LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 00, 2015, 00–00

doi: 10.1002/lno.10085

Trophic structure and food resources of epipelagic and mesopelagic fishes in the North Pacific Subtropical Gyre ecosystem inferred from nitrogen isotopic compositions

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Abstract

We used bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values and $\delta^{15}N$ values of individual amino acids (AA) to characterize the trophic structure of a pelagic fish assemblage from the North Pacific Subtropical Gyre (NPSG) ecosystem. We focus on energy flow between fishes inhabiting distinct epipelagic, mesopelagic, and upper bathypelagic habitats and on predatory fish foraging across and within these depth habitats. Trophic positions (TPs) estimated from a combination of trophic and source AA $\delta^{15}N$ values ($TP_{Tr\text{-}Src}$) spanned a narrow range of 0.7 TP for 10 species of large fishes, including tunas, billfishes, and gempylids ($TP_{Tr\text{-}Src}$ 4.3-5.0). Similarly, 13 species of small micronekton fishes encompassed a range of 1.2 TP ($TP_{Tr\text{-}Src}$ 2.6-3.8). The $\delta^{15}N$ values of three source AAs were found to increase with increasing depth of capture across the 13 micronekton fish species ($\delta^{15}N_{Phe}$ range = 6.6‰; $\delta^{15}N_{Gly}$ range = 13.4‰; $\delta^{15}N_{Ser}$ range = 13.6‰), indicating that some species from epipelagic, mesopelagic, and upper bathypelagic communities access distinct food resources, such as suspended particles. These isotopic depth trends are consistent with previous observations in particulate organic matter and zooplankton from the NPSG, providing new evidence that large pelagic and micronekton fishes access a food web fueled by particles formed in surface waters but that are highly modified by microbes as they slowly settle to remote depths. On the contrary, no significant relationships between the $\delta^{15}N$ values of source AAs and habitat depth were observed in the large predator fish group, of which many species move and forage across large depth gradients.

Pelagic marine environments away from continental shelves comprise the largest ecosystems on Earth (Robison 2009). These open-ocean ecosystems house diverse microbial and animal assemblages in which top predator sharks and large fishes provide great commercial value and sustenance to global societies. Due to selected, sustained removal of top predator biomass from pelagic species assemblages, widespread changes to global marine food webs have been observed (e.g., Pauly et al. 1998; Micheli 1999). Restructuring of marine food webs occurs primarily through complex predator–prey feeding interactions among species. Thus, continued harvesting of marine top predators necessitates detailed knowledge of trophic structure and accurate characterization of overall energy flow within pelagic ecosystems (Young et al., in press).

Ecosystem-based fishery management (EBFM) explicitly calls for characterization of trophic structure by delineating food web pathways and linking exploited species with their prey and other potential competitors (Grumbine 1994). EBFM differs from narrow single-species management by accounting for all ecosystem components and their interactions with target species. More frequently, ecosystem models have been used to directly inform EBFM efforts by contributing ecosystem-level outputs of food-web linkages, energy cycling, and changes in the biomasses of different species groups. However, ecosystem models require reliable inputs of trophic linkages and overall food web structure to better couple physical–biogeochemical and climate models exploring the population dynamics of exploited marine fishes (Mackinson et al. 2009; Howell et al. 2013).

The North Pacific Subtropical Gyre (NPSG) ecosystem is the largest interconnected biome on Earth, and within this open-ocean system, micronekton form a critically important food web component. Micronekton are small fishes, crustaceans, and cephalopods ~2-20 cm in size and are the

Additional Supporting Information may be found in the online version of this article.

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decisive trophic link bridging marine food webs from bottom-to-top (Brodeur and Yamamura 2005). Fishes are by far the dominant micronekton group, comprising at least half of the numbers and biomass in open-ocean waters (Maynard et al. 1975; Drazen et al. 2011). Many species of top predators including sharks, marine mammals, and commercially harvested pelagic fishes such as tunas (bigeye, yellowfin, skipjack, albacore) and swordfish feed directly on diverse oceanic micronekton assemblages (e.g., King and Ikehara 1956; Watanabe et al. 2009). Existing data highlighting important predator–prey pathways within the NPSG pelagic ecosystem are limited, but suggest that large pelagic fishes exploit unique combinations of very different micronekton prey resources (e.g., Choy et al. 2013). Recent analysis of Hawaii longline fishery data suggests large-scale alterations to open-ocean food webs of the NPSG, in which decadal-scale reductions in the abundance of large, high trophic-level fishes such as marlins, sharks, and tunas, have been accompanied by increases in nontarget mesopelagic fish species such as gempylids and alepisaurids (Polovina et al. 2009; Polovina and Woodworth-Jefcoats 2013). Understanding the ecological reasons behind these changes is hampered by sparse information on predator–prey interactions, which ultimately trace the flow of energy and biomass.

In addition to identifying key trophic relationships within pelagic ecosystems, the partitioning of food resources and predator–prey interactions across extremely large depth gradients (e.g., epi-, meso-, and bathypelagic habitats) can also structure energy flow (Sutton 2013). Primary production originating in surface waters is rapidly consumed by zooplankton, including vertical migrators, and a portion is transported to the deep sea as vertical particle flux (Buesseler et al. 2008). Particle remineralization by microbial communities results in drastic, exponential decreases in organic carbon concentrations with depth (Martin et al. 1987; Buesseler et al. 2008). The portion of sinking and suspended particulate organic matter (POM) exported out of surface waters is rapidly utilized and degraded by microbial communities, or directly consumed by migrating zooplankton, which are in turn direct food resources for micronekton fishes, cephalopods, and crustaceans (Steinberg et al. 2008). Partitioning the relative importance of these different food resources to ecologically complex pelagic organisms to elucidate primary energy pathways in open-ocean ecosystems is limited by traditional methods such as gut content analysis, requiring alternative approaches.

Anchored in the predictable behaviors of stable carbon (C) and nitrogen (N) isotopic compositions within food webs, stable isotope analysis (SIA) has proven to be particularly useful for illuminating trophic dynamics in remote environments like the deep sea (e.g., Drazen et al. 2008). Consumers preferentially retain varying levels of ^{13}C and ^{15}N in their tissues during metabolic processing and thus, $\delta^{13}C$ and $\delta^{15}N$ values can guide quantitative estimates of trophic position (TP) for food web components (Deniro and Epstein 1978, 1981). More recently this technique has been combined with amino acid (AA) compound-specific isotope analysis (AA-CSIA) to account for complex primary production dynamics and produce more accurate measures of consumer TP (e.g., Hannides et al. 2009; Choy et al. 2012; Seminoff et al. 2012). More specifically, AA-CSIA is utilized to clarify complex isotope dynamics at or near the base of the food web. For example, seasonal shifts in the NPSG between nitrate-fueled and N_2-fixation-based production affect baseline $\delta^{15}N$ values differently (Dore et al. 2002), ultimately affecting interpretations at higher trophic levels (Hannides et al. 2009). AA-CSIA of consumers has been shown to account for seasonal or spatial fluctuations in isotopic baseline by recording the $\delta^{15}N$ values of individual "source" AAs (e.g., phenylalanine [Phe], serine [Ser], glycine [Gly]) that track $\delta^{15}N$ values at the base of the food web (McClelland and Montoya 2002; Popp et al. 2007; Hannides et al. 2009). Additionally, certain AAs ("trophic" AAs) incorporate the history of trophic transfers in animals (e.g., glutamic acid [Glu], alanine [Ala], valine [Val]), increasing by up to ~8‰ with each step in TP (McClelland and Montoya 2002; Chikaraishi et al. 2009). Using bulk tissue SIA in conjunction with AA-CSIA of consumers can thus provide unique insights into both trophic structure and the importance of different N sources fueling production in large marine ecosystems. A few pelagic studies have applied SIA techniques specifically to expansive vertical ecosystems, demonstrating stark changes in the $\delta^{15}N$ values of zooplankton and particle food-resources with depth (Hannides et al. 2013), and increases in the $\delta^{15}N$ values of consumers with depth that do not necessarily align with known trophic ecology (Bergmann et al. 2009; Zintzen et al. 2013; Trueman et al. 2014).

