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Midwater zooplankton and suspended particle dynamics in the North Pacific Subtropical Gyre: A stable isotope perspective

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Abstract

We used amino acid (AA) compound-specific isotope analysis ($\delta^{15}N_{AA}$ and $\delta^{13}C_{AA}$ values) of midwater zooplankton and suspended particles to examine their dynamics in the mesopelagic zone. Suspended particle $\delta^{15}N_{AA}$ values increased by up to 14‰ with depth, whereas particle trophic status (measured as trophic position, TP) remained constant at 1.6 ± 0.07. Applying a Rayleigh distillation model to these results gave an observed kinetic isotope fractionation of 5.7 ± 0.4‰, similar to that previously measured for protein hydrolysis. AA-based degradation index values also decreased with depth on the particles, whereas a measure of heterotrophic resynthesis (ΣV) remained constant at 1.2 ± 0.3. The main mechanism driving ^{15}N enrichment of suspended particles appears to be isotope fractionation associated with heterotrophic degradation, rather than a change in trophic status or N source with depth. In zooplankton the "source" AA phenylalanine (Phe) became ^{15}N enriched by up to 3.5‰ with depth, whereas zooplankton TP increased by up to 0.65 between the surface ocean and midwaters. Both changes in the $\delta^{15}N$ values of food resources at the base of the zooplankton food web and changes in zooplankton TP drive observed zooplankton ^{15}N enrichment with depth. Midwater zooplankton $\delta^{15}N_{Phe}$ values were lower by 5–8‰ compared with suspended particles, indicating this organic matter pool is not a significant zooplankton food resource at depth. Instead, 62–88% of the N sustaining midwater zooplankton is surface derived, obtained through consumption of sinking particles, carnivory of vertical migrants, or direct feeding in surface waters at night.

Mesopelagic zooplankton communities are important mediators of particle dynamics in the ocean's interior and are the forage base for many mesopelagic micronekton. Zooplankton consume particles sinking from the surface ocean, thus controlling in part the rapid attenuation of particle flux observed in midwaters (beneath the sunlit euphotic zone to approximately 1000 m depth; Steinberg et al. 2008*b*). Zooplankton also contribute to the exchange between sinking and suspended particle pools in midwaters through physically fragmenting sinking particles (Dilling and Alldredge 2000) and consuming and repackaging the suspended pool (Wilson et al. 2008). A large component of the mesopelagic zooplankton community migrates to feed in surface waters at night and resides at midwater depths during the day, further contributing to the vertical transfer of organic material through excretion and egestion in the deep ocean (Longhurst and Harrison 1989; Hannides et al. 2009*a*). Finally, both resident and migrant zooplankton in the mesopelagic zone form a food resource for carnivorous micronekton (Clarke 1978) and thus are a gateway for biomass transfer up the mesopelagic food web to commercially important fish species (Brodeur and Yamamura 2005). The sum of these processes results in the transfer of carbon (C) produced through photosynthesis in surface waters into the midwater zooplankton community and associated consumers, thus attenuating vertical C fluxes and ultimately limiting C sequestration in the deep ocean interior.

Despite the significant role of mesopelagic zooplankton in particle cycling and as food for harvestable fish populations, very little is known concerning the structure and dynamics of these communities because of difficulties in sampling and manipulating them (Robinson et al. 2010). One approach that has shown promise for understanding midwater trophic dynamics is stable nitrogen (N) isotope analysis. This technique does not require in situ manipulations, is not subject to the uncertainties of gut content analysis (e.g., unidentifiable remains), and can indicate the integrated trophic history of zooplankton over weeks to months, depending on zooplankton growth dynamics and tissue turnover rates. Although stable isotope analysis has been used sparingly for deep-sea populations, zooplankton in several regions of the world's oceans appear to become enriched in ^{15}N with depth in the mesopelagic and bathypelagic zones, for example, in the Arabian Sea (Koppelmann and Weikert 2000), the Mediterranean Sea (Koppelmann et al. 2009), and the south Atlantic Ocean (Laakmann and Auel 2010). This has been ascribed to increasing trophic level, or carnivory, in deep-water zooplankton (Koppelmann et al. 2003), as animal $\delta^{15}N$ values can increase by ~ 3.4‰ with each step in trophic position (Minagawa and Wada 1984). Thus, larger $\delta^{15}N$ values could indicate an elongation of deep-sea food webs, which has ecological implications for trophic transfer and ultimately higher trophic level production.

The observed ^{15}N enrichment in midwater zooplankton could, alternatively, indicate a shift in N isotopic composition at the base of the food web. For deep-sea zooplankton communities, this food web base largely comprises particulate organic matter (POM) that sinks to mesopelagic depths from surface waters ("marine snow")

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or remains suspended in midwaters. Early studies documented a substantial increase in the ^{15}N content of suspended particles from surface waters through the mesopelagic (Saino and Hattori 1980). This increase was interpreted to have resulted from degradation of labile, ^{15}N-depleted organic material, leaving a ^{15}N-enriched pool of suspended material at midwater depths. In contrast, the $\delta^{15}N$ values of larger sinking particles have been observed to increase (Casciotti et al. 2008) or decrease (Altabet et al. 1991) only slightly with depth, despite their flux attenuation in midwaters. Thus, depth increases in zooplankton $\delta^{15}N$ values could suggest feeding by zooplankton on ^{15}N-enriched suspended particles in midwaters.

It is difficult to distinguish between these two alternative drivers of ^{15}N enrichment in midwater zooplankton based on analysis of whole-animal, or "bulk," stable isotopic compositions. To do so, we used compound-specific isotope analysis (CSIA) of individual amino acids (AA; $\delta^{15}N_{AA}$ analysis), which can significantly increase the interpretive power of N isotope studies. The power of CSIA derives from the different AA responses to trophic transfers (McClelland and Montoya 2002). One group of AAs ("trophic" AAs [Popp et al. 2007]: alanine [Ala], aspartic acid [Asp], glutamic acid [Glu], isoleucine [Ile], leucine [Leu], proline [Pro], valine [Val]) become significantly ^{15}N enriched with each trophic transfer (e.g., $\delta^{15}N_{Glu}$ values can increase by 8‰ for each step in trophic position between consumer and producer; Chikaraishi et al. 2009). In contrast, the $\delta^{15}N$ values of another group of AAs ("source" AAs [Popp et al. 2007]: glycine [Gly], lysine [Lys], methionine [Met], phenylalanine [Phe], serine [Ser], threonine [Thr], tyrosine [Tyr]) change very little during a trophic transfer and thus retain their source isotopic value (i.e., the $\delta^{15}N_{AA}$ value acquired by producers at the base of the food web). For example, $\delta^{15}N_{Phe}$ values increase by only 0.4‰ per trophic level (Chikaraishi et al. 2009). Thus, variability in source $\delta^{15}N_{AA}$ values can indicate a change in N source isotopic composition at the base of zooplankton food webs, and differences between trophic and source $\delta^{15}N_{AA}$ values can indicate a change in zooplankton trophic status (Hannides et al. 2009*b*). Comparison of different $\delta^{15}N_{AA}$ values within the same type of organism or sample could thus be used to ascertain the mechanisms driving change in N isotopic composition in midwater food webs.

A different perspective on animal nutrition can be gleaned from AA-specific analysis of C isotope compositions ($\delta^{13}C_{AA}$ analyses), as $\delta^{13}C_{AA}$ values have recently been used to identify the biosynthetic origins of material supporting animal food webs. Application of this new technique is based on highly conserved modes of C acquisition and AA biosynthesis in different types of producers that produce unique patterns of C isotopic fractionation (Keil and Fogel 2001). These $\delta^{13}C$ patterns are preserved in essential AAs (EAAs) during trophic transfers and thus can act as "fingerprints" of producer source in consumers. Larsen et al. (2009) first used this technique to distinguish between terrestrial plant, bacterial, and fungal sources of EAAs to lichens and fruit fly eggs. More recently, Larsen et al. (2012) differentiated between marine and terrestrial plant sources of EAAs in mangrove food webs on the basis of $\delta^{13}C_{EAA}$ values. Thus, analysis of $\delta^{13}C_{EAA}$ values in zooplankton should complement $\delta^{15}N_{AA}$ analyses and allow one to distinguish between marine producer vs. bacterial sources of EAAs to midwater consumers.

In this study, we investigate zooplankton trophic status and potential linkage to particle cycling in midwaters of the oligotrophic North Pacific Subtropical Gyre (NPSG) for the first time using CSIA. We determine $\delta^{15}N_{AA}$ and $\delta^{13}C_{AA}$ values for epi- and mesopelagic zooplankton and suspended particles and use this information to (1) investigate the drivers of change in suspended particle $\delta^{15}N$ values with depth, (2) determine whether depth variability in zooplankton $\delta^{15}N$ values is driven by changes in food source N isotope composition or changes in trophic position, and (3) establish the biosynthetic origin of material supporting zooplankton food webs and contributing to suspended particle cycling in midwaters.

Methods

Zooplankton were collected in late August 2011 at Sta. ALOHA (22.45°N, 158°W), the Hawaii Ocean Time-series station in the NPSG, using a multiple opening–closing net and environmental sensing system (MOCNESS) fitted with 1 m^2 of 0.2 mm mesh plankton nets. Plankton were collected during the daytime (10:00–14:00 h) and nighttime (22:00–02:00 h) by oblique tows at the following depth intervals between the surface and 1000 m: 0–50 m, 50–100 m, 100–150 m, 150–200 m, 200–250 m, 250–300 m, 300–500 m, 500–700 m, and 700–1000 m. Onboard, zooplankton were rapidly wet-sieved in filtered seawater using 0.2, 0.5, 1.0, 2.0, and 5.0 mm mesh sieves into different size fractions and frozen at −20°C. Suspended particles were collected at the same site (Sta. ALOHA) using 10 liter polyvinyl chloride (PVC) bottles. The particles were collected at 24 standard depths between the surface and 2000 m, fed into multiple 2 liter high-density polyethylene (HDPE) Nalgene bottles, and 8 liters of water vacuum filtered onto precombusted (400°C, 8 h) 25 mm GF/F filters for bulk stable isotope analysis. For CSIA, particles were collected at 14 standard depths between 0 and 1500 m by closing multiple PVC bottles at each depth, collecting the water in multiple 25 liter HDPE carboys, and filtering 100 liters of water using pressurized canisters onto precombusted 142 mm GF/F filters.

