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# Understanding Nitrogen Isotopes in Deep Sea Corals; A Potential Tracer of Paleoceanographic Conditions

A Thesis Presented by Sylvia Bergerud

To the Keck Science Department of Claremont McKenna, Pitzer, and Scripps Colleges

In partial fulfillment of the degree of Bachelor of Arts

Senior thesis in Organismal Biology

Dec12, 2022

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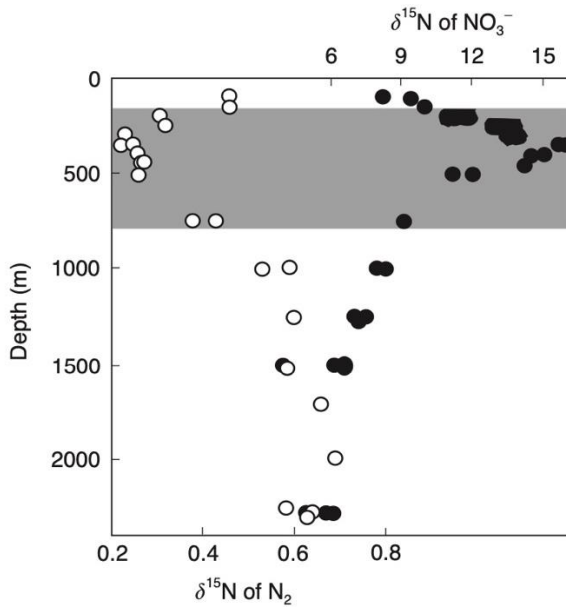
### Abstract

In the face of anthropogenic climate change, increasing pressure is being mounted on contextualizing current climate alterations compared to those of the past. One key tracer of climate variation is the isotopic ratio of  $^{15}\text{N}$  compared to  $^{14}\text{N}$ , which tracks biological and chemical reactions throughout the N cycle. However, it is difficult to find accurate palaeoceanographic records of N isotope fractionation, as most sedimentary and organic samples are subject to extensive diagenesis, degradation, and erosive processes. Scleractinian corals present a potential solution to this lack of accurate data. Multiple studies indicate that, due to their protective aragonite skeletons and quality of being rooted to the seafloor, these corals are exempt from many of the contamination and deterioration issues of other sample types. Yet challenges persist when trying to interpret coral-bound nitrogen in corals as proxies of their environment. Unlike other organisms, corals do not display a consistent  $\sim 3.5\text{‰}$  trophic offset in N isotope ratio compared to their diet of POM (particulate organic matter). Instead, their offset is approximately  $\sim 8.5\text{‰}$ , which is 2-3  $\text{‰}$  higher than the ubiquitous trophic offset of  $\sim 3.5\text{‰}$ , but it is unclear what factors are responsible for this difference. Although multiple theories exist, this study examines the potential influence of preferential uptake of suspended PON (particulate organic nitrogen) by corals as a source of higher  $\delta^{15}\text{N}$  relative to sinking PON. Combined with the expected  $\sim 3.5\text{‰}$  trophic offset, we hypothesized that because suspended PON has high  $\delta^{15}\text{N}$ , this preferential feeding could result in higher  $\delta^{15}\text{N}$  in corals. However, our results indicate that this is not the case. Instead, the unexpected offset could result from high  $\delta^{15}\text{N}$  in starting nitrate in addition to a higher trophic diet than expected. This study provides a starting point from which to pursue nitrate and zooplankton as determining factors in CB-  $\delta^{15}\text{N}$ .

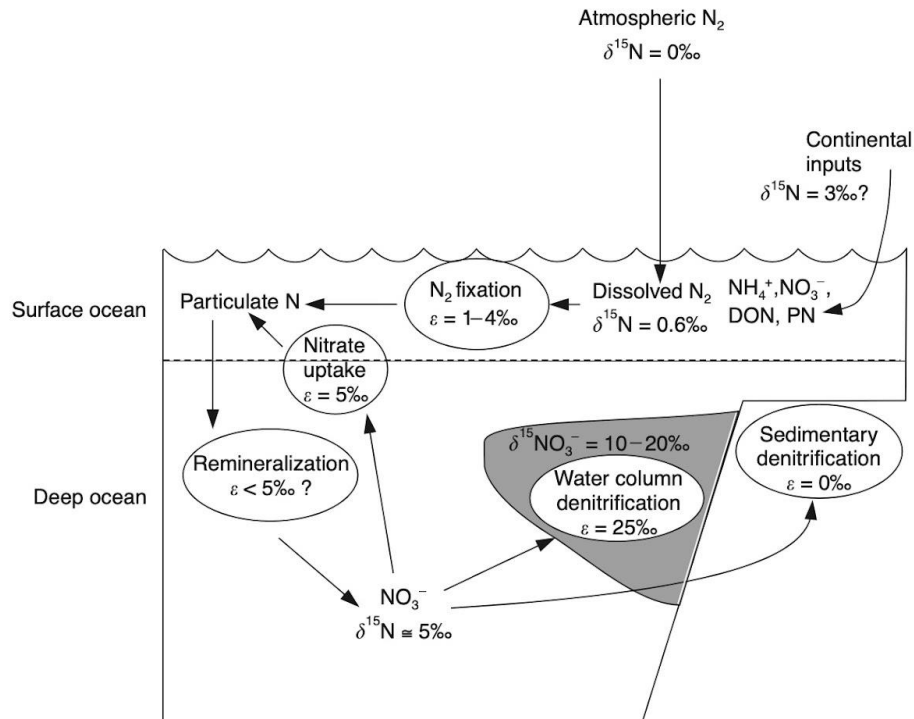
## Background

### The Nitrogen Cycle

The global biogeochemical nitrogen cycle is composed of five key processes: 1) fixation, 2) nitrification, 3) assimilation, 4) ammonification, and 5) denitrification (Altabet, 1988). For the purposes of this study, I will focus on the oceanic version of this cycle, which has slight variations from the terrestrial nitrogen cycle. The marine nitrogen cycle begins with molecular nitrogen gas (N<sub>2</sub>) dissolving into the ocean (Gruber, 2008) where it is converted into organic matter (largely in the form of amino acids) by specific “diazotrophic” bacteria, the only organisms capable of biological N<sub>2</sub> fixation (Middleburg et al., 2015). When these organisms die, their amino acids are decomposed (mineralized) into ammonium. The ammonium is then converted into nitrite and subsequently nitrate through nitrification by two other groups of bacteria, “ammonium oxidizing” and “nitrifying” bacteria. As nitrate, nitrogen is highly biologically accessible to primary producers such as plankton and is quickly assimilated by organic matter (Altabet, 1988). As primary producers are subsequently eaten by organisms higher up the food chain, nitrogen is gradually ingested by higher trophic level organisms, until eventually the organism containing it dies of other causes and is allowed to decompose, once again releasing ammonium in the process of ammonification. At this point, either the ammonium can be either directly taken up as a nutrient (N-source) or re-oxidized into nitrate and re-assimilated by primary producers, thus repeating the stages of the nitrogen cycle before ammonification. In the oxygen deficient zones of the ocean, often in regions of upwelling, where O<sub>2</sub> concentration of subsurface water masses is less than ~ 5% of saturation, nitrate is used as an electron acceptor, converted into N<sub>2</sub> gas by denitrifying bacteria, and re-released into the atmosphere (Altabet, 1988; Gruber, 2008; Middleburg et al., 2015).



**Figure 1.**  $\delta^{15}\text{N}\%$  of  $\text{N}_2$  (white dots) versus Nitrate (black dots) across depth in the Eastern Tropical North Pacific ( $22^\circ\text{N}$ ,  $107^\circ\text{W}$ ), adapted from (Sigman et al., 2001).  $\delta^{15}\text{N}\%$  of Nitrate reaches its maximum near the surface, where  $\text{N}_2$  reaches its minimum, as most  $\text{N}_2$  is converted to Nitrate at the surface before this Nitrate is consumed by organic matter.



**Figure 2.** Adapted from (Sigman & Casciotti., 2001). The marine nitrogen cycle and isotope fractionation processes, including inputs from  $\text{N}_2$  fixation in the surface ocean and continental deposits of Nitrogen. Marine cycling processes include surface ocean nitrate uptake and deep-sea remineralization. Ammonium uptake and regeneration in the surface ocean are excluded from the diagram, as is isotope fractionation of nitrification due to its complete reaction in the water column. Areas of uncertainty are indicated by question marks (Sigman & Casciotti, 2001).

### Relevance of Nitrogen Isotope Fractionation

As previously mentioned, nitrogen in the form of nitrate ( $\text{NO}_3^-$ ) is taken up by phytoplankton as a necessary component of photosynthesis (Altabet, 1988). As all N transformations are accompanied by a certain amount of isotopic fractionation, the  $\delta^{15}\text{N}$  of organic matter can provide information on where nitrate was a limiting factor in primary production, what forms of nitrogen supported primary production (e.g. nitrate vs.  $\text{N}_2$  fixation), and what nitrogen transformations occurred in a particular region. All biological processes in which nitrogen is metabolized or changes form tend to favor lighter  $^{14}\text{N}$  isotopes (with one exception of biological  $\text{N}_2$  fixation), creating products depleted of nitrogen-15 and reactants that are consequently enriched (Sigman & Casciotti, 2001; Mariotti et al., 1981). The ratio of isotopes in products compared to reactants, known as the kinetic fractionation factor, varies among chemical and biological processes and can therefore be used to examine the processes of nitrogen cycling in question (Mariotti et al., 1981). Ocean circulation redistributes the nitrogen isotopic signatures transporting diagnostic  $\delta^{15}\text{N}$  values in various water masses, in which different processes of the nitrogen cycle occur.

