



# Carbon and Nitrogen Isotopes from Top Predator Amino Acids Reveal Rapidly Shifting Ocean Biochemistry in the Outer California Current

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## Abstract

Climatic variation alters biochemical and ecological processes, but it is difficult both to quantify the magnitude of such changes, and to differentiate long-term shifts from inter-annual variability. Here, we simultaneously quantify decade-scale isotopic variability at the lowest and highest trophic positions in the offshore California Current System (CCS) by measuring  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of amino acids in a top predator, the sperm whale (*Physeter macrocephalus*). Using a time series of skin tissue samples as a biological archive, isotopic records from individual amino acids (AAs) can reveal the proximate factors driving a temporal decline we observed in bulk isotope values (a decline of  $\geq 1\text{‰}$ ) by decoupling changes in primary producer isotope values from those linked to the trophic position of this toothed whale. A continuous decline in baseline (i.e., primary producer)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values was observed from 1993 to 2005 (a decrease of  $\sim 4\text{‰}$  for  $\delta^{15}\text{N}$  source-AAs and  $3\text{‰}$  for  $\delta^{13}\text{C}$  essential-AAs), while the trophic position of whales was variable over time and it did not exhibit directional trends. The baseline  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  shifts suggest rapid ongoing changes in the carbon and nitrogen biogeochemical cycling in the offshore CCS, potentially occurring at faster rates than long-term shifts observed elsewhere in the Pacific. While the mechanisms forcing these biogeochemical shifts remain to be determined, our data suggest possible links to natural climate variability, and also corresponding shifts in surface nutrient availability. Our study demonstrates that isotopic analysis of individual amino acids from a top marine mammal predator can be a powerful new approach to reconstructing temporal variation in both biochemical cycling and trophic structure.

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**Data Availability:** The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

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## Introduction

The California Current System (CCS) contains one of the five major coastal upwelling zones in the world's oceans, and hosts a great diversity and abundance of marine life [1]. The oceanographic state of this large ecosystem is dynamic. Natural climate variation and anthropogenic stressors alter biochemical cycling, food web dynamics, and the fitness of species [1–3]. Known interannual and decadal changes are related both to the El Niño–Southern Oscillation (ENSO) and to basin-scale processes associated with the Pacific Decadal Oscillation (PDO) [1]. The latter, is an index of interannual sea surface temperature (SST) variability in the North Pacific, that is related to physical and biochemical variations and influences community changes in plankton, fish and other taxa [4,5]. In addition to this natural variability, humans have perturbed climate by increasing atmospheric  $\text{CO}_2$  concentrations, which have increased ocean temperatures, water column stratification, hypoxia, and water column

anoxia and have decreased surface ocean pH [6,7]. These environmental factors may negatively impact populations of species, increasing mortality and decreasing reproductive success due to habitat compression and metabolic constraints [8]. Other anthropogenic pressures, such as intensive fisheries and the past whaling industry (which principally targeted sperm whales, *Physeter macrocephalus*) might have triggered top-down effects. Given the lack of detailed proxy records to trace simultaneously biochemical baselines and length of food webs, assessing the extent to which biogeochemical cycling and community structure in pelagic ecosystems have changed over the past century is difficult, as is attributing change to natural cycles versus anthropogenic disturbances.

The isotopic values of marine primary producers are sensitive to environmental variation, such as change in temperature, and  $\text{CO}_2$  or nitrate concentrations, as well as biological differences such as physiology and growth rate [9–11]. Hence, the carbon and nitrogen isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively) of

primary producers, also known as “baseline isotope values”, vary in space and time as a function of these fundamental ecosystem properties [12]. Baseline isotope values are then integrated into consumers’ tissues through diet, typically with metabolic fractionation leading to enrichment in the heavier isotope (especially  $^{15}\text{N}$ ) in consumers [13,14]. Therefore, isotopic values of marine consumers could be used to reconstruct changes in diet and/or ecosystem biogeochemistry. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from a resident animal can potentially provide an integrated record of the biogeochemical characteristics of its habitat, as well as its trophic position [15]. However, because multiple factors influence the bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ultimately recorded in consumer tissues, it is often difficult to disentangle the effects of changing trophic position from shifts in baseline values.

Studies in different ocean basins have shown that bulk tissue  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values have declined over the last century, but interpretations of these trends have varied widely [16]. For example, declining bulk tissue  $\delta^{15}\text{N}$  values are sometimes attributed to a drop in consumer trophic level [17,18] or to baseline shifts due to either changes in foraging zone or biogeochemical cycles [16]. In particular, two recent studies in the Pacific have revealed pervasive declines in  $\delta^{15}\text{N}$  values in the offshore Central Pacific [18] and North Pacific Subtropical Gyre (NPSG) [19], but offered diametrically opposing interpretations as to underlying mechanism. In the highly productive CCS, despite accumulating evidence for oceanographic changes since the 1950s [2], isotopic data from plankton species have been contradictory. Bulk  $\delta^{15}\text{N}$  values from three zooplakton species have exhibited no long-term trends, whereas data for a specialized zooplankton feeder decreased by approximately 3‰ [20,21]. Declines in  $\delta^{13}\text{C}$  values over the 20<sup>th</sup> century are expected due to the combustion of fossil fuels (i.e., the Suess effect), and have been observed in many records and ecosystems [22]. However, variability in the magnitude and timing of  $\delta^{13}\text{C}$  declines has suggested that other factors, such as declining primary productivity, could also contribute in some regions [16]. In the offshore CCS, there are currently no  $\delta^{13}\text{C}$  time series for organic or inorganic material.

