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USING STABLE ISOTOPES TO INVESTIGATE FORAGING VARIATION AND HABITAT USE OF SPERM WHALES FROM THE EASTERN TROPICAL PACIFIC

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ABSTRACT

Using stable isotopes to investigate foraging variation and habitat use of sperm whales from the Eastern Tropical Pacific

by

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Female sperm whales (*Physeter macrocephalus*) are top predators in mesopelagic ecosystems, integrating chemical information about ecosystem structure through their diet. By studying proxies for diet and habitat use, we may be able to learn about how sperm whales' foraging and environment change through time. We measured stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) from individual growth layer bands from the teeth of 10 female sperm whales, to track changes in diet and habitat use from ca. 1930 to 1960, and to investigate relationships with major environmental events. While El Niño events can strongly affect food webs, particularly in the Eastern Tropical Pacific, we found no clear linkage between bulk $\delta^{13}C$ and $\delta^{15}N$ records and El Niño records, possibly due to high variability among dentinal isotopic records or weak effects of El Niño events during the 20th century. However, we found that bulk $\delta^{13}C$ and $\delta^{15}N$ records fall into three temporal patterns, suggesting distinct groupings of whales with clearly differentiated life-long foraging strategies. Average bulk $\delta^{13}C$ and $\delta^{15}N$ values for each tooth were positively correlated, and we found individual whales generally separated in isotopic space according to temporal pattern groupings.

To determine if whales from each temporal pattern foraged in different regions with distinct isotopic baselines, we measured $\delta^{13}C$ and $\delta^{15}N$ values from individual amino acids (AAs) in a subset of samples. Amino acid isotope results clearly indicate that the bulk isotopic trend is due to baseline differences, as opposed to differences in diet or ecosystem structure. Specifically, our results indicate that whales from each of our identified groupings used different geographic regions, but had similar trophic positions, because essential- and source-AA isotope values correlated with bulk isotopic values, while both non-essential- and trophic-AAs had no relationship to bulk $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Considering the bulk isotopic records together with CSIA data, we suggest that female sperm whales inhabiting the eastern Tropical Pacific likely had three different life-long foraging strategies under similar large-scale environmental constraints. Together, these results provide novel insight into social bonds among female sperm whales, since each social group shared the same habitat and diet over their life-time, but had separate trophic niches between adjacent social groups possibly due to environmental gradients.

DEDICATION

This thesis is dedicated to Meeko and The Dorthies et al. who spent many hours by my side as I was writing and kept me company during my journey through grad school.

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INTRODUCTION

Sperm whales (*Physeter macrocephalus*), the largest of the toothed whales, are found throughout most of the world's oceans (Rice 1989). Adult males typically forage at high latitudes, migrating to low latitudes during the breeding season (Rice 1989). Conversely, life-long units of related females and their young offspring are found in temperate, subtropical, and tropical zones and remain within their natal waters throughout their lives (Rice 1989). Stomach content analysis of hunted whales reveals that mesopelagic squid, primarily Humboldt squid, are the largest component of the diet of sperm whales from the south Eastern Tropical Pacific (ETP; Clarke et al. 1976). However, investigating the feeding ecology of highly mobile top predators such as sperm whales is challenging because they can be hard to track and follow in their remote habitats. Direct observation has provided valuable information about their foraging behavior (Whitehead & Hope 1991, Whitehead 1996, Whitehead & Rendell 2004, Mate & Ortega-Ortiz 2008), but collection of such data is limited by ship time.

Stable isotope analysis has been increasingly refined to study a wide range of ecological topics including food web structure, diet, and the movement of organisms within marine systems (Fry 1981, Hobson & Welch 1982, Rau et al. 1982, Minagawa &Wada 1984, Schell et al. 1989, Hobson 1999). The $\delta^{13}C$ values of marine primary producers (which set baseline $\delta^{13}C$ values) vary predictably with latitude and between onshore versus open ocean regions (Rau et al. 1982, Goericke & Fry 1994), and there is a relatively weak $\delta^{13}C$ increase of approximately 1‰ with trophic transfer (e.g.,

DeNiro & Epstein 1978, Peterson & Fry 1987, Post 2002). Thus, $\delta^{13}C$ values can be particularly useful as indicators of autotrophic carbon source, because they are often linked to foraging regions (e.g., Rau et al. 1982). In contrast, stable nitrogen isotope ($\delta^{15}N$) values are typically used to assess relative trophic structure of food webs, as they enrich in ^{15}N by approximately 3‰ with each trophic transfer (Minagawa & Wada 1984, Michner & Schell 1994, Post 2002). Yet as with carbon, phytoplankton $\delta^{15}N$ values (baseline $\delta^{15}N$) show strong spatial variation, reflecting a combination of nutrient source and plankton growth rate among other factors (Dugdale & Goering 1967, Altabet 2001, Montoya 2007, Somes et al. 2010).

Top predators integrate isotopic values throughout the food webs in which they fed. Therefore, isotopic values preserved in their tissues reflect variability at both the base of the food web and at the top predator's relative trophic position. For sperm whales, analysis of $\delta^{13}C$ and $\delta^{15}N$ values from soft and hard tissues has been used to identify main prey items, habitat use, migration, and dietary differences related to their complex social structure (Rendell & Whitehead 2003, Ruiz-Cooley et al. 2004, Marcoux et al. 2007a and b, Mendes et al. 2007a and b). In particular, for studies targeting the life history of an animal, teeth represent a long-term bio-archive of isotopic information. Sperm whales lay down annual growth layer bands (GLBs) throughout their entire lives, so teeth provide high-resolution life-history records of isotopic variation. Paired measurements of $\delta^{13}C$ and $\delta^{15}N$ values from GLBs can be used to identify foraging region, food web interactions, and possibly differences in

behavior between clans of sperm whales (Mendes et al. 2007a) and other odontocetes such as killer whales (Matthews & Ferguson 2014).

Teasing apart the relative influence of baseline and trophic effects on bulk $\delta^{13}C$ and $\delta^{15}N$ values of a consumer is difficult when samples from primary producers and prey items are unavailable. Compound specific isotope analysis of amino acids (CSIA-AA) is rapidly being integrated into ecological studies to address many inherent issues with bulk SIA. Specific amino acids (AA) or AA groupings are now commonly used as proxies to separate the relative contribution of baseline and trophic position to bulk isotopic values, effectively allowing both factors to be quantified from a single biological sample (Fantle et al. 1999, McClelland & Montoya 2002, Popp et al. 2007, Sherwood et al. 2014). For carbon, the side-chains of essential AAs (EAA) cannot be synthesized by most animals (e.g., Rawn 1983) and must be assimilated from diet. Therefore, $\delta^{13}C$ values of EAA do not undergo fractionation with trophic transfer and can be used as a direct proxy for $\delta^{13}C$ values at the base of food webs (e.g., Vokhshoori et al. 2014, Schiff et al. 2014, McMahon et al. 2015). Conversely, animals are capable of re-synthesizing the side-chains of the non-essential AAs (NEAA), so $\delta^{13}C$ values these AAs can be used to make inferences about metabolic processes and diet quality (McMahon et al. 2013). For nitrogen, 'source' AA $\delta^{15}N$ values ($\delta^{15}N_{Sr}$) remain essentially unchanged with trophic transfer, and thus reflect baseline $\delta^{15}N$ values (McClelland & Montoya 2002, Popp et al. 2007). In contrast, 'trophic' AAs ($\delta^{15}N_{Tr}$) become strongly ^{15}N-enriched with trophic transfer. The difference between trophic and source AAs ($\Delta\ ^{15}N_{Tr\text{-}Sr}$) can therefore be

used to assess change in trophic position. These methods have been applied to sperm whale skin from the California Current system (Ruiz-Cooley et al. 2014) and killer whale teeth from the arctic (Matthews & Ferguson 2014) to distinguish trophic effects from temporal variation in baseline values. As top predators in mesopelagic ecosystems, sperm whales not only exert top down control on oceanic ecosystems, but their trophic position and the chemical information preserved within their tissues represent a broad integration of mesopelagic food webs. Therefore, by studying stable isotope proxies for diet and habitat use preserved in their tissues, sperm whales may be used as indicators of how mesopelagic food webs change through time.

Because female sperm whales exhibit long-term site fidelity (Whitehead et al. 1997, Mate & Ortega-Ortiz 2008), stable isotope data from their teeth can be particularly useful for investigating changes over time within a specific habitat. Our isotopic study focused on female sperm whales from the south ETP (Fig. 1). The ETP is highly productive, accounting for approximately 10% of the global ocean's primary productivity, driven by a vast upwelling system that supports extensive fisheries (Fiedler & Talley 2006, Kessler 2006, Pennington et al. 2006). Productivity in this ecosystem is sensitive to the El Niño-Southern Oscillation (ENSO). El Niño events can have negative impacts on ecosystems because they can drive dramatic changes in nutrients, productivity, and food webs (e.g., Wang & Fiedler 2006).

The main objective of this study was to examine variability in isotopic records from sperm whale teeth to better understand foraging strategies and intra-specific feeding variability among female sperm whales from the ETP. Furthermore, we

analyzed a subset of dentin samples using CSIA-AA to differentiate baseline from trophic effects on patterns observed in bulk stable isotope records. These CSIA-AA data were then compared to an isoscape of $\delta^{15}N$ values derived from sediment core tops collected throughout the ETP (Tesdal et al. 2013) to identify possible foraging habitats of our whales. Complementary bulk and CSIA-AA $\delta^{13}C$ and $\delta^{15}N$ revealed long-term variability in female sperm whale diet or baseline values and were used to identify the presence of distinct foraging regions of female sperm whales from the ETP.

MATERIALS AND METHODS

Tooth collection. Between 1959 and 1960, Clarke (1968) recorded data such as length and sex of hunted sperm whales and collected biological samples such as stomach contents and teeth to examine diet (Clarke et al. 1976) and determine age (Clarke 1968), respectively. We selected 10 half-teeth from Paita, Peru for sampling based on the following criteria: 1) the final band around the pulp cavity was clear, distinguishable, and able to be sampled, 2) the individual was at least 10 years old at time of death, and 3) GLBs were clear throughout the tooth.

Tooth Preparation. The 10 half-teeth were polished with subsequently decreasing carbide powder grit to reveal GLBs (Ruiz-Cooley and Herzo, *unpublished*). Briefly, the polishing lap was wetted, liberally dusted with carbide powder, then the inside surface of each tooth was moved against the lap in a back and forth motion. To ensure even polishing, teeth were periodically rotated by 180˚. A

different polishing lap was used for each grit to avoid contamination. Teeth were washed in a Branson 3510 ultrasonicator for 10 minutes between each grit and again once polishing was completed.

After polishing, teeth were mounted onto 2x3 in glass slides by their outer surface using Crystal Bond, a synthetic substitute for Canada balsam which can be removed from surfaces by heating, chipping, or acetone upon completion of sampling. To mount each tooth, Crystal Bond chips were placed on a glass slide, which was then set on a hot plate at ~140˚C until completely liquid. The glass slide was then removed from the hot plate and the tooth placed on the Crystal Bond and held in place until it solidified again. Wooden beverage stirrers were stacked together and used as supports when necessary. A bubble level was set on the sampling surface of each tooth to make sure the polished surface was level.

Images of teeth were printed on 8.5x11 in paper at a resolution of 720 pixels/in to identify and count GLBs. Auxiliary bands, banding in excess of the typical one dark and one light band per year pattern, and neonatal regions, distinguished by banding that is markedly narrower than bands leading up to this region, were also identified. Distinguishing features, such as osteodentin deposits, cracks, and chips, were used as reference points when comparing enlarged images to actual teeth. Neonatal and weaning period bands, identified by their $\delta^{15}N$ values and band widths, were excluded from this study, because they represent periods during which whales were dependent on their mothers for food, but are identified and plotted in Fig. S1.

Sample collection. Each GLB was sampled using a New Wave Research micromill paired with an Olympus SZ61 microscope and fitted with Brasseler carbide drill bits ranging in size from 0.4 mm to 1.2 mm, depending on GLB width. GLB widths were measured using a 90˚ guide to ensure consistency. Recorded widths were used to discriminate auxiliary bands from annual growth layer bands, for example, if the width of a particular band was substantially smaller or larger than the previous band. Sampling depth was restricted to 600 μm to avoid milling into the next GLB.

Bulk Stable Isotope Analysis. Dentin aliquots of 1600 +/- 5μg from each band were weighed into 5x9 mm tin capsules (Costech, Valencia, CA, USA) in preparation for stable isotopic analysis following Brault et al. (2014). Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ values in the Stable Isotope Laboratory at the University of California, Santa Cruz on an EA 1108 elemental analyzer (Carlo Erba, Milan, Italy) coupled with a Thermo Finnigan Delta^{Plus} XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The $\delta^{13}C$ values were referenced to Vienna-Pee Dee Belemnite (V-PDB), while $\delta^{15}N$ were referenced to atmospheric N_2. Internal standards PUGEL and Acetanilide were used. Weighing surfaces and tools were wiped down with Kimwipes and methanol between each sample. Tins were folded and crushed into cubes and placed in 96 well plates in a desiccator for storage until analysis.

