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Environmental perturbation effects on baseline δ^{15}N values and zooplankton trophic flexibility in the southern California Current Ecosystem

Moira Décima,[a,*] Michael R. Landry,[a] and Brian N. Popp [b]

[a] Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California
[b] Department of Geology and Geophysics, University of Hawaii, Honolulu, Hawaii

Abstract

Nitrogen isotopic compositions of zooplankton in the California Current Ecosystem (CCE) are known to vary over inter-annual scales of climate variability, but the extent to which those changes are driven by variations in baseline phytoplankton δ^{15}N values vs. zooplankton trophic position (TP) is poorly resolved. We use field samples collected during a large natural environmental perturbation, the 1998–1999 alternation between El Niño and La Niña states, to test the ability of large dominant CCE zooplankton *Euphausia pacifica* and *Calanus pacificus* to alter their TPs in response to environmental variability. To distinguish trophic changes from variations of δ^{15}N values at the base of the food web, the zooplankton were assayed by Compound Specific Isotope Analysis of Amino Acids (CSIA-AA). Linear mixed-effect models were developed to utilize data from all amino acids (AAs), providing greater statistical power than the typical CSIA-AA approach of using only phenylalanine and glutamic acid. We confirm a significant ^{15}N enrichment of ~ 2‰ at the base of the food web for all AAs and all zooplankton groups during the 1998 El Niño. This baseline enrichment in ^{15}N has been speculated to occur during El Niño events but never conclusively shown. We also demonstrate a significantly elevated TP, implying increased carnivory during 1998, for *E. pacifica* while *C. pacificus* did not alter their TP between years. Lastly, TPs calculated from the standard CSIA-AA equation with laboratory-derived constants gave unrealistically low estimates, suggesting an assessment of these variables in situ is needed for an accurate application in natural systems.

Mesozooplankton occupy a central position in marine food webs between diverse assemblages of protists and microbes at the base of the web and larger metazoans, including human-exploited stocks, at the upper levels. As the first level of pelagic consumers with long histories of oceanographic sampling, taxonomic characterization, and archived collections, the mesozooplankton represent a rich source of integrative information about biomass and structural and biogeochemical responses of the food web to environmental variability. This information resides in the observed fluctuations of biomass and community composition in time-series sampling in many ocean systems (Beaugrand 2003; Lavaniegos and Ohman 2003; Mackas and Beaugrand 2010), as well as in the body composition, especially stable nitrogen isotopic composition, of preserved archived specimens (Rau et al. 2003; Chiba et al. 2012). In the southern California Current Ecosystem (CCE), for example, recent work using preserved historical collections has shown significant linkages between bulk tissue δ^{15}N values of zooplankton species (copepods and chaetognaths) and climate variability related to El Niño Southern Oscillation (ENSO; Ohman et al. 2012). However, the underlying mechanisms are ambiguous. Climate-related trends in zooplankton δ^{15}N values may simply reflect variations at the base of the food web from the source nutrient or metabolic fractionation of ^{15}N by phytoplankton. Alternatively, or in addition, species may alter their dietary compositions and trophic positions (TPs) within the food web as a consequence of changes in the size structure and availability of phytoplankton, either as a passive response to relative availability of alternate prey or an active switching between suspension-feeding and predatory feeding modes (Landry 1981). The extent to which climate variations can alter TPs of zooplankton in marine systems is important to understand because any expansions or contractions of trophic linkages will directly affect the efficiency of energy transfer in food webs, in addition to other climate effects.

Here, we use zooplankton samples collected during a natural environmental perturbation of the CCE region to test the capacity of two large dominant species, the copepod *Calanus pacificus* and the euphausiid *Euphausia pacifica,* to alter their TPs in response to environmental forcing. To distinguish trophic structure effects from shifts in the baseline δ^{15}N values, we used Compound Specific Isotope Analysis of Amino Acids (CSIA-AA). The CSIA-AA method is based on differential ^{15}N enrichment of AAs in organism tissues, with some AAs retaining a δ^{15}N value similar to the mean baseline value (*source* AAs), whereas other AAs are strongly enriched in ^{15}N with each trophic step (*trophic* AAs [McClelland and Montoya 2002; Hannides et al. 2009]). Studies using this method have typically focused on one AA each as representative of baseline (phenylalanine) and trophic (glutamic acid) values (McClelland and Montoya 2002; Chikaraishi et al. 2009). To enhance statistical power for hypothesis testing in the present application, we developed three-level Linear Mixed-Effect (LME) models that utilize the isotopic data from all AAs analyzed.

The large environmental perturbation that we studied was the 1998–1999 alternation between El Niño and La Niña states. During the 1998 El Niño, the southern CCE was characterized by warmer waters, higher stratification,

* Corresponding author: mdecima@ucsd.edu

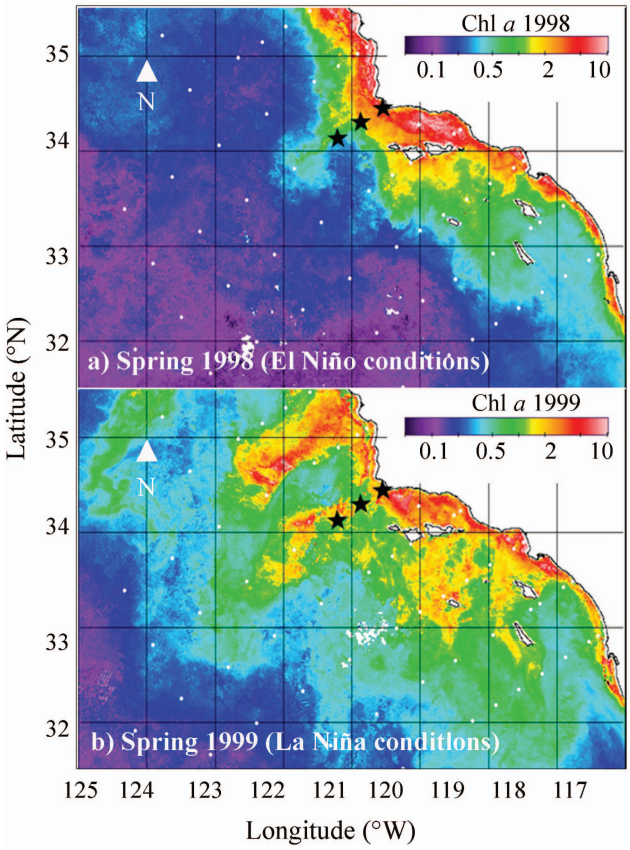

Fig. 1. Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua monthly averaged maps of surface Chl *a* for (a) April 1998, and (b) April 1999. Black stars indicate stations sampled, and white dots show the Southern California CalCOFI sampling grid. Figure courtesy of Mathi Kahru.

lower average chlorophyll *a* (Chl *a* [Bograd et al. 2000; Hayward 2000]), and the lowest zooplankton standing stocks measured over the previous 50 yr (Hayward 2000). In contrast, strong La Niña conditions during 1999 brought abnormally cold and high-nutrient waters, resulting in very high Chl *a* concentrations (Bograd et al. 2000; Kahru and Mitchell 2000), and a return of zooplankton standing stocks close to the long-term mean (Bograd et al. 2000). Although neither year is representative of normal CCE conditions, the dramatic contrast between adjacent years provided an excellent opportunity to determine whether measureable differences in zooplankton TP can be assessed using nitrogen isotope analyses in a natural ecosystem.

