



# Compound specific amino acid $\delta^{13}\text{C}$ patterns in a deep-sea proteinaceous coral: Implications for reconstructing detailed $\delta^{13}\text{C}$ records of exported primary production



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## ABSTRACT

Deep-sea proteinaceous corals represent high-resolution paleoarchives, extending biogeochemical time series far beyond recent instrumental data. While recent studies have applied compound specific amino acid  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N-AA}$ ) measurements of their organic skeletal layers to investigate Holocene nitrogen cycling, potential applications of amino acid  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C-AA}$ ) in proteinaceous corals have not yet been examined. Here we developed  $\delta^{13}\text{C-AA}$  analysis in deep-sea bamboo coral (*Isidella* sp.) from the Monterey Canyon to reconstruct exported primary production over an ~80 year record. Preserved deep-sea coral essential amino acid  $\delta^{13}\text{C-AA}$  patterns ( $\delta^{13}\text{C-EAA}$ ) closely matched those expected from natural and cultured phytoplankton, supporting the hypothesis that deep-sea coral  $\delta^{13}\text{C-EAA}$  values represent unaltered signatures of exported primary production sources. The coral bulk  $\delta^{13}\text{C}$  record showed cyclic 0.5‰ variations over the last century, with a shift to lower  $\delta^{13}\text{C}$  values in the early 1960s. Variations in coral  $\delta^{13}\text{C-EAA}$  values closely followed bulk  $\delta^{13}\text{C}$  signatures, although both the range and the magnitude of change in the bulk  $\delta^{13}\text{C}$  record were highly attenuated compared to the  $\delta^{13}\text{C-EAA}$  record. Our results indicate that  $\delta^{13}\text{C-EAA}$  in proteinaceous corals represent a new, direct proxy for  $\delta^{13}\text{C}$  in primary production that is more sensitive and accurate than bulk  $\delta^{13}\text{C}$ . To test this idea, we used existing phytoplankton  $\delta^{13}\text{C-AA}$  data to calculate an offset between bulk  $\delta^{13}\text{C}$  and  $\delta^{13}\text{C-EAA}$ . When applied to our data, a reconstructed record of  $\delta^{13}\text{C}$  values for exported organic matter was consistent with regional phytoplankton dynamics and expected trophic transfer effects, suggesting significant AA resynthesis only in the non-essential AA pool. Together, these results indicate that  $\delta^{13}\text{C-EAA}$  in deep-sea proteinaceous corals provide a powerful new long-term, high resolution tool for investigating variations in exported primary production and biogeochemistry.

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## 1. Introduction

During the last 40 years, stable isotope analysis has proven to be a powerful tool for tracing organic carbon in the geosphere. The  $\delta^{13}\text{C}$  signature of detrital organic matter has been widely applied to trace carbon sources, such as when comparing terrestrial and marine carbon (e.g., Goni et al., 1997; Meyers, 1994, 1997), chemosynthetic biosynthate (e.g., Rau and Hedges, 1979; McCarthy et al., 2011), and relative mixtures of different major biochemical classes (e.g., Roland et al., 2008). Variations in carbon isotopic fractionation during  $\text{CO}_2$  uptake have been linked to plankton growth rate, cell size, and  $p\text{CO}_2$ , such that  $\delta^{13}\text{C}$  values vary widely in marine microalgae from different

latitudes and/or characteristic ocean regions (Goericke and Fry, 1994; Rau et al., 1990, 1992, 2001). As a result,  $\delta^{13}\text{C}$  values for sedimentary organic matter are important paleoceanographic proxies, because variations can provide information about shifts in oceanographic regime, productivity, organic matter source, and  $p\text{CO}_2$  over widely varying timescales.

Proteinaceous corals in the orders Alcyonacea, Antipatharia and Zoantharia represent promising new archives for high-resolution paleoceanographic reconstruction (Roark et al., 2005; Sherwood et al., 2005; Williams and Grotto, 2010a, 2010b; Sherwood et al., 2011; Guilderson et al., 2013; Sherwood et al., 2014). These corals secrete endoskeletons composed of diagenetically resistant, fibrillar protein (e.g., Sherwood et al., 2006). Alcyonacean skeletons are reinforced with carbonate to variable extents. While the skeletal carbonate derives from DIC at depth, radiocarbon evidence indicates that the skeletal

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protein is synthesized from particulate organic matter (POM) recently exported from surface waters (Griffin and Druffel, 1989; Roark et al., 2005; Roark et al., 2006; Sherwood and Edinger, 2009; Hill et al., 2014). This means that the chemical and isotopic composition of proteinaceous skeletal material reflects an integration of exported primary production (EPP). These corals deposit laminar growth layers and lifespans range from decades to millennia (e.g., Roark et al., 2009; Sherwood and Edinger, 2009). Together, these factors mean deep-sea corals can provide long-term, high-resolution (annual or even sub-annual) paleoceanographic records that are not possible with sedimentary archives, thus extending biogeochemical time series far beyond the time limits of current instrumental capabilities.

Most geochemical work involving proteinaceous corals has focused on stable isotopic measurements of total (“bulk”) skeletal material, as a proxy for changes in surface ocean conditions (e.g., Sherwood et al., 2005; Williams et al., 2007; Sherwood et al., 2009; Williams and Grottoli, 2010a,b). However, bulk isotopic analysis has multiple inherent drawbacks. One of the biggest challenges to interpreting bulk stable isotope data is determining whether changes in a consumer’s stable isotope values are due to changes in diet or variability at the base of the food web ( $\delta^{13}\text{C}_{\text{baseline}}$ ,  $\delta^{15}\text{N}_{\text{baseline}}$ ), change in trophic position or variability in trophic fractionation ( $\Delta^{13}\text{C}_{\text{C-D}}$ ,  $\Delta^{15}\text{N}_{\text{C-D}}$ ), or some combination of all of these factors (McMahon et al., 2013). For example, the bulk tissue stable isotope values of deep-sea coral colonies reflect variability in the sources of primary and secondary production contributing to sinking POM. These signatures will be further modified by trophic transfers both during POM export and incorporation of POM into coral biomass. While a relatively modest 1‰ change per trophic transfer ( $\Delta\delta^{13}\text{C}$ ) is often assumed (DeNiro and Epstein, 1978), trophic fractionation values can vary widely and are poorly constrained (McMahon et al., 2013). Additionally, while deep-sea corals may be assumed to represent a single trophic transfer from the exported POM they consume, selective feeding or changes in planktonic community structure (induced by environmental change) might also alter the average trophic position between phytoplankton and exported POM. As a result, bulk tissue  $\delta^{13}\text{C}$  values in coral skeletal material and polyp tissue are often substantially removed from primary production values: indeed there is biochemical fractionation between the polyp (rich in lipids) and the proteinaceous skeleton (Roark et al., 2009; Sherwood et al., 2008). Together, these factors introduce substantial uncertainty when interpreting bulk  $\delta^{13}\text{C}$ .

Compound specific isotope amino acid analysis (CSI-AA) is a rapidly evolving analytical tool that can resolve much of the uncertainty in bulk isotopic data.  $\delta^{15}\text{N}$  CSI-AA has now been measured in many different types of organisms in order to analyze trophic dynamics and decouple baseline  $\delta^{15}\text{N}$  values from food web effects (e.g., McClelland and Montoya, 2002; McClelland et al., 2003; Popp et al., 2007; Sherwood et al., 2011, 2014). In contrast,  $\delta^{13}\text{C}$ -AA applications have not been investigated in as much detail. Research to date suggests that  $\delta^{13}\text{C}$ -AA patterns show promise as a tracer for specific C and protein sources within food webs (e.g., Fantle et al., 1999; McMahon et al., 2011a; Larsen et al., 2012; Larsen, 2013). However, in paleoceanographic applications,  $\delta^{13}\text{C}$ -AA may also be useful in reducing uncertainty related to tissue offsets and trophic effects. This idea is based on the division between non-essential and essential amino acids (NEAA and EAA, respectively). Generally, the molecular carbon skeletons of EAA cannot be synthesized by higher organisms (e.g., Rawn, 1983), and these structures are not altered during incorporation into biomass (i.e., “salvage” uptake, or direct incorporation of a molecular structure into a consumer). As a result, assuming there are no extensive diagenetic effects, it seems likely that the  $\delta^{13}\text{C}$ -EAA values are not altered during trophic transfer (e.g., O’Brien et al., 2002; McMahon et al., 2010).