Zooplankton and suspended particle filters were lyophilized and the plankton weighed to determine dry weight biomass. Mixed zooplankton material in the different size fractions was then ground using a mortar and pestle, and the samples were weighed or rolled into tin capsules. Bulk isotope composition of plankton and particles from all collection depths was determined using a Costech elemental combustion system (Model 4010) coupled to a Thermo-Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS) through a Conflo IV interface. Isotope values are reported in standard δ-notation relative to the atmospheric N_2 standard (AIR) as $\delta^{15}N$ (‰) = $[(^{15}N:{}^{14}N_{sample}/^{15}N:$

$^{14}N_{AIR}) - 1] \times 1000$. To ensure accuracy, glycine and ground tuna reference samples with well-characterized $\delta^{15}N$ values were analyzed every 10 samples. The standard deviation on samples analyzed in duplicate was $\leq 0.2‰$.

CSIA was performed on zooplankton samples collected from 50–100 m, 300–500 m, and 700–1000 m depth intervals and suspended particles collected at 25, 75, 400, and 750 m depth horizons. The plankton and particle samples were hydrolyzed and derivatized according to the methods of Popp et al. (2007) and Hannides et al. (2009*b*). Briefly, 5–10 mg of plankton or particle filters were hydrolyzed using trace metal–grade 6 mol L^{-1} HCl and the hydrolysate purified using low protein–binding filters and cation exchange chromatography. The purified samples were then esterified using 4 : 1 isopropanol : acetyl chloride and derivatized using 3 : 1 methylene chloride : trifluoroacetyl anhydride. Finally, trifluoroacetyl and isopropyl ester (TFA) derivatives were purified using solvent extraction and stored at −20°C up to 1 month before analysis.

TFA derivatives of AAs were analyzed for stable N isotopic composition using a Thermo Scientific Delta V Plus IRMS interfaced to a trace gas chromatograph (GC) fitted with a 60 m BPx5 *forte* capillary column (0.32 mm internal diameter with 1.0 μm film thickness) through a GC–C III combustion furnace (980°C), reduction furnace (680°C), and liquid nitrogen cold trap as described by Hannides et al. (2009*b*) and Dale et al. (2011). The analysis consisted of three to five injections for each sample, with norleucine and aminoadipic acid internal reference compounds co-injected in each run. The $\delta^{15}N$ values of the pure norleucine and aminoadipic acid internal reference compounds were previously measured using the bulk isotope technique described above. A suite of pure AAs of known N isotopic composition (Ala, Thr, Ile, Pro, Glu, and Phe) was also analyzed every three injections to bracket each sample and provide an additional measure of instrument accuracy. For replicate zooplankton injections, $\delta^{15}N_{AA}$ standard deviations averaged 0.46‰ and ranged from 0.04‰ to 2.24‰. For suspended particle samples, standard deviations averaged 0.33‰ and ranged from 0.03‰ to 0.72‰. Full AA reference suites (15 AAs) were analyzed with each batch of samples, and the corresponding response factors (Vs [nmol AA]$^{-1}$) were then used to determine AA concentrations and AA mol% (i.e., mol AA_i/Σ mol AA × 100% for each AA sample *i*).

Stable C isotopic composition of AAs in suspended particles and zooplankton (selected size fractions from the 50–100 m, 300–500 m, and 700–1000 m collections) was also determined using GC-C-IRMS. Samples were prepared in batches of eight, with an additional vial containing a mixture of 15 pure AAs purchased commercially (Sigma Scientific; i.e., a reference suite). Before creating the reference suite, the $\delta^{13}C$ values of these individual compounds were measured using the bulk isotope technique described above. The $\delta^{13}C$ values of AAs in each sample were then determined using a MAT 253 IRMS interfaced with a Trace GC Ultra via a combustion furnace (1030°C) and ConFlo IV interface (Thermo Scientific). Derivatized AAs were injected using a pressure–temperature–volume injector, held at 40°C for three seconds, heated to 87°C (400°C min^{-1}), heated again to 200°C, and transferred at 200°C using a 1 : 10 split. Helium (1 mL min^{-1}) was used as the carrier gas. AAs were separated using a 30 m BPX5 *forte* capillary column (0.32 mm internal diameter with 1.0 μm film thickness). The oven temperature for the GC started at 40°C and was held for 1 min before heating at 15°C min^{-1} to 120°C, then 3°C min^{-1} to 190°C, and finally 5°C min^{-1} to 300°C, where it was held for an additional 10 min. Isotope values are reported in standard δ-notation relative to Vienna Pee Dee belemnite. CSIA of zooplankton and suspended particles consisted of three or four injections per sample with an *n*-C_{20} alkane with a well-characterized $\delta^{13}C$ value co-injected as an internal reference. The 15 AA reference suite was analyzed every four injections, and sample $\delta^{13}C_{AA}$ values were corrected relative to this AA suite following Silfer et al. (1991). For our samples, the correction equation was $\delta^{13}C_{AA} = [\delta^{13}C_{CSIA} - (1 - X) \times \delta^{13}C_{ISO}]/X$, where $\delta^{13}C_{AA}$ is the corrected isotope value for the AA of interest, $\delta^{13}C_{CSIA}$ is the isotope value initially determined by GC-C-IRMS, X is the mole fraction of C in each AA, and $\delta^{13}C_{ISO}$ is the isotope value of the isopropanol added to each AA during esterification (the correction factor). Additionally, norleucine and aminoadipic acid reference compounds prepared with each sample batch were co-injected with all samples, their $\delta^{13}C$ values corrected using the above equation, and the results analyzed to establish instrument accuracy. For replicate zooplankton injections, average $\delta^{13}C_{AA}$ standard deviations were 0.59‰ and ranged from 0.12‰ to 0.99‰. For POM samples, standard deviations averaged 0.68‰ and ranged from 0.06‰ to 2.55‰. Process blanks analyzed in parallel with samples for stable N and C isotope composition never included detectable AAs.

Composite source (Sr-AA) and trophic (Tr-AA) $\delta^{15}N_{AA}$ values were calculated by averaging specific suites of AAs (i.e., $\delta^{15}N_{Sr\text{-}AA}$ = the average of Gly, Phe, and Ser $\delta^{15}N_{AA}$ values, and $\delta^{15}N_{Tr\text{-}AA}$ = the average of Ala, Glu, Ile, Leu, Pro, and Val $\delta^{15}N$ values). We also calculated zooplankton trophic positions (TPs). Zooplankton TPs were determined for $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values following Chikaraishi et al. (2010) as $TP_{Glu\text{-}Phe} = 1 + (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta)/TEF$, where the isotopic difference between Glu and Phe in marine producers (β) is 3.4‰ and the trophic enrichment factor (TEF) is 7.6‰. In the literature, TP has also been calculated using different suites of Tr-AAs and Sr-AAs (Hannides et al. 2009*b*; Sherwood et al. 2011). We calculated a Tr-AA– and Sr-AA–based measure of trophic position as $TP_{Tr\text{-}Sr} = 1 + (\delta^{15}N_{Tr\text{-}AA} - \delta^{15}N_{Sr\text{-}AA} - \beta)/TEF$, where β for this suite of Tr-AA and Sr-AA is also 3.4‰ and the TEF is 5.6‰ (derived from Chikaraishi et al. [2009]). Standard deviations were calculated for $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ considering propagation of error following Dale et al. (2011).

We calculated AA mol%–based degradation index values (DI values) for suspended particles based on Dauwe et al. (1999) and values for the ΣV parameter based on McCarthy et al. (2007). That is, DI values were calculated using the formula $DI = \Sigma[(AA_i - AVG\ AA_i)/SD\ AA_i] \times$

Table 1. Zooplankton phenylalanine (Phe) $\delta^{15}N$ values and trophic positions ($TP_{Glu\text{-}Phe}$) at Sta. ALOHA, weighted by standard deviation and averaged over all size fractions. The change (Δ) in $\delta^{15}N_{Phe}$ value and $TP_{Glu\text{-}Phe}$ between plankton in surface waters (50–100 m) and plankton at mesopelagic depths (300–1000 m) is also shown.

		Weighted mean		Δ (x–75 m)	
Time	Depth (m)	$\delta^{15}N_{Phe}$ (‰)	$TP_{Glu\text{-}Phe}$	Δ $\delta^{15}N_{Phe}$ (‰)	Δ $TP_{Glu\text{-}Phe}$
Day	50–100	−2.1±0.1	2.2±0.1	na	na
Night	50–100	−2.4±0.2	2.3±0.1	na	na
Day	300–500	−1.0±0.1	2.5±0.1	1.1±0.2	0.3±0.1
Night	300–500	−0.6±0.2	2.9±0.1	1.8±0.3	0.5±0.1
Day	700–1000	1.4±0.3	2.9±0.1	3.5±0.3	0.6±0.1
Night	700–1000	0.3±0.2	2.7±0.1	2.6±0.3	0.4±0.1

na, not applicable.

factor coefficient$_i$, where AVG AA_i and SD AA_i are our averages and standard deviations for mol% of AA sample i, and factor coefficients are those of Dauwe et al. (1999; Table 1). DI values decrease with decreasing organic matter (OM) lability (Dauwe et al. 1999); thus, this parameter can be used as an indicator of OM degradation state (Sheridan et al. 2002). The ΣV parameter was calculated using the formula $\Sigma V = 1/n \times \Sigma\ \mathrm{Abs}(\chi_{AAi})$, where n = the total number of AA summed and χ_{AAi} is the deviation of each trophic AA from their mean (i.e., $\delta^{15}N_{Tr\text{-}AAi}$ − AVG $\delta^{15}N_{Tr\text{-}AA}$ for Ala, Leu, Ile, Pro, Asp, and Glu). ΣV increases with increasing deviation in $\delta^{15}N_{Tr\text{-}AA}$ values, for example, through de novo heterotrophic reprocessing. Thus, this parameter has been used as a proxy for total heterotrophic resynthesis (McCarthy et al. 2007).