Methods

Zooplankton collection—Samples for CSIA were collected in the vicinity of Point Conception (California) as part of the California Cooperative Oceanic Fisheries Investigations (CalCOFI) time-series program (Fig. 1a, b). Routine sampling of zooplankton has been conducted in the southern region of the CCE since 1949, and standard CalCOFI protocols can be found online (www.calcofi.org). Briefly, zooplankton are collected using oblique tows with paired 0.71 m diameter Bongo frames fitted with 505 μm Nitex mesh nets. The Bongo net is lowered to depth at 50 m min^{-1}, allowed to settle for 30 s, and retrieved at 20 m min^{-1}. To achieve the target tow depth of 210 m, 300 m of wire are let out. Vessel speed, typically 2–4 km h^{-1}, is adjusted to keep the wire angle at 45° (± 8°), which is recorded every 10 m to estimate actual tow depth. Upon recovery, the net is rinsed and the codend samples preserved in 1.8% buffered formaldehyde.

Samples for the present study were collected at three nearshore stations on CalCOFI line 80: stations 80.55, 80.60, and 80.70 (Table 1). These stations were sampled on 15 April 1998 and 13 April 1999 (Fig. 1a, b). In 2008, after approximately a decade of temperature-controlled storage in the Scripps Institution of Oceanography Pelagic Invertebrates Collections, the preserved samples were sorted to obtain specimens of the two target species: the copepod *Calanus pacificus* and the euphausiid *Euphausia pacifica.* For each sample, we analyzed groups of 20 adult female and 100 copepodite stage Vs (CV) of *C. pacificus* separately. We restricted our euphausiid analyses to juveniles (5–10 specimens per sample), which presumably had N tissue contents that better reflected contemporaneous food conditions than did those of larger adults. Prior to the AA processing described below, each group was rinsed three times in Milli-Q water and dried at 60°C for 24 h. All specimens were gently handled and transferred using ethanol-cleaned forceps.

Long-term storage effects of formaldehyde-fixed specimens on $\delta^{15}N$ values of individual AAs have not been

Table 1. Summary of conditions at each station during 1998 and 1999, at each station sampled. Temperature, salinity, nitrate, and Chl *a* are surface values. Nitracline is defined as the depth at which the concentration of nitrate exceeded 10 μmol L^{-1}, and MLD is the approximate mixed-layer depth.

Year	Station	Distance (km)	Temperature (°C)	Salinity	Nitrate (μmol L^{-1})	Chl *a* (mg m^{-3})	MLD (m)	Nitracline (m)
1998	80.55	29	13.7	33.17	0.0	2.06	50	60
	80.60	61	14.2	33.10	0.1	0.37	70	85
	80.70	125	14.2	33.09	0.0	0.32	70	85
1999	80.55	29	11.8	33.67	9.8	3.15	20	20
	80.60	61	11.0	33.82	10.2	10.60	none	surface
	80.70	125	11.5	33.63	7.7	6.44	40	30

systematically studied. Rau et al. (2003) has shown, however, that 11 yr of storage of similarly preserved samples had no appreciable effect on the nitrogen isotopic composition of whole zooplankton. In addition, Hannides et al. (2009) has reported similar $\delta^{15}N$ values of individual AAs in unpreserved, frozen zooplankton (1–2 mm size fraction) and formalin-preserved copepods (*Euchaeta rimana,* 2.5 mm length) from the same net tows. Although the topic of the effects of storage on the $\delta^{15}N$ values of individual AAs needs to be investigated further, here we make the logical assumption that at least the relative differences in AA $\delta^{15}N$ values will be retained in samples preserved and stored similarly for a comparable period of time.

Hydrolysis and derivatization of samples for CSIA—Samples were prepared for CSIA as detailed in previous studies (Popp et al. 2007; Hannides et al. 2009; Dale et al. 2011). Prior to AA analysis, zooplankton specimens were subject to acid hydrolysis, esterification of the carboxyl terminus, and trifluoroacetylation of the amine group (Macko et al. 1997; Popp et al. 2007). Samples were hydrolyzed by adding Sequanal grade 6 mol L^{-1} HCl to each sample vial (containing 1–2 mg of zooplankton). Each vial was then flushed with N_2, capped with a Teflon-lined cap, and heated at 150°C for 70 min. Acid hydrolysis destroys tryptophan and cystine, and converts asparagine to aspartic acid and glutamine to glutamic acid. The resulting hydrolysate was evaporated to dryness under N_2 at 55°C, re-dissolved in 1 mL 0.01 mol L^{-1} HCl, purified by filtration (0.45 μm hydrophilic filter), and washed with 1 mL of 0.01 mol L^{-1} HCL. The hydrolysate was further purified using cation-exchange chromatography with a 5 cm column of resin (Dowex 50WX8-400) prepared in a glass Pasteur pipette. AAs were eluted with 4 mL of 2 mol L^{-1} NH_4OH and evaporated to dryness under a stream of N_2 at 80°C. Samples were then re-acidified with 0.5 mL of 0.2 mol L^{-1} HCl, flushed with N_2, heated to 110°C for 5 min, and evaporated to dryness under N_2 at 55°C. Hydrolyzed samples were esterified with 2 mL of 4 : 1 isopropanol : acetyl chloride, flushed with N_2, and heated to 110°C for 60 min. After drying at 60°C under N_2, the samples were acylated by adding 1 mL of 3 : 1 methylene chloride : trifluoracetic anhydride (TFAA) and heated to 100°C for 15 min. The derivatized AAs were further purified by solvent extraction. The acylated AA esters were evaporated at room temperature under N_2 and re-dissolved in 3 mL of 1 : 2 chloroform : P-buffer ($KH_2PO_4 + Na_2HPO_4$ in Milli-Q water, pH 7). Vigorous shaking ensured that the derivatized AAs were partitioned into chloroform and that contaminants remained in the P-buffer. The solvents were separated by centrifugation (10 min at 600 × *g*), the chloroform was transferred to a clean vial, and the solvent extraction process repeated. Finally, to ensure derivatization, the acylation step was repeated. Samples were stored at −20°C in 3 : 1 methylene chloride : TFAA for up to 6 months until isotope analysis.