Isotopic analysis of individual amino acids (AAs) can effectively separate trophic effects from shifts in baseline isotope values [23,24]. Regardless of an animal’s trophic position, the original  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from primary producers are relatively well preserved within the group of ‘source-AAs’ for nitrogen [25] and the ‘essential-AAs’ for carbon [26]. In contrast, isotopic values from the ‘trophic-AAs’ for nitrogen, and ‘non-essential-AAs’ for carbon, undergo significant metabolic fractionation, and vary in association with a consumer’s diet [23,24], tissue turnover rates, and possibly metabolism [27]. Hence, isotopic analysis of amino acids from apex marine mammal predators offers a unique opportunity to simultaneously investigate temporal variation at the lowest and highest trophic levels of their food web. Sperm whales are top predators of the mesopelagic ocean. Mark-recapture studies, morphology, and acoustic analysis indicate that female sperm whales forage within the same oceanic region year round [28]. Consequently, they can function as natural biological samplers, broadly integrating biogeochemical information from their home ecosystem. In this study, we use sperm whale skin as a novel biological archive of time series data. Our data combine bulk tissue and AA isotope analysis to examine temporal variation in baseline values (reflecting ecosystem biogeochemistry) and whale trophic position (indicating trophic structure) from offshore waters of the California Current ecosystem.

## Results and Discussion

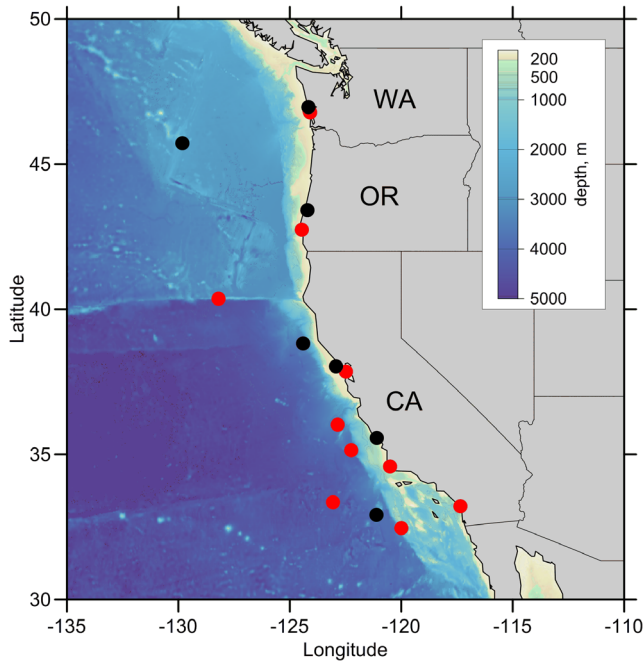
### Foraging zone of sperm whales sampled in CCS

In the CCS off the US west coast, sperm whales are found in oceanic waters from California to Washington [29]. Their habitat therefore excludes the coastal upwelling system that exhibits strong latitudinal isotopic gradients [30]. Mitochondrial and nuclear markers reveal that the CCS whales are an independent population and a single genetic stock [31]. Our isotopic data from skin biopsies (Figure 1) indicate that whales fed homogeneously within the offshore northern and central CCS. First, the variation in bulk isotope values ( $n = 18$ ;  $\text{SD} = 1.2\text{‰}$  for  $\delta^{13}\text{C}$  and  $1.2\text{‰}$  for  $\delta^{15}\text{N}$ ) is similar to the variation observed in other sperm whale populations that are considered to be resident (i.e., Gulf of Mexico and Gulf of California,  $\text{SD} \leq 0.8\text{‰}$  for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  [15]; SE Pacific,  $\text{SD} = 3.5\text{‰}$  for  $\delta^{15}\text{N}$  and  $0.7\text{‰}$  for  $\delta^{13}\text{C}$  [32]). In addition, the  $\delta^{15}\text{N}$  values for phenylalanine (Phe;  $n = 12$ ; mean (SD) =  $10.9\text{‰}$  (0.9)) are relatively consistent with expected nitrate and particulate organic matter  $\delta^{15}\text{N}$  values from the oceanic northern CCS ( $\sim 6$  to  $10\text{‰}$ ) [12], and also with published Phe  $\delta^{15}\text{N}$  values from muscle of the jumbo squid (*Dosidicus gigas*; potential prey of sperm whales) [33]. Phe  $\delta^{15}\text{N}$  values are a proxy for primary producer values [25] as they exhibit only minor  $^{15}\text{N}$ -enrichment with trophic transfer [23]. In top predators (such as sperm whales), this likely results in slightly higher Phe  $\delta^{15}\text{N}$  values versus baseline inorganic N sources. Lastly, because latitudinal trends in the  $\delta^{15}\text{N}$  values from predator source-AAs can indicate their geographic residency [24,33], the lack of any latitudinal variation in Phe  $\delta^{15}\text{N}$  values ( $r^2 = 0$ ;  $n = 12$ ) strongly suggests that the individual sperm whales sampled here were not foraging in different localized regions, but rather foraged over a broad latitudinal range within the northern and central CCS. While the isotopic incorporation rate for extremely large animals like whales is not well known, the thick skin of sperm whales likely integrates information for at least three and possibly more than six months prior to sampling [34]. Our data set encompasses information mainly from the fall and winter, except for the samples collected in 2001 and 2003, which also integrate information from the summer.

### Coupled decadal declines in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values

Bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in whale skin decreased from 1993 to 2005 by  $1.1\text{‰}$  and  $1.7\text{‰}$ , respectively. These decreases were statistically significant at the  $\alpha = 0.05$  level. Inclusion of a single sample available from 1972 further suggests possible longer-term temporal declines for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values by  $\geq 4\text{‰}$  and  $> 3\text{‰}$ , that are also statistically significant (Table 1). Together, these coupled time-series declines in bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values suggest coincident biogeochemical or trophic system perturbation (Table 1, Figure 2). In particular, the rate of decrease for bulk  $\delta^{15}\text{N}$  values since the 1970’s is at least five times greater than the rate for the long-term  $\delta^{15}\text{N}$  decrease recently documented in the central Pacific from proteinaceous corals ( $2.3\text{‰}$  in 150 years; annual decrease calculated at  $0.015\text{‰}$ ) [19], and it is more similar to the rate of change observed for a single zooplankton  $\delta^{15}\text{N}$  record from southern California ( $\sim 3\text{‰}$  in 50 years) [21].