Statistics conducted on zooplankton and particle isotope compositions included linear discriminant analysis (LDA) and principal components analysis (PCA). LDA was conducted on $\delta^{13}C_{AA}$ values normalized to their means as $N(\delta^{13}C_{AAi}) = \delta^{13}C_{AAi} - (\sum_{i=1}^{n}\delta^{13}C_{AAi})/n$, where n is the number of samples used to calculate the mean and AA_i is an AA for sample i. The training set used for the LDA was composed of marine producer and bacterial $N(\delta^{13}C_{AA})$ values from Larsen et al. (2009, 2012) where "marine producers" include seagrasses, seaweeds, and microbial mats (mats of cyanobacteria, photosynthetic sulfur bacteria, and Archaea). PCA was conducted on $\delta^{15}N_{AA}$ (Phe, Sr-AA, and Tr-AA), $TP_{Glu\text{-}Phe}$, and $N(\delta^{13}C_{AA})$ values standardized by dividing each AA_i or TP by its standard deviation. Statistics were conducted using R (R Core Team 2012).

Results

Zooplankton biomass—The biomass of zooplankton we collected at Sta. ALOHA in surface waters (0–150 m) ranged from 3.5 to 12.2 mg dry weight (dry wt) m^{-3} (Fig. 1) and on an areal basis from 1.2 (day) to 1.3 g dry wt m^{-2} (night). This is within the range determined for zooplankton in the upper 150 m at Sta. ALOHA by the Hawaii Ocean Time-series program (0.25–2.4 g dry wt m^{-2} from 1996 to 2011; http://hahana.soest.hawaii.edu/hot/hotdogs/). Zooplankton biomass decreased exponentially with depth (Fig. 1), as has been observed in many other regions of the world's oceans (Angel and Baker 1982), and at Sta. ALOHA, was 0.3 mg m^{-3} at 700–1000 m. Steinberg et al. (2008*a*) document very similar biomass values for surface and midwater zooplankton collected with a 1 m^2 MOCNESS system at Sta. ALOHA in July 2004. Over the entire epi- and mesopelagic water column, zooplankton biomass was 1.9 (night) to 2.2 g dry wt m^{-2} (day). Migrant zooplankton biomass in each depth strata was calculated by subtracting day from night biomass values. In the upper 100 m, migrant biomass was 0.17 g dry wt m^{-2} and was negative in all other depth ranges, indicating movement from depth into the upper mesopelagic and surface layers of the water column at night. This diel vertical migration was dominated by 0.5–1.0 and 1.0–2.0 mm zooplankton, which together had a migrant biomass of 0.26 g dry wt m^{-2} in the upper 100 m. Vertical migration was minimal (0.021 g dry wt m^{-2} migrants) in the lower mesopelagic zone at 700–1000 m depth.

Bulk $\delta^{15}N$ values—The $\delta^{15}N$ values of suspended particles and zooplankton clearly increased with depth from the surface ocean through the mesopelagic zone. For suspended particles collected on GF/F filters, $\delta^{15}N$ values increased significantly and rapidly (linear regression, $F_{1,13} = 30.4$, $p < 0.001$) from 0.5‰ ± 0.7‰ in the upper 50 m to 8.7‰ ± 0.9‰ below 125 m depth (Fig. 2a). Zooplankton $\delta^{15}N$ values also increased with depth (Fig. 2b,c). The increase in zooplankton $\delta^{15}N$ values from the epipelagic (0–150 m: 1.4–4.5‰) through the upper mesopelagic (150–300 m: 3.0–5.9‰) and mid to lower mesopelagic (300–1000 m: 2.8–8.9‰) was in most cases significant (analysis of variance [ANOVA], $F_{2,6} = 5.8$–22.8, $p < 0.05$), the exceptions being the 1.0–2.0 mm and 2.0–5.0 mm size fractions during the day (ANOVA, $F_{2,6} = 3.9$–5.1, $p > 0.05$). These significant depth changes in stable N isotope composition were driven by dissimilar epipelagic compared with mid to lower mesopelagic plankton $\delta^{15}N$ values, which differed by 2.4–4.5‰ during the day and by 2.7–4.0‰ at night. Maximum differences in bulk $\delta^{15}N$ values (up to 6.5‰ during the day and 5.6‰ at night) were observed between plankton in surface waters (0–50 m) and plankton at our deepest sampling depth (700–1000 m). Within each depth interval zooplankton stable N isotope compositions also changed with size (Fig. 2b,c). In the upper 300 m, an increase of 1.3 ± 0.3‰ between the smallest size class (0.2–0.5 mm) and the largest size classes (> 2 mm) was observed. Below this depth the size distinction was negligible or even

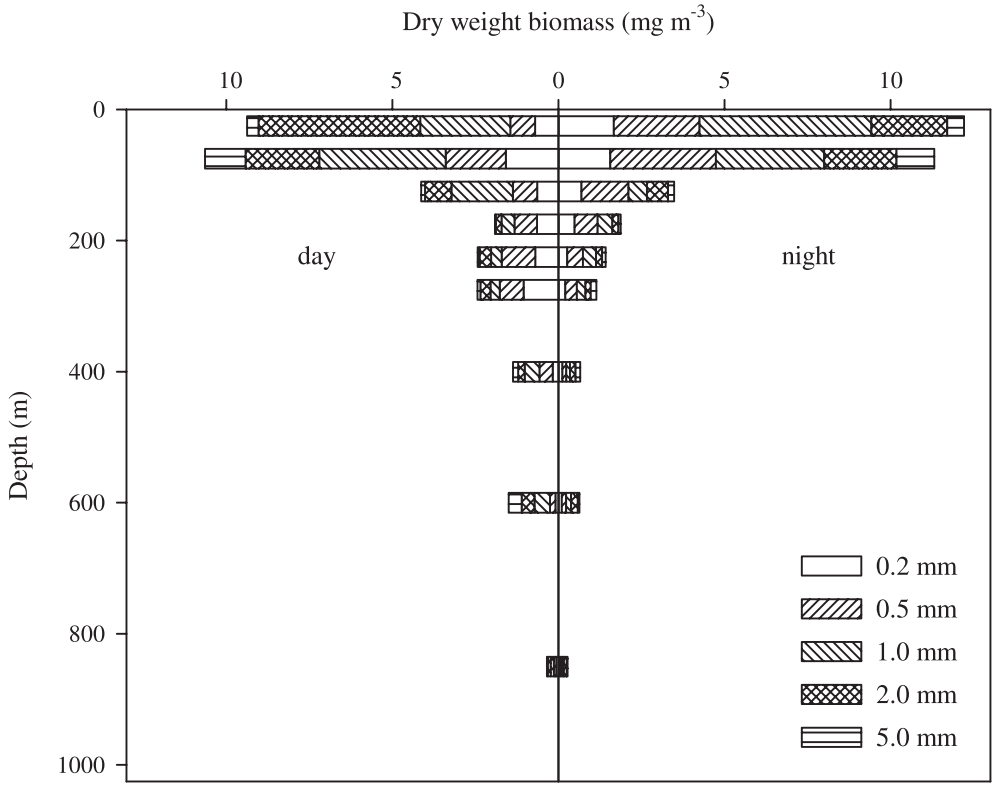

Fig. 1. Day and night size-fractionated zooplankton biomass at Sta. ALOHA.

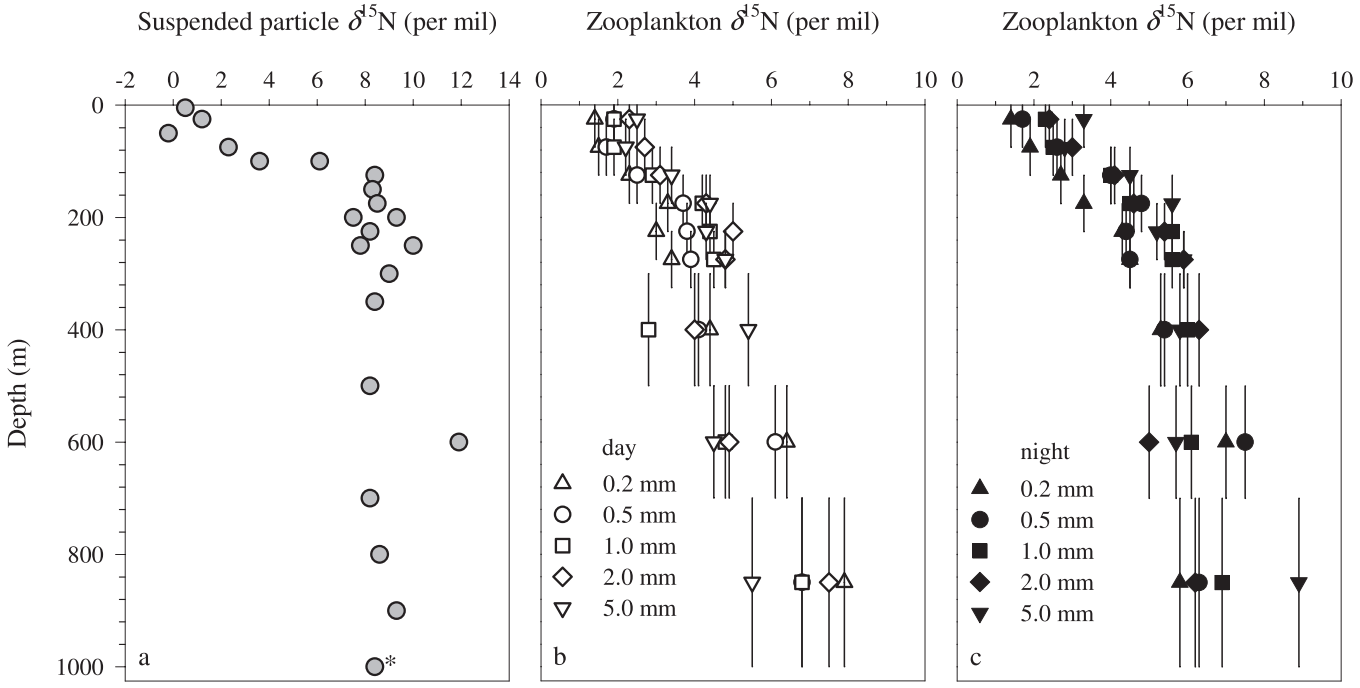

Fig. 2. Plankton and particle stable N isotope composition at Sta. ALOHA in late August 2011. (a) Suspended particle N isotope composition (*δ^{15}N values from 1000–2000 m averaged 8.5‰ ± 0.3‰). (b) Size-fractionated zooplankton δ^{15}N values from collections during the day. (c) Size-fractionated zooplankton δ^{15}N values from nighttime collections. Vertical bars indicate the depth range through which each MOCNESS net sampled.

reversed, particularly in the daytime collections; that is, below 500 m large zooplankton (> 2 mm) $\delta^{15}N$ values were less than small zooplankton (0.2–0.5 mm) $\delta^{15}N$ values by 1.6‰ ± 0.2‰ (except for plankton at 700–1000 m at night, where bulk $\delta^{15}N$ values increased by 1.8‰ in the > 2 mm relative to the 0.2–0.5 mm size class).