<b>Significance</b>	<b>Equation</b>
Isotope Ratio	$R = {}^{15}\text{N} / {}^{14}\text{N}$ $R_p = R_{\text{product}}$ $R_s = R_{\text{substrate}}$
Definition of $\delta^{15}\text{N}$ based on the $^{15}\text{N}/^{14}\text{N}$ ratio in a sample compared to the standard (which is atmospheric $\text{N}_2$ in parts per thousand, ‰)	$\delta^{15}\text{N}\text{‰} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$
Enrichment Factor (in parts per thousand, ‰)	$\epsilon_{p/s} = (\delta_s - \delta_{s,0}) / \ln f$ $\delta_s = \delta^{15}\text{N} \text{ of product}$ $\delta_{s,0} = \delta^{15}\text{N} \text{ of initial substrate}$
Unreacted fraction of substrate remaining	$f = N_s / N_{s,0}$

	$N_s$ = concentration of product $N_{s,0}$ = concentration of substrate
Kinetic (Isotopic) Fractionation Factor:	$\alpha_{p/s} = R_p / R_s$

**Table 1.** Isotopic notations and expressions of isotopic fractionation; Equations for  $\delta^{15}\text{N}$  from Mariotti et al., (1981).

### Nitrogen Isotope Fractionation in the Marine N Cycle

As marine nitrogen moves from different sources and sinks and is transferred between different components of the marine nitrogen cycle, it undergoes isotopic fractionation, the amount of which can be indicative of the processes involved (Gruber, 2008; Altabet, 1998). Two separate but related nitrogen cycling processes involving isotopic fractionation pertain to this study. As atmospheric  $\text{N}_2$  gas with  $\delta^{15}\text{N}$  of 0‰ (see Table 1 for definitions) first enters the ocean and dissolves, its  $\delta^{15}\text{N}$  is 0.6‰ at equilibrium with air (Sigman & Casciotti., 2001). The subsequent biological fixation of  $\text{N}_2$  causes negligible isotopic fractionation-- newly fixed nitrogen is roughly ~0‰ (Sigman & Casciotti, 2001). However, this low isotopic composition of source nitrogen, which exists predominantly in the ocean as nitrate, is also balanced by denitrification. Approximately 1/4 of marine nitrate is denitrified in the water column, which contributes a large fractionation of ~ -20‰. The remaining 3/4 of nitrate is denitrified in marine sediments with effective isotopic fractionation of ~ 0‰ due to the reaction going until completion (Sigman et al., 2003; Sigman & Casciotti, 2001). However, water mixing ensures that hardly any regions are fully isolated—most marine  $\delta^{15}\text{N}$  is not 0‰ (due to fixation) or 20‰ (due to denitrification) but somewhere in between. As the result of the isotopic mass balance between the net source ( $\text{N}_2$  fixation at 0‰) and two net sinks, water column denitrification (of -20‰) and sedimentary denitrification (at 0‰), the mean ocean nitrate is ~ 5‰ (Gruber, 2008).

Branching off from the marine nitrogen cycle is another critical process of nitrogen fractionation, which begins with uptake by organic matter and moves up through the food chain.



Once in the form of nitrate, nitrogen is taken up by primary producers such as phytoplankton, which have an approximate fractionation factor of 5‰ (Gruber, 2008). Consequently, in accordance with Rayleigh fractionation relationships, as phytoplankton preferentially absorb  $N^{14}$  over  $N^{15}$ ,  $\delta^{15}N$  of Nitrate in surrounding water is elevated by a certain value, while phytoplankton  $N^{15}$  is depleted by the same amount (Sigman & Casciotti, 2001), with  $\delta^{15}N$  of the remaining nitrate and the product depending on the degree of uptake (Wang et al., 2014). The isotopic fractionation between the source nitrate and resulting phytoplankton organic matter is only expressed in the situation of “incomplete” nitrate uptake, in the regions where factors other than nitrate limit phytoplankton growth (Gruber, 2008). In contrast, in regions where nitrogen is the limiting nutrient for photosynthesis and all nitrate is consumed by this process, the product phytoplankton approaches the initial  $\delta^{15}N$  of the source nitrate, which is roughly ~5‰ throughout much of the ocean (Sigman & Casciotti, 2001; Gruber, 2008). To summarize, in the regions of incomplete nitrate consumption, phytoplankton  $\delta^{15}N$  is lower than source nitrate, while in areas where nitrate consumption is complete, phytoplankton exhibits the same  $\delta^{15}N$  as source nitrate. By examining  $\delta^{15}N$  of phytoplankton, we can therefore discern regions of upwelling, where nitrate consumption is often incomplete, gaining invaluable insight into climate interactions and ocean productivity.

#### Marine Nitrate in Different Regions of the Ocean

N isotope fractionation has already revealed much about modern ocean circulation and nitrogen cycling. Nitrogen fixation only occurs in areas of complete nitrate consumption, in which microbes must produce their own biologically accessible nitrogen to survive. However, in order for these microbes to “fix” nitrogen, they need adequate sources of iron. Iron from the continents is deposited in the surface ocean from atmospheric dust, enabling nitrogen fixation

and subsequently photosynthesis. Therefore, where iron is sufficient, which includes much of the surface ocean (except for the poles), the rate of nitrogen fixation determines how much nitrate is biologically available, and practically all produced nitrate is immediately consumed by organic matter. Because of complete nitrate consumption,  $\delta^{15}\text{N}$  of phytoplankton approaches that of source nitrate, which is relatively low in most areas (Sigman & Casciotti, 2001).

In contrast with surface fixation, denitrification occurs in oxygen minimum zones, typically in the 300-500 depth range, below the upwelling regions, and at the edge of gyres where the circulation is relatively stagnant (Gruber, 2008). Examples of such locations can be found in the Eastern Tropical North Pacific, the Eastern Tropical South Pacific, and the Arabian sea (Gruber, 2008). Denitrification is accompanied by a large isotope effect of  $\sim -20\text{‰}$  (Sigman & Casciotti, 2001). Consequently, water that undergoes partial denitrification exhibits high  $\delta^{15}\text{N}$ , and in areas where equatorial, seasonal, or coastal upwelling brings this water to the surface, such as the California margin (of special interest to this thesis) surface nitrate consequently exhibits higher  $\delta^{15}\text{N}$  relative to the mean ocean nitrate of  $5\text{‰}$  (Aguñiga et al., 2010; Sigman & Casciotti, 2001). As a result, regional deviation of nitrate  $\delta^{15}\text{N}$  from the mean  $5\text{‰}$  value suggests either a source (e.g.  $\text{N}_2$  fixation) or a sink (e.g. denitrification) influence, and can therefore be used as a robust indicator of the type and magnitude of the processes occurring within a particular region of the ocean. Examining starting  $\delta^{15}\text{N}$  of phytoplankton as recorded in various paleoceanographic nitrogen “archives” can help reconstruct marine nitrogen cycling processes of the past.

