 1997). Remotely sensed Chl *a*, SSH, and SST were acquired as monthly means through NOAA's OceanWatch program (http://oceanwatch.pifsc.noaa.gov/). See Supporting Information 3 for more detailed information on oceanographic data acquisition.

To evaluate the variability in phytoplankton cell size across our study area, which could indicate potential differences in dominant phytoplankton functional groups and trophic structure, we used Chl *a* and SST values to estimate median phytoplankton cell mass (M_{B50}), using the following predictive equation derived from a global dataset of 361 water samples (Barnes et al. 2011):

$$\log_{10}(M_{B50}) = 1.340 - 0.043\ (\mathrm{SST}) + 0.929\ (\log_{10}(\mathrm{Chl}\ a)). \quad (2)$$

Median cell diameter (M_{D50}) was calculated from M_{B50} estimates using the following equation from Polovina and Woodworth (2012):

$$M_{D50} = 2.14\ (M_{B50})^{0.35}. \quad (3)$$

We calculated a denitrification index, N^*, using the follow equation by Deutsch et al. (2001), which was modified from that of Gruber and Sarmiento (1997):

$$N^* = (\mathrm{N} - 16\mathrm{P} + 2.90\ \mu\mathrm{mol\ kg^{-1}}), \quad (4)$$

where N and P are nitrate and phosphate concentrations ($\mu g\ L^{-1}$), respectively. Negative N^* values indicate complete utilization of nitrate in regions with a phosphate deficit (denitrification) and positive values are indicative of a gain in nitrate concentration (nitrogen fixation). We utilized N^* values at both 0 m and 200 m to assess differences between surface values and those below the photic zone, where denitrification is likely more dominant compared with surface waters.

Data analysis

Spatial trends in nitrogen isotope values

We used linear regression analyses to evaluate whether variability in bulk $\delta^{15}N$ values could be attributed to spatial variability in regional biogeochemistry, changes in food web structure, or both. To evaluate the spatial variability in $\delta^{15}N$ values, we fitted a multiple linear regression model to the bulk isotope data of each species with latitude and longitude as predictor variables. Estimated slopes from these models were compared among species using a *t*-test. We used R (R Development Core Team 2008) for this and all other statistical analyses. To evaluate the influence of trophic differences and variability in nitrogen cycling processes on bulk $\delta^{15}N$ values across our sampling area, we fitted a univariate linear model to data of all species for $\delta^{15}N_{Phe}$, a source amino acid, with bulk $\delta^{15}N$ as the predictor variable. Species differences were not considered in this analysis because, in theory, the relationship between bulk $\delta^{15}N$ and $\delta^{15}N_{Phe}$ should be the same across all taxa.

Spatial structure in TP estimates was evaluated in two ways. First, we evaluated spatial trends in TP computed from Eq. 1 across latitude and longitude separately, for each species, using univariate, rather than multivariate, linear regressions due to limited sample sizes per species. Second, using data for all species, we fitted linear mixed effects models (LMEs; Pinheiro and Bates 2004) to the replicate AA $\delta^{15}N$ measurement data, as LMEs have been shown to be useful for modeling data with replicate AA $\delta^{15}N$ measurements (Lorrain et al. 2009; Décima et al. 2013). The use of LMEs made it possible to account for variability among samples even though our sampling design was unbalanced as a result of the variability in the number of assays run. Based on preliminary analyses and the limited sample sizes per species, we included latitude and longitude as linear terms (fixed effects), and sample and species as random effects, where sample was nested within species. LMEs were fitted with the "nlme" package in R (Pinheiro et al. 2016). We fitted LMEs using two proxies for TP, $\delta^{15}N_{glu}$-$\delta^{15}N_{phe}$, and $\delta^{15}N_{trophic}$-$\delta^{15}N_{source}$, the latter of which allowed us to incorporate data for several amino acids. We selected a suite of trophic and source amino acids that were detected in samples from all species, where $\delta^{15}N_{trophic}$ = mean of Ala, Glu, Leu, and Pro $\delta^{15}N$ values, and $\delta^{15}N_{source}$ = mean of Gly, Phe, Lys, and Ser $\delta^{15}N$ values. This approach is similar to using Eq. 1 but circumvents the dependence on TDF and β values (Décima et al. 2013).

We compared CSIA-AA derived TP estimates to independent TP outputs from an ecosystem model of the ETP food web (Olson and Watters 2003). We empirically estimated TDFs for the different taxa by rearranging Eq. 1 following Bradley et al. (2015):

$$\Delta^{15}N_{Glu-Phe} = TDF_{AA}(TP_{OW} - 1) + 3.4, \quad (5)$$

where $\Delta^{15}N_{Glu\text{-}Phe} = \delta^{15}N_{Glu} - \delta^{15}N_{Phe}$, and TP_{OW} represents independent trophic position estimates from an ecosystem model for the ETP (Olson and Watters 2003). The slope of this linear equation, is an empirical estimate of TDF for each trophic group. The TDF of 7.6‰, based on Chikaraishi et al. (2009), provides a reference for comparison across taxa. We used *t*-tests to compare the slopes of each trophic group to the line with a slope (TDF) of 7.6‰, as recent studies have indicated that this value is not appropriate for all taxa (Bradley et al. 2015; McMahon et al. 2015a,b; Nielsen et al. 2015).

Oceanographic conditions and their relation to isotope values

To summarize oceanographic conditions in our study area, we evaluated pairwise correlations of the eight aforementioned oceanographic variables by creating a correlation matrix using the Pearson product-moment correlation coefficient (see Supporting Information) and maps of each oceanographic variable.

We fitted a multiple linear regression model with oceanographic variables as the predictors in a stepwise manner (using forward and backward selection) to the bulk $\delta^{15}N$ data of each species to evaluate relationships between bulk $\delta^{15}N$ and oceanographic conditions. Some of our oceanographic variables were correlated with each other (see Supporting Information 2), so we limited the variables in the multiple linear regression to surface nitrate concentration, Chl *a*, and thermocline depth. We used Akaike's Information Criterion (AIC; Burnham and Anderson 2004) to evaluate the relative performance of each model, with a ΔAIC threshold value of 2. The cephalopod species were not included in this analysis as a result of small sample sizes and limited spatial range of sample locations.

We evaluated spatial patterns in $\delta^{15}N$ values and the relationship of $\delta^{15}N$ to oceanography by fitting LME models and creating a nitrogen isoscape of source AA $\delta^{15}N$ values. For all LMEs, sample and species were considered random effects, where sample was nested within species. To test for spatial effects on baseline $\delta^{15}N$ values using source amino acids, $\delta^{15}N_{phe}$ and $\delta^{15}N_{source}$, we fit LMEs in which latitude and longitude were included as linear terms (fixed effects to test for environmental effects, we chose the oceanographic variable that best described bulk $\delta^{15}N$ values in the stepwise model selection for the bulk isotope analysis (see Results below) and fitted LMEs to evaluate the relationship between source amino acid (Gly, Phe, Lys, and Ser) $\delta^{15}N$ values and nitrate concentration (linear fixed effect). We created a spatial isotope map, or isoscape, of $\delta^{15}N_{Phe}$ values for our study area using Ocean Data View 4.5.2 (http://odv.awi.de, Schlitzer, R. 2012), which interpolates areas between sampling locations by means of Data-Interpolation Variational Analysis (DIVA) gridding.

Temporal mismatch caveats

We performed preliminary comparisons of *Dosidicus gigas* bulk $\delta^{15}N$ values from this study with previously collected and analyzed *D. gigas* mantle tissue samples from 2003 and 2010. All samples were collected at the Costa Rica Dome onboard the National Oceanic and Atmospheric Administration's research vessels. We utilized these samples to evaluate potential temporal variability in bulk $\delta^{15}N$ values for a single species. We found no significant differences in $\delta^{15}N$ values between years, which provided some justification for utilizing samples collected from different years.

There was a temporal mismatch in our sample collection between tunas (2003–2005) and other taxa (August–December 2006) due to opportunistically collected samples from two independent sources. Additionally, our study was designed to examine large-scale time integrated processes, not to evaluate temporal variability in $\delta^{15}N$ over time.

We also evaluated oceanographic data from 2003 to 2006 to determine if anomalous conditions were present during our sampling period. We tested whether there were differences in El Niño Southern Oscillation (ENSO) conditions during 2003–2006, using the Oceanic Niño Index (ONI; http://www.cpc.ncep.noaa.gov/), which is based on sea-surface temperature anomalies in the equatorial Pacific. Although 2004 was characterized as a weak El Niño year (defined as 0.5–0.9°C SST anomaly for five consecutive months), we found no significant ONI differences between sampling years (ANOVA: $F = 1.71$, $p = 0.18$). ENSO is the strongest source of variability in the tropical Pacific (Pennington et al. 2006), and temporal stability in this index provides some justification for utilizing samples collected in different years, while applying oceanographic data for one representative year. We chose 2005 annual averages for our oceanographic analyses because those data were available for all nine of our oceanographic variables of interest.

