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Impact of skeletal heterogeneity and treatment method on interpretation of environmental variability from the proteinaceous skeletons of deep-sea gorgonian octocorals

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ABSTRACT

The stable isotope geochemistry of gorgonian octocoral skeletons facilitates detailed time series reconstructions of nutrient biogeochemistry. However, comparisons among reconstructions from different locations require realistic estimates of the uncertainty surrounding each measured geochemical value. Here, we determine quantitative uncertainties related to 1) standard skeletal pretreatment in preparation for stable isotopic analysis and 2) biological variability associated with a heterogeneous isotopic composition of the gorgonin skeleton. We found that the 5% HCl pretreatment required for the $\delta^{13}C$ measurements does not significantly impact the $\delta^{15}N$ values of the skeleton nor the reproducibility of the $\delta^{15}N$ measurements. In contrast, while 5% HCl pretreatment significantly altered bulk $\delta^{13}C$ values via removal of $CaCO_3$, it did not change amino acid $\delta^{13}C$ values in the organic skeleton. We found that the variance of repeat measurements of skeleton samples formed contemporaneously and homogenized skeleton for both $\delta^{13}C$ and $\delta^{15}N$ exceeded that of instrumental uncertainty of an acetanilide standard. This indicates that instrumental uncertainty underestimates the true precision of an isotopic measurement of the organic skeleton. Furthermore, measurements of contemporaneous skeleton around the circumference of an octocoral colony yielded variability exceeding that of homogenized skeleton. Based on these results, we find that 1) both $\delta^{13}C$ and $\delta^{15}N$ values can be measured simultaneously in pretreated skeleton, 2) growth bands should be homogenized prior to analysis, and 3) reported error should include uncertainty due to biological effects determined from repeat analysis of homogenized skeleton and not just instrument error to reduce false significant differences. Our results present an important protocol for processing proteinaceous octocoral skeletons and propagating uncertainty to more accurately reconstruct nutrient dynamics from proteinaceous deep-sea octocoral skeletons.

1. Introduction

1.1. Gorgonian octocorals

Projecting future changes in nutrient regimes in the ocean necessitates the understanding of the mechanisms that drive variability in past oceanic biogeochemical cycling (e.g., Gordon and Morel, 2012; Henderson, 2002; Rothwell and Rack, 2006). However, there is often a significant data gap between the high-resolution instrumental records spanning the recent past and the lower resolution paleoceanographic sediment records that cover much longer time frames. Long-term, high resolution geochemical information extracted from accretionary, biogenic skeletons of long-lived deep-sea octocorals can fill this critical information gap (e.g., Druffel, 1997; Ehrlich, 2010; Robinson et al., 2014). For instance, time series measurements of the stable isotope composition across the skeletal axes of proteinaceous octocorals have

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revealed insights into nutrient dynamics including increased terrestrial effluent to the deep-sea, changing planktonic communities, and variable export production from oceanic surface waters (e.g., Baker et al., 2010b; McMahon et al., 2015; Sherwood et al., 2014; Ward-Paige et al., 2005; Williams and Grottoli, 2010). These insights have in turn yielded critical information about ecosystem drivers related to anthropogenic versus natural climate variability.

The deep-sea gorgonian octocorals (> 50 m depth) capture geochemical signals of changing biogeochemistry in their skeletons, which comprised organic, proteinaceous "gorgonin" material in some combination with calcite in concentric, coeval (i.e., the skeleton material deposited during the same time period) growth rings (Fig. 1) (Roberts, 2010) . The carbon source to the calcite skeleton is ambient dissolved inorganic carbon (Roark et al., 2006) . In contrast, the carbon and nitrogen in the gorgonin skeleton is primarily sourced from sinking particulate organic matter (POM), recently exported from the surface mixed layer (Griffin and Druffel, 1989; Sherwood et al., 2005) . Octocorals consume food via their metabolically active polyps (Orejas et al., 2003; Ribes et al., 1999; Roark et al., 2009) and the isotopic composition of that dietary signal is then faithfully preserved in the proteinaceous gorgonian skeleton of the octocorals (McMahon et al., 2018) . The isotopic composition of the skeletal material is preserved once it is laid down as the skeleton is metabolically inert post deposition (Sherwood et al., 2006) . These corals can live for hundreds of years (Prouty et al., 2015; Williams et al., 2007a) . Therefore, isotopic measurements of the concentric growth rings of the proteinacous skeleton (Fig. 1) provide a chronological record of past nutrient sources to the octocorals over the past centuries (Sherwood et al., 2005) .

The time series of nutrient geochemistry extracted from the gorgonin skeleton of the deep-sea octocorals are often interpreted such that fluctuations and secular changes in the skeletal stable isotopic composition exceeding instrumental error reflect environmental variability. However, there are two potential additional sources of uncertainty that should be considered when interpreting time series reconstructions: 1) uncertainty associated with changes in the isotopic composition of the skeleton due to pretreatment of the skeleton in preparation for stable isotopic analysis and 2) biological uncertainty associated with a heterogeneous isotopic composition of the skeleton within a single specimen reflecting the biology of the organism. While these sources of error have been recognized in previous studies that interpret geochemical records extracted from these octocorals (e.g., Heikoop et al., 2002; Sherwood et al., 2005), there has been no systematic quantification of their impacts on the measured isotopic composition of the skeleton.

Fig. 1. Polished cross-section of the trunk of a gorgonian *Primnoa pacifica* colony. The concentric, coeval growth rings formed of gorgonin skeletal material interspersed with calcite are visible.

Pretreatment of the gorgonian octocorals is needed for carbon stable isotope ($\delta^{13}C$) reconstructions of export production from the gorgonin skeleton. This is because the calcite fraction of the skeleton, sourced from ambient dissolved inorganic carbon, needs to be removed to isolate the organic gorgonin skeleton. This isolation procedure involves bathing the octocoral skeleton in hydrochloric acid (HCl) for hours to weeks to dissolve the calcite skeleton. However, the impact of this pretreatment on the isotopic integrity of the remaining organic matrix, and thus the ability of pretreated skeleton to faithfully preserve the isotopic composition of the octocoral's food geochemistry (i.e., sinking POM) is not well understood. Furthermore, the impact of acidification pretreatment on the $\delta^{15}N$ composition of other organic materials has been highly variable in previous studies: pretreatment with high concentrations of HCl significantly changes the bulk $\delta^{15}N$ values yet pretreatment with lower concentrations of HCl (1 M HCl) has an inconsistent impact, if any, on $\delta^{15}N$ values (Jacob et al., 2005; Kennedy et al., 2005) . In terms of gorgonian octocorals, previous studies report higher $\delta^{15}N$ values of 0.7–0.9‰ in acidified pretreated material than untreated material (Heikoop et al., 2002; Sherwood et al., 2010) . The potential impact of the acidification pretreatment on the repeatability of these measurements in gorgonian octocorals is unknown. If there is a systematic impact on either the mean or the reproducibility of a measurement, then this impact needs to be considered during statistical analyses to accurately distinguish environmental-caused variability in the skeleton from analytical-induced variability.

Typically, time series reconstructions exploring the variability in nutrient dynamics through time measure the stable isotopic composition of the gorgonian octocoral skeleton along a single radial transect of an octocoral colony cross-section (e.g., Williams et al., 2007b). Conversely, studies exploring spatial variability in nutrient geochemistry typically measure either the polyp tissue and/or outer growth layers of the skeletal axis (e.g., Baker et al., 2010a). The isotopic value measured for each point in time is assumed to reflect coeval skeleton for that octocoral colony. However, $\delta^{15}N$ values within a coeval ring may vary up to 1.5‰, far exceeding instrumental error (typically 0.1 to 0.3‰) Sherwood et al. (2005). This suggests that the isotopic composition of the gorgonian skeleton may vary around the circumference of a coeval skeleton growth ring, which also must be reconciled when interpreting environmental variability from deep-sea octocoral skeletal geochemistry.