In this study, we examine the trophic structure and energy flow within an assemblage of abundant large pelagic and micronekton fishes inhabiting epipelagic, mesopelagic, and upper-bathypelagic waters of the NPSG. We investigate ecological mechanisms for how this diverse species assemblage partitions resources within the NPSG pelagic ecosystem, specifically probing different modes of animal nutrition and how they may differ according to known depth preferences of the different fish species. Using bulk tissue SIA ($\delta^{15}N$ and $\delta^{13}C$ values) alongside AA-CSIA, we investigate relative differences in TPs, as well as the trophic connectivity of these animals across a large depth gradient (~0-2500 m). Specifically, we explore the interconnectivity of representative fishes from multiple trophic levels and multiple depth habitats, examining potential broad-scale vertical differences in isotopic values at the base of the food web(s) across the epipelagic (~0-200 m), mesopelagic (~200-1000 m) and upper bathypelagic zones (~1000-2500 m). Our results suggest that micronekton and large pelagic fishes from different depth habitats utilize POM, zooplankton, and micronekton

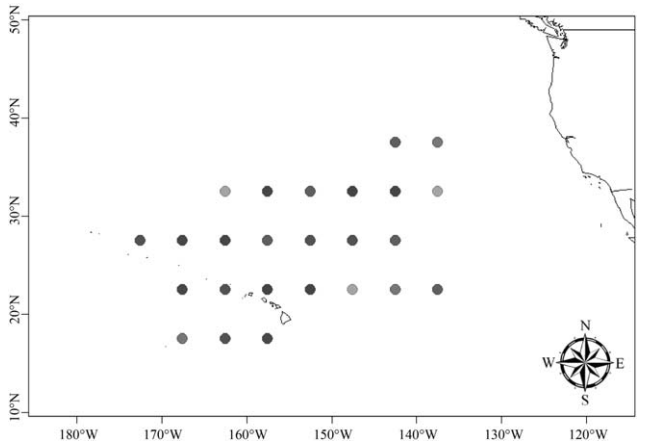

Fig. 1. Map showing the general capture locations of fishes from this study. Non-confidential catch locations are reported as the centers of 5 × 5 degree cells. Level of circle transparency scales with the relative number of individuals captured from that 5 × 5 degree cell.

food-resources to varying degrees, and yet there is surprisingly little difference in the relative TPs of these fish groups. We discuss how to link these results to improving the biological input parameters of ecosystem models, and increasing overall understanding of model outputs for pelagic systems.

Materials and Procedures

Sample collection

Large pelagic fish tissue samples were collected at sea by trained longline fishery observers of the National Oceanic and Atmospheric Administration's (NOAA) Pacific Islands Regional Observer Program during the years 2009-2011 (e.g., Choy et al. 2009). Longline observers aboard commercial fishing vessels operating in the NPSG removed ~5-10 g of white muscle tissue from each large fish specimen. These tissue samples were immediately frozen at −20°C at sea, transported frozen, and stored at −80°C until analysis. For each specimen, observers recorded species, forklength (cm), sex (if known), and sampling date. Approximate catch locations were reported in accordance with NOAA's Fisheries Operations Data Confidentiality Policy (Figure 1).

The majority of micronekton fish samples were collected in August 2011 at two sites in offshore waters to the north and west of the island of O'ahu in the NPSG. Station ALOHA is the Hawaii Ocean Time-series site located north of O'ahu (22.45°N, 158°W) and Station KAHE is an offshore site located west of O'ahu at (~21.3°N, 158.3°W). A 10-m^2 Multiple Opening and Closing Nets and Environmental Sensing System (MOCNESS; B.E.S.S., Falmouth, MA, USA) was fished during the daytime (08:00-14:00) and nighttime (19:00-04:00) at preset discrete depth intervals 100-500 m wide, from surface waters to as deep as 2500 m. Onboard, micronekton fish samples were quickly identified to the lowest taxonomic level possible, after which each specimen was measured to the nearest millimeter, and was then immediately frozen in a sealed cryovial with liquid nitrogen. Samples were stored at −80°C until analysis. A small number of micronekton fish ($n = 3$) were collected in March 2011 at Station KAHE in oblique midwater trawls using a 3-m-Tucker Trawl (Research Nets, Seattle, WA, USA). Juvenile *Thunnus albacares* were collected off small boats using hook and line methods in July 2011. A total of 10 species of large pelagic and 13 species of micronekton fishes were targeted to encompass species representing a range of TPs and habitat depths, based on existing diet and electronic tagging information (*see* the Supporting Information, Table S1).

Laboratory analysis

Outer layers of large pelagic fish tissue samples were removed with clean tools to avoid any potential sampling contamination. Only dorsal white muscle tissue of micronekton fish specimens was used for analysis; bones, skin, and internal organs were avoided. If sufficient white muscle tissue was not available internal organs were removed and samples were acidified with 12 N HCl to remove carbonate prior to analysis (after Yamamuro and Kayanne 1995). Skeletal carbonate is typically formed from inorganic carbon sources and can result in carbon isotope values higher than those of organic animal tissues (*see* Post 2002). All tissue samples were lyophilized, ground and homogenized with a ceramic mortar and pestle, weighed and packed into tin capsules.

Bulk sample nitrogen (N) and carbon (C) isotope compositions of all fishes were determined using an isotope ratio mass spectrometer (IRMS; Delta ^{Plus}XP) coupled to an elemental analyzer (Conflo IV/Costech ECS 4010). Isotope values are reported in conventional δ-notation relative to the international standards atmospheric N_2 and V-PDB, for N and C, respectively. Accuracy and precision were <0.2‰, as determined from reference materials analyzed every 10 samples (glycine and a tuna muscle homogenate, both extensively characterized with NIST certified reference materials and their $\delta^{13}C$ and $\delta^{15}N$ values verified independently in other laboratories). All $\delta^{13}C$ values were normalized for lipid-content (based on molar C : N ratios) using isotope mass balance approaches based on comparable pelagic fishes (for micronekton, Hoffman & Sutton 2010; for large fishes, Logan et al. 2008). Twelve of 222 individuals (from three large fish species) had molar C : N ratios >8 and the associated $\delta^{13}C$ values were removed from the bulk tissue isotopic dataset, as recommended by Hoffman and Sutton (2010).

A subset of large pelagic and micronekton fishes was selected for AA-CSIA (60 of 222); three individuals of each large pelagic fish species and one to three individuals of each micronekton species were chosen in a manner that minimized intraspecific temporal, spatial, and size-related variability. Samples were prepared according to the methods of Hannides et al. (2009) and Choy et al. (2012). Briefly, ~5-10 mg of each dried sample was subjected to acid hydrolysis

with trace-metal grade 6 N HCl. The hydrolysate was purified (low protein-binding filters and cation exchange chromatography), followed by esterification of the carboxyl terminus (4 : 1 isopropanol : acetyl chloride) and trifluoroacetylation of the amine group (3 : 1 methylene chloride : trifluoroacetyl anhydride). Sample derivatives were purified with solvent extraction and stored at −20°C prior to analysis. Lastly, samples were redissolved in 50-100 μL of ethyl acetate and the δ^{15}N values of individual AAs were measured using an IRMS (Delta ^{Plus}XP, Delta V Plus or MAT 253) interfaced with a gas chromatograph (Trace GC) through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid-N cold trap. One to two microliters of each sample was injected (split/splitless injector using a 10 : 1 split ratio) onto a capillary column (BPx5 forte, micronekton samples on 60 m × 0.32 mm × 1.0 μm film thickness and large fish samples on 30 m × 0.32 mm × 1.0 μm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.2 mL min^{-1}.

All samples were analyzed in triplicate and the measured AA-δ^{15}N values were normalized to known δ^{15}N values of two coinjected internal reference compounds (norleucine [Nor] and aminoadipic acid [AAA] with δ^{15}N reference values of 19.06‰ and −5.8‰, respectively). For the micronekton fish samples only, a combination of six commercially available pure AAs (Ala, threonine [Thr], isoleucine [Ile], proline [Pro], Glu, Phe) with equimolar concentration and δ^{15}N values determined using the bulk sample isotopic methods described above was coinjected with Nor and AAA between triplicate analyses of individual samples, bracketing each sample to provide an additional measure of instrument performance, and accuracy and precision of isotope analysis. Analysis of this suite of pure AAs provided an additional method for the normalization of measured sample isotope values. Reproducibility of isotopic analysis of Glu and Phe in samples averaged 0.4‰ ± 0.2‰ (range: 0.1-0.9‰) and 0.6‰ ± 0.4‰ (range: 0.1-2.4‰), respectively. Reproducibility was either determined using the internal reference compounds Nor and AAA, or using a regression analysis of known δ^{15}N values against measured δ^{15}N values in the suites of pure AAs used to bracket individual samples. Standard deviations for all AAs averaged ±0.4‰ (range: 0.1-2.4‰). Accuracy of all AA-CSIA analyses was estimated using the known δ^{15}N value of Nor to determine a measured δ^{15}N value of AAA both coinjected with samples, treating AAA as an unknown. Accuracy averaged 0.7‰ ± 0.5‰ (range: 0-3.4‰).