In this study, our ultimate goal was to explore the potential for deep-sea coral  $\delta^{13}\text{C}$ -EAA as a new, high-resolution proxy for  $\delta^{13}\text{C}$  of exported primary production (EPP). We analyzed  $\delta^{13}\text{C}$ -AA in gorgonin skeletal material from bamboo coral (*Isidella* sp.) collected live in 2007 that

recorded approximately 80 years of prior ocean history from the Monterey Canyon. We compared coral  $\delta^{13}\text{C}$ -AA patterns with those known from marine zooplankton and phytoplankton to establish a relationship between coral and primary producer  $\delta^{13}\text{C}$ -AA signatures. We hypothesized that  $\delta^{13}\text{C}$ -EAA in a deep-sea coral should represent an integrated average of EAA from all autotrophic sources. If this is the case, the  $\delta^{13}\text{C}$ -EAA measured in the coral paleoarchive should represent a precise record of exported production that is decoupled from trophic fractionation and tissue-specific offsets that limit the utility of conventional bulk stable isotope analysis. Our development of deep-sea coral CSI-AA will have important implications for extending our understanding of past ocean conditions well beyond the modern instrumental record.

## 2. Methods

### 2.1. Sampling

One bamboo coral specimen, T1104-A07, (*Isidella* sp.) was live-collected in 2007 in the Monterey Canyon, Monterey Bay, California at 870 m depth (36°44′39.2280″, −122°02′13.9740″). Bamboo coral skeletons consist of ~5–15 cm long carbonate segments, separated by ~5–20 mm thick nodes of proteinaceous gorgonin (Noé et al., 2008). The coral was sectioned and dried at UC Davis, and an ~11 mm thick basal nodal disk was shipped to UC Santa Cruz for isotopic analysis. The skeletal disk was immersed in 10% HCl to dissolve the carbonate from the node, and the remaining node was rinsed in distilled water. Using scalpel and tweezers under a binocular microscope, approx. 0.5–1 mm thick peels were separated, following the concentric growth rings, starting from the outer margin of the node and working inward (see Sherwood et al., 2009). Sample peels (n = 45) were placed in 5 ml polyethylene vials and re-dried at 50 °C for 24 h. Each peel was subsampled for radiocarbon, bulk  $\delta^{13}\text{C}$ , and  $\delta^{13}\text{C}$ -AA analysis.

### 2.2. Radiocarbon analysis

Radiocarbon analysis to construct a coral age model was conducted on eight selected peel subsamples (~700 µg each) at the Center for Accelerator Mass Spectrometry (CAMS) at Lawrence Livermore National Laboratory (LLNL). CO<sub>2</sub> from the coral samples was graphitized after methods outlined in Vogel et al. (1987); method details used at CAMS have also recently been described in more detail by Roark et al. (2006) and Walker et al. (2008). The <sup>14</sup>C data were corrected for  $\delta^{13}\text{C}$  fractionation, and include a matrix-specific background subtraction and are presented in concordance with standard conventions defined by Stuiver and Polach (1977) using the 5568 yr Libby half-life.

### 2.3. Bulk $\delta^{13}\text{C}$ analysis

Peel subsamples (n = 45, ~300 µg each) were loaded into tin capsules and analyzed for bulk  $\delta^{13}\text{C}$  values with a standard dumas (flash) combustion Carlo Erba 1108 elemental analyzer coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (EA-IRMS), following standard protocols in the University of California, Santa Cruz Stable Isotope Laboratory (<http://es.ucsc.edu/~silab/index.php>). We also performed a second, independent sampling from the same peels to assess sampling variability (see Fig. 1, Supplementary information). Stable isotope values are reported using standard delta ( $\delta$ ) notation in parts per thousand (‰) using the following equation:

$$\delta^{13}\text{C} = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

where R is the <sup>13</sup>C/<sup>12</sup>C ratio. The reference standard for carbon is Vienna PeeDee Belemnite (VPDB). We split the bulk results into two groups, pre- and post-1960s and used one-way analysis of variance (ANOVA) to test the null hypothesis that there is no statistically significant

difference between these two groups. ANOVA was performed in Excel with Data Analysis ToolPak.

#### 2.4. Compound specific $\delta^{13}\text{C}$ amino acid analysis

Based on the main features of the bulk  $\delta^{13}\text{C}$  record, peel subsamples ( $n = 10$ , ~10 mg each) were selected for  $\delta^{13}\text{C}$ -AA analysis. Eight peels were analyzed individually and, due to lack of sufficient material in the smallest diameter rings, the four innermost peels were combined into two individual composites. Sample aliquots were hydrolyzed in 6 N HCl (110°, 20 h) and derivatized to isopropyl-TFA esters using established protocols (e.g., Silfer et al., 1991; McCarthy et al., 2013).  $\delta^{13}\text{C}$ -AA measurements were made with a Thermo Trace gas chromatograph (DB-5 ms 0.32 mm ID  $\times$  50 m length  $\times$  0.25  $\mu\text{m}$  film thickness) coupled with a Finnigan Delta Plus XP isotope ratio mass spectrometer (GC-IRMS). Samples were analyzed in triplicate, bracketed by analysis of an in-house AA standard mixture with known  $\delta^{13}\text{C}$  values. Final amino acid  $\delta^{13}\text{C}$  values were corrected for added carbon following the approach of Silfer et al (1991). Peak identification and manual baseline correction were performed with Isodat 2.0 software.  $\delta^{13}\text{C}$  values were determined for twelve AA: the essential amino acid (EAA) group, consisting of phenylalanine (Phe), threonine (Thr), isoleucine (Ile), leucine (Leu), valine (Val), and lysine (Lys); and the non-essential amino acid (NEAA) group, consisting of aspartic acid + aspartate (Asx), glutamic acid + glutamate (Glx), proline (Pro), alanine (Ala), serine (Ser), and glycine (Gly). Standard deviation for triplicate injections ranged from 0.0 to 1.8‰; the average standard deviation across the entire data set (i.e., for all AA measured) was  $0.3\text{‰} \pm 0.3$  (Table 1). While analytical precision can vary run to run, and is typically also linked strongly to sample type (e.g., McCarthy et al., 2007), such high precision is not unusual for TFAA derivatives in our laboratory when analyzing pure biological materials (e.g., Vokhshoori et al., 2014).

#### 2.5. Normalization and CSI-AA pattern comparisons with marine phytoplankton

We compared CSI-AA biosynthetic patterns in samples having different bulk  $\delta^{13}\text{C}$  values by normalizing the data to the average  $\delta^{13}\text{C}$  of the EAA group (e.g., Larsen et al., 2012) using the following equation:

$$\delta^{13}\text{C-AA}_n = \delta^{13}\text{C-AA}_m - \delta^{13}\text{C-EAA}_{\text{Avg}} \quad (2)$$

where  $\delta^{13}\text{C-AA}_n$  is the final normalized  $\delta^{13}\text{C}$  value of a given AA,  $\delta^{13}\text{C-AA}_m$  is the measured value for the same AA, and  $\delta^{13}\text{C-EAA}_{\text{Avg}}$  is the average (mean)  $\delta^{13}\text{C}$  value for all EAA measured in a given sample. We used this normalization approach to compare  $\delta^{13}\text{C}$ -EAA patterns in our *Isidella* specimen to literature marine algal  $\delta^{13}\text{C}$ -EAA patterns

(compiled by Vokhshoori et al., 2014), as well as selected zooplankton  $\delta^{13}\text{C}$ -EAA values from McMahon et al. (2011a).

#### 2.6. Linear discriminant analysis

To assess the phylogenetic origin of essential amino acids measured in coral tissue, we applied the linear discriminant function analysis (LDA) approach of Larsen et al. (2009, 2012, 2013). This approach uses LDA coupled with a published “training set” of  $\delta^{13}\text{C}_{\text{EAA}}$  data from evolutionarily distinct groups of potential primary producers, including eukaryotic marine microalgae, axenically cultured bacteria, and brown macroalgae (Larsen, 2013), in order to predict likely group membership based on measured  $\delta^{13}\text{C}_{\text{EAA}}$  values in coral peels. The LDA predictive analyses were performed in R version 2.12.1 (R-Development-Core-Team, 2012) using RStudio interface version 0.98.501 and R package MASS (Venables and Ripley, 2002).