We addressed the following questions to work towards more robust interpretations of gorgonian octocoral $\delta^{13}C$ and $\delta^{15}N$ reconstructions: 1) Does acidification pretreatment to remove the calcified portion of the skeleton impact the resulting stable isotopic composition of the gorgonin skeleton? (Experiment One) 2) Does gorgonin skeletal stable isotopic composition vary circumferentially around a single coeval growth band? (Experiment Two). These experiments tested whether pretreatment increases variance in the isotopic composition of the skeleton and/or if there is heterogeneity in the isotopic composition of coeval skeleton exceeding that of instrumental uncertainty. The additional uncertainties revealed from these experiments need to be propagated into the resulting statistical analyses for robust interpretation of environmental reconstructions derived from the gorgonian octocoral skeletons. To illustrate this point, we evaluated differences in octocoral skeleton geochemistry among different regions in the northeast Pacific Ocean with and without the propagation of the uncertainty resulting from analytical treatment and biological variability in octocoral skeletons.

2. Methods

2.1. Specimens

Octocorals were collected from multiple locations in the northeastern Pacific Ocean (Fig. 2). Three *Acanthogorgia* sp. colonies, two *Eugorgia rubens* colonies, and two *Adelogoria* sp. colonies, all within the suborder Holaxonia were, were collected in 2015 from the Channel Islands National Marine Sanctuary (Table 1) Caldow et al. (2015). One *Callogorgia* sp. and two *Thouarella* sp. colonies, both within the suborder Calcaxonia, were collected in 2014 from Sur Ridge. Fifteen *Primnoa pacifica* colonies within the suborder Calcaxonia were collected from the NE Pacific in 2010, 2013, and 2015 (Table 1) (Rooper et al., 2017) . Specimens were air dried and stored at ambient room temperature prior to sample preparation.

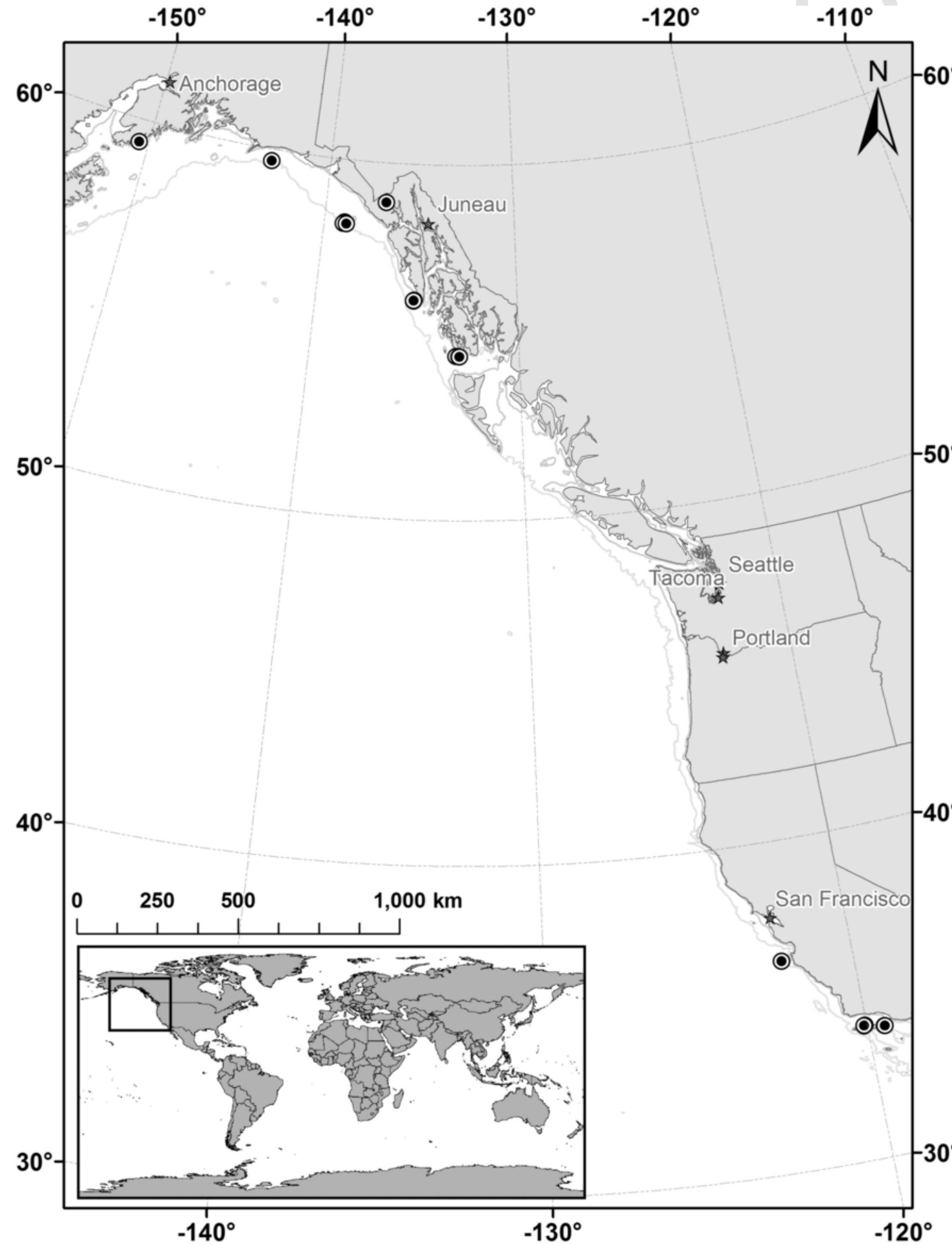

Fig. 2. Locations of specimens used in the present study.

Table 1
Gorgonian specimens used in the present study. Bulk indicates specimens analyzed for bulk stable isotopic composition in Experiment One, CSIA indicates compound-specific stable isotope analysis of amino acids in Experiment One, and Heterogeneity indicates specimens analyzed in Experiment Two.