Compound Specific Stable Isotope Analysis of Amino Acids. Six whales, two from each pattern (described below), were selected for further investigation. For five whales, 2 mg of dentin from GLBs corresponding to the years 1948 to 1952 were

combined to form composite samples. For one whale, 3.33 mg dentin from GLBs corresponding to the years 1950 to 1952 were combined. These GLBs were selected because mean values from this range of years was representative of the observed pattern and spread of average tooth isotopic values.

These six dentin samples were demineralized with 0.25N HCl and prepared for CSIA-AA as described in Brault et al. (2014). Demineralization is critical to remove the inorganic constituents of dentin, which inhibit analysis by gas chromatography isotope-ratio mass spectrometry (GC-IRMS). The remaining collagen was hydrolyzed using 6M HCl for 22 hr at 110˚C and then stored in a 4˚C freezer. For acetylation and derivitization, HCl was evaporated under a stream of nitrogen gas until the samples were dry. Individual AAs were converted to trifluoroacetic acid anhydride derivatives following Silfer et al. (1991) and their $\delta^{13}C$ and $\delta^{15}N$ values were measured using a Thermo Trace gas chromatograph coupled to a Thermo Finnigan Delta^{Plus} XP isotope ratio mass spectrometer (oxidation furnace at 940˚C for carbon or 980˚C for nitrogen and reduction furnace at 630˚C for carbon or 650˚C for nitrogen). For $\delta^{13}C$ analyses, a DB-5 column (50 m x 0.32 mm, 0.52 μm film thickness; Agilent Technologies, Santa Clara, CA, USA) was used. For $\delta^{15}N$ analyses, a BPX5 column (60 m x 0.32 mm, 1 μm film thickness; SGE Analytical Science, Trajan, Austin, TX, USA) was used. Using this approach, the $\delta^{13}C$ and $\delta^{15}N$ values of the following AAs could be reproducibly quantified in all six samples of sperm whale dentin: alanine (Ala), aspartic acid + asparagine (Asp), glycine (Gly), glutamic acid + glutamine (Glu), isoleucine (Ile), leucine (Leu), lysine (Lys),

phenylalanine (Phe), proline (Pro), serine (Ser), and valine (Val). We were able to measure the $\delta^{15}N$ value of threonine (Thr), but not its $\delta^{13}C$ value. Co-elution of Val with Ser prevented accurate peak integration, thus $\delta^{13}C$ values calculated for these AAs were unlikely to be correct and were excluded from our results. Throughout this paper, we present glutamic acid + glutamine as Glu and aspartic acid + asparagine as Asp, which is common in CSIA-AA literature. However, note that some studies report these amino acids as Glx and Asx, respectively.

Analysis of data. To ensure that we analyzed only GLBs reflecting whale diet consumed from the ecosystem, we did not use isotopic values from the neonatal bands and first 3 to 4 years of each whale's life since these bands include information from gestation and nursing. We used correlation analysis to identify any long term trends that were consistent between individual whales. These trends were analyzed as a function of age to investigate ontogenetic patterns in stable isotopes. To look at the influence of El Niño events on isotopic variability, we calculated yearly averages, 95% confidence intervals, and variance, and then compared these parameters between El Niño and non-El Niño years (Quinn et al. 1987) using Student's t-test ($a = 0.05$).

We also calculated average $\delta^{13}C$ and $\delta^{15}N$ values along with standard deviation for each whale. The relationship between average $\delta^{13}C$ and $\delta^{15}N$ values was compared using linear regression analysis. CSIA-AA was used to identify the underlying cause of the relationship between bulk carbon and nitrogen values. We used linear regression analysis to compare $\delta^{13}C_{EAA}$ to bulk $\delta^{13}C$ values, and $\delta^{15}N_{Sr}$ and $\Delta^{15}N_{Tr\text{-}Sr}$ to bulk $\delta^{15}N$ values. To constrain the possible foraging locations of

these whales, we compared the $\delta^{15}N$ values of Phe from six whales to an isoscape constructed from published sedimentary records from the ETP (Tesdal et al. 2013).

RESULTS

Bulk stable isotope analysis. We sampled approximately 232 GLBs from the teeth of 10 female sperm whales. Individuals ranged from 12 to 34 years of age, with the oldest dating back to approximately 1926. Isotopic values averaged across all 10 teeth in this sample set were -11.5 ± 0.4‰ for $\delta^{13}C$ and 15.1 ± 0.6‰ for $\delta^{15}N$. Averages for all bands within individual teeth ranged from -12.1 ± 0.2‰ to -10.7 ± 0.3‰ for $\delta^{13}C$ values and from 14.4 ± 0.5‰ to 15.8 ± 0.4‰ for $\delta^{15}N$ values.

Correlation analysis of bulk $\delta^{13}C$ and $\delta^{15}N$ values through the temporal GLB record for each whale revealed three patterns of isotopic change (Fig. S2). While six records showed no obvious trend, four whales had generally similar trends through time in both $\delta^{13}C$ and $\delta^{15}N$ values. Furthermore, whales with similar temporal trends also showed greater overlap in isotopic values than those with differing trends. Therefore, we grouped whales by isotopic trends into three broad patterns (Fig. 2). We defined Pattern 1 (Pa541 and Pa418) as whales in which both $\delta^{13}C$ and $\delta^{15}N$ values generally increased between 1930 and 1960. These two whales had the lowest average $\delta^{13}C$ (-12.1±0.2, -11.7±0.3‰) and $\delta^{15}N$ (14.3±0.5, 14.5±0.4‰) values in our sample set. Although the record from Pa418 represents only half of the time period of Pa541, the inter-annual variation in $\delta^{13}C$ and $\delta^{15}N$ values within GLBs of both whales were similar during the period when both whales were alive.

Pattern 2 (Pa734 and Pa665) is defined as whales for which $\delta^{13}C$ values neither increased nor decreased from ca. 1930 to 1960, while $\delta^{15}N$ values tended to decrease during the same time interval. The average $\delta^{13}C$ (-11.7±0.1, -11.2±0.2‰) and $\delta^{15}N$ (15±0.5, 15.2±0.3‰) values for this pattern were intermediate within the entire data set. Pattern 2 whales had a narrower spread in $\delta^{13}C$ values than Pattern 1 and Pattern 3 (defined below) whales. Interestingly however, the $\delta^{13}C$ values for these two whales did not overlap at any point during the time series. In contrast, the $\delta^{15}N$ values of these whales overlapped for much of the record.

Pattern 3 contained the remaining six whales, which did not exhibit consistent increasing or decreasing temporal trends for either $\delta^{13}C$ or $\delta^{15}N$ values. The two whales with the highest overall isotopic values, Pa15 and Pa700 ($\delta^{13}C$: -10.7±0.3, -11.2±0.3‰ and $\delta^{15}N$: 15.8±0.4, 15.5±0.3‰), are plotted in Fig. 2 while the remaining Pattern 3 whales are plotted in Fig. S3. These whales had the widest spread of isotopic values out of the three temporal patterns such that for any given year, isotopic values of both $\delta^{13}C$ and $\delta^{15}N$ differed by up to 2‰ among individuals. Conversely, differences in isotopic values for a given year between individuals within both Pattern 1 and Pattern 2 were ≤1‰. Furthermore, Pattern 3 whales had isotopic values that overlapped with those of Pattern 2 whales.

We calculated average isotopic values for each tooth and observed a significant linear relationship between tooth averaged bulk $\delta^{13}C$ and $\delta^{15}N$ values among the sampled whales (n=10; $R^2 = 0.59$; $p = 0.0095$; Fig. 3). The range in average isotopic values among the teeth was approximately 1.5‰ for both $\delta^{13}C$ and

$\delta^{15}N$ values. Further, whales with different patterns (as defined above) tended to separate in terms of average bulk $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 3). A summary of bulk isotopic data for all whales, including the remaining Pattern 3 whales not shown in Fig. 2, can be found in Table S1.

Compound specific isotope analysis of amino acids. Carbon isotope values of individual amino acids ($\delta^{13}C_{AA}$) from the dentin of six sperm whales selected for CSIA-AA (see Materials and Methods) ranged from -32.4 to 9.0‰ (Table S2). The EAA were substantially ^{13}C-depleted ($\delta^{13}C_{EAA}$: -21.0‰ to -26.4‰, Fig. S4) relative to both bulk and NEAA $\delta^{13}C$ values ($\delta^{13}C_{NEAA}$: -11.9 to -8.7‰). The ^{13}C-enrichment in NEAAs relative to EAAs reflects the increase in the degree of re-synthesis of amino acid R-groups in heterotrophs (e.g., McMahon et al. 2013). The nitrogen isotope values of individual amino acids ($\delta^{15}N_{AA}$) from the six whales ranged from -38.8 to 27.4‰ (Table S3). The overall $\delta^{15}N_{AA}$ pattern was similar to that expected for heterotrophs. Trophic-AA values were uniformly ^{15}N-enriched relative to source-AA ($\delta^{15}N_{Sr}$) values, with bulk $\delta^{15}N$ values falling between those for trophic-AA and source-AA. Additionally, Thr was strongly ^{15}N-depleted relative to all other AAs (Fig. S5). The $\delta^{15}N$ values for Glu, the canonical trophic-AA, ranged from 21.9 to 24.7‰, while $\delta^{15}N$ values for Phe, the canonical source-AA, ranged from 6.6 to 9.6‰. Overall, broad patterns for both $\delta^{13}C_{AA}$ and $\delta^{15}N_{AA}$ values in all sperm whale teeth were consistent with expectations from earlier studies of different consumers, including zooplankton, tuna, penguins, and corals (McClelland & Montoya 2002,

Schmidt et al. 2004, Popp et al. 2007, Lorrain et al. 2009, McMahon et al. 2013, Schiff et al. 2014).

With the exception of a single individual all whales showed a strong and significant linear correlation between $\delta^{13}C_{EAA}$ and bulk $\delta^{13}C$ values (R^2= 0.63, Fig. 4). The relative ordering of whales in terms of their pattern designations (as defined above, based on bulk $\delta^{13}C$ and $\delta^{15}N$ values) also corresponded directly with increasing $\delta^{13}C_{EAA}$ values. The $\delta^{13}C_{EAA}$ values of the two Pattern 3 whales were, however, very different to one another. While Pa15 had the highest $\delta^{13}C_{EAA}$ values of all six whales, and also fell within the same linear relationship between bulk $\delta^{13}C$ and $\delta^{13}C_{EAA}$, Pa700 did not. For this individual, the bulk $\delta^{13}C$ value was either decoupled from $\delta^{13}C_{EAA}$ values, or had a very different relationship than that observed in all other whales. Finally, $\delta^{13}C_{NEAA}$ values were ^{13}C-enriched relative to bulk $\delta^{13}C$ values (Fig. S4), however, unlike the EAAs, there was no clear relationship between bulk $\delta^{13}C$ and $\delta^{13}C_{NEAA}$ values (Fig. S6). Note that Ser, Val, and Lys were excluded from average calculations of EAA and NEAA as described in Materials and Methods.

The $\delta^{15}N_{Sr}$ values had a strong and significant linear correlation with average bulk $\delta^{15}N$ values from the same teeth (R^2 = 0.89, Fig. 5A), and the whale groupings followed the same order for $\delta^{15}N_{Sr}$ values as previously observed for average bulk $\delta^{15}N$ values (Fig. 3). In contrast, the difference between average trophic- and source-AAs ($\Delta^{15}N_{Tr\text{-}Sr}$), which is a broad proxy for relative trophic position (Sherwood et al. 2014, Batista et al. 2014), showed no relationship with bulk $\delta^{15}N$ values (R^2 = 0.1; Fig. 5B).

We found no statistically significant relationship (Student's t-test, $p>>0.05$) between average yearly isotopic values among whales from each pattern and known strong El Niño events (1940-1941 and 1957-1958; identified by Quinn et al. 1987) for any of the identified patterns. However, for the two whales belonging to Pattern 1, bulk $\delta^{13}C$ and $\delta^{15}N$ values show consistent coupled changes relating to strong El Niño Events (Quinn et al. 1987). For example, isotopic values for both whales in Pattern 1 decrease from 1939 (non-El Niño) to 1940 (strong El Niño), increase from 1940 to 1941 (strong El Niño), and decrease from 1941 to 1942 (non-El Niño). Although, these isotopic shifts are less than 0.5‰. There are no consistencies or specific trends relating to El Niño events among any other patterns (Fig. S8).

