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Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes

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Abstract

We quantify the trophic positions of subtropical open-ocean zooplankton species using amino acid–specific (AA) stable nitrogen isotopic compositions. We model animal trophic position by computing trophic ^{15}N enrichment of glutamic acid relative to phenylalanine, and find that trophic position for primary copepod consumers (*Oithona* spp., *Neocalanus robustior*) and secondary copepod consumers (*Pleuromamma xiphias* and *Euchaeta rimana*) varied little over a 5–10-yr period in the North Pacific Subtropical Gyre (NPSG; mean ± SD: 2.1 ± 0.1 and 2.9 ± 0.1, respectively). Comparison of AA ^{15}N enrichment patterns in different copepod species suggests that trophic ^{15}N enrichment is most consistent in glutamic acid, aspartic acid, and alanine, "trophic" AAs that are intimately involved in the citric acid cycle and energy production. We further test equations involving these trophic AAs and "source" AAs (which appear to retain the nitrogen isotopic composition of the food-web base), and find that such compound-specific models give results that are identical to those calculated using whole-animal (bulk) stable isotopic compositions. However the benefits of our AA-based approach (i.e., the relatively few samples needed for precise TP estimation, elimination of the need for concurrent prey isotopic analyses, and the ability to utilize formalin-preserved specimens from archived collections), make this a powerful technique for the quantitative assessment of trophic position within the pelagic food web. We further discuss how stable isotopic analyses provide a new perspective on the structure of open-ocean food webs and can be used to trace large seasonal fluctuations in nitrogen source in the NPSG.

The ability to position and compare individual species in trophic networks is critical to understanding energy flow and ecological relationships. Assignment of trophic position within complex aquatic food webs has been facilitated in recent years by stable isotope techniques (Kling et al. 1992; Post 2002). Stable nitrogen (N) isotopes, in particular, can be used to determine trophic structure because the ^{15}N content of consumers increases with transfer path length through the food web. On average, N isotopic composition, measured as $\delta^{15}N$, increases by ~3.4‰ in a consumer relative to its diet (DeNiro and Epstein 1981; Vander Zanden and Rasmussen 2001; Post 2002). Thus larger consumer $\delta^{15}N$ values should reflect higher trophic status within a community. However interpretations of $\delta^{15}N$-based indices of trophic position are often complicated by changes in isotopic composition of prey resources. For example, spatial heterogeneity or seasonal cycles in N dynamics can drive significant and rapid fluctuations in $\delta^{15}N$ values at the base of the food web (Rolff 2000; Syväranta et al. 2006).

To determine animal trophic position accurately, temporal or spatial isotopic variability in N sources at the base of the food web must be standardized relative to a baseline indicator (e.g., a long-lived primary consumer such as a mussel or snail [Post 2002]). For many ecosystems, finding an appropriate baseline indicator can be difficult. Zooplankton in oligotrophic systems, for example, may feed

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selectively on a diverse array of autotrophic and heterotrophic organisms. However isotopic assessment of their "food web base" usually relies on analyses of particulate material filtered indiscriminately from the environment. Moreover, because protists at the food-web base have high growth rates and N turnover times, and consequently vary their isotopic compositions on much faster time scales than zooplankton, "isotopic mismatches" often occur between zooplankton predators and their microplankton prey. Following an upwelling event in Lake Tanganyika, for example, $\delta^{15}N$ values of phytoplankton and zooplankton were found to be identical because phytoplankton rapidly incorporated ^{15}N-enriched deep-water nitrate, while the lagging zooplankton $\delta^{15}N$ values reflected an average of pre- and post-upwelling phytoplankton isotopic compositions (O'Reilly et al. 2002). Similar isotopic mismatches have been observed for krill and food particles in the Southern Ocean (Schmidt et al. 2006), and make accurate determination of zooplankton trophic position using whole-tissue, or "bulk," $\delta^{15}N$ values difficult unless a large amount of data is available (e.g., from a spatial survey or time-series).

In this study we assess a new approach for quantitatively determining trophic position in lower pelagic food webs: compound-specific isotope analysis (CSIA), a technique based on stable N isotope ratios of individual amino acids (AAs). From seminal laboratory experiments, McClelland and Montoya (2002) suggested that the isotopic compositions of "trophic" AAs ($\delta^{15}N_{AA}$ values) are enriched by as much as ~7‰ in marine predators relative to their prey, while other "source" AAs are little affected by trophic status and retain N isotopic compositions indicative of N sources at the base of the food web. Because AA analyses provide information on both trophic ^{15}N enrichments and basal N isotope compositions, trophic position can be quantified without the need to characterize baseline $\delta^{15}N$ values for an ecosystem. In initial explorations of the potential of CSIA for trophic studies, results were roughly consistent with expectations of zooplankton herbivory from mixed community analyses in the subtropical Atlantic (McClelland et al. 2003) and krill omnivory in the Southern Ocean (Schmidt et al. 2006). CSIA has further been used by Chikaraishi et al. (2007) to assess the trophic status of coastal herbivores and by McCarthy et al. (2007) as a trophic level indicator. The models for these latter field applications, however, require knowledge of $\delta^{15}N_{AA}$ values for both consumers and food sources.

Here we develop a quantitative model of animal trophic position based on the internal trophic index of McClelland and Montoya (2002). The model utilizes differences in trophic ^{15}N enrichment in glutamic acid (glu) relative to phenylalanine (phe) from collected specimens of individual species, and thus does not require separate analysis of prey isotopic composition. We use glu and phe because they are considered "canonical" trophic and source AAs that represent extremes of metabolic cycling. Glu is the primary intermediate for AA transamination reactions, for which kinetic isotopic fractionation results in significant ^{15}N enrichment of glu amino groups (Macko et al. 1997). In contrast, phe is an essential AA for crustaceans and other animals (Claybrook 1983) and is incorporated from food material with negligible isotopic alteration. We are thus able to calculate trophic position ($TP_{glu\text{-}phe}$) as

$$\text{Trophic Position}_{(glu-phe)} = \left[\frac{\left(\delta^{15}N_{glu} - \delta^{15}N_{phe}\right) - 4‰}{7‰}\right] + 1 \quad (1)$$

which assumes that $\delta^{15}N_{glu}$ is enriched by 7‰ with each trophic transfer above phytoplankton, whose $\Delta\delta^{15}N_{glu\text{-}phe}$ is 4‰ (McClelland and Montoya 2002). A similar trophic position model, based on glu and glycine $\delta^{15}N$ values, was applied to yellowfin tuna (*Thunnus albacares*) in the eastern tropical North Pacific by Popp et al. (2007). However, here we use CSIA for the first time to quantify the trophic position of zooplankton within a complex open-ocean food web. This represents an advance over previous bulk isotope–based models because it eliminates errors previously associated with analyses of prey isotopic composition, and because it is amenable to retrospective analyses of animals from preserved collections which lack contemporaneous prey samples.

We apply our CSIA-based model to zooplankton in the North Pacific Subtropical Gyre (NPSG). Food-web relationships in such oligotrophic open-ocean ecosystems are difficult to characterize due to their extreme high diversity and inaccessibility, and thus CSIA potentially offers a new perspective on these trophic interactions. We focus on a suite of target plankton including the copepods *Neocalanus robustior*, *Pleuromamma xiphias*, *Euchaeta rimana*, and *Oithona* spp., and the euphausiid *Thysanopoda* spp. We determine $TP_{glu\text{-}phe}$ for these species and compare our CSIA-based estimates with inferences from bulk (whole-animal) $\delta^{15}N$ values. We also evaluate zooplankton trophic position relative to the limited information on species-level feeding behaviors and preferences in the open ocean. Differences in AA trophic ^{15}N enrichment patterns between primary and secondary consumers are further used to develop models of trophic position using alternate AAs. Finally, we use CSIA to assess temporal stability in zooplankton trophic position and identify seasonal fluctuations in food-web N source in the NPSG.