Specimen ID	Location	Taxa	Latitude	Longitude	Depth (m)	Collection year	Experiment
CIMNS33C	Channel Islands, CA	*Acanthogorgia* sp.	33.95534	−119.46715	203	2015	Bulk
CINMS44A	Channel Islands, CA	*Acanthogorgia* sp.	33.9595	−119.47561	174	2015	Bulk
CINMS32C	Channel Islands, CA	*Acanthogorgia* sp.	33.95498	−119.4667	203	2015	Bulk
CINMS35A	Channel Islands, CA	*Eugorgia rubens*	34.08747	−120.06872	61	2015	Bulk
CINMS40A	Channel Islands, CA	*Eugorgia rubens*	34.07521	−120.09156	63	2015	Bulk
CINMS38A	Channel Islands, CA	*Adelogorgia* sp.	34.07923	−120.09187	68	2015	Bulk
CINMS39A	Channel Islands, CA	*Adelogorgia* sp.	34.07671	−120.09193	63	2015	Bulk
BSR1	Sur Ridge, CA	*Callogorgia* sp.	36.43145	−122.294221	1557	2014	Bulk
BSR2	Sur Ridge, CA	*Thouarella* sp.	36.43145	−122.294221	1557	2014	Bulk
BSR3	Sur Ridge, CA	*Thouarella* sp.	36.43145	−122.294221	1557	2014	Bulk
GB2	Glacier Bay, AK	*Primnoa pacifica*	58.88433	−136.79017	>100	2010	CSIA
GOA004	Nula Bay, AK	*Primnoa pacifica*	59.35453	−150.52772	290	2013	CSIA
GOA005	Middleton Ridge, AK	*Primnoa pacifica*	59.66517	−143.22141	248	2013	CSIA
GOA011	Shutter Ridge, AK	*Primnoa pacifica*	56.17494	−135.11639	196	2013	Bulk, CSIA, Heterogeneity
GOA022	Shutter Ridge, AK	*Primnoa pacifica*	56.17217	135.11604	206	2013	Bulk, Heterogeneity
GOA046	Shutter Ridge, AK	*Primnoa pacifica*	56.19202	−135.11023	167	2013	Bulk, CSIA
GOA060	Shutter Ridge, AK	*Primnoa pacifica*	56.17932	135.11948	205	2013	Bulk
GOA067	Shutter Ridge, AK	*Primnoa pacifica*	56.17837	−135.11802	216	2013	Bulk, Heterogeneity
WPA1	Fairweather Ground, AK	*Primnoa pacifica*	58.24574	−138.90448	142	2015	Bulk
WPA2	Fairweather Ground, AK	*Primnoa pacifica*	58.24577	−138.90443	142	2015	Bulk
WPA3	Fairweather Ground, AK	*Primnoa pacifica*	58.20335	−138.97746	393	2015	Bulk
WPA4	Fairweather Ground, AK	*Primnoa pacifica*	58.20468	−138.81752	162	2015	Bulk
GOA-195	Dixon Entrance, AK	*Primnoa pacifica*	54.64080	−133.00505	339	2015	Bulk
GOA-209	Dixon Entrance, AK	*Primnoa pacifica*	54.63376	−132.85332	160	2015	Bulk
GOA-212	Dixon Entrance, AK	*Primnoa pacifica*	54.63447	−132.85103	165	2015	Bulk

2.2. Experiment One: pretreatment effect

The trunk of each octocoral colony was cross-sectioned, polished, and imaged using a microscope with a digitally controlled x, y, and z stage to create a high-resolution photo mosaic of the skeleton (Fig. 1). A Taig micromill controlled using CNC software facilitated careful drilling of the colony skeleton along the path of the growth bands so that only coeval skeleton was removed. The resulting powdered skeletal material (~20 mg) was homogenized and separated into two splits of ~10 mg each. The first split of the powdered skeleton was bathed with 5% HCl for 5 days, with the acid refreshed daily by centrifuging until the powder formed a pellet, decanting the old acid, and then adding new 5% HCl followed by manually shaking the samples to resuspend the sample. After 5 days, the skeleton was rinsed thoroughly with MQ water three times by centrifuging, decanting the old MQ water, and then adding fresh MQ water and manually shaking the samples to resuspend the sample. Samples were then dried overnight at 60 °C. The second split of powdered skeleton was kept dry for the duration of the experiment. The splits were individually homogenized and then ten sub-samples of ~0.7 mg of material were separated from each split, weighed, and packaged for stable isotopic analysis. We evaluated the effect of a standard 5% acidification pretreatment on the bulk $\delta^{13}C$ and $\delta^{15}N$ values of the gorgonin skeleton. We also evaluated the isotopic composition of individual amino acids in a subset of skeleton samples before and after pretreatment to explore potential underlying mechanisms of isotopic offsets during the standard 5% acidification pretreatment. For these samples, each octocoral skeleton sample was homogenized and subdivided into two aliquots, one that was directly processed for compound-specific stable isotope analysis of amino acids (CSIA-AA) and another that underwent a 5% acidification pretreatment process and was then processed for CSIA-AA.

2.3. Experiment Two: skeletal heterogeneity

To evaluate the heterogeneity in the isotopic composition of the skeleton within a single growth band, we measured the stable isotopic composition of the bulk gorgonin skeleton at different points around the trunk of one set of growth bands for each of three separate colonies of one species, *Primnoa pacifica* (Table 1). To isolate samples for analysis, the skeletal cross-sections from the trunk were bathed in 5% HCl, refreshed every other day, for 15 days to remove the calcite fraction. The longer duration for the pretreatment was used here since the octocoral cross-sections were thicker and thus contained more calcite. After 10 days of dissolution, sections were transferred to a glass petri dish and immersed in MQ water. Using forceps, the skeletal bands were peeled off the main cross section under a binocular microscope. Using forceps, the outermost skeletal band immediately adjacent to the tissue was discarded to eliminate the incorporation of surface damaged outer band. A second and then third layer of growth bands were then removed, rinsed three times in MQ water, and dried overnight at 60 °C. Since only full growth bands were used, there should be no aliasing of time along a single layer. Thus, each layer represents coeval skeleton. One growth layer was homogenized into a fine powder and then divided into ten equal ~0.7 mg sub-samples for stable isotopic analysis. The other growth layer was broken into sequential pieces around the circumference of the growth layer. Each piece was individually homogenized into a powder and then packaged into ~0.7 mg sub-samples for stable isotopic analysis.

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2.4. Bulk stable isotopic analysis

The stable isotope (δ ^{13}C and δ ^{15}N) values of each sample were measured using a CE Instruments NC2500 elemental analyzer interfaced to a ThermoFinningan Delta Plus XP isotope ratio mass spectrometer (IRMS) at the University of California Santa Cruz Stable Isotope Laboratory (UCSC-SIL). Tin encapsulated samples were flash (Dumas) combusted at 950°C in a quartz column containing chromium oxide (acting as an oxygen source to aid combustion) and silvered colbatous/cobaltic oxide (acting as a scrubber to clean the combustion products of sulfur bearing compounds and halides). Following combustion, excess oxygen and oxides of nitrogen are reduced in a reduction column (reduced copper at 650°C). Helium carrier flows through a water trap containing magnesium perchlorate. N_2 and CO_2 are separated on a Carbosieve GC column (45°C, 100 mL/min) before introduction to the IRMS.

During analysis, the calibrated in-house standard Pugel preceded and was interspersed between samples to correct for linearity (size) effects and drift. A second calibrated laboratory standard, Acetanalide, was run as a sample to monitor quality control and long-term performance. These standards were compositionally similar to the coral samples and have been previously calibrated against NIST Standard Reference Materials (IAEA-N2, IAEA-N3, IAEA-USGS25, and IAEA-USGS26 of δ ^{15}N and IAEA-CH7, and NBS-22 and IAEA-USGS25 for δ ^{13}C).

δ ^{13}C values are reported relative to Vienna Peedee Belemnite Standard (V-PDB) (δ ^{13}C = permil deviation of the ratio of stable carbon isotopes ^{13}C:^{12}C relative to V-PDB (Coplen, 1994)). δ ^{15}N values are reported relative to air (δ ^{15}N = per mil deviation of the ratio of stable nitrogen isotopes ^{15}N:^{14}N relative to air (Mariotti et al., 1984)). The standard deviation of the mean of repeated measurements of the Acetanilide standard (n = 85) was 0.06‰ for δ ^{13}C and 0.15‰ for δ ^{15}N. The average standard deviation of samples run in duplicate (10% of all samples) was 0.37‰ for δ ^{13}C and 0.43‰ for δ ^{15}N.