TP designations

Fractional TP estimates were based on two established formulations, both of which rely on the predictable manner in which different types of AAs are transferred from prey to predator (e.g., Hannides et al. 2013; Vander Zanden et al. 2013). "Trophic" AAs (Tr-AA; e.g., Glu, leucine [Leu], Ala, Pro; Popp et al. 2007) predictably enrich in ^{15}N relative to source AAs with each step in TP as C—N bonds are broken in transamination and deamination reactions (Chikaraishi et al. 2007). The δ^{15}N values of "source" AAs (Src-AA; e.g., Phe, lysine [Lys], Gly, Ser; Popp et al. 2007) change little with each step in TP, likely undergoing metabolic processes that do not break C—N bonds (Chikaraishi et al. 2007). Glu and Phe have been the two canonical Tr-AA and Src-AAs most commonly used to calculate TP across diverse groups of marine organisms (e.g., Chikaraishi et al. 2009), but a recent meta-analysis of diverse groups of marine teleosts recommended a specific combination of Tr-AAs and Src-AAs for the best agreement between known TP estimated from stomach content analysis and AA-CSIA estimated TP (Bradley 2013), and we adopt this approach here (Eq. 1).

$$TP_{Tr-Src} = \frac{(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Src-AA} - \beta_{Tr-Src})}{TEF_{Tr-Src}} + 1 \quad (1)$$

Briefly, $TP_{Tr\text{-}Src}$ incorporates the weighted mean δ^{15}N values of three specific Tr-AAs ($\delta^{15}N_{Tr\text{-}AA}$; Ala, Leu, Glu) and three specific Src-AAs ($\delta^{15}N_{Src\text{-}AA}$; Phe, Lys, Gly). Nine of 60 samples were missing one or more of these six AAs and thus were not included in this calculation. A weighted mean approach considers analytical uncertainty in such a way that AAs with more variability in δ^{15}N values (larger standard deviations) have less emphasis on TP estimates than those AAs with lower variability or uncertainty (Hayes et al. 1990). Trophic and source AA designations are the same as those used by McClelland and Montoya (2002) and Sherwood et al. (2011). $\beta_{Tr\text{-}Src}$ is calculated as the difference between the weighted means of three Tr-AAs and three Src-AAs reported from a suite of different marine photoautotrophs (Chikaraishi et al. 2010; McCarthy et al. 2013) and for the six AAs used is 3.6‰ (±0.5‰). We adopt a $TEF_{Tr\text{-}Src}$ for these AAs of 5.7‰ (±0.3‰), as determined from the difference between the weighted means of the three Tr-AAs and three Src-AAs per trophic level from the results of Bradley (2013). The standard deviation of $TP_{Tr\text{-}Src}$ was calculated by propagation of errors (as previously in Gelwicks and Hayes 1990; Phillips and Gregg 2001; Blum et al. 2013), which combines the analytical uncertainty in AA δ^{15}N values measured in triplicate with the uncertainty in $\beta_{Tr\text{-}Src}$ and $TEF_{Tr\text{-}Src}$ determined above.

The second formulation of TP estimated from AA-CSIA data is a proxy indicator of relative TP expressed as $\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ (e.g., Décima et al. 2013). These two proxy measures of relative TP are calculated as the difference between weighted averages of δ^{15}N values of the three trophic and source AAs identified above, and the difference between the δ^{15}N values of Glu and Phe, respectively. $\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ calculations require no a priori literature-based assumptions about the β and trophic enrichment factor (TEF) values used to estimate TP from AA-CSIA other than the assumption that these values remain constant

among the samples analyzed and thus, provide an unbiased proxy of fish relative TP. These relative TP estimates essentially remove the isotopic effect of food web baseline, focusing on relative differences in food web position based on two different combinations of Tr-AAs and Src-AAs. Both proxy estimates of relative TP were made for all 60 large pelagic and micronekton samples; if any $\delta^{15}N$ values of specified Tr-AAs and Src-AAs were missing, the values were simply excluded from the individual averages of these two groups of AAs.

Data analysis

Linear regression was used to examine the relationship between fish length (log-transformed) with bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values (all species pooled), as well as with AA-CSIA TP (species means of $TP_{Tr\text{-}Src}$). For species with sufficient sample sizes across a range of fish lengths (five large pelagic fishes, two micronekton fishes) linear regression was used to examine intraspecific relationships between length and bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values. Across group differences were evaluated with permutational analysis of variance (PERMANOVA) using species means of bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values, $\delta^{15}N$ values of individual AAs, and $TP_{Tr\text{-}Src}$. Multivariate cluster analysis (Ward's minimum variance method) was used to examine trophic groupings of fish species based on different isotopic parameters averaged per species (bulk tissue $\delta^{15}N$ values, $\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ values, and $\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$, and $\delta^{15}N_{Ser}$ values). Ward's method uses an analysis of variance approach to evaluate distances between clusters, producing the smallest possible increase in the error sum of squares. Similarity profile routines were used to determine significant groupings from cluster analysis output at a significance level of 0.05. Median habitat depth is the median of the day and nighttime depth range from tagging and trawling studies for large pelagic fishes, and median depth of capture is the midpoint of known collection depth range for micronekton fishes (*see* Supporting Information Tables S1 and S2 for depths). PERMANOVA analyses were conducted with PERMANOVA+ (Anderson et al. 2008), the add-on for PRIMER v6. All other data analysis and statistics were performed using R version 2.15.1 (R Development Core Team 2012).

Results

Bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values

The bulk tissue $\delta^{15}N$ values of large pelagic fishes (12 species, $n = 159$) were significantly greater than those of the micronekton fish group (13 species, $n = 75$; Mann–Whitney U test, $p < 0.001$, $U = 1459.0$; *see* Table 1 for ranges). Bulk tissue $\delta^{13}C$ values for the two broad fish groups overlapped, but the large pelagic fishes had significantly higher $\delta^{13}C$ values than the micronekton group (Mann–Whitney U test, $p < 0.001$, $U = 2837.0$; *see* Table 1 for ranges). The mean bulk tissue $\delta^{15}N$ values of two micronekton fishes, *Cyclothone pallida* and *Serrivomer sector*, overlapped with the bulk $\delta^{15}N$ values of large pelagic fishes (10.4 and 9.2‰, respectively; Figure 2). Assuming a uniform food web isotopic baseline value and an average ^{15}N TEF of 3.4‰ (Vanderklift and Ponsard 2003), the 10 large pelagic species occupy ~1.2 TP range and the 13 micronekton fish species ~1.4 TP range.

Species mean bulk tissue $\delta^{15}N$ values of the 10 large pelagic fishes were significantly different from one another (PERMANOVA, $p = 0.001$; df = 134, pseudo-$F = 8.08$). Likewise, species mean bulk tissue $\delta^{15}N$ values of the 13 micronekton fishes were significantly different from one another (PERMANOVA, $p = 0.001$; df = 74, pseudo-$F = 4.21$). Intraspecific variability in bulk tissue $\delta^{15}N$ values was also evident, especially for the large pelagic fish species *T. albacares* (6.8‰ range, from minimum to maximum values) and *Alepisaurus ferox* (6.9‰ range), and the micronekton fishes *Chauliodus sloani* (6.7‰ range) and *C. pallida* (7.4‰ range). In general, within species differences in bulk tissue $\delta^{13}C$ values were small, with the exception of *A. ferox* (6.9‰ range) and *C. pallida* (6.1‰ range), neither species of which showed high and/or variable molar C : N ratios. All bulk tissue isotopic results and general sample information are available through the Biological & Chemical Oceanography Data Management Office (BCO-DMO) (http://www.bco-dmo.org/project/491309).

AA $\delta^{15}N$ values

The $\delta^{15}N$ values of AAs measured in micronekton ranged from −29.4 to 26.3‰ and in large pelagic fishes from −35.9 to 32.8‰. The $\delta^{15}N$ values of all trophic AAs (Ala, Val, Leu, Ile, Pro, aspartic acid [Asp], Glu) were significantly higher than that of all source AA (Phe, Ser, Gly, Lys) $\delta^{15}N$ values (t-test, $t = 44.86$, df = 672, $p < 0.001$). Furthermore, the Tr-AA $\delta^{15}N$ values of large pelagic fish were significantly higher than that of all micronekton Tr-AA $\delta^{15}N$ values (t-test, $t = 18.24$, df = 383, $p < 0.001$). There was no significant difference between Src-AA $\delta^{15}N$ values of large pelagic fishes and micronekton fishes (t-test, $t = 0.87$, df = 287, $p > 0.05$). However, Src-AA $\delta^{15}N$ values spanned considerable ranges within and between fish species. For example, average large pelagic fish $\delta^{15}N_{Phe}$ values had a range of 7.3‰ while average micronekton $\delta^{15}N_{Phe}$ values had a range of 5.3‰, equaling a 9.1‰ range in mean $\delta^{15}N_{Phe}$ values across all 23 species. Mean micronekton species $\delta^{15}N_{Ser}$ values were the most variable in terms of Src-AA $\delta^{15}N$ values between species, with a range of 11.8‰. All results of AA-CSIA are available through the BCO-DMO (http://www.bco-dmo.org/project/491309).