Results

Isotope analyses

Across our sampling area, we found spatial variability and a large range in bulk $\delta^{15}N$ values by species. Species-specific mean $\delta^{15}N$ values over the entire region were highest for the cephalopod *Dosidicus gigas* (13.4‰), and lowest for the euphausiid species *Euphausia tenera* (8.4‰; Table 2). Euphausiid $\delta^{15}N$ values by sample ranged from 4.8‰ to 15.0‰ across the sampling region, with the highest value above the mean values for the tunas and squids. Five of the seven multiple regression models for $\delta^{15}N$, with predictors latitude and longitude were significant (Fig. 2; Table 3, "ddf": denominator degrees of freedom): *Thunnus albacares* ($R^2 = 0.62$, $F = 8.38$, ddf = 7, $p = 0.01$), *Katsuwonus pelamis* ($R^2 = 0.78$, $F = 15.07$, ddf = 6, $p < 0.01$), *T. obesus* ($R^2 = 0.72$, $F = 14.07$, ddf = 8, $p < 0.01$), *Myctophum nitidulum* ($R^2 = 0.59$, $F = 8.34$, ddf = 8, $p = 0.01$), and *Symbolophorus reversus* ($R^2 = 0.52$, $F = 5.82$, ddf = 7, $p = 0.03$). Model significance was largely driven by a significant relationship between $\delta^{15}N$ and latitude; no significant effect of longitude on bulk $\delta^{15}N$ values was identified for any of the species (Table 3). Using *t*-tests, we found no differences in the estimated latitude slopes among species ($p > 0.05$ for all comparisons). We found no significant relationships between $\delta^{15}N$ values vs. latitude and longitude for the euphausiids *E. distinguenda* ($p = 0.06$) and *E. tenera* ($p > 0.1$).

Table 2. Species names, mean $\delta^{15}N_{Bulk}$ values, $\delta^{15}N_{Bulk}$ range for each species, mean amino acid $\delta^{15}N$ values ± standard deviations, and trophic position estimates from CSIA-AA $\delta^{15}N$ values (TP_{AA}) ± propagated errors. Abbreviations for amino acids are: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), leucine (Leu), and proline (Pro), and source amino acids: glycine (Gly), lysine (Lys), phenylalanine (Phe), Serine (Ser), and Threonine (Thr). nd = not detected on chromatographs.

		Source amino acids					Trophic amino acids					
Bulk $\delta^{15}N$ (‰; Mean ± SD, Range)	Species (Samp No.)	Gly	Lys	Phe	Ser	Thr	Ala	Asp	Glu	Leu	Pro	TP*
12.6 ± 2.3	T.a. (4)	−1.9 ± 1.3	6.9 ± 0.6	7.6 ± 0.7	4.3 ± 0.2	−25.1 ± 0.7	26.5 ± 0.9	26.3 ± 0.2	25.9 ± 0.1	24.9 ± 0.4	21.0 ± 0.7	3.0 ± 0.4
8.1–14.6	T.a. (5)	−1.9 ± 0.1	5.8 ± 0.2	8.7 ± 0.4	3.8 ± 0.6	−25.3 ± 0.6	26.4 ± 0.1	24.5 ± 0.3	26.1 ± 0.1	25.1 ± 0.2	21.3 ± 0.4	2.8 ± 0.2
	T.a. (7)	−0.9 ± 0.1	6.4 ± 0.0	7.7 ± 0.2	4.9 ± 0.3	−25.4 ± 0.5	27.3 ± 0.1	28.4 ± 0.2	27.9 ± 0.3	25.9 ± 0.3	23.6 ± 0.3	3.2 ± 0.1
	T.a. (8)	−0.7 ± 0.5	6.2 ± 0.2	6.0 ± 0.6	4.6 ± 0.0	−23.8 ± 0.4	26.7 ± 0.6	26.1 ± 0.2	25.6 ± 0.2	24.0 ± 0.2	21.8 ± 0.3	3.1 ± 0.3
	T.a. (9)	0.4 ± 0.5	5.8 ± 0.4	5.6 ± 0.2	5.1 ± 0.3	−20.3 ± 0.7	25.3 ± 0.2	24.6 ± 0.3	24.3 ± 0.8	22.9 ± 0.1	19.9 ± 0.2	3.0 ± 0.4
	T.a. (12)	−4.4 ± 0.3	2.1 ± 0.2	2.3 ± 0.9	−1.1 ± 0.6	−28.0 ± 2.5	21.6 ± 0.6	21.6 ± 0.2	20.9 ± 0.3	19.3 ± 0.3	18.2 ± 0.9	3.0 ± 0.5
11.9 ± 1.6	K.p.(2)	−1.9 ± 0.4	3.7 ± 0.3	7.7 ± 0.6	3.0 ± 0.7	−22.4 ± 0.3	23.3 ± 0.4	24.6 ± 0.2	24.0 ± 0.4	22.7 ± 0.1	19.0 ± 0.2	2.7 ± 0.4
8.3–14.4	K.p.(5)	−1.4 ± 0.2	6.4 ± 0.3	10.2 ±0.2	4.6 ± 0.7	−22.8 ± 0.4	25.9 ± 0.3	27.3 ± 0.1	27.3 ±0.3	25.1 ± 0.3	22.7 ± 0.5	2.8 ±0.1
	K.p.(6)	−3.2 ± 0.5	4.3 ± 0.2	7.4 ± 0.5	2.8 ± 0.3	−25.7 ± 0.4	25.2 ± 0.5	26.6 ± 0.3	25.4 ± 0.3	24.3 ± 0.3	21.5 ± 0.2	2.9 ± 0.3
	K.p.(8)	−3.3 ± 0.1	3.5 ± 0.1	7.4 ± 0.5	2.3 ± 0.4	−25.6 ± 0.6	23.6 ± 0.1	23.2 ± 0.2	23.3 ± 0.3	22.1 ± 0.4	17.5 ± 0.4	2.6 ± 0.3
	K.p. (11)	−0.1 ± 0.4	6.7 ± 0.2	10.5 ± 0.1	4.6 ± 0.5	−20.5 ± 0.5	25.1 ± 0.3	25.2 ± 0.1	24.5 ± 0.1	23.2 ± 0.3	17.6 ± 0.3	2.4 ± 0.1
	K.p. (13)	−5.3 ± 0.2	0.5 ± 0.1	4.8 ± 0.3	−0.5 ± 0.6	−23.7 ± 0.4	21.6 ± 0.5	21.6 ± 0.1	21.2 ± 0.2	19.4 ± 0.1	14.2 ± 0.6	2.7 ± 0.2
12.7 ± 1.4	T.o. (3)	1.7 ± 0.2	7.4 ± 0.2	10.1 ± 0.7	6.5 ± 0.5	−19.4 ± 0.2	27.8 ± 0.2	26.9 ± 0.2	26.7 ± 0.2	25.1 ± 0.1	23.0 ± 0.3	2.7 ± 0.4
8.7–14.2	T.o. (5)	1.0 ± 0.2	6.3 ± 0.5	7.2 ± 1.0	5.0 ± 0.2	−22.0 ± 0.8	26.7 ± 0.1	26.2 ± 0.4	25.6 ± 0.4	24.0 ± 0.2	21.9 ± 0.5	3.0 ± 0.6
	To. (7)	−2.3 ± 0.4	6.2 ± 0.1	8.0 ± 0.3	3.5 ± 0.9	nd	nd	27.3 ± 0.2	26.1 ± 0.2	24.0 ± 0.2	23.2 ± 0.4	2.9 ± 0.2
	T.o. (11)	−2.5 ± 0.3	5.9 ± 0.6	6.7 ± 0.6	1.8 ± 0.3	−23.6 ± 1.1	26.1 ± 0.1	26.4 ± 0.2	24.9 ± 0.1	22.7 ± 0.2	22.1 ± 0.4	2.9 ± 0.3
	T.o. (13)	−0.8 ± 0.3	nd	6.0 ± 0.5	4.5 ± 0.7	−25.5 ± 0.4	nd	25.7 ± 0.2	26.0 ± 0.4	24.4 ± 0.4	21.0 ± 0.6	3.2 ± 0.3
	T.o. (15)	0.3 ± 0.1	6.1 ± 0.2	7.3 ± 0.2	4.3 ± 0.7	nd	26.4 ± 0.2	26.5 ± 0.1	25.8 ± 0.3	23.6 ± 0.2	21.8 ± 0.5	3.0 ± 0.1
13.4 ± 0.9	D.g. (1)	1.3 ± 0.9	7 ± 0.3	6.2 ± 0.7	9.6 ± 0.9	nd	25.8 ± 0.7	21.0 ± 0.3	25.2 ± 0.2	23.7 ± 0.4	23.9 ± 0.8	3.1 ± 0.4
12.5–14.8	D.g. (4)	1.9 ± 1.1	nd	5.8 ± 0.2	2.0 ± 0.7	−15.8 ± 0.7	26.5 ± 0.6	21.2 ± 0.5	25.9 ± 0.7	25.5 ± 0.6	22.0 ± 0.8	3.2 ± 0.3
	D.g. (6)	2.8 ± 0.3	5.7 ± 0.4	4.6 ± 0.6	9.3 ± 0.1	−16.2 ± 0.8	25.1 ± 0.7	18.4 ± 0.3	23.4 ± 0.2	23.5 ± 0.1	20.5 ± 0.7	3.0 ± 0.3
12.9 ± 1.1	S.o. (1)	−2.2 ± 0.5	nd	4.8 ± 0.3	9.3 ± 0.7	−21.1 ± 0.7	25.3 ± 0.4	20.4 ± 0.3	24.3 ± 0.2	25.0 ± 0.5	24.0 ± 0.5	3.1 ± 0.2
11.7–13.7	S.o. (2)	−4.0 ± 0.1	4.8 ± 0.1	4.6 ± 0.6	5.6 ± 0.5	−19.5 ± 0.5	25.4 ± 0.2	19.4 ± 0.2	23.3 ± 0.2	23.3 ± 0.3	21.3 ± 0.7	3.0 ± 0.3
	S.o. (3)	−1.8 ± 0.6	nd	7.2 ± 0.3	8.6 ± 1.0	−19.0 ± 0.8	28.4 ± 0.2	22.0 ± 0.4	26.2 ± 0.2	25.8 ± 0.4	23.3 ± 0.4	3.1 ± 0.2
10.8 ± 2.0	M.n. (1)	1.8 ± 0.8	4.5 ± 0.6	5.4 ± 0.3	5.2 ± 0.7	−10.0 ± 0.7	21.6 ± 0.4	15.6 ± 0.1	19.0 ± 0.5	18.3 ± 0.3	15.7 ± 0.8	2.3 ± 0.3
5.8–12.4	M.n. (3)	−1.0 ± 0.1	nd	−0.5 ± 0.7	1.8 ± 0.6	−13.5 ± 0.9	18.3 ± 0.3	12.3 ± 0.3	15.7 ± 0.1	14.7 ± 0.3	12.3 ± 1.2	2.7 ± 0.4
	M.n. (5)	2.8 ± 0.1	5.4 ± 0.3	3.6 ± 0.3	4.9 ± 0.5	−11.3 ± 0.5	23.2 ± 0.5	16.2 ± 0.3	19.8 ± 0.5	19.3 ± 0.1	16.5 ± 0.5	2.7 ± 0.3
	M.n. (6)	−0.6 ± 0.5	4.8 ± 0.3	2.7 ± 0.2	3.6 ± 0.4	−11.2± 0.5	21.5 ± 0.3	16.0 ± 0.4	19.8 ± 0.1	19.3 ± 0.4	16.6 ± 0.3	2.8 ± 0.1
	M.n. (7)	3.4 ± 0.3	6.1 ± 0.6	3.1 ± 1.6	6.3 ± 0.4	−9.3 ± 0.4	22.2 ± 0.3	15.7 ± 0.3	18.7 ± 0.2	18.2 ± 0.4	16.1 ± 0.4	2.6 ± 0.8
	M.n. (11)	−2.8 ± 0.2	0.7 ± 0.4	−0.7 ± 0.5	−0.2 ± 0.8	−18.0 ± 0.3	17.7 ± 0.3	11.1 ± 0.2	14.8 ± 0.2	13.6 ± 0.2	10.8 ± 0.2	2.6 ± 0.3
12.2 ± 2.5	S.r. (1)	1.9 ± 0.3	5.6 ± 0.3	3.0 ± 0.5	4.5 ± 0.1	−13.6 ± 1.0	20.8 ± 0.7	14.4 ± 0.2	19.4 ± 0.2	17.6 ± 0.5	15.3 ± 0.3	2.7 ± 0.3
5.6–15.8	S.r. (2)	4.2 ± 0.6	7.9 ± 0.2	6.1 ± 1.6	5.3 ± 0.0	−9.8 ± 0.9	23.3 ± 0.4	17.7 ± 0.3	21.1 ± 0.2	21.6 ± 0.4	17.8 ± 0.5	2.5 ± 0.8
	S.r. (5)	3.0 ± 0.3	6.4 ± 0.1	3.5 ± 0.6	5.4 ± 0.7	−13.0 ± 1.4	21.3 ± 0.7	15.8 ± 0.1	20.3 ± 0.2	18.1 ± 0.1	15.2 ± 0.4	2.8 ± 0.3
	S.r. (6)	6.7 ± 0.3	10.5 ± 0.2	7.4 ± 0.5	8.8 ± 0.8	−10.8 ± 0.8	25.3 ± 0.5	19.6 ± 0.2	23.9 ± 0.1	22.6 ± 0.6	20.5 ± 0.4	2.7 ± 0.3