2.5. Compound specific stable isotopic analysis

All samples were processed through standard CSIA-AA procedures outlined in McMahon et al. (2015) and Sherwood et al. (2014). Briefly, gorgonin skeleton samples (3 mg for δ ^{13}C and 6 mg for δ ^{15}N) underwent protein hydrolysis followed by standard clean up in cation exchange columns (Dowex 50WX* 400 ion exchange resin) to isolate individual AAs. Samples were then derivatized by esterification with acidified iso-propanol and acylation with trifluoroacetic anhydride and dichloromethane (Silfer et al., 1991) . Derivatized samples were extracted with P-buffer (KH2PO4 + Na22HPO4 in Milli-Q water, pH 7) and chloroform three times with centrifugation (600 g) and organic phase extraction between each round (Ueda et al., 1989) .

For AA δ ^{13}C analyses, the derivatized AAs were injected in split mode at 250°C and separated on a DB-5 column (50 m × 0.5 mm inner diameter; 0.25 m film thickness; Agilent Technologies, Santa Clara, California, USA) in a Thermo Trace Ultra gas chromatograph (GC) at the UCSC-SIL. The separated AA peaks were analyzed on a Finnegan MAT DeltaPlus XL isotope ratio mass spectrometer (IRMS) interfaced to the GC through a GC-C III combustion furnace (960C) and reduction furnace (630°C). For AA δ ^{15}N analyses, the derivatized AAs were injected in splitless mode at 250° C and separated on a BPX5 column (60 m × 0.32 mm inner diameter, 1.0 m film thickness; SGE Analytical Science, Austin, Texas, USA) in the same GC-C-IRMS interfaced through a combustion furnace (980°C), reduction furnace (650°C), and a liquid nitrogen trap.

Standardization of runs was achieved using intermittent pulses of a CO2 or N2 reference gas of known isotopic value and internal nor-Leucine standards. All CSIA-AA samples were analyzed in triplicate along with AA standards of known isotopic composition (Sigma-Aldrich Co.). The long-term reproducibility of stable isotope values in a laboratory algal standard provides an estimate of full protocol reproducibility (replicate hydrolysis, wet chemistry, and analysis): 13C = 0.7‰ and 15N = 0.3‰ (calculated as the long-term SD across >100 separate full analyses, averaged across all individual AAs).

2.6. Statistical analysis

To evaluate the potential impact of the acidification pretreatment on the octocoral geochemistry, first we ran separate paired t-tests comparing the C:N, 13C, and 15N values for all samples before and after pretreatment. Next, to test if there were differences in the response of the isotopic composition by region/taxa, we used separate One-Way Analyses of Variance (ANOVAs) to assess significant differences in C:N ratios and stable isotope composition of gorgonin skeleton with and without 5% acidification pretreatment. Testing for differences among taxa within a single region was not feasible because of low statistical power; however, the results from different taxa tended to group together by site suggesting that site was more important of a factor than taxa. To examine the impact of 5% acidification pretreatment on AA stable isotope values, we calculated offsets in individual AA 13C and 1515N values for the gorgonin skeleton subsamples before and after the acidification pretreatment. We then used separate one-sample t-tests to see if the 13C and 15N offsets were significantly different from 0. All statistics were performed in R using RStudio interface (R Core team 2013).

To evaluate skeletal heterogeneity, we calculated the standard deviation of repeat analysis of 1) homogenized coeval skeleton and 2) coeval skeleton sampled from around the circumference of a colony trunk (not homogenized). We did this for both pretreated and not pretreated skeleton. Bartlett's test was used to determine if variance differed among the four different treatments: 1) homogenized and pretreated, 2) homogenized with no pretreatment, 3) pretreated and not homogenized, and 4) not pretreated and not homogenized.

To examine how incorporation of varying degrees of analytical uncertainty and biological variability impacted comparisons of octocoral geochemistry, we tested for significant differences in the skeletal stable isotopic composition among octocorals collected in different regions in the northeast Pacific Ocean with only instrumental uncertainty measured by an acetanilide standard and the full error propagation. To do this, we conducted an error propagation simulation for 13C and 15N. Using empirical uncertainty estimates derived from Experiment Two for each of the four treatments, we simulated 100 sets of sample values for each specimen in the original data set from Experiment One. The simulations were drawn from normal distributions with mean zero and standard deviation equal to: (1) the empirical uncertainty estimates for the homogenization and pretreatment errors, (2) the empirical uncertainty estimates for the homogenization and no pretreatment errors, (3) the empirical uncertainty estimates for the no homogenization and pretreatment errors, and (4) the empirical uncertainty estimates for the no homogenization and no pretreatment errors. The error standard deviations in (1) through (4) were calculated using the standard error propagation formula, with total uncertainty equal to:

$$u_{total} = \pm\sqrt{\Sigma_{i=1}^{L} u_i^2}$$

where u*i* are the individual component uncertainties, indexed i = 1, 2, ..., L. Simulated errors were added to the original 13C and 15N values for each specimen. We then calculated the mean values and standard deviations of 13C and 15N values from each simulated sample for every geographical region with multiple specimens (Channel Islands, Sur Ridge, Gulf of Alaska - Shutter Ridge, Gulf of Alaska - Fairweather

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Ground, and Gulf of Alaska - Dixon Entrance (Table 2). This yielded 100 error-propagated samples for each region. To obtain estimates of the expected "region/taxa effect" we calculated the mean values for 13C and 15N and their standard deviations, respectively, from the 100 samples for each region and determined 95% confidence intervals using a t-distribution. We chose the conservative t-distribution as opposed to a normal distribution since the total variance for each region is unknown. Following this step, we were able to compare geographical regions and determine which comparisons showed statistically significant differences by inspecting whether the confidence intervals overlap.

3. Results

3.1. Effect of pre-treatment

The C:N ratios of octocoral skeletons in our study ranged from 3.0 to 6.7 in the skeleton for the material with no pretreatment and then significantly decreased to the range of 2.8 to 4.6 (p = 0.0003, df = 18) for the pretreated skeleton. This difference was significant for the sites SR, GOA-SR, WPA, and GOA-DE (Fig. 3). δ^{13}C values ranged from −21.45 to −10.35‰ for the material with no pre-treatment and then converged to −20.85 to −17.29‰ for the pretreated skeleton (significant difference between means = 0.0001, df = 18). This difference was significant for the sites SR, GOA-SR, WPA, and GOA-DE (Fig. 3). The δ^{15}N values ranged from 9.27 to 14.66‰ in the gorgonin skeleton for the material with no pretreatment and 10.60 to 15.29‰ for the material with the acidification pretreatment. The δ^{15}N values did not significantly differ between the acidification pretreated material and that with no pretreatment at any site (p = 0.58, df = 19) (Fig. 3).

Table 2
Mean (no acidification treatment minus acidification treatment SD) offset in amino acid 13C and 15N values from the gorgonin skeletons of *Primnoa pacifica* before and after 5% acidification pretreatment (n = 5 octocorals, na = not analyzed). Separate one-sample t-tests were used to determine if individual amino acid 13C and 15N offsets were significantly different from 0‰ (= 0.05) (df = 4 for all amino acids except 15N Met where df = 1).