Deep sea corals as Paleoceanographic Archives of Marine N Cycling

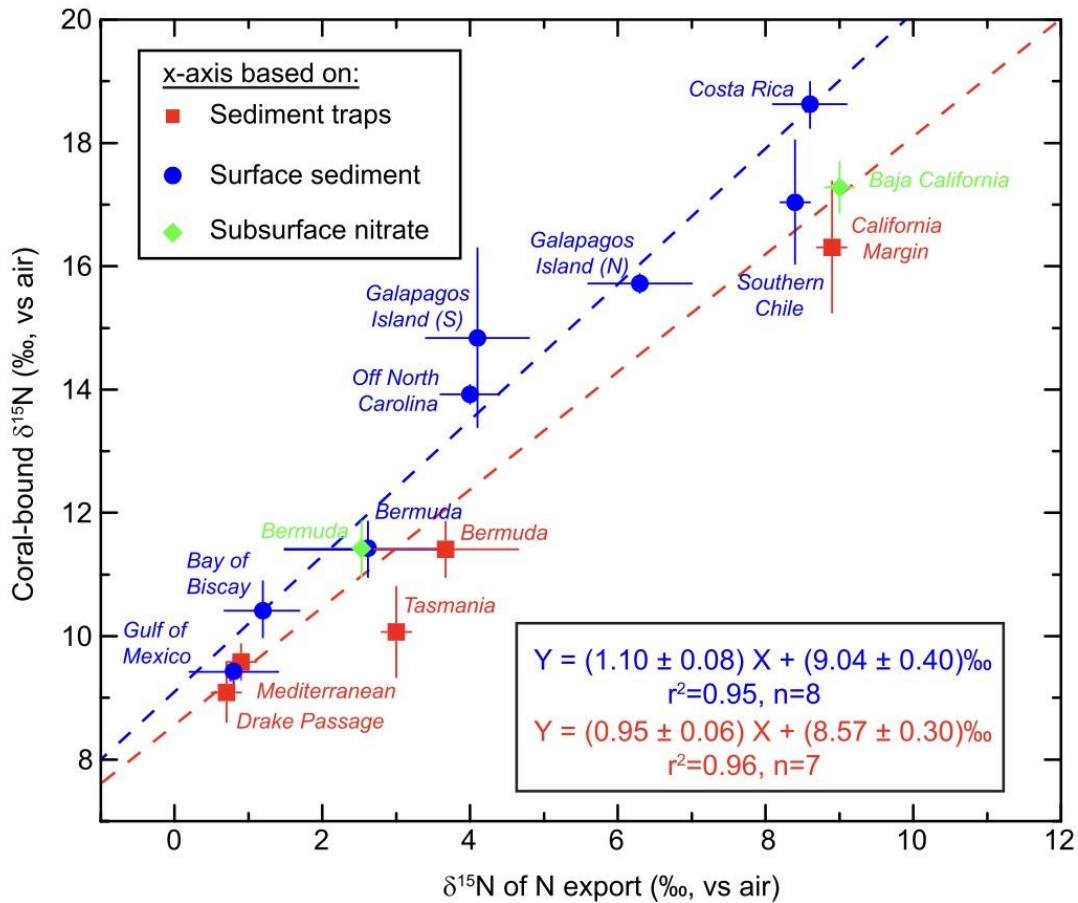
When well-preserved, nitrogen isotopes can provide information about ocean productivity in the past. Marine sediment samples have previously been used in assessments of historic  $\delta^{15}\text{N}$  but are subject to diagenesis and allochthonous N contamination due to ocean circulation (Wang et al., 2014; Gruber, 2008). Deep-sea cold-water corals present a potentially improved alternative for  $\delta^{15}\text{N}$  sampling, as they are subject to less degradation and are rooted to the surfaces on which they originated. Not all corals are reliable proxies of their environment, however. Organic layers of proteinaceous corals are exposed to their environment and consequently experience the same issues of diagenesis and natural degradation; Even the best-preserved samples are not accurate indicators of their surroundings beyond a few thousand years (Wang et al., 2014). In contrast, the organic matter of scleractinian corals is protected by carbonate skeletons, which act as a mineral matrix to protect organic matter from decomposition (Wang et al., 2014). These corals make ideal records of ocean environments because they inhabit a vast range of ocean ecosystems and depths (surviving even deeper than 5,000 meters), populations persist for thousands of years. In addition, individual scleractinian corals can live for up to 100 years and are well-preserved since the Last Glacial Maximum, 20,000 years ago (Robinson et al., 2005) (Robinson et al., 2014). Scleractinian corals grow as individuals or colonially, with each individual specimen consisting of a single cylindrical polyp with a hard aragonite skeleton, which grows outwards from the “center of calcification” (Constantz & Weiner, 1988). To precipitate their calcium carbonate, they absorb DIC (dissolved inorganic carbon) and pump it through a tissue membrane consisting of aboral endoderm and calicoblastic cells using  $\text{Ca}^{2+}$ -ATPase exchangers (Constantz & Weiner, 1988; Puvarel et al., 2005). These

exchangers simultaneously remove protons from  $\text{H}_2\text{CO}_3$  to facilitate the production of Bicarbonate,  $\text{HCO}_3^-$ , maintaining the basic pH necessary within the subcalicoblastic space to forward the reaction and enabling precipitation of calcium carbonate. Individual polyps then externally secrete this calcium carbonate to protect the growing organic tissue inside (Constantz & Weiner, 1988).

Scleractinian corals are thought to feed on various types of particulate organic matter (POM), which is also their source of nitrogen (Mueller et al., 2014) (Kiriakoulakis et al., 2005) (Duineveld et al., 2007) (Wang et al., 2014). It is widely accepted that, when organisms of any given trophic level ingest food, their tissue will exhibit an enrichment factor of roughly 3.5‰ relative to the  $\delta^{15}\text{N}$  of their diet. In other words, for every trophic level between prey and predator, the trophic offset is  $\sim 3.5\%$  (Hannides et al., 2013) (Duineveld et al., 2007). For instance, zooplankton have 3.5‰ higher  $\delta^{15}\text{N}$  than their food source, phytoplankton. Similar to the assimilation process, this phenomenon is once again due to metabolic reactions favoring lighter isotopes and can be used to trace an organism's place in the food chain (Mariotti et al., 1981).

Based on the enrichment level of their supposed POM diet, we would expect corals to exhibit a  $\sim 3.5\%$  enrichment compared to POM (the exact values depending on geographic location) (Hannides et al., 2013). Instead, corals display an unexpected offset of 8.5‰ in  $\delta^{15}\text{N}$  relative to regional POM (phytoplankton organic matter "exported" from the surface) (Wang et al., 2014). This value, which is more than double the expected trophic offset, presents a conundrum in using scleractinian corals as a proxy of surrounding nitrogen. Without understanding the mechanism(s) that lead to the 8.5‰ offset, we do not know if this enrichment is constant across time, space, and environmental conditions (Doi et al., 2019). Factors such as

diet, feeding schedules, seasonal access to nutrients, symbionts, effects of current, and anatomical characteristics of corals are not well understood in relation to coral nitrogen fractionation (Doi et al., 2019) (Dodds et al., 2009) (Lourey et al., 2003) (Middelburg et al., 2016). While multiple theories have been proposed to explain this offset, none have been proven, and combined factors are likely responsible.



**Figure 3.** CB- $\delta^{15}\text{N}$  varied with  $\delta^{15}\text{N}$  of export (algae), indicating a linear relationship between the two with an offset of roughly 8.5‰ (Wang et al., 2014).

## Introduction

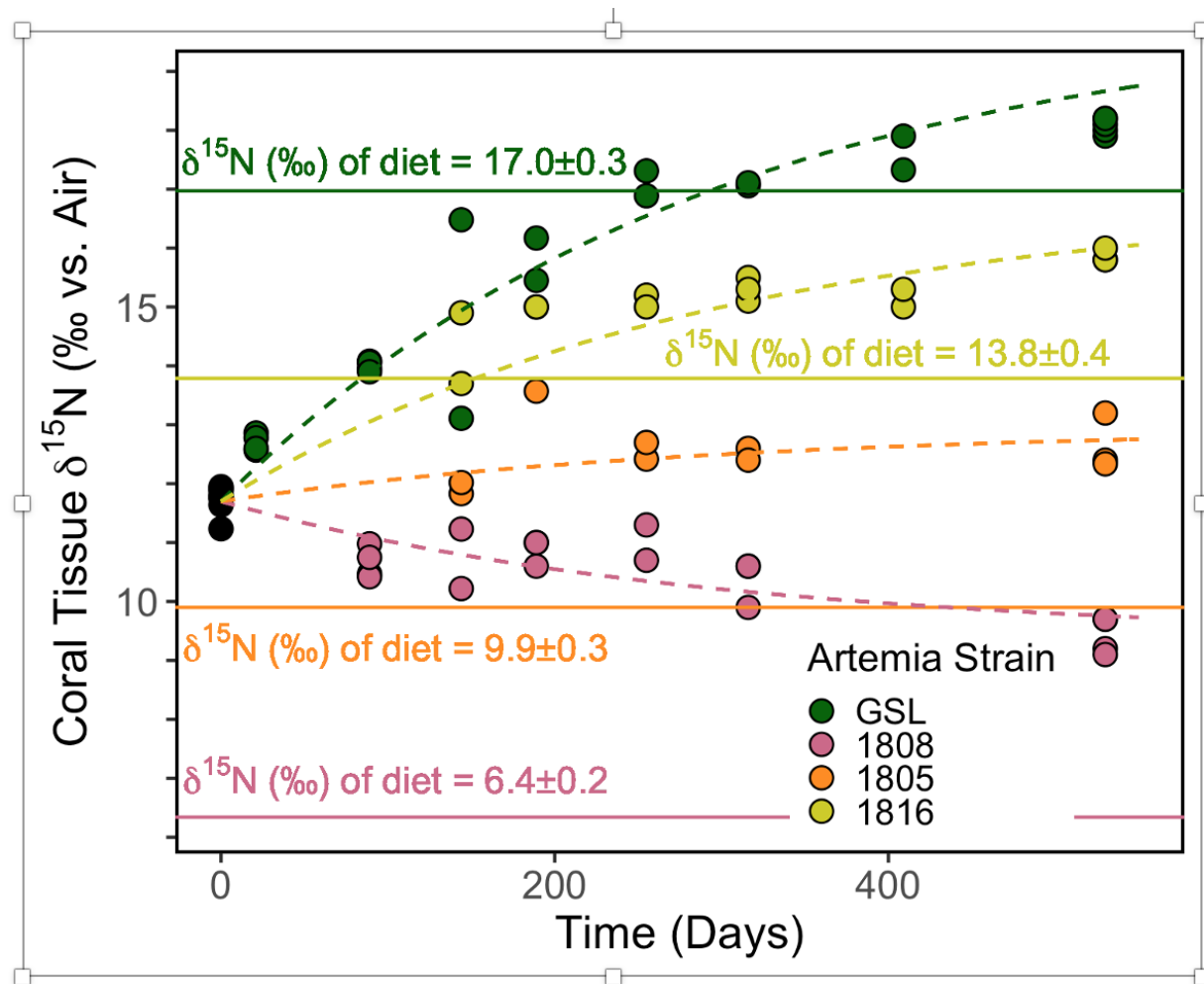
### Nitrogen and Climate Cycling

Contextualizing current climate changes in the history of Earth's climate variability is arguably one of the most difficult and critical objectives of science today. Undertaking such an endeavor requires a complex analysis of interactions between the atmosphere, nutrient cycling, ocean circulation, the marine biological carbon pump, and external climate forcing--and how these interactions repeat and shift over hundreds of thousands of years (Altabet, 1988) (Sigman & et al., 2001). One method of studying these climate dynamics through time is by investigating the distribution of elements and isotopes being recycled at different points in history (Sigman & et al., 2001) (Gruber, 2008). Nitrogen is particularly useful in this respect, as it is one of the most prevalent elements in the atmosphere (constituting 78% of the modern atmosphere) and is a critical nutrient for biosynthetic processes (Sigman & et al., 2001). The nitrogen cycle drives many of the aforementioned climate interactions and can be tracked based on the flux of specific forms of nitrogen in the ocean and the atmosphere (Wang et al., 2019). However, the concentration of nitrogen trapped in each stage of the nitrogen cycle may have varied in past. Understanding historic nitrogen exchange can provide valuable insight into paleo-oceanographic conditions and how the current climate may evolve in the future.