To disentangle the factors driving the declines in bulk isotope values, we analyzed individual AA isotope values, focusing on AAs that have been demonstrated to track baseline changes (as noted above, essential AA for  $\delta^{13}\text{C}$  values, source AA for  $\delta^{15}\text{N}$  values). Linear regression models for average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from the most accurately measured essential- and source-AAs both exhibited strong negative temporal trends across all the data (i.e. for both 1972 and 1993 to 2005; Table 1, Figure 2), with drops of



**Figure 1. Sperm whales are distributed year-round in offshore deep waters (~>150 km off the US west coast [29]).** Skin samples (○) from free-ranging sperm whales were collected together with skin from stranded individuals. Tissue samples were used for bulk (in black) and amino acid (in red) stable isotope analysis. doi:10.1371/journal.pone.0110355.g001

$\geq 3\%$  and  $>4\%$  respectively indicated by compound-specific isotope data. Residuals for all regressions exhibited a random pattern. In contrast, average  $\delta^{15}\text{N}$  values for the trophic-AA were much variable, resulting in a lower  $r^2$ , but overall they paralleled the source-AA trend (Table 1, Figure 2A). These results are not consistent with any significant drop in sperm whale trophic level as the primary driver of decreases in bulk isotope ratios, and instead strongly implicate coupled changes in baseline  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values.

These negative trends in baseline  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values might relate to changes in biochemical cycling, rates of primary production, or primary producer species composition. In particular, the decline in average essential-AA  $\delta^{13}\text{C}$  values (Figure 2B), which are a direct proxy for primary producers, is far too high to be explained solely by the Seuss effect ( $\sim 0.2\%$  per decade since 1960 [35]), and it also coincides with the decline in average source-AA  $\delta^{15}\text{N}$  values. This suggests that the mechanism explaining a drop in primary producer  $\delta^{15}\text{N}$  values should be consistent with a concurrent large decline in  $\delta^{13}\text{C}$  values. One possibility, which would represent a direct analogy to changes in other ocean regions, would be a shift towards more oligotrophic conditions for the outer CCS. This explanation would be consistent with coupled declines in both isotopes, linked to decreased primary production and a shift in species composition that is typically associated with warmer and more stratified ocean conditions [36]. Oligotrophy in the world ocean is increasing due to climate shifts [37] and is projected to continue increasing in the North Pacific [38]. Recent isotopic records from deep sea proteinaceous corals, for example, provide strong support for such linked trends associated with warming of the NPSG [39]. The nitrogen isotope record from deep sea coral indicate that the long-term declines in baseline  $\delta^{15}\text{N}$  values are likely linked to progressive increases in seasonal gyre

extent, leading to steady increases in N contribution from diazotrophy [19]. Therefore, an analogous explanation would imply that oceanographic conditions in the offshore CCS region (which have conditions more similar to the open ocean and represent the base of sperm whales' food web) might have shifted toward more "gyre-like" conditions, driving baseline isotope values toward those more typical of the oligotrophic open ocean.