	δ^{13}C offset (‰)		δ^{15}N offset (‰)	
	Mean SD	tdf (p value)	Mean SD	tdf (p value)
Alanine	−0.2 ± 0.5	1.04 (0.36)	0.0 ± 0.4	−0.10 (0.92)
Aspartic acid	0.0 ± 0.4	0.00 (1.00)	0.2 ± 0.6	0.56 (0.60)
Glutamic acid	0.0 ± 0.4	−0.25 (0.82)	0.0 ± 0.4	0.23 (0.83)
Glycine	0.0 ± 0.4	−0.1 (0.92)	0.2 ± 0.3	−1.23 (0.29)
Isoleucine	0.0 ± 0.5	0.1 (0.93)	0.2 ± 0.4	0.87 (0.43)
Leucine	0.3 ± 0.4	1.69 (0.17)	0.1 ± 0.1	2.06 (0.11)
Lysine	na	na	0.4 ± 0.3	3.64 (0.02)
Methionine	na	na	−0.1 ± 0.1	−3.00 (0.20)
Phenylalanine	0.0 ± 0.4	−0.23 (0.83)	0.3 ± 0.3	2.76 (0.06)
Proline	0.0 ± 0.4	−0.31 (0.77)	−0.4 ± 0.3	−2.54 (0.06)
Serine	−0.3 ± 0.3	−1.76 (0.15)	0.0 ± 0.5	−0.16 (0.88)
Threonine	0.2 ± 0.5	0.91 (0.42)	−0.1 ± 0.4	−0.86 (0.44)
Valine	0.3 ± 0.3	2.74 (0.05)	−0.2 ± 0.4	−1.36 (0.25)

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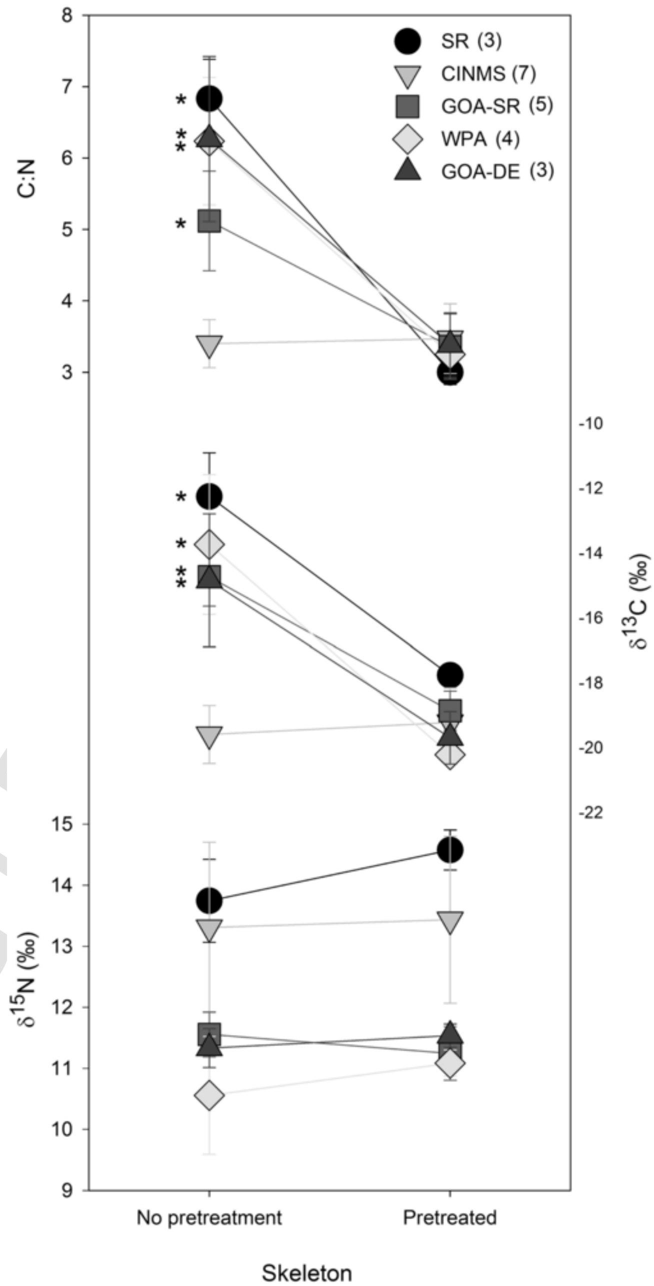

Fig. 3. Differences between no pretreatment (skeleton with $CaCO_3$) and acidification pretreatment (skeleton with no $CaCO_3$) on C:N, δ^{13}C, and δ^{15}N for the octocoral gorgonin skeleton. The number in parentheses indicates the sample N. * indicates significant differences between treatments. Data plotted by region: Channel Islands (CINMS), Sur Ridge (SR), Gulf of Alaska - Shutter Ridge (GOA-SR), Gulf of Alaska - Fairweather Ground (WPA), and Gulf of Alaska - Dixon Entrance (GOA-DE).

The 5% acidification pretreatment did not significantly alter the 13C nor the 15N values of the amino acids in the *Primnoa pacifica* gorgonin skeleton away from the non-acidified mean: mean offset between non-acidified and acidified octocoral skeletons (averaged across all amino acids) was 0.0 ± 0.4‰ for both 13C and 15N. Only Lysine showed a significant 15N offset (0.4 ± 0.3‰) but even that offset was close to the instrumental error of the CSIA-AA procedure (0.3‰).

3.2. Skeletal heterogeneity

For the δ^{13}C values, the average standard deviation for repeat analyses increased from 0.03 (Acetanalide standard) to 0.10 (pretreated and homogenized skeleton) to 0.20 (homogenized and not pretreated skeleton) to 0.33 for (pretreated with no homogenization) to 0.40 (homogenization and not pretreated) (Fig. 4). For the $\delta^{15}N$ values, the average standard deviation increased from 0.02 (Acetanalide standard) to 0.13 (homogenized and pretreated) to 0.16 (homogenized with no pretreatment) to 0.30 for (pretreated with no homogenization) to 0.29 (no pretreatment and no homogenization) (Fig. 4). In comparing if pretreatment impacted the variance, the average standard deviation of the $\delta^{13}C$ values in homogenized samples differed between the pretreated samples and samples with no pretreatment (test statistic = 0.003) while there was no difference in the variance of the $\delta^{15}N$ values (Table 3). In contrast, the variance of the $\delta^{15}N$ values in samples that were not homogenized did differ between the pretreated samples and samples with no pretreatment (test statistic = 0.005), however there was no difference in the variance of the $\delta^{13}C$ values (Table 3). In comparing if homogenization had an impact on the variance, the variance differed for both $\delta^{13}C$ and $\delta^{15}N$ values for the pretreated skeleton (test statistics = < 0.0001 and 0.019, respectively) and the skeleton with no pretreatment (test statistics = < 0.0001 and 0.022, respectively) (Table 3).

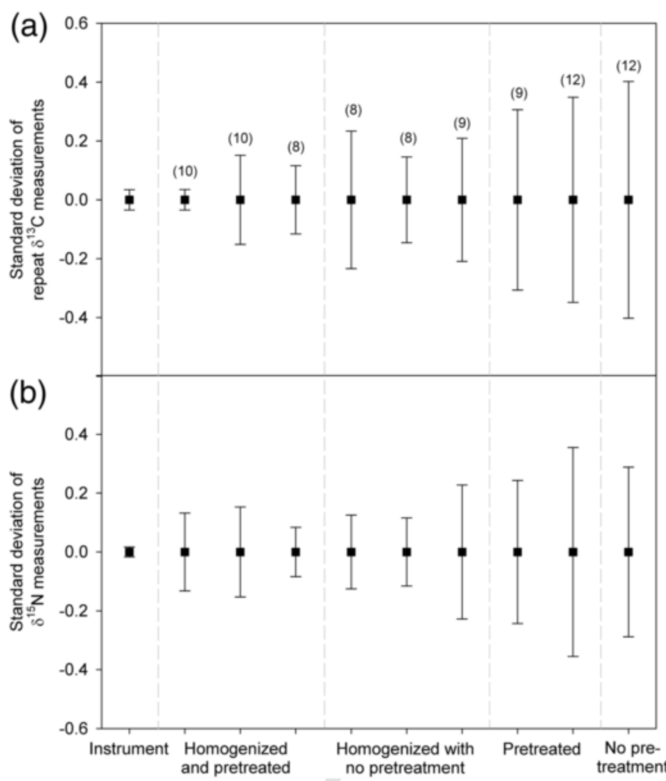

Fig. 4. Standard deviation of (a) carbon stable isotopic composition and (b) nitrogen stable isotopic composition of repeated measurements. The number in parentheses indicates N for each treatment. See Table 3 for significance of Bartlett's test comparing variance among the treatments.