Methods

Zooplankton collection—Zooplankton analyzed in this study were collected at the Hawaii Ocean Time-series (HOT) Station (Sta.) ALOHA (22.45°N, 158°W), a site 100 km north of the island of Oahu in the NPSG (Karl and Lukas 1996). Routine time-series sampling for zooplankton was conducted on approximately monthly cruises from 1994 to 2005. Samples analyzed for CSIA were collected on "winter" cruises (Feb 1995 [HOT 60], Apr 2000 [HOT 114], and Jan 2005 [HOT 167]) and on "summer" cruises (Aug 1995 [HOT 65], Aug 2000 [HOT 118], and Aug 2005 [HOT 172]). For each cruise, we analyzed zooplankton samples collected at night (22:00–02:00 h) using a 1-m^2, 200-μm-mesh plankton net towed obliquely through the ~160-m euphotic zone (Landry et al. 2001). The samples were immediately split on shipboard and one-half of each tow

preserved in 5% borate-buffered formalin. The other half of each tow was wet-sieved, and the resulting size fractions (0.2–0.5, 0.5–1, 1–2, 2–5 and >5 mm) were frozen in liquid nitrogen and stored at −80°C.

Sample processing—Stable isotope analysis was conducted on frozen and preserved zooplankton. Frozen material was only available for time-series cruises from 1998 to 2005. For these cruises, the 1–2-mm zooplankton size fractions were oven-dried (60°C for ≤5 d) and ground using a mortar and pestle. The homogenates were then packaged in tin capsules (for bulk isotope analyses) or combusted glass reaction vials (for compound-specific analyses).

Formalin-preserved animals were sorted from one of three replicate nighttime tows from each cruise. Adult females of three target copepod species were sorted from each tow: *Euchaeta rimana*, *Pleuromamma xiphias*, and *Neocalanus robustior*. Ten *E. rimana* were sorted from each nighttime tow on two cruises in 2005 to test inter-tow variability. *Oithona* spp. and *Thysanopoda* sp. (8-mm) were picked from the 1995 and 2000 collections (HOT 60, 65, 114, and 118). Preserved zooplankton were rinsed three times in Milli-Q water after sorting, and 1 (*E. rimana*, *P. xiphias*, *N. robustior*, *Thysanopoda* sp.) to 15 (*Oithona* spp.) were packaged in tin capsules and dried (60°C for 3 d) for bulk isotope analyses. Only abdominal segments of *Thysanopoda* spp. were analyzed for bulk isotope analysis. For CSIA, 1 (*Thysanopoda* sp.) to 500 (*Oithona* spp.) specimens were placed in combusted reaction vials and dried at 60°C for 3 d. On average, 20 specimens of *E. rimana*, *P. xiphias*, and *N. robustior* were used for CSIA. All transfers were conducted gently with dissecting probes to minimize contamination.

Bulk stable nitrogen isotope analysis—Bulk stable isotope analyses were performed using an on-line carbon–nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus). 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-shrimp reference samples with well-characterized $\delta^{15}N$ values were run every 10 samples. For each time point, we ran 2–4 replicates per species. The standard deviation on samples analyzed in duplicate was generally ≤0.2‰.

AA hydrolysis and derivatization—Prior to AA isotope analysis, zooplankton were subject to acid hydrolysis, esterification of the carboxyl terminus and trifluoroacetylation of the amine group (Macko et al. 1997; Popp et al. 2007). Acid hydrolysis destroys tryptophan and cystine, and coverts asparagine to aspartic acid and glutamine to glutamic acid. Sequanal grade 6 N HCl (1 mL) and an isotopic internal compound, norleucine (0.984 μmol sample^{-1}), were added to 5 mg ground plankton or dried individual zooplankton species in each reaction vial. The vials were flushed with N_2, and all material was hydrolyzed at 150°C for 70 min. The resulting hydrolysate was evaporated to dryness under N_2 at 55°C, redissolved in 1 mL 0.01 N HCl and filtered with low protein-binding filters to remove particles. The hydrolysate was further purified using cation-exchange chromatography (Metges et al. 1996) with a 5-cm column of resin prepared in a glass Pasteur pipette. AAs on the column were eluted with repeated rinses of 2 N NH_4OH, and the eluant was evaporated to dryness under N_2 at 80°C. Finally, the samples were re-acidified with 2 mL 0.2 N HCl, heated at 110°C for 5 min and evaporated to dryness under N_2 at 110°C.

Hydrolyzed plankton samples were esterified with 2 mL of 4 : 1 isopropanol : acetyl chloride, heated to 110°C for 60 min following a N_2 flush. The esterified samples were dried under N_2 at 60°C, and 1 mL of 3 : 1 methylene chloride : trifluoracetic anhydride (TFAA) was added. Samples were acylated by heating to 100°C for 15 min after a N_2 flush. The derivatized samples were further subject to purification by solvent extraction following Ueda et al. (1989). The TFA derivatives were evaporated at room temperature (RT) and redissolved in 3 mL 1 : 2 chloroform : P-buffer (KH_2PO_4 + Na_2HPO_4 in Milli-Q water, potential Hydrogen [pH] 7). After sonication and centrifugation (10 min at 600 *g*), the chloroform fraction containing solely the acylated AA esters was removed and the solvent extraction process repeated. Finally, to ensure complete derivatization, the chloroform was evaporated at RT, and the acylation step was repeated. All samples were stored in 3 : 1 methylene chloride : TFAA at ≤4°C and analyzed within 1 month. With this procedure, ~9 samples can be prepared for AA analysis in 2 d.

Amino acid identification—AAs from select plankton samples were identified using a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 mass selective detector (GC–MS). Before analysis, TFA derivatives were evaporated at RT under N_2 and redissolved in 1000-μL ethyl acetate. Samples (1 μL) were injected (split/splitless, 50 : 1 split ratio) onto a 30-m HP-5ms column (0.25-mm inner diameter [i.d.], 0.25-μm film thickness) at an injector temperature of 180°C and a constant helium flow rate of 1 mL min^{-1}. The column oven was initially held at 50°C for 1 min, ramped to 185°C at 5°C min^{-1} and then to 300°C at 10°C min^{-1}, and finally held at 300°C for 10 min. MS inlet temperature was 300°C. AA composition of each sample was determined by comparison with a mixed AA standard (Standard H; Pierce Biotechnology).

Compound-specific stable nitrogen isotope analysis—The stable N isotopic compositions of AAs were analyzed using a Delta XP mass spectrometer interfaced to a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (680°C), and liquid nitrogen cold trap. All TFA derivatives were evaporated at RT under N_2 and redissolved in 100–1000-μL ethyl acetate. Samples (3 μL) were injected (split/splitless, 5 : 1 split ratio) onto a 50-m HP Ultra-2 column (0.32 mm i.d., 0.52-μm film thickness) at an injector temperature of 180°C and a constant helium flow rate of 2 mL min^{-1}. The column oven was initially held at 50°C for 2 min, ramped to 190°C at 8°C min^{-1} and then to 280°C at 10°C min^{-1}, and finally held at 280°C for 10 min. Samples were analyzed in triplicate, and the measured N isotopic compositions

corrected relative to the known $\delta^{15}N$ value of the norleucine internal reference. A cocktail of AAs with known $\delta^{15}N$ values (glycine, leucine, phe, and L-2-aminoadipic acid) was injected every three samples to evaluate accuracy. Standard deviation for triplicate injections of each sample averaged 0.5‰, and ranged from 0.03‰ to 4.8‰.