Compound-specific isotope analysis (CSIA)—The nitrogen isotopic compositions of the TFAA derivatives of amino acids were analyzed by isotope ratio monitoring gas chromatography–mass spectrometry. We used a Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid nitrogen cold trap. The samples (1–2 μL) were injected (split–splitless injector, using a 10 : 1 split ratio) onto a Forte BPx5 capillary column (30 m × 0.32 mm × 1.0 μm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.4 mL min^{-1}. The column was initially held at 50°C for 2 min and then increased to 190°C at a rate of 8°C min^{-1}. Once at 190°C, the temperature was increased at a rate of 10°C min^{-1} to 300°C, where it was held for 7.5 min. Internal reference compounds, aminoadipic acid and norleucine of known nitrogen isotopic composition, were co-injected with samples and used to normalize the measured $\delta^{15}N$ values of unknown amino acids. All samples were analyzed at least in triplicate. The average standard deviation of the multiple runs per amino acid was 0.69‰, ranging from 0.01‰ to 2.5‰.

Statistical analysis: Linear mixed-effects (LME) models—For CSIA-AA, the number of machine runs (assays) was variable to resolve occasional analytical issues (e.g., optimize signal-to-noise ratio, minimize peak co-elution) and to improve analytical precision. The minimum and maximum number of assays per sample was 3 and 6, respectively, and standard deviations were typically ≤ 1‰ for each AA. We also analyzed replicate samples when possible (one-third of samples) to account for within-tow variability. The sampling design of this study is therefore multi-leveled (with precision variability arising from multiple machine assays within samples and ecological variability among replicate samples) and unbalanced (due to different number of machine and sample replicates). In order to account for both the different sources of variability and the unbalanced design, we used LME models for our analyses (Pinheiro and Bates 2000; West et al. 2007). These were coded in R using the 'nmle' package (R Development Core Team 2007), which can be freely downloaded through the R software (http://www.r-project.org). LMEs differ from the common generalized linear models (GLMs) by including, in addition to fixed-effects, random effects associated with one or more covariates. The fixed-effects parameters (later mentioned simply as parameters) are analogous to least-squares estimates of standard linear models. LME models are well-suited for dealing with correlated variables and have been used previously to combine analytical and replicate errors to obtain species population estimates (Lorrain et al. 2009).

We analyzed patterns in individual AAs using a two-level LME model in order to account for both machine and replicate variability. This approach is identical in concept to focusing only on phenylalanine and glutamic acid; yet, it uses the LME approach to handle more accurately the different sources of variability. The data were clustered into assays per AA (level 1) and AA per zooplankton group (level 2). We tested for differences within zooplankton groups in $\delta^{15}N$ values of phenylalanine ($\delta^{15}N_{phe}$), as well as the difference between the nitrogen isotopic values of

glutamic acid ($\delta^{15}N_{glu}$) and phenylalanine ($\delta^{15}N_{glu} - \delta^{15}N_{phe}$), a proxy for trophic position. We investigated the effect of year and station sampled, as well as the interaction of the two (stations by year). We used the 'step-up' model strategy—building our model by adding covariates one at a time, and conducting *F*-tests (for fixed factors) and likelihood ratio tests (for random effects and variance structures) at each step to determine fit improvements of the reference vs. nested models. For details on LME model building and diagnostics, *see* West et al. (2007).

As an alternative to using only two AAs to investigate baseline and trophic-structure variability, we developed a three-level LME to consider changes measured in all AAs. Given the multiple levels of data and the unbalanced design of the analyses, taking simple averages of the trophic and source AAs would be inappropriate. However, the three-level model for clustered data allows the main predictors of nitrogen isotope measurements to be determined for each zooplankton group. For this application, we used all AAs except for threonine, which displays a unique behavior and probably does not belong among the source AAs (Sherwood et al. 2011).

Data were clustered into three levels: assays (machine runs) within each AA (level 1, same as in the two-level LME), AAs within each sample (level 2), and samples within zooplankton group (level 3). The important difference in this model is the implementation of the 'AAs within sample' level as an added category of analysis. In the absence of any fixed factors, all AAs will vary randomly around a mean sample value. We know from previous studies that the expected $\delta^{15}N$ value of an AA will largely depend on its 'source' or 'trophic' grouping, so we included this difference as a fixed-effect parameter at the level 2 of analysis. Within this level of analysis, source AAs should vary randomly around their group mean and trophic AAs around a different mean that is significantly higher than the source group. Both parameters, the trophic enrichment and the random effects, are part of the model output. We considered fixed-effects associated with the years and stations sampled at the third level of analysis (zooplankton group). Finally, we added a parameter to investigate the interaction between year and trophic enrichment. Significance of this parameter indicates a significant between-year difference in enrichment of trophic AAs. As above, we used the 'step-up' model strategy, building our model by adding level 2, then level 3 covariates one at a time, and conducting likelihood ratio tests at each step to determine the improved fits of the reference vs. nested models (West et al. 2007).

To evaluate the validity of the model assumptions, we investigated the distribution of three types of residuals that emerged from the fitted models (Nobre and Singer 2007; West et al. 2007). The random effects at each level of clustering were estimated using the empirical best linear unbiased predictors (EBLUP). An explanation of how these estimators are obtained can be found in West et al. (2007) and further discussion of their properties can be found in Jiang (1999). We investigated normality of random effects associated with the different levels of clustering by looking at Quantile–Quantile (QQ) plots of the associated EBLUPs (the approximate real random effects), and we also investigated the normality of the residuals. We generated a 95% confidence envelope and tested for outliers using a two-sided outlier test. Sample effects (level 3) showed no significant departures from normality for any of the zooplankton groups. *C. pacificus* females had two outliers at the tail end of the distribution of the AA effects (level 2), both due to alanine estimates. Removal of these points and re-estimation of model parameters led to very similar estimates, differing by 3%. Model results for *C. pacificus* CV had EBLUPs (at level 2 and 3) and residuals (level 1) that were all normally distributed. Finally, QQ plots of the EBLUPS of level 2 (AA effects) for *E. pacifica* juveniles showed 5 outliers falling out of the 95% envelope at the high tail end of the distribution: alanine (3 outliers), glycine (1 outlier), and isoleucine (1 outlier). Removal of these outliers changed the year parameter by 7%. We interpret this analysis as indicating that other processes may be linked to AA ^{15}N content, notably to alanine, but are inadequately known to include in the model. Given that effects on parameter estimates and statistical significance were relatively minor after removal of these outliers, we consider the model an adequate representation of the *main* mechanisms shaping $\delta^{15}N$ values in the tissues of our zooplankton species. Lastly, we investigated normality of the residuals, which correspond to the random effects of the machine estimates for individual AAs at level 1 of clustering. Departures from normality of these values may arise from errors in chromatograph background estimations and corrections, leaving outliers that are not linked to ecological variability. Both *C. pacificus* females and *E. pacifica* had some estimates fall outside of the 95% envelope, which corresponded to some specific assays of specific AAs. Deleting these values changed the parameter estimates of *C pacificus* females by 2% and *E. pacifica* by 7%, and only involved deleting one of multiple machine runs for a specific AA (when necessary). Re-estimation of these parameters did not change any of the main results of this study. The estimates and *p*-values in Table 2 were obtained after the removal of outliers.