AA $\delta^{15}N$ values—Stable N isotopic composition of AAs in zooplankton ranged from −18.0‰ to 21.3‰, and in suspended particles ranged from −4.9‰ to 17.8‰ (data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5fn76). Overall source AAs, such as Phe, had significantly lower $\delta^{15}N_{AA}$ values (zooplankton mean $\delta^{15}N_{Sr\text{-}AA}$ value: 2.2‰) than trophic AAs, such as Glu (zooplankton mean $\delta^{15}N_{Tr\text{-}AA}$ value: 12.8‰; *t*-test, t = 21.5, degrees of freedom [df] = 334, $p < 0.001$). At each depth interval examined, zooplankton $\delta^{15}N_{AA}$ values differed between the different size classes (ANOVA, $F_{3,10}$ = 167.1–4.2, $p < 0.05$). For source AAs, these size class differences were relatively small (e.g., the difference in $\delta^{15}N_{Sr\text{-}AA}$ value between the smallest [0.2–0.5 mm] and largest [2.0–5.0 mm] size classes averaged −0.19‰; range, −1.8‰ to 0.69‰). Size class differences for trophic AAs were generally larger than was observed for source AAs (e.g., differences in $\delta^{15}N_{Tr\text{-}AA}$ values between the largest and smallest zooplankton size class averaged 1.7‰ [range, −0.17‰ to 3.8‰]). The "canonical" source AA Phe was the only AA in which significant size class differences in stable N isotope composition were not observed. $\delta^{15}N_{Phe}$ values were similar across all size classes at all depths (ANOVA, $F_{3,10}$ = 0.7–3.2, $p > 0.05$), with the exception of one comparison (0.2 vs. 0.5 mm $\delta^{15}N_{Phe}$ value at 50–100 m during the day; pairwise *t*-test, t = 7.1, df = 4, $p < 0.05$). This finding indicates that, within each depth interval, food web N sources were similar for all zooplankton size fractions.

Depth changes in $\delta^{15}N_{AA}$ values of source and trophic AAs matched the pattern seen in bulk zooplankton $\delta^{15}N$ values, with significant ^{15}N enrichment in AAs at midwater depths. For zooplankton collected during the day, $\delta^{15}N_{Sr\text{-}AA}$, $\delta^{15}N_{Tr\text{-}AA}$, and $\delta^{15}N_{Phe}$ values increased through all depths (50–100 m vs. 300–500 m vs. 700–1000 m; Fig. 3a–c; ANOVA, $F_{2,7}$ = 44.0 to $F_{2,6}$ = 1442.6, $p <$ 0.05), with the exception that $\delta^{15}N_{Phe}$ at 75 m was similar to that at 400 m in 1.0–2.0 mm zooplankton (pairwise *t*-test, t = 3.3, df = 4, $p > 0.05$). Averaged over all size fractions, the difference in daytime zooplankton $\delta^{15}N_{Phe}$ values between the epipelagic (50–100 m) and lower mesopelagic (700–1000 m) was 3.5‰ ± 0.3‰ (Table 1). For zooplankton collected at night, $\delta^{15}N_{Sr\text{-}AA}$, $\delta^{15}N_{Tr\text{-}AA}$, and $\delta^{15}N_{Phe}$ values increased significantly from surface waters to mesopelagic depths (Fig. 3a–c; ANOVA, $F_{2,7}$ = 12.2–1304.9, $p < 0.05$), but $\delta^{15}N_{AA}$ values at mid mesopelagic (300–500 m) and lower mesopelagic depths were similar (e.g., mid mesopelagic = lower mesopelagic $\delta^{15}N_{Phe}$ for 0.2, 0.5, and 1.0 mm zooplankton; pairwise *t*-test, t = 1.8, df = 6, $p > 0.05$ to t = 2.0, df = 5, $p >$ 0.05). Averaged over all size fractions, the difference in zooplankton $\delta^{15}N_{Phe}$ values between the epipelagic and lower mesopelagic at night was 2.6‰ ± 0.3‰ (Table 1).

Suspended particle AAs also became ^{15}N enriched with depth. Significant differences were observed for all $\delta^{15}N_{AA}$ values between all depths (25 vs. 75 vs. 400 vs. 750 m; ANOVA, $F_{3,10}$ = 7.1–2192.0, $p < 0.01$ for all AAs), with the exception of Leu, Ile, and Asp (400 = 750 m) and Thr (25 = 75 m, 25 = 750 m, and 75 = 750 m; pairwise *t*-tests, t = 0.9, df = 6, $p > 0.05$ to t = 2.4, df = 5, $p > 0.05$). The change in suspended particle $\delta^{15}N_{AA}$ values between the surface and midwaters was large for all AAs (except Thr). For example, the difference in suspended particle $\delta^{15}N_{Sr\text{-}AA}$, $\delta^{15}N_{Tr\text{-}AA}$, and $\delta^{15}N_{Phe}$ values between the surface (25 m) and lower mesopelagic depths (750 m) was 11.5‰, 10.4‰, and 9.7‰, respectively (Fig. 3a–c).

Trophic position—Overall, zooplankton trophic position ranged from 2.1 to 3.1 (for $TP_{Glu\text{-}Phe}$) and from 1.7 to 2.8 (for $TP_{Tr\text{-}Sr}$); thus, zooplankton in the NPSG can be considered primary to secondary consumers (Fig. 3d). Both $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ varied by size fraction; however, when considering propagation of error (Dale et al. 2011) and the resulting TP standard deviations, size differences were not found to be significant for either $TP_{Glu\text{-}Phe}$ or $TP_{Tr\text{-}Sr}$ (i.e., TP were within 2 SD of each other).

Zooplankton TP increased with depth (Fig. 3d), with significant differences in $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ observed between epipelagic zooplankton (50–100 m) and mesopelagic zooplankton (300–1000 m) during both the day and night (*t*-test, t = 3.4 and 4.4, df = 10, $p < 0.01$). The difference in $TP_{Glu\text{-}Phe}$ between epipelagic zooplankton and those in the lower mesopelagic zone (700–1000 m) was on the order of 0.65 ± 0.13 during the day and 0.37 ± 0.13 at night (Table 1). Within the mesopelagic zone, zooplankton $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ increased significantly between the two depth intervals studied (300–500 m and 700–1000 m), but only during the day (*t*-test, t = 5.1, df = 6, $p < 0.05$). This $TP_{Glu\text{-}Phe}$ difference was on the order of 0.37 ± 0.13. At night no significant differences were detected in $TP_{Glu\text{-}Phe}$ or $TP_{Tr\text{-}Sr}$ within the mesopelagic zone. In stark contrast to the trends observed for zooplankton, the trophic status of suspended particles as measured by $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ did not change significantly with depth (Fig. 3d; *t*-test, t = 1.0, df = 2, $p > 0.05$). Suspended particle $TP_{Glu\text{-}Phe}$ averaged 1.6 ± 0.07 throughout the water column from 0 to 1000 m.

AA $\delta^{13}C$ values—The stable C isotopic composition of AAs in zooplankton ranged from −28.3‰ to −2.9‰, and in suspended particles ranged from −28.1‰ to −0.7‰ (data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5fn76). EAAs were significantly depleted in ^{13}C (mean zooplankton $\delta^{13}C_{EAA}$ value: −20.8‰) compared with nonessential AAs (mean zooplankton $\delta^{13}C_{NAA}$ value: −15.0‰; *t*-test, t = 6.04, df = 110, $p < 0.001$). For zooplankton, depth changes in $\delta^{13}C_{AA}$ values were significant for all AAs (ANOVA, $F_{2,17}$ = 5.0–32.6, $p < 0.05$) except Ile, Val, and Tyr, but consistent trends in $\delta^{13}C_{AA}$ values with depth were not found. For suspended particles, depth changes in $\delta^{13}C_{AA}$ values were also significant (ANOVA, $F_{3,11}$ = 13.7–138.1, $p < 0.001$) with $\delta^{13}C_{EAA}$ and $\delta^{13}C_{NAA}$ values tending to increase from