In the interest of using deep-sea scleractinian corals as indicators of historic climate conditions, this study aims to clarify what factors influence CB- $\delta^{15}\text{N}$ , specifically the ~8.5 ‰ offset in  $\delta^{15}\text{N}$  as compared with POM. Four hypotheses for this offset have been proposed:

- a. Naturally occurring biosynthetic N isotope fractionation between CWC tissue and skeleton (Horn et al., 2011) (Puverel et al., 2005).
- b. An atypically large N isotope fractionation associated with the trophic level transfer between the N-15 of CWC tissue and diet, communicated to the coral skeleton (Duineveld et al., 2007; Kiriakoulakis et al., 2005; Wang et al., 2014; Dodds et al., 2009).
- c. The combined factors of CWC eating deep suspended PON, which has higher  $^{15}\text{N}$  compared to PON exported from the surface in addition to the typical single trophic level transfer of 3-3.5‰ (Hannides et al., 2013; Sherwood et al., 2008; Altabet, 1988).
- d. CWC eating zooplankton, which could induce an approximately 7‰ offset between  $^{15}\text{N}$ -PON from the surface and coral skeleton  $^{15}\text{N}$  due to two trophic level transfers (Wang et al., 2014; Duinevald et al., 2007; Kiriakoulakis et al., 2005; Saino & Hattori, 1987).

To test hypotheses (a),  $\delta^{15}\text{N}$  of CWC collected live from the ocean were sampled to observe any differences in biosynthetic enrichment between tissue and skeleton. However, only a ~1-2 ‰ offset between tissue and skeleton was found, indicating that corals distribute nitrogen relatively evenly throughout their structures (Mottram et al., in prep). Hypothesis (b) was disproven by testing lab-grown corals, which were fed controlled diets containing known  $\delta^{15}\text{N}$  ‰. The trophic enrichment of the corals compared to the known  $\delta^{15}\text{N}$  ‰ of their diets was consistent with the normal 3.5‰ amount, indicating that corals are not merely an exception to the ubiquitous  $^{15}\text{N}$  enrichment processes in this way (see figure 4).



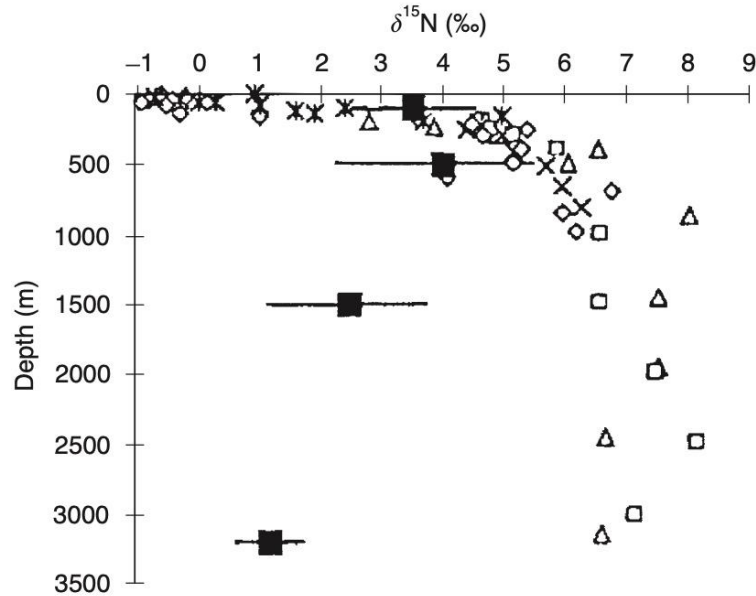
**Figure 4.** Shifts in CB- $\delta^{15}\text{N}$  in response to diets of different trophic levels (Mottram et al., in prep).

Hypotheses (c) and (d) are currently being examined through comparison of  $\delta^{15}\text{N}$  ‰ of CWC at different depths with vertical transects of PON and zooplankton  $\delta^{15}\text{N}$  ‰ (Prokopenko, personal communications). Assuming that coral diet consists of POM, and that coral nitrogen therefore derives from PON (particulate organic nitrogen), CWC  $\delta^{15}\text{N}$  ‰ should track closely with  $\delta^{15}\text{N}$  ‰ of PON profiles, with an additional 3.5 ‰ offset (Wang et al., 2014). However, hypothesis (c) projects that preferential consumption of deep-suspended POM, which has higher  $\delta^{15}\text{N}$  than sinking POM, (Casciotti et al., 2008) may contribute to heightened CB- $\delta^{15}\text{N}$ . However, if hypothesis (d) is correct, and zooplankton diet is accountable for the offset instead,



we can expect CWC  $\delta^{15}\text{N}$  to more closely model the zooplankton profiles, which generally have higher isotopic compositions than PON, though this varies by location (Kiriakoulakis et al., 2005) (Hannides et al., 2013). While this thesis mainly focuses on hypothesis (c) of the greater study, it does not exclude the possible influence of zooplankton on CB-  $\delta^{15}\text{N}$ .

This hypothesis will currently be harder to prove due to gaps in consistent data regarding zooplankton  $\delta^{15}\text{N}$  across depth at two of our three sample sites, including the California Margin. Differentiating between hypotheses (c) and (d) is further complicated by the fact that zooplankton is encompassed by POM—if deep suspended PON is responsible for the trophic offset, then so is zooplankton to some extent. The reasoning behind hypothesis (c) is that deep suspended PON is  $\delta^{15}\text{N}$  enriched compared to PON sinking directly from the surface due to natural isotope fractionation and bacterial consumption processes, which accumulate in suspended PON over time (Sherwood et al., 2008). Therefore, even though deep suspended PON derives from surface PON, it has been through more degradation and N-cycling, causing significant  $\delta^{15}\text{N}$  enrichment. This thesis will mainly focus on hypothesis (c) and interpreting the influence of suspended PON on CB-  $\delta^{15}\text{N}$  in the California Margin, though it also briefly examines the potential influence of zooplankton in this region.



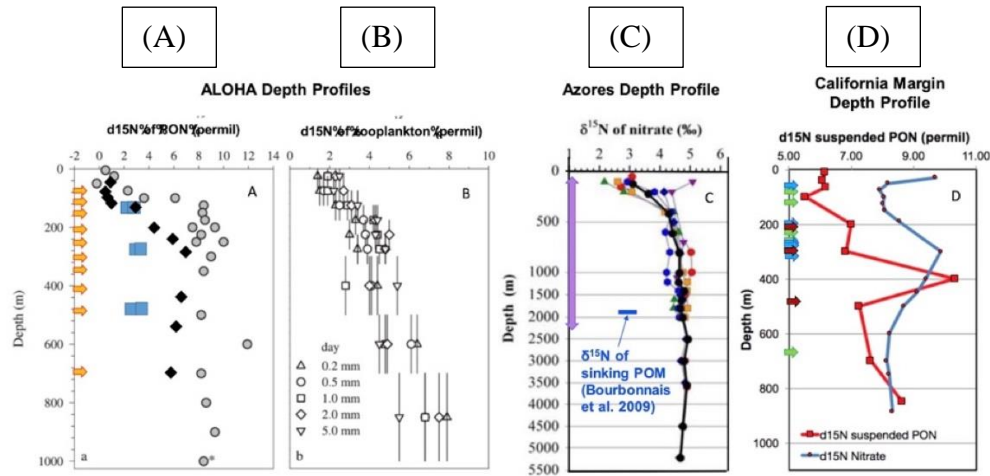
**Figure 5.**  $\delta^{15}\text{N}$  ‰ of PON across depth in the Atlantic. Sediment traps (sinking PON) are indicated by the black squares, whereas suspended PON samples are indicated by all other shapes. While sinking PON maximizes at around 500 meters at approximately 4‰, suspended PON shows an initially steep increase in  $\delta^{15}\text{N}$  ‰ in the first ~500 meters before levelling off at around ~7-8 ‰ beyond this point. (Sigman & et al., 2001).