DISCUSSION

The range in $\delta^{13}C$ values for the present study is similar to those measured in skin of sperm whales around the Galapagos Islands (approximately -17.6 to -14.2 ‰, Marcoux et al. 2007a). Furthermore, $\delta^{15}N$ values from dentin samples overlap with those from sperm whale skin, however Marcoux et al. (2007a) measured a much wider range of values (approximately 8‰ to 20‰). Differences in isotopic values between the two studies are likely due to isotopic differences between tissue types (dentin vs. skin) and spatial range inhabited by the whales in each study. Additionally, $\delta^{15}N$ values from our dentin are approximately 3.5‰ heavier than Humboldt squid muscle collected off Peru (Ruiz-Cooley & Gerodette 2012) as expected, because squid are the main prey item of sperm whales in Peru and Chile

(Clarke et al. 1976). The $\delta^{13}C$ values for these whales are ^{13}C-enriched relative to squid by about 5‰ (Ruiz-Cooley & Gerodette 2012). While this enrichment is larger than the approximately 1‰ expected with each trophic transfer, $\delta^{13}C$ of dentinal collagen is typically enriched relative to both skin and muscle tissue by approximately 4‰ (Koch 2007) therefore, a 5‰ difference between sperm whales and squid is reasonable.

Intra-specific variability in sperm whale dentinal stable isotopes. Bulk stable isotopic values from the tissues of top predators reflect information from both habitat biochemistry and animal diet (e.g., Newsome et al. 2010, Ruiz-Cooley & Gerrodette 2012, Ruiz-Cooley et al. 2012). We suggest that the three different temporal isotopic patterns in our data set reflect three distinct foraging strategies used by sperm whales living in the ETP. In other words, whales foraged in different habitats with distinct baseline isotopic values or had differing diet compositions. Four clans, identified by characteristic behavior and vocal codas, have been detected in the southeastern Pacific using acoustic methods and photo identification. Each clan spans thousands of kilometers (Jaquet & Whitehead 1996) and has specific diving and foraging behavior that is consistent among clan members through both time and space (e.g., Whitehead & Rendell 2004, Marcoux et al. 2007a). For example, members of the 'Regular' clan were observed around the Galapagos Islands in 1987 and 1989, and in 2000 different members of the same clan were observed off the coast of Chile (Whitehead & Rendell 2004). Additionally, members of the 'Short' clan were found along a much wider range of the South American coast than members of two other

sympatric clans (Whitehead & Rendell 2004). Differences in foraging behavior among clans have been detected in the $\delta^{13}C$ and $\delta^{15}N$ values of sperm whale skin collected around Chile, Peru, and the Galapagos Islands. Sperm whales foraging relatively inshore tended to have higher $\delta^{13}C$ values than whales foraging offshore, while $\delta^{15}N$ values in whales are inversely related to latitude (Marcoux et al. 2007a). The dentinal time series of $\delta^{13}C$ and $\delta^{15}N$ values from the present study represent coupled records of both baseline primary production isotope values and diet composition throughout the lives of each individual. Therefore, whales with similar isotopic patterns through time likely belonged to the same clan and had similar behavior and/or fed within the same region as one another.

Differences in foraging habitat among sperm whales. Differences in bulk isotopic values averaged across the lifetime of each whale do not support the hypothesis that the sampled whales are derived from a population with homogeneous distribution or life history. Instead, the isotopic separation observed among whales based on averaged $\delta^{13}C$ and $\delta^{15}N$ values and the significant linear relationship between bulk $\delta^{13}C$ and $\delta^{15}N$ values suggest that the sperm whales had distinct foraging areas and/or trophic positions that were maintained throughout the lifetime of each whale. The slope of the regression of average bulk $\delta^{13}C$ values versus $\delta^{15}N$ values ($m = 0.91$) suggests that differences among whales in trophic position alone cannot explain the observed isotopic co-variation. If trophic position were the driver of this relationship, we would expect a slope of ~3. However, it is possible that both isotopic baseline and trophic position differences may underlie differences in average

isotopic values among whales. Bulk isotopes alone cannot be used to assess the relative importance of these two factors (Post 2002, McMahon et al. 2013). Therefore, we used CSIA-AA to assess the importance of baseline versus trophic differences as drivers of the observed relationships.

Both $\delta^{13}C$ and $\delta^{15}N$ values of amino acids strongly support the interpretation that isotopic baseline variation, and not food web or trophic position, was the underlying cause for differences in bulk isotopic values among these whales. For carbon, $\delta^{13}C_{EAA}$ values have been shown to derive directly from primary producers (McMahon et al. 2013). Thus, the correlation between bulk $\delta^{13}C$ and $\delta^{13}C_{EAA}$ values (Fig. 4) suggests that baseline variation was the primary driver of bulk $\delta^{13}C$ differences. Because the EAAs in consumers derive from primary producers, the relationship between bulk $\delta^{13}C$ and the $\delta^{13}C$ for each EAA should exhibit the same trend. Interestingly, Lys had a negative relationship, while all other EAA had positive relationships, to bulk $\delta^{13}C$ values (Fig. S7). This is possibly indicative of multiple baseline sources within our data set however, an in depth evaluation is beyond the scope of this study. In contrast to EAAs, consumers typically re-synthesize the carbon skeletons of NEAAs to varying degrees (Schiff et al. 2014, Vokhshoori et al. 2014), and so bulk $\delta^{13}C$ and $\delta^{13}C_{NEAA}$ values are often decoupled (McMahon et al. 2013). As a consequence, the relationship between bulk $\delta^{13}C$ and $\delta^{13}C_{NEAA}$ values is expected to become progressively weaker with successive trophic transfer (e.g., Schiff et al. 2014). For these reasons, while $\delta^{13}C_{NEAA}$ values have rarely been

measured in high trophic position organisms, the lack of correlation between bulk $\delta^{13}C$ and $\delta^{13}C_{NEAA}$ values (Fig. S6) is not surprising.

For nitrogen, the strong correlation between bulk $\delta^{15}N$ and $\delta^{15}N_{Sr}$ values, as well as the lack of relationship between bulk $\delta^{15}N$ and $\delta^{15}N_{Tr\text{-}Sr}$ values, indicate that whales integrated discrete $\delta^{15}N$ values related to primary productivity, which was likely linked to specific geographic regions. This is similar to a recent study by Ruiz-Cooley et al. (2014) which detected coupled changes in isotopic values over a 12-year time period. CSIA-AA data revealed this was due to shifting baselines throughout the study period. While Phe is used as the canonical source-AA (recently reviewed in McMahon & McCarthy, *in press*), in many environments the average $\delta^{15}N$ value from all source-AAs likely represents a better estimate of the baseline, due to potential variation in measurements from any single AA (McCarthy et al. 2007, Batista et al. 2014, McMahon & McCarthy, *in press*). This may be especially true when analyzing samples with a complex matrix, such as sperm whale teeth, for which extraction and cleanup steps are required prior to hydrolysis (Brault et al. 2014). While the relationship between bulk $\delta^{15}N$ and $\delta^{15}N_{Phe}$ values in the present study mirrored the positive relationship between bulk $\delta^{15}N$ and $\delta^{15}N_{Sr}$ values, the correlation was not as strong. This comparison provides further support for the idea that $\delta^{15}N_{Sr}$ may be a more effective tracer for differences in baseline values among animals than $\delta^{15}N_{Phe}$ alone.

Together, data from CSIA-AA support our interpretation that the distinct temporal patterns defined above, which correlate with different individual mean

values, indicate that whales in our sample set represent individuals with different social bonds (discussed above) that maintained geographically segregated life histories. Baseline $\delta^{13}C$ and $\delta^{15}N$ values are known to exhibit spatial variation (e.g., McMahon et al. 2013) linked to biogeochemical and oceanographic factors (e.g., Graham et al. 2010, McMahon et al. 2013). Over large spatial scales at mid-latitudes, coupled differences in $\delta^{13}C$ and $\delta^{15}N$ values are typically observed between nearshore and offshore ocean regions, with a gradient from high to low $\delta^{13}C$, and often $\delta^{15}N$ values, toward the open ocean. The $\delta^{13}C$ values are typically ^{13}C-enriched in productive coastal regions due to faster growth rates and larger cell size of phytoplankton relative to oligotrophic offshore regions (e.g., Rau et al. 1982, Goericke & Fry 1994, Popp et al. 1998). For nitrogen, differences between ocean regions are largely due to differences in nitrogen source; oligotrophic gyres typically have lower $\delta^{15}N$ values due to fixation of ^{15}N-depleted atmospheric nitrogen (Dore et al. 2002, Montoya 2007), in contrast to highly productive coastal regions, which typically have baseline $\delta^{15}N$ values that more directly reflect ^{15}N-enriched subsurface nitrate values. Therefore, if feeding locations of the sampled whales ranged from coastal to offshore regions, one interpretation of the observed patterns in bulk $\delta^{13}C$ and $\delta^{15}N$ values is that higher isotopic values represent whales that foraged closer to shore than those with lower isotopic values, a possibility previously discussed for the California Current system (Ruiz-Cooley et al. 2014).

While an onshore versus offshore explanation may be reasonable in the context of basin-scale oceanographic features, if all whales sampled here fed

relatively close to the Paita whaling station, then the oceanographic complexity of this region likely necessitates a more nuanced interpretation. Within the most likely foraging region of our sperm whales (Fig. 1), complex interactions of denitrification, incomplete nitrate utilization, and advected fractionated nitrate from the equatorial upwelling cold-tongue, together result in regional nitrogen isotope gradients that are essentially opposite to the general basin-scale tends described above (Codispoti & Christensen 1985, Altabet 2001, Mollier-Vogel et al. 2012). The lowest bulk $\delta^{15}N$ values are found nearest to shore within the Peru Coastal Upwelling system (PCU), due to strong persistent upwelling resulting in incomplete utilization of nutrients by phytoplankton (Montoya 2007). Here, bulk $\delta^{15}N$ values are typically between 5‰ and 6‰, with values dropping to 4‰ where upwelling is most persistent. Along the equatorial cold tongue, where upwelling occurs to a lesser degree than within the PCU, bulk $\delta^{15}N$ values range from 7‰ to 8‰. To the north and south of the cold tongue, bulk $\delta^{15}N$ values increase (to > 9‰) due to a combination of a strong oxygen minimum zone and attendant denitrification (which produces ^{15}N-enriched nitrate) in this region (Wada & Hattori 1976, Saino & Hattori 1987). Hence, baseline $\delta^{13}C$ and $\delta^{15}N$ isoscapes may be useful to constrain likely foraging zones of the whales we sampled.

The ETP has been subject to intensive paleoceanographic study, and regional bulk $\delta^{15}N$ isoscapes (based on sediment core-top records; Tesdal et al. 2013) can provide an invaluable tool to assess potential foraging areas. While we only have data from CSIA-AA for six whales, the $\delta^{15}N_{Phe}$ values from these individuals (Table S3)

correspond well to $\delta^{15}N$ ranges in core-top isoscapes (Fig. 6). Studies of $\delta^{15}N_{Phe}$ values in multiple systems suggest that it is a reasonable proxy for the $\delta^{15}N$ value of export primary production (e.g., Vokhshoori & McCarthy 2014, Sherwood et al. 2014, Ruiz-Cooley et al. 2014). Therefore, a comparison between $\delta^{15}N_{Phe}$ values from our sampled whales, and a nitrogen isoscape of the ETP (Fig. 6) may provide reasonable estimates of the likely regions in which different sperm whales foraged. The low $\delta^{15}N_{Phe}$ values of Pattern 1 individuals suggest these whales most likely foraged nearest to the PCU. The intermediate $\delta^{15}N_{Phe}$ values of Pattern 2 individuals correspond best with values expected for the equatorial cold tongue. Finally, the $\delta^{15}N_{Phe}$ values in Pattern 3, as with all other isotopic measurements, were more variable. However, the highest $\delta^{15}N_{Phe}$ value measured within this group was also the highest in the entire data set, suggesting foraging within regions offshore, and likely either to the north or south of the equatorial cold tongue (Fig 6).

These geographical assignments are clearly hypotheses, however the reasonable match between $\delta^{15}N_{Phe}$ and core-top bulk $\delta^{15}N$ values demonstrates the potential for values from CSIA-AA to be coupled with detailed isoscapes in order to constrain foraging regions of highly mobile marine top predators. Additional data, as well as ongoing refinements in CSIA-AA, could substantially increase the confidence of such results in future studies. For example, as noted above, single $\delta^{15}N_{AA}$ values are subject to possible analytical variations. If calibrations between $\delta^{15}N$ values of primary production and $\delta^{15}N_{Sr}$ values are developed (similar to calibrations for $\delta^{13}C_{EAA}$ reported by Vokhshoori et al. (2014)) this may represent a more accurate

means to characterize foraging region. Additionally, the nitrogen isoscape represents $\delta^{15}N$ values averaged over tens of years (nearshore) to thousands of years (offshore; Tesdal et al. 2013), while $\delta^{15}N_{Sr}$ values of our whales represent averages from 1948 to 1952 (five whales) and 1950 to 1952 (one whale). Actual oceanic conditions during these years, as indicated by $\delta^{15}N$ values, may have departed from the average bulk $\delta^{15}N$ values presented in Fig. 6.