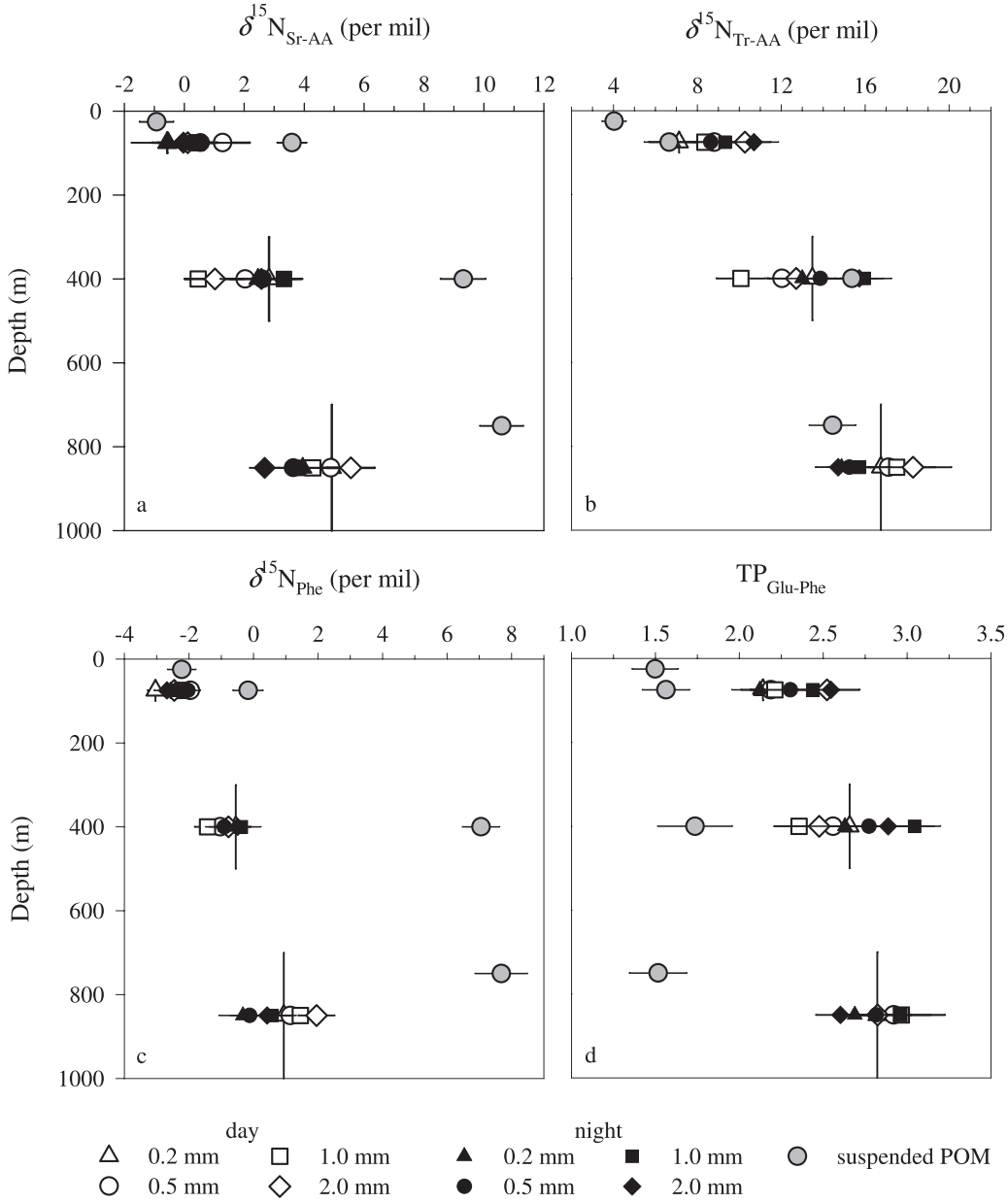

Fig. 3. Stable N isotope composition of "source" and "trophic" AAs (Sr-AA and Tr-AA), the canonical source AA phenylalanine (Phe), and trophic positions of zooplankton and suspended particles at Sta. ALOHA. (a) $\delta^{15}N_{Sr\text{-}AA}$ values for different size fractions of zooplankton collected during the day and night and for suspended particles. (b) $\delta^{15}N_{Tr\text{-}AA}$ values of zooplankton and suspended particles. (c) $\delta^{15}N_{Phe}$ values of zooplankton and suspended particles. (d) The trophic position of zooplankton and suspended particles, calculated using Phe and glutamic acid (Glu; $TP_{Glu\text{-}Phe}$). Vertical bars indicate the depth range through which each MOCNESS net sampled.

the surface (25–75 m) to mesopelagic depths (400–750 m) by 3.2‰ (range, 0.8–8.6‰) and 3.4‰ (range, 1.9–7.8‰), respectively (except for $\delta^{13}C_{Lys}$ and $\delta^{13}C_{Val}$ values, which decreased by 0.1− and 1.1‰, respectively).

DI index and ΣV values—Values of the AA mol%-based DI index for suspended particles decreased significantly from 0.27 ± 0.02 in surface waters to −0.27 ± 0.12 in the mesopelagic zone (*t*-test, $t = 6.6$, df = 2, $p < 0.05$). Values for the ΣV parameter for suspended particles varied from 0.8–1.6 at the four measured depths, or 1.2 ± 0.3 over the entire water column (0–1000 m). No significant trends in ΣV with depth were observed.

Multivariate analyses—LDA of essential AA $\delta^{13}C$ values distinguished between marine producers and bacteria with > 99.99% certainty, when conducted on a training set derived from Larsen et al. (2009, 2012). Applied to our samples, the LDA classified all suspended particles and zooplankton as marine producers with > 99.99% certainty (Fig. 4). This indicates that marine producers are the main source of EAA to suspended detrital OM pools and to

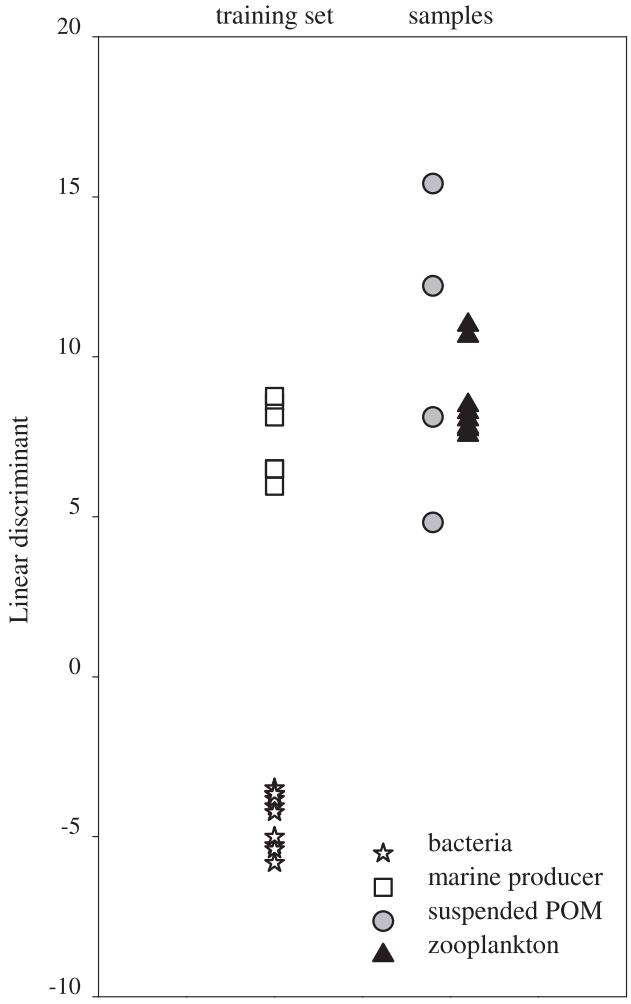

Fig. 4. Linear discriminant analysis (LDA) based on a training set built with normalized essential AA $\delta^{13}C$ values from marine producer and bacteria from Larsen et al. (2009, 2012). Suspended POM and zooplankton from surface and midwater depths at Sta. ALOHA were classified as marine producers with > 99.99% probability.

consumers in the surface and midwaters of the NPSG. When bacterial $\delta^{13}C_{EAA}$ data from Scott et al. (2006) was added to the analysis, this extended training set could only distinguish between marine producers and bacteria with 96% and 67% certainty, respectively, and thus was a poor predictor of EAA origin. Issues with this extended analysis likely included the diversity of bacterial metabolisms included in the extended LDA and the fact that not all EAAs (i.e., Met) could be included in the analysis.

PCA of suspended particle and zooplankton $\delta^{15}N_{AA}$, $TP_{Glu\text{-}Phe}$, and $N(\delta^{13}C_{EAA})$ values yielded two significant principal components (PC1 and PC2; based on the broken stick model), which together explained 70.1% of the variance in the data (Fig. 5a). Along PC1, suspended particles at 400 and 750 m depth differed from suspended particles in the euphotic zone and zooplankton at all depths (Fig. 5b). The variables driving PC1 were primarily $\delta^{15}N_{Phe}$, $\delta^{15}N_{Sr\text{-}AA}$, $N(\delta^{13}C_{Met})$, and $N(\delta^{13}C_{Thr})$, which tended to increase in $\delta^{15}N$ or $\delta^{13}C$ value with depth. PC2 distinguished between small, epipelagic zooplankton and all suspended particles vs. larger or mesopelagic zooplankton. This axis was driven primarily by variation in $TP_{Glu\text{-}Phe}$, $\delta^{15}N_{Tr\text{-}AA}$, $N(\delta^{13}C_{Ile})$, and $N(\delta^{13}C_{Leu})$, with values of zooplankton $TP_{Glu\text{-}Phe}$, $\delta^{15}N_{Tr\text{-}AA}$, and $N(\delta^{13}C_{Ile})$ increasing with depth and $N(\delta^{13}C_{Leu})$ decreasing with depth. Cluster analysis of the data defined three groups: (1) suspended particles at 25 m and all zooplankton, (2) suspended particles at 75 m, and (3) suspended particles at 400 and 850 m.

Discussion

Depth changes in suspended particle stable isotope compositions—Bulk $\delta^{15}N$ values increased by more than 8‰ between the euphotic zone and midwater depths at Sta. ALOHA, with the majority of this change occurring in the upper 150 m. Similar rapid changes in suspended particle $\delta^{15}N$ values with depth have been observed in the Indian Ocean (~ 10‰; Saino and Hattori 1980), the North Atlantic Ocean (~ 7‰; Altabet et al. 1991), and the Mediterranean Sea (~ 6–7‰; Emeis et al. 2010) and were documented in 2004 at Sta. ALOHA by Casciotti et al. (2008; ~ 6‰). This isotopic transformation is accompanied by changes in particle composition. Surface water POM is dominated by microplankton populations, whereas particles in midwaters tend to include amorphous detrital material as well as diatom frustules and coccoliths (Bishop et al. 1977; Gowing and Wishner 1992).

The apparently global phenomenon of increasing suspended particle $\delta^{15}N$ value with depth could result from three factors: (1) inclusion of material of a higher trophic status in the midwater suspended particle pool, (2) fractionation associated with the heterotrophic degradation of suspended particles, or (3) a change in the N source contributing to suspended POM at depth. Here we evaluate the mechanistic basis for change in suspended particle $\delta^{15}N$ values for the first time by characterizing the isotope composition of POM at the molecular level. Our CSIA indicates the stable N isotope composition of suspended particle source and trophic AAs paralleled the change in bulk values with depth, with $\delta^{15}N_{AA}$ values increasing by up to 14‰ between the surface and lower mesopelagic zone. However, at the same time, suspended particle $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ remained constant. Thus, it is not likely that inclusion of high-TP material in the suspended particle pool (or some other mechanism driving a change in trophic status) is responsible for the ^{15}N enrichment of midwater suspended particles in the NPSG.