3.3. Propagated error

When we compared the average $\delta^{13}C$ and $\delta^{15}N$ values of octocorals among regions using only instrumental error determined using the acetanilide standard, we found significant differences among each region, whether pretreated or not pretreated values were investigated (Fig. 5). For the $\delta^{13}C$ values, the average values significantly varied among the regions using the propagated errors determined for pretreated and homogenized treatment. The $\delta^{13}C$ values of the octocorals from the Channel Islands and Shutter Ridge (GOA) did not significantly differ if the propagated error for the pretreatment but not homogenized treatment was used. For the $\delta^{15}N$ values, only the octocorals from the Channel Islands and Sur Ridge, both from California, significantly differed consistently from the other sites when the propagated errors were used for all treatments. From the Alaskan sites, the octocorals from Dixon Entrance significantly differed with the homogenization and pretreatment values, and the octocorals from Fairweather Ground significantly differed with the no homogenization and pretreatment values and the no homogenization and no pretreatment values (Fig. 5).

4. Discussion

4.1. Experiment One: pretreatment effect

In the octocorals within the suborder Calcaxonia (Sur Ridge octocorals *Callogorgia* sp. and *Thouarella* sp., and Alaskan octocorals *Primnoa* sp), the acidification pretreatment significantly decreased the amount of carbon in the skeleton and reduced the C:N ratios (Fig. 3). This suggests that the acidification pretreatment removed the calcitic portion of the octocoral skeleton. As a result, the pretreatment process also significantly decreased the $\delta^{13}C$ values of the bulk skeleton, since the carbon source of the calcitic skeleton is ambient DIC with isotopically higher $\delta^{13}C$ values than marine organic matter (Fig. 3). These results are consistent with previous studies that used the HCl pretreatment to remove calcite in the *Primnoa* sp. skeleton (e.g., Sherwood et al., 2009, Williams et al., 2007b). While the standard 5% acidification pretreatment did significantly alter octocoral skeleton bulk $\delta^{13}C$ values, it did not significantly impact the $\delta^{13}C$ values of the individual AAs that make up that bulk material (Table 2). This suggests that the pretreatment acidification process is doing what it was intended to do: remove the inorganic fraction of the skeleton while preserving the integrity of the remaining organic fraction. In contrast to the Calcaxonian colonies, the acidification pretreatment did not significantly change the C:N ratio and the $\delta^{13}C$ values in the Holaxonian octocorals from the Channel Islands (Fig. 3). This suggests that there may be minimal calcite contribution to these octocorals, and thus pretreatment may not be required in this taxon.

We evaluated the impact of the pretreatment on the repeatability of the $\delta^{13}C$ measurement. For homogenized material, the variance of repeated measurements was significantly higher in samples that were not pretreated than in samples that were pretreated (Table 3). This sug-

Table 3
Results from Bartlett's test comparing variance of repeated measurements of the C:N ratios and stable isotopic composition of different treatments of gorgonian octocoral skeleton.

	Experiment One:		Experiment Two:	
	Pretreatment vs. No pretreatment		Homogenized vs. Not homogenized	
	Homogenized	Not homogenized	Acidification pretreatment	No pretreatment
CN	0.210	< 0.0001	< 0.0001	0.020
$\delta^{13}C$	0.003	0.409	< 0.0001	< 0.0001
$\delta^{15}N$	0.835	0.005	0.019	0.022

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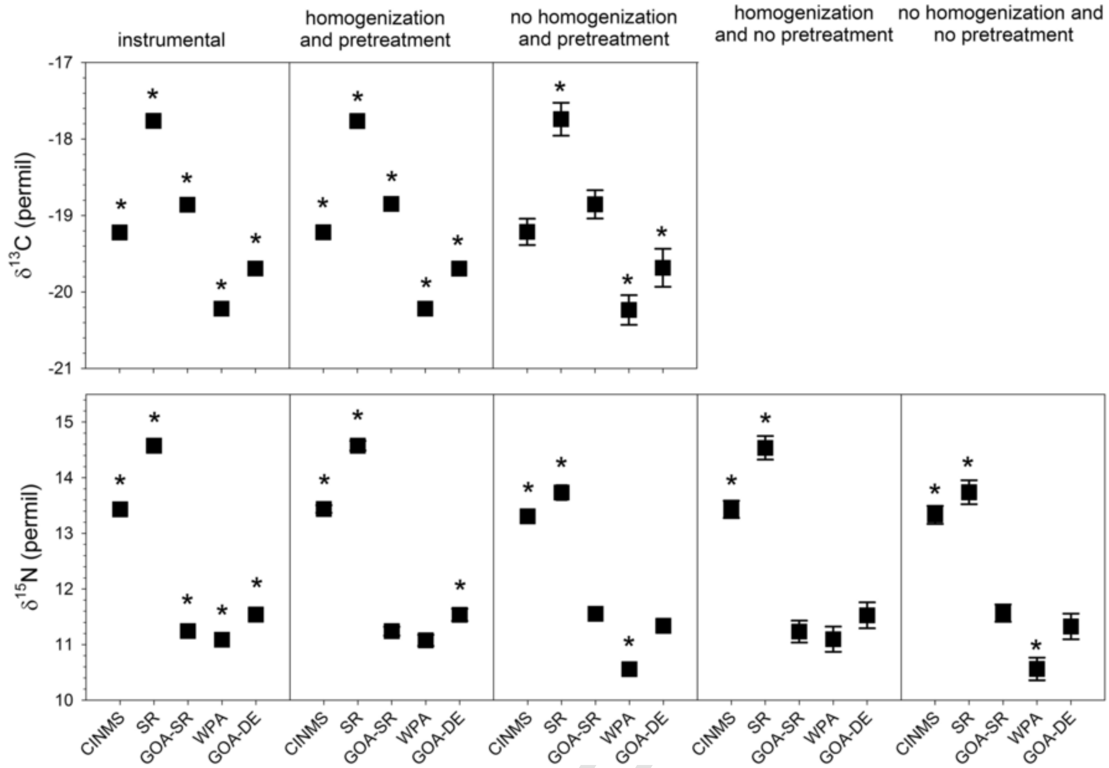

Fig. 5. Comparison of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values among regions using propagated error. Only pretreated results are shown for $\delta\ ^{13}C$ values. * indicates significantly different means among the regions for that treatment. Regions included are Channel Islands (CINMS), Sur Ridge (SR), Gulf of Alaska - Shutter Ridge (GOA-SR), Fairweather Ground (WPA), and Gulf of Alaska - Dixon Entrance (GOA-DE).

gests that the presence of the dual skeleton, gorgonin and calcite, may physically interfere with the drilling process that removes coeval skeleton from the octocoral cross section. One mechanism that might explain this interference is the different mechanical characteristics of the calcite crystals and the gorgonin fibular protein (Ehrlich, 2010) . Both of these materials may not become completely pulverized at the ultra-small scale during the drilling process, preventing complete homogenization. Alternatively, the drilling process itself could induce physical fractionation of the isotopic composition of the material, altering the isotopic composition of the drilled skeleton. However, since the isotopic values of the amino acids were not impacted (Table 2, this second explanation is unlikely to contribute to the higher variance found in the non-pretreated samples relative to the pretreated samples. As a result, we recommend pretreating the skeleton of Calcaxonian octocorals to avoid this increased variability in repeat measurements.