### 3. Results and discussion

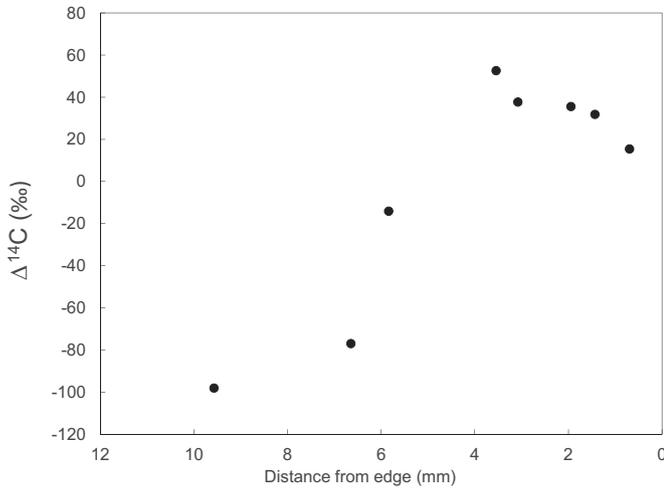
#### 3.1. Coral age, growth rate, and bulk $\delta^{13}\text{C}$ record

Eight sample peels were measured for radiocarbon (Fig. 1). The innermost peel had a  $\Delta^{14}\text{C}$  value of  $-98 \pm 3\text{‰}$  (peel #39) and the next oldest sample had a value of  $-76.9 \pm 3\text{‰}$  (peel #21). The value for peel #39 reflects pre-bomb levels of  $^{14}\text{C}$  in the water column. The increase in  $^{14}\text{C}$  reached a peak value of  $+52.6 \pm 3.3\text{‰}$  in peel #11, consistent with oceanic uptake of bomb produced radiocarbon beginning in the late 1950s (Mahadevan, 2001; Kerr et al., 2005). Taking  $1958 \pm 2$  as the year in which the ocean began taking up bomb- $^{14}\text{C}$  (Mahadevan, 2001; Kerr et al., 2005), we assigned this year to peel number 21 (distance = 6.65 mm). Combined with the year of collection (2007; distance = 0 mm), we defined a strictly linear age model (e.g., Roark et al., 2005; Sherwood and Edinger, 2009). This age model (based on Fig. 1 data) indicates that the *Isidella* record (radius = 10.76 mm) spans ~80 years. The calculated linear growth rate ( $136 \mu\text{m yr}^{-1}$ ) is consistent with measured bamboo coral growth rates from similarly highly productive environments (Roark et al., 2006; Thresher et al., 2007; Tracey et al., 2007; Sherwood et al., 2009). Overall, this growth rate indicates that each peel represents  $1.8 \pm 1.5$  yrs (range <1 yr to 8 yrs).

The average  $\delta^{13}\text{C}$  for the bulk record (from the primary run only, values plotted in Fig. 2) was  $-16.01 \pm 0.2\text{‰}$ . The  $\delta^{13}\text{C}$  values from a duplicate sub-sampling of the same peels also showed excellent agreement, demonstrating the reproducibility of this peel-based sampling approach (Fig. 1, Supplementary information). Most time intervals in the record indicate relatively similar  $\delta^{13}\text{C}$  value in Monterey Bay, with sample-to-sample variability typically  $<0.5\text{‰}$ . However, a significant

**Table 1**  
Mean  $\delta^{13}\text{C}$  values ( $\text{‰} \pm 1\sigma$  analytical variation) for measured individual amino acids for peels from *Isidella* specimen (T1104-A07). The estimated year for each sample peel # was derived from the radiocarbon age model (see Methods). Essential amino acids\*: Phe (phenylalanine), Thr (threonine), Ile (isoleucine), Leu (leucine), Val (valine), and Lys (lysine). Non-essential amino acids: Asp (aspartic acid and aspartate), Glu (glutamic acid and glutamate), Pro (proline), Ala (alanine), Ser (serine), and Gly (glycine).

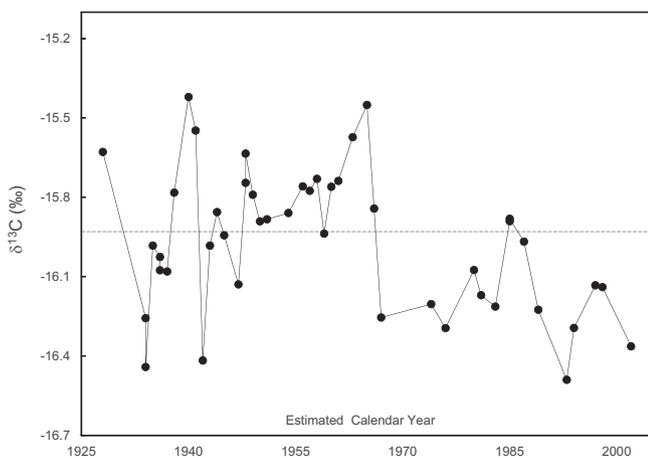
	1984	1975	1966	1965	1962	1961	1959	1956	1947	1942
	Peel 9	Peel 13	Peel 15	Peel 16	Peel 18	Peel 19	Peel 20	Peel 23	Peels 30–31	Peels 34–35
Phe*	$-22.2 \pm 0.4$	$-22.4 \pm 0.6$	$-23.0 \pm 0.2$	$-14.9 \pm 0.3$	$-22.3 \pm 0.2$	$-17.7 \pm 0.3$	$-18.2 \pm 0.2$	$-22.6 \pm 0.1$	$-21.4 \pm 1.1$	$-16.3 \pm 1.5$
Thr*	$-11.8 \pm 0.1$	$-11.6 \pm 0.3$	$-12.8 \pm 0.3$	$-13.7 \pm 0.1$	$-13.5 \pm 0.3$	$-11.5 \pm 0.1$	$-15.2 \pm 0.2$	$-15.0 \pm 0.3$	$-20.9 \pm 0.4$	$-19.5 \pm 1.2$
Ile*	$-17.7 \pm 0.3$	$-17.8 \pm 0.4$	$-19.2 \pm 0.2$	$-18.0 \pm 0.2$	$-18.3 \pm 0.1$	$-17.3 \pm 0.3$	$-18.7 \pm 0.1$	$-18.9 \pm 0.3$	$-18.7 \pm 0.5$	$-19.4 \pm 0.3$
Leu*	$-25.7 \pm 0.2$	$-26.5 \pm 0.2$	$-26.0 \pm 0.2$	$-25.1 \pm 0.2$	$-25.8 \pm 0.2$	$-22.5 \pm 0.2$	$-25.2 \pm 0.3$	$-26.2 \pm 0.4$	$-23.9 \pm 0.4$	$-24.3 \pm 0.4$
Val*	$-21.8 \pm 0.4$	$-22.0 \pm 0.1$	$-23.3 \pm 0.1$	$-22.4 \pm 0.1$	$-22.4 \pm 0.1$	$-19.5 \pm 0.7$	$-22.4 \pm 0.2$	$-23.8 \pm 0.5$	$-25.7 \pm 0.6$	$-24.6 \pm 0.8$
Lys*	$-29.0 \pm 0.9$	$-29.5 \pm 0.3$	$-27.3 \pm 0.1$	$-30.5 \pm 0.1$	$-18.5 \pm 0.4$	$-29.0 \pm 0.3$	$-29.5 \pm 0.1$	$-23.5 \pm 0.3$	$-23.9 \pm 0.3$	$-18.8 \pm 1.8$
Asp	$-5.6 \pm 0.2$	$-16.7 \pm 0.1$	$-2.9 \pm 0.3$	$-15.6 \pm 0.1$	$-2.3 \pm 0.2$	$-17.8 \pm 0.2$	$1.0 \pm 0.1$	$-0.6 \pm 0.6$	$-16.8 \pm 0.0$	$-18.1 \pm 0.2$
Glu	$-16.9 \pm 0.1$	$-17.0 \pm 0.2$	$-16.4 \pm 0.2$	$-15.7 \pm 0.1$	$-17.1 \pm 0.1$	$-16.9 \pm 0.2$	$-15.0 \pm 0.1$	$-16.3 \pm 0.1$	$-16.7 \pm 0.0$	$-16.0 \pm 0.2$
Pro	$-5.3 \pm 0.4$	$0.0 \pm 0.6$	$3.8 \pm 0.2$	$-17.8 \pm 0.0$	$2.5 \pm 0.3$	$7.1 \pm 0.4$	$-16.6 \pm 0.1$	$3.3 \pm 0.2$	$9.3 \pm 0.3$	$11.6 \pm 0.5$
Ala	$-16.4 \pm 0.2$	$-17.5 \pm 0.1$	$-21.0 \pm 0.1$	$-19.3 \pm 0.1$	$-19.4 \pm 0.1$	$-15.2 \pm 0.2$	$-17.9 \pm 0.1$	$-20.8 \pm 0.1$	$-20.8 \pm 0.4$	$-20.3 \pm 0.2$
Ser	$-10.1 \pm 0.3$	$-7.4 \pm 0.4$	$-11.1 \pm 0.2$	$-4.8 \pm 0.8$	$-8.6 \pm 0.2$	$-1.6 \pm 0.5$	N/A	$-9.4 \pm 0.1$	$-8.6 \pm 0.9$	$-8.3 \pm 0.8$
Gly	$-18.3 \pm 0.1$	$-16.5 \pm 0.1$	$-17.4 \pm 0.2$	$-16.7 \pm 0.1$	$-16.1 \pm 0.4$	$-13.7 \pm 0.2$	$-12.8 \pm 0.1$	$-18.1 \pm 0.1$	$-21.9 \pm 0.4$	$-20.5 \pm 0.3$