Isotope fractionation associated with heterotrophic degradation could also drive change in suspended particle $\delta^{15}N$ values. Saino and Hattori (1980, 1987) and Altabet and McCarthy (1986) both attributed ^{15}N enrichment of deep-water suspended particles to fractionation associated with the oxidative degradation of particulate N, based on their observations of parallel increases in particle $\delta^{15}N$ values, NO_3^- concentration, apparent O_2 utilization, and particle C : N ratios. To further investigate isotope fractionation as a possible driver of ^{15}N enrichment in the midwater suspended particle pool, we applied a Rayleigh

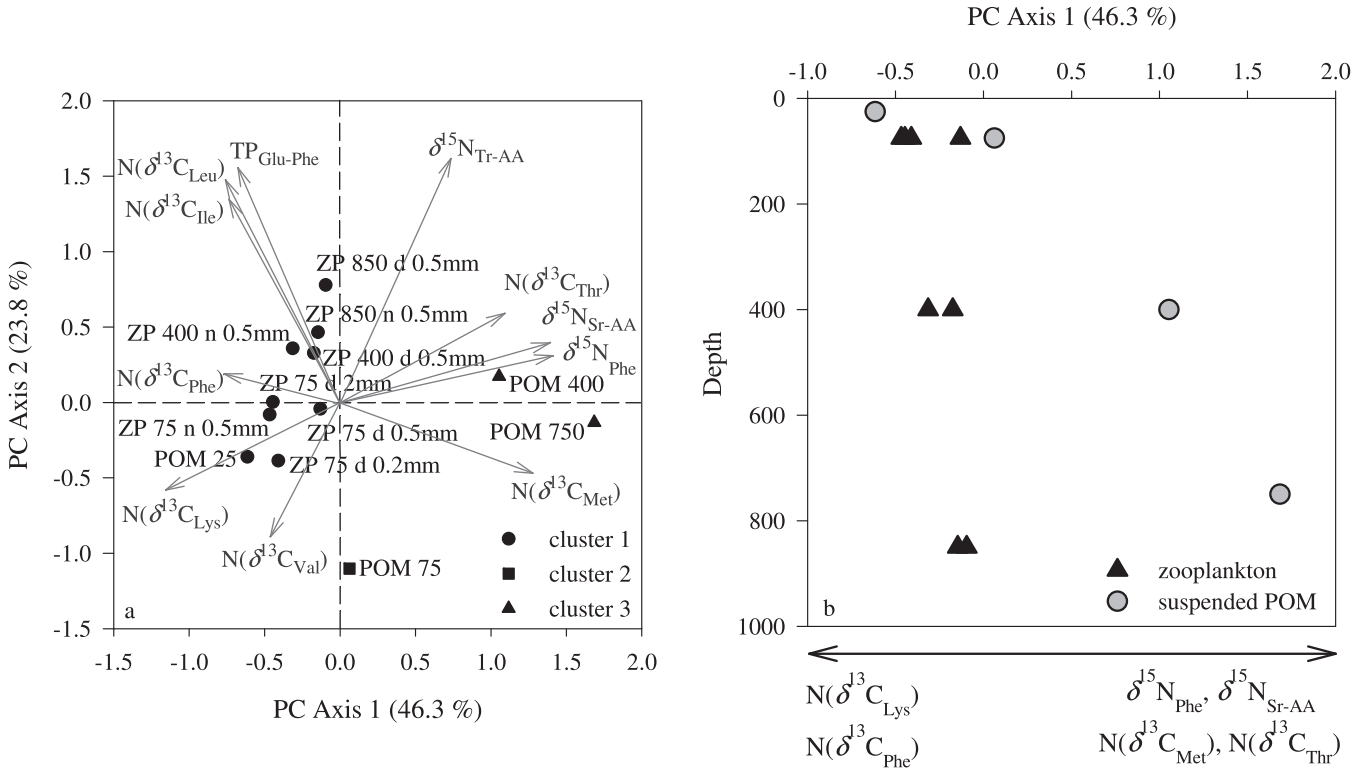

Fig. 5. Principal components analysis (PCA) of suspended particle and zooplankton $\delta^{15}N_{AA}$, normalized $\delta^{13}C_{AA}$, and trophic position (TP) values at Sta. ALOHA. (a) Biplot of the first two PCA axes (PC1 and PC2) showing site scores (black circles, squares, and triangles) and variable loadings (grey arrows). Site scores are labeled as "POM–depth" for suspended particles or "ZP–median of the depth interval–time (d = day, n = night)–size" for zooplankton. The three groups resulting from cluster analysis are shown. (b) Change in zooplankton and suspended particle PC1 scores with depth.

fractionation model (Mariotti et al. 1981) to our observed depth increase in particle $\delta^{15}N_{AA}$ values, as $\delta^{15}N_{AAi(d)} = \delta^{15}N_{AAi(s)} - \varepsilon_{AAi} \times \{\ln(f)\}$, where $\delta^{15}N_{AAi(d)}$ is the $\delta^{15}N$ value of AA sample i at depth d, $\delta^{15}N_{AAi(s)}$ is the $\delta^{15}N$ value of AA sample i at the surface (25 m), f is the fraction of AA sample i remaining at depth, and ε_{AAi} is the kinetic isotope fractionation associated with the particle becoming more protein-poor (here a decrease in particle ng AA [mg C]$^{-1}$). Significant isotope fractionation (ε_{AA}) was measured for five AAs (Asp, Glu, Ile, Phe, and Ser; linear regression, $p < 0.05$), with a mean ε_{AA} (± SEM) of 5.5 ± 0.5‰ determined using analysis of covariance (ANCOVA; $p < 0.001$). This ε_{AA} value is similar to that measured by Gaebler et al. (1966) for deamination (i.e., the removal of NH_2 from AAs) and to ε values measured by Silfer et al. (1992) for protein hydrolysis (ε = 2.5–4‰). Both processes likely drive protein degradation at depth. Transamination, a related mechanism involving the transfer of NH_2 groups between AAs, has also been observed to occur with an ε value of 1.7–8.3‰ (Macko et al. 1986). Thus, the preferential cleavage of ^{14}N-C bonds in proteins during bacterial degradation or with other heterotrophic activity could contribute to the ^{15}N enrichment observed for residual AA within midwater suspended particle pools. It is interesting to note that when less significant trends in AA isotope composition are included in the model (i.e., Gly, Leu, and Pro; linear regression, $p < 0.1$), the mean ε_{AA} is still 5.5 ± 0.4‰ (ANCOVA, $p < 0.001$). The only AA exhibiting a significantly lower kinetic isotope effect was Lys (ε_{AA} = 2.1‰; multiple linear regression, $p < 0.05$), which is also the only AA we modeled with two amino groups. Lys would be expected to have a relatively low ε_{AA} if a residual $^{14}NH_2$ group remained intact at the ε-C after protein hydrolysis (i.e., isotope mass balance requires that ε_{AA} values would be lower if only one of two ^{14}N-C bonds were broken).

Previous studies have found degradation of POM to be accompanied by compositional changes as AAs associated with labile cellular material (e.g., Glu) are removed and AAs associated with more refractory calcareous and siliceous shell material remain (e.g., Asp and Gly; Lee et al. 2000; Sheridan et al. 2002). In fact, our increase in suspended particle $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Sr\text{-}AA}$ values with depth paralleled decreases in mol% Tyr (linear regression, $p < 0.01$), a primary driver of change in AA degradation state (Dauwe et al. 1999). More significantly, DI values decrease between surface waters and the mesopelagic zone, indicating that midwater suspended particle OM is more degraded and less labile than OM found in surface water particles. This decrease in DI values was accompanied by increases in particle $\delta^{15}N_{Phe}$, $\delta^{15}N_{Sr\text{-}AA}$, and $\delta^{15}N_{Tr\text{-}AA}$ values at depth (linear regression, $F_{1,2}$ = 7.0–27.7, p = 0.05, 0.12, 0.03, respectively). Degradation of suspended particle proteins could therefore drive both organic compositional changes and changes in N isotopic composition. Overall, our data indicate that all modeled AAs (except Lys) fractionate in a

similar manner regardless of their usual contribution to degradation state, whether they are considered source or trophic AAs, or considered essential or nonessential. ^{15}N enrichment of suspended POM at depth thus does not appear to be caused by the removal of specific ^{15}N-depleted AAs.

Source AAs such as Phe clearly become dramatically ^{15}N enriched in midwater suspended particle pools; however it is most likely not a change in the N source contributing to suspended POM that drives this enrichment. Deep-water NO_3^- is enriched in ^{15}N, with $\delta^{15}N$ values of 5–7‰ (Casciotti et al. 2008), yet it is not clear how this nutrient source would be accessed to produce a significant pool of suspended OM at depth. Our LDA of $\delta^{13}C_{EAA}$ values does not indicate that bacteria provide a significant source of organic material to suspended particles in the mesopelagic zone. Archaea are another important contributor to midwater microbial populations (Karner et al. 2001), and we cannot rule out an archaeal contribution to suspended POM because our LDA training set included Archaea-containing microbial mats in the marine producer category (Larsen et al. 2012). Moreover suspended particle $\delta^{15}N$ values increase between 100 and 150 m depth at Sta. ALOHA, the depth interval at which Archaea populations start to become prominent (Karner et al. 2001) and where microbial (potentially archaeal) nitrification rates are maximal (Dore and Karl 1996). Although deep-water Archaea appear to be metabolically active (based on rRNA hybridization studies), the mode of their metabolism is still poorly understood (DeLong and Karl 2005). Some are autotrophic ammonia oxidizers (Konneke et al. 2005; Beman et al. 2008), but others may be heterotrophic or mixotrophic (Herndl et al. 2005; Ingalls et al. 2006); for example Archaea appear to utilize dissolved amino acids (Ouverney and Fuhrman 2000) or urea (Alonso-Saez et al. 2012). Regardless, it is not clear how these nutritional modes would result in a significant pool of ^{15}N-enriched material at depth or how Archaea would produce sufficient biomass to dominate suspended POM given their relatively small size (e.g., 0.2 μm cell width and 0.5–0.9 μm cell length; Konneke et al. 2005) and the potential for many cells to pass through our GF/F filters (nominal pore size, 0.7 μm; Altabet 1990). While elucidating metabolic pathways involving deep-water Archaea remains an important research goal, our findings suggest a change in N source might not be a significant contributor to observed depth trends in suspended particle isotope composition.