TPs estimated from AA-CSIA

$TP_{Tr\text{-}Src}$ of individuals ranged from 2.6 in a 25 mm long *C. alba* specimen to 5.6 in a 166 cm long *Xiphias gladius* specimen. Mean $TP_{Tr\text{-}Src}$ for micronekton fish species ranged from 2.6 in the gonostomatid *C. alba* to 3.8 in the myctophid *Bolinichthys distofax* (Table 1). Mean $TP_{Tr\text{-}Src}$ for large pelagic fish species ranged from 4.3 in *Gempylus serpens*, *Coryphaena*

Table 1. Sample sizes for bulk tissue SIA, lengths (*AA-CSIA), bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values, AA-CSIA TPs ($TP_{Tr\text{-}Src}$), and the differences between average trophic AAs and average source AAs ($\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$). AA-CSIA sample size was $n = 3$ per species, except for *Exocoetus volitans* and *Idiacanthus fasciola* ($n = 1$). Values are mean ± SD.

	n	Length (mm)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$TP_{Tr\text{-}Src}$	$\Delta\delta^{15}N_{Tr\text{-}Src}$	$\Delta\delta^{15}N_{Glu\text{-}Phe}$
Large Pelagic Species							
Alepisaurus ferox	30	965±367; 1153±274*	9.7±1.9	−18.4±1.7	4.9±0.8	26.6±3.7	26.4±4.5
Coryphaena hippurus	15	900±135; 960±200*	10.3±1.8	−15.4±0.5	4.3±0.5	22.0±1.4	19.5±2.1
Gempylus serpens	18	983±169; 1088±221*	10.0±1.4	−17.0±0.7	4.3±0.7	22.4±3.5	23.3±3.5
Katsuwonus pelamis	6	545±144; 560±184*	9.2±1.7	−16.2±0.6	4.3±0.5	22.3±2.7	17.6±1.9
Lampris guttatus	24	1003±123; 1013±133*	12.1±1.0	−17.5±0.5	4.5±0.1	23.8±2.0	23.3±1.0
Lepidocybium flavobrunneum	16	626±121; 723±185*	10.8±1.7	−16.4±0.7	4.6±0.4	24.3±2.3	21.6±3.9
Makaira nigricans	7	2048±559; 1880±368*	11.8±1.9	−15.7±1.5	4.4±0.2	22.5±3.5	19.4±3.4
Thunnus albacares	9	804±396; 1213±40*	9.2±2.1	−15.6±0.6	4.6±0.1	22.7±0.8	21.0±3.1
Thunnus obesus	9	950±213; 1010±231*	11.1±1.0	−16.3±0.6	5.0±0.6	24.5±2.0	21.4±1.7
Xiphias gladius	13	1329±372; 1417±529*	13.4±1.9	−17.0±0.7	4.9±0.9	25.8±2.9	25.2±3.4
Micronekton Species							
Anoplogaster cornuta	5	75±24; 89±1*	7.8±1.2	−18.7±0.5	3.5±0.5	17.7±0.6	20.7±0.5
Bolinichthys distofax	3	52±25; 63±21*	7.2±1.1	−18.1±0.5	3.8±0.3	19.3±1.7	20.1±2.2
Bolinichthys longipes	13	40±8; 34±9*	6.3±1.0	−18.3±0.6	3.2±0.5	15.4±2.2	15.8±1.2
Chauliodus sloani	7	80±36; 103±45*	8.2±2.9	−17.1±1.4	3.5±0.5	18.2±2.5	19.01±2.0
Cyclothone alba	3	25±1; 25±1*	5.9±0.7	−18.5±0.5	2.6±0.1	12.8±0.5	14.7±0.9
Cyclothone pallida	8	47±9; 48±13*	10.4±3.1	−16.0±2.4	3.2±0.4	16.8±2.1	18.4±2.5
Cyema atrum	3	115±21; 115±21*	7.0±2.0	−17.3±0.3	3.0±0.2	15.4±1.2	17.3±1.9
Exocoetus volitans	2	57±26; 75*	6.3±3.0	−18.0±0.7	3.5	16.7	17.4
Hygophum proximum	5	30±13; 33±14*	5.6±1.7	−17.0±1.6	2.8±0.1	14.3±0.1	16.3±0.4
Idiacanthus fasciola	7	170±81; 178*	6.6±1.4	−17.7±0.9	3.7	19.2	19.6
Myctophum lynchnobium	3	60±42; 65±58*	7.8±2.2	−18.3±0.4	3.4±0.5	16.9±2.5	16.9±2.4
Serrivomer sector	11	400±112; 415±81*	9.2±1.7	−17.1±1.9	3.2±0.1	16.3±0.7	17.7±0.9
Thunnus albacares (J)	5	158±20; 165±37*	5.5±0.2	−17.7±0.2	3.2±0.3	16.2±1.2	16.7±1.1

hippurus, and *Katsuwonus pelamis* to 5.0 in *T. obesus*. $TP_{Tr\text{-}Src}$ was not available for the largest specimen of *X. gladius* (178 cm) due to missing data for some Tr-AAs and Src-AAs. Large pelagic fish $TP_{Tr\text{-}Src}$ estimates were significantly higher than micronekton fish $TP_{Tr\text{-}Src}$ estimates (*t*-test, $t = 10.46$, $df = 49$, $p < 0.001$). Mean $TP_{Tr\text{-}Src}$ for large pelagic fishes and micronekton spanned similar ranges, that is, 0.7 and 1.2 TP, respectively. Mean $TP_{Tr\text{-}Src}$ of the 10 different species of large pelagic fishes were not significantly different from one another (PERMANOVA, $p > 0.05$, $df = 21$, pseudo-$F = 0.58$). Likewise, the 13 micronekton fish species were not significantly different from one another in terms of mean $TP_{Tr\text{-}Src}$ estimates (PERMANOVA, $p > 0.05$, $df = 28$, pseudo-$F = 1.80$).

The two AA-CSIA proxy relative TP metrics ($\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ values) were not entirely in agreement with the relative rankings of mean $TP_{Tr\text{-}Src}$ for the 23 fish species (Table 1). However, for both metrics the large pelagic fish *A. ferox* had the highest relative TP, followed by *X. gladius*. The micronekton fish *C. alba* had the lowest relative TP in terms of both proxy metrics, whereas, among the micronekton, *Anoplogaster cornuta* had the highest mean $\Delta\delta^{15}N_{Glu\text{-}Phe}$ value and *B. distofax* had the highest mean $\Delta\delta^{15}N_{Tr\text{-}Src}$ value. Figure 3a presents multivariate cluster analysis based on these two proxy relative TP metrics. The 10 species of large pelagic fishes were completely separated from the 13 species of micronekton fish at the greatest linkage (Figure 3a). Additionally, there were six significantly different subclusters of large pelagic fishes and eight within the micronekton fish group.

Broad groupings and depth-related patterns

Bulk tissue $\delta^{15}N$ values of the micronekton fishes showed significant increases with increasing median habitat depth (*see* the Supporting Information, Table S2 for capture depths; linear regression, $F_{1,73} = 18.75$, $R^2 = 0.19$, $p < 0.001$). To explain this trend, there were clear increases in the $\delta^{15}N$ values of three Src-AAs (Phe, Gly, Ser) with depth of capture for individual micronekton fishes (Figure 4; *see* the Supporting Information, Table S2 for capture depths), but only $\delta^{15}N_{Gly}$ and $\delta^{15}N_{Ser}$ values significantly increased with median depth of capture (linear regression, $F_{1,28} = 8.72$, $R^2 = 0.21$, $p < 0.01$ for $\delta^{15}N_{Gly}$; $F_{1,28} = 13.3$, $R^2 = 0.30$, $p < 0.01$ for $\delta^{15}N_{Ser}$). One specimen of *C. alba* was captured between 2000 and 2500 m, a bathypelagic depth range far exceeding that of the next nearest capture depth of 1000-1500 m. By excluding this

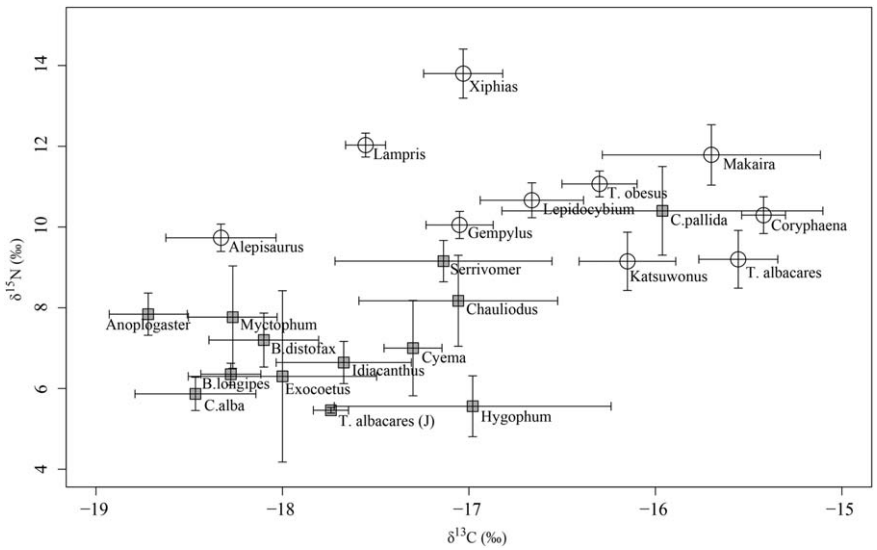

Fig. 2. Biplot of mean bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values for 23 species of large pelagic (empty circles) and micronekton fishes (filled squares). Error bars are standard error.

specimen from the analysis, the $\delta^{15}N$ values of Phe, Gly, and Ser all increased with increasing median depth of capture of micronekton fishes (linear regression, $F_{1,27} = 5.36$, $R^2 = 0.14$, $p < 0.05$ for $\delta^{15}N_{Phe}$; $F_{1,27} = 21.19$, $R^2 = 0.42$, $p < 0.001$ for $\delta^{15}N_{Gly}$; $F_{1,27} = 30.57$, $R^2 = 0.51$, $p < 0.001$ for $\delta^{15}N_{Ser}$; *see* Figure 4).