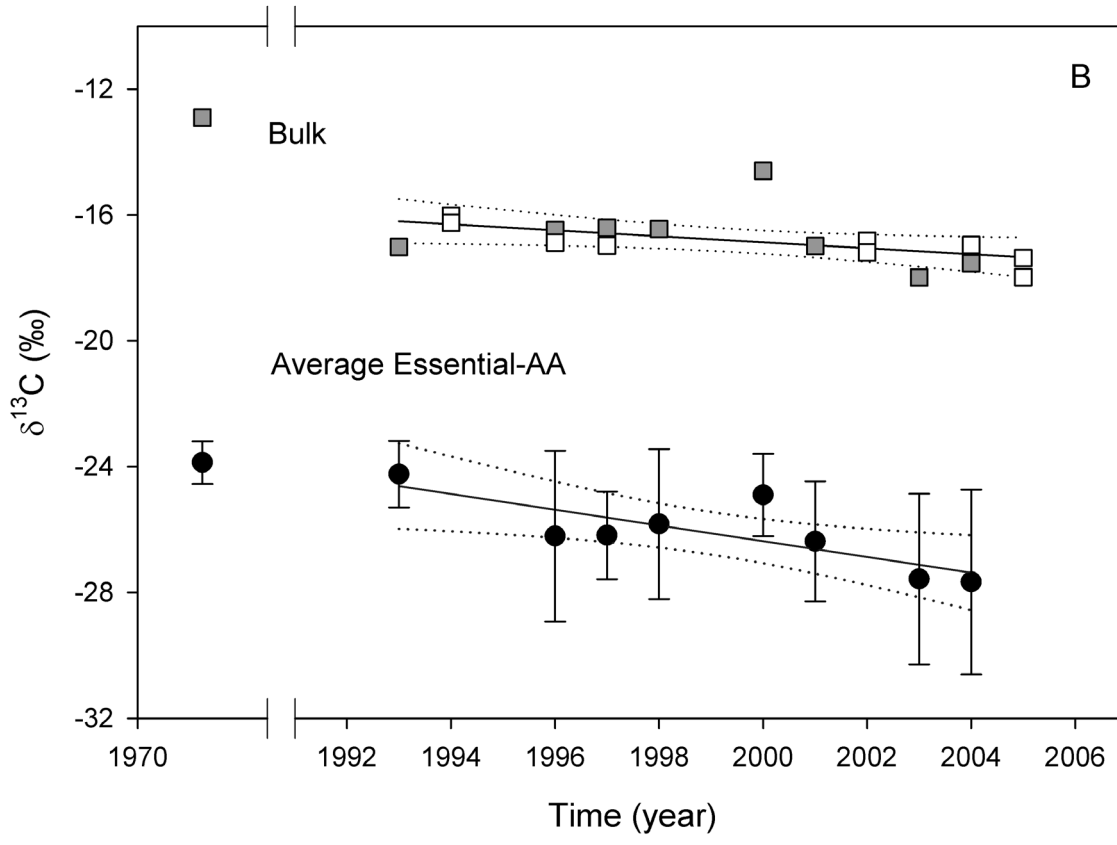
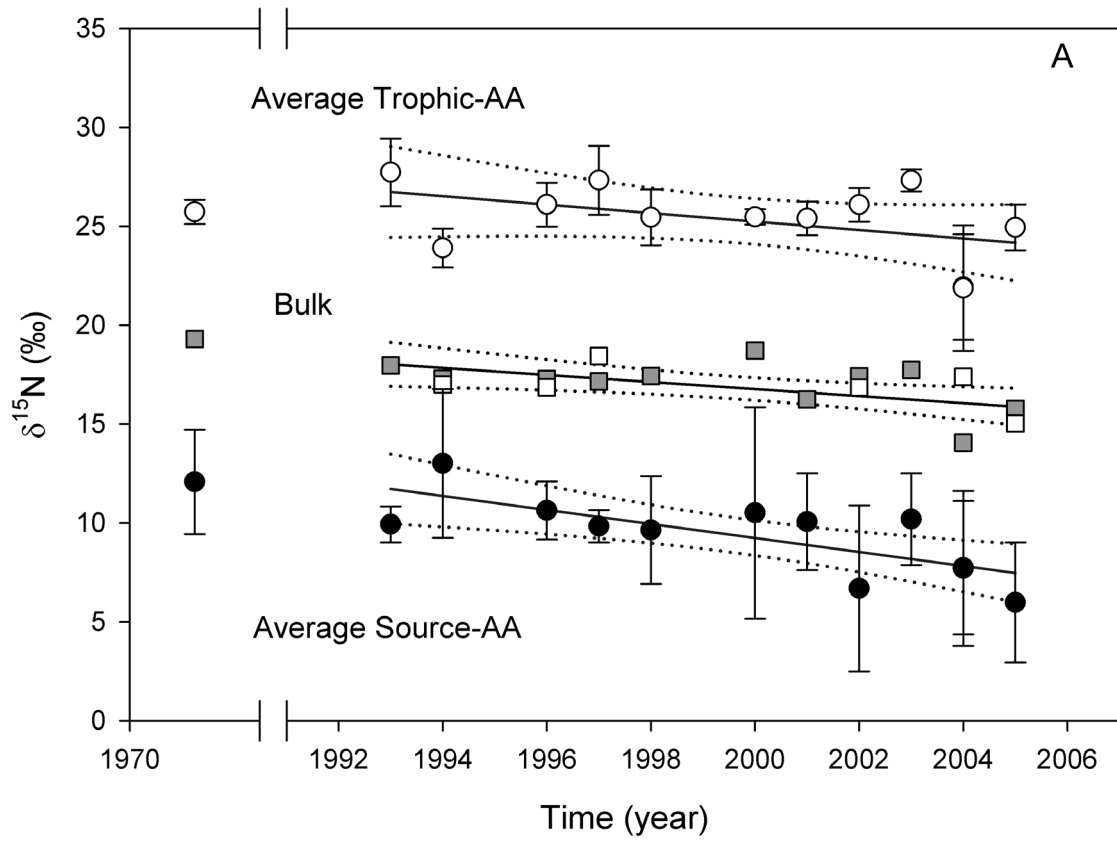
However, to our knowledge, there is currently no evidence for substantially increasing SST and diazotrophy in the CCS itself. Instead, recent analyses suggest largely the opposite: overall, the thermocline weakened and shoaled in the offshore CCS between 1950 and 1993 [7], possibly increasing nutrient availability in the euphotic zone despite increased stratification [40]. Additionally, the offshore CCS has cooled (not heated) since the early 1990s (Figure 3), and this trend is also reflected in the present "cool" PDO regime. Furthermore, the generalization that global warming will universally increase stratification and thus decrease surface nutrient supply has been recently challenged for some regions including the CCS [41]. For example, one recent model projects increases in nitrate supply and productivity in the CCS during the 21st century despite increases in stratification and limited change in wind-driven upwelling [42]. In the southern CCS, coastal surface nutrients have increased possibly linked to a general shoaling of the nutricline [43]. In the Southern California Bight, the most intensively monitored region of the CCS, nutrients in source waters have also increased over the last three decades, but the N:P and Si:N ratios were greatly reduced, possibly shifting phytoplankton species composition and abundance [44]. Whether or not these trends in nutrient dynamics extend to other regions of the CCS is unclear, because the oceanographic state of this ecosystem varies regionally [1,45].

In particular, shifts in offshore and onshore oceanographic conditions appear to be decoupled. Coastal upwelling has recently increased, as expected for enhanced alongshore winds [46], but has decreased offshore where upwelling is driven by wind-stress curl [47]. Since 1997, trends in satellite chlorophyll estimates, an index of phytoplankton biomass, have been positive in coastal upwelling waters but tend to be zero or negative in offshore waters [48]. Together, this current evidence indicates cooling, but not increases in productivity, in the offshore CCS concurrent with the observed 1993–2006 trends in sperm whale  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. Lower temperatures increase the solubility of  $\text{CO}_2$  and change the fractionation associated with carbon fixation, often resulting in lower phytoplankton  $\delta^{13}\text{C}$  values [49]; lower temperatures might have also changed phytoplankton growth and species composition. If surface nitrate also increased along the outer CCS region sampled by these whales, then the degree of nitrate utilization by primary producers (and so their  $\delta^{15}\text{N}$  values) could have also changed, since phytoplankton preferentially assimilate  $^{14}\text{NO}_3^-$  [50]. In general, proportional nitrate utilization is lower where surface  $\text{NO}_3^-$  concentrations are higher [50]. Therefore, lower  $\text{NO}_3^-$  utilization during seasonal upwelling might also be expected to depress the  $\delta^{15}\text{N}$  values of primary producers, propagating the  $^{15}\text{N}$ -depleted signal into food webs during their most productive periods. At present, there simply are not enough detailed data on nutrient concentrations and other oceanographic factors in the outer CCS to deduce a mechanism. However, the observed declining baseline values revealed by sperm whales do indicate a recently progressive shift in primary producer dynamics, likely associated with changes in SST, average state of surface nutrients and/or primary production.