The calcite in gorgonian octocorals includes only small amounts of nitrogen trapped in the carbonate matrix (ranging from ~ 2000 to 7000 nmols/g for gorgonian octocorals, Williams, B and Prokopenko, M, unpublished data). In addition, this nitrogen in the carbonate matrix is expected to derive from the same source as the nitrogen contributing to the gorgonin proteinaceous skeleton, as has been reported in deep-sea scleractinian octocorals (Wang et al., 2014) . Therefore, removing the calcite fraction of the octocoral is not expected to change the bulk $\delta\ ^{15}N$ composition. Consistent with this, we found no significant difference in the $\delta\ ^{15}N$ values between the pretreated octocoral material and that with no pretreatment (Fig. 3). Furthermore, the similar $\delta\ ^{15}N$ values of the AAs in non-pretreated and pretreated skeletal material support the absence of a systematic impact of pretreatment on skeletal $\delta\ ^{15}N$ values of the organic skeleton. The small changes in bulk $\delta\ ^{15}N$ values, for example in the WPA octocorals (Fig. 3), may reflect minimal leaching of some of the AAs. This may cause a small change in composition of the bulk skeleton but no alteration of the isotopic fractionation of biogeochemical signal.

4.2. Experiment Two: skeletal heterogeneity

For both the $\delta\ ^{13}C$ values and the $\delta\ ^{15}N$ values, the average standard deviation of replication homogenized coeval samples was significantly larger than the reported instrumental reproducibility based on an Acetanilide standard (Fig. 4). This indicates that even with homogenization, the reproducibility of the measurements of the gorgonian skeleton is higher than instrumental uncertainty. Furthermore, the reproducibility of the skeleton that was not homogenized was larger than the homogenized material for both $\delta\ ^{13}C$ values and $\delta\ ^{15}N$ values. Therefore, the isotopic composition of coeval skeletal material is not consistent around the entire circumference of a gorgonian colony trunk. This heterogeneity around the circumference of the octocoral skeleton may relate to the morphology of the colonies and localized incorporation of the geochemical signature in their food.

The gorgonian octocorals can have an asymmetric gross morphology resembling a large fan, with the widest portion of the colony facing into the prevalent current to maximize food collection (Tong et al., 2012) . As such, the observed heterogeneity in isotopic values around the circumference of the trunk could reflect uneven growth patterns. The expansion of skeleton growth bands could be uneven radially given the asymmetric gross morphology of the polyp structure around the trunk. As a result, the standard sampling plan of equidistant circumferential milling may be reflecting different amounts of temporal incorporation, aliasing the resulting isotopic values. However, here sampling was guided by the growth bands for both the pretreated material and the non-pretreated material. An equidistant strategy was not used. Therefore, this process is unlikely to explain the heterogeneity present in the isotopic composition. Alternatively, the asymmetrical polyp orientation may bring different food particles to polyps on separate sides of the octocoral colony. For example, while sinking organic matter clearly is a major source of nutrients to the deep-sea octocorals

(Griffin and Druffel, 1989) , resuspended material may comprise at least some of the food to the octocorals (Roberts, 2006) depending on the prevailing currents. These different food particles have different environmental histories and thus different isotopic composition (e.g., Altabet, 1988). Local incorporation of this input signal into the skeleton as opposed to homogenization of the input signal through connected polyps would generate heterogeneity in the isotopic composition of coeval skeleton. As a result of this biological variability within a specimen, a larger amount of material needs to be sampled along coeval bands then is required for stable isotope analysis. This material can be homogenized and analyzed to obtain a measurement representative of the octocoral skeleton.

4.3. Application of propagated error

When comparing differences in stable isotope values of octocoral skeletons, either among colonies or within colonies through time, it is critical to accurately incorporate uncertainty in the comparison. This prevents false attribution of significant differences beyond the inherent uncertainty in the analyses. We found that the propagated error from analytical uncertainty and biological variability had little impact on the interpretation of variability in δ^{13}C values of octocorals collected from different regions in comparison to using instrumental error alone (Fig. 5). In only one treatment did using the propagated error result in a change of significance of the statistical test: the average δ^{13}C values between the sites Channel Islands and Gulf of Alaska - Shutter Ridge octocorals might not be in fact different. In contrast, using the propagated error showed that there was little to no significant variability in the δ^{15}N values among all the Alaskan octocorals. This contradicts statistical analysis using the standard approach of instrumental error only, which yielded results showing that the δ^{15}N values of octocorals differed significantly among all the regions examined in this study, including variability among the Alaskan sites. As a result, this study supports using propagated errors when comparing among colonies from different locations.

5. Implications

Based on our results in this study, we make the following recommendations:

1) The acidification pretreatment that is required for the δ^{13}C measurements does not significantly impact the δ^{15}N values of the skeleton nor the reproducibility of the δ^{15}N measurements. Thus, we propose that it is appropriate to measure δ^{13}C and δ^{15}N values on the same pretreated skeletal material.
2) Repeat measurements of homogenized octocoral skeleton should be reported in addition to instrumental reproducibility to accurately reflect the precision of an individual measurement.
3) The heterogeneity in the skeletal material around the circumference of an octocoral colony suggests that more complete sampling of the circumference of the skeleton growth band followed by homogenization provides a more representative sample of the entire colony at that point in time than a single point along the growth band.