TABLE 2. Continued

Bulk $\delta^{15}N$ (‰; Mean ± SD, Range)	Species (Samp No.)	Source amino acids					Trophic amino acids					
		Gly	Lys	Phe	Ser	Thr	Ala	Asp	Glu	Leu	Pro	TP*
	S.r. (9)	−2.6 ± 0.6	nd	−1.5 ± 1.0	−1.3 ± 1.0	−14.4 ± 0.7	17.6 ± 1.1	9.9 ± 0.4	13.1 ± 0.3	13.4 ± 0.3	10.9 ± 0.5	2.5 ± 0.5
	S.r. (10)	0.6 ± 0.6	4.7 ± 0.1	1.4 ± 0.2	2.3 ± 0.2	−16.8 ± 0.4	20.7 ± 0.4	14.3 ± 0.2	19.0 ± 0.2	17.5 ± 0.5	15.0 ± 0.4	2.9 ± 0.1
9.0 ± 3.2	E.d. (2)	10.8 ± 0.2	11.1 ± 0.2	7.8 ± 0.3	13.2 ± 0.5	−2.3 ± 0.5	24.8 ± 0.0	20.0 ± 0.5	24.6 ± 0.3	20.2 ± 0.4	17.3 ± 0.3	2.8 ± 0.2
5.0–15.0	E.d. (3)	1.7 ± 0.2	1.8 ± 0.6	−0.4 ± 0.3	3.9 ± 0.2	−11.0 ± 0.3	13.8 ± 0.0	9.7 ± 0.2	13.3 ±0.2	10.4 ± 0.4	7.0 ± 0.3	2.4 ±0.2
	E.d. (4)	1.0 ± 0.3	1.8 ± 0.3	−1.1 ± 0.6	2.7 ± 0.6	−9.5 ± 0.2	12.5 ± 0.6	8.5 ± 0.1	13.0 ± 0.1	9.4 ± 0.5	5.3 ± 0.6	2.4 ± 0.3
	E.d. (5)	3.8 ± 0.1	4.5 ± 0.2	0.3 ± 0.4	7.4 ± 0.6	−10.2 ± 0.3	18.7 ± 0.1	14.2 ± 0.7	20.3 ± 0.1	14.2 ± 0.2	11.0 ± 0.3	3.2 ± 0.2
	E.d. (6)	5.8 ± 1.4	5.0 ± 0.6	3.1 ± 0.4	8.3 ± 0.7	−9.7 ± 0.6	19.7 ± 1.2	15.8 ± 0.4	19.6 ± 0.4	16.0 ± 0.8	13.8 ± 0.5	2.7 ± 0.3
	E.d. (7)	7.4 ± 0.5	5.7 ± 0.5	2.3 ± 0.1	7.6 ± 0.9	nd	nd	15.7 ± 0.1	20.3 ± 0.3	13.7 ± 1	11.2 ± 0.9	2.9 ± 0.1
8.4 ± 3.1	E.t. (1)	2.5 ± 0.5	2.1 ± NA	0.3 ± 0.9	4.4 ± 0.6	−10.6 ± 0.8	15.7 ± 0.5	9.9 ± 0.7	14.3 ± 0.5	10.9 ± 0.1	7.3 ± 0.6	2.4 ± 0.6
4.8–13.7	E.t. (2)	11.9 ± 0.8	nd	7.9 ± 0.7	12.0 ± 0.8	−4.5 ± 0.6	nd	20.5 ± 0.9	22.9 ± 0.4	19.3 ± 0.3	17.4 ± 0.4	2.5 ± 0.4
	E.t. (4)	4.2 ± 0.6	4.1 ± 0.5	2.8 ± 0.7	6.1 ± 0.4	nd	17.9 ± 0.4	11.8 ± 0.5	14.7 ± 0.8	11.1 ± 0.5	10.8 ± 0.4	2.1 ± 0.7
	E.t. (5)	6.0 ± 0.8	6.4 ± 0.9	3.1 ± 0.4	7.9 ± 0.9	nd	18.8 ± 0.7	1.3 ± 0.9	17.9 ± 0.6	14.8 ± 0.2	12.9 ± 0.5	2.5 ± 0.4
	E.t. (6)	5.3 ± 0.4	nd	3.1 ± 0.3	9.2 ± 0.8	−8.3 ± 0.3	19.2 ± 0.5	14.7 ± 0.3	17.4 ± 0.3	14.6 ± 0.5	12.9 ± 0.6	2.4 ± 0.2

We analyzed the isotope values of 18 AAs, but report data for 10 AAs that were consistently detected on all chromatographs (Table 2). As anticipated, trophic AAs had higher $\delta^{15}N$ values compared with the source amino acids. Thr $\delta^{15}N$ values in all species were substantially lower than the values of the other AAs (Table 2). We found highly significant positive linear relationships between the source amino acid $\delta^{15}N_{phe}$ and $\delta^{15}N_{bulk}$ across all taxa ($p < 0.001$; $R^2 = 0.69$; Fig. 3). Additionally, we found similar relationships between $\delta^{15}N_{bulk}$ and $\delta^{15}N$ values of other source amino acids. The $\delta^{15}N$ values of glutamic acid ($\delta^{15}N_{glu}$), the trophic AA we used to calculate TP, mirrored patterns in $\delta^{15}N_{phe}$ between taxa and locations, indicating that trophic amino acid $\delta^{15}N$ values were driven by variability in source amino acid $\delta^{15}N$ values.