Furthermore, adding the available information from both bulk tissue and amino acid $\delta^{13}C$ values should increase confidence. The most direct way to evaluate our $\delta^{13}C$ data would be an analogous sediment core-top isoscape. However, an extensive database of $\delta^{13}C$ from sedimentary organic carbon from this region is not available. While cores from this region have been examined for organic carbon content (e.g., Wefer et al. 1990, Loubere et al. 2003), few studies provide $\delta^{13}C$ values and fewer still examine core-tops. Nonetheless, we note that coupling between $\delta^{13}C$ and $\delta^{15}N$ values, as in our data set, is commonly observed in isoscape records (e.g., McMahon et al. 2013) and has also been identified in sperm whale and other bio-archive studies (Ruiz-Cooley et al. 2014, McMahon et al. 2015) further supporting our overall interpretations.

Temporal variability in sperm whale habitats. Strong shifts in baseline values, animal diet, or both can drive isotopic changes or trends through time. While ontogenetic shifts due to changes in diet composition or trophic position with age can cause similarly coupled shifts in $\delta^{13}C$ and $\delta^{15}N$ values (Lesage et al. 2001, Overman & Parrish 2001, Ruiz-Cooley et al. 2014), none of the sperm whales in this study

showed positive shifts in either carbon or nitrogen with age. Thus, long-term changes in the bulk $\delta^{13}C$ and $\delta^{15}N$ temporal records of these 10 sperm whales were either due to shifts in prey of the sperm whales or shifts in the biochemistry of their habitat. Stomach content analysis has shown that Humboldt squid are a primary component of sperm whale diet in the ETP (Clarke & Paliza 2001) and Humboldt squid are highly abundant throughout the ETP (Nigmatullin et al. 2001). While changes in prey composition through the lifetime of a sperm whale can influence isotopic variation in temporal trends, the continuous high abundance of Humboldt squid in the ETP suggests that whales maintain similar trophic status over many years. Assuming sperm whales from Peru had a stable trophic status throughout their lives, the observed isotopic patterns in sperm whale teeth could indicate shifts in baseline values.

To the best of our knowledge, there is no record of changes in primary productivity or biochemical cycling in the ETP for the duration of this study. However, recent studies have shown that changes in baseline values are currently occurring in different ecosystems within the Pacific Ocean and that these baseline shifts can be distinct between adjacent habitats or ecosystems (e.g., inshore versus offshore ecosystems). Decadal-scale decreases in $\delta^{13}C$ and $\delta^{15}N$ values of EAAs and source-AAs from sperm whale skin suggest shifts in the carbon and nitrogen biochemical cycling along the offshore California Current System (CCS; Ruiz-Cooley et al. 2014). Similar baseline trends have been observed in the North Pacific Gyre (NPG) using $\delta^{13}C$ (McMahon et al. 2015) and $\delta^{15}N$ (Sherwood et al. 2014)

values from bulk deep sea coral tissue as well as EAAs and source-AAs, respectively. Previous authors attributed the temporal isotopic trend in corals to increases in nitrogen fixation since the mid-1800s for nitrogen (Sherwood et al. 2014), and multiple shifts in phytoplankton community composition since the mid-900s for carbon (McMahon et al. 2015), while Ruiz-Cooley et al. (2014) suggested the observed decadal declines in both bulk $\delta^{13}C$ and $\delta^{15}N$ values were due to decreases in primary productivity in the offshore CCS. Between adjacent habitats, shifts in $\delta^{13}C$ and $\delta^{15}N$ values due to baseline difference can be quite large and closely linked to local oceanographic factors that can be difficult to predict (McMahon et al. 2013). For example, in Hawaiian petrels, large differences in $\delta^{13}C$ and $\delta^{15}N$ values among breeding groups are directly related to differences in foraging location, determined by satellite tracking, and strongly linked to latitude (Wiley et al. 2013). Therefore, the habitats use by our three groups of whales could have responded differently to large-scale perturbation. Further investigation using time series CSIA-AA will help to better constrain the underlying mechanism leading to long-term shifts observed in the bulk isotopic records of these whales.

Evaluating effects of El Niño events on sperm whale isotopic patterns. We explored the effects of El Niño events on the present time series, because El Niño events exert strong atmospheric and oceanographic forcing on the ETP (Wang & Fiedler 2006). However, we did not observe large, significant, or consistent shifts in isotopic values associated with El Niño events among all three recognized patterns (Fig. S8). The consistent small shifts in both $\delta^{13}C$ and $\delta^{15}N$ values from Pattern 1

whales associated with 'strong' El Niño events suggest that this relatively inshore habitat could be influenced by El Niño events to a higher degree than habitats further offshore. Studies show that El Niño events have the greatest impact on coastal upwelling regions, effectively shutting off the supply of nutrients to the surface and resulting in shifts in phytoplankton communities from large to small cells (Pennington et al. 2006). This may explain why El Niño events have a stronger influence on whales feeding closer to shore than those feeding offshore. A larger sample size of whales from the same foraging region paired with CSIA-AA on individual years is necessary to further explore El Niño effects.

Because isotopic variation in dentinal GLBs is expected to broadly reflect variation in habitat baseline and sperm whale diet, we might expect that large ecosystem changes, driven by El Niño, would be reflected in these values. The fact that our time series show no apparent anomalies relating to El Niño in either $\delta^{13}C$ and $\delta^{15}N$ values may seem surprising given the abundance of evidence on the impacts of El Niño events on both the biology and ecology of organisms living in the ETP (Wang & Fiedler 2006). In particular, a decline in the feeding rates, determined by defecation rates, of Galapagos sperm whales was observed during the 'very strong' 1987 El Niño event (Smith & Whitehead 1993, Whitehead 1996), indicating that sperm whales can be heavily affected by the warm phase of the ENSO.

There are a number of reasons why a clear El Niño signal might be difficult to observe in the isotopic time series of these whales. First, while we expect that signals of an El Niño event should be prominent in planktonic food webs (Pennington et al.

2006), it is less certain how the changes in bulk isotopic baseline would propagate into an apex predator. This would require a relatively short-lived isotopic baseline perturbation to be propagated though an entire food web to the integrated record at the top of the food chain. The strength of El Niño events might therefore be an important factor. Our data set coincides with eight El Niño events, of which three are classified as 'moderate +' and five as 'strong'. However, there are no events in this time series classified as 'very strong' (Quinn et al. 1987), such as the 1982/83 and 1997/98 events which are rare, but have intense impacts at both regional and large scales. Since El Niño events of 'moderate +' and 'strong' classification tend to be less intense and impact a smaller area than 'very strong' events (Boisea et al. 1998), it is possible that such events may simply not be pervasive enough in either time or space to be recorded in the yearly growth layer bands of sperm whale teeth.

Second, it is possible that impacts of El Niño events on mesopelagic ecosystems are not as disruptive as those observed in other systems. The impacts of El Niño events on mesopelagic food webs are not well characterized, especially within the ETP. A few studies note changes in community structure within the mesopelagic related to El Niño events such as shifts in abundance of fish larvae and *Hydromedusae sp.* and expanded ranges of fish larvae (Raskoff 2001, Funes-Rodriguez et al. 2006, Funes-Rodriguez et al. 2011). In contrast, Humboldt squid, a primary prey item of sperm whales in the ETP (Clarke et al. 1976), are highly adaptable to environmental fluctuations (Argüelles and Tafur 2010, Bazzino et al. 2010). If Humboldt squid remained in the area during an El Niño event, as generalist

feeders (Field et al. 2013, Markaida 2006), they may not be as constrained by changes in mesopelagic prey community as other organisms since they can switch prey items when necessary and can retain a stable trophic position (Tam et al. 2008).

Finally, the lack of apparent El Niño signal in the present bulk isotopic data is likely related to the limitations of the bulk stable isotope analysis and also to differing clan foraging strategies. We have explained that bulk isotopes cannot distinguish variation due to changes in habitat from differences in diet. Clan structure and variation in foraging behavior add complexity and increase variability in isotopic records from teeth. For example, off the coast of the Galapagos Islands, two distinct groups of sperm whales belonging to different clans show clear differences in habitat use and movement patterns (Whitehead & Rendell 2004). Whitehead & Rendell (2004) note that each clan maintains the same foraging strategy regardless of oceanographic conditions. It is possible that different clans of sperm whales could be impacted by El Niño events in distinct ways, related to their particular foraging behavior and fidelity to a specific foraging habitat.

SUMMARY AND CONCLUSIONS

We measured bulk carbon and nitrogen stable isotopes in dentin from annual GLBs in the teeth of 10 female sperm whales that were processed at a whaling station in Paita, Peru. Our data provide stable isotope records of the life histories of these whales between ca. 1926 and 1960. Three distinct temporal patterns were identified in the bulk $\delta^{13}C$ and $\delta^{15}N$ records from individual whales, suggesting that individuals

from each pattern may have belonged to specific clans each with a distinct foraging strategy and/or feeding area. Furthermore, the strong positive correlation between average bulk $\delta^{13}C$ and $\delta^{15}N$ values from individual teeth, in which whales with similar temporal patterns also grouped together, provides strong evidence of differences in habitat use by sperm whales with different temporal patterns. Integrating compound specific isotope analysis of amino acids on a subset of samples indicated that the trend in bulk isotopic values was due to differences in isotopic baselines, rather than differences in trophic structure among whales. Specifically, a positive correlation in both bulk $\delta^{13}C$ versus $\delta^{13}C_{EAA}$ values and bulk $\delta^{15}N$ versus $\delta^{15}N_{Sr}$ values, as well as a lack of relationship in bulk $\delta^{15}N$ versus $\Delta^{15}N_{Tr\text{-}Sr}$ values support the hypothesis that the sampled whales lived and foraged within different regions of the ETP throughout their lifespans.

To further investigate potential geographic ranges, we also compared sperm whale $\delta^{15}N_{Phe}$ values with bulk $\delta^{15}N$ values from a nitrogen isoscape derived from sediment core-top records for the ETP region near Paita. The close correspondence of $\delta^{15}N_{Phe}$ values and isoscape $\delta^{15}N$ values suggests that foraging regions can potentially be identified using this approach. The lowest $\delta^{15}N_{Phe}$ values were consistent with whales foraging nearest to the coastal upwelling center, while individuals with higher $\delta^{15}N_{Phe}$ values corresponded to several distinct offshore regions, within and to the south of, the equatorial upwelling cold tongue.

Since El Niño events are associated with dramatic oceanographic and ecosystem shifts in this region, we expected that annual stable isotopic records might

show the influence of El Niño events on mesopelagic food webs. However, we did not detect any isotopic evidence of changes associated with El Niño years in our collective time series across all 10 Paita whales nor among the three isotopic patterns. We hypothesize that this might be due to a lack of 'very strong' El Niño events during the historical study period, the ability of sperm whales to travel large distances to find abundant prey, or differences in foraging strategy (e.g., behavior, foraging area, prey type) between individuals belonging to different clans.

The results of this study illustrate the power of using bulk stable isotopes coupled with CSIA-AA to assist in defining foraging strategies of highly mobile top predators such as sperm whales, especially for ecological studies using historical or archaeological samples. This approach may be one of the very few for which hypotheses about foraging region, trophic structure, and clan divisions can be addressed. The correspondence between $\delta^{15}N_{Phe}$ data from the present sperm whales and $\delta^{15}N$ values from isoscapes near to the Paita whaling station are particularly compelling, suggesting that such CSIA-AA data may offer a window into the detailed movement and ecology of past whale populations. We suggest that establishing a better mechanistic understanding of the correlations between compound specific carbon and nitrogen isotope proxies and primary production, as well as understanding their propagation into top predators, will be an important research area for further developing this approach.