Results

Preservation and replication—To determine formalin preservation effects on $\delta^{15}N$ values, we compared N isotope composition time-series of unpreserved, frozen zooplankton (1–2-mm size fraction) and formalin-preserved copepods (*Euchaeta rimana*, 2.5-mm length). Previous studies have found that formalin does not affect copepod $\delta^{15}N$ values, even after 2 yr (Mullin et al. 1984) to 11 yr (Rau et al. 2003) of storage. Consistent with these findings, bulk $\delta^{15}N$ values of our preserved and unpreserved samples were significantly correlated ($R = 0.773$, $p < 0.01$, $n = 70$), and Model II regression (Bartlett's three-group method; Sokal and Rohlf 1995) gave an ~1 : 1 linear relation between their isotope values (slope = 0.92 [95% Confidence Level (CL) = 1.1, 0.78], $n = 70$). The y-axis intercept for the regression was slightly positive (0.63), but not statistically different from zero. As with the bulk isotope measurements, $\delta^{15}N$ values of AAs did not differ appreciably between formalin-preserved *E. rimana* and unpreserved 1–2-mm zooplankton (linear regression: Er $\delta^{15}N_{AA} = 1.1 \times$ (zooplankton $\delta^{15}N_{AA}$) + 0.52; [slope 95% CL = 1.17, 1.00], $n = 30$). Thus our comparison of these time-series samples indicates negligible formalin preservation effects for bulk and AA-specific isotope compositions, which allows for our CSIA-based TP technique to be used on archived collections.

Within- and between-tow variations in bulk $\delta^{15}N$ values were assessed by stable isotope analyses of 10 *E. rimana* from three replicate plankton tows in January and August 2005. The average (± standard deviation [SD]) within-tow difference in *E. rimana* $\delta^{15}N$ values (0.4 ± 0.1‰) was identical to the between-tow difference for replicate tows taken 1–24 h apart (0.4 ± 0.1‰). These intra-cruise differences in $\delta^{15}N$ values were much less than average difference between January and August cruises in 2005 (1.4 ± 0.2‰; Mann–Whitney *U*-test, $p < 0.05$) and seasonal changes in *E. rimana* $\delta^{15}N$ values of 0.9–8.1‰. Thus differences in copepod $\delta^{15}N$ values through the time-series were clearly greater than within-cruise variability.

Bulk $\delta^{15}N$ values—Bulk stable N isotope ratios of zooplankton at Sta. ALOHA ranged from 1.7‰ to 11.5‰ in 1995 and 2000 (Fig. 1; *see* Web Appendix 1, www.aslo.org/lo/toc/vol_54/issue_1/0050a1.pdf). For all plankton, bulk $\delta^{15}N$ values were higher in the winter than in the summer. In 1995 and 2000, this annual difference averaged 5.6‰ for *E. rimana* and *N. robustior*, 3.5‰ for *P. xiphias*, 3.3‰ for *Thysanopoda* spp., 2.8‰ for *Oithona* spp., and 2.4‰ for mixed 1–2-mm zooplankton. The seasonal changes were larger than long-term average winter–summer differences in $\delta^{15}N$ values (i.e., 2.2‰ for *E. rimana*, 2.1‰ for *N. robustior*, 1.8‰ for *P. xiphias*, and 1.6‰ for 1–2-mm mixed zooplankton [$n = 12$]).

AA $\delta^{15}N$ values—The N isotopic compositions of AAs ($\delta^{15}N_{AA}$) isolated from individual zooplankton taxa collected in 1995 and 2000 ranged from −14.7‰ to 20.8‰ (*see* Web Appendix 1). AAs considered "source AAs" by Popp et al. (2007; i.e., those whose $\delta^{15}N$ value is defined only by N sources at the base of the zooplankton food web [McClelland and Montoya 2002]), included phe, glycine, lysine, serine, and threonine. These AAs had relatively low $\delta^{15}N_{AA}$ values (mean: −1.6‰ [range: −15–6.7‰]). In contrast, AAs defined by Popp et al. (2007) as "trophic AAs," (i.e., those whose stable isotope compositions change with both N source and trophic level [McClelland and Montoya 2002]), had relatively high $\delta^{15}N_{AA}$ values (mean: 12‰ [range: 3.9–21‰]). These trophic AAs included glu, alanine, aspartic acid, isoleucine, leucine, proline, and valine.

Annual changes in trophic and source $\delta^{15}N_{AA}$ values matched the seasonal pattern in bulk $\delta^{15}N$ values at Sta. ALOHA. A time-series of the canonical trophic and source AAs, glu ($\delta^{15}N_{glu}$) and phe ($\delta^{15}N_{phe}$), best illustrates the seasonal changes in AA isotopic composition (Fig. 1). For both glu and phe, winter $\delta^{15}N$ values were consistently higher than summer $\delta^{15}N$ values. The average seasonal differences in $\delta^{15}N_{glu}$ for *E. rimana* and *N. robustior* (4.1‰ and 4.3‰, respectively) were higher than for *P. xiphias* (2.4‰) and mixed 1–2-mm zooplankton (2.5‰; Fig. 1; $n = 2$). Seasonal changes in $\delta^{15}N_{phe}$ followed a similar pattern, ranging from 5.1‰ (*N. robustior*) to 2.6‰ (*P. xiphias*). Within each season, however, the absolute values of $\delta^{15}N_{phe}$ were strikingly similar for most zooplankton. For all taxa except the euphausiid *Thysanopoda* spp., average $\delta^{15}N_{phe}$ was 0.04‰ in the winter and −3.2‰ in summer (Fig. 1; $n = 2$). This annual change in $\delta^{15}N_{phe}$ indicates a consistent seasonal shift in N source at the base of the zooplankton food web at Sta. ALOHA during the time period of this study.

Trophic position—In contrast to the uniformity in source AA $\delta^{15}N$ values, the isotope composition of trophic AAs differed significantly with taxa (Fig. 1; ANOVA, $p < 0.001$). For example, $\delta^{15}N_{glu}$ values for *Euchaeta rimana* and *Pleuromamma xiphias* were consistently higher than those for *Neocalanus robustior* and *Oithona* spp. (Fig. 1).

Trophic position (TP) calculated for zooplankton species using $\delta^{15}N_{glu}$ and $\delta^{15}N_{phe}$ values (Eq. 1) ranged from 2.0 to 3.0, and were thus within the range of primary and secondary consumers (Fig. 2; *see* Web Appendix 1). On average, TP increased in the following order: *Oithona* spp. (2.1 ± 0.1 [SD], $n = 4$) < *N. robustior* (2.2 ± 0.1, $n = 3$) < *Thysanopoda* sp. (2.3 ± 0.2, $n = 4$) < 1–2-mm mixed zooplankton (2.6 ± 0.2, $n = 3$) < *P. xiphias* (2.8 ± 0.1, $n = 4$) < *E. rimana* (2.9 ± 0.1, $n = 6$). The trophic positions of *E. rimana* and *N. robustior* did not change significantly throughout the time-series (Mann–Whitney *U*-test, $p \gg 0.05$, $n = 3$; Fig. 2). However, significant changes in TP were found for mixed 1–2-mm zooplankton in 2005 (TP = 2.8) as compared to 2000 (TP = 2.4; Mann–Whitney *U*-test, $p < 0.03$, $n = 4$). Other significant differences in TP were observed for *Thysanopoda* (winter 1995 > any other time, summer 1995 > winter 2000), *Oithona* (winter 1995 > summer 1995 and 2000), and *P. xiphias* (winter 1995 < summer 1995 and winter 2000; Mann–Whitney *U*-test, $p < 0.05$, $n = 3$).