Multivariate cluster analysis based on four isotopic parameters that increased with micronekton fish depth of capture (bulk tissue $\delta^{15}N$ values, and $\delta^{15}N_{Phe}$, $\delta^{15}N_{Ser}$, $\delta^{15}N_{Gly}$ values) mixed four species of large pelagic fishes with the micronekton fishes (Figure 3b). The substructures of both clusters (Figure 3a,b) appear to be highly influenced by known depth preferences for the different fish species. For instance, significant subgroupings in Figure 3a included *C. hippurus* and *Makaira nigricans*. Both species have median depths of occurrence of 50 m, and are known epipelagic species that forage above the thermocline (*see* the Supporting Information, Table S1). Another group comprised *G. serpens* and *Lampris guttatus, both species* have median depths of occurrence of 131.5 and 225 m, respectively, and are known to occur at least throughout the upper half of the mesopelagic zone (*see* the Supporting Information, Table S1). *Lepidocybium flavobrunneum* and *T. obesus* have median depths of occurrence slightly deeper in the mesopelagic zone, 500 and 250 m, respectively, and are species known to occur and forage throughout the upper and lower mesopelagic zone (*see* the Supporting Information, Table S1). Of the micronekton prey, there are also clear food web groupings influenced by habitat depth in Figure 3a. Two migrating species of myctophids, *B. longipes* and *Hygophum proximum* have median depths of 387.5 and 362.5 m, respectively. Micronekton species with median depths within or near the lower mesopelagic zone (~600-1000 m) also formed two significant subclusters (*B. distofax* and *Idiacanthus fasciola*, *A. cornuta* and *C. sloani*; *see* the Supporting Information, Table S1). *Exocoetus volitans* and *S. sector* have median depths of 0 and 800 m, respectively, and do not represent a subcluster influenced by depth. This is also true for the subcluster containing *Cyema atrum* and *T. albacares* (J), which have median depths of 1250 and 50 m, respectively. In Figure 3b, these significant subgroupings of both large pelagics and micronekton shift away from those in Figure 3a but the influence of depth on isotopic source indicators remains evident.

Large pelagic fish species were grouped according to four significant subclusters in Figure 3a. Again, these groups approximated species inhabiting or utilizing the epipelagic, upper mesopelagic, lower mesopelagic zones, and a group with a known deep-diver *X. gladius*, and *A. ferox*. Large pelagic fish $\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$, and $\delta^{15}N_{Ser}$ values were not significantly different between the four cluster-based depth groupings (ANOVA; $\delta^{15}N_{Phe}$: $F_{3,23} = 1.89$, $p = 0.16$; $\delta^{15}N_{Gly}$: $F_{3,19} = 0.92$, $p = 0.46$; $\delta^{15}N_{Ser}$: $F_{3,21} = 0.99$, $p = 0.42$). Large pelagic fish bulk tissue $\delta^{15}N$ values did, however, show a weakly significant increase with increasing median habitat depth (*see* the Supporting Information, Table S1 for depths; linear regression, $F_{1,145} = 8.63$, $R^2 = 0.05$, $p < 0.01$). Neither large pelagic fish nor micronekton bulk $\delta^{13}C$ values showed any significant relationships with median habitat depth.

Size-based differences

Bulk tissue $\delta^{15}N$ values significantly increased with log-transformed fish size (as total or fork-length) across all 23 species (linear regression, $F_{1,204} = 149.8$, $R^2 = 0.42$, $p < 0.001$). Bulk $\delta^{13}C$ values also significantly increased with fish length

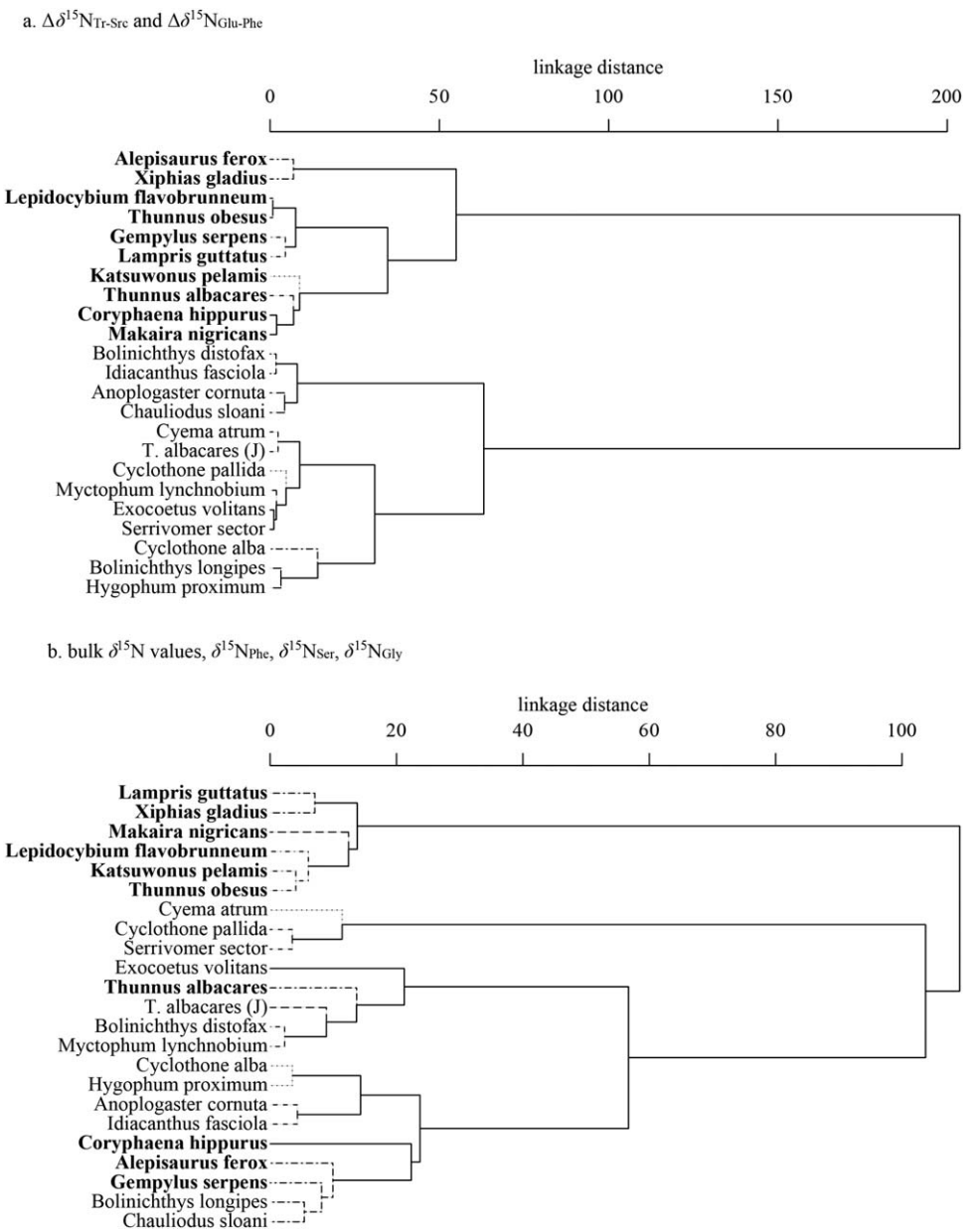

Fig. 3. Cluster analysis dendrogram of large pelagic and micronekton fish species (each node represents a species) using a) species mean $\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ values (trophic indicators), and b) species mean bulk tissue $\delta^{15}N$ values, $\delta^{15}N_{Phe}$, $\delta^{15}N_{Ser}$, $\delta^{15}N_{Gly}$ values (source indicators). Shared line types indicate significantly similar subgroups. Large predator fish species are shown in bold.

across all species (linear regression, $F_{1,204} = 5.2$, $R^2 = 0.02$, $p < 0.05$) but the relationship was weaker than with bulk tissue $\delta^{15}N$ values and fish length. Intraspecific relationships were weak and insignificant except for four cases: *A. ferox* and *X. gladius* lengths and bulk tissue $\delta^{15}N$ values (linear regression, $F_{1,28} = 7.3$, $R^2 = 0.17$, $p < 0.05$ for *A. ferox*; $F_{1,9} = 53.1$, $R^2 = 0.84$, $p < 0.001$ for *X. gladius*), *A. ferox* and *G. serpens* lengths and bulk $\delta^{13}C$ values (linear regression, $F_{1,28} = 14.3$, $R^2 = 0.31$, $p < 0.001$ for *A. ferox*; $F_{1,16} = 23.8$, $R^2 = 0.57$, $p < 0.001$ for *G. serpens*). $TP_{Tr\text{-}Src}$ significantly increased with log-transformed animal length (across all species, linear regression, $F_{1,60} = 103.8$, $R^2 = 0.63$, $p < 0.001$), providing clear evidence for size-related increases in trophic status within this assemblage of pelagic animals (Figure 5). For these same specimens with both bulk tissue and AA-CSIA data, bulk tissue $\delta^{15}N$ values also significantly increased with log-transformed animal length (linear regression, $F_{1,60} = 60.2$, $R^2 = 0.50$, $p < 0.001$) but were not as strongly correlated as $TP_{Tr\text{-}Src}$ was with animal length. Bulk size-fractionated zooplankton collected at the same time and