Acknowledgments

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References

Altabet, M., 1988. Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean. Deep Sea Res. Part A 35, 535–554. https://doi.org/10.1016/0198-0149(88)90130-6.
Baker, D., Jordan-Dahlgren, E., Maldonado, M., Harvell, C., 2010. Sea fan corals provide a stable isotope baseline for assessing sewage pollution in the Mexican Caribbean. Limnol. Oceanogr. 55, 2139–2149.
Baker, D., Webster, K., Kim, K., 2010. Caribbean octocorals record changing carbon and nitrogen sources from 1862 to 2005. Glob. Chang. Biol. 16, 2701–2710. https://doi.org/10.1111/j.1365-2486.2010.02167.x.
Caldow, C., Etnoyer, P.J., Kracker, L., 2015. Patterns in deep-sea corals expedition: Noaa ship bell m. shimada sh-15-03. In: NOAA Technical Memorandum NOS NCCOS 200, 15 pp..
Coplen, T.B., 1994. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances (technical report). Pure Appl. Chem. 66, 273–276.
Druffel, E.R., 1997. Geochemistry of corals: proxies of past ocean chemistry, ocean circulation, and climate. Proc. Natl. Acad. Sci. 94, 8354–8361.
Ehrlich, H., 2010. Gorgonin. In: Biological Materials of Marine Origin. pp. 257–270.
Gordon, H.R., Morel, A.Y., 2012. Remote Assessment of Ocean Color for Interpretation of Satellite Visible Imagery: A Review. 4, Springer Science & Business Media.
Griffin, S., Druffel, E., 1989. Sources of carbon to deep-sea coral skeletons. Radiocarbon 31, 533–542.
Heikoop, J., Hickmott, D., Risk, M., Shearer, C., Atudorei, V., 2002. Potential climate signals from the deep-sea gorgonian coral Primnoa resedaeformis. Hydrobiologia 471, 117–124.
Henderson, G.M., 2002. New oceanic proxies for paleoclimate. Earth Planet. Sci. Lett. 203, 1–13.
Jacob, U., Mintenbeck, K., Brey, T., Knust, R., Beyer, K., 2005. Stable isotope food web studies: a case for standardized sample treatment. Mar. Ecol. Prog. Ser. 287, 251–253.
Kennedy, P., Kennedy, H., Papadimitrious, S., 2005. The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. Rapid Commun. Mass Spectrom. 19, 1063–1068. https://doi.org/10.1002/rcm.1889.
Mariotti, A., Lancelot, C., Billen, G., 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt Estuary. Geochim. Cosmochim. Acta 48, 549–555.
McMahon, K., McCarthy, M., Sherwood, O., Larsen, T., Guilderson, T., 2015. Millennial-scale plankton regime shifts in the subtropical north Pacific Ocean. Science 350, 1530–1533.
McMahon, K.W., Williams, B., Guilderson, T.P., Glynn, D.S., McCarthy, M.D., 2018. Calibrating amino acid 13c and 15n offsets between polyp and protein skeleton to develop proteinaceous deep-sea corals as paleoceanographic archives. Geochim. Cosmochim. Acta 220, 261–275. https://doi.org/10.1016/j.gca.2017.09.048.
Orejas, C., Gili, J.M., Arntz, W., 2003. Role of small-plankton communities in the diet of two antarctic octocorals (Primnoisis antarctica and Primnoella sp.). Mar. Ecol. Prog. Ser. 250, 105–116.
Prouty, N., Roark, E., Andrews, A., Robinson, L., Hill, T., Sherwood, O., Willams, B., Guilderson, T., Fallon, S., 2015. Age, growth rates, and paleoclimate studies of deep sea corals. In: Hourigan, T., Etnoyer, P., SD, C. (Eds.), The State of Deep-Sea Coral and Sponge Ecosystems of the United States: 2015. NOAA Technical Memorandum X. NOAA, Silver Spring, 10–1-10-21.
Ribes, M., Coma, R., Gili, J.M., 1999. Heterogeneous feeding in benthic suspension feeders: the natural diet and grazing rate of the temperate gorgonian paramuricea clavata (cnidaria: Octocorallia) over a year cycle. Mar. Ecol. Prog. Ser. 125–137.
Roark, E.B., Guilderson, T.P., Dunbar, R.B., Fallon, S.J., Mucciarone, D.A., 2009. Extreme longevity in proteinaceous deep-sea corals. Proc. Natl. Acad. Sci. 106, 5204–5208.
Roark, E.B., Guilderson, T.P., Dunbar, R.B., Ingram, B.L., 2006. Radiocarbon-based ages and growth rates of Hawaiian deep-sea corals. Mar. Ecol. Prog. Ser. 327, 1–14.
Roberts, J., 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. Science 312, 543–547. https://doi.org/10.1126/science.1119861.
Roberts, J.M., 2010. Cold-water Corals the Biology and Geology of Deep-sea Coral Habitats. Cambridge University Press.
Robinson, L.F., Adkins, J.F., Frank, N., Gagnon, A.C., Prouty, N.G., Roark, E.B., van de Flierdt, T., 2014. The geochemistry of deep-sea coral skeletons: a review of vital effects and applications for palaeoceanography. Deep-Sea Res. II Top. Stud. Oceanogr. 99, 184–198.
Rooper, C., Stone, R., Etnoyer, P., Salgado, E., Conrath, C., Reynolds, J., Greene, H., Williams, B., 2017. Deep-sea coral research and technology program alaska deep-sea coral and sponge initiative: Final report. In: NOAA Technical Memorandum XX, 63 pp..
Rothwell, R.G., Rack, F.R., 2006. New Techniques in Sediment Core Analysis: An Introduction. 267, Geological Society, London, Special Publications, 1–29.

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Sherwood, O., Heikoop, J., Scott, D., Risk, M., Guilderson, T., McKinney, R., 2005. Stable isotopic composition of deep-sea gorgonian corals Primnoa spp.: a new archive of surface processes. Mar. Ecol. Prog. Ser. 301, 135–148.

Sherwood, O., Lapointe, B., Risk, M., Jamieson, R., 2010. Nitrogen isotopic records of terrestrial pollution encoded in floridian and Bahamian gorgonian corals. Environ. Sci. Technol. 44, 874–880.

Sherwood, O., Thresher, R., Fallon, S., Davies, D., Trull, T., 2009. Multi-century time-series of 15n and 14c in bamboo corals from deep Tasmanian seamounts: evidence for stable oceanographic conditions. Mar. Ecol. Prog. Ser. 397, 209–218.

Sherwood, O.A., Guilderson, T.P., Batista, F.C., Schiff, J.T., Mccarthy, M.D., 2014. Increasing subtropical North Pacific Ocean nitrogen fixation since the little ice age. Nature 505, 7881. https://doi.org/10.1038/nature12784.

Sherwood, O.A., Scott, D.B., Risk, M.J., 2006. Late Holocene radiocarbon and aspartic acid racemization dating of deep-sea octocorals. Geochim. Cosmochim. Acta 70, 2806–2814. https://doi.org/10.1016/j.gca.2006.03.011.

Silfer, J., Engel, M., Macko, S., Jumeau, E., 1991. Stable carbon isotope analysis of amino acid enantiomers by conventional isotope ratio mass spectrometry and combined gas chromatography/isotope ratio mass spectrometry. Anal. Chem. 63, 370–374.

Tong, R., Purser, A., Unnithan, V., Guinan, J., 2012. Multivariate statistical analysis of distribution of deep-water gorgonian corals in relation to seabed topography on the norwegian margin. PLoS ONE 7, e43534.

Ueda, K., Morgan, S.L., Fox, A., Gilbart, J., Sonesson, A., Larsson, L., Odham, G., 1989. D-alanine as a chemical marker for the determination of streptococcal cell wall levels in mammalian tissues by gas chromatography/negative ion chemical ionization mass spectrometry. Anal. Chem. 61, 265–270. https://doi.org/10.1021/ac00178a015.

Wang, X.T., Prokopenko, M.G., Sigman, D.M., Adkins, J.F., Robinson, L.F., Ren, H., Oleynik, S., Williams, B., Haug, G.H., 2014. Isotopic composition of carbonate-bound organic nitrogen in deep-sea scleractinian corals: a new window into past biogeochemical change. Earth Planet. Sci. Lett. 400, 243–250. https://doi.org/10.1016/j.epsl.2014.05.048.

Ward-Paige, C., Risk, M., Sherwood, O., 2005. Reconstruction of nitrogen sources on coral reefs: δ ^{15}n and δ ^{13}c in gorgonians from Florida reef tract. Mar. Ecol. Prog. Ser. 296, 155–163.

Williams, B., Grottoli, A., 2010. Recent shoaling of the nutricline and thermocline in the western tropical Pacific. Geophys. Res. Lett. 37, L22601. https://doi.org/10.1029/2010GL044867.

Williams, B., Risk, M., Stone, R., Sinclair, D., Ghaleb, B., 2007. Oceanographic changes in the North Pacific Ocean over the past century recorded in deep-water gorgonian corals. Mar. Ecol. Prog. Ser. 335, 85–94.

Williams, B., Risk, M., Stone, R., Sinclair, D., Ghaleb, B., 2007. Oceanographic changes in the North Pacific Ocean over the past century recorded in deep-water gorgonian corals. Mar. Ecol. Prog. Ser. 335, 85–94.

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