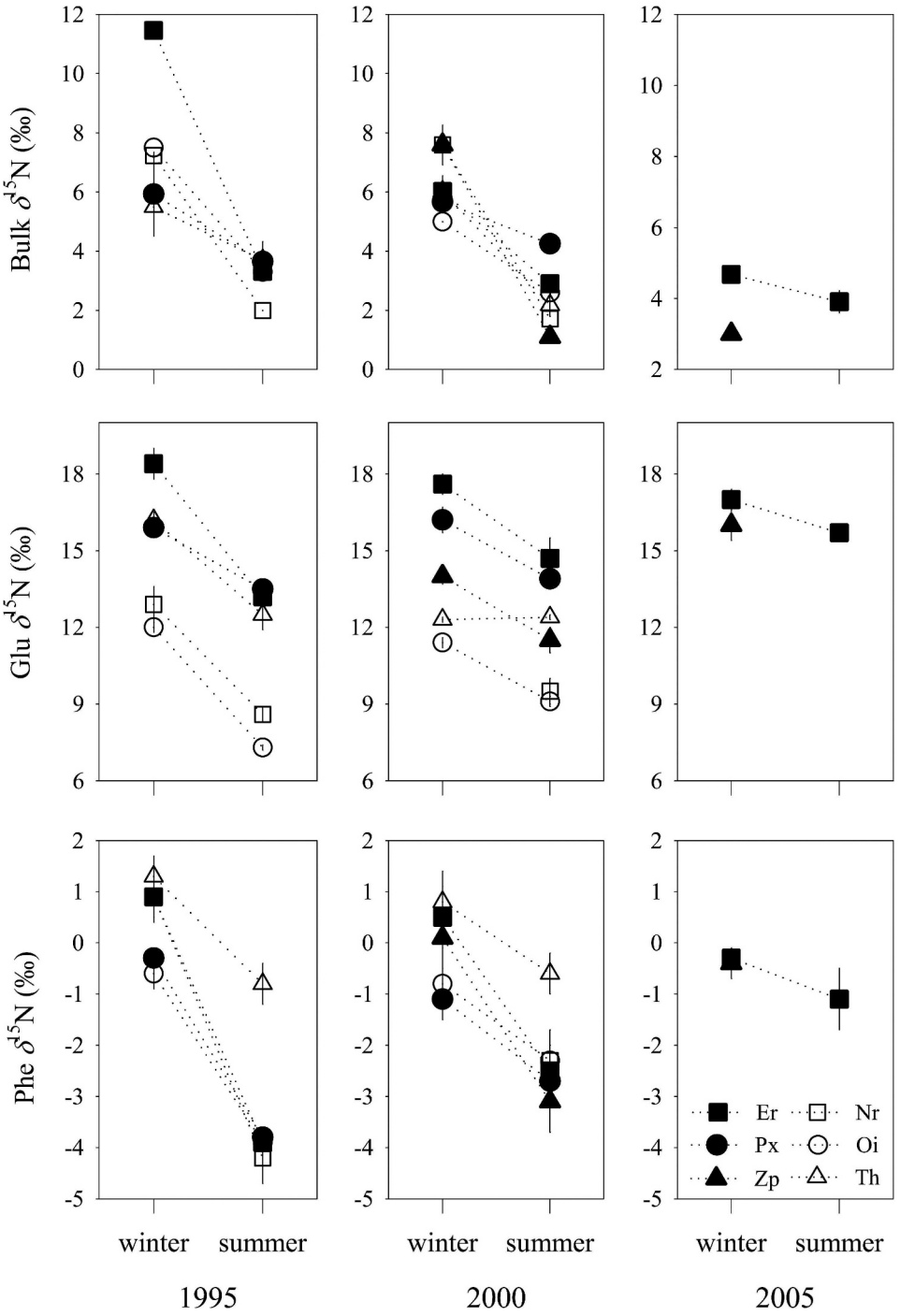

Fig. 1. Stable nitrogen isotope values of whole animals (Bulk δ^{15}N), glu (Glu δ^{15}N), and phe (Phe δ^{15}N) for *E. rimana* (Er), *P. xiphias* (Px), 1–2-mm zooplankton (Zp), *N. robustior* (Nr), *Oithona* spp. (Oi), and *Thysanopoda* spp. (Th) at Sta. ALOHA. Error bars are standard deviations ($n = 3$).

Trophic alteration of $\delta^{15}N_{AA}$ values was evaluated by comparing AA ^{15}N enrichment in a primary (*Oithona* spp.) and a secondary consumer (*Euchaeta rimana*) relative to the base of their food webs (i.e., $\delta^{15}N_{phe}$ values; Fig. 3). The source AAs glycine, serine, and lysine deviated little from the food-web base ($\Delta_{phe}\delta^{15}$N) at both trophic positions (Fig. 3). In contrast, the trophic AAs exhibited large ^{15}N enrichments (Fig. 3). The trophic AAs for *Oithona* spp. all exhibited similar trophic ^{15}N enrichments (Mann–Whitney *U*-test, $p \gg 0.05$, $n = 12$). For *E. rimana*, however, alanine, aspartic acid, and glu (trophic II AAs) were significantly more ^{15}N enriched than proline, valine, leucine, and isoleucine (trophic I AAs; Fig. 3; Mann–Whitney *U*-test, $p < 0.005$, $n = 12$). The differentiation among trophic I and

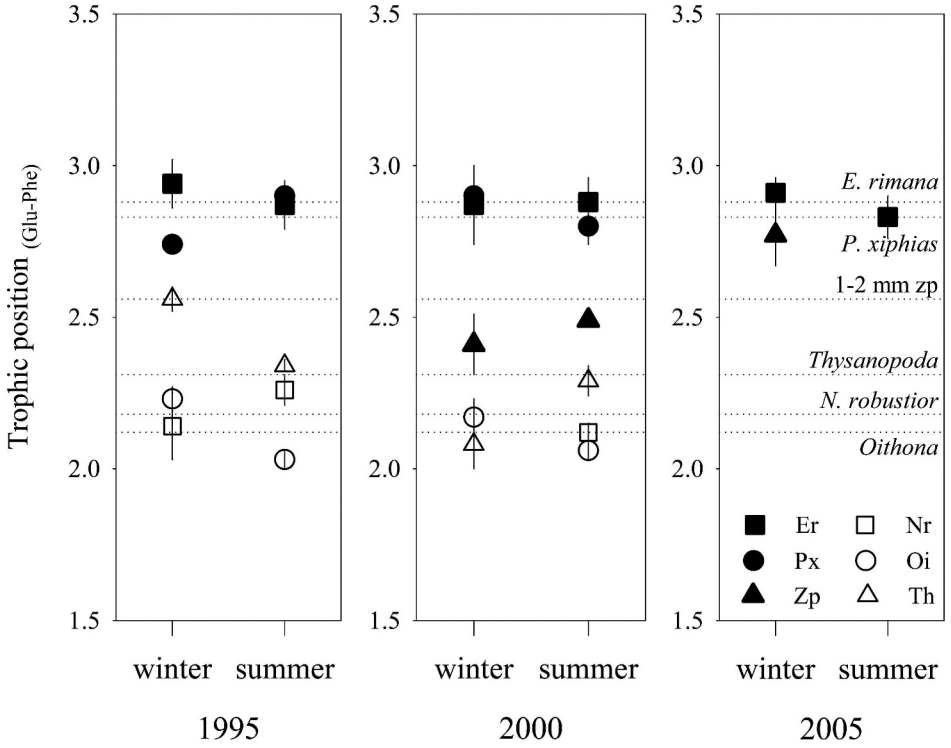

Fig. 2. Trophic position of *E. rimana* (Er), *P. xiphias* (Px), 1–2-mm zooplankton (Zp), *N. robustior* (Nr), *Oithona* spp. (Oi), and *Thysanopoda* spp. (Th) at Sta. ALOHA calculated using glu and phe δ^{15}N values ($TP_{glu\text{-}phe}$; Eq. 1). Mean $TP_{glu\text{-}phe}$ for each zooplankton is labeled and indicated with a dotted line. Error bars are standard deviations ($n = 3$).