**Fig. 1.** Measured radiocarbon ( $\Delta^{14}\text{C}$  ‰; y-axis) as a function of sampled distance from the coral edge (mm; x-axis) for *Isidella* coral peels. The maximum value measured ( $+52.6 \pm 3.3\%$ ) corresponds very well with the expected bomb spike value, fixing this sampled interval at  $1958 \pm 2$  years in the age model (see text). The overall shape of the  $\Delta^{14}\text{C}$  curve corroborates the expectation that organic matter in the *Isidella* coral originates from surface-derived sinking particulate organic matter.

shift to lower  $\delta^{13}\text{C}$  values appears to have occurred in the early 1960s. While there are also some excursions to lower values in the earlier part of the record (which would require additional specimens to verify), values between the 1930s and the 1960s are generally higher than those from the 1970s to present (Fig. 2). Pre-1960s  $\delta^{13}\text{C}$  values averaged  $-16.2 \pm 0.27\%$ , while post-1964  $\delta^{13}\text{C}$  averaged  $-15.9 \pm 0.15\%$  (groups are significantly different at 95% confidence via one-way ANOVA;  $p = 0.004$ ). While the average offset between these periods does not appear large, there are consistently lower bulk  $\delta^{13}\text{C}$  values after the mid-1960s (without the larger variation seen in the pre-1960s data), which continue to the end of the record (2007). Because, as noted above (Fig. 1), the chronology of the  $\Delta^{14}\text{C}$ -based record is best constrained around 1960, the timing of this shift in  $\delta^{13}\text{C}$  values seems very likely to be in the early to mid 1960s.

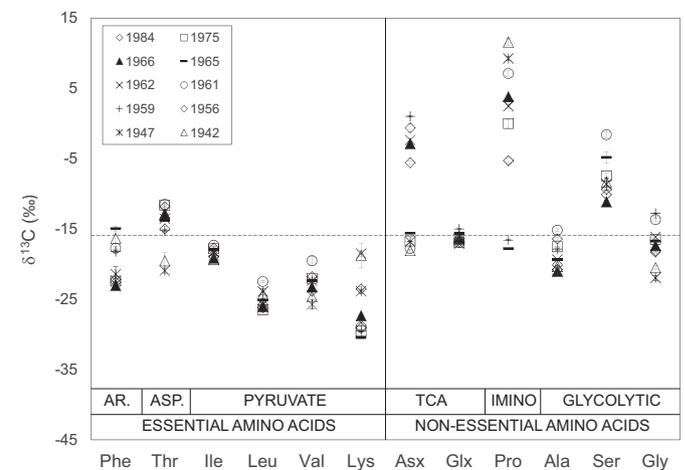
Bulk isotope records are a work-horse of the paleoceanographic community used to explore carbon/biogeochemistry dynamics from the near-instrumental to deep-time. Bulk proteinaceous deep-sea coral records can be generated with small ( $\sim 300 \mu\text{g}$ ) samples quickly and with relative ease compared with the sample size, preparation,



**Fig. 2.** Bulk  $\delta^{13}\text{C}$  record for the *Isidella* coral specimen from the Monterey Canyon in central California, with estimated calendar years calculated via a linear  $^{14}\text{C}$  age model. Dashed line represents the average bulk  $\delta^{13}\text{C}$  value.

and analytical requirements of compound-specific isotope analysis. With regard to this study, the bulk records serve two important functions: the first is to characterize isotopic variability at high (approximately annual) temporal resolution over the length of the available record. The second is to identify optimal time periods in the record where measurement of  $\delta^{13}\text{C}$ -AA will yield the most amount of information about ecosystem change, since currently  $\delta^{13}\text{C}$ -AA cannot be sampled at the same temporal (mass) resolution as that of bulk  $\delta^{13}\text{C}$ .

The reconstruction of specific paleoceanographic trends is not the main goal of this study and in general one should not make large a regional interpretation from a single specimen. However, previous work on bulk isotope records from deep-sea proteinaceous corals has shown that bulk  $\delta^{13}\text{C}$  records in particular are typically reproducible between specimens in similar locations, and can provide highly valuable records of broad ecosystem change (Sherwood et al., 2005; Williams et al., 2007; Williams and Grotoli, 2010a; Hill et al., 2014). Therefore, the bulk record from our specimen can at least provide a preliminary view of approximately centennial  $\delta^{13}\text{C}$  trends in primary production in Monterey Bay. In general, the bulk  $\delta^{13}\text{C}$  record suggests that  $\delta^{13}\text{C}$  of EPP in Monterey Bay has remained relatively stable over much of the last century. While the overall downward trend in  $\delta^{13}\text{C}_{\text{bulk}}$  appears to be consistent with the oceanic uptake of fossil fuel derived  $\text{CO}_2$  (i.e., the Suess effect), the magnitude of this change is less than what has been observed in other areas of the ocean (e.g., Keeling, 1979; Quay and Stutsman, 2003; Swart et al., 2010). Superimposed on this overall trend, there are clear but also relatively minor fluctuations in  $\delta^{13}\text{C}$  on the order of  $0.5\%$  (i.e., multiple excursions in Fig. 2, each data point representing  $\sim 2$  yr integrations). Very close to 1964, however, the data suggests that a monotonic shift may have occurred toward more negative  $\delta^{13}\text{C}$  values. This implies that a state shift in the Monterey Bay biogeochemical carbon cycling may have occurred around this time. Because the Monterey Bay is a highly productive region that is sensitive to numerous climatic and oceanographic processes, there could be multiple explanations for this apparent excursion. A more detailed exploration of this possible shift, including potential explanations, is beyond the scope of this paper, and will require collection and analysis of additional regional coral specimens. We suggest that this Monterey Bay bulk  $\delta^{13}\text{C}$  record will serve as a valuable reference as additional corals become available which together can provide more detailed interpretation.



**Fig. 3.** Compound specific isotope amino acid (CSI-AA)  $\delta^{13}\text{C}$  values from the *Isidella* specimen across a nearly 80 year record (different symbols represent different years from a linear  $^{14}\text{C}$  age model). Amino acids are grouped by their metabolic pathway (essential or non-essential) and their carbon skeleton biosynthetic families (Ar. = Aromatic; Asp. = Aspartic). Dashed line indicates average bulk  $\delta^{13}\text{C}$  value for all samples ( $-15.93\%$ ). The average  $\delta^{13}\text{C}$  value for measured amino acids after adjusting for molar composition ( $-15.88 \pm 1.51\%$ ) was statistically indistinguishable from the bulk value. This indicates that hydrolyzable amino acids represent essentially all organic carbon in the skeletal material.