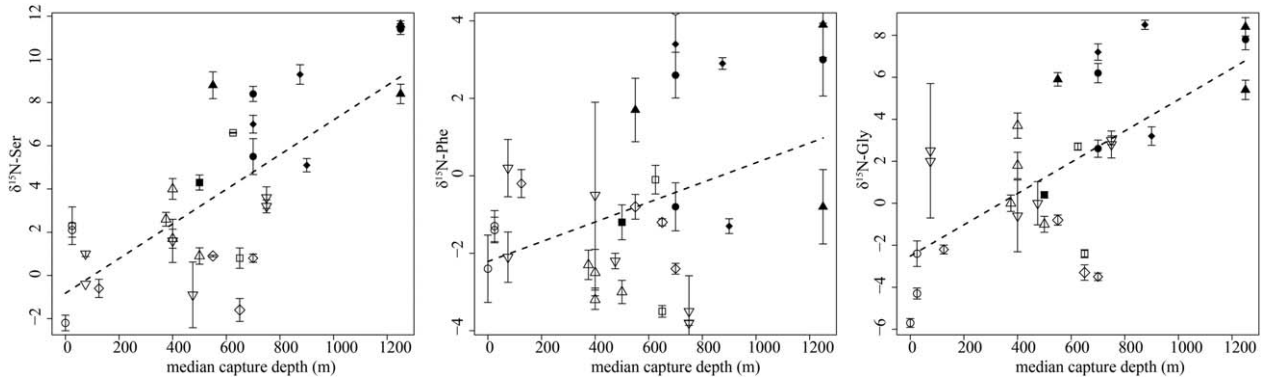

Fig. 4. Stable nitrogen isotope composition (δ^{15}N) of the "source" AAs serine (Ser), phenylalanine (Phe), and glycine (Gly) plotted against median capture depth for 13 species of micronekton fishes. Each symbol type represents a unique species, open symbols are micronekton fishes that perform DVM. Table S2 provides median capture depths (*see* the Supporting Information). All three source AA δ^{15}N values significantly increase with increasing median depth of micronekton capture ($R^2 = 0.51$ for $\delta^{15}N_{Ser}$, $R^2 = 0.14$ for $\delta^{15}N_{Phe}$, $R^2 = 0.42$ for $\delta^{15}N_{Gly}$). Vertical bars are SDs of replicate injections.

location from Hannides et al. (2013) are also included in these relationships, representing a total of three primary pelagic food web components.

Discussion

Isotopic variability with depth

The δ^{15}N values of Src-AAs in marine animals are determined by the specific nitrogenous inputs used by primary producers, and thus have been shown to vary in consumers according to seasonal (temporal) shifts in nutritional sources (e.g., Hannides et al. 2009), spatial differences in productivity and ocean biogeochemistry (e.g., Choy et al. 2012), and microbial trophic processing (e.g., Gutierrez-Rodriguez et al. 2014). For example, highly migratory leatherback sea turtles ranging the expanse of the Pacific Ocean expressed ~4‰ spatial differences in $\delta^{15}N_{Phe}$ values (Seminoff et al. 2012). Additionally, the δ^{15}N values of Src-AAs in pelagic copepods from both NPSG (seasonal range of ~2-5‰ in Phe; Hannides et al. 2009) and California Current (interannual range of ~2‰; Décima et al. 2013) ecosystems tracked seasonal and interannual fluctuations in N sources. However, the magnitude of variability in the δ^{15}N values of Src-AAs evident for fishes in this study has only been observed by Choy et al. (2012; $\delta^{15}N_{Phe}$ values ranging ~10‰), who examined myctophid and stomiid micronekton fishes from five mesopelagic ecosystems across the globe. These wide-ranging Src-AA δ^{15}N values were attributed to N-baseline differences in oceanographic regimes, which were preserved in the higher-order fish consumers. We propose that depth related changes in average $\delta^{15}N_{Phe}$, $\delta^{15}N_{Ser}$, and $\delta^{15}N_{Gly}$ values in the NPSG are suggestive of variation in nutrition sources used by this pelagic fish assemblage. Few existing studies have applied AA-CSIA across large vertical gradients in the ocean, but those that have report comparably large variation in Src-AA δ^{15}N values (e.g., $\delta^{15}N_{Phe}$ range of ~9‰ in suspended POM sampled from 25 to 750 m; Hannides et al. 2013).

Suspended and sinking POM can be accessed as a direct food resource for deep water animals (e.g., via filter feeding), or indirectly (e.g., via consumption of filter feeding animals; Alldredge and Silver 1988). Carnivory of resident and diel-vertically migrating zooplankton is another, likely more important, source of nutrition for midwater fish communities (e.g., Clarke 1978), which is complicated by various diel-vertical migratory behaviors and depth preferences. Stable isotope approaches have more recently begun to provide new insights into midwater food web structure and energy flow by demonstrating isotopic differences in food resources with depth. For instance, the bulk N isotope values of both POM and bulk or species-specific zooplankton pelagic food web components are systematically enriched in ^{15}N with increasing depth through the mesopelagic and upper bathypelagic zones of the global ocean (e.g., Saino and Hattori 1980; Altabet et al. 1991; Koppelmann et al. 2003). Interestingly, slope-based demersal food web studies from the Atlantic and Antarctic have also used bulk stable isotopes to examine the variability of food resources with depth (Iken et al. 2001; Mintenbeck et al. 2007; Trueman et al. 2014). All of these studies show distinct isotopic separation of benthic suspension feeders that loosely mirror observed increases in suspended POM bulk δ^{15}N values with depth. Additionally, negligible depth-dependent ^{15}N enrichment is observed in bentho-pelagic feeding demersal species, suggesting the importance of surface production over suspended POM food resources.

In terms of more recent studies utilizing both bulk stable isotopes and AA-CSIA in pelagic ecosystems, Hannides et al. (2013) demonstrated that Src-AA δ^{15}N values of zooplankton and suspended POM increase with depth through the

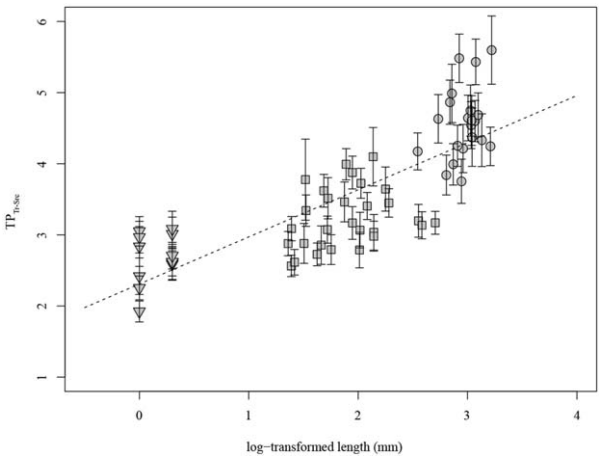

Fig. 5. Relationships between animal length (log-transformed) and pelagic animal TPs estimated from AA-CSIA ($TP_{Tr\text{-}Src}$). Large pelagic fishes are shown with circles, micronekton fishes with squares, and size-fractionated bulk zooplankton with triangles (data from Hannides et al. 2013). $TP_{Tr\text{-}Src}$ significantly increases with fish length (dashed line; $R^2 = 0.63$; $y = 0.66x + 2.31$).

epipelagic and mesopelagic zones of the NPSG, providing initial evidence for changes in food resources between epipelagic and mesopelagic zooplankton communities. More specifically, suspended POM was on average, more enriched in ^{15}N than the mixed zooplankton. Large differences in the Src-AA $\delta^{15}N$ values of zooplankton and suspended POM pools implied that suspended POM was not an important food resource for mixed midwater zooplankton communities at depth. Instead, the majority of N used by midwater zooplankton communities inferred through isotope mass balance of Src-AA $\delta^{15}N$ values is surface-derived (e.g., sinking particles formed in the euphotic zone or direct feeding in surface waters). Zooplankton $\delta^{15}N_{Phe}$ values did, however, increase by $\sim$3.5‰ with depth (between 75 m and 850 m), indicating an increasing amount of suspended POM in the diets of deeper-dwelling zooplankton. Additionally, the large changes in Src-AA $\delta^{15}N$ values with depth were primarily attributed to microbial degradation of suspended particles. Large differences in the Src-AA $\delta^{15}N$ values (Phe, Gly, Ser) of micronekton fishes in this study extend these results to higher order consumers of the NPSG, suggesting that they too increasingly consume food with a suspended POM AA isotopic signature with depth.