PON and Zooplankton  $\delta^{15}\text{N}$  profiles vary widely by location due to different sources of nitrate as well as chemical and biological processes. Therefore, comparing CB- $\delta^{15}\text{N}$  depth profiles to vertical transects of PON and zooplankton at different sample sites should help to establish which of these factors has a greater influence on nitrogen in corals. Three locations were chosen to study depth profiles of  $\delta^{15}\text{N}$  ‰ for coral bound organic nitrogen: The North Pacific near Hawaii, the North Atlantic on the slopes of the Azores Islands, and the California Margin. Although this paper focuses only on the California Margin due to data from the other locations still being processed, I will discuss all three regions here for context. These locations were chosen because they are each areas of complete nitrate consumption, thus mitigating the confounding variable of inconsistent isotopic composition of starting nitrate (Sigman & Casciotti, 2001). However, the unique properties of the water column in each location still create

different starting isotopic values, which must be accounted for in analysis of CB- $\delta^{15}\text{N}$ . The Azores Islands are located within the North Atlantic Subtropical Gyre, an area marked by downwelling. As such, this is an oligotrophic region, with nitrate being a limiting nutrient for phytoplankton (Gruber, 2008). This is simultaneously a region of  $\text{N}_2$  fixation, meaning that due to the slow  $\text{N}$  fixation rate, all freshly fixed nitrate is immediately consumed by phytoplankton at the surface (Sigman et al., 2001). The result of this complete consumption in addition to some influence from deep water mixing is a starting  $\delta^{15}\text{N}$  in PON of approximately 2.5 - 3‰, which remains unchanged for sinking PON at depth, but for suspended PON, increases to ~6‰ below the thermocline as biological processes accumulate (Altabet, 1988; Bourbonnais et al., 2009). In contrast, conditions near the Hawaiian Islands are more representative of mean ocean processes, in which nitrification is occurring at the surface, but  $\text{N}_2$  is not being downwelled (Hannides et al., 2013). Unlike in the Azores, Hawaii is not in the center of a subtropical gyre. Here,  $\delta^{15}\text{N}$  of sinking and suspended PON is roughly equal until about 200 meters below the surface, where sinking PON  $\delta^{15}\text{N}$  exists at ~2-3‰, whereas suspended PON is approximately ~8‰ (Hannides et al., 2013). For Hawaii, we already have depth profiles of zooplankton, which indicate a steady increase in  $\delta^{15}\text{N}$  from roughly ~2‰ at the surface to about ~7‰ at 1000 meters (Casciotti et al., 2008). This data will enable valuable comparisons between PON and zooplankton influence in future studies. However, because of the similar trend in PON  $\delta^{15}\text{N}$  and zooplankton  $\delta^{15}\text{N}$  in this region, it will be hard to differentiate which of these factors is responsible for  $\delta^{15}\text{N}$  in corals based on this sample site alone.

Finally, in the California Margin, coastal upwelling brings thermocline nitrate to the surface. This thermocline nitrate is supplied by the California Current from the north, characterized by low  $\delta^{15}\text{N}$ , and the California Undercurrent from the south, which runs closer to

the shore and carries denitrified elevated  $\delta^{15}\text{N}$  from the nearby OMZ (oxygen minimum zone) (DiGiacomo et al., 2002; Sigman et al., 2005). Once again, as the limiting ingredient for photosynthesis, all nitrate is consumed here. However, because the  $\delta^{15}\text{N}$  of starting nitrate is highly influenced by whatever currents are present in a given location, although the starting  $\delta^{15}\text{N}$  of phytoplankton is generally around 8‰, seasonal and annual variations in water mixing make this approximation highly inconsistent (Tems & Prokopenko, 2015). Despite the challenge of interpreting two sources of nitrate, the California Margin has great potential in this study because we can use its unique depth profile, which results from these two isotopically distinct currents, to determine whether the profile of CB- $\delta^{15}\text{N}$  aligns with that of suspended PON. The high  $\delta^{15}\text{N}$  of the undercurrent in this region is most concentrated at 300-400 meters (Prokopenko, unpublished). Therefore, if PON influences CB- $\delta^{15}\text{N}$ , we would expect maximal  $\delta^{15}\text{N}$  at this same depth for corals. If CB- $\delta^{15}\text{N}$  consistently matches the trends of PON  $\delta^{15}\text{N}$  in the California Margin and that of profiles from the other two locations, coincidental correlation is doubtful, and PON is likely the source of CB- $\delta^{15}\text{N}$ . This study of the California Margin will then provide a starting point from which to compare CB- $\delta^{15}\text{N}$  from other sites.

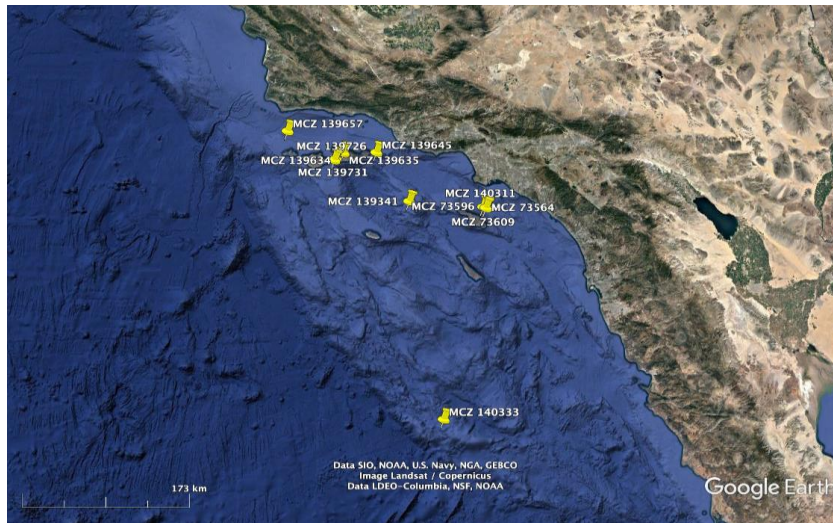


**Figure 6.** (A)  $\delta^{15}\text{N}$  of PON at Station ALOHA (Hawaii), showing a compilation of  $\delta^{15}\text{N}$  from suspended PON samples (grey circles) from Hannides et al. (2013) and  $\delta^{15}\text{N}$  of sinking and suspended PON indicated by blue squares and black diamonds respectively, from Casciotti et al. (2008). Orange arrows indicate the depths at which we have coral samples for this study. (B) indicates  $\delta^{15}\text{N}$  of zooplankton at Station ALOHA from Hannides et al. (2013). (C) The depth profile of Nitrate  $\delta^{15}\text{N}$  in the Azores (North Atlantic), from Bourbonnais et al. (2009). The blue bar indicates the  $\delta^{15}\text{N}$  of sinking POM measured at 2000 meters, while the range of depths from which we have coral samples is indicated by the purple arrow because the number of samples is too high to represent each individually. (D) Unpublished pilot data from MP displaying  $\delta^{15}\text{N}$  of Nitrate and PON in the California Margin during the summers of 2013 and 2014 at San Pedro Ocean Time series site, 33°33' N, 118°24' W. *Carophyllia* corals are indicated by green arrows, *D. dianthus* corals are indicated by blue arrows, and *L. pertusa* corals are indicated by red arrows.

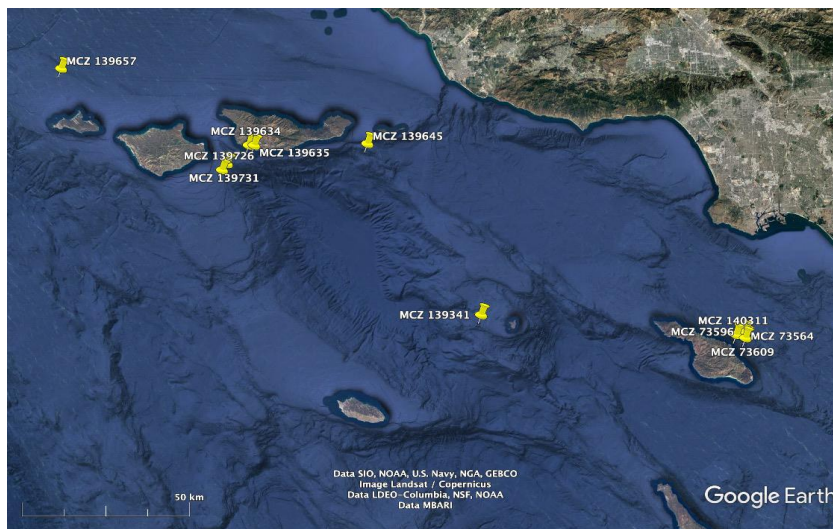
By comparing  $\delta^{15}\text{N}$  ‰ patterns in Scleractinia across depth with vertical profiles of  $\delta^{15}\text{N}$  ‰ of suspended PON, we hope to clarify whether suspended PON is responsible for the isotopic composition of corals. Resolving this question is essential in order to use stony corals as proxies of historic nitrogen and nitrogen processes in surrounding water, which are critical in the context of paleoceanographic studies and contextualizing modern climate change. Once we can accurately interpret  $\delta^{15}\text{N}$  records in corals, the revelations trapped in their carbonate skeletons are potentially colossal.

## Methods

Scleractinian coral samples were collected from the California Margin (see Figures 5 & 6) and were prepared and measured for  $\delta^{15}\text{N}$  following the methodology of Wang et al., (2014).



**Figure 7.** MCZ coral sample locations.



**Figure 8.** MCZ coral sample locations, excluding samples 141529 and 140333 to better view the more clustered samples.

Pieces of coral theca were cleaned mechanically using a diamond disk and/or diamond drill bit using a Dremel or small dental drill. Once the patches of coral were initially cleaned, smaller pieces of the coral septum were identified, cut out, and cleaned again with a small drill bit. With smaller septa, the entire septum was cut out and cleaned as one sample (Wang et al., 2014).