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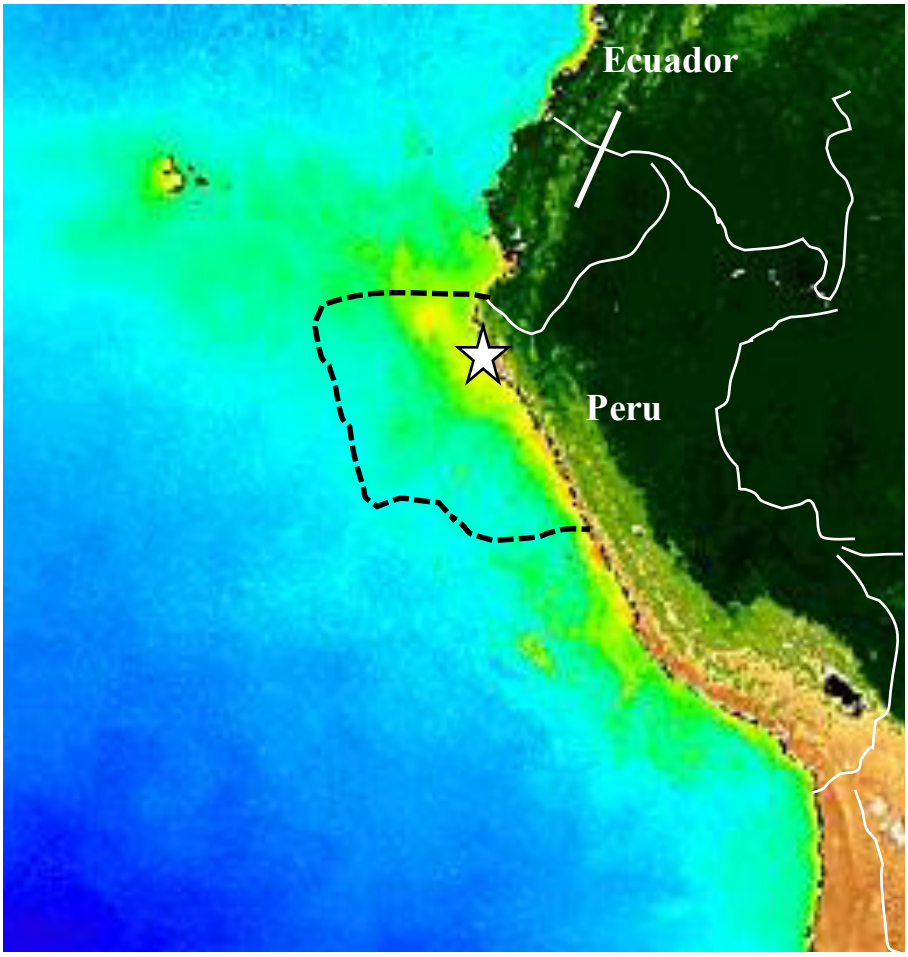

Figure 1: South Eastern Tropical Pacific (ETP) adapted from SeaWiFS. Teeth used in this study were collected at a whaling station in Paita, Peru (white star) in 1959 and 1960. The extent of whaling efforts for this station is outlined in the dashed black line (Whitehead et al. 1997).

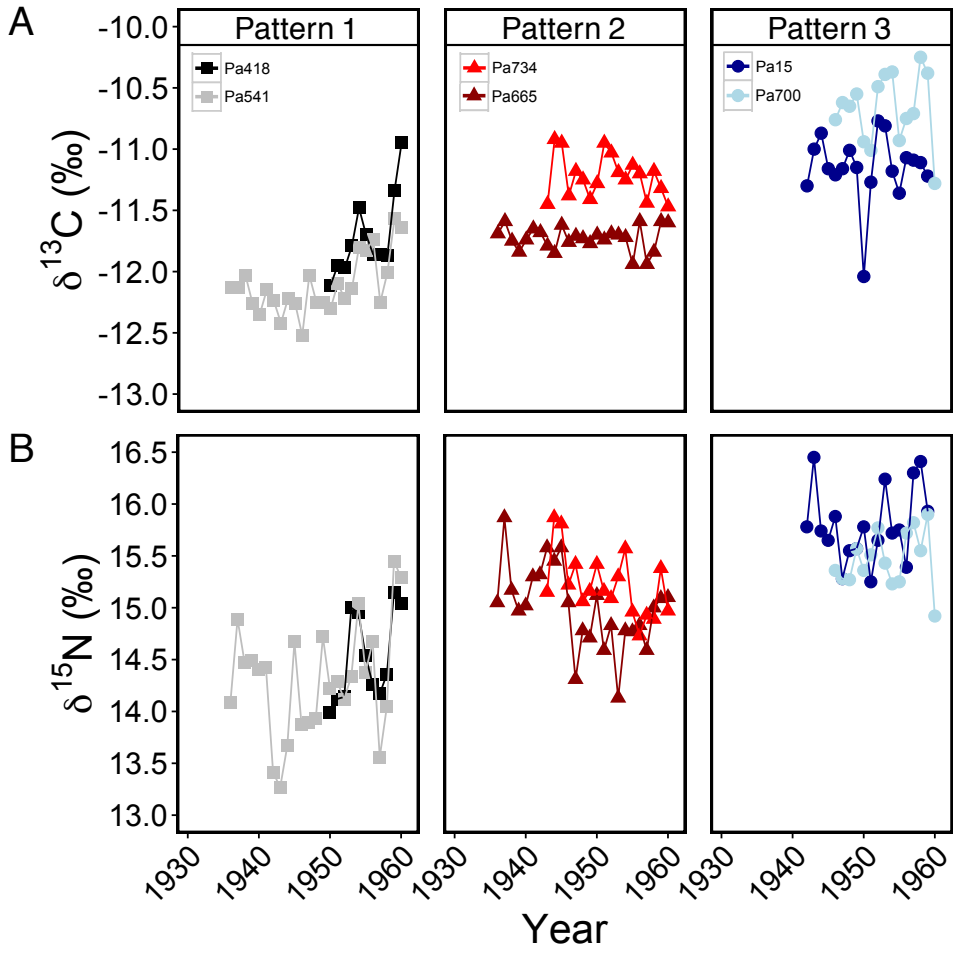

Figure 2: Different temporal patterns in (A) $\delta^{13}C$ values and (B) $\delta^{15}N$ values through time among ETP sperm whales, with two whales shown for each. Pattern 1 (black and grey squares) had increasing $\delta^{13}C$ and $\delta^{15}N$ values throughout the record. Pattern 2 (red triangles) had no long term shifts in $\delta^{13}C$ and decreasing $\delta^{15}N$ values. Pattern 3 (blue circles) had no long term shifts in either $\delta^{13}C$ or $\delta^{15}N$ values. Bands related to weaning and nursing are not plotted.

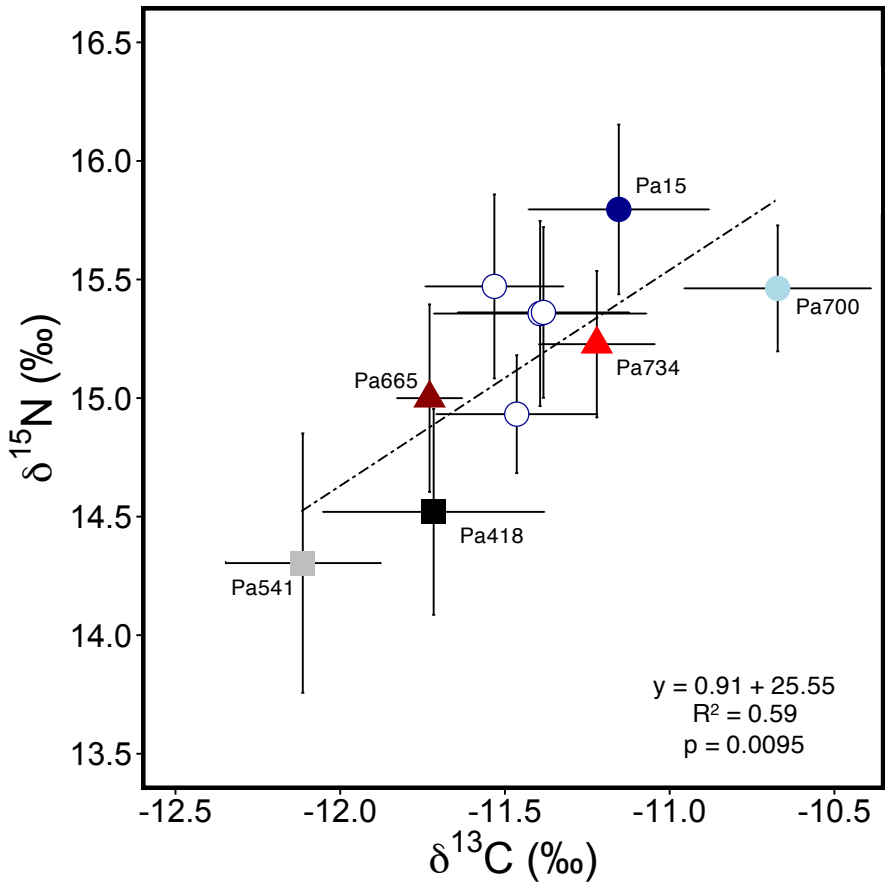

Figure 3: Averaged $\delta^{13}C$ and $\delta^{15}N$ values (± 1 standard deviation) of all adult GLBs sampled from individual whales. Symbols and colors correspond to patterns defined in Fig. 2. Closed symbols represent individuals that were selected for CSIA-AA. Open symbols represent individuals that fall into Pattern 3, but were not used in CSIA-AA. There is a significant positive linear relationship (dashed line) between $\delta^{13}C$ and $\delta^{15}N$ values.

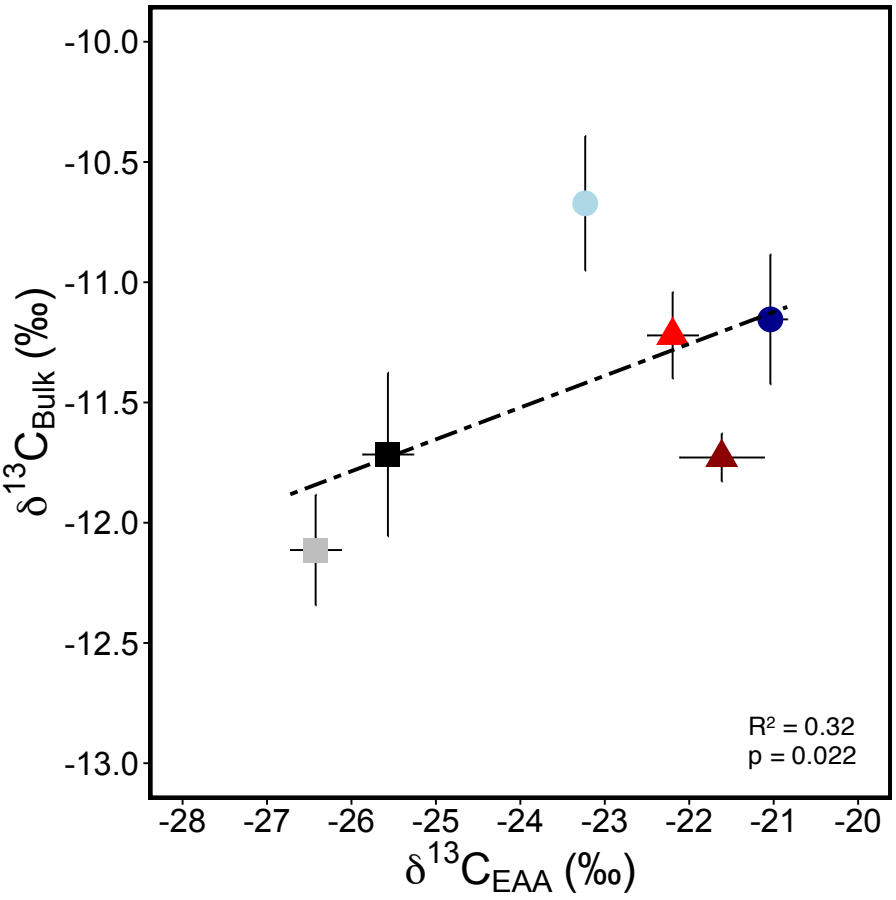

Figure 4: Compound specific $\delta^{13}C$ values of essential amino acids (EAA) versus bulk $\delta^{13}C$ (± 1 standard deviation) values. Symbols and colors correspond to patterns defined in Fig. 2. Bulk $\delta^{13}C$ values increase as EAA $\delta^{13}C$ values increase. Individual Pa700 (light blue circle) has higher than expected EAA value given its bulk value. When Pa700 is excluded from the linear regression, the correlation between bulk $\delta^{13}C$ and EAA $\delta^{13}C_{EAA}$ is stronger ($R^2 = 0.62$, $p = 0.0079$).