Similar to Hannides et al. (2013), we applied a simple two-source isotope mass balance mixing model to quantify the proportion of surface-derived N sources entering meso- and upper bathypelagic food webs. Since AA-CSIA data for suspended POM and zooplankton in Hannides et al. (2013) were determined on samples collected from the same location (St. ALOHA) and same time point as the fish consumers in this study, we can approximate the importance of surface-derived suspended POM food resources (f) to epipelagic, mesopelagic, and upper bathypelagic micronekton fishes. Hannides et al. (2013) clearly demonstrated that surface (25 m) suspended POM food resources differ substantially in Src-AA stable N isotope composition ($\delta^{15}N_{Phe\text{-}surface} = -2.2‰$; $\delta^{15}N_{Gly\text{-}surface} = 0.4‰$; $\delta^{15}N_{Ser\text{-}surface} = -0.9‰$) from suspended particle resources in midwaters (750 m; $\delta^{15}N_{Phe\text{-}deep} = 7.5‰$; $\delta^{15}N_{Gly\text{-}deep} = 12.2‰$; $\delta^{15}N_{Ser\text{-}deep} = 12.1‰$). The zooplankton food resources carry mixed isotopic signals of surface and deep water food resources, and thus for simplicity, we have chosen to focus on suspended POM as a basal food resource. Our mixing model is applied to fish Src-AA $\delta^{15}N$ values (Phe, Gly, Ser) as: $\delta^{15}N_{Src\text{-}AA\text{-}Fish} = \delta^{15}N_{Src\text{-}AA\text{-}surface} \times f + \delta^{15}N_{Src\text{-}AA\text{-}deep} \times (1 - f)$. We applied this model to four different fish consumers known for distinct depth and feeding preferences (Table 2). As expected, the lowest values of $\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$, and $\delta^{15}N_{Ser}$ were measured in the neustonic, zooplanktivorous flying fish (*E. volitans*), where the $\delta^{15}N$ Src-AA values matched those of surface (euphotic zone) suspended POM and zooplankton reported in Hannides et al. (2013). Food resources for *E. volitans* were estimated to be entirely surface-derived, in line with the fact that this fish does not leave the surface layer of the ocean. In contrast, the $\delta^{15}N$ values of Src-AAs become increasingly scattered as fish depth of occurrence increases (Table 2). Deep-dwelling fishes that do not exhibit diel-vertical migration (DVM) had some of the highest Src-AA $\delta^{15}N$ values (e.g., *C. atrum*, *S. sector*, *C. pallida* in Tables 1 and 2). However, at least for the zooplanktivorous *C. atrum* and *Cyclothone* spp., substantial proportions of their diets were linked by isotope mass balance to surface-derived particles (range 19-61%, Table 2). If these fishes are thought to not move into surface waters, they must be consuming prey items who themselves feed on surface-derived particles and preserve these isotopic values characteristic of surface waters. These results also provide evidence for the consumption of suspended particles within deep layers of the ocean and suggest that some species obtain as much as 80% of nitrogen from this highly altered source of organic matter. Meanwhile, *A. cornuta*, the piscivorous fangtooth fish, exhibits mild DVM and typically inhabits lower-mesopelagic and upper-bathypelagic waters (*see* the Supporting Information, Table S1). Mixing model results suggest that this deep-dwelling fish expresses source isotopic values that are mostly linked to surface particles (>67%, Table 2). Thus, *A. cornuta* likely receives a large portion of its nutrition from consuming animals feeding in large part on surface-derived food resources, despite residing primarily at deeper mesopelagic and upper bathypelagic depths.

The isotope mixing model results reflect multiple modes of nutrition for pelagic micronekton fishes, some of which are well known but one of which may be underappreciated. One clear trophic mode is to consume resident and/or migrant zooplankton in surface waters, ultimately preserving low Src-AA $\delta^{15}N$ values likely derived exclusively from the surface (as seen in the flying fish, *E. volitans* and juvenile

Table 2. Source AA $\delta^{15}N$ values (glycine (Gly), serine (Ser), and phenylalanine (Phe)) of suspended particles (from Hannides et al. 2013) and micronekton fishes (this study). f is the proportion of surface-derived suspended POM food resources fueling epipelagic, mesopelagic, and upper bathypelagic micronekton fishes ($\delta^{15}N_{Src\text{-}AA\text{-}Fish}$) as estimated from the isotope mixing model: $\delta^{15}N_{Src\text{-}AA\text{-}Fish} = \delta^{15}N_{Src\text{-}AA\text{-}surface} \times f + \delta^{15}N_{Src\text{-}AA\text{-}deep} \times (1 - f)$, using three different source AAs (Gly, Ser, Phe).

Food Web Component	Depth (m)	$\delta^{15}N_{Gly}$ (‰)	$\delta^{15}N_{Ser}$ (‰)	$\delta^{15}N_{Phe}$ (‰)	f_{Gly}	f_{Ser}	f_{Phe}
Surface suspended particles	25	0.4	−0.9	−2.2	—	—	—
Deep suspended particles	750	12.2	12.1	7.5	—	—	—
Epipelagic fish (*Exocoetus volitans*)	0	−5.7	−2.2	−2.4	1.52	1.10	1.02
Lower-mesopelagic fish, some DVM (*Anoplogaster cornuta*)	712.5	2.9	3.4	−3.6	0.79	0.67	1.14
Lower-mesopelagic fish, no DVM (*Cyclothone* spp.)	800	5.5	8.4	1.6	0.57	0.28	0.61
Upper-bathypelagic fish, no DVM (*Cyema atrum*)	1250	6.6	9.6	1.6	0.47	0.19	0.61

yellowfin tuna, *T. albacares* (J)). Some micronekton fishes exhibiting DVM also often consume resident and/or DVM zooplankton in near surface waters at night (e.g., Clarke 1980), and therefore, have surface-derived Src-AA $\delta^{15}N$ values, such as the myctophids *H. proximum* ($\delta^{15}N_{Phe} = -1.8‰$) and *Myctophum lynchnobium* ($\delta^{15}N_{Phe} = -1.3‰$). Other nonmigratory lower-mesopelagic and bathypelagic fishes expressing isotopic baseline values indicative of surface sources of nutrition, such as *A. cornuta* probably feed mainly on migratory micronekton, which in turn appear to feed on surface zooplankton or POM (Vinogradov 1962). This type of trophic coupling between the surface and deeper waters has been called the ladder of vertical migrations (Vinogradov and Tseitlin 1983). Our results also indicate that some deeper water micronekton fishes may be accessing food derived from suspended particles because they have high Src-AA $\delta^{15}N$ values very close to those of suspended particles from 400 and 750m (e.g., $\delta^{15}N_{Phe}$ ~7‰; Hannides et al. 2013). Suspended particles are very small so it is unlikely that fish directly consume them but suspension feeders such as salps, pyrosomes, and larvaceans are known to filter particles as small as 2 μm (e.g., Harbison and McAlister 1979; Sutherland et al. 2010). It is certainly possible that micronekton fishes consume these animals. For example, the gonostomatid fish, *Cyclothone*, is numerically the most dominant micronekton fish in most pelagic systems (Brodeur and Yamamura 2005) and diet studies report unknown detrital material, fecal pellets, and large quantities of unrecognizable material in the stomachs of deep nonmigrating species (Marshall 1960; DeWitt and Cailliet 1972). In this way, organic material in very small particles may re-enter the larger animal component of the pelagic food web.

Depth-dependent increases in the $\delta^{15}N$ values of source AAs in the 10 species of large pelagic fishes were not observed though there was some clustering of these fishes by depth but this clustering occurred using TP metrics rather than source AA metrics (Figure 3a vs. b). The lack of a strong trend with depth could be due to their mobility and opportunistic feeding on lower trophic level animals across large vertical and horizontal distances.

Trophic structure and ecological niche partitioning of the pelagic fish assemblage

What is perhaps most striking about the trophic structure of the fish assemblage from an AA-CSIA perspective is that within each of the two main fish groups (large pelagics and micronekton fishes), there is a high degree of trophic overlap. Independent ecological diet and life history studies point toward apparent niche specialization within this speciose fish assemblage (*see* Table S1 and references therein), but isotopic niches are not as clearly resolved as dietary niches evident in the literature. We show that regardless of whether absolute or relative TPs are used to estimate isotopic niches, resulting trophic niche widths are generally very narrow, and thus necessitate complementary direct observations from consumer diet.