Nitrogen isotope analysis was conducted on coral samples based on previous methods for foraminifera shell-bound organic N analysis (Ren et al., 2009, 2012). Samples were first prepared at Pomona College by Maria Prokopenko and Austin Cordova before being sent to the University of Connecticut to undergo Isotope Ratio Mass Spectrometry (IRMS). In preparation, 10-15 mg samples of coral skeleton (each containing approximately 2  $\mu\text{mol/g}$  of N to maintain at least 20-30 nmol of coral-bound-organic-nitrogen (CBON) were ground into a granular powder (grain size was approximately a few hundred micrometers) and sonically cleaned in 2% sodium polyphosphate for 5 minutes. The purpose of sonification was to purify the sample by removing any detrital material clinging to it (Wang et al., 2014). Samples were then rinsed three times with deionized water by filling, centrifuging, and decanting processes before being reductively cleaned with sodium bi-carbonate buffered dithionite-citrate reagent to remove potential metal coatings (Mehra, 1958), based off the method developed by Lomitschka and Mangini (1999). After 3-4 more rinses with deionized water, samples were placed in 13% sodium hypochlorite for 24 hours to clear organic N contamination before another 3-4 rinses (Wang et al., 2014). Samples were then oven-dried at 60 °C and dissolved in 4 N hydrochloric acid to initiate the release of organic matter, which is then oxidized into nitrate with a basic potassium per-sulfate solution (Nydahl, 1978). The dissolved nitrate emitted by this process was then bacterially converted into  $\text{N}_2\text{O}$  (nitrous oxide) (Sigman & Casciotti, 2001) to avoid the potential



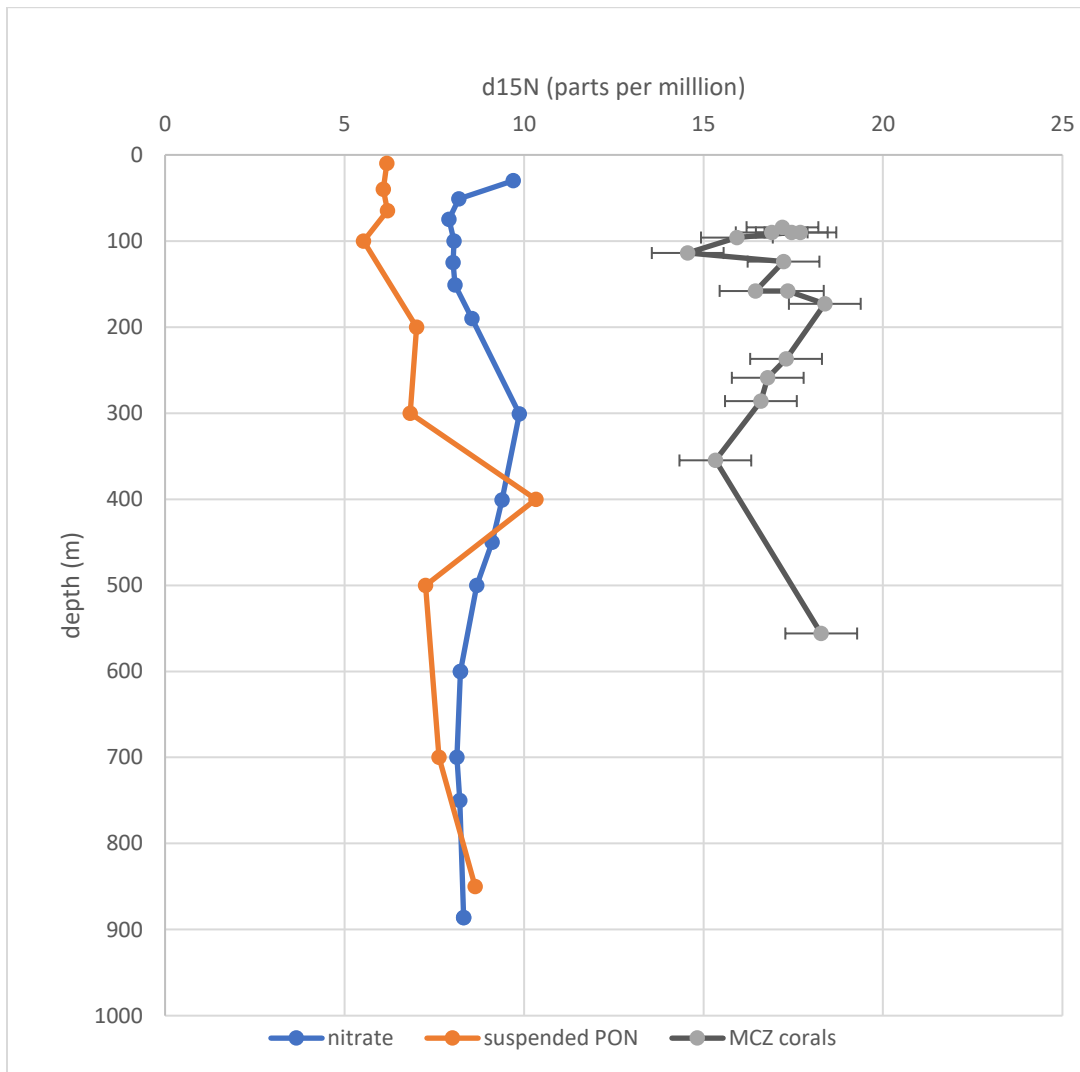
contamination levels of N<sub>2</sub> (the usual gas used in these calibrations), which would overwhelm the accuracy of the miniscule sample sizes in this study (Prokopenko, personal communications). Resulting N<sub>2</sub>O was subsequently analyzed for  $\delta^{15}\text{N}$  isotopic composition by the “denitrifier” method, which uses automated extraction and gas chromatography-isotope ratio mass spectrometry (Sigman & Casciotti, 2001). Each sample was run in duplicates or triplicates, and multiple blanks were employed in attempt to characterize and account for potential contamination: Gas standards for the mass-spectrometer, Nitrate standards for checking the N<sub>2</sub>O standard, standards for monitoring oxidation and bacterial conversion using commercially available Glutamate, and a *Lophelia* coral standard from Princeton University with known isotopic composition with which to compare final results (Prokopenko, personal communications). Quality control was ensured by the use of an in-house coral standard in each batch of analysis in order to guarantee precision of 0.3‰ (1 $\sigma$ ) or greater.



Results:

<b>Sample ID</b>	<b>Depth</b>	<b>Longitude</b>	<b>Latitude</b>	<b>D15N</b>	<b>Standard Deviation</b>
MCZ 139635*	84	33.56	119.50	17.20	0.5
MCZ 139731*	90	33.54	119.56	17.46	0.1
MCZ 139725*	90			17.70	0
MCZ 139726*	90	33.54	119.56	16.90	0.3
MCZ 139634*	96	33.57	119.50	15.93	0.1
MCZ 139657*	114	34.10	120.26	14.56	
MCZ 140311	124	33.24	118.22	17.23	0.1
MCZ 73564	158	33.29	119.08	16.45	0.3
MCZ 139341	158	33.29	119.08	17.35	0.4
MCZ 139645	173	33.57	119.29	18.38	
MCZ 73609	237	33.24	118.20	17.30	1.9
MCZ 73564	259	33.40	118.33	16.79	0.3
MCZ 141529*	286	38.55	123.56	16.60	0.8
MCZ 73596	355	33.24	118.20	15.33	0.0
MCZ 140333	556	31.23	118.49	18.28	0.4
Average*	265			16.6	0.3
Average	133			17.1	0.4
Total Average	194			16.9	0.4
Stdev of Mean*				0.7	
Stdev of Mean				0.5	

**Table 2.** The depth, longitude, latitude, CB- $\delta^{15}\text{N}$ , and standard deviation for all coral samples. Samples marked with \* are from the north and are more exposed to the California Current, while samples without the \* are from the south and are more exposed to the California Undercurrent.



**Figure 9.** A comparison of  $\delta^{15}\text{N}$  of nitrate, suspended PON, and CB- $\delta^{15}\text{N}$ . Values for nitrate and suspended PON were measured by MP in the California Margin during the summers of 2013 and 2014 at San Pedro Ocean Time series site, 33°33' N, 118°24' W. Samples of CB- $\delta^{15}\text{N}$  were collected in June 2014.

Corals within the first 100 meters from the surface exhibited  $\delta^{15}\text{N}$  values between 15.93 ‰ and 17.20 ‰, with an average value of 16.87 ‰ and an average standard deviation of 0.25 ‰. Between 100 meters and 200 meters, average CB- $\delta^{15}\text{N}$  ranged from 14.56 ‰ to 18.38 ‰, with an average of 16.86 ‰, with an average standard deviation of 0.27 ‰. However, the standard

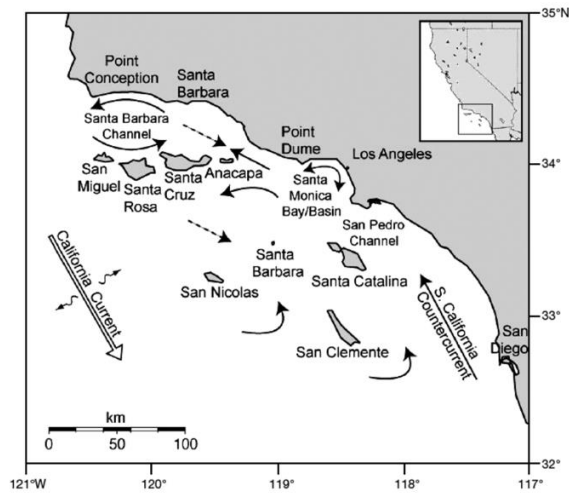
deviation here was only calculated based on the middle three samples, as the corals with the maximum and minimum  $\delta^{15}\text{N}$  values only had one run each. In this depth range, samples generally decreased in  $\delta^{15}\text{N}$  with increasing depth. For samples between 200 and 400 meters, the range was 15.33 ‰ to 17.30 ‰, the average was 16.41 ‰, and the average standard deviation was 0.90 ‰. These three samples each increased in  $\delta^{15}\text{N}$  with depth, though the sample at 355 meters with  $\delta^{15}\text{N}$  of 15.33 ‰ was also measured in one run only. In addition, two of the runs for the 17.30 ‰ sample were excluded from the calculations because they were more than three times the standard deviation of the mean from the mean from the whole population, possibly due to contamination. Only one sample was measured below 400 meters. This sample, at 556 meters, had  $\delta^{15}\text{N}$  of 18.28 ‰ and a standard deviation of 0.40 ‰, showing an increase in  $\delta^{15}\text{N}$  from the next deepest sample at 355 meters of 2.95 ‰. The average  $\delta^{15}\text{N}$  for the entire population was calculated to be  $16.9 \pm 0.4$  ‰. Despite these apparent trends in CB-  $\delta^{15}\text{N}$ , we must also account for spatial context beyond depth, which will be elaborated on in the discussion.