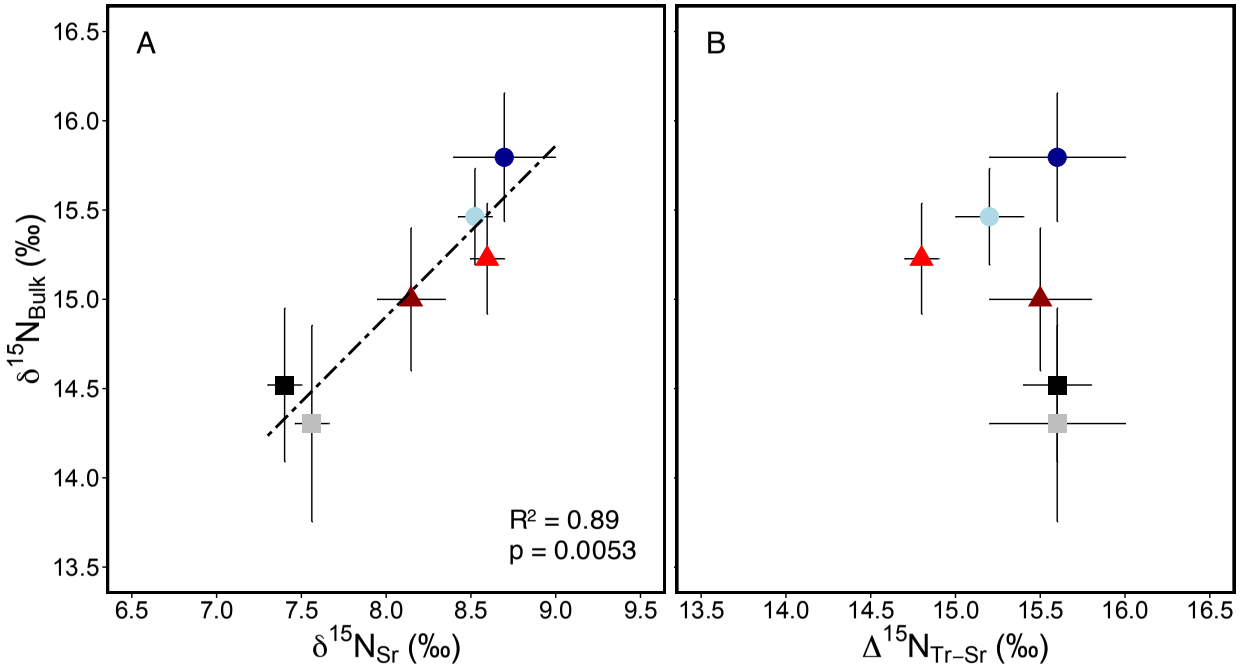

Figure 5: Compound specific amino acid nitrogen isotope parameters for (A) the base of the food web ($\delta^{15}N_{Sr}$) and (B) trophic position ($\Delta^{15}N_{Tr\text{-}Sr}$) versus bulk $\delta^{15}N$ values. Symbols and colors correspond to patterns defined in Fig. 2. (A) dashed line represents significant linear regression, indicating that bulk isotope variation is strongly coupled to baseline $\delta^{15}N$ values. (B) Lack of correlation between bulk $\delta^{15}N$ and $\Delta^{15}N_{Tr\text{-}Sr}$ values ($R^2 = 0.1$, $p > 0.05$) indicates that variation in trophic position is not a strong contributor to the observed trend in bulk isotopic data (Fig. 3).

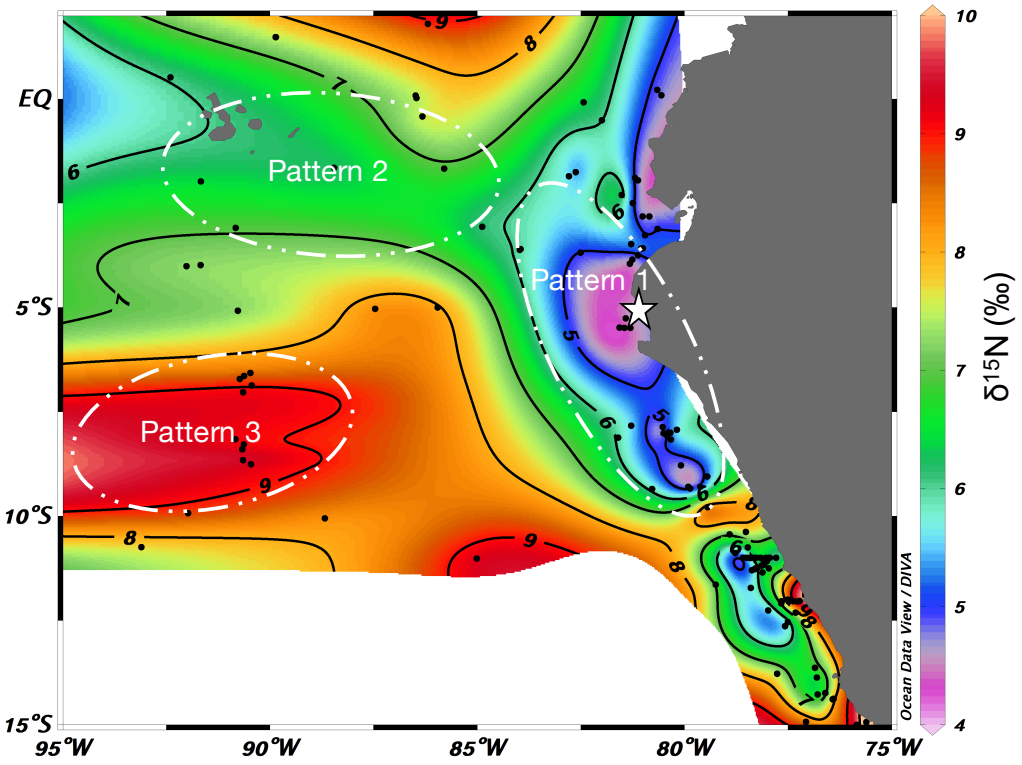

Figure 6: Core-top $\delta^{15}N$ isoscape of the ETP from Tesdal et al. (2013). White star marks Paita, Peru. Black dots represent sediment core sample sites. Contours indicate lines of the same isotopic values. Possible foraging regions of the three identified patterns were determined based on the $\delta^{15}N$ value of Phe from each whale (labelled in white). Pattern 1 whales likely foraged within the Peru Coastal Upwelling (PCU) system. Pattern 2 whales may have foraged within the Equatorial Upwelling (EU) region. Pattern 3 whales correspond best with the offshore region, southwest of the EU.

SUPPLEMENTARY MATERIALS

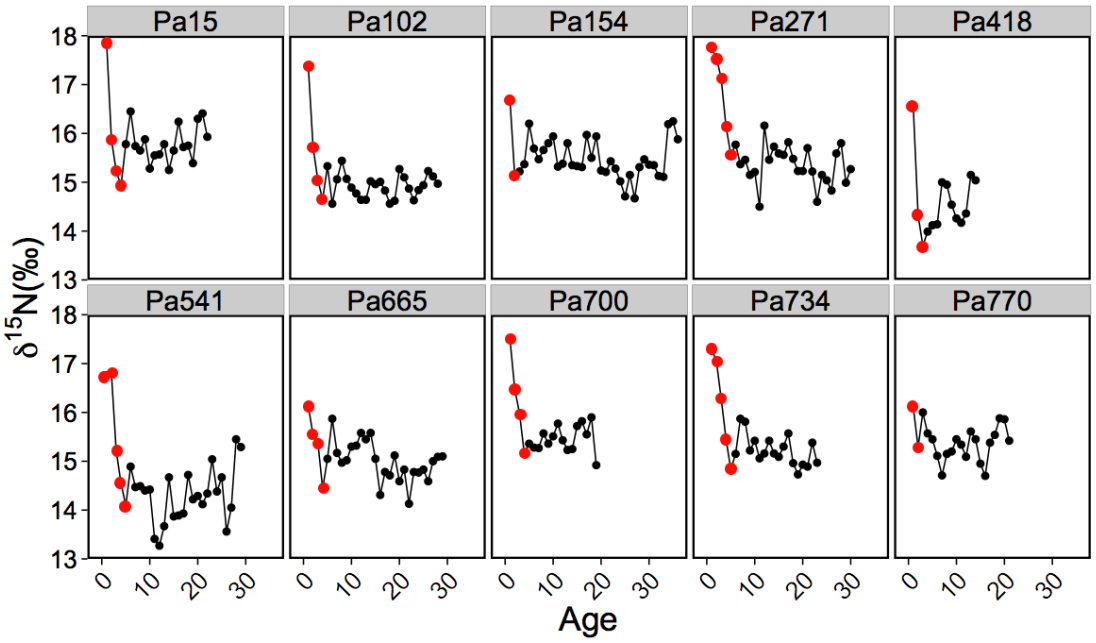

Figure S1: Identification of GLBs that may have been associated with gestation or nursing from 10 sperm whales. Ages determined based on number of GLBs present in each tooth. We excluded samples indicated with red dots from further analysis to ensure that we only examined isotopic values from the post-weaning life of each whale. Weaning periods are typically identified by a gradual decrease in nitrogen isotopic values during the first years of life. While orcas are weaned at about 3 years (Newsome et al. 2009), the weaning age of sperm whales is highly variable and can be anywhere from 2 to 15 years (Rice 1989).

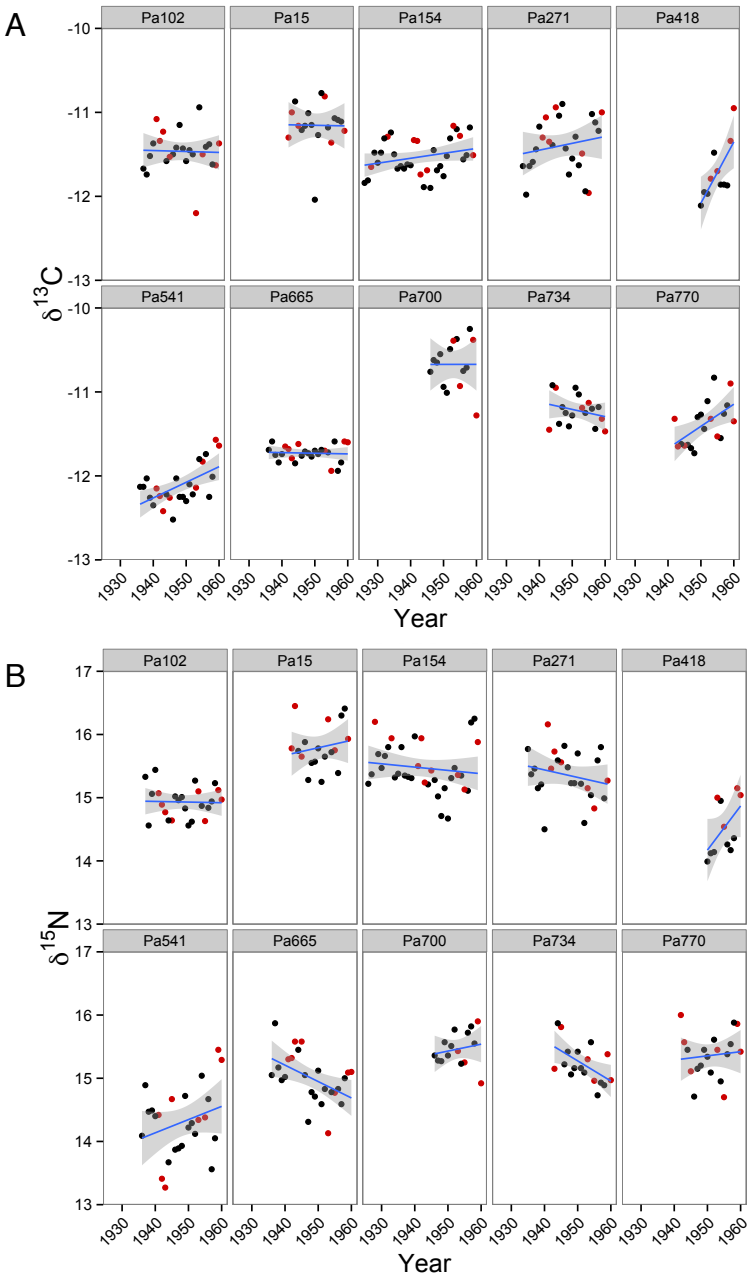

Figure S2: Linear regressions of carbon (A) and nitrogen (B) isotope values through time for each whale. Gray shading represents 95% confidence intervals. Slope of regression was used to sort whales into pattern designations.