Ten ecologically different species of large pelagic fishes of the NPSG occupy a relatively narrow range of TPs (0.7 TPs) based on AA-CSIA data. The 13 micronekton fish species occupied a slightly broader trophic range, ~1.2 TPs from AA-CSIA data and ~1.4 TPs from bulk tissue $\delta^{15}N$ values. More specifically, within the large pelagic group, the most massive carnivorous billfishes like the broadbill swordfish (*X. gladius*) and the blue marlin (*M. nigricans*) are expected to have higher TPs than smaller, micronektonivorous fishes like the lancetfish (*A. ferox*), yellowfin tuna (*T. albacares*), and even the moonfish or opah (*L. guttatus*). Both the broadbill swordfish and the blue marlin are known to feed on large, mid- to high-trophic level ommastrephid and onychoteuthid squids and finfish (Brock 1984; Watanabe et al. 2009). However, there is less than a ~0.5 TP difference between these ecologically different groups of fish species and the estimated TPs of the large pelagic fish group were statistically indistinguishable, as estimated from both AA-CSIA and bulk tissue $\delta^{15}N$ values. Similarly, within the micronekton fish assemblage, there is a range of known feeding preferences, including piscivorous dragonfishes (*I. fasciola*, *C. sloani*; Clarke 1982), and zooplanktivorous myctophids (*H. proximum*, *B. distofax*, *B. longipes*; Clarke 1973) and flying fish (*E. volitans*; Van Noord et al. 2013). However, once more, there is generally a smaller than ~0.5 TP difference between piscivorous and zooplanktivorous

micronekton fishes, with some of the average TPs of the piscivorous species estimated to be actually lower than the zooplanktivorous species (Table 1). Choy et al. (2012) estimated the TPs of piscivorous dragonfishes and zooplanktivorous myctophids from AA-CSIA, and also found a smaller than expected difference in TPs. The widths of the trophic guilds represented in this study are based on absolute TPs calculated from assumed representative values for TEFs (AA-CSIA TEF=5.5‰, bulk tissue δ^{15}N TEF=3.4%, *see* Materials and Procedures). Thus, the emphasis should be on comparisons of relative TPs that do not require assumed β or TEF values to estimate TP (e.g., our $\Delta\delta^{15}N_{Tr\text{-}Src}$ and $\Delta\delta^{15}N_{Glu\text{-}Phe}$ values).

As expected from diet studies and ecological theory, there is clear separation in average trophic levels between the large pelagic and micronekton fish groups, with the lowest trophic level ($TP_{Tr\text{-}Src} = 2.6$) measured in the micronekton fish *C. alba*, and the highest in the bigeye tuna, *T. obesus* ($TP_{Tr\text{-}Src} = 5.0$). This overall range in relative TPs is in line with expectations from diet studies, which suggest that the 23 fish species sampled here span between 2 and 3 TPs, approximating zooplanktivores (TP 3), piscivores (TP 4), micronektonivores and nektonivores (TPs 4-5; *see* the Supporting Information, Table S1 and references therein).

Ecological niche partitioning in terms of vertical feeding and type of feeding habits (e.g., selective vs. opportunistic feeding) is also supported by detailed diet data on large pelagic fishes from the NPSG. Oligotrophic subtropical gyres are generally thought to house diverse assemblages of species (reviewed in Angel 1993), arguing for a diversity of trophic roles to reduce competition. While stable isotope data provide a valuable, time-integrated picture of trophodynamics, one of its primary limitations is the inability to identify species level trophic interactions, which are provided only by complementary direct dietary observations (Ramos and González-Solís 2012). For example, Choy et al. (2013) recently examined the diets of five large mesopelagic fishes included in this study, with similar AA-CSIA TPs (two species of opah or moonfish [$TP_{Tr\text{-}Src} = 4.5 \pm 0.1$], lancetfish [$TP_{Tr\text{-}Src} = 4.9 \pm 0.8$], escolar [$TP_{Tr\text{-}Src} = 4.6 \pm 0.4$], and snake mackerel [$TP_{Tr\text{-}Src} = 4.3 \pm 0.7$]; *see* Table 1). However, diet studies reveal that each predator species fills a rather unique ecological niche, both in terms of prey selection and depth of feeding. For example, lancetfish feed heavily on hyperiid amphipods, pelagic polychaete worms, and mesopelagic fishes like hatchetfish (*Sternoptyx* spp.) and juvenile fangtooth fishes (*A. cornuta*; e.g., Choy et al. 2013). Many of these species are strictly mesopelagic and happen to be relatively immobile or slow-moving. Snake mackerel, conversely, feeds primarily on highly mobile fishes (e.g., exocoetids, molids) and muscular ommastrephid squids from epipelagic waters (Choy et al. 2013). Similar to the lancetfish, the two related species of opah feed primarily in mesopelagic waters, but focus on consuming generally more mobile fishes, onychoteuthid squids, and pelagic octopods. Therefore, combining stable isotope and direct feeding observations (e.g., stomach contents, video) provides for stronger and clearer ecological interpretations as ecological niche separation cannot be resolved from isotopic data alone.

Synthesis

We have examined the trophic structure of a speciose assemblage of large pelagic and micronekton fishes using bulk C and N stable isotope analysis and AA-CSIA approaches. Following recent findings that suspended particle and zooplankton food resources are enriched in ^{15}N with increasing depth across epipelagic and mesopelagic habitats (Hannides et al. 2013), we found a similar pattern in micronekton fishes accessing these food resources. Significant increases in the Src-AA δ^{15}N values Phe, Gly, and Ser were observed with increasing micronekton fish habitat depth, which implies a midwater food web fueled in part by suspended particles along with previously recognized sinking particles and zooplankton. Understanding the relative importance of a suspended particle food web to meso- and bathypelagic fishes will require more detailed investigation of a greater diversity of animals including suspension feeders. Decades earlier, Vinogradov (1962) proposed a trophic "ladder of migrations" in pelagic systems, whereby diel vertically migrating animals and their predators actively transport (i.e., through feeding and DVM) organic material across large depth gradients with overlapping habitat depths. Results from this study provide new isotopic evidence for such trophic flow(s) across epipelagic, mesopelagic, and bathypelagic zones of the ocean. Lastly, bringing relevant diet studies into observations of trophic structure in this pelagic fish assemblage provides direct evidence for ecological niche partitioning, some of which occurs across vertical depth gradients. These findings were only possible with the unique determination of the nitrogen isotopic composition of individual AAs in micronekton and large pelagic fishes, and provide direct evidence for shifting isotope food web baselines across large depth gradients in the ocean.

Future refinement of trophic ecosystem models characterizing pelagic food web energy flow and predator–prey interactions can be improved by incorporating isotopically derived predator–prey length ratios and including suspended particles as an important food resource to midwater communities (e.g., Howell et al. 2013; Polovina and Woodworth-Jefcoats 2013). Such large-scale ecosystem models can incorporate empirical ecological data from multiple perspectives (isotopes, diet, etc.), thus lending insight to overall system functioning, the flow of energy and carbon, and potential perturbations to these ecosystem dynamics from perceived anthropogenic impacts.

Finally, by refining our understanding of trophic relationships between midwater fish consumers and surface-derived zooplankton and particle food resources, we indirectly show that midwater fish communities may attenuate carbon flux

and affect subsequent burial to deep waters. While we are not able to directly assess the metabolic demands of this midwater fish community, Hannides et al. (2015) have recently done so for midwater zooplankton communities from the same region and clearly show that by consuming and respiring substantial amounts of surface-derived particulate matter zooplankton inhabiting lower mesopelagic depths (700-1000 m) affect particle attenuation. Midwater fish-mediated carbon export out of surface waters has been estimated as ~15% of total carbon export in the northeast Pacific Ocean (Davison et al. 2013). Few other studies have attempted to estimate the same carbon requirements for midwater fish communities, but Trueman et al. (2014) demonstrated that demersal fishes feeding bentho-pelagically in the North Atlantic fulfill an important ecological role sequestering carbon below the remineralization zone by consuming diel-vertical migrating prey items. In addition to potentially attenuating carbon flux burial, it is possible that conversely, midwater consumers may positively affect deep water carbon burial through fecal production generated by the consumption of surface water production. Both possibilities necessitate future work that investigates the dynamics of carbon flux through midwater communities, while also incorporating implications and findings from our study.

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Acknowledgments

We thank the fishery observers of the NOAA Pacific Islands Regional Observer Program for help with sample collection, and K. Busscher, L. Jantz, M. Burns, and R. Humphreys of NOAA for their assistance with fishery data and samples. We thank the crew and scientists of R/V Kilo Moana for their assistance with midwater trawling (M. Donohue, J. Calderwood), and B. Mundy for his taxonomic expertise. We thank N. Wallsgrove, C. Lyons, and E. Gier for laboratory assistance and bulk isotope measurements. Lastly, two anonymous reviewers provided helpful criticisms to help improve this manuscript.

This research was funded by the Pelagic Fisheries Research Program via Cooperative Agreement NA09OAR4320075 between the Joint Institute for Marine and Atmospheric Research and the National Oceanic and Atmospheric Administration, and was also partially supported by the National Science Foundation under Grant OCE-1041329 (to BNP and JCD). CAC's time was partially supported by the Monterey Bay Aquarium Research Institute.

The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subdivisions. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. This study was carried out in accordance with the animal use protocols of the University of Hawai'i (protocol #10-984). This is SOEST contribution number 9283.

Received 4 February 2015
Accepted 2 March 2015

Associate editor: Mikhail Zubkov