### 3.2. Compound specific amino acid $\delta^{13}\text{C}$ values

The CSI-AA  $\delta^{13}\text{C}$  results for replicate coral subsamples produced similar patterns and values (Fig. 3). The average bulk  $\delta^{13}\text{C}$  for the subsamples used for CSI-AA was  $-15.93 \pm 0.3\text{‰}$ , and the average total hydrolyzable amino acid  $\delta^{13}\text{C}$  value (the molar percent weighted  $\delta^{13}\text{C}$  value;  $\delta^{13}\text{C}_{\text{THAA}}$ ) across the same samples was within the  $1\sigma$  error ( $\delta^{13}\text{C}_{\text{THAA}} = -15.88 \pm 1.5\text{‰}$ ). Since the gorgonin is dominated by protein, the close match between these values indicates that  $\delta^{13}\text{C}$ -AA values are accurately measuring all major amino acids in the gorgonin skeleton. This also indicates for the first time that  $\delta^{13}\text{C}$ -AA values can provide a molecular-level understanding of variation in gorgonin bulk  $\delta^{13}\text{C}$  values.

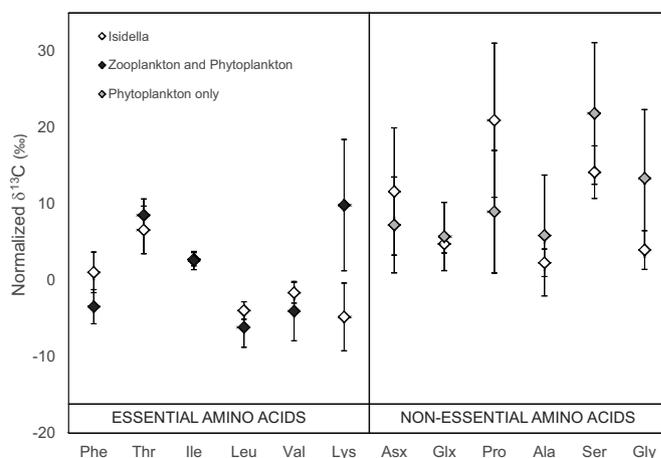
When considering the *Isidella*  $\delta^{13}\text{C}$ -AA results with respect to other heterotrophic organisms, both the range and offsets of individual AA  $\delta^{13}\text{C}$  values conform to expectations. The overall range between individual AA  $\delta^{13}\text{C}$  values is large ( $>40\text{‰}$ ), consistent with previous studies (McMahon et al., 2010). The EAA had depleted  $\delta^{13}\text{C}$  values (average  $-21.16 \pm 0.9\text{‰}$ ) relative to the mean bulk  $\delta^{13}\text{C}$  value while the average NEAA  $\delta^{13}\text{C}$  value was much less depleted ( $-11.74 \pm 1.6\text{‰}$ ). This is consistent with  $^{13}\text{C}$  in NEAA being further enriched during trophic transfer (Vizzini and Mazzola, 2003; France and Peters, 1997). Therefore, the overall  $\delta^{13}\text{C}$ -AA patterns in our *Isidella* specimen are consistent with the hypothesis that EAA directly reflects primary production sources, although we note that value comparisons alone cannot conclusively make this linkage.

### 3.3. Comparison to phytoplankton $\delta^{13}\text{C}$ -AA patterns

We compared the  $\delta^{13}\text{C}$ -AA patterns in *Isidella* to  $\delta^{13}\text{C}$ -AA patterns in marine algae and zooplankton to test the underlying hypothesis that EAA  $\delta^{13}\text{C}$  values derive directly from primary production sources, without influence from trophic transfers. Previous work has indicated that  $\delta^{13}\text{C}$ -AA patterns for different marine plankton sources are very similar (McCarthy et al., 2004), and those patterns are passed on to upper trophic level consumers virtually unmodified (McMahon et al., 2010; McMahon et al., 2011a). After normalizing measured  $\delta^{13}\text{C}$ -EAA values, the resulting pattern should therefore correspond with the marine algal pattern, despite having been transferred through the food web. The  $\delta^{13}\text{C}$ -EAA pattern in the *Isidella* specimen closely matches the expected pattern from published plankton  $\delta^{13}\text{C}$ -AA data (Fig. 4; Vokshoori et al., 2014) with the exception of Lys, which shows high variability between the two patterns. However, we caution drawing conclusions from Lys  $\delta^{13}\text{C}$  values; Lys is notoriously challenging to accurately analyze (co-elutes with tyrosine and has poor chromatography).

While the overall match between the normalized EAA patterns in the *Isidella* and plankton species seems remarkably close, the patterns for NEAAs are much further apart (Fig. 4). In a heterotroph, NEAA offsets from the autotrophic pattern indicate that resynthesis has occurred, and is generally expected (McMahon et al., 2010, 2011b). The much greater ranges for NEAA in autotrophic sources is also consistent with the high variability in specific AA (e.g., Ser and Gly; McMahon et al., 2011b). Together, this comparison strongly supports the expectation that  $\delta^{13}\text{C}$ -EAA values are maintained through trophic transfer, while  $\delta^{13}\text{C}$ -NEAA values are subject to potentially extensive heterotrophic alteration (O'Brien et al., 2002; McMahon et al., 2010).

One possible caveat with respect to *Isidella*  $\delta^{13}\text{C}$ -EAA patterns is that EAA and NEAA groupings have not been directly studied in proteinaceous corals. In general, cnidarians are phylogenetically distant from metazoan species that the canonical "essential" and "non-essential" dichotomy is based upon. To our knowledge there have been no direct studies on obligate amino acid synthesis in deep-sea proteinaceous corals, although some previous studies have suggested that shallow water hermatypic zooxanthellate scleractinian corals can synthesize EAA to a limited degree (Fitzgerald and Szmant, 1997; and related Wang and Douglas, 1999). However, in these studies the canonically



**Fig. 4.** A comparison of amino acid  $\delta^{13}\text{C}$  values, normalized to mean essential amino acid (EAA)  $\delta^{13}\text{C}$  value ( $\delta^{13}\text{C}_{\text{Normalized}} = \delta^{13}\text{C}_{\text{AA}} - \text{Avg. } \delta^{13}\text{C}_{\text{EAA}}$ ), between *Isidella* and literature data for plankton. The strong similarity of normalized EAA patterns between *Isidella* (open diamonds) and phytoplankton (gray diamonds; Supplementary information) as well as mixed tows of phytoplankton and zooplankton together (black diamonds; Supplementary information) confirms that EAA patterns are passed on to upper trophic level consumers virtually unmodified. In contrast, much poorer correspondence between normalized non-essential amino acid (NEAA) patterns for phytoplankton compared to *Isidella* indicates significant resynthesis. For zooplankton and phytoplankton, error bars are  $\pm 1\sigma$  across the chosen data sets and for *Isidella* the error bars are  $\pm 1\sigma$  across peels. Note that because substantial resynthesis and fractionation are expected for NEAAs with trophic transfer, only pure phytoplankton data are shown for NEAAs.

"essential" amino acids were not synthesized to the extent of "non-essential" amino acids. Furthermore, we note that caution is warranted in applying the results of experiments using scleractinian corals and cnidaria that harbor symbiotic bacteria and other microorganisms to azooxanthellate proteinaceous deep-sea corals. While the basis of the EAA group in these corals is an interesting topic for further research, the correspondence between phytoplankton and  $\delta^{13}\text{C}$ -EAA patterns clearly does not suggest any significant EAA resynthesis in *Isidella*.

### 3.4. Linear discriminant analysis of $\delta^{13}\text{C}$ -EAA sources: autotrophic algae vs. bacteria

One important process that could alter the  $\delta^{13}\text{C}$ -EAA patterns of sinking POM available to deep-sea proteinaceous corals is microbial degradation and reworking during water column transit. It is well known that the large majority of sinking POM exiting the euphotic zone is remineralized during water column transit, resulting in dramatically reduced yields of hydrolyzable amino acids, as well as other major biochemical classes (e.g. Wakeham et al., 1997). However, at the same time sinking particles in general retain relatively "fresh" biogeochemical signatures, as opposed to the often more degraded suspended POM pool. For example, previous studies have shown that sinking POM has modern radiocarbon ( $\Delta^{14}\text{C}$ ) signatures (e.g., Druffel et al., 1992), and solid state NMR data has demonstrated broad non-selective degradation of exported POM reaching the deep ocean in multiple basins (Hedges, 2001), and perhaps most directly relevant to this study, prior CSI-AA data has shown that deep ocean sediments trap material with "fresh" algal patterns, lacking characteristic signatures of microbial degradation (McCarthy et al., 2004, 2007). For a highly productive, high sediment-accumulation margin regime with a relatively shallow water column (such as Monterey Bay), one would generally expect sinking particles to be strongly dominated by recently exported surface algal material.