Trophic position calculation—Based on Chikaraishi et al. (2009), we calculated TP, using the difference in $\delta^{15}N$ of glutamic acid and phenylalanine, as

$$TP = \frac{\left(\delta^{15}N_{glu} - \delta^{15}N_{phe} - 3.4\right)}{7.6} + 1 \qquad (1)$$

where 3.4‰ is $\delta^{15}N_{glu} - \delta^{15}N_{phe}$ in aquatic primary producers, and 7.6‰ is the ^{15}N enrichment of glutamic acid with respect to phenylalanine at each trophic step (also called the trophic enrichment factor, TEF).

Results

Oceanographic conditions—Waters off the southern California coast were very different in the springtime of 1998 compared with 1999 (Fig. 1a, b). The nitracline (here defined as the shallowest depth at which nitrate concen-

Table 2. Amino acid stable nitrogen isotopic composition and trophic position (TP) for California Current zooplankton collected in 1998 and 1999. Some AAs values could not be obtained due to peak co-elution, indicated by 'nd.' Zooplankton groups included in the table are: *Calanus pacificus* CV, *Calanus pacificus* ♀, and *Euphausia pacifica* juveniles.

		Trophic AAs*							Source AAs*					
		Ala	Asp	Glu	IsoL	Leu	Pro	Val	Gly	Lys	Phe	Ser	Thr	
Zooplankton group	Year	δ^{15}N (‰±SD)‡	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	δ^{15}N (‰±SD)	$TP_{glu\text{-}phe}$†
C. pacificus CV	1998	18.4±0.5	15.4±0.3	17.3±0.5	13.9±0.8	12.1±0.6	15.9±0.1	16.4±0.8	10.7±0.4	8.9±0.2	7.7±0.2	6.4±0.7	4.3±0.5	1.8±0.4
C. pacificus CV	1998	19.2±0.7	15.5±0.5	17.8±0.8	13.4±0.9	11.5±1.3	16.4±0.3	15.5±0.5	10.2±1.2	7.8±0.9	8.0±0.2	6.9±0.2	4.7±0.4	1.9±0.5
C. pacificus CV	1998	19.1±1.2	15.4±0.6	15.6±1.0	15.5±0.6	12.9±0.6	15.4±0.5	17.2±0.6	10.2±0.7	7.2±0.5	6.0±1.6	11.5±0.5	−1.4±0.5	1.8±0.6
C. pacificus CV	1998	17.4±0.2	14.9±0.3	16.6±0.6	12.9±0.7	13.5±0.5	14.3±0.4	15.9±0.8	8.9±0.6	8.8±0.2	5.5±0.2	8.7±0.2	−1.6±0.7	2.0±0.5
C. pacificus CV	1999	15.9±0.7	11.4±1.2	14.4±1.5	10.8±0.4	7.0±1.0	10.8±1.3	12.6±1.2	6.9±0.6	4.5±0.5	3.7±0.6	4.4±0.9	−1.9±0.9	1.9±0.5
C. pacificus CV	1999	16.5±0.9	12.8±0.9	15.1±0.3	9.2±0.9	8.0±0.7	12.9±1.2	13.9±0.6	7.8±0.5	6.8±0.2	4.4±0.5	4.1±0.6	−3.3±0.9	1.9±0.4
C. pacificus CV	1999	18.8±0.2	13.6±0.5	16.4±0.2	12.0±0.5	9.5±0.7	14.5±0.4	14.3±0.7	9.3±0.6	5.7±0.9	6.1±0.7	5.9±1.2	1.0±0.5	1.9±0.5
C. pacificus ♀	1998	21.0±0.8	13.6±0.6	15.9±0.5	12.8±0.6	11.8±0.4	14.4±0.4	17.3±0.4	12.9±1.2	7.8±0.4	6.1±0.4	7.9±0.2	4.3±0.5	1.8±0.5
C. pacificus ♀	1998	20.6±0.6	14.5±0.2	16.9±0.9	14.8±0.5	13.3±0.1	15.8±0.4	18.1±1.0	13.1±0.9	7.1±0.3	5.4±1.7	9.7±0.6	5.3±0.4	2.1±0.6
C. pacificus ♀	1998	18.9±0.2	15.8±0.8	18.8±0.7	15.5±0.1	11.8±0.7	11.8±0.8	15.6±0.6	10.6±0.6	6.0±1.0	6.3±0.4	10.3±1.3	0.3±0.7	2.2±0.5
C. pacificus ♀	1998	15.3±0.7	14.1±0.7	11.4±1.8	13.6±0.7	10.1±0.7	9.4±1.6	12.2±0.7	8.4±0.8	2.7±1.7	2.1±0.9	3.6±1.6	−1.9±1.3	1.8±0.6
C. pacificus ♀	1999	20.4±0.3	12.6±0.3	nd	11.1±0.3	9.9±0.5	13.5±0.7	16.4±1.0	11.4±1.0	4.2±0.7	5.9±0.7	6.9±0.4	0.9±0.1	nd
C. pacificus ♀	1999	14.2±0.8	11.0±0.7	nd	13.4±0.9	6.0±0.9	7.7±1.5	10.6±0.9	6.2±1.1	0.3±0.9	0.9±0.4	6.7±0.1	−2.2±0.7	nd
C. pacificus ♀	1999	14.5±1.1	10.4±1.0	13.0±0.7	6.2±0.7	7.3±0.6	8.6±0.5	10.7±1.1	5.7±1.4	3.3±0.9	3.9±0.9	3.6±0.7	−3.3±0.5	2.5±0.5
C. pacificus ♀	1999	16.7±0.5	11.2±0.6	13.3±1.0	8.8±0.1	7.4±0.7	10.5±0.7	12.9±0.7	9.2±0.6	1.6±2.5	2.2±0.8	3.2±1.1	−0.2±0.6	2.0±0.5
C. pacificus ♀	1999	16.3±0.2	13.6±0.1	15.5±0.8	12.8±1.0	9.5±0.9	11.1±0.4	14.2±0.9	9.3±0.6	2.0±0.1	3.8±1.0	8.1±0.8	0.8±0.4	2.1±0.5
E. pacifica	1998	17.6±0.5	14.3±0.4	16.0±0.9	12.3±0.9	13.3±0.2	16.3±0.6	16.1±1.0	8.2±0.6	8.2±0.2	6.1±0.1	8.6±0.3	1.7±1.0	1.8±0.5
E. pacifica	1998	19.0±0.7	15.2±0.3	18.1±0.4	15.0±0.4	15.6±1.0	16.3±0.7	19.5±1.5	7.8±1.0	8.0±0.2	5.8±0.7	7.1±0.9	−1.1±0.7	2.2±0.5
E. pacifica	1998	19.6±0.3	13.1±0.7	15.7±0.6	15.2±0.6	14.9±0.7	13.0±0.5	16.4±0.5	10.3±0.4	4.2±0.5	7.6±0.9	6.3±1.0	0.4±0.8	1.6±0.6
E. pacifica	1998	22.7±0.5	16.7±0.5	19.9±0.3	14.8±0.7	15.6±0.7	16.5±0.8	15.7±0.7	10.2±1.0	8.9±0.7	6.3±0.5	7.0±0.4	−2.3±0.8	2.3±0.5
E. pacifica	1999	16.5±0.7	10.6±0.5	12.4±0.2	9.4±0.3	9.3±1.0	8.7±0.0	13.9±0.7	5.3±0.4	2.3±0.6	2.0±0.6	6.3±0.7	−0.8±0.5	1.9±0.5
E. pacifica	1999	nd	12.5±0.5	14.2±0.5	10.3±0.6	11.7±0.4	12.5±0.8	13.00	10.90	5.6±1.2	3.7±0.8	7.0±0.5	−1.2±0.8	1.9±0.5
E. pacifica	1999	16.7±0.8	12.1±0.4	13.0±0.5	7.3±0.8	9.9±0.3	12.5±0.7	12.8±0.5	7.7±0.9	4.3±0.8	4.9±0.5	6.0±0.8	−2.1±0.6	1.6±0.5
E. pacifica	1999	16.8±0.4	11.9±0.2	13.4±0.5	8.0±0.5	8.8±0.5	11.3±0.7	14.2±0.1	7.1±0.2	3.4±0.4	4.0±0.9	6.4±0.4	0.2±0.7	1.8±0.5

* Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; IsoL, isoleucine; Leu, leucine; Pro, proline; Val, valine; Gly, glycine; Lys, lysine; Phe, phenylalanine; Ser, serine; Thr, threonine.

† $TP_{glu\text{-}phe} = [(\delta^{15}N_{glu} - \delta^{15}N_{phe} - 3.4)/7.6 + 1]$.

‡ Standard deviation for multiple machine measurements.

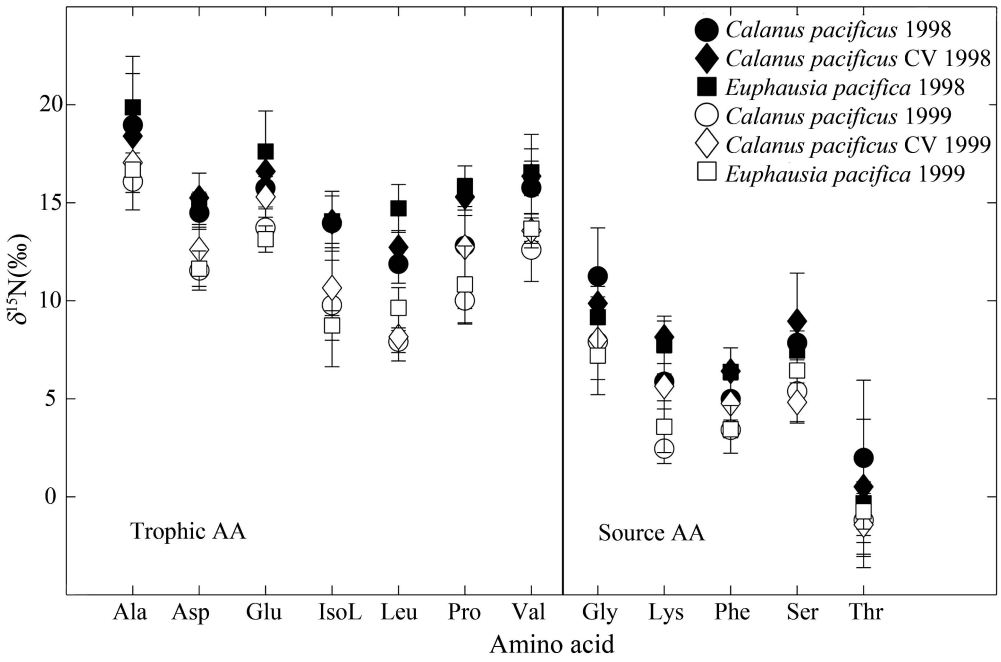

Fig. 2. δ^{15}N values of individual AAs for all zooplankton groups of this study. Amino acid abbreviations: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), isoleucine (IsoL), leucine (Leu), proline (Pro), valine (Val), glycine (Gly), lysine (Lys), phenylalanine (Phe), serine (Ser), and threonine (Thr). Filled symbols correspond to samples collected during the 1998 El Niño event, and open symbols correspond to 1999. Error bars are ± SD of multiple samples collected during each year.

tration exceeded 10 μmol L^{-1}) was much deeper at every station during 1998 compared with 1999 (Table 1), and was reported to be notably deeper than the long-term mean in the entire southern California Current region (Hayward 2000). Surface waters were warmer (2–3°C), with lower salinities and lower nitrate concentrations during 1998 vs. 1999 (Table 1). In contrast, surface waters were cold and salty in 1999 (Table 1), which indicates intense upwelling of high-nutrient waters, which led to shallower mixed layers and higher standing stocks of Chl *a* (Table 1) as well as a greater areal extent of surface waters with high chlorophyll (Fig. 1a, b) in 1999 compared with 1998.