We found no significant differences in TPs estimated using $\delta^{15}N_{phe}$ and $\delta^{15}N_{glu}$ values (Eq. 1) as a function of latitude or longitude for any species (Fig. 4a). LME models also showed no significant differences in our proxies for TP ($\delta^{15}N_{glu}$ - $\delta^{15}N_{phe}$ and $\delta^{15}N_{trophic}$ - $\delta^{15}N_{source}$) across latitude or longitude. There were, however, significant changes in source amino acid $\delta^{15}N$ values as a function of latitude (Table 4a), which indicates that there was spatial variability in $\delta^{15}N$ at the base of the food web across our study area as opposed to changes in trophic structure.

Although CSIA-AA was useful to evaluate spatial variability in TP, using this technique for estimating absolute TP was problematic for some species. Average TP estimates (Eq. 1) for macrozooplankton *Euphausia tenera* and *E. distinguenda* were 2.4 and 2.7, respectively, while TP estimates for both myctophid species, *Myctophum nitidulum* and *Symobolophorus reversus*, macrozooplankton predators, were the same as that for *E. distinguenda* and much lower than that expected based on diet data (3.45, Olson and Watters 2003). Mean TP estimates for both cephalopod species, *Dosidicus gigas* (3.1) and *Sthenoteuthis oualaniensis* (3.1), were lower than expected based on diet data (4.40, Ehrhardt 1991; Shchetinnikov 1992; Olson and Watters 2003). Mean TP estimates were particularly low for the micronektonivore tunas, *Katsuwonus pelamis* (2.7), *Thunnus albacares* (3.0), and *T. obesus* (3.0). Our TP estimates for the tunas were comparable to or lower than those for the squids, which are tuna prey, and similar to that of the euphausiid *E. distinguenda*.

Empirical TDF estimates (based on Eq. 5) showed differences between trophic groups (Fig. 5). The slopes of the lines fitted to our 12 myctophid (TDF = 5.0) and 6 squid samples (TDF = 4.6‰) were similar to each other, whereas the TDF for the 18 samples comprising the tunas (TDF = 4.0‰) was lower than the slope for the 11 euphausiid samples (TDF = 7.0‰; Fig. 5). Using *t*-tests, we determined that the slope of the line fitted to the data for our euphausiid samples was not significantly different ($t = 0.38$, $p > 0.1$) from the TDF value of 7.6‰ estimated by Chikaraishi et al. (2009), but the TDFs for tunas ($t = 7.74$, $p < 0.0001$), squids

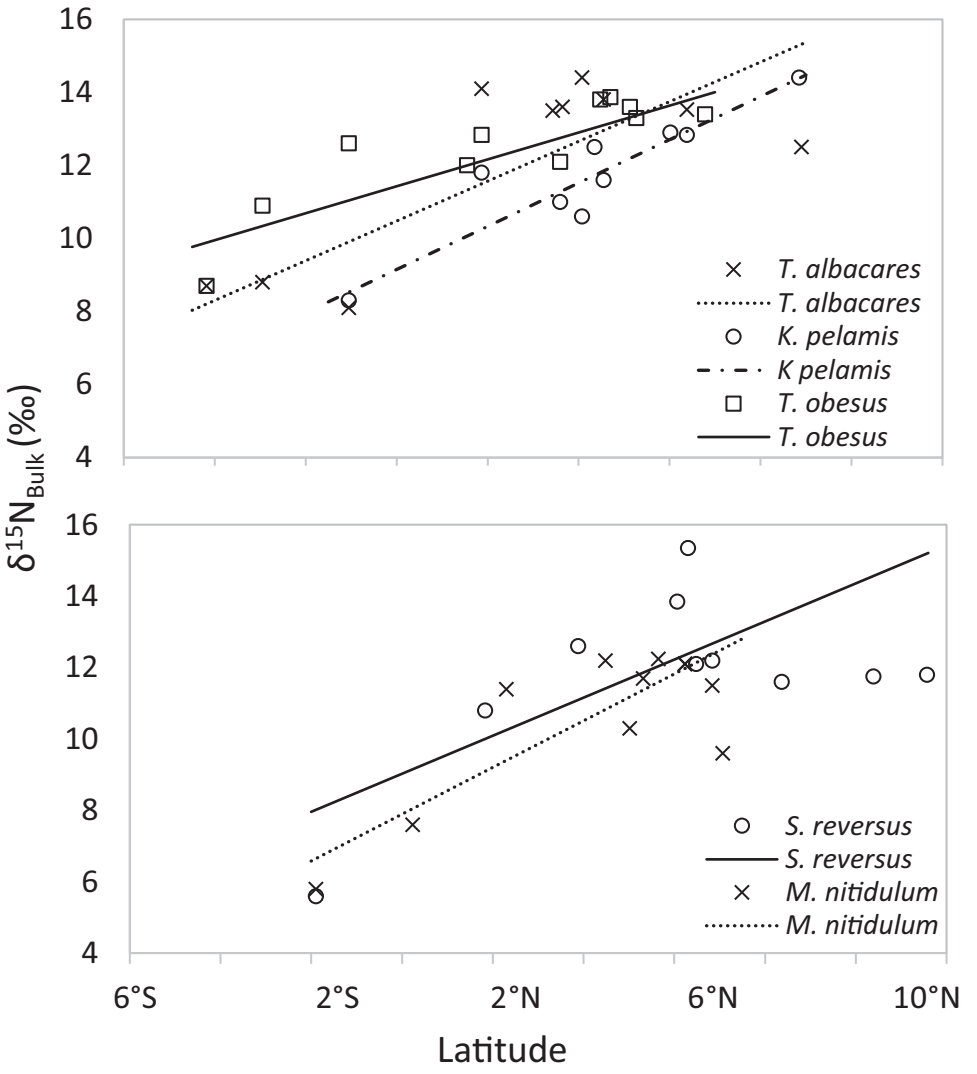

Fig. 2. Significant relationships between $\delta^{15}N$ of bulk tissue ($\delta^{15}N_{bulk}$) and latitude for five of seven species analyzed using multiple linear regressions. (**a**) three species of tunas, *Thunnus albacares*, *Katsuwonus pelamis*, and *T. obesus*, and (**b**) two species of Myctophidae, *Myctophum nitidulum*, and *Symbolophorus reversus*. Regression lines were computed at a fixed longitude (110°W), using the equation from the multiple linear regression output (Table 3).

($t = 16.87$, $p < 0.0001$), and myctophids ($t = 5.45$, $p = 0.001$) were each significantly lower than 7.6‰.

Oceanographic analyses

Since there were no observed changes in trophic structure with latitude and longitude across our study area, we utilized oceanographic data to better understand regional oceanographic conditions and their relationships to $\delta^{15}N$ values. A correlation matrix of the eight oceanographic variables showed that some variables were highly correlated, including nitrate concentration, phosphate concentration, and SST (Supporting Information Table 2). We found a deepening of the thermocline from east to west, and generally low or negative N^* values throughout the study area. Chl *a* values and phytoplankton cell diameter estimates were enhanced in coastal regions and along the equator, but there were no longitudinal or latitudinal gradients across the sampling area (Fig. 4c), further substantiating our CSIA-AA result that there were no changes in food-chain length across our study area. See Supporting Information for a more detailed description of oceanographic conditions.

Stepwise multiple linear model procedures for each species revealed that nitrate concentration was the best descriptor of variability in bulk $\delta^{15}N$ values, as nitrate was the first variable selected in all significant models. Univariate linear models were generally the best fit for our data. Adding additional oceanographic variables to the stepwise procedure did not lead to a meaningful decrease in AIC values for most species, except for *T. albacares*, and *E. tenera*¸ for which the best model fits included nitrate and Chl *a* concentrations.