II AAs was a regular feature of comparisons involving secondary consumers and food-source (phe) δ^{15}N values. These trophic enrichment patterns for primary and secondary consumers also remained constant despite a large shift in food-web basal isotopic values ($\delta^{15}N_{phe}$) from ~0‰ in the winter to −4‰ in the summer of 1995 (Fig. 3).

The size of the species analyzed in our study was not a good indicator of zooplankton trophic position (Fig. 4). Although the smallest copepod examined (*Oithona* spp., 0.6 mm) occupied the lowest TP, two similarly sized copepods, *N. robustior* and *E. rimana* (3.2 and 2.5 mm, respectively) differed by 0.7 TP units on average. Moreover, in 2000, the largest zooplankton, *Thysanopoda* spp. (8 mm), was not significantly different in trophic position from the smallest copepod, *Oithona* spp., despite the fact they differed by over an order of magnitude in body length (Mann–Whitney *U*-test, $p \gg 0.05$, $n = 3$).

Discussion

CSIA and zooplankton trophic position—Our study demonstrates for the first time that CSIA can be used to quantitatively identify the trophic positions of individual zooplankton species in open-ocean food webs. Our approach, based on ^{15}N enrichment of the "canonical" trophic and source AAs glu and phe, shows $TP_{glu\text{-}phe}$ in the NPSG ranging from 2.0 to 3.0 (Fig. 2). That is, the lower food web in this oligotrophic open-ocean habitat includes primary consumers that feed on components of the

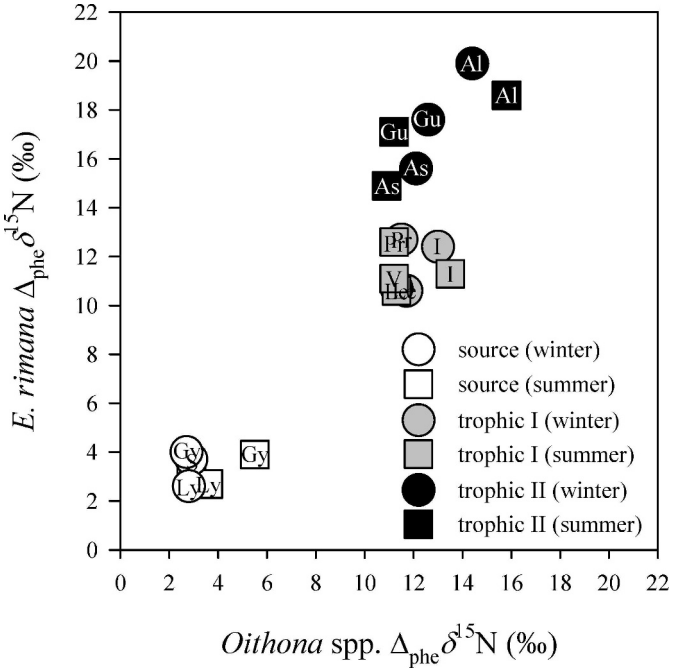

Fig. 3. The difference between AA and food-source (phenylalanine) δ^{15}N values ($\Delta_{phe}\delta^{15}$N) for *Oithona* spp. (trophic position 2) and *E. rimana* (trophic position 3) in winter and summer 1995. Source AAs [serine (S), glycine (Gy), and lysine (Ly)] and trophic AAs in groups I [proline (Pr), leucine (Le), isoleucine (I), and valine (V)] and II [glutamic acid (Gu), aspartic acid (As), and alanine (Al)] are shown.

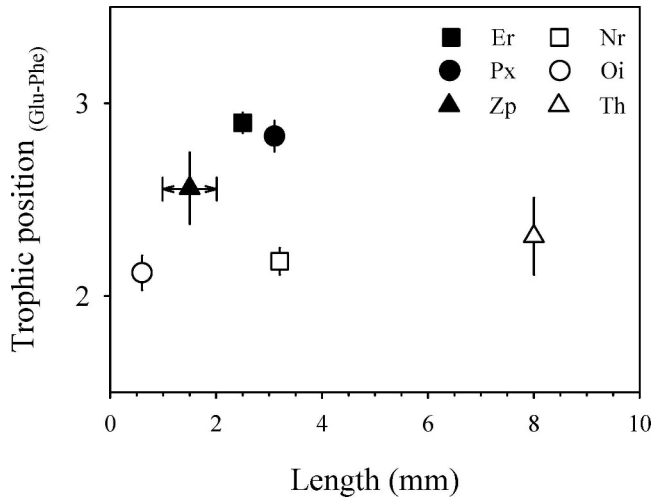

Fig. 4. Zooplankton length and trophic position for *E. rimana* (Er), *P. xiphias* (Px), 1–2-mm zooplankton (Zp), *N. robustior* (Nr), euphausiids (Eu), and *Oithona* spp. (Oi) at Sta. ALOHA. Lengths were measured from the rostrum to the end of the prosome for copepods and from the rostrum to the end of the telson for euphausiids. Error bars are standard deviations ($n = 3$).

microbial loop (*Oithona* spp, *Neocalanus robustior*), omnivores (*Thysanopoda* spp.), and primary carnivores (*Pleuromamma xiphias*, *Euchaeta rimana*).

Our estimates of zooplankton $TP_{glu\text{-}phe}$ can be compared to previous, though limited, trophic categorizations based on direct observations of the feeding behavior of open-ocean taxa such as *Oithona* spp. and *Euchaeta rimana*. Oithonidae do not generate feeding currents (Paffenhöfer 1993) but ambush prey after detection of hydrodynamical signals (Svensen and Kiorboe 2000; Paffenhöfer and Mazzocchi 2002). Prey targeted by *Oithona* spp. include small motile and sinking particles, such as flagellates, ciliates, and fecal pellets (Gonzalez and Smetacek 1994; Nielsen and Sabatini 1996; Castellani et al. 2005). In a Hawaiian coastal embayment, for example, *Oithona* spp. fed on small (2–5-μm) autotrophic and heterotrophic nanoflagellates (Calbet et al. 2000). In contrast, the predator *E. rimana* responds to larger mobile prey and has been observed attacking the subtropical copepods *Acartia fossae* and *Acrocalanus inermi* (Doall et al. 2002). Gut contents of *Euchaeta* spp. are also known to contain more crustacean body parts than other copepods in the central North Pacific gyre (Hayward 1980). Previous TP inferences based on AA $\delta^{15}N$ values for zooplankton in the Southern Ocean also agree with the results of stomach content and fatty acid analyses (Schmidt et al. 2006). Thus "traditional" trophic assessment methods (e.g., gut contents, body composition, or feeding behaviors) corroborate CSIA-based estimates of trophic position, and specifically our $TP_{glu\text{-}phe}$ estimates for *Oithona* spp. (2.0–2.2) and *E. rimana* (2.8–3.0) at Sta. ALOHA.