**Table 1.** Temporal variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from the offshore California Current System in sperm whale skin samples.

Time period	Tracer	Linear Regression	n	$r^2$	p-value	Isotopic shift (%)	Annual decrease
1993–2005	$\delta^{15}\text{N}$						
	Bulk	$y = 302 - 0.143 * \text{year}$	17	0.25	<0.05	1.7	0.14
	Mean Source-AA	$y = 717 - 0.354 * \text{year}$	11	0.52	=0.01	4.2	0.35
1972–2005	Mean Trophic-AA	$y = 311 - 0.143 * \text{year}$	11	0.12	>0.05	1.7	
	$\delta^{15}\text{N}$						
	Bulk	$y = 218 - 0.101 * \text{year}$	18	0.37	<0.05	3.3	0.10
1993–2005	Mean Source-AA	$y = 298 - 0.145 * \text{year}$	12	0.39	<0.05	4.7	0.14
	Mean Trophic-AA	$y = 88 - 0.031 * \text{year}$	12	0.03	>0.05	1.0	
	$\delta^{13}\text{C}$						
1993–2005	Bulk	$y = 174 - 0.095 * \text{year}$	17	0.24	<0.05	1.1	0.09
	Mean Essential-AA	$y = 474 - 0.250 * \text{year}$	8	0.62	<0.05	3.0	0.25
	$\delta^{13}\text{C}$						
1972–2005	Bulk	$y = 242 - 0.129 * \text{year}$	18	0.67	<0.01	4.2	0.12
	Mean Essential-AA	$y = 184 - 0.105 * \text{year}$	9	0.58	<0.05	3.4	0.10

For mean calculations: Source-AAs are phenylalanine, glycine, lysine, tyrosine; Trophic-AA: glutamic acid, alanine, isoleucine, leucine, proline; Essential-AA: phenylalanine, valine, leucine. Isotopic shifts were calculated using the corresponding linear regression equations listed in this table. The annual decrease was calculated for shifts that exhibited a  $p$ -value  $\leq 0.05$ .  
doi:10.1371/journal.pone.0110355.t001



**Figure 2. Time series of isotopic data from sperm whale skin.** (A)  $\delta^{15}\text{N}$  values from bulk skin, average source-AAs and average trophic-AAs ( $\pm$  SD); and (B)  $\delta^{13}\text{C}$  values from bulk skin and average essential-AAs ( $\pm$ SD). Bulk isotope data are plotted with a square symbol ( $\square$ ), filled grey squares indicate the samples that were also analyzed for amino acid stable isotope analysis. The corresponding linear regression equations are provided in Table 1, as are the amino acids included within each AA-group. doi:10.1371/journal.pone.0110355.g002

### Implications of Rapid Change for offshore CCS Biogeochemistry

Although our time series data are limited for both elements, the compound-specific AA data identify a parallel decline in both baseline  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the outer CCS from 1992 to 2005, likely indicative of major recent shifts in biochemical cycling. At the same time, however, the overall similarity in whale trophic position signifies that the broad trophic structure is relatively unaffected. We note that in comparison with the recent deep sea coral data from the gyre offshore of this region [19], our data suggest that both the rate and scale of biochemical change on the CCS margin may be far greater than in the open Pacific Ocean. The coral record from the NPSG indicates a fairly steady  $\delta^{15}\text{N}$  annual decrease of  $\sim 0.015\text{‰}$  over the last 150 years with a total drop of 2.3 ‰ in exported primary production  $\delta^{15}\text{N}$  values over that period. In contrast, our molecular-level proxies for  $\delta^{15}\text{N}$  values at the base of the food chain (the source AAs) indicate more rapid annual declines of 0.35 ‰ since the 1990's. The independent molecular proxies for primary production  $\delta^{13}\text{C}$  values (the essential AAs) indicate relatively similar declines.

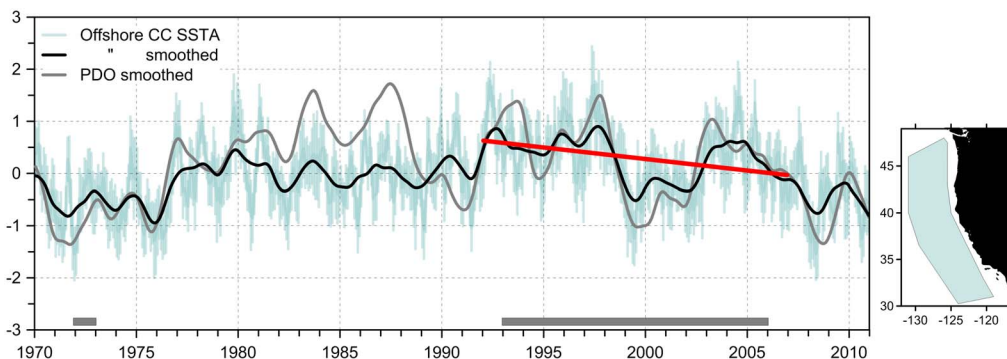
Together with the CCS observations discussed above, the contrast with the NPSG coral data (while not directly comparable in terms of time scale), suggests that despite the fact that baseline  $\delta^{15}\text{N}$  declines are observed in both data sets, different biogeochemical mechanisms may underlie the changes in these very different oceanographic regions. Climate variability likely affects the biochemistry of ecosystems differently depending on the oceanographic properties, microbial and phytoplankton communities, and species assemblages. In the eastern Pacific Ocean, the structure of the pycnocline varies strongly among the known biogeochemical provinces [51]. This likely influences geographic variation in surface nutrient availability, and therefore stable isotope ratios in POM, primary producers [12] and consumers [14]. Temporal trends in pycnocline depth, SST, stratification, and mixed layer depths also differ between these biogeochemical provinces [40]. For example, while SST decreased overall since 1958 in many parts of the California Current, SST increased in

the easternmost southern subtropical gyre and equatorial Pacific [40]. Ultimately, more detailed data that couple integrated measures of ecosystem baseline with oceanographic state will be required to understand the substantial biogeochemical changes our data indicate.