In addition to the trends we observed in $\delta^{15}N_{AA}$ values, the stable C isotope composition of AAs in suspended particles also changed with depth, with $\delta^{13}C_{AA}$ values increasing by 3.3‰, on average, between the surface ocean and the mesopelagic zone. In the Mediterranean, $\delta^{13}C$ values have been observed to increase with particle size (Rau et al. 1990), and this was attributed to increased TP (and therefore modest ^{13}C enrichment) in the larger suspended particles. However, we did not find the $TP_{Glu\text{-}Phe}$ or $TP_{Tr\text{-}Sr}$ of suspended particles to change with depth; thus, a change in trophic status is not likely responsible for ^{13}C enrichment of suspended particles in the mesopelagic zone. Instead, it is more likely that alteration of proteinaceous material in midwater suspended particles explains this trend because C isotope fractionation has been observed during peptide bond hydrolysis (Silfer et al. 1992). Thus, ^{12}C-containing bonds could be preferentially hydrolyzed and ^{13}C-enriched material remain in suspended pools at depth. In summary, given the changes we have observed in $\delta^{15}N_{AA}$ and $\delta^{13}C_{AA}$ values and our results concerning kinetic isotope effects and OM degradation state, it is likely that fractionation associated with the heterotrophic degradation of suspended particles is a major driver of change in particulate stable isotope composition with depth.

Suspended particle degradation in midwaters—Suspended particles are a key component of the marine OM size–reactivity continuum (Amon and Benner 1996) and may, through precursor–product relationships (Walker and McCarthy 2012) act as a conduit for degradation pathways between large particles and dissolved organic matter (DOM). Such pathways likely drive the reduction of large particle fluxes in midwaters and the formation of recalcitrant DOM, a major repository of fixed C in the world's oceans. Additionally, small, slowly settling particles were recently found to be a significant component of the total sinking flux (Alonso-Gonzalez et al. 2010) and likely overlap in size and composition with the suspended particles measured in our study. Thus, our investigation of the drivers of change in suspended particle degradation state and isotope composition has major implications in regard to particle cycling and carbon sequestration in deep waters.

To advance our knowledge of midwater particle dynamics, we can investigate the fractionating mechanisms controlling suspended particle isotope composition. We have focused on protein hydrolysis as a dominant driver of change in suspended particle $\delta^{15}N$ values, with this mechanism a key step in degradation processes including OM remineralization and the selective removal of labile components such as AAs. However "degradation" also encompasses the transformation of OM through incorporation of new molecules, for example, those resynthesized by bacteria (McCarthy et al. 2007). Calleja et al. (2013) observed that the incorporation of bacterial biomass or degradation products into high molecular weight DOM (HMWDOM), as evidenced by changes in enantiomeric (*D* and *L* isomer) AA ratios, is accompanied by an increase in $\delta^{15}N$ value of 3–6‰. This shift in isotope composition was demonstrated to occur primarily through the bacterial resynthesis of proteins. Thus, it may be possible that a similar mechanism (incorporation of bacterial degradation products) drives ^{15}N enrichment in the suspended particle pool at depth, particularly because the fractionation we measured for midwater suspended particles ($\varepsilon_{AA} = 5.5$‰) is of the same magnitude as that observed by Calleja et al. (2013) for HMWDOM.

Complimentary OM degradation proxies can help resolve the effect of different mechanisms in altering the midwater suspended particle pool. As discussed above, suspended particle DI values in the NPSG decreased with depth, indicating the midwater pool to be more degraded than fresh, surface ocean particles. However at the same

time, ΣV, a measure of heterotrophic AA resynthesis, remained low and stable with depth. Compared with our observed value of ~ 1.2, ΣV for sinking particles in the equatorial Pacific has been shown to range from 1.4 to 3.4 (McCarthy et al. 2007) and ΣV in degraded HMWDOM to range from 3.0 to 3.7 (Calleja et al. 2013). Although ΣV might not be "linear" with degradation, our observed lack of change in this parameter indicates bacterial resynthesis might not be an important suspended particle degradation mechanism. Moreover LDA of our suspended particle $\delta^{13}C_{AA}$ values indicates the major source of OM to this pool is marine producers rather than bacteria. This application relies on unique biosynthetic patterns in C isotope composition that are conserved, for example, with trophic transfers between producers and consumers. Although the LDA approach is a new technique and should be further developed with a larger training database of marine producer and bacterial $\delta^{13}C_{AA}$ values, our results to date further suggest incorporation of bacterial biomass or degradation products (e.g., cell wall remnants or recalcitrant cellular proteins) might not be the dominant driver of ^{15}N enrichment in midwater suspended particles.

In summary, our findings indicate that isotope fractionation associated with heterotrophic degradation, likely driven by a hydrolytic mechanism, controls midwater suspended particle $\delta^{15}N_{AA}$ and $\delta^{13}C_{AA}$ values. However our interpretation of observed isotopic changes must be reconciled with previous research on organic biomarker compositions. For example, research on enantiomeric AA ratios and concentrations of muramic acid, a peptidoglycan component, found suspended particulate N to be composed of ~ 50% bacterial OM in subtropical gyres (Kaiser and Benner 2008). More recent work indicates this percentage increases in the mesopelagic zone (Kaiser and Benner 2012), although the contribution of living cells to the "bacterially derived" OM at these depths may be minimal (Kawasaki et al. 2011). Bacterial branched chain fatty acids and β-alanine, a microbial degradation product, also increase with depth in midwater suspended particle pools (Lee et al. 2000; Sheridan et al. 2002). Furthermore, correlations between microbial respiratory activity and particulate organic carbon concentration in the meso- and bathypelagic zone of the subtropical North Atlantic (Baltar et al. 2009) supports a major role for marine microbes in degrading suspended particles and potentially contributing degradation-resistant (Amon et al. 2001) bacterially sourced OM. One possible resolution of our findings with these studies could be that cyanobacteria provide a marine producer source of suspended OM that is resistant to degradation and accumulates in the suspended particle pool, similar to the "cyanobacterial shunt" identified by McCarthy et al. (2004) for DOM. Ultimately a holistic approach, evaluating compound-specific isotope and organic biomarker compositions together with midwater metabolic activity, may be necessary to resolve fully the suspended particle degradation pathways at a mechanistic level.

Depth changes in zooplankton stable isotope compositions—Surface and midwater zooplankton communities at Sta. ALOHA are a diverse assemblage dominated by calanoid and poecilostomatoid copepods, with an increasing contribution of harpacticoid copepods at depth (Steinberg et al. 2008*a*). Despite such relatively modest changes in plankton composition, we found bulk zooplankton $\delta^{15}N$ values at Sta. ALOHA to increase by 4.6‰ (range, 3.2–6.4‰) between the surface waters and the lower mesopelagic zone. This depth increase is on the same order of magnitude as that observed in other regions of the world's oceans. In the Mediterranean Sea, Koppelmann et al. (2003) observed an increase of 2–2.9‰ between the surface waters and 1000 m, and in the Arabian Sea, Koppelmann and Weikert (2000) observed increases of 1.8–4.3‰ between the surface ocean and mesopelagic zone. Between 0–250 m and 250–500 m in April 1997, Koppelmann and Weikert (2000) actually observed a decrease of 3.5–4.8‰ in zooplankton $\delta^{15}N$ values in the Arabian Sea, and this phenomenon was attributed to the input of ^{15}N-depleted material derived from the N_2-fixing cyanobacteria *Trichodesmium* spp. However, for the most part, zooplankton $\delta^{15}N$ values have been observed to increase with depth, with this trend attributed mainly to increasing food chain length (Koppelmann et al. 2003).

Our results from CSIA demonstrate that zooplankton $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Sr\text{-}AA}$ values increased from surface waters through the lower mesopelagic zone by up to 3.5‰ and 4.9‰, respectively. Because the $\delta^{15}N$ value of source AAs such as Phe changes little with trophic transfers (McClelland and Montoya 2002), the increase in these values indicates that food resources at the base of the zooplankton food web become ^{15}N enriched with depth. Some studies have suggested the forage base for zooplankton is suspended particles; for example, Koppelmann et al. (2009) link high $\delta^{15}N$ values in the bathypelagic copepod *Lucicutia longiserrata* to feeding on ^{15}N-enriched suspended particles. However Steinberg et al. (2008*b*) observe that suspended particle C is insufficient to sustain the metabolic demands of zooplankton in the mesopelagic zone at Sta. ALOHA. Our results support this latter argument, as suspended particle $\delta^{15}N_{Phe}$ values are 6–8‰ higher than zooplankton $\delta^{15}N_{Phe}$ in midwaters, indicating a significant disconnect between the dynamics of zooplankton and suspended particles at depth. Results from our PCA also imply that midwater zooplankton feed primarily on surface-derived material, not on the relatively protein-poor suspended particles found at depth. Thus, we hypothesize that the relatively low ^{15}N contents of source AAs in mesopelagic zooplankton most likely originate from surface-derived sinking particles or from vertical migration and feeding in surface waters at night. These source AAs become ^{15}N enriched with depth, indicating feeding on more degraded (and therefore isotopically altered) sinking particles in the lower mesopelagic zone or feeding at least in part on ^{15}N-enriched suspended particles in deeper waters.

While changes in stable N isotope composition at the base of the food web clearly drive much of the depth variability in bulk zooplankton $\delta^{15}N$ values, plankton $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ also increase by up to 0.6 from surface waters through the lower mesopelagic zone. At the surface, zooplankton $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ ranged from 2.1

to 2.6 and 1.7 to 2.3, respectively. Low $TP_{Tr\text{-}Sr}$ values ($<$ 2.0) were not statistically different from 2.0 (within 2 SD), indicating that surface ocean zooplankton are herbivorous or omnivorous. In the mesopelagic zone, zooplankton $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$ increased to 2.4–3.0 and 2.1–2.8. Thus, food chain length could increase in zooplankton communities at depth, as suggested by Koppelmann et al. (2003). In fact carnivory as an important mode of deep-water zooplankton nutrition was indicated in early studies (Vinogradov and Tseitlin 1983) and is supported by research showing "carnivore layers" consisting of predatory amphipods, chaetognaths, and gelatinous zooplankton in the mesopelagic zone (Steinberg et al. 2008*a*). However, the increase in TP could also occur if there was a shift in the trophic status of particles at the base of the food web in midwaters. As we observed above, zooplankton in the mesopelagic zone likely feed on particles sinking rapidly from surface waters. McCarthy et al. (2007) indicate that such sinking particles can have a higher trophic status than plankton in the surface ocean. Using their $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values (McCarthy et al. 2007), sinking particle $TP_{Glu\text{-}Phe}$ at 2°N in the equatorial Pacific differed by 0.5 compared with 26–850 μm plankton collected at the surface, and by 1.4 compared with a bloom of the phytoplankton *Rhizosolenia* spp. Thus, although an increase in TP clearly contributes to the trends seen in bulk zooplankton $\delta^{15}N$ values with depth, either increased carnivory in midwater zooplankton or a shift in the trophic status of their forage base, or both, could contribute to the observed changes in zooplankton $TP_{Glu\text{-}Phe}$ and $TP_{Tr\text{-}Sr}$.