Our results using CSIA also compare well with TP_{bulk} estimates from bulk zooplankton $\delta^{15}N$ values (Post 2002; Table 1), although this more standard approach is strongly affected by choice of trophic enrichment factor (TEF). If the canonical TEF of 3.4‰ is chosen (DeNiro and Epstein 1981), TP_{bulk} are lower than $TP_{glu\text{-}phe}$ by 0.4–0.8 trophic units. However, if we use the trophic shift for ammonotelic consumers raised on algal or invertebrate diets (~2‰; McCutchan et al. 2003), TP_{bulk} for *E. rimana*, *N. robustior* and 1–2-mm mixed zooplankton are not significantly different from those generated from CSIA (Table 1). Despite the similarity in average TP values, the range in TP_{bulk} is much larger than is generated by CSIA (Table 1). This is likely due to temporal decoupling of zooplankton and particle N isotopic compositions, because particle time-series can miss short-lived events that significantly influence zooplankton $\delta^{15}N$ values (Post 2002). For example, in the winter of 2000, *E. rimana* $\delta^{15}N$ values were very high (6.0‰) during a nitrate injection event (i.e., high modeled vertical nitrate fluxes; Fei Chai, U. Maine pers. comm.). One month after this event, *E. rimana* $\delta^{15}N$ values remained high (4.6‰, in the top quartile), while the suspended particle $\delta^{15}N$ values dropped to 0.3‰, below the 2000–2004 average of 0.6‰. Clearly rapid turnover in suspended pools diminished the effect on particle $\delta^{15}N$ values while slower N turnover caused *E. rimana* to retain ^{15}N-enriched body tissue. The effect of mesoscale events, such as eddies (Letelier et al. 2000) or large phytoplankton blooms (Dore et al. 2008), on suspended particle composition can also be seen in the larger variability of this pool ($\delta^{15}N = 0.6 \pm 1.0$‰, $n = 46$) relative to that of zooplankton (*E. rimana* $\delta^{15}N = 4.0 \pm 0.6$‰, $n = 49$). It is unlikely that the observed isotopic mismatch between zooplankton and particle $\delta^{15}N$ values resulted from

Table 1. Trophic position (TP; mean ± SD) of target zooplankton calculated from AA-specific isotope analyses ($TP_{glu\text{-}phe}$, $TP_{glu\text{-}gly}$, $TP_{TII\text{-}S}$) and from bulk isotope analyses using a trophic enrichment factor of 2‰ (TP_{bulk}).

Zooplankton	$TP_{glu\text{-}phe}$*	*n*	TP_{bulk}†	*n*	$TP_{glu\text{-}gly}$‡	*n*	$TP_{TII\text{-}S}$§	*n*
Euchaeta rimana	2.9±0.05	7	2.9±0.4	35	2.9±0.09	7	3.0±0.07‖	7
Pleuromamma xiphias	2.8±0.08	4	3.3±0.4‖	33	2.7±0.1	4	2.8±0.08	4
Neocalanus robustior	2.2±0.07	3	2.3±0.4	29	2.0±0.08	3	2.3±0.08	3
Mixed 1–2-mm zooplankton	2.6±0.2	3	2.8±0.4	34	2.6±0.07	3	2.7±0.05	3

* Calculated using Eq. 1.
† Calculated following Post (2002) as $TP_{bulk} = 1 + (\delta^{15}N_{zooplankton} - \delta^{15}N_{PM})/2$‰, where $\delta^{15}N_{PM}$ is the mean $\delta^{15}N$ value of suspended particulate material collected during that season (i.e., on each cruise and the two before it; from the Hawaii Ocean Time-series Data Organization System [HOT-DOGS]: hahana.soest.hawaii.edu/hot/hot-dogs/interface.html).
‡ Calculated using Eq. 2.
§ Calculated using Eq. 3.
‖ TP differs significantly from $TP_{glu\text{-}phe}$ (Mann–Whitney *U*-test, $p < 0.05$).

formalin preservation, because both preserved (*E. rimana*, *P. xiphias*, and *N. robustior*) and unpreserved (1–2-mm) zooplankton exhibited the same large range in TP_{bulk} and similar temporal isotopic fluctuations (1–2-mm zooplankton $\delta^{15}N$ values were significantly correlated with those of all target species; Pearson's $r = 0.54$–0.77, $p < 0.01$, $n = 56$–70). Thus CSIA appears to estimate trophic position in the lower food web more precisely than bulk isotope–based models, even when animals from only a few cruises or preserved material are available for analysis.

Zooplankton trophic position estimated using AA and bulk $\delta^{15}N$ values differed only for *P. xiphias*, a diel vertical migrator (Table 1). This discrepancy is likely due to changes in copepod physiology and N isotopic composition during vertical migration. *P. xiphias* must survive on food consumed in surface waters at night while swimming to and from their daytime resting depths (~400–600 m). Thus, for a portion of their diel cycle, dietary proteins would not replace the ^{14}N-containing compounds that are preferentially metabolized and excreted as a by-product of energy generation (Gannes et al. 1997). Whole-body $\delta^{15}N$ values for *P. xiphias* (4.9 ± 0.7‰, $n = 114$) are high relative to other copepods (e.g., the carnivore *E. rimana* [4.2 ± 1.0‰]); consequently, TP_{bulk} for *P. xiphias* (3.3 ± 0.4) is much higher than would be predicted from previous behavioral studies or gut-content analyses (Moore et al. 1999; Schnetzer and Steinberg 2002*a*). Our AA-based estimates ($TP_{glu\text{-}phe} = 2.7$–$2.9$) are consistent with research that has found *P. xiphias* to be an opportunistic omnivore with a largely animal-based diet (Schnetzer and Steinberg 2002*b*). Metabolic control of animal isotopic composition should be more thoroughly explored in future studies, particularly in regard to the physiology of vertical migration. However our comparison of *P. xiphias* $TP_{glu\text{-}phe}$ and TP_{bulk} suggests that CSIA can produce an accurate representation of food-web structure even when whole-organism $\delta^{15}N$ values are affected by changes in animal body condition.

CSIA-based assessment of animal TP relies on regular ^{15}N enrichment in trophic AAs relative to source AAs. Although we have derived TP estimates from glu and phe, alternate trophic and source AA combinations could potentially be used to assess marine food-web structure. In particular, our assessment of AA $\delta^{15}N$ values for primary and secondary consumers (Fig. 3) indicates that ^{15}N enrichment is most consistent in the trophic "group II" AAs glu, alanine, and aspartic acid. These AAs are intermediates in the citric acid cycle and energy production (Bender 1985), and have high rates of turnover in crustacean proteins (Claybrook 1983). Our data indicate that frequent metabolic recycling of amino groups in these trophic II AAs results in regular ^{15}N enrichment with trophic level. In contrast, trophic I AAs show inconsistent ^{15}N enrichment (Fig. 3), likely because they are either essential dietary components (valine, isoleucine, and leucine) or have slow metabolic turnover rates (proline; Claybrook 1983). CSIA-based TP calculations should therefore incorporate only trophic II AAs as well as the source AAs phe, serine, glycine, and lysine (Fig. 3).