Table 3. Significant multiple linear regression model results for bulk $\delta^{15}N$ values vs. latitude (Lat) and longitude (Lon), with the estimated coefficient (Est. Co.), standard errors for coefficients (SE), and *p*-values for each variable, where bold values indicate significance ($p < 0.05$).

	Est. Co.	SE	*p*-value
T. albacares			
Intercept	3.55	4.61	0.47
Lat	0.54	0.13	**<0.01**
Lon	−0.06	0.04	0.15
K. pelamis			
Intercept	8.26	3.50	0.06
Lat	0.59	0.14	**<0.01**
Lon	−0.01	0.03	0.76
T. obesus			
Intercept	8.71	2.63	0.01
Lat	0.37	0.07	**<0.001**
Lon	−0.02	0.02	0.30
M. nitidulum			
Intercept	4.45	3.49	0.24
Lat	0.65	0.16	**<0.01**
Lon	−0.03	0.03	0.32
S. reversus			
Intercept	−0.06	4.31	0.99
Lat	0.53	0.16	**0.01**
Lon	−0.08	0.04	0.06

Multiple linear regression models that were used to evaluate relationships between $\delta^{15}N$ values and oceanographic variables were significant for six of seven species analyzed: all tuna species: *Thunnus albacares* ($R^2 = 0.88$, $F = 34.47$, $p < 0.001$), *Katsuwonus pelamis* ($R^2 = 0.48$, $F = 8.42$, $p = 0.02$), and *T. obesus* ($R^2 = 0.69$, $F = 22.9$, $p < 0.001$), both myctophid species *Myctophum nitidulum* ($R^2 = 0.60$, $F = 14.68$, $p < 0.01$), and *Symbolophorus reversus* ($R^2 = 0.57$, $F = 13.04$, $p < 0.01$), and a weak relationship for the euphausiid species *Euphausia tenera* ($R^2 = 0.94$, $F = 26.37$, $p = 0.04$).

We found spatial variability in source AA $\delta^{15}N$ values, which was related to nitrate concentrations. Using LMEs, we found significant relationships between the $\delta^{15}N$ values of all four source amino acids (phenylalanine, glycine, lysine, and serine) and nitrate concentrations (Table 4b), indicating that nitrate concentrations can explain spatial variability in source amino acid $\delta^{15}N$ values. The isoscape of $\delta^{15}N_{Phe}$ values was used to visualize the spatial variability in baseline nitrogen isotopic composition (Fig. 6a) and compare with spatial maps of nitrate concentrations and N^* (Fig. 6b,c). Our results indicate generally lower $\delta^{15}N_{Phe}$ values south of the equator and higher values in the northern portion of our transect and along the coast, which is consistent with spatial patterns in nitrate concentration and N^*. Higher $\delta^{15}N_{Phe}$ values were detected in areas with lower N^*.

Discussion

In addition to evaluating spatial variation in trophic structure in the eastern tropical Pacific (ETP), our study contributes to the ongoing debate on variability in CSIA-AA trophic discrimination factors when using this technique to estimate TPs of species. Most previous CSIA-AA studies involving single components of disparate ecosystems have provided conflicting results. Our results bolster recent arguments (Germain et al. 2013; Bradley et al. 2015; McMahon et al. 2015a,b) that Chikaraishi et al.'s (2009) commonly used methodology for estimating TP is not appropriate for all species. Our results supported a hypothesis that a TDF of 7.6‰ provided unrealistic TP estimates for higher trophic level species.

The $\delta^{15}N$ composition of bulk tissues and amino acids of nine species comprising four trophic groups from a pelagic food web in the ETP indicate that spatial variability in bulk nitrogen isotopic values is largely explained by nitrogen cycling processes and not variability in trophic structure. Our study was designed to sample across a presumed productivity gradient to capture potential trophic structure variability due to differences in phytoplankton size composition. Using CSIA-AA, we found little support for our hypotheses regarding changes in TPs, phytoplankton cell size, and food web structure. Instead, we found constant TP estimates across our sampling area (Fig. 4a), which were consistent with estimates of fairly uniform phytoplankton cell size. Our data demonstrate that variability in bulk nitrogen isotope values was related to regional oceanography, particularly nitrate concentrations and SST. Source amino acid data were also related to oceanographic conditions, and thus can provide information about past environmental conditions and dynamics at the base of the food web.

Trophic structure based on CSIA-AA

TP estimates based on CSIA-AA for *Euphausia distinguenda* and *E. tenera*, (mean TP = 2.6) were comparable to previous estimates for macrozooplankton in this region (2.70, Chai et al. 2002; Olson and Watters 2003). Given Brinton's (1979) estimate that euphausiids comprise 50% of the total zooplankton biomass in the ETP, understanding diet and trophic interactions of euphausiids and other macrozooplankton is important for understanding energy transfer to higher trophic levels.

Our TP estimates for *Myctophum nitidulum* and *Symbolophorus reversus* (mean TP = 2.6) were slightly lower than estimates for other myctophids based on CSIA-AA and stomach contents (2.9 and 3.2 respectively, Choy et al. 2012) and lower than the estimates from the ecosystem model (Olson and Watters 2003) based on diet data (3.45). Myctophids, however, were grouped with other small mesopelagic fishes (the bristlemouths, Phosichthyidae) in the ecosystem model, and the diet was assumed to comprise meso- and microzooplankton. Van Noord et al. (2013, 2016) found mesozooplankton (TP 2.7, Olson and Watters 2003) almost exclusively in the gut contents of three

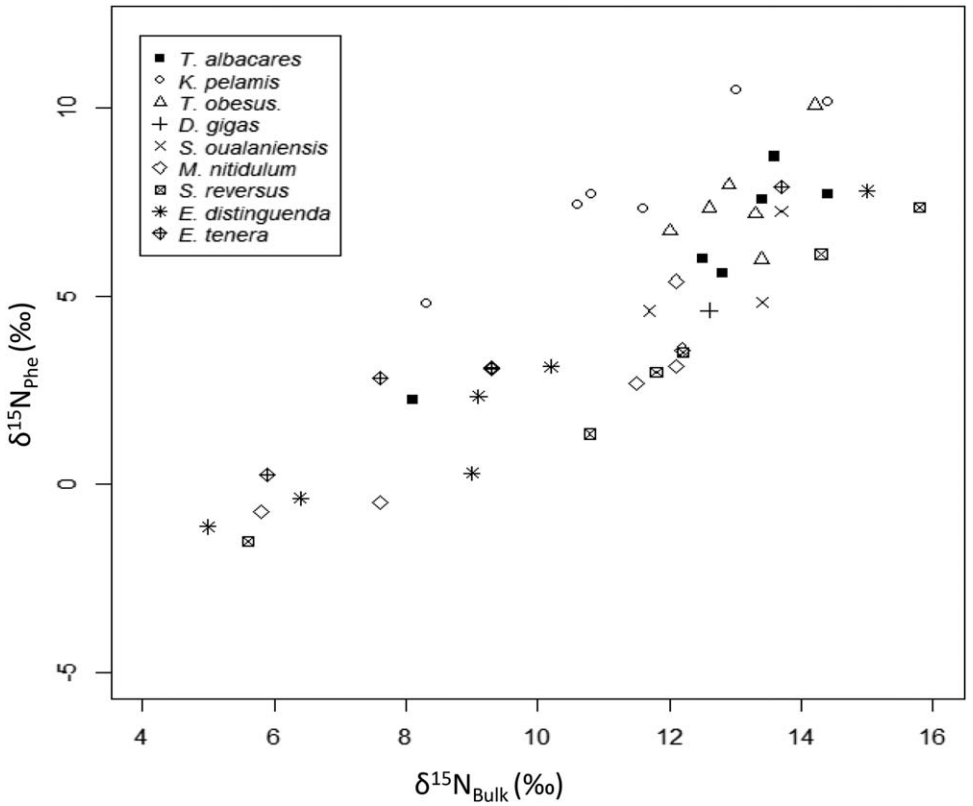

Fig. 3. $\delta^{15}N$ values of phenylalanine ($\delta^{15}N_{phe}$) vs. $\delta^{15}N$ values of bulk tissue ($\delta^{15}N_{Bulk}$) for 1) small micronektonivores: *Thunnus albacares*, *Katsuwonus pelamis*, and *T. obesus*, 2) cephalopods: *Dosidicus gigas* and *Sthenoteuthis oualaniensis*, 3) mesopelagic micronekton: *Myctophum nitidulum* and *Symbolophorus reversus*, and 4) macrozooplankton: *Euphausia distinguenda* and *E. tenera*. $\delta^{15}N_{phe}$ values represent means from multiple assays, and SDs (Table 2) were $\leq \pm$ 1.0‰ for all values.

myctophid species in the same region, so our TP estimates based on CSIA-AA are not ecologically realistic. Myctophids comprise an abundant and ecologically important family of mesopelagic fishes that occupy lower-middle trophic levels in the ETP food web (Gjøsaeter and Kawaguchi 1980), and provide a trophic link between zooplankton and higher-level species.