To test this expectation, we applied an isotopic fingerprinting LDA model to our EAA  $\delta^{13}\text{C}$  values, based on the approach and molecular-isotopic training set in Larsen (2013). The LDA approach is based on evolutionary divergence in EAA  $\delta^{13}\text{C}$  patterns, and has now been increasingly applied to fingerprint relative phylogenetic sources in a

variety of both living and detrital OM pools (e.g., Larsen et al., 2012; Larsen, 2013; Vokhshoori et al., 2014). This is a particularly effective approach for separating bacterially synthesized AA from those derived from algal AA (Hannides et al., 2013). The LDA results for the Monterey Bay *Isidella* samples predicted marine microalgae as the most likely source for EAA (probability =  $96 \pm 3\%$ ) for all coral peel samples (Fig. 5). Importantly, none of the coral peel samples showed indication of either bacterial or macroalgal  $\delta^{13}\text{C}_{\text{EAA}}$  patterns, indicating that protein contributions from microbially re-worked detritus were negligible, at least when compared to the microalgal sources. This result corresponds closely with conclusions from the more general pattern comparison discussed above (Section 3.3), as well as the basic organic geochemical expectations for POM source and flux in this high production margin system. It also further supports the basic assumption that deep-sea proteinaceous corals' primary food source is "fresh" sinking particles (as opposed to potentially far more degraded suspended particles), based on  $\Delta^{14}\text{C}$  data (Roark et al., 2005, 2006; Sherwood et al., 2005, 2009), as well as prior  $\delta^{15}\text{N}_{\text{AA}}$  data (Sherwood et al., 2011, 2014).

### 3.5. $\delta^{13}\text{C}$ temporal trends in essential amino acids compared to bulk gorgonin

Based on our hypothesis that surface primary production is the main C source for these corals, the variations in  $\delta^{13}\text{C}$ -EAA would be expected to follow those in *Isidella* bulk  $\delta^{13}\text{C}$  values. However, as noted above, the exact bulk  $\delta^{13}\text{C}$  values could also be substantially decoupled from the original primary production signal, for example due to cumulative food web effects. Therefore, we directly compared the bulk  $\delta^{13}\text{C}$  to the average  $\delta^{13}\text{C}$ -EAA for the samples on which CSI-AA was performed, both in terms of relative variation,  $\delta^{13}\text{C}$  values, and dynamic range (Fig. 6). Due to the high variability in NEAA values (Fig. 4), and also the potential for complex and somewhat unpredictable resynthesis effects noted below, we have focused our direct comparison only to the more stable EAA values.

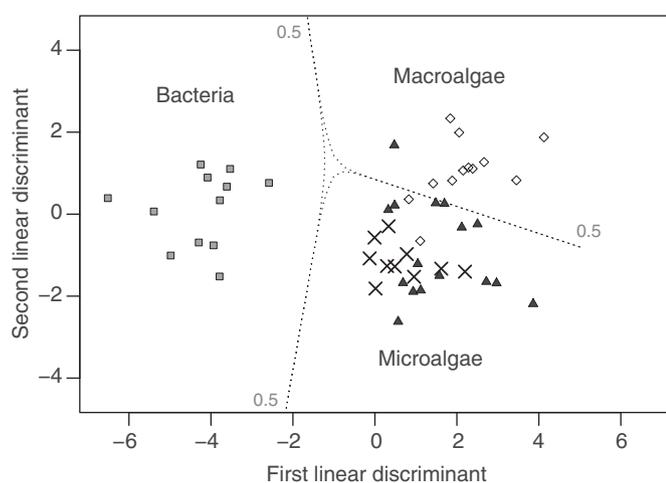
Temporal variations in average  $\delta^{13}\text{C}$ -EAA values closely tracked the bulk record (Fig. 6). When including all data points in a regression analysis,  $r^2 = 0.3$  and  $p = 0.099$ ; however, removing the earliest data points from peels (i.e., the region most likely to exhibit overlap sampling errors; see Fig. 1, Supplementary information) produces an improved

correlation ( $r^2 = 0.6$  and  $p = 0.02$ ). This shows a potential limitation of larger sample requirements for CSI-AA based analysis when working with very small specimens. However, overall, good correspondence is evident for the 1960s excursion, as well as relative shifts occurring later (Fig. 6). We note that the overall pattern in both bulk and  $\delta^{13}\text{C}$ -EAA shown in Fig. 6 appears different compared to the entire bulk record, due to containing far fewer data points. However, our goal here was only to compare how relative offsets in bulk and  $\delta^{13}\text{C}$ -EAA track each other, rather than to construct a time series record.

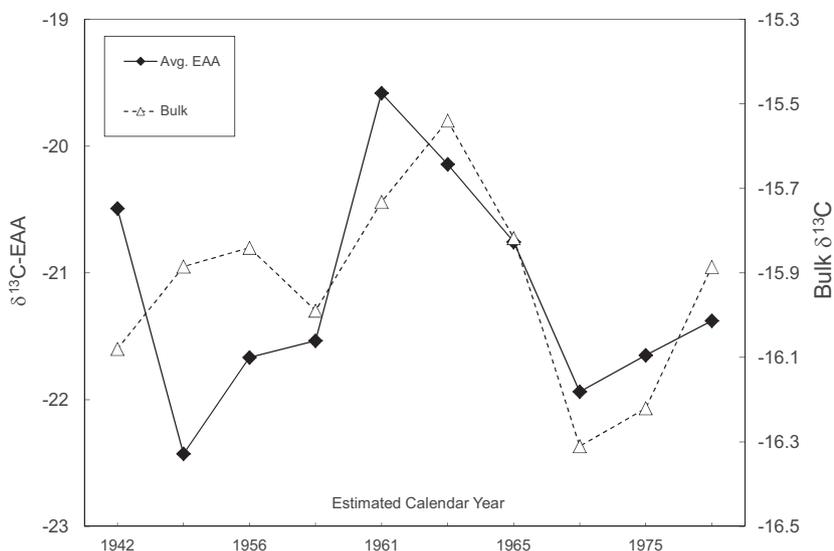
Overall, the similarity between these records confirms that changes in *Isidella* bulk  $\delta^{13}\text{C}$  values closely follow  $\delta^{13}\text{C}$  values of EPP. However, at the same time, the substantial offset in the relative magnitude of the variations between the bulk and EAA records suggests that either other carbon sources, or the resynthesis of NEAA has strongly muted primary production  $\delta^{13}\text{C}$  changes recorded in the bulk coral  $\delta^{13}\text{C}$  record. Because of the close match between reconstructed  $\delta^{13}\text{C}$ -THAA and bulk values noted above (Fig. 3) we hypothesize that resynthesis of NE-AA is the more likely scenario. In heterotrophic consumers, the  $\delta^{13}\text{C}$  values of NEAAs biosynthesized from glycolytic (e.g. glycine) or Krebs cycle (e.g. glutamate) precursors are not closely related to their respective dietary amino acids, but rather the bulk diet  $\delta^{13}\text{C}$  value (Howland et al., 2003; Jim et al., 2006; Newsome et al., 2011). As a result,  $\delta^{13}\text{C}$  variations in the bulk record could become strongly muted compared to those in the average EAA record (Fig. 6, primary vs. secondary axis), due to the averaging effect of NEAA biosynthesis from a bulk carbon pool. The gorgonin skeleton, like most tissues, is comprised mostly of NEAA (~75 mol% NEAA vs. ~25 mol% EAA), consistent with the hypothesis that trophic changes could be responsible for the offset observed. Overall, we suggest that this hypothesis (that NE-AA resynthesis, linked to trophic effects) can dampen bulk  $\delta^{13}\text{C}$  records in deep proteinaceous corals and will be an important topic for future research. If correct, this implies that the actual shifts in  $\delta^{13}\text{C}$  values may be substantially greater than are apparent from bulk  $\delta^{13}\text{C}$  coral analysis. If correct, this would imply that  $\delta^{13}\text{C}$ -EAA represents both a more accurate and more sensitive indicator of shifts in primary production  $\delta^{13}\text{C}$  values.