Our work highlights that detailed time-series of biochemical baseline and trophic structure records among different ecosystems will be crucial to identify rapid ecosystem shifts in response to climate change. In particular, in the face of uncertain coupling of natural and anthropogenic climate forcing, understanding the timing, extent and especially the mechanistic basis for baseline shifts now represents an urgent challenge. However, despite many efforts to unravel the linkage and feedback controls between the carbon and nitrogen cycles, and the effect of their variability on primary production and food-web dynamics, they are still not well understood. This study has demonstrated the great potential in coupling molecular isotopic tools with the unique bioarchive of sperm whales (or other top predators), as sentinels of offshore ecosystems. This may allow, for the first time, decoding of the factors that underlie temporal trends in bulk isotopic records, while simultaneously monitoring changes at both the highest and lowest trophic levels. We suggest that integrating this approach with detailed oceanographic data will be a major new tool to identify the effects of natural climate variability versus anthropogenic global warming on ecosystem biochemistry and primary production. Elucidating such patterns from this and other ocean margin regions, in particular their relationships with oceanographic and climatic variations and shifts in primary production, will be an essential part of the critical task of predicting future trends in both ecosystem biochemistry and trophic dynamics.

### Material and Methods

A total of 18 skin samples (Figure 1) were analyzed for bulk stable isotope analysis. Skin tissue samples with enough material (3.5 mg) were selected for CSIA-AA. Data from 12 samples were obtained for individual AA  $\delta^{15}\text{N}$  values, and 9 samples for  $\delta^{13}\text{C}$  values. The Southwest Fisheries Science Center/Pacific Islands



**Figure 3. Time series data of sea surface temperature anomaly (SSTA) from the offshore California Current (inset map) and the Pacific Decadal Oscillation (PDO).** Monthly SSTA was computed in 0.5-deg fields from the Simple Ocean Data Assimilation version 2.2.4 reanalysis ([http://coastwatch.pfeg.noaa.gov/erddap/griddap/hawaii\\_d90f\\_20ee\\_c4cb.html](http://coastwatch.pfeg.noaa.gov/erddap/griddap/hawaii_d90f_20ee_c4cb.html)), and then averaged in the offshore area (the plot shows  $\pm 1\text{sd}$ ). Monthly SSTA ( $^{\circ}\text{C}$ ) and PDO values were smoothed with a 25-month lowess smooth. The linear fit is for 1992–2006 (red line, slope  $-0.044^{\circ}\text{C y}^{-1}$ ). Sample periods are indicated along the time axis. doi:10.1371/journal.pone.0110355.g003

Fisheries Science Center Institutional Animal Care and Use Committee (IACUC) approved the original animal work that produced the samples. Sex was determined genetically using qPCR sexing assay by the PRD-Genetic Lab at NOAA [52]. These samples consisted of 5 females, 2 males and 2 unidentified individuals possibly corresponding to females or juvenile males. Large adult males were not included. Bulk isotope values were analyzed by continuous flow isotope ratio mass spectrometry (IRMS; Thermo Finnigan) and standardized relative to Vienna-Pee Belemnite (V-PDB) for carbon and atmospheric N<sub>2</sub> for nitrogen. Results are expressed in part per thousand (‰) and standard notation:  $\delta^H X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where H is the mass number of the heavy isotope, X is either C or N, and R<sub>sample</sub> and R<sub>standard</sub> are the ratio of <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N in the sample and standard, respectively.

We hydrolyzed and prepared approximately 3.5 mg of skin as well as a control (Cyano; bacteria tissue) [53] to quantify  $\delta^{15}\text{N}$  values from source- and trophic-AAAs and  $\delta^{13}\text{C}$  values from essential- and non-essential-AAAs. All derivatives were injected with an AA control, N-leucine, to verify accuracy during each run, and analyzed via gas chromatography-IRMS to obtain  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from individual AAAs. Each sample was run 3–4 times to maximize accuracy among chromatograms. The associated analytical error among replicates was <1.0 ‰. For all samples,  $\delta^{15}\text{N}$  values were obtained from a total of four source-AAAs (phenylalanine, glycine, lysine, tyrosine), and five trophic-AAAs (glutamic acid, alanine, isoleucine, leucine, proline) (Figure S1A). For  $\delta^{13}\text{C}$  values, the essential-AAAs that we consistently determined were phenylalanine, valine and leucine, and the non-essential-AAAs were alanine, proline, aspartic acid, glutamic acid and tyrosine (Figure S1B).

The relative pattern of AA  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values was highly consistent with past work from other organisms and tissues [23,25,54]. We grouped data as source- or trophic-AAAs for  $\delta^{15}\text{N}$  values, and essential- or non-essential-AAAs for  $\delta^{13}\text{C}$  values to increase power in the analysis and evaluate temporal variation. We calculated average values for each AA group and they are reported in Table S1. Regression analyses were conducted to evaluate linear relationship between time and each isotopic tracer for both bulk and individual-AA  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Table 1).