Our TP estimates for cephalopods (3.1) and tunas (2.7, 3.0) based on CSIA-AA were lower than estimates from previous studies based on stomach contents analyses (Olson and Watters 2003; Markaida et al. 2008; Olson et al. 2010) and stable isotope analysis of bulk muscle tissue and amino acids (yellowfin tuna Popp et al. 2007; Olson et al. 2010). One possible explanation is that stomach contents analysis failed to elucidate all prey items in the diet, as it provides information about only the most recent prey consumed and likely overestimates the dietary importance of large prey that are evacuated slowly and small prey that are evacuated quickly (Olson and Boggs 1986). Stomach contents analysis is particularly challenging for squids, as they macerate their prey. The CSIA-AA based TP estimates for tunas were lower than or nearly the same as those for the squids, which are common prey of tunas.

Underestimated TPs based on CSIA-AA have also been reported for penguins, two elasmobranchs, and the harbor seal (Lorrain et al. 2009; Dale et al. 2011; Germain et al. 2013; Hoen et al. 2014; McMahon et al. 2015a,b). TP estimates from our study support the contention of previous work that a TDF of 7.6‰, based on Chikaraishi et al. (2009), is adequate only for species with TPs < 3.0 (Chikaraishi et al. 2009; Hannides et al. 2009; Dale et al. 2011; Germain et al. 2013; Bradley et al. 2015). We empirically estimated TDF values using the methodology of Bradley et al. (2015) and found decreasing TDF values with increasing TPs. The mean empirical TDF for tunas was significantly lower than our TDF estimate for the euphausiids but not for the myctophids and squids. Our study is the first to demonstrate sequentially decreasing TDF values with increasing TP for multiple taxa in a single pelagic ecosystem.

It is unclear why TDFs vary among marine consumers (McCarthy et al. 2013; Bradley et al. 2015; McMahon et al. 2015a,b), although it may be related to protein assimilation efficiency (Choy et al. 2012; Germain et al. 2013; McMahon et al. 2015a,b). Organisms assimilate $\sim$ 75% of the protein they consume, while the remainder is catabolized and excreted (Popp et al. 2007; Choy et al. 2012). During

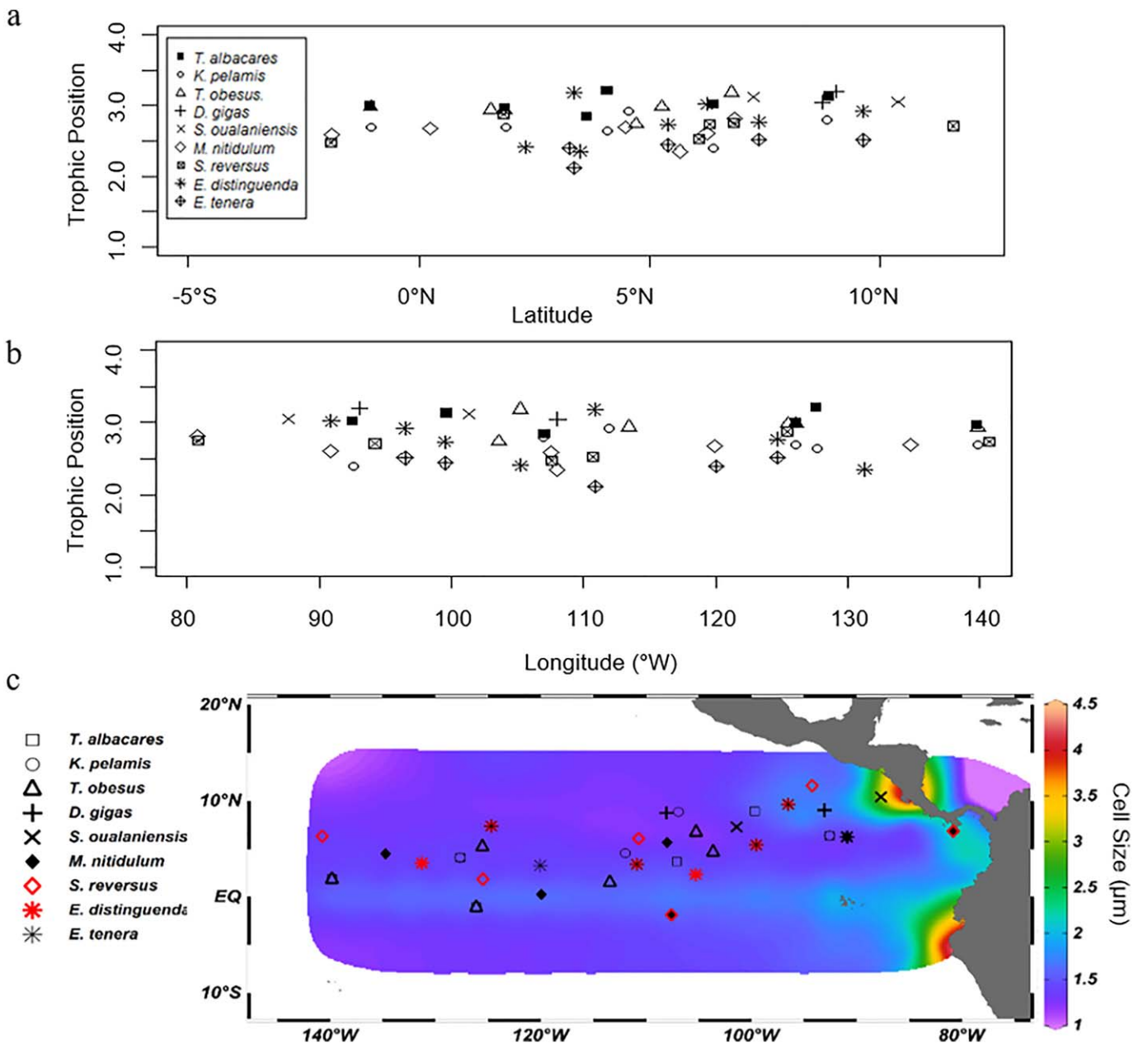

Fig. 4. Trophic position estimates (Eq. 1) from compound-specific nitrogen isotope analysis vs. (**a**) latitude and (**b**) longitude for 1) small micronektonivores: *Thunnus albacares*, *Katsuwonus pelamis*, and *T. obesus*, 2) cephalopods: *Dosidicus gigas* and *Sthenoteuthis oualaniensis*, 3) mesopelagic micronekton: *Myctophum nitidulum* and *Symbolophorus reversus*, and 4) macrozooplankton: *Euphausia distinguenda* and *E. tenera*. Panel (**c**) is median annual phytoplankton cell diameter estimates with sample locations overlayed.

assimilation there is preferential excretion of ^{14}N, which leads to enrichment in ^{15}N in the consumer's tissue. If a predator's diet consists of little protein and has a different amino acid composition than the predator itself, there will be more catabolism, excretion of ^{14}N, and thus fractionation. Conversely, if a predator's diet has a similar amino acid composition to itself (high protein), more protein is assimilated without catabolism and there is less fractionation. Germain et al. (2013) suggested that the form of nitrogen excretion, ammonia vs. urea, might influence TDF values. Ammonia production occurs in one deamination step and results in no isotopic fractionation, whereas urea production requires two steps and leads to fractionation (Champe and Harvey 2010). Germain et al. (2013) hypothesized that organisms that produce urea have lower TDF values compared with ammonia-producing organisms. Our results do not support their hypothesis, as our empirical TDF estimates were lowest for the tunas, followed by the squids and myctophids, all of

Table 4. Estimated parameters from the LME models for a) of glutamic acid minus phenylalanine $\delta^{15}N$ values ($\delta^{15}N_{Glu}$- $\delta^{15}N_{Phe}$), trophic minus source amino acids $\delta^{15}N$ values ($\delta^{15}N_{Trp}$- $\delta^{15}N_{Src}$) vs. latitude and longitude, and b) $\delta^{15}N$ values of phenylalanine, glycine, serine, and lysine ($\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$, $\delta^{15}N_{Ser}$, and $\delta^{15}N_{Lys}$) vs. surfaces nitrate concentrations. Shown for the fixed effect component of each LME are the estimated coefficients, and in parentheses the standard errors and *p*-values. Shown for the random effect component of each LME are the estimated standard deviations of the random effect distributions, and in parentheses the approximate 95% confidence intervals. For the LMEs for $\delta^{15}N_{Lys}$ and $\delta^{15}N_{Src}$, the full model estimates of $\sigma_{species}$ were nearly zero, and hence the model was re-fitted with only a random effect for sample.