Temporal variability in baseline δ^{15}N values in the CCE—The difference in AA δ^{15}N values between years was apparent for all zooplankton groups (Table 2; Fig. 2). The ^{15}N content was generally enriched during 1998 compared with 1999 for all AAs, regardless of where they fell in the source or trophic groups (Table 2; Fig. 2). The LME models for each zooplankton group showed improved fits when a parameter associated with year was included, confirming a statistically significant pattern of differential ^{15}N enrichment by year for all zooplankton groups. No significant spatial pattern (associated with the CalCOFI stations) was evident. The ^{15}N enrichment between years was ~ 2‰ for all zooplankton, with the LME year parameter equal to 2.8‰ and 2.6‰ for *C. pacificus* females and CVs, respectively, and 1.7‰ for *E. pacifica* (although none of these were statistically different from each other). Results from the LME models are detailed in Tables 3 and 4.

In previous studies using CSIA-AA, variability in baseline ^{15}N content has been investigated primarily from changes in the δ^{15}N values of phenylalanine. Here, the results for phenylalanine were inconsistent among groups. Female *C. pacificus* showed no visible or statistically significant difference ($p > 0.05$) between years (Table 2; Fig. 3). In contrast, mean δ^{15}N values for phenylalanine were significantly higher for *C. pacificus* CV in 1998, despite the small overlap between years, and the difference in δ^{15}N values of phenylalanine between years was even more pronounced for juvenile euphausiids (Table 2; Fig. 3). Patterns in phenylalanine ^{15}N content therefore suggest a change in baseline δ^{15}N for the younger stages of zooplankton (juvenile *E. pacifica* and *C. pacificus* CV), but not for adult females of *C. pacificus.*

Trophic structure and temporal variability in the CCE—Assessing TP based on ^{15}N differences in glutamic acid and phenylalanine (McClelland and Montoya 2002; Chikaraishi et al. 2009), we found no statistical differences in TP ($p > 0.05$) between years for any of the zooplankton groups (Table 2; Fig. 4). For each group, however, the highest TP estimate was from a sample collected during the 1998 El Niño (Table 2; Fig. 4). In absolute terms, calculated TP values were lower, on average, than expected for primary consumers, with approximately two-thirds of values falling below TP = 2 and approximately one-fourth of values above (Table 2; Fig. 4).

The three-level LME model, with an added interaction parameter between year and trophic enrichment, enhanced

Table 3. Fixed effects of the LME model results for the each group of zooplankton analyzed. The final model that predicts the δ^{15}N of any AA is given by: $\delta^{15}N = \beta_{intercept} + \beta_{year} \times year + \beta_{trophic} \times trophic_{AA} + \beta_{year\times trophic} \times trophic_{AA} + \mu_{sample} + \mu_{AA/sample} + \varepsilon_{run/AA/sample}$. β parameters represent fixed effects (covariates), while μ and ε represent random effects and residuals, respectively. Parameter estimates (± SE) are presented along with significance levels. Year sampled was represented as a categorical value: 1998 = 0, 1999 = 1. Trophic and source AAs were represented in the same manner: Source = 0, Trophic = 1. Year × trophic indicates the interaction parameter representing differential trophic enrichment per year. 'ns' indicates terms that did not significantly improve the model fit. Dashed lines are included when the LME parameter did not improve the model fit (i.e., was not significant). The final model used does not include values detected as outliers.

	C. pacificus ♀		*C. pacificus* CV		*E. pacifica* juveniles	
Parameter	Value±SE	*p*-value	Value±SE	*p*-value	Value±SE	*p*-value
$\beta_{intercept}$	7.65±0.92	<0.001	8.4±0.49	<0.001	5.9±0.64	<0.001
β_{year}	−2.8±1.13	0.04	−2.62±0.56	0.005	−1.66±0.9	0.0004
$\beta_{trophic}$	7.1±0.58	<0.001	7.14±0.52	<0.001	10.3±0.84	<0.001
$\beta_{year\times trophic}$	—	ns	—	ns	−2.4±1.1	0.03

our ability to resolve trophic enrichment effects compared with using only δ^{15}N values of glutamic acid and phenylalanine. Although the model did not provide calculated values of trophic position, using all AAs substantially improved the statistical power for hypothesis testing of inter-annual differences. Juvenile euphausiids were the only group that provided evidence of significantly elevated TP in 1998 (Table 3). Females and CVs of *C. pacificus* had remarkably similar parameters for both trophic and year variables (Table 3) and neither differed in TP between years.

Discussion

Baseline and trophic variability in the CCE—Variability in the N isotopic composition of phytoplankton at the base of the food web has been invoked as a mechanism in previous studies (Rau et al. 2003; Ohman et al. 2012) to explain variability in zooplankton bulk tissue δ^{15}N values in the California Current during ENSO years. Until now, however, it has not been possible to differentiate between nitrogen changes at the base of the food web vs. altered trophic structure. Using CSIA-AA, we unambiguously show a ^{15}N enrichment in baseline nitrogen during the 1998 El Niño. Increased nitrate utilization relative to supply during 1998 (Table 1) could have led to ambient NO_3^- enriched in ^{15}N due to preferential uptake of ^{14}N by phytoplankton (Waser et al. 1998) and Rayleigh distillation of the residual nitrogen pool (Wu et al. 1997). Phytoplankton uptake of NO_3^- from the ^{15}N-enriched pool would have resulted in higher δ^{15}N values that would propagate up the food web through their zooplankton consumers (Fig. 2; Table 2). Although we did not include 'normal' years in our study, our findings are consistent with previous studies linking this phenomenon to ENSO perturbations (Rau et al. 2003; Ohman et al. 2012).

Table 4. Standard deviations for random effects at different levels of clustering for each zooplankton group. $\sigma_{residual}$ indicates random effects due to machine error, σ_{AA} refers to variation in δ^{15}N of AAs not due to trophic enrichment, and σ_{sample} represents random effects at the sample level, unexplained by year effects.