## References

1. Checkley JDM, Barth JA (2009) Patterns and processes in the California Current System. *Prog Oceanogr* 83: 49–64.
2. Bograd SJ, William JS, Barlow J, Broth A, Brodeur RD, et al. (2010) Status and trends of the California Current region, 2003–2008. *PICES Special Publication*. 106–141 p.
3. McGowan JA, Bograd SJ, Lynn RJ, Miller AJ (2003) The biological response to the 1977 regime shift in the California Current. *Deep Sea Res II* 50: 2567–2582.
4. Brinton E, Townsend A (2003) Decadal variability in abundances of the dominant euphausiid species in southern sectors of the California Current. *Deep Sea Res II* 50: 2449–2472.
5. Chavez FP, Ryan J, Lluch-Cota SE, Niquen CM (2003) From anchovies to sardines and back: Multidecadal change in the Pacific Ocean. *Science* 299: 217–221.
6. Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, et al. (2008) Emergence of anoxia in the California Current Large Marine Ecosystem. *Science* 319: 920.
7. Palacios DM, Bograd SJ, Mendelssohn R, Schwing FB (2004) Long-term and seasonal trends in stratification in the California Current, 1950–1993. *J Geophys Res* 109: C10016.
8. Bograd SJ, Castro CG, Di Lorenzo E, Palacios DM, Bailey H, et al. (2008) Oxygen declines and the shoaling of the hypoxic boundary in the California Current. *Geophys Res Lett* 35: L12607.
9. Farrell JW, Pedersen TF, Calvert SE, Nielsen B (1995) Glacial-interglacial changes in nutrient utilization in the equatorial Pacific Ocean. *Nature* 377: 514–517.
10. Rau GH, Sweeney RE, Kaplan IR (1982) Plankton <sup>13</sup>C:<sup>12</sup>C ratio changes with latitude: differences between northern and southern oceans. *Deep Sea Res* 29: 1035–1039.
11. Goericke R, Fry B (1994) Variations of marine plankton  $\delta^{13}\text{C}$  with latitude, temperature, and dissolved CO<sub>2</sub> in the World Ocean. *Global Biogeochem Cy* 8: 85–90.
12. Somes CJ, Schmittner A, Galbraith ED, Lehmann MF, Altabet MA, et al. (2010) Simulating the global distribution of nitrogen isotopes in the ocean. *Global Biogeochem Cy* 24.
13. Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. *Annu Rev Ecol Evol Syst* 18: 293–320.
14. Ruiz-Cooley RI, Gerrodette T (2012) Tracking large-scale latitudinal patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  along the eastern Pacific using epi-mesopelagic squid as indicators. *Ecosphere* 3: 63.
15. Ruiz-Cooley R, Engelhaupt D, Ortega-Ortiz J (2012) Contrasting C and N isotope ratios from sperm whale skin and squid between the Gulf of Mexico and Gulf of California: effect of habitat. *Mar Biol*: 1–14.
16. Schell DM (2001) Carbon isotope ratio variations in Bering Sea biota: The role of anthropogenic carbon dioxide. *Limnol Oceanogr Methods* 46: 999–1000.
17. Emslie SD, Patterson WP (2007) Abrupt recent shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in Adélie penguin eggshell in Antarctica. *Proc Natl Acad Sci USA* 104: 11666–11669.
18. Wiley AE, Ostrom PH, Welch AJ, Fleischer RC, Gandhi H, et al. (2013) Millennial-scale isotope records from a wide-ranging predator show evidence of recent human impact to oceanic food webs. *Proc Natl Acad Sci USA* 110: 8972–8977.
19. Sherwood OA, Guilderson TP, Batista FC, Schiff JT, McCarthy MD (2013) Increasing subtropical North Pacific Ocean nitrogen fixation since the Little Ice Age. *Nature* 505: 78–81.

There was a weak correlation between average source-AA and trophic-AA ( $r^2 = 0.13$ ;  $p = 0.67$ ), indicating that trophic-AA  $\delta^{15}\text{N}$  values could not be predicted by the variability in source-AAAs, and vice versa. However, the correlation between average essential-AA and non-essential-AA  $\delta^{13}\text{C}$  values was moderate ( $r^2 = 0.63$ ,  $p = 0.06$ ). Since the controls on isotopic patterns for non-essential-AA  $\delta^{13}\text{C}$  values are complex and dependent on diet quality and quantity, including *de novo* synthesis and routing of AAAs from diet-to-tissue, this group was not considered in the linear regression analysis.

## Supporting Information

**Figure S1 Stable isotope values of individual amino acids (AAAs) in skin samples of sperm whales (*Physeter macrocephalus*).** (A) Four  $\delta^{15}\text{N}$  Source-AAAs: phenylalanine (phe), glycine (gly), lysine (lys), tyrosine (tyr), and five Trophic-AAAs: glutamic acid (glx), alanine (ala), isoleucine (ile), leucine (leu), proline (Pro); and (B) Three  $\delta^{13}\text{C}$  essential-AAAs: phe, leu, and valine (val). (TIF)

**Table S1 Average values and one standard deviations (SD) were calculated for Source-AAAs (phenylalanine, glycine, lysine, tyrosine), Trophic-AAAs (glutamic acid, alanine, isoleucine, leucine, proline) and Essential-AAAs (phenylalanine, valine, leucine).** (DOCX)

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## Author Contributions

Conceived and designed the experiments: RIRC MDM. Performed the experiments: RIRC MDM. Analyzed the data: RIRC PCF. Contributed reagents/materials/analysis tools: MDM. Contributed to the writing of the manuscript: RIRC PLK PCF MDM.