### 3.6. $\delta^{13}\text{C}$ EAA values: a new approach to reconstruct $\delta^{13}\text{C}$ of EPP

We show that  $\delta^{13}\text{C}$ -EAA values in paleoarchives provide a powerful new tool to reconstruct the bulk  $\delta^{13}\text{C}$  values of EPP. A conceptual diagram (Fig. 7) summarizes the relationship between  $\delta^{13}\text{C}$ -EAA and bulk  $\delta^{13}\text{C}$  values in a simplified deep-sea coral food web, showing both issues with bulk analysis, and potential of directly measuring  $\delta^{13}\text{C}$ -EAA. The cartoon indicates the major trophic enrichment factors (TEFs), as well as tissue-specific biosynthetic offsets, that underlie the offset ( $\epsilon$ ) between  $\delta^{13}\text{C}$  values in primary production and in  $\delta^{13}\text{C}$  of bulk gorgonin. We hypothesized that the substantial  $^{13}\text{C}$  enrichment in bulk gorgonin skeletal material observed compared to the expected marine primary production (i.e., ~ $\delta^{13}\text{C}$  4 to 6‰ greater in gorgonin material vs. likely plankton sources) is due to a series of trophic transfer fractionations between phytoplankton, zooplankton, and the coral polyp (TEF 1, TEF 2). As noted above, bulk  $\delta^{13}\text{C}$  values are elevated with each trophic transfer, however the exact magnitude of each TEF is very difficult to predict. In our cartoon we have used  $\delta^{13}\text{C}$  TEF values of ~1‰ (e.g., Fry and Sherr, 1984), although as noted above, TEF values in practice can be highly variable. Further, the coral's food source (sinking POM) is a mixture of primary and secondary production (with relative proportions varying with exact location), whose proportions could change based on overlying planktonic food web variations. Therefore, the cartoon also describes a third pathway representing the effective simultaneous coral feeding on the phytoplankton portion of sinking POM (TEF 3). Overall, consideration of these contributing factors shows that the effective TEF for a given skeletal band would be almost impossible to predict with any precision, due to the dependence on multiple TEF values and the relative composition of labile material in sinking POM.



**Fig. 5.** Linear discriminant phylogenetic source analysis based on essential amino acid (EAA)  $\delta^{13}\text{C}$  values in *Isidella*. First two linear discriminants are plotted, based on  $\delta^{13}\text{C}$  values of five essential amino acids (EAA: Ile, Leu, Phe, Thr, Val). Training set data for three categorical variables, marine microalgae (filled triangles,  $n = 16$ ), bacteria (gray squares,  $n = 12$ ), and marine macroalgae (open diamonds,  $n = 12$ ) are from Larsen (2013). The training data set is used to predict the biosynthetic origins of EAAs among coral peel samples (solid X,  $n = 10$ ). All coral samples classified as microalgae, with an average of 96% probability.



**Fig. 6.** Average essential amino acid values ( $\delta^{13}\text{C}$ -EAA) of *Isidella* coral peels (solid line, left y-axis) compared to the bulk  $\delta^{13}\text{C}$  record in from those sample samples (dashed line, right y-axis) across an 80 year time series. The close correspondence of changes in both records shows the direct link between coral bulk  $\delta^{13}\text{C}$  values and primary production. However, the substantial offset in dynamic ranges (note different scales on the left and right y axis) suggests that  $\delta^{13}\text{C}$  variation in exported production has been strongly attenuated in the bulk  $\delta^{13}\text{C}$  record.

Finally, due to characteristic compound-class fractionations, we would also expect substantial  $\delta^{13}\text{C}$  offsets between the gorgonin skeleton and the total animal (coral polyp). Protein has heavier  $\delta^{13}\text{C}$  values than other major biochemical compound classes (e.g., Hayes, 2001), implying expected heavier  $\delta^{13}\text{C}$  values for the proteinaceous gorgonin skeleton. However, gorgonin is also a unique structural protein, with an AA composition different than that found in muscle or other tissues (Goodfriend, 1997; Sherwood et al., 2006). As a result, a substantial offset between the structural gorgonin and the polyp tissue (“ $\epsilon$  Gorgonin”) is also likely.

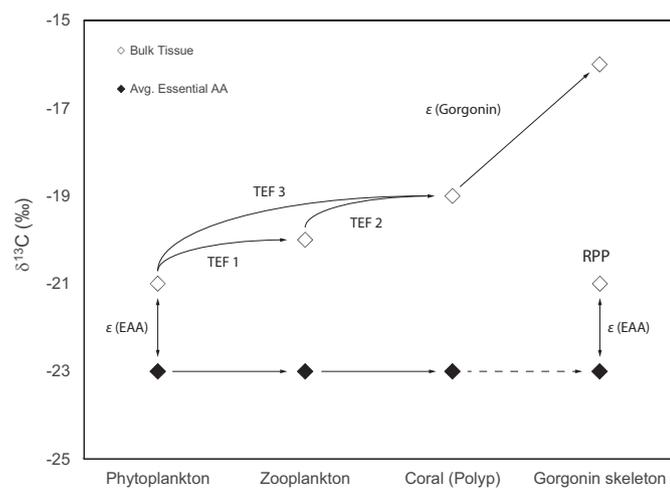
Overall, the conceptual cartoon makes it clear that while trends in bulk  $\delta^{13}\text{C}$  of gorgonin skeletal material might mirror those in primary

production sources, the *actual*  $\delta^{13}\text{C}$  values of primary production can probably be only very roughly estimated from bulk values, because the exact offsets depend on many variables that are both organism and location specific. The net offset might also change through a coral record, and the effective TEF value is likely linked to changes in POM composition. However, even if bulk TEF factors vary greatly, the EAA pass through trophic levels with little or no change in  $\delta^{13}\text{C}$  values (O’Brien et al., 2002; McMahon et al., 2010). As a result,  $\delta^{13}\text{C}$ -EAA values in gorgonin should represent a direct record of average  $\delta^{13}\text{C}$ -EAA values from surface ocean primary production sources. If a predictable offset between  $\delta^{13}\text{C}$ -EAA and bulk  $\delta^{13}\text{C}$  for phytoplankton exists, then this could allow a direct calculation of  $\delta^{13}\text{C}$  for exported primary production (Fig. 7). This concept is supported by our results presented above (Fig. 4), as well as previous research suggesting similar phytoplankton  $\delta^{13}\text{C}$ -AA patterns (e.g., McCarthy et al., 2004).

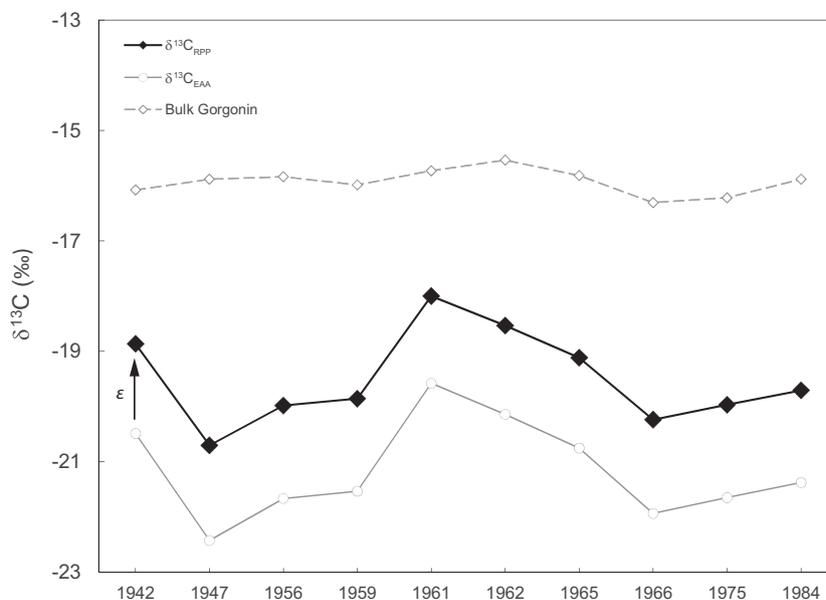
### 3.7. Using $\delta^{13}\text{C}$ -EAA to reconstruct bulk primary production