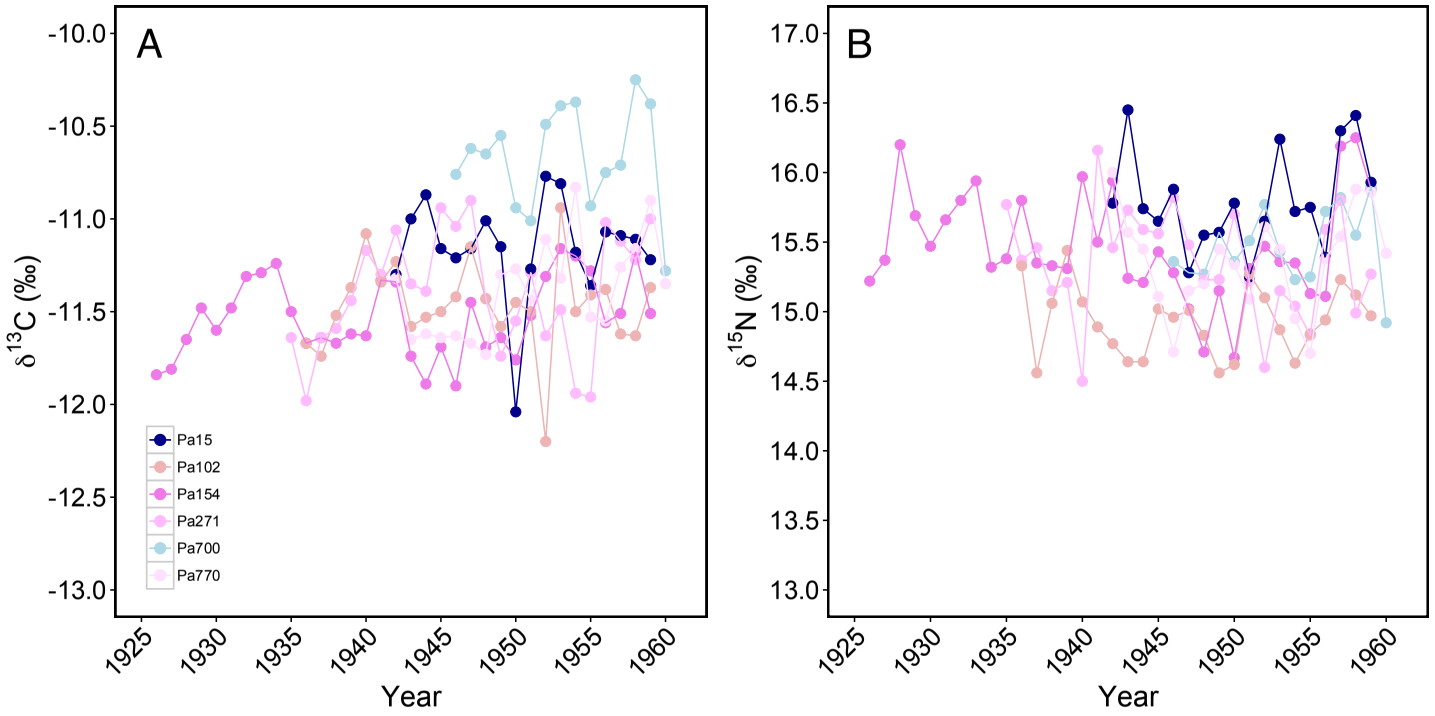

Figure S3: Temporal carbon (A) and nitrogen (B) isotope values among all six Pattern 3 whales. Circles mark isotopic values for a given year. Each whale is represented by a different color. These whales show no long-term increase or decrease in either $\delta^{13}C$ or $\delta^{15}N$ values. Bands possibly associated with pre-weaning years have been removed.

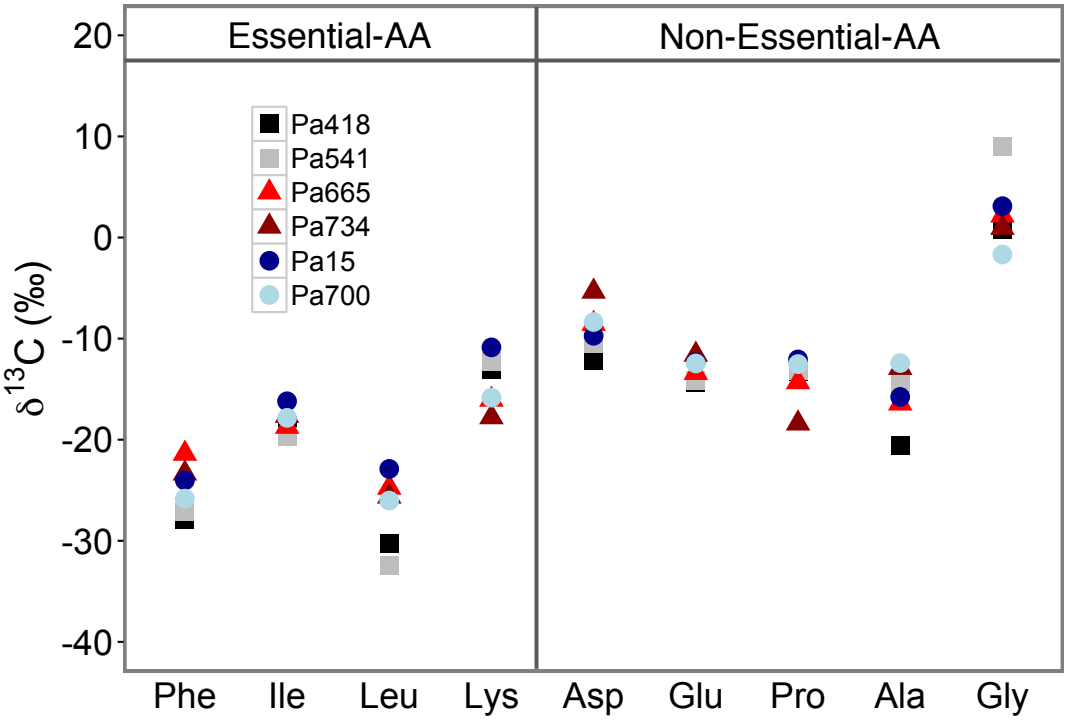

Figure S4: Individual amino acid $\delta^{13}C$ values from composite samples of six sperm whales. Each color represents an individual whale. Symbols represent whale's pattern designations. Square = Pattern 1, triangle = Pattern 2, circle = Pattern 3. Amino acids are arranged according to their essential and non-essential groupings. While recovery of specific AA varies from study to study, we do see similarities in relative isotopic values among AAs recovered in the present study versus previous carbon CSIA-AA work. Frequently, Gly values are enriched and Leu depleted in ^{13}C relative to other AAs recovered. Values of Glu, Pro, and Ala tend to overlap. Finally, values of Phe often fall in between those of Ile and Leu.

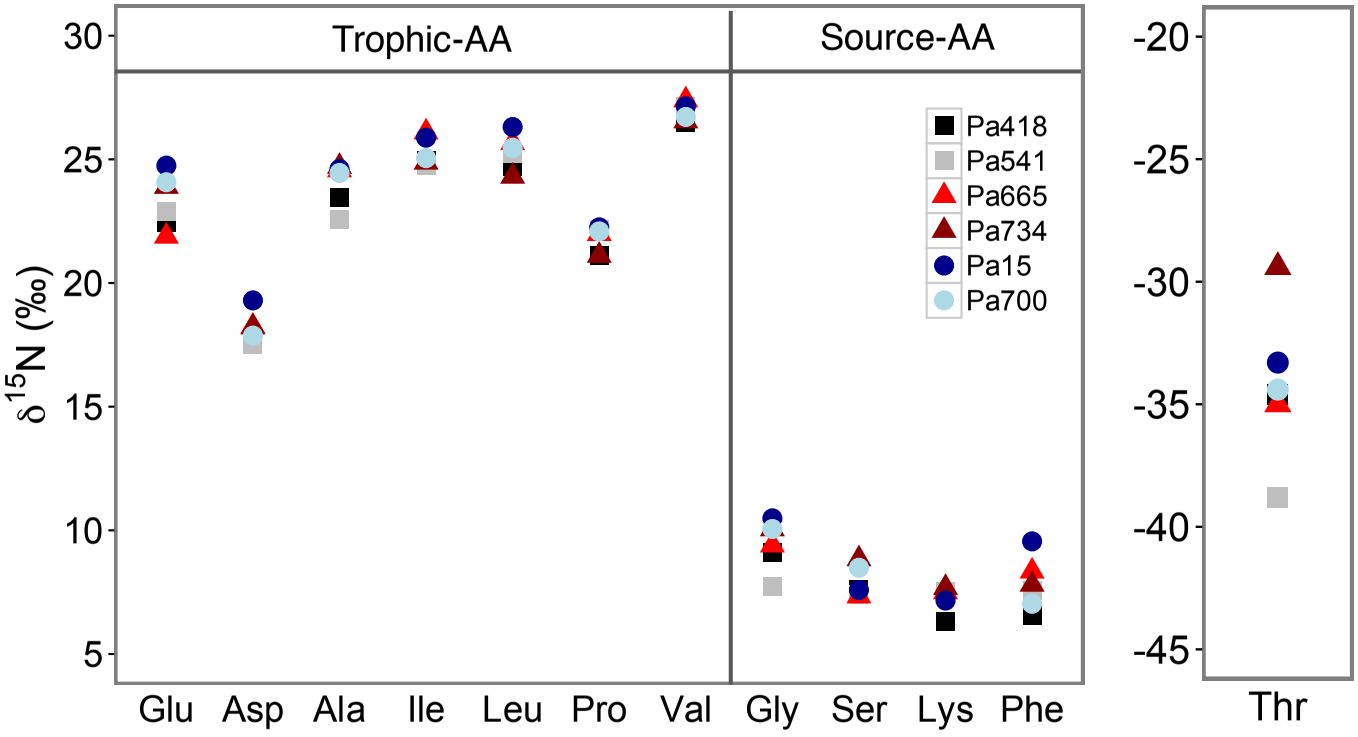

Figure S5: Individual amino acid $\delta^{15}N$ values from composite samples of six female sperm whales. Symbols and colors are consistent with previous figures. Amino acids are arranged according to typical groupings: trophic, source, and metabolic (Thr). The recovered AAs follow the expected pattern, with trophic-AA ^{15}N-enriched relative to source-AAs and Thr ^{15}N-depleted relative to both trophic- and source-AAs.

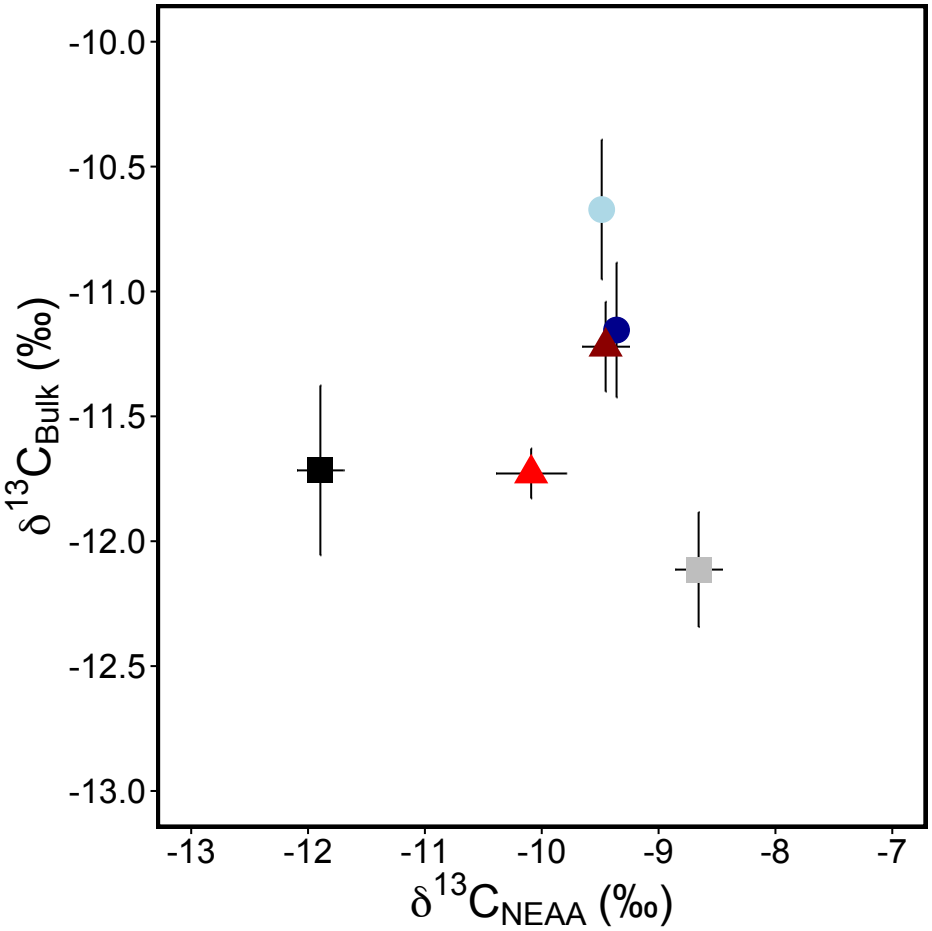

Figure S6: Average bulk $\delta^{13}C$ versus $\delta^{13}C$ values averaged across 5 non-essential amino acids (Asp, Glu, Pro, Gly). These two parameters are not well correlated (R^2 = 0.01).