Assessment of trophic status based on alternative AAs was first suggested by Popp et al. (2007), who calculated yellowfin tuna TP in the North Pacific to be ~4.5 using differences in glu and glycine $\delta^{15}N$ values:

$$\text{Trophic Position}_{(\text{glu}-\text{gly})} = \left[\frac{\left(\delta^{15}N_{\text{glu}} - \delta^{15}N_{\text{gly}}\right)}{7‰}\right] + 1 \quad (2)$$

When applied to our data from Sta. ALOHA, this combination of trophic II and source AAs provides estimates of zooplankton $TP_{glu\text{-}gly}$ that are not significantly different from $TP_{glu\text{-}phe}$ (Table 1). As in Popp et al. (2007) and McCarthy et al. (2007), quantitative trophic assessment can also be based on the averages of trophic II and source AAs

$$\text{Trophic Position}_{(\text{TII}-\text{S})} = \left[\frac{\left(\text{AVERAGE } \delta^{15}N_{\text{TII}} - \text{AVERAGE } \delta^{15}N_{\text{S}}\right)}{7‰}\right] + 1 \quad (3)$$

where trophic II AAs (TII) are glu, alanine, and aspartic acid and source AAs (S) are serine, glycine, and lysine. When applied to the food web at Sta. ALOHA, $TP_{TII\text{-}S}$ estimates again do not differ significantly from $TP_{glu\text{-}phe}$, with the exception of *E. rimana* (Table 1). $TP_{TII\text{-}S}$ for this species is significantly higher than $TP_{glu\text{-}phe}$, largely due to high-alanine $\delta^{15}N$ values (Fig. 3). However, the ranges in *E. rimana* $TP_{glu\text{-}phe}$ (2.8–3.0) and $TP_{TII\text{-}S}$ (2.9–3.1) are similar and consistent with what is known about the behavior and feeding patterns of this carnivorous copepod (Doall et al. 2002). Thus CSIA-based equations involving trophic "group II" and source AAs appear to model zooplankton TP equally accurately, and furthermore can be used for TP estimates over a range of more than three trophic levels (i.e., *Oithona* spp. to yellowfin tuna). Moreover our results indicate that a 7‰ TEF for AAs (McClelland and Montoya 2002) may be a consistent characteristic of marine pelagic food webs. The universality of this TEF should be tested more rigorously in the laboratory, particularly in regard to deviations involving animal condition (*see* above) and trophic I AAs (proline, valine, leucine, and isoleucine). However the benefits of the CSIA approach (i.e., the relatively few samples needed for precise estimates, elimination of the need for prey isotopic analyses, and the ability to utilize specimens from archived collections) make this a powerful technique for the quantitative assessment of trophic position within pelagic food webs.

Zooplankton food webs in the NPSG—Our isotopic analysis of food-web structure in the North Pacific provides a unique window into the ecology of open-ocean zooplankton communities. Mesozooplankton in this oligotrophic gyre system are extremely diverse, including at least 175 species of copepods, the dominant taxa, and many other Crustacea and gelatinous organisms (McGowan and Walker 1985; Landry et al. 2001). Zooplankton in this community differ by almost two orders of magnitude in size (from 0.2 mm to >5 mm; Landry et al. 2001) and exhibit a large number of feeding modes. For example copepods are known to feed omnivorously, exhibit ontogenetic changes

in feeding strategy, feed on their competitors (intraguild predation) and can even be cannibalistic (Landry and Fagerness 1988). In the NPSG, this feeding diversity translates into opportunistic grazing on small adult zooplankton, copepodites, nauplii, phytoplankton and heterotrophic or mixotrophic components of the microbial food web (Hayward 1980; Calbet and Landry 1999). Given the breadth of mesozooplankton diets and the diversity of available prey, one might expect a priori that the zooplankton in this system would register as omnivores or vary in TP as food availability fluctuates. However copepods clearly align as either primary consumers or primary carnivores, and their trophic positions were remarkably constant regardless of season over a 5–10-yr period at Sta. ALOHA (Fig. 2).

Our low TP estimates for subtropical mesozooplankton are another unexpected finding of this study. $TP_{glu\text{-}phe}$ for the species examined ranged from 2.0 (*Oithona* spp.) to 3.0 (*E. rimana*). However, Calbet and Landry (1999) have documented that NPSG mesozooplankton feed on microplankton prey, which in turn graze at the top of a tightly coupled microbial food web containing at least three trophic levels (from picoplankton to <5 μm nanoheterotrophs to 5–20-μm nanoplankton). How, then, can we reconcile these multiple trophic linkages, and plankton diversity in subtropical food webs, with our apparently contradictory TP estimates from stable isotope analyses?

It is unlikely that primary consumers in the NPSG rely solely and directly on phytoplankton food sources, as would be implied by a strict interpretation of the *Oithona* spp. $TP_{glu\text{-}phe}$ of 2.0, for example. The picoautotrophs that dominate at Sta. ALOHA are too small for efficient mesozooplankton consumption (Calbet and Landry 1999), and mesozooplankton grazing on autotroph populations is low unless large phytoplankton bloom (e.g., during relatively rare events such as the passage of mesoscale eddies [Landry et al. 2008]). Mixotrophy should theoretically be important in the NPSG because of the competitive advantage it affords for acquisition of limiting N (Paffenhöfer et al. 2007). However, the extent to which mixotrophs might dominate microbial food webs at Sta. ALOHA, and thus provide a trophically mixed ^{15}N source to the food-web base, is unknown. More generally, the lack of significant isotopic enrichment for microzooplankton grazing pathways may be a characteristic of rapid and tightly coupled growth, grazing, and remineralization processes, as is suggested by the low ^{15}N discrimination found for continuous culture systems (Hoch et al. 1996). With only small amounts of new N entering the euphotic zone of the NPSG (Karl et al. 1996), rapid N recycling and the corresponding homogenization of N isotope values by the microbial food web could make it impossible to distinguish between consumers feeding on a combination of auto- and heterotrophic protists and those feeding exclusively on phytoplankton. Determining how mixotrophy or microbial N cycling relate to primary consumer trophic position in the oligotrophic open ocean is a rich and unexplored area for future research.

Our analysis thus indicates that the NPSG primary consumers identified by isotopic techniques should technically be considered omnivores, which feed on autotrophic, heterotrophic, and mixotrophic components of the microbial food web. This breadth of possible prey items likely contributes to the order of magnitude size range observed for zooplankton consumers at $TP_{glu\text{-}phe}$ 2.0–2.3 (Fig. 4). The largest of these, the euphausiid *Thysanopoda* spp., had a $TP_{glu\text{-}phe}$ 2.6 ± 0.04 in winter 1995 (Fig. 2), indicating opportunistic feeding on animal prey (Schnetzer and Steinberg 2002*a*). Switching between animal- and protistan-based food sources is one way in which larger zooplankton like *Thysanopoda* spp. may adapt their behaviors to relative prey abundances (Paffenhöfer et al. 2007). Different copepod food webs may also operate simultaneously in the NPSG. For example, we observed that N sources for *E. rimana* and *N. robustior* were significantly more ^{15}N enriched (high $\delta^{15}N_{phe}$ values) than those supporting *P. xiphias* and *Oithona* spp. in the winters of 1995 and 2000 (Fig. 1; Mann–Whitney *U*-test, $p < 0.05$). This indicates the simultaneous existence of different trophic pathways, which may derive from vertical stratification (McGowan and Walker 1979) or selective feeding on recycled sources (e.g., fecal pellets depleted by 1–9‰ relative to zooplankton $\delta^{15}N$ values [Tamelander et al. 2006]).

CSIA and food-web nitrogen source—Our AA isotopic analyses indicate that copepod TP was essentially constant over a 5–10-yr period at Sta. ALOHA (Fig. 2). The observed shift of only 0.2 trophic units for all copepod species translates into a change in bulk isotopic composition of 0.4‰, maximum, over a decade (given a TEF of 2‰). Such a temporal shift is indistinguishable from intra- and inter-tow differences in $\delta^{15}N$ values or ''isotopic static'' in the temporal record (*see* Results). Our conclusion is that significant ($\geq$0.5‰) changes in bulk copepod $\delta^{15}N$ values, at the species level, are solely caused by changes in N source at the base of the food web. Thus, time-series or spatial surveys of bulk copepod $\delta^{15}N$ values could be used as a record of change in nutrient isotopic composition at the base of pelagic food webs on a larger scale. Given the importance of understanding long-term climate impacts on nutrient delivery and marine ecosystem function, the use of copepod $\delta^{15}N$ time-series as proxies for change in N sources must be tested using both AA-specific and bulk isotope techniques in systems other than the NPSG.