### Discussion

We conducted this study to test the relationship between  $\delta^{15}\text{N}$  of suspended PON and  $\delta^{15}\text{N}$  of MCZ coral skeletons across depth. We hypothesized that preferential uptake of suspended PON rather than freshly fixed sinking PON by corals in conjunction with the usual 3-3.5 ‰ offset between trophic levels could be responsible for the apparent ~ 8.5 ‰ offset between coral  $\delta^{15}\text{N}$  and that of their expected food source, POM (Hannides et al., 2013, & Prokopenko, unpublished). Although we have not yet analyzed all three prospective sample sites, data from the California Margin indicates that suspended PON in surrounding water of corals does not determine their  $\delta^{15}\text{N}$ , as coral isotopic composition does not track with the patterns of suspended PON. Whereas  $\delta^{15}\text{N}$  of PON reaches its maximum of 10.33 ‰ at approximately 400 meters, CB- $\delta^{15}\text{N}$  at this same depth is only 15.33 ‰, near its minimum value. When CB- $\delta^{15}\text{N}$  is highest, at 18.38 ‰ and 18.28 ‰ at 173 meters and 556 meters respectively,  $\delta^{15}\text{N}$  of PON is relatively low, at approximately ~8.5 ‰ in each location. Apparently, the areas in which CB- $\delta^{15}\text{N}$  is consistently increasing or decreasing do not track with that of suspended PON. In addition, the average CB- $\delta^{15}\text{N}$  in this dataset is ~16.9 ‰, while that of PON is only 7.7 ‰, indicating an offset of ~9 ‰. This value is much greater than the 3 – 3.5 ‰ expected value given that trophic enrichment accounts for the remaining offset between PON and skeletal CB- $\delta^{15}\text{N}$ . According to our dataset, this is clearly not the case; Some other factor or factors must be driving the heightened isotopic offset between corals and their food.

As suspended PON does not drive CB  $\delta^{15}\text{N}$ , we considered other potential factors. Our corals were widely distributed across the California Bight, a region impacted by multiple dynamic currents and oceanic processes (DiGiacomo et al., 2002; Tems & Prokopenko, 2015).

Consequently, we decided to reanalyze our data, this time splitting our samples based on currents and the resulting isotopic composition of the water our samples are exposed to.



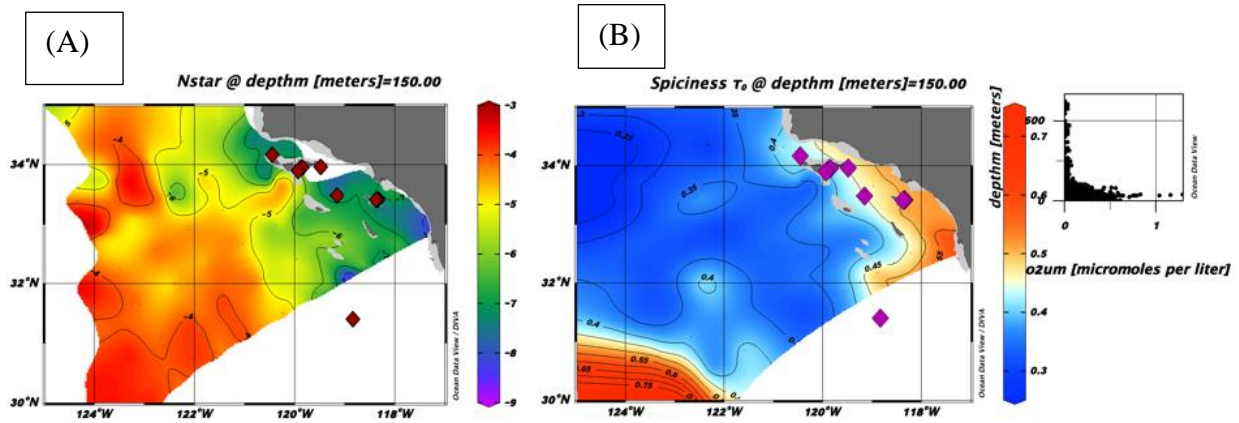
**Figure 10.** Surface circulation (~10 meters deep) in the Southern California Bight (from DiGiacomo et al., 2002). Solid arrows portray flows to the north nearshore and flows toward the equator offshore. The California Current in general comes closer to the shore in the spring and summer. Dashed arrows represent the transition of the Southern California Bight away from shore in the spring.

The two main currents in the California Bight Region are the California Undercurrent and the California Current (DiGiacomo et al., 2002). The California Undercurrent carries denitrified water coming from the south north up the coast. This water is higher in  $\delta^{15}\text{N}$  because it is coming from a large Oxygen Minimum Zone in the north Pacific, one of the three major water column denitrification regions of the ocean (Townsend-small et al., 2014; Gruber, 2008). In contrast, the California Current has lower  $\delta^{15}\text{N}$  because it carries surface flows from the arctic, which have not had time to undergo the nitrogen processes of the undercurrent (Prokopenko, personal communications). We wondered if these two contrasting sources of nitrate could be changing the starting isotopic composition of phytoplankton, which would then have effects higher up the food chain in zooplankton and other potential components of POM before being translated into CB-  $\delta^{15}\text{N}$ .

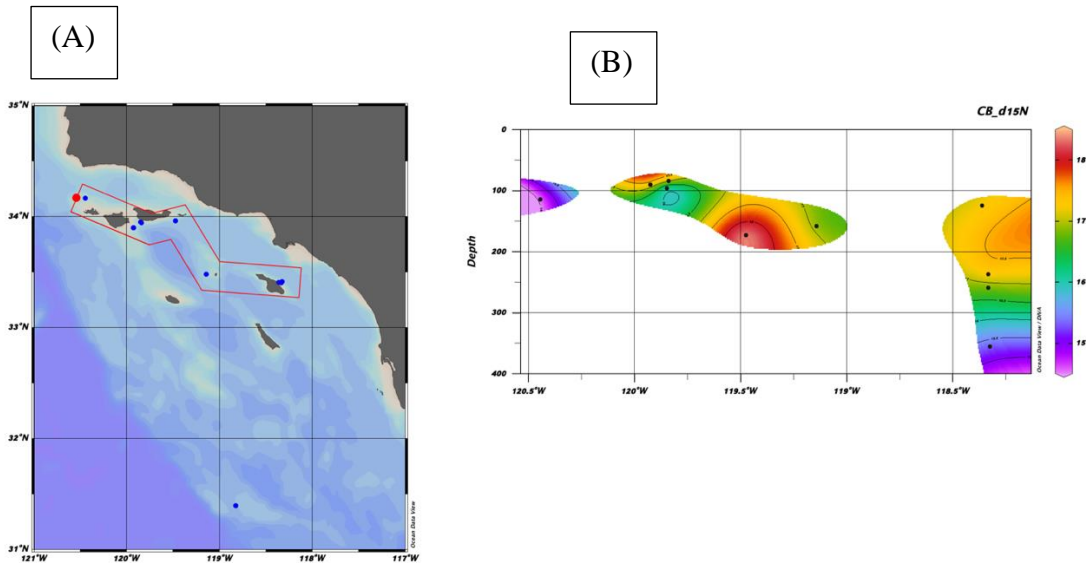
Water from the two currents is distinguishable by its “spiciness,” an oceanographic property calculated from temperature and salinity. Because these properties are physical attributes unperturbed by biological reactions, they are important “conservative” markers of water masses (Prokopenko, personal communications). In the California Bight, the water coming from the south was part of the North Pacific Subtropical Gyre surface water before entering the OMZ and denitrification region. Gyre water sits on the surface and accumulates heat and salinity before being downwelled, retaining these properties even after undergoing multiple nitrogen processes (Prokopenko, personal communications). In this case, therefore, high spiciness is expected to correspond with high  $\delta^{15}\text{N}$  deriving from denitrification. We can use spiciness to track water movement from the North Pacific Subtropical Gyre as it flows north with the California Undercurrent. We can also use  $\text{N}^*$  (defined in Figure 11) as a proxy of denitrified water, as lower  $\text{N}^*$  values indicate less nitrate relative to phosphate based on the consistent mean relationship between these two nutrients. Low  $\text{N}^*$  indicates denitrification and likely corresponds to high  $\delta^{15}\text{N}$  of nitrate. Together, these two characteristics enable us to distinguish California Undercurrent water from California Current water, which is colder, has lower salinity, higher  $\text{N}^*$ , and lower  $\delta^{15}\text{N}$  (Townsend-small et al., 2014, & CALCOFI 2015). While the California Undercurrent runs beside and up the coast, the California Current lies above and to the west. We can therefore re-analyze our samples based on positionality relative to these currents and the source nitrate they carry.