	C. pacificus ♀	*C. pacificus* CV	*E. pacifica* juveniles
σ_{sample}	1.47	0.30	0.0004
σ_{AA}	2.73	2.16	2.14
$\sigma_{residual}$	0.96	0.72	0.86

The magnitude of both the El Niño and La Niña events constituted, in principle, an ideal scenario to test the flexibility of the plankton food web under varying environmental conditions. The warmest temperatures on record occurred during the height of El Niño in late 1997–1998, and the system was characterized by deep nitraclines and low surface Chl *a* (Table 1), changes in the populations of many marine organisms, and the lowest zooplankton standing stock of the past 50 yr (Hayward 2000). SSTs subsequently dropped in 1999 to low temperatures not seen in decades (3°C below normal), constituting an unprecedented rapid cooling driven by the record intensification of coastal upwelling (Schwing et al. 2000). The pervasive cooling that characterized the southern CCE region during 1999, with higher nitrate concentrations and shallower nitraclines, likely had an important effect on phytoplankton community size structure (Table 1). Previous studies in the region have shown that absolute biomass as well as the relative contributions of > 8 μm phytoplankton increase as the depth of the nitracline decreases (Mullin 1998). Given the preference of zooplankters for larger cells (Frost 1972),

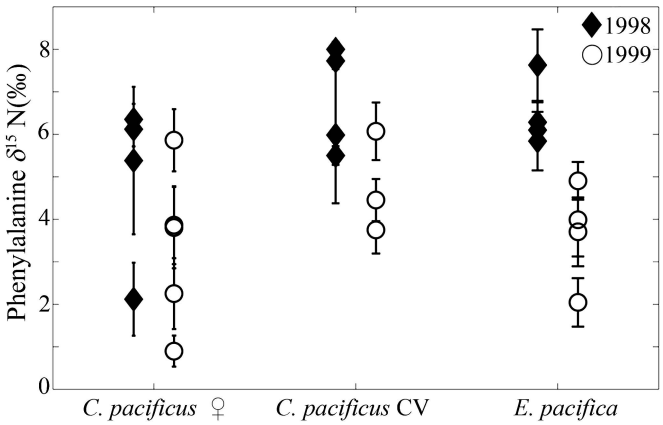

Fig. 3. δ^{15}N values of phenylalanine for all CCE zooplankton groups analyzed. Error bars are ± SD of multiple analytical runs.

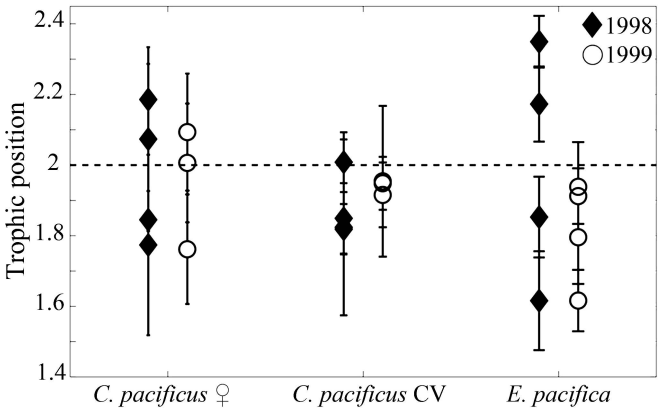

Fig. 4. Trophic positions (TP) of all zooplankton groups. Note that TP estimates are fewer than phenylalanine due to two glutamic acid values confounded by peak co-elution (Table 2). Dotted line represents the minimum position for a primary consumer (TP = 2). Error bars of estimates are ± SD of multiple analytical runs.

their ability to switch diets with changes in relative abundances of phytoplankton and animal prey (Landry 1981; Ohman and Runge 1994), and their tendency toward omnivory when phytoplankton are small (Calbet and Landry 1999), the large 1998–1999 ENSO perturbation provided a strong field test of the ability of CCE-dominant zooplankton to alter their TPs in response to environmental effects on the structure of lower trophic levels. The juvenile euphausiids did shift their TP significantly, consistent with our view of *E. pacifica* as an omnivorous zooplankter. These euphausiids are known to consume a variety a food items, including ciliates (Nakagawa et al. 2004), copepod carcasses (Park et al. 2011), and marine snow (Dilling et al. 1998). However, the magnitude of the LME interaction parameter was smaller than the trophic parameter, implying a TP change of < 1 full trophic level (Table 3). Thus, the change in the heterotrophic N contribution to their nutrition was not as dramatic as we might have expected from their dietary breadth.

In light of the large differences between years, the relative stability of *C. pacificus* TPs was surprising. *Calanus* spp. are known to be omnivores that opportunistically consume protozoo- and metazoan plankton (Fessenden and Cowles 1994; Ohman and Runge 1994). Trophic ^{15}N enrichment of protozoans with respect to phytoplankton could be low when their production, grazing, and nutrient re-mineralization is tightly coupled (Hoch et al. 1996), and Hannides et al. (2009) suggested this as the cause of the apparent invisibility of protozoans in TP estimates of zooplankton from the oligotrophic subtropical Pacific. This factor could explain the lack of an apparent trophic switch if we were considering protozoan prey alone. However, metazoan plankton (such as eggs and nauplii) have also been shown to be an important, albeit variable, source of nutrition for omnivorous copepods in the field (Bonnet et al. 2004). *C. pacificus* has been shown in a laboratory setting to remove nauplii at high rates (Landry 1980), and switch between predatory and suspension feeding modes when the relative abundances of phytoplankton and naupliar prey are varied (Landry 1981). In the present case, however, it could also be that the ratios of phytoplankton to alternate protistan or small metazoan prey in the appropriate size range for *Calanus* were not altered dramatically by the ENSO perturbation. If these food resources stay in reasonably comparable proportions (e.g., by dramatic El Niño suppression of egg and nauplii production that matches the decline in available phytoplankton food) the main perturbation effect may be the absolute decline in food biomass, rather than altered proportions of alternative prey. In addition, as discussed further below, it is possible that the timing of our samplings did not capture the full effects of the ENSO perturbations due to response lags.

Transient ENSO responses and isotopic steady state—It is known that El Niño episodes can cause 'transient responses,' with systems persisting in an altered state even after the perturbation has subsided (Overland et al. 2010). In terms of readily measured environmental variables at our sampling stations, temperatures, salinities, nitrate, and chlorophyll concentrations all point to a dramatic difference between years (Table 1), which would seem to discount lag effects. However, the relevant time scales of biological responses differ significantly, from days for phytoplankton to weeks or months for crustacean zooplankton (Vidal 1980, Ross 1982). Strong upwelling in the California Current in 1999 did not begin until April (Schwing et al. 2000). By mid-April then, the time of our sampling, ^{15}N contents of the zooplankton would likely not have reached steady state with newly produced food. Lagged responses in the $\delta^{15}N$ values of zooplankton body tissues to rapidly improving feeding conditions in 1999 could consequently have contributed to the relatively small trophic differences observed between years, and the lack of significant year effects in phenylalanine for female *Calanus.* Although we are unaware of any studies documenting the turnover time of AA ^{15}N specifically in zooplankton, laboratory-derived carbon-weight–specific growth rates of *C. pacificus* CV at temperatures encountered during this study (Table 1) are $\sim 10\%$ d^{-1} (Vidal 1980), while juvenile *E. pacifica* tend to grow much faster (20% d^{-1}; Ross 1982), which suggests that a lag effect (if present) would be more important for *C. pacificus.* However, because significant shifts in baseline $\delta^{15}N$ values were evident in body tissue AAs between years for all groups, clearly not all of the smaller trophic effects can be ascribed to response lags.