a	Parameter	$\delta^{15}N_{Glu}$- $\delta^{15}N_{Phe}$	$\delta^{15}N_{Trp}$- $\delta^{15}N_{Src}$	$\delta^{15}N_{Phe}$	$\delta^{15}N_{Src}$
	Fixed effects				
	Intercept	15.72 (1.81; <0.01)	15.03 (2.32; <0.01)	−4.25 (2.69; 0.12)	−5.08 (2.23; 0.02)
	Latitude	0.12 (0.07; 0.10)	0.04 (0.07; 0.58)	0.54 (0.10; <0.01)	0.58 (0.09; <0.01)
	Longitude	−0.01 (0.01; 0.61)	−0.01 (0.01; 0.46)	−0.06 (0.02; 0.01)	−0.06 (0.02; <0.01)
	Random effects				
	$\sigma_{species}$	1.62(0·93,2.81)	4.33 (2.63 ,7.14)	2.16 (1.23, 3.08)	----------
	$\sigma_{sample\ within\ species}$	1.20(0.93, 1.54)	1.30(1.03, 1.65)	1.86 (1.46, 2.36)	σ_{sample}: 1.63(1·31, 2.03)

b	Parameter	$\delta^{15}N_{Phe}$	$\delta^{15}N_{Gly}$	$\delta^{15}N_{Ser}$	$\delta^{15}N_{Lys}$
	Fixed effects				
	Intercept	6.75 (0.90; <0.01)	3.42 (1.02; <0.01)	8.41 (0.77; <0.01)	7.30 (0.49; <0.01)
	Nitrate	−0.82 (0.15; <0.01)	−0.84(0.16; <0.01)	−1.02 (0.14; <0.01)	−0.83 (0.15; <0.01)
	Random effects				
	$\sigma_{species}$	2.24(1.29, 3.88)	2.62 (1.52, 4.52)	1.87(1.03, 3.39)	----------
	$\sigma_{sample\ within\ species}$	1.82(1.44, 2.34)	1.93 (1.52, 2.43)	1.69(1.32, 2.15)	σ_{sample}:1.72 (1.37, 2.16)

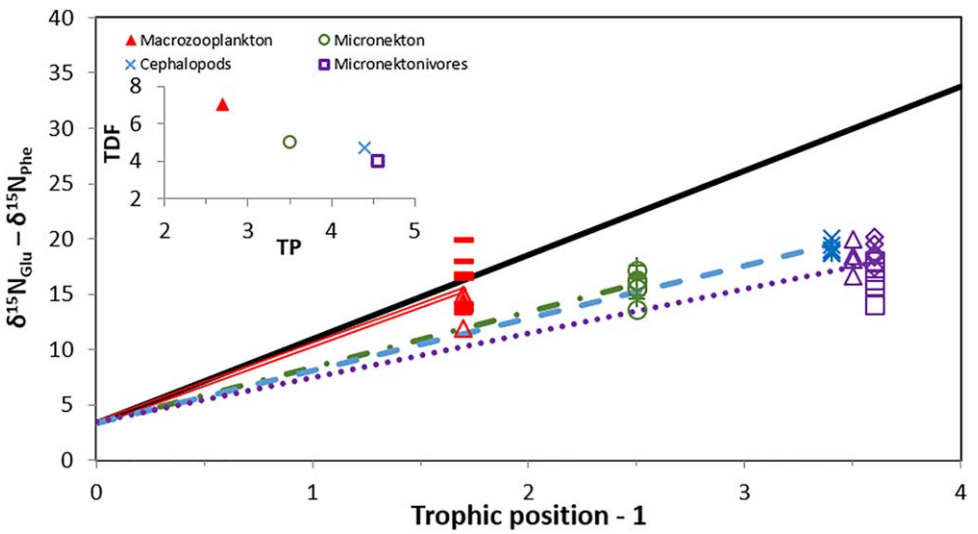

Fig. 5. Linear relationships (Eq. 5) between $\delta^{15}N$ values of glutamic acid ($\delta^{15}N_{glu}$) minus $\delta^{15}N$ values of phenylalanine ($\delta^{15}N_{phe}$) vs. trophic position minus 1, where independent trophic position estimates are from Olson and Watters (2003). The solid black line represents the trophic discrimination factor (TDF, 7.6‰) and the β-value (3.4‰) of Chikaraishi et al. (2009), which are the slope and *y*-intercept. The equations for the purple [small micronektonivores (tunas)], blue [Cephalopods (squids)], green [micronekton (myctophids)], and red [Macrozooplankton (euphausiids)] lines yield slopes that indicate TDF values (in parenthesis) for these taxa. Different symbols within each trophic group represent the different species within the group. The average TDF values for each trophic group are represented in the insert.

which are ammonia-producing organisms. Further, Hoen et al. (2014) found no difference in TDF values between urea producing elasmobranches and ammonia producing teleosts in semi-controlled feeding studies.

Recent studies have proposed an alternative method to estimate TP, using mean $\delta^{15}N$ values of three source amino acids (glycine, lysine, phenylalanine) and three trophic amino acids (glutamic acid, leucine, and alanine), rather than

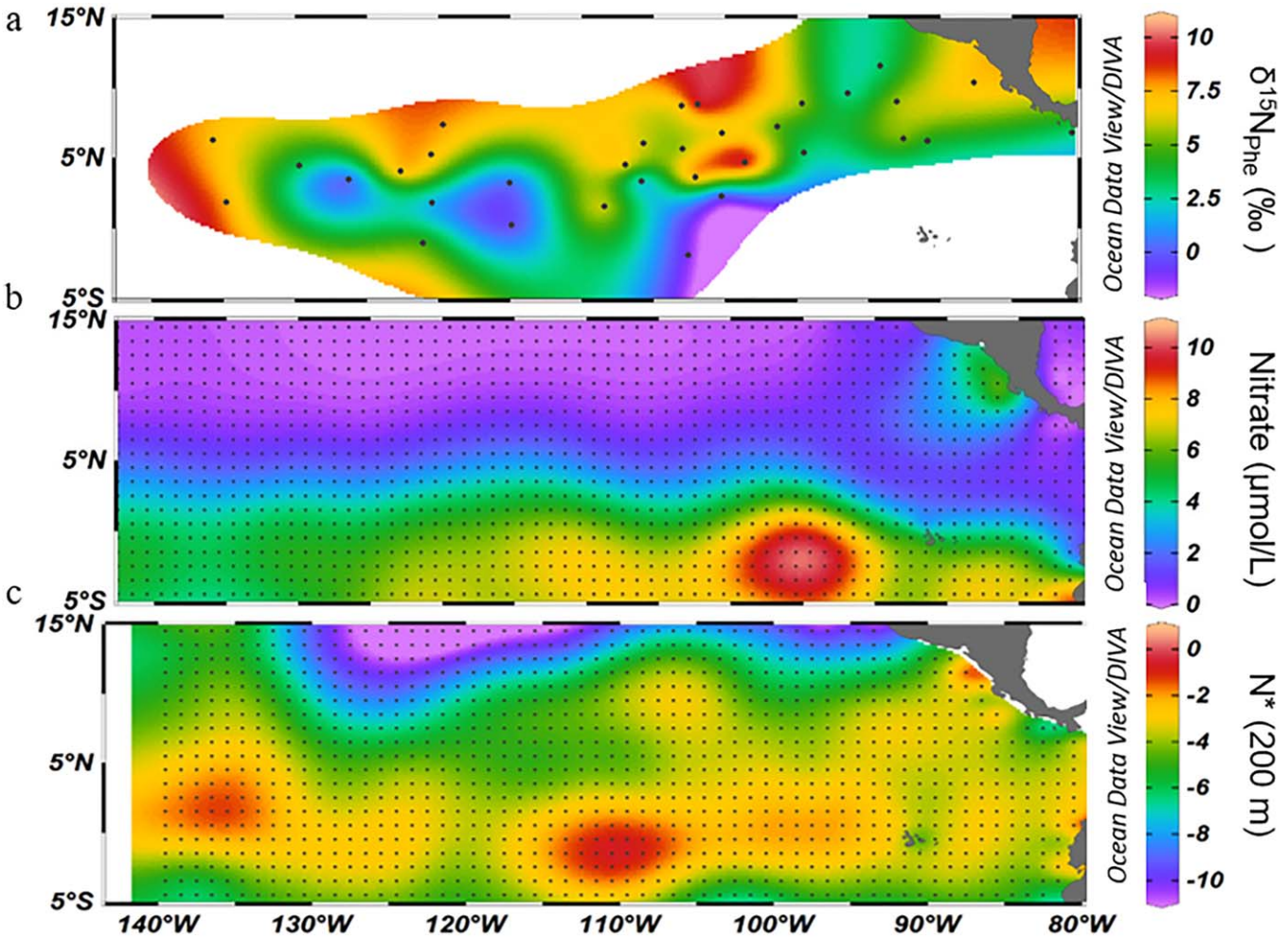

Fig. 6. Maps of spatial variability in (**a**) $\delta^{15}N_{phe}$ values from 49 samples of nine species, (**b**) annual nitrate concentrations averaged from 0 m to 50 m, and (**c**) a denitrification index, *N**, where positive values indicate nitrogen fixation and negative values indicate denitrification. Black dots in panel a indicate sample locations from this study. Nitrate and *N** values are from the World Ocean Atlas (see Methods for more details).

relying solely on $\delta^{15}N_{phe}$ and $\delta^{15}N_{glu}$ values (Bradley et al. 2015; Lorrain et al. 2015; Nielsen et al. 2015). Although Bradley et al. (2015) focused on teleosts, we applied their equation to our data and found more realistic mean TP estimates for all taxa [micronektonivores (4.1), cephalopods (4.2), micronekton (3.1), and macrozooplankton (2.5)], which may suggest that one ecosystem-level TDF value is plausible. However, the mechanisms driving differences in $\delta^{15}N_{glu}$-$\delta^{15}N_{phe}$ remain elusive, and future experiments are needed to investigate and re-examine TDF variability among taxa.