We used spiciness at 150 meters of depth to inform how we grouped our samples according to currents. This depth was chosen because it is deep enough to avoid the influence of surface modification on nitrate and spiciness, but shallow enough to represent the characteristics of source water masses being upwelled from below. This “source” nitrate at 150 meters is

eventually taken up at the surface by phytoplankton, which constitutes part of the theoretical POM diet of corals. With respect to these new groupings, we expected CB- $\delta^{15}\text{N}$  in the California Undercurrent to be higher than that of the California Current due to higher starting values of source nitrate in this region. Our values indicate that this prediction was correct, as the average of CB-  $\delta^{15}\text{N}$  in the California Undercurrent region was 17.1 ‰, while for the California Current region it was only 16.6 ‰. In addition, in the California Undercurrent group, all samples but one contained  $\delta^{15}\text{N}$  of 16.79 ‰ or higher, and it is worth re-emphasizing that the one exception, MCZ 73596 at 15.33 ‰, did not have any duplicate runs—only two identical samples of the same oxidation were processed, meaning that this sample could well have been contaminated. If this is the case, and this sample is inaccurate, the difference between groupings would have been even greater, as the Undercurrent average would have been ~17.4 ‰. However, because we only have one run for this potentially inaccurate sample, we have no way of knowing if contamination occurred or whether we can exclude this point. Nevertheless, although our averages are only based on seven and eight samples for northern and southern groups respectively, it appears that starting nitrate may indeed have an influence over CB-  $\delta^{15}\text{N}$ .

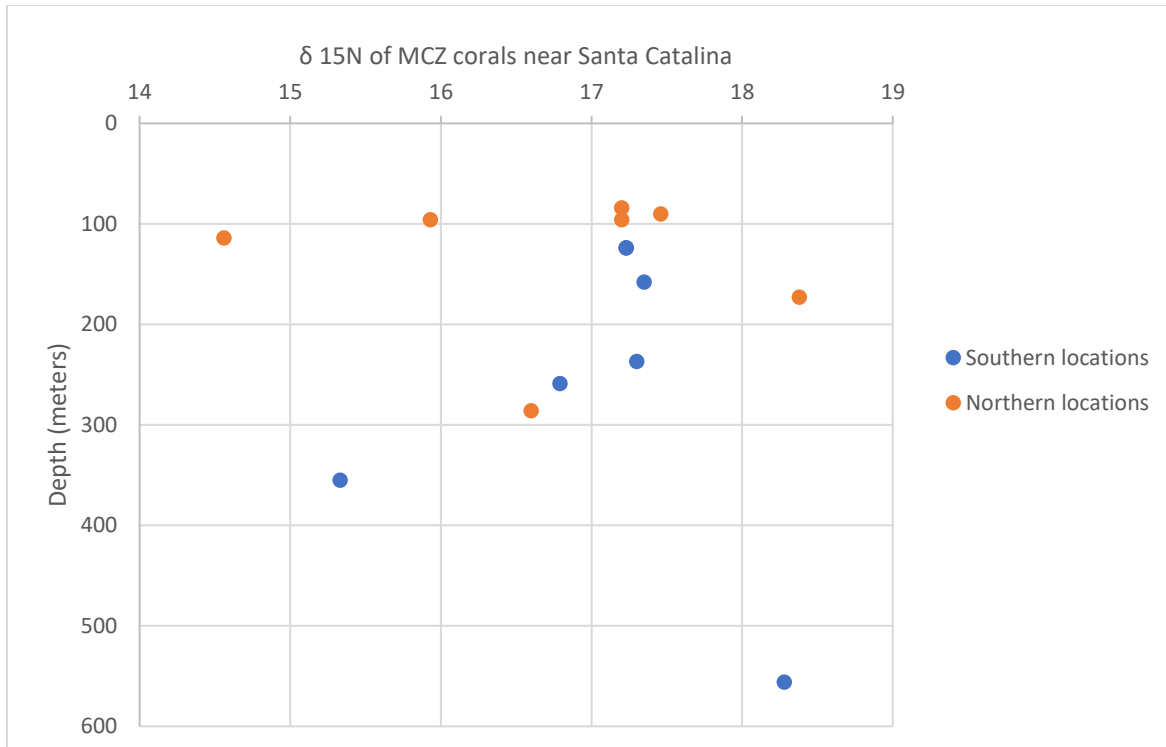


**Figure 11.** (A)  $N^*$  ( $(16 \times PO_4^{3-}) - NO_3^-$ ) at 150 meters of depth. (B) Spiciness at 150 meters of depth. These metrics were used to distinguish high  $\delta^{15}N$  water from the California Undercurrent from low  $\delta^{15}N$  water from the California Current.  $N^*$  and Spiciness were calculated and plotted using data from California Cooperative Oceanic Fisheries Investigations (Available at <https://calcofi.org/>) and ODV software.



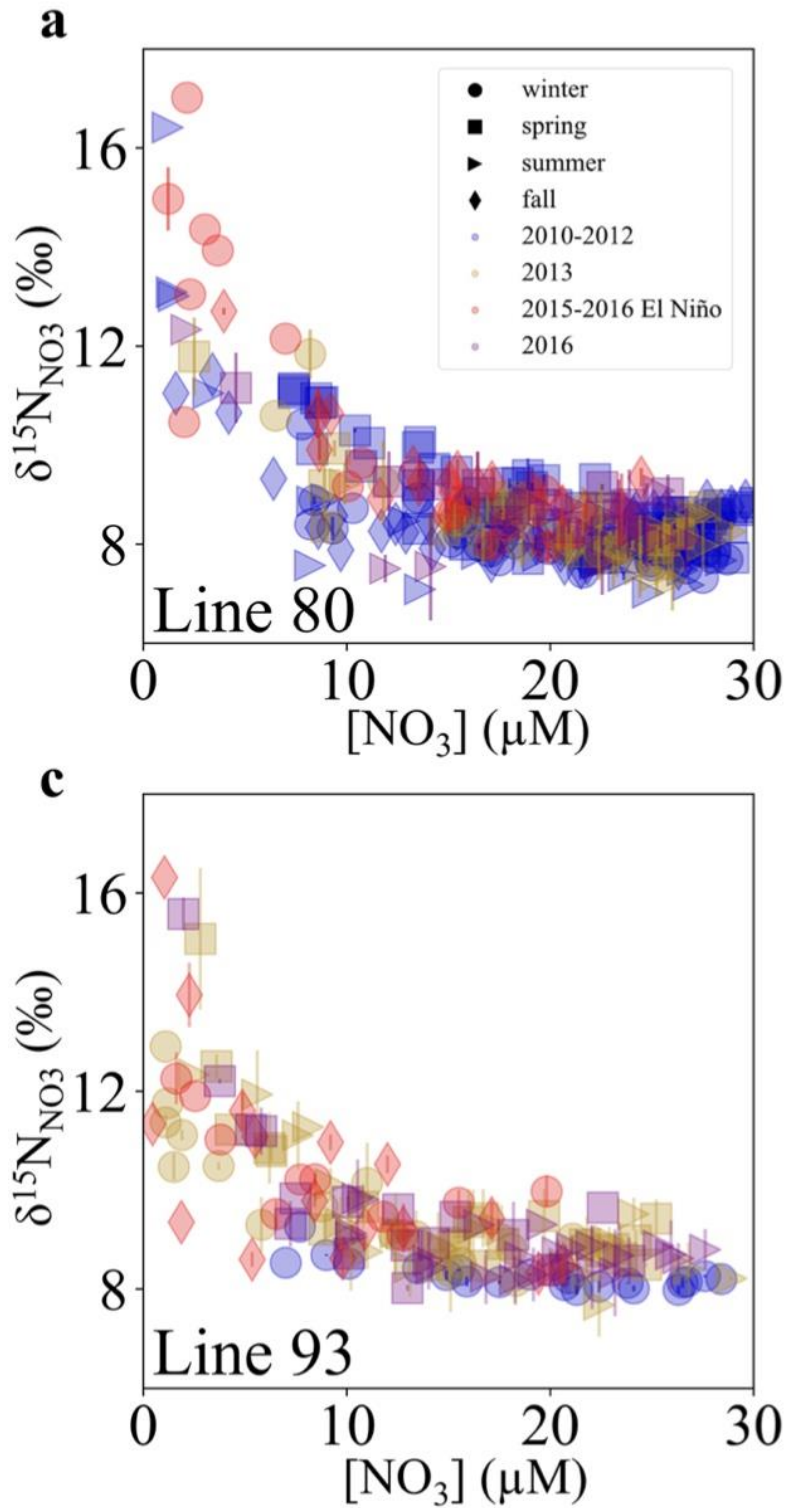
**Figure 12.** (A) Spatial map of transect for (B) CB-  $\delta^{15}N$  showing actual values in addition to theoretical values for areas surrounding these samples. Plots constructed using Ocean Data View (Schlitzer et al., 2021).