Temporal change in bulk copepod and source AA $\delta^{15}N$ values at Sta. ALOHA indicate large seasonal fluctuations in N dynamics in an ecosystem once considered ''monotonous'' in comparison to dynamic regimes such as the California Current (McGowan and Walker 1979). On average, NPSG zooplankton $\delta^{15}N$ values are low (3–5‰) when compared with California Current zooplankton (10–14‰; Mullin et al. 1984; Rau et al. 2003), reflecting intense recycling of N in the subtropics and more nitrate-replete conditions in coastal upwelling systems. However our observed seasonal shifts in bulk N isotopic composition of $\sim$2‰ in the NPSG are significant when compared to the 2–3‰ changes observed for zooplankton in the California Current during El Niño conditions (Rau et al. 2003).

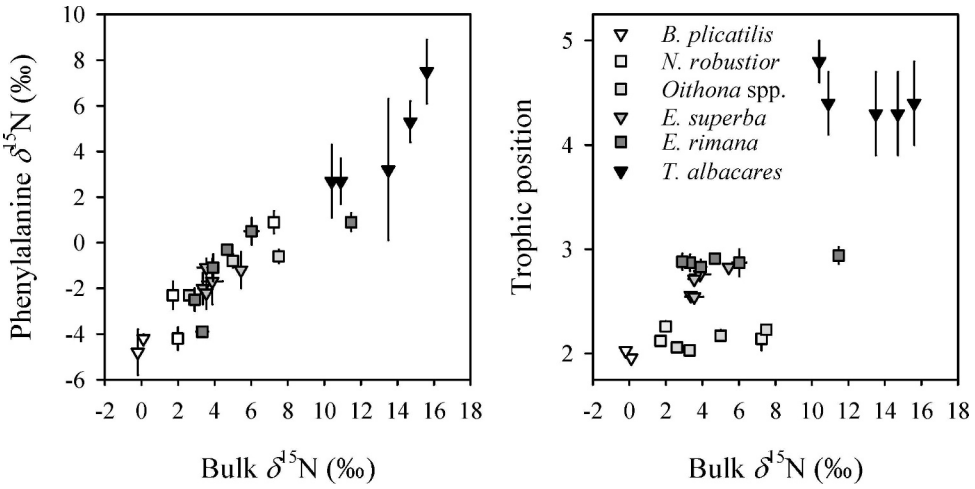

Fig. 5. Phenylalanine δ^{15}N values and trophic position (based on $\delta^{15}N_{AA}$ values) along a gradient of bulk tissue δ^{15}N values. Data for primary consumers (*Brachionus plicatilis*, *Oithona* spp., and *Neocalanus robustior*), an omnivore (*Euphausia superba*), a carnivore (*Euchaeta rimana*) and a top pelagic predator (*Thunnus albacares*) from this (squares) and previous studies (diamonds; McClelland and Montoya 2002; Schmidt et al. 2006; Popp et al. 2007) are shown.

Moreover seasonal changes in isotopic composition at Sta. ALOHA can be very large. For example, *E. rimana* bulk δ^{15}N values decreased by up to 9‰, and $\delta^{15}N_{phe}$ decreased by almost 5‰, between winter and summer seasons in 1995 (Fig. 1). Thus the magnitude of isotopic variability at Sta. ALOHA indicates significant intra-annual change in N sources at base of the NPSG food web.

Seasonal changes in new production (i.e., production supported by the entrainment of deep-water nitrate [NO_3^-] or the biological fixation of atmospheric dinitrogen gas [N_2; Karl et al. 1997]), likely drive our observed intra-annual shifts in N isotopic composition. These new nitrogen sources have distinct δ^{15}N values ($\delta^{15}N\text{-}NO_3^- \equiv$ 5–7‰ vs. $\delta^{15}N\text{-}N_2 \equiv 0$‰; Miyake and Wada 1967; K. Casciotti unpubl.), and seasonal changes in their relative contribution will affect the isotopic composition of particulate material and zooplankton (Dore et al. 2002). In the winter to spring, erosion of the upper pycnocline and deepening of isolumes facilitates entrainment of nitrate into the euphotic zone, whereas in the summer to early autumn the surface ocean stratifies and isolumes shoal into waters stripped of their nutrients (Letelier et al. 2004). N_2-fixation activity by diazotrophic plankton such as *Trichodesmium* spp. (Karl et al. 1997), *Richelia*-associated diatoms (Scharek et al. 1999), and unicellular diazotrophs (Montoya et al. 2004) also peaks during the late summer at Sta. ALOHA (Dore et al. 2002). Thus observed high-bulk and source AA δ^{15}N values during the winter (Fig. 1) indicates, as expected, that the relative importance of NO_3^- as a nutrient source increases during this time period (Jan–Apr). Correspondingly, low zooplankton δ^{15}N values in the late summer (Aug) indicate a greater reliance on N_2-fixation and recycled N sources. Phenylalanine δ^{15}N values for copepods during this time period range from −4.2‰ to −1.1‰, which bracket $\delta^{15}N_{phe}$ for the diazotroph *Trichodesmium* in cultures (−3.6‰; McClelland et al. 2003) and a *Trichodesmium* bloom at Sta. ALOHA (−2.8‰; this study, data not shown). Thus N_2-fixing organisms likely contribute significantly to NPSG zooplankton production in the summer, even if the primary route of N transfer from diazotrophs to the mesozooplankton community is through the microbial food web (Capone et al. 1997). Our data also indicates that new N from N_2-fixation likely stimulates the increase in zooplankton biomass that has been observed consistently during summer months at Sta. ALOHA over the past decade (Landry et al. 2001; Sheridan and Landry 2004).

In conclusion, our isotopic analyses of zooplankton at Sta. ALOHA demonstrate that compound-specific techniques provide a powerful new approach for assessment of marine food-web structure and function. By analyzing the trophic status of a number of species by both CSIA and bulk isotope techniques, we demonstrate for the first time that trophic position within the lower pelagic food web can be reliably quantified using source (phe, serine, glycine, and lysine) and trophic II (glu, alanine, aspartic acid) AA δ^{15}N values. Can this approach be used to evaluate food-web structure and N source in other ecosystems? A compilation of the available data for marine species demonstrates the potential wide application of this method (Fig. 5). We include animals from the subtropical and eastern-tropical North Pacific (this study; Popp et al. 2007), the Southern Ocean (Schmidt et al. 2006), and a culture system (McClelland and Montoya 2002). Source AA (phe) and bulk animal δ^{15}N values are significantly correlated (Pearson's $r = 0.94$, $p < 0.01$, $n = 29$; Fig. 5), and both reflect the dominant N transformation processes in each region (i.e., N recycling and N_2-fixation in the NPSG and denitrification in the eastern tropical Pacific). Despite the diversity in N source isotopic composition ($\delta^{15}N_{phe}$ of −4.8‰ to 7.5‰), animals can be positioned using trophic II and source AAs in a manner largely consistent with expectations regarding pelagic ecosystem structure. Our collection of published AA δ^{15}N values illustrates the

potential utility of CSIA for comparative studies of marine ecosystems in space and time, and further suggests our method may be widely applicable to questions bridging both aquatic and terrestrial food webs. Widespread application of CSIA techniques would benefit from more field and laboratory testing of predator and prey pairs, further evaluation of AA trophic enrichment factors, and a greater understanding of the effects of animal body condition and metabolic processing on ^{15}N enrichment of AAs.

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