20. Rau GH, Ohman MD, Pierrot-Bults A (2003) Linking nitrogen dynamics to climate variability off central California: a 51 year record based on  $^{15}\text{N}/^{14}\text{N}$  in CalCOFI zooplankton. *Deep Sea Res Part II* 50: 2431–2447.
21. Ohman MD, Rau GH, Hull PM (2012) Multi-decadal variations in stable N isotopes of California Current zooplankton. *Deep Sea Res Part I* 60: 46–55.
22. Sonnerup RE, Quay PD, McNichol AP, Bullister JL, Westby TA, et al. (1999) Reconstructing the oceanic  $^{13}\text{C}$  Suess Effect. *Global Biogeochem Cy* 13: 857–872.
23. Chikaraishi Y, Ogawa NO, Kashiyama Y, Takano Y, Suga H, et al. (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol Oceanogr Methods* 7: 740–750.
24. Popp BN, Graham BS, Olson RJ, Hannides CCS, Lott MJ, et al. (2007) Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. In: Dawson TD, Siegwolf, R. T. W., editor. *Stable isotopes as indicators of ecological change*. New York: Elsevier Academic Press. pp. 173–190.
25. McClelland JW, Montoya JP (2002) Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* 83: 2173–2180.
26. O'Brien DM, Fogel ML, Boggs CL (2002) Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. *Proc Natl Acad Sci USA* 99: 4413–4418.
27. Germain LR, Koch PL, Harvey JT, McCarthy MD (2013) Nitrogen isotopic fractionation of amino acids in harbor seals (*Phoca vitulina*): Differential trophic enrichment factors based on ammonia vs. urea excretion. *Mar Ecol Prog Ser* 482: 265–277.
28. Default S, Whitehead H, Dillon M (1999) An examination of the current knowledge on the stock structure of sperm whales (*Physeter macrocephalus*) worldwide. *J Cetac Res Manage* 1: 1–10.
29. Carretta JV, Forney KA, Lowry MS, Barlow J, Baker J, et al. (2010) U.S. Pacific marine mammal stock assessments: 2009. California, USA. 336 p.
30. Sigman DM, Casciotti KL (2001) Nitrogen Isotopes in the Ocean. In: Editor-in-Chief: John HS, editor. *Encyclopedia of Ocean Sciences*. Oxford: Academic Press. pp. 1884–1894.
31. Mesnick SL, Taylor BL, Archer FI, Martien KK, Treviño SE, et al. (2011) Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA. *Mol Ecol Resour* 11: 278–298.
32. Marcoux M, Whitehead H, Rendell L (2007) Sperm whale feeding variation by location, year, social group and clan: Evidence from stable isotopes. *Mar Ecol Prog Ser* 333: 309–314.
33. Ruiz-Cooley RI, Ballance LT, McCarthy MD (2013) Range expansion of the jumbo squid in the NE Pacific:  $\delta^{15}\text{N}$  decrypts multiple origins, migration and habitat Use. *PLoS ONE* 8: e59651.
34. Ruiz-Cooley RI, Gendron D, Aguiniga S, Mesnick S, Carriquiry JD (2004) Trophic relationships between sperm whales and jumbo squid using stable isotopes of C and N. *Mar Ecol Prog Ser* 277: 275–283.
35. Francey RJ, Allison CE, Etheridge DM, Trudinger CM, Enting IG, et al. (1999) A 1000-year high precision record of  $\delta^{13}\text{C}$  in atmospheric  $\text{CO}_2$ . *Tellus B* 51: 170–193.
36. Karl DM, Bidigare RR, Letelier RM (2001) Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: The domain shift hypothesis. *Deep Sea Res Part II* 48: 1449–1470.
37. Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. *Geophys Res Lett* 35: L03618.
38. Polovina JJ, Dunne JP, Woodworth PA, Howell EA (2011) Projected expansion of the subtropical biome and contraction of the temperate and equatorial upwelling biomes in the North Pacific under global warming. *ICES J Mar Sci* 68: 986–995.
39. Guilderson TP, McCarthy MD, Dunbar RB, Englebrecht A, Roark EB (2013) Late Holocene variations in Pacific surface circulation and biogeochemistry inferred from proteinaceous deep-sea corals. *Biogeosciences* 10: 3925–3949.
40. Fiedler PC, Mendelsohn R, Palacios DM, Bograd SJ (2012) Pycnocline Variations in the Eastern Tropical and North Pacific, 1958–2008. *J Climate* 26: 583–599.
41. Dave AC, Lozier MS (2013) Examining the global record of interannual variability in stratification and marine productivity in the low-latitude and mid-latitude ocean. *J Geophys Res-Oceans* 118: 3114–3127.
42. Rykaczewski RR, Dunne JP (2010) Enhanced nutrient supply to the California Current Ecosystem with global warming and increased stratification in an earth system model. *Geophys Res Lett* 37: L21606.
43. Aksnes DL, Ohman MD (2009) Multi-decadal shoaling of the euphotic zone in the southern sector of the California Current System. *Limnol Oceanogr* 54: 1272–1281.
44. Bograd SJ, Buil MP, Lorenzo ED, Castro CG, Schroeder ID, et al. (2014) Changes in source waters to the Southern California Bight. *Deep Sea Res Part II*. Available: <http://dx.doi.org/10.1016/j.dsr2.2014.04.009>.
45. McClatchie S (2013) Regional fisheries oceanography of the California Current System: the CalCOFI Program. Dordrecht: Springer. 253 p.
46. García-Reyes M, Largier J (2010) Observations of increased wind-driven coastal upwelling off central California. *J Geophys Res* 115.
47. Jacox MG, Moore AM, Edwards CA, Fiechter J (2014) Spatially resolved upwelling in the California Current System and its connections to climate variability. *Geophys Res Lett* 41: 3189–3196.
48. Kahru M, Kudela RM, Manzano-Sarabia M, Greg Mitchell B (2012) Trends in the surface chlorophyll of the California Current: Merging data from multiple ocean color satellites. *Deep Sea Res Part II* 77–80: 89–98.
49. Rau GH, Takahashi T, Marais DJD (1989) Latitudinal variations in plankton  $\delta^{13}\text{C}$ : implications for  $\text{CO}_2$  and productivity in past oceans. *Nature* 341: 516–518.
50. Wada E, Hattori A (1991) Nitrogen in the sea: forms, abundances, and rate processes. Boca Raton: CRC Press. 208 p.
51. Longhurst AR (2007) *Ecological Geography of the Sea*; Press. EA, editor. 542 p.
52. Morin PA, Nestler A, Rubio-Cisneros NT, Robertson KM, Mesnick S (2005) Interfamilial characterization of a region of the ZFX and ZFY genes facilitates sex determination in cetaceans and other mammals. *Mol Ecol* 14: 3275–3286.
53. McCarthy MD, Benner R, Lee C, Fogel M (2007) Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochim Cosmochim Acta* 71: 4727–4744.
54. Sherwood OA, Lehmann MF, Schubert CJ, Scott DB, McCarthy MD (2011) Nutrient regime shift in the western North Atlantic indicated by compound-specific  $\delta^{15}\text{N}$  of deep-sea gorgonian corals. *Proc Natl Acad Sci USA*.