LME parameters (and their significance levels) provide some information about different food environments experienced in combination with tissue turnover times. In Table 4, σ_{sample} quantifies the level 3 variability among samples once the effect of the year sampled is taken into account. Juvenile euphausiids show the smallest σ_{sample}, followed by *C. pacificus* CVs, and finally *C. pacificus* females, which indicates increasing levels of heterogeneity among the samples in each one of these groups. Although this does not indicate non–steady-state conditions in zooplankton with respect to ^{15}N in phytoplankton *per se,* organisms residing in different areas of the coastal ocean probably experienced variable food levels associated with patchy resources, resulting in varying degrees of isotopic

steady state and larger heterogeneity among samples. The higher significance of the year parameter for *E. pacifica* (Table 3), and their lower σ_{sample} values (Table 4) suggest that they integrated environmental variability faster than CV and female *C. pacificus* during the study years, which is consistent with greater weight-specific growth rates.

Because both years were anomalous, we are not able to distinguish between a shift to higher TP during poor environmental conditions in 1998 vs. a shift to lower TP during more productive oceanographic conditions of 1999, or to determine whether a combination of the two occurred. Regardless, although the absolute magnitude and specific environmental drivers of the trophic shift were not fully resolved, this study was able to demonstrate significant trophic plasticity for zooplankton under natural field conditions using CSIA-AA, especially in combination with LME statistical models. To the extent that all variations in trophic position are measureable with the CSIA-AA method, the present results suggest that *C. pacificus* and *E. pacifica* responded differently to environmental perturbations in the CCE. *C. pacificus* appears mainly to live herbivorously without markedly altering its dietary preferences. In contrast, *E. pacifica* displays a more flexible dietary behavior. The ecological implications of this trophic flexibility may include a greater resilience to varying conditions in the field, aiding the survival of regional populations during less favorable environmental conditions and/or the ability to exploit favorable conditions more rapidly, as also suggested by the very high abundance of this species recorded in 1999 (Brinton and Townsend 2003).

Analytical methods—ENSO-related perturbations in the CCE are evident in the nitrogen isotopic composition of all zooplankton AAs (Table 2; Fig. 2). Although phenylalanine, usually considered the best indicator of source nitrogen (Chikaraishi et al. 2009), did not show this for *C. pacificus* females, the overall pattern for all AAs was consistent with enrichment of phytoplankton at the base of the food web (Figs. 2, 3). One possibility for reduced response of phenylalanine in *Calanus* females is mixing of food sources with different δ^{15}N values (mentioned above). Because adult copepods mainly express growth as egg production, we would expect slower turnover rates in body constituents and greater mismatch between contemporaneous δ^{15}N values in food and body tissues. This is suggested by the standard deviation of the LME random effects at the sample level for *Calanus* females (Table 4). Another plausible explanation involves ontogenetic changes in AA metabolism. For example, significant alterations in AA composition, specifically serine and phenylalanine, are associated with reproduction in freshwater zooplankton (Ventura and Catalan 2010). Statistical analyses, such as the LME model, that can use the isotope information from all AAs may consequently circumvent potential problems associated with any given AA, which differ in biochemical pathways among species (and stages within one species) and in their tissue turnover times. In addition, the random effects of these different groups might be indicative of the degree to which they have reached isotopic steady state with ambient food (a consequence of tissue turnover times). This parameter was orders of magnitude smaller for the juvenile euphausiids (Table 4); this is the only group for which we were able to detect a trophic shift (Table 3) and which have laboratory-derived growth rates that are at least double those of *C. pacificus* CV and females (Vidal 1980, Ross 1982).

TP calculation—Nonsymmetrical variability around the theoretical minimum TPs for large suspension-feeding zooplankton, as observed in our TP calculations (Fig. 4), is puzzling. As noted above, such animals readily feed on proto-zooplankton and small metazoans (Stoecker and Capuzzo 1990; Ohman and Runge 1994), which would imply a TP of no less than 2.0. Given errors due to analytical methods alone, we would still expect estimates to be symmetrically distributed around 2. The pattern observed therefore suggests a systematic underestimation of TP.

While the number of our observations is small (Table 2; $n = 22$ for TPs), underestimation of TP values for field-collected animals by the CSIA-AA method has been suggested elsewhere. For example, TP estimates for sharks and penguins based on stomach contents were invariably higher than, and sometimes double, the estimates obtained from CSIA-AA (Lorrain et al. 2009; Dale et al. 2011). Effects of urea retention on glutamate metabolism, TEF differences in different tissues, and interspecific differences in TEF are among explanations put forth to explain these discrepancies. None apply easily, however, to whole-body analyses of marine zooplankton, for which this method was first applied.

Our data consequently add to previous speculations that an assumed constant TEF—a 7.6‰ enrichment of ^{15}N in glutamic acid with respect to phenylalanine per trophic level—might not hold uniformly, or at all, under field conditions. Evidence exists that ^{15}N fractionation increases with decreasing similarity of AA composition between predator and prey (Ventura and Catalan 2010); therefore, the unnatural diets in laboratory experiments that provided this TEF may explain the reason that it performs less well in the field. To obtain TPs that are consistent with the known ecology of these zooplankters, this laboratory-derived parameter would have to be decreased by $\geq$ 1‰ (assuming average TP = 2) and up to 3‰ considering a mean zooplankton trophic level of 2.5. Regardless of the magnitude and cause of this discrepancy, it is clear from our results and previous studies that the universality of the TEF relationship needs to be more carefully evaluated if absolute TPs of organisms in their natural environments are to be obtained.

In summary, we found baseline ^{15}N enrichment in the CCE during the 1998 El Niño compared with 1999 La Niña conditions, which suggests that observed patterns of δ^{15}N values in bulk tissues for CCE zooplankton (Rau et al. 2003; Ohman et al. 2012) are driven mainly by changes in phytoplankton ^{15}N contents at the base of the food web rather than by marked alteration of dietary composition (enhanced carnivory) by zooplankton. Our analysis does confirm, however, that at least some large zooplankton species (*E. pacifica* but not *C. pacificus*) have sufficient dietary flexibility to alter their trophic position significantly

in response to environmental perturbation, although it is possible that insufficient time elapsed for us to observe the full extent of the trophic shifts. Detection of these results is mainly attributable to LME statistical models that utilize data from all analyzed AAs rather than limiting isotopic data to only two. Finally, zooplankton TPs calculated from the nitrogen isotopic compositions of glutamic acid and phenylalanine and laboratory-derived TEF estimates gave unrealistically low values for the field-collected animals, which suggests that a reassessment of the approach is needed for accurate application in natural systems.

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