Despite general trends of bulk $\delta^{15}N$ values increasing from south to north (Fig. 2), TP estimates based on CSIA-AA were uniform across the study transect (Fig. 4), and we found a significant relationship between $\delta^{15}N_{Bulk}$ values and $\delta^{15}N_{Phe}$ (Fig. 3). Therefore, the $\delta^{15}N$ variability we observed across our study region was governed primarily by differences in biogeochemical processes in the ETP, not trophic differences. Furthermore, LME models, which were used to evaluate spatial and temporal variability in proxies for TP ($\delta^{15}N_{Glu}$ - $\delta^{15}N_{Phe}$ and $\delta^{15}N_{trophic}$ - $\delta^{15}N_{source}$) showed no changes across latitude or longitude. The LME models illustrated that source amino acid $\delta^{15}N$ varied as a function of latitude and longitude, and differences in baseline $\delta^{15}N$ values were driving the variability in bulk $\delta^{15}N$, which underscores the utility of the CSIA-AA method to evaluate multiple components of a food web within a single ecosystem.

Oceanography and $\delta^{15}N$ values

Overall, we found highly significantly negative relationships between bulk $\delta^{15}N$ values and nitrate concentrations, which supports our hypothesis that nutrient concentration data could explain variability in $\delta^{15}N$ values. We found significant relationships between $\delta^{15}N$ values and SST and phosphate concentrations, which were highly correlated with nitrate concentrations. We focused our analyses on

nitrate concentrations, as they have a direct influence on nitrogen isotope values. Our analyses suggest that nitrate concentration are useful for evaluating $\delta^{15}N$ patterns, which is in agreement with previous research (e.g., Somes et al. 2010). We found few relationships between bulk isotope values and most other oceanographic variables, including thermocline depth, Chl *a*, and phytoplankton cell diameter, which indicates that nitrate concentrations (and SST, and phosphate concentrations) provide the most useful information about spatial variability in $\delta^{15}N$.

Our analyses were limited by the number of samples per species that we analyzed. We used a multiple regression analysis approach for evaluating relationships between bulk isotope data and oceanographic variables because it was not appropriate to group data from multiple species together given that the relationships between $\delta^{15}N$ values and oceanographic parameters were not uniform across taxa. Given the limited sample sizes for each species (Table 1), there were not enough data in this study to fit more complex models. We assumed that bulk isotope values for each species varied linearly with latitude and longitude, and an independent and identically distributed Gaussian error structure was adequate. This approach provided a useful overall summary of the relationships between $\delta^{15}N$ and oceanography for each species. However, given the spatial complexity of the oceanographic environment, more complex models should be considered if more data for each species were available.

The $\delta^{15}N_{Phe}$ values of the samples we analyzed for CSIA-AA were highly variable across our study area, which may be a result of our large spatial coverage and variability in nitrogen biogeochemistry in the ETP. Several biogeochemical processes influence $\delta^{15}N$ values, including isotopic fractionation as a result of nitrate utilization by phytoplankton, nitrogen fixation, and denitrification, and the relative influences of these factors varies spatially and temporally (Cline and Kaplan 1975; Saino and Hattori 1987; Liu and Kaplan 1989; Gruber and Sarmiento 1997; Waser et al. 1998; Altabet 2001; Deutsch et al. 2001). Denitrification discriminates against ^{15}N, which results in a residual nitrate pool with high $\delta^{15}N$ values (Deutsch et al. 2001; Voss et al. 2001). This phenomenon has been documented in areas with pronounced oxygen minimum zones (OMZ) such as the ETP (Voss et al. 2001; Somes et al. 2010; Lorrain et al. 2015). Therefore, $\delta^{15}N$ values in regions of the ETP with a pronounced OMZ are higher than areas dominated by nitrogen fixation processes (Cline and Kaplan 1975; Liu and Kaplan 1989; Somes et al. 2010).

We used the denitrification index, N^*, to evaluate the relative influence of nitrogen fixation (addition of nitrate, positive values) and denitrification (subtraction of nitrate, negative values). Our N^* values were largely negative, which is congruent with published values and patterns in the ETP (Gruber and Sarmiento 1997). There was a strong denitrification signal in certain portions of our study area. Since N^* values on a local level vary owing to mixing and transport of different water masses (Gruber and Sarmiento 1997; Deutsch et al. 2001), creating isotope landscapes (or isoscapes) in biogeochemically diverse areas is useful for the interpretation of stable isotopes in ecological studies. Our $\delta^{15}N_{Phe}$ isoscape, spatial maps of nitrate and N^*, and relationships between source AA $\delta^{15}N$ values and nitrate concentrations indicate that high rates of denitrification were likely the mechanism driving spatial trends in isotope composition.

Despite our relatively small sample sizes for CSIA-AA analyses, we found strongly significant relationships in source amino acid $\delta^{15}N$ as a function of latitude and longitude. $\delta^{15}N_{Phe}$ values were significantly related to $\delta^{15}N_{Bulk}$ values, indicating that variability in bulk $\delta^{15}N$ in this study can partially be explained by nitrogen cycling and physical forcing, rather than changes in trophic structure. LMEs showed that source amino acid $\delta^{15}N$ values were related to nitrate concentrations, which demonstrates that CSIA-AA can be used to successfully evaluate trophic structure and fluctuations in biogeochemical cycling at the base of the food web.

Although CSIA-AA provides baseline $\delta^{15}N$ values, the cost of analysis limits sample sizes. Using CSIA-AA in conjunction with bulk $\delta^{15}N$ analysis of lower trophic level species (e.g., Jennings and Warr 2003) and modeling (e.g., Somes et al. 2010) will provide a better understanding of spatial gradients in oceanic $\delta^{15}N$ values.

Productivity and $\delta^{15}N$ values

Previous research has documented spatial variability in ETP oceanography (Fiedler and Talley 2006; Pennington et al. 2006) with an east-west productivity gradient which we hypothesized might relate to changes in phytoplankton cell size and changes in TP of higher level consumers. The data we compiled showed a deepening of the thermocline from east-to-west across our study area, indicating more upwelling and higher primary productivity in the eastern portion of our study area (Supporting Information). Although coastal upwelling regions showed phytoplankton cell diameter values > 2 um, our remotely sensed phytoplankton cell size analysis showed overall small median cell diameter < 2 um, corresponding to a region dominated by pico-phytoplankton and oligotrophic conditions. Thus both phytoplankton cell sizes and CSIA-AA data agree that a marked east-west productivity gradient across our study area did not exist. Given the size based nature of pelagic food webs, this suggests uniform food-web length across our study area. Targeted sampling efforts in highly eutrophic coastal waters contrasted with offshore oligotrophic waters are necessary to adequately address relationships between food-web length and productivity in the open ocean.

Conclusions

Our results suggest that nitrogen cycling, particularly denitrification, in the ETP is a driver of variability in $\delta^{15}N_{Bulk}$ and source amino acid $\delta^{15}N$ values at the base of the food web, which propagated up to higher-trophic-level predators.

Evaluating relationships between nitrogen isotope values, oceanography, and biogeochemistry is crucial for understanding isotopic variability in marine ecosystems. This finding highlights the utility of the CSIA-AA method in differentiating causal relationships of $\delta^{15}N$ variability in an ecosystem, trophic ecology vs. spatial variability in nitrogen cycling processes. Our results show that CSIA-AA is a broadly applicable, useful tool for evaluating food webs, trophic structure, and energy flow, particularly in biogeochemically diverse areas.

We report CSIA-AA TDFs based on empirical estimates for multiple taxa (tunas: 4.0‰, squids: 4.6‰, myctophids: 5.0‰, and euphausiids: 7.0‰) within the same pelagic ecosystem. These values are consistent with those found in recent studies (Bradley et al. 2015; Nielsen et al. 2015). Our results suggest that a better understanding of mechanisms influencing TDFs is critical for accurately estimating absolute TPs in food webs. Combining CSIA-AA data with diet studies and ecosystem models will help elucidate food web structure in different oceanographic regions and is a useful approach for ecosystem-based management of fisheries.

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Conflict of Interest

None declared.

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