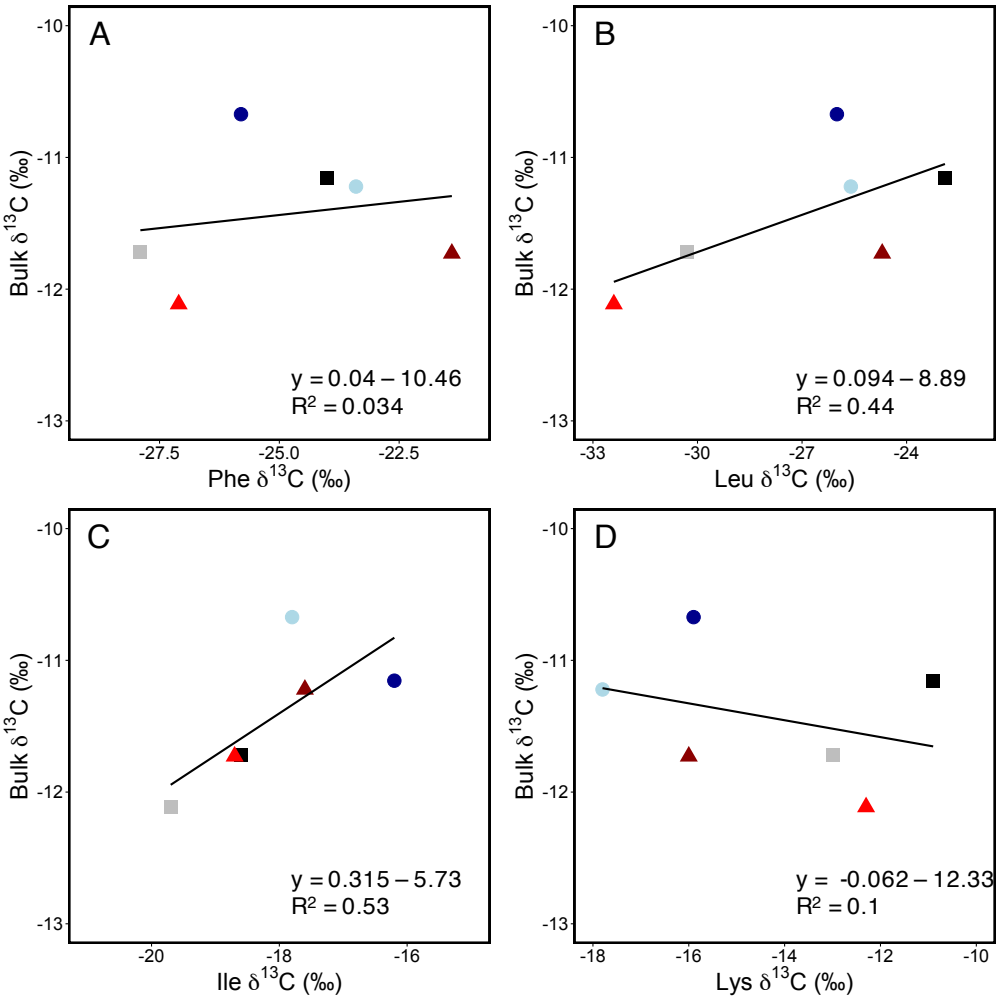

Figure S7: Bulk $\delta^{13}C$ values versus (A) Phe, (B) Leu, (C) Ile, and (D) Lys $\delta^{13}C$ values. Symbols and colors are consistent with previous figures. Black lines represent linear regressions. The $\delta^{13}C$ values of Phe, Leu, and Ile are each positively correlated to bulk $\delta^{13}C$ values, while the $\delta^{13}C$ values of Lys is negatively correlated with bulk $\delta^{13}C$ values. Because Lys does not follow the same relationship with bulk $\delta^{13}C$ values as the other recovered essential amino acids, we excluded this AA from calculations of average EAA $\delta^{13}C$ values.

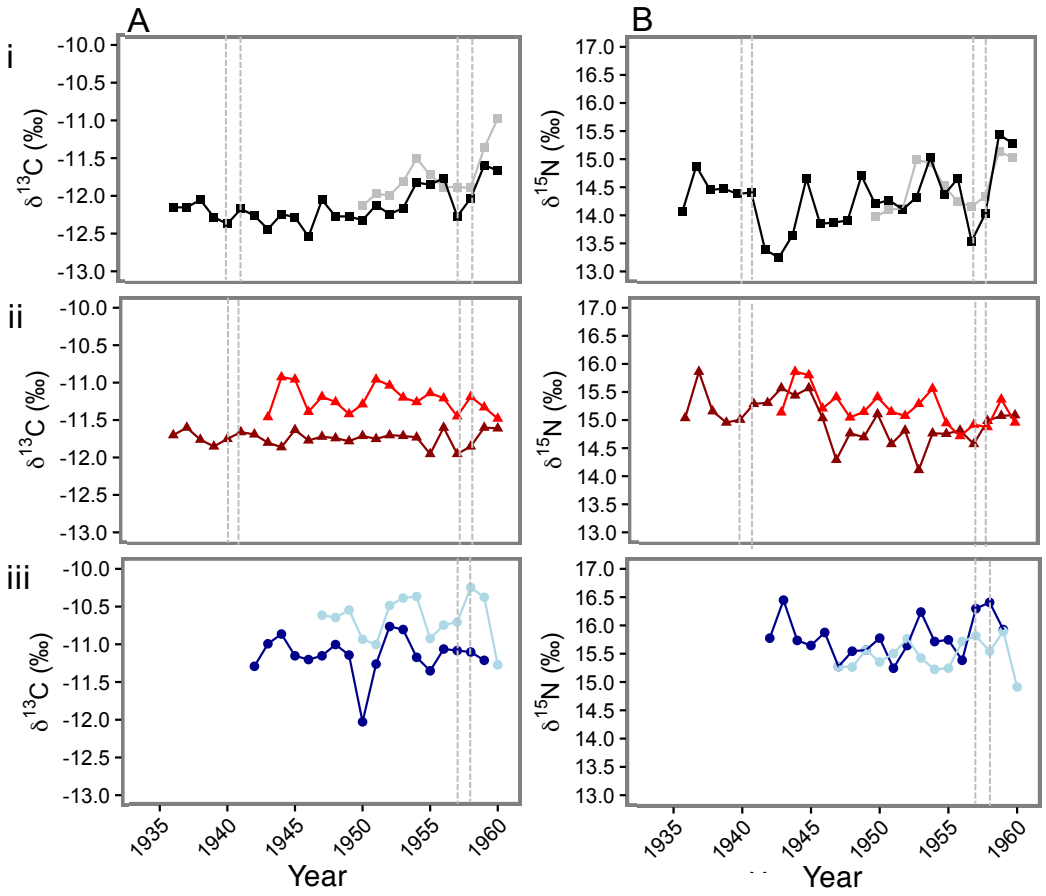

Figure S8: Different temporal patterns in (A) $\delta^{13}C$ values and (B) $\delta^{15}N$ values through time among ETP sperm whales, with two whales shown for each. (i) Pattern 1, (ii) Pattern 2, (iii) Pattern 3. Dotted grey lines indicate strong El Niño events (Quinn et al. 1987). There is no significant difference in average value or standard deviation between El Niño and non-El Nino years. Bands related to weaning and nursing are not plotted.

Table S1: Summary of bulk $\delta^{13}C$ and $\delta^{15}N$ values from 10 female sperm whales. Pattern designations determined by temporal isotopic trends. GLB is the number of growth layer bands sampled for each whales; also closely related to age of each whale. Isotopic values were averaged across all adult GLBs from each tooth, presented ± 1 standard deviation.

			Nitrogen			Carbon		
Whale ID	*Pattern*	*# GLB*	*Min*	*Mean ± sd*	*Max*	*Min*	*Mean ± sd*	*Max*
Pa541	1	24	13.3	14.3 ± 0.5	15.5	-12.5	-12.1 ± 0.2	-11.6
Pa418		12	14	14.5 ± 0.4	15.2	-12.1	-11.7 ± 0.3	-11
Pa665	2	25	14.1	15 ± 0.5	15.9	-11.9	-11.7 ± 0.1	-11.6
Pa734		18	14.7	15.2 ± 0.3	15.9	-11.5	-11.2 ± 0.2	-10.9
Pa15	3	18	15.9	15.8 ± 0.4	16.5	-12	-11.2 ± 0.3	-10.8
Pa700		16	14.9	15.5 ± 0.3	15.9	-11.3	-10.7 ± 0.3	-10.3
Pa102		23	14.6	14.9 ± 0.2	15.4	-12.2	-11.5 ± 0.3	-10.9
Pa271		25	14.5	15.4 ± 0.4	16.2	-12	-11.4 ± 0.3	-10.9
Pa770		19	14.7	15.4 ± 0.4	16	-11.7	-11.4 ± 0.3	-10.8
Pa154		34	14.7	15.5 ± 0.4	16.3	-12.5	-11.5 ± 0.2	-11.6

Table S2: Compound specific $\delta^{13}C$ values of amino acids for composite samples from 6 female sperm whales. Amino acids are ordered by their essential and non-essential groupings. Abbreviations can be found in the main text (see Materials and Methods). Ser and Val are excluded due to co-elution through the GC-IRMS. While Lys is typically used as a baseline indicator, this AA did not follow the same relationship with bulk $\delta^{13}C$ values as the other EAA. Therefore, we did not include Lys in calculations of average EAA.

	Essential								Non-Essential									
Whale ID	*Phe*	±	*Ile*	±	*Leu*	±	*Lys*	±	*Asp*	±	*Glu*	±	*Pro*	±	*Ala*	±	*Gly*	±
Pa541	-27.1	0.7	-19.7	0.5	-32.4	0.2	-12.3	ND	-10.4	0.5	-14.2	0.6	-13.2	0.2	-14.5	0.8	9	0.5
Pa418	-27.9	0.9	-18.6	0.1	-30.3	0.1	-13	0.5	-12.1	0.1	-14.3	0.1	-13.3	0.1	-20.6	0.9	0.8	0.2
Pa734	-23.3	0.6	-17.6	0.5	-25.6	0.1	-17.8	0.5	-5.4	0.4	-11.6	0.9	-18.4	0.1	-12.9	0.1	1	0.1
Pa665	-21.4	1.6	-18.7	0.1	-24.8	0.1	-16	0.2	-8.5	0.2	-13.4	0.1	-14.3	0.1	-16.4	0	2.2	0.8
Pa700	-25.9	0.3	-17.8	0.1	-26	0.3	-15.8	0.3	-8.4	0.2	-12.5	0.1	-12.5	0.4	-12.4	0.4	-1.7	0.3
Pa15	-24	0.3	-16.2	0.3	-22.9	0.1	-10.9	0.1	-9.7	0.2	-12.3	0.2	-12.1	0.2	-15.8	0.1	3.1	0.4

Table S3: Compound specific $\delta^{15}N$ values of amino acids for composite samples from 6 female sperm whales. Amino acids are grouped by Trophic, Source, and Metabolic designations. Abbreviations are included in the main text (see Materials and Methods).

	Trophic													Source								Metabolic	
Whale ID	*Glx*	±	*Asx*	±	*Ala*	±	*Ile*	±	*Leu*	±	*Pro*	±	*Val*	±	*Gly*	±	*Ser*	±	*Lys*	±	*Phe*	±	*Thr*	±
Pa541	22.9	1.1	17.5	1.4	22.6	1	24.7	0.2	25.2	1.2	22.2	0.6	27.1	1.4	7.7	0.5	7.4	0	7.5	0.4	7.6	0.1	-38.8	0.3
Pa418	22.4	0.2	17.7	0.8	23.5	0.1	25	0.4	24.7	0.2	21.1	0.3	26.5	0.2	9.1	0.1	7.6	0.4	6.3	0.1	6.6	0.4	-34.6	0.5
Pa734	23.9	0.2	18.2	0.2	24.7	0.1	24.9	0.2	24.3	0.2	21.1	0.1	26.6	0.5	10.1	0.1	8.8	0.2	7.7	0.2	7.8	0.4	-29.4	0.4
Pa665	21.9	0.1	18.2	0.5	25.6	0.1	26.1	0.1	25.7	0.7	22	0.2	27.4	0.2	9.4	0.1	7.3	0.4	7.5	0.8	8.3	0.8	-35	0.7
Pa700	24.1	0.8	17.9	0.3	24.5	0.1	25.1	0.9	25.5	0.5	22.1	0.1	26.7	0.6	10.1	0.2	8.5	0.3	ND	ND	7	0.2	-34.4	0.6
Pa15	24.8	0.2	19.3	0	24.6	0.2	25.9	1	26.3	0.4	22.3	0.2	27.2	0.7	10.5	0.1	7.6	0.4	7.2	1.2	9.6	0.6	-33.3	1.2