We measured  $\delta^{13}\text{C}$ -EAA values from our *Isidella* specimen, together with a compilation of literature phytoplankton  $\delta^{13}\text{C}$ -AA data, to calculate “reconstructed” bulk EPP values for Monterey Bay. This calculation is based on the scheme outlined above (Fig. 7). While marine phytoplankton  $\delta^{13}\text{C}$ -AA data are not yet extensive, we followed the approach of Vokhshoori et al. (2014). These authors showed a strong linear relationship between bulk  $\delta^{13}\text{C}$  and  $\delta^{13}\text{C}$ -EAA across wide range of plankton species. Using this regression relationship (see Fig. 2, Supplementary information), our measured  $\delta^{13}\text{C}$ -AA yields a mean  $\delta^{13}\text{C}$  value of  $-19.5 \pm 0.8\%$  for reconstructed EPP production, across all intervals for which we measured  $\delta^{13}\text{C}$ -AA (Fig. 8). This value is much lighter compared to the corresponding average bulk gorgonin value of  $-15.9 \pm 0.3\%$ , and heavier than the average  $\delta^{13}\text{C}$ -EAA ( $-21.16 \pm 0.9\%$ ). These offsets correspond directly with the expected relationships described above (Fig. 7). Our “reconstructed” primary production (RPP) using this approach mirrors all changes in average EAA presented above, since it is offset by a constant (Fig. 7). All main changes in the primary production  $\delta^{13}\text{C}$  pattern therefore track bulk record features, however with the much greater amplitude of  $\delta^{13}\text{C}$  change indicated by the  $\delta^{13}\text{C}$ -EAA data (Fig. 6).

Overall, the RPP  $\delta^{13}\text{C}$  values predicted by this approach correspond well with  $\delta^{13}\text{C}$  values in previous research on phytoplankton production in this region (Rau et al., 2001). As noted above, phytoplankton in



**Fig. 7.** Conceptual diagram of reconstruction of primary production  $\delta^{13}\text{C}$  values based on measured essential amino acid (EAA)  $\delta^{13}\text{C}$  in the *Isidella* coral gorgonin skeleton. The illustration indicates relative offsets between EAA and bulk  $\delta^{13}\text{C}$  values ( $\epsilon$ ), trophic discrimination factors (TEF) associated with trophic transfer in a simplified deep coral food web. The measured bulk  $\delta^{13}\text{C}$  value (open diamond) of gorgonin skeletal material (“gorgonin skeleton”) can only be related back to initial primary production through a series of poorly constrained bulk fractionation changes. These include changes related to direct trophic transfers, complicated by variable fraction of primary versus secondary production in labile sinking POM (TEF 1 vs. TEF 2 + 3), as well as tissue specific ( $\epsilon$  Gorgonin) biosynthetic  $\delta^{13}\text{C}$  offsets. In contrast,  $\delta^{13}\text{C}$ -EAA values (black diamonds) are constant through trophic transfer.



**Fig. 8.** A record of bulk  $\delta^{13}\text{C}$  reconstructed primary production ( $\delta^{13}\text{C}_{\text{RPP}}$ ) compared to bulk  $\delta^{13}\text{C}$  for *Isidella* sp. gorgonin (bulk gorgonin). The  $\delta^{13}\text{C}_{\text{RPP}}$  is derived from measured average essential amino acid  $\delta^{13}\text{C}$ -EAA values, and is based on the offset ( $\epsilon$ ) between plankton bulk  $\delta^{13}\text{C}$  and  $\delta^{13}\text{C}$ -EAA, which is assumed as a constant (indicated by arrow). The offset was calculated using a published linear regression relationship, as described in the text. While pattern of change is similar for all records, estimated primary production  $\delta^{13}\text{C}$  values are substantially depleted relative to gorgonin skeleton, consistent with the mechanisms outlined in Fig. 7.

coastal upwelling zones typically have heavier  $\delta^{13}\text{C}$  values, in contrast with offshore samples. However, ranges also substantially overlap, and exact values are tied to the ocean processes of specific locations. [Rau et al. \(2001\)](#) found that POC from the Monterey Bay had  $\delta^{13}\text{C}$  values with a general range of  $-18\text{‰}$  to  $-24\text{‰}$  in the 1990s. While these comparisons cannot address any specific feature of this preliminary record, they do support our hypothesis that reasonable  $\delta^{13}\text{C}$  values for exported primary production can be calculated from  $\delta^{13}\text{C}$ -EAA data.

#### 4. Overview and conclusions

We have presented the first  $\delta^{13}\text{C}$  CSI-AA data from a deep-sea proteinaceous coral. Our high-resolution ( $\sim 2$  yr intervals) bulk  $\delta^{13}\text{C}$  record from a nearly century old specimen exhibited  $\sim 0.5\%$  fluctuations on decadal scales, including an apparent shift to lower  $\delta^{13}\text{C}$  values in the early 1960s. Overall, measured  $\delta^{13}\text{C}$ -AA patterns indicate excellent preservation of AA isotope values in the gorgonin skeletal material. In particular, the  $\delta^{13}\text{C}$  patterns for the EAA closely matched patterns expected from marine phytoplankton, while NEAA patterns were far more variable, and showed clear evidence for AA resynthesis. This strongly supports the basic hypothesis that  $\delta^{13}\text{C}$ -EAA values derive from surface algal production, without significant isotopic fractionation.

Taken together, our results suggest that  $\delta^{13}\text{C}$ -EAA in deep sea proteinaceous corals constitutes a direct record of  $\delta^{13}\text{C}$  of surface export production, which would be unaffected by variations in plankton trophic structure, POM composition, or likely coral species. The correspondence in timing of  $\delta^{13}\text{C}$ -EAA vs. bulk  $\delta^{13}\text{C}$  change in our *Isidella* is also consistent with a plankton surface source for all AA making up gorgonin skeletal carbon. However, we also observed substantial differences in amplitude of the bulk vs.  $\delta^{13}\text{C}$ -EAA records, most likely linked to resynthesis of NEAA that constitutes the majority of the total AA pool. This finding implies that much of the isotopic variability in EPP may be obscured in proteinaceous coral bulk  $\delta^{13}\text{C}$  records, and that  $\delta^{13}\text{C}$ -EAA data may represent both a more accurate and more sensitive record of primary production  $\delta^{13}\text{C}$  changes.

Based on the direct link between the EAA and algal sources, we propose that  $\delta^{13}\text{C}$ -EAA data can be used to calculate  $\delta^{13}\text{C}$  values for average EPP. Our finding that a calibration derived from phytoplankton cultures

could recreate expected bulk  $\delta^{13}\text{C}$  values for POM has potentially wide implication in paleoceanographic studies. It suggests that  $\delta^{13}\text{C}$ -EAA measurements may be developed as a new tool for more accurate reconstruction of primary production  $\delta^{13}\text{C}$  records in any archive where proteinaceous material is well preserved. Further, despite our focus on  $\delta^{13}\text{C}$ -EAA values, we note that  $\delta^{13}\text{C}$  values of the NEAA pool may also carry significant, complementary information. Specifically, because AA resynthesis linked to trophic transfer should occur only in the NEAA pool, we hypothesize that comparing relative change in both groups might provide complementary information about shifts in food web structure over time. Understanding the information potential in NEAA  $\delta^{13}\text{C}$  in living organisms is now in early stages (e.g., [McMahon et al., 2010](#)), however we suggest that this area may also have substantial potential in paleoarchives.

Finally, we suggest that an important area of future work should be more firmly establishing the offset between  $\delta^{13}\text{C}$  values in bulk phytoplankton relative to  $\delta^{13}\text{C}$ -EAA, and also investigating if this offset varies among major plankton types. This information will be necessary to more accurately interpret proteinaceous coral  $\delta^{13}\text{C}$ -EAA records in different oceanic regions. The extreme longevity of these organisms suggests that if robust calibrations can be developed,  $\delta^{13}\text{C}$ -EAA measurements may allow extremely high resolution  $\delta^{13}\text{C}$  records of EPP, potentially spanning the Holocene. The wide-distribution of deep-sea corals further suggests far reaching potential application, to construct detailed records of oceanic baseline isotopic changes.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marchem.2014.09.008>.

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