Our CSIA of zooplankton at Sta. ALOHA thus indicates that two main processes likely drive observed distributions of $\delta^{15}N_{Phe}$ and $TP_{Glu\text{-}Phe}$. On the one hand, resident zooplankton in midwaters could feed carnivorously on vertically migrating plankton, which would deliver ^{15}N-depleted, surface-derived N into midwaters during the day. Alternatively, resident zooplankton could feed on sinking particles, which are significantly ^{15}N depleted compared with the suspended material (Casciotti et al. 2008), especially during the summer at Sta. ALOHA (Karl et al. 2012). To further investigate these mechanisms, it is instructive to compare the dynamics of zooplankton populations at 300–500 m to those at 700–1000 m. At 300–500 m in the NPSG, zooplankton populations include a significant proportion (0.7 mg dry wt m^{-3}) of vertical migrants (Hannides et al. 2009*a*). These migrators include calanoid copepods, ostracods, euphausiids, and decapods (Al-Mutairi and Landry 2001; Steinberg et al. 2008*a*). We can derive a $\delta^{15}N_{Phe}$ value for the migrant zooplankton assemblage of $-1.4‰ \pm 0.3‰$ using a mass balance approach, which is not statistically different from (within 2 SD) $\delta^{15}N_{Phe}$ values for night-collected resident zooplankton ($-0.6‰ \pm 0.2‰$) in this depth interval. Thus, vertically migrating zooplankton could be a significant carrier of ^{15}N-depleted, surface-derived material to biological communities residing in the upper and mid mesopelagic zone. However at 700–1000 m, zooplankton $\delta^{15}N_{Phe}$ values are significantly higher (0.3–1.3‰) than those calculated for migrant zooplankton, indicating that diel vertical migration does not provide a significant source of ^{15}N-depleted material to the lower mesopelagic zone. Instead, sinking particles that are slightly elevated in $TP_{Glu\text{-}Phe}$ compared with surface ocean particulate matter likely dominate the delivery of surface-derived N at these depths. This finding is corroborated by our measurement of migrant biomass, which was very low at 700–1000 m. Previous studies have also found diel vertical migration to be negligible in the lower mesopelagic zone (Angel et al. 1982). Our conclusions are further supported by research on fatty acid biomarkers at Sta. ALOHA (Wilson et al. 2010), which has shown that both indicators of carnivory (e.g., oleic acid) and indicators of particle feeding (e.g., % MUFA) increase with depth in zooplankton at Sta. ALOHA. We conclude that both carnivory and consumption of sinking particles drive observed trends in mesopelagic zooplankton $\delta^{15}N_{AA}$ values, with consumption of sinking material becoming more important to midwater plankton communities with depth.

Synthesis—Values of $\delta^{15}N_{Phe}$ for zooplankton and suspended particles differed by 7.3–8.3‰ at 300–500 m and by 5.5–7.8‰ at 700–1000 m, indicating a significant disconnect in the dynamics of these two OM pools in midwaters. Our PCA reinforced this disconnect, separating all zooplankton and surface ocean particles from suspended particles collected in the mesopelagic zone. At the same time, our LDA indicated a common marine producer source of EAA to plankton and particles in surface waters and at depth. The sum of these observations indicates a surface ocean marine producer source of OM to zooplankton in the mesopelagic zone, delivered via vertical migration or by particles sinking from surface waters. We posit that disaggregation and heterotrophic alteration of this marine producer source (sinking particles or egested migrant fecal pellets) eventually produces suspended particles in midwaters, which differ substantially in stable isotopic composition from the original source material. Such a scenario would be consistent with the size–reactivity continuum proposed for marine POM and DOM (Amon and Benner 1996) and potentially with the precursor–product scenario recently proposed by Walker and McCarthy (2012), if such a relationship could be extended to sinking POM. Moreover, previous organic geochemical and stable isotope research has underscored differences in the chemical composition of sinking and suspended particles, supporting our conclusions. For example, Sheridan et al. (2002) found suspended and sinking particles in the equatorial Pacific to differ in terms of OM degradation state, with suspended particles becoming progressively more degraded and comprising more zooplankton and microbial indicator lipids with depth in the mesopelagic zone. Abramson et al. (2010) also found suspended particles in the Mediterranean Sea to differ significantly in amino acid and chloropigment composition from sinking particles, suggesting limited reaggregation of suspended POM back into the sinking particle phase. In terms of bulk stable isotope composition, both Altabet et al. (1991) and Casciotti et al. (2008) have observed midwater suspended particles to be significantly ^{15}N enriched compared with sinking particles collected from the same depths and

locations. Together with our findings, these previous studies suggest that the composition and dynamics of sinking particles (and therefore the zooplankton that feed on them; Wilson et al. 2010) differ significantly from the composition and dynamics of suspended particles, and that reaggregation mechanisms contributing to exchange between the two pools may be more limited than was suggested by earlier studies (Bacon and Anderson 1982; Bacon et al. 1985).

Results of our study indicate that a significant proportion of the N-sustaining zooplankton in the NPSG is surface derived, even at depth in the mesopelagic zone. We have demonstrated that food resources in surface waters differ substantially in source AA stable N isotope composition ($\delta^{15}N_{Phe\text{-}surface} \equiv -2‰$) from potential food resources (suspended particles) in midwaters ($\delta^{15}N_{Phe\text{-}deep} \equiv 7‰$); thus, to quantify the amount of surface N entering midwater food webs, we can apply a two-source mass balance mixing model to zooplankton (ZP) $\delta^{15}N_{Phe}$ values, as: $\delta^{15}N_{Phe\text{-}ZP} = \delta^{15}N_{Phe\text{-}surface} \times f + \delta^{15}N_{Phe\text{-}deep} \times (1 - f)$, where f is the fraction of N entering midwater food webs that is surface derived. This model may be considered conservative, as here we assume that surface-derived food resources (i.e., sinking particles and migrant food resources) have $\delta^{15}N_{Phe}$ values similar to that of surface particles in the upper euphotic zone. For migrant zooplankton, this is likely a good approximation, as most of the migrant biomass we measured at night at Sta. ALOHA was found in the upper 50 m of the water column. However for sinking particles, bulk $\delta^{15}N$ values at Sta. ALOHA can be somewhat ^{15}N enriched compared with surface ocean particles and, moreover, can increase slightly in $\delta^{15}N$ value with depth (Casciotti et al. 2008). Thus, sinking particle $\delta^{15}N_{Phe}$ values may be slightly higher than those we have measured for surface ocean particles. However, this would indicate a smaller amount of feeding on deep-water suspended particles and would consequently increase our estimate of the proportion of surface-derived N entering midwater food webs (f). At 300–500 m, f values range from 0.80 ± 0.09 to 0.92 ± 0.10, and average 0.82 ± 0.02 (daytime collections) and 0.88 ± 0.03 (nighttime collections). In the lower mesopelagic zone (700–1000 m), f values are slightly lower, ranging from 0.56 ± 0.09 to 0.80 ± 0.12 and averaging 0.62 ± 0.05 (daytime collections) and 0.75 ± 0.05 (nighttime collections). We can further apply this approach to our PCA results, with the surface-derived fraction of OM entering midwater food webs (f) expressed as $PC1_{ZP} = PC1_{surface} \times f + PC1_{deep} \times (1 - f)$. Here, $PC1_{ZP}$, $PC1_{surface}$, and $PC1_{deep}$ are the PC1 scores for zooplankton, surface ocean food resources, and potential midwater food resources (suspended particles), respectively. This model suggests that f values range from 0.80 to 0.86, averaging 0.83 ± 0.03 over the mesopelagic zone at Sta. ALOHA. In summary, given the conservative nature of our approach, we suggest our estimate of 62–88% is a lower limit to the amount of surface-derived N entering zooplankton food webs in the mesopelagic zone at Sta. ALOHA.

We have shown that a substantial fraction of zooplankton food resources at depth are surface derived; however, whereas some studies have focused on the link between mesopelagic zooplankton nutrition and sinking particles (Koppelmann et al. 2003), zooplankton C demand exceeds particle remineralization fluxes in midwaters (Steinberg et al. 2008*b*). Thus, other food resources must contribute to zooplankton metabolic needs in the mesopelagic zone. Migration and feeding in surface waters, and the consequent carnivorous consumption of migrant zooplankton at depth, is one potential alternate source of C to the midwater resident zooplankton pool. As discussed above, both of these mechanisms (feeding on sinking particles and carnivory of migrant zooplankton) are indicated by the results of our CSIA. Our study has also focused on zooplankton collected with 0.2 mm mesh nets and thus likely did not effectively sample smaller zooplankton (e.g., *Oncaea* spp.) that can significantly contribute to populations at midwater and greater depths (Böttger 1987; Böttger-Schnack 1994). CSIA of these organisms could reveal a stronger trophic link to deep-water suspended particles (i.e., through higher $\delta^{15}N_{Phe}$ values). The zooplankton-suspended particle link might also be greater deeper in the water column. Koppelmann et al. (2009) found $\delta^{15}N$ values of the bathypelagic copepod *L. longiserrata* to be almost 10‰ greater than sinking particle $\delta^{15}N$ values measured at depths > 3000 m. It is not likely that this copepod was feeding at a TP of four or above (assuming a shift of 3.4‰ per trophic level); instead, as these authors suggest, it is more likely that its nutrition source at depths > 3000 m was ^{15}N-enriched suspended particles. Regardless, our results indicate that the dominant zooplankton trophic pathways in midwaters are based on surface-derived food resources, despite the potential imbalance between C supply and demand. To further investigate midwater plankton and particle dynamics, future studies should focus simultaneously on CSIA and organic biomarker analysis of multiple OM pools (zooplankton and the sinking to suspended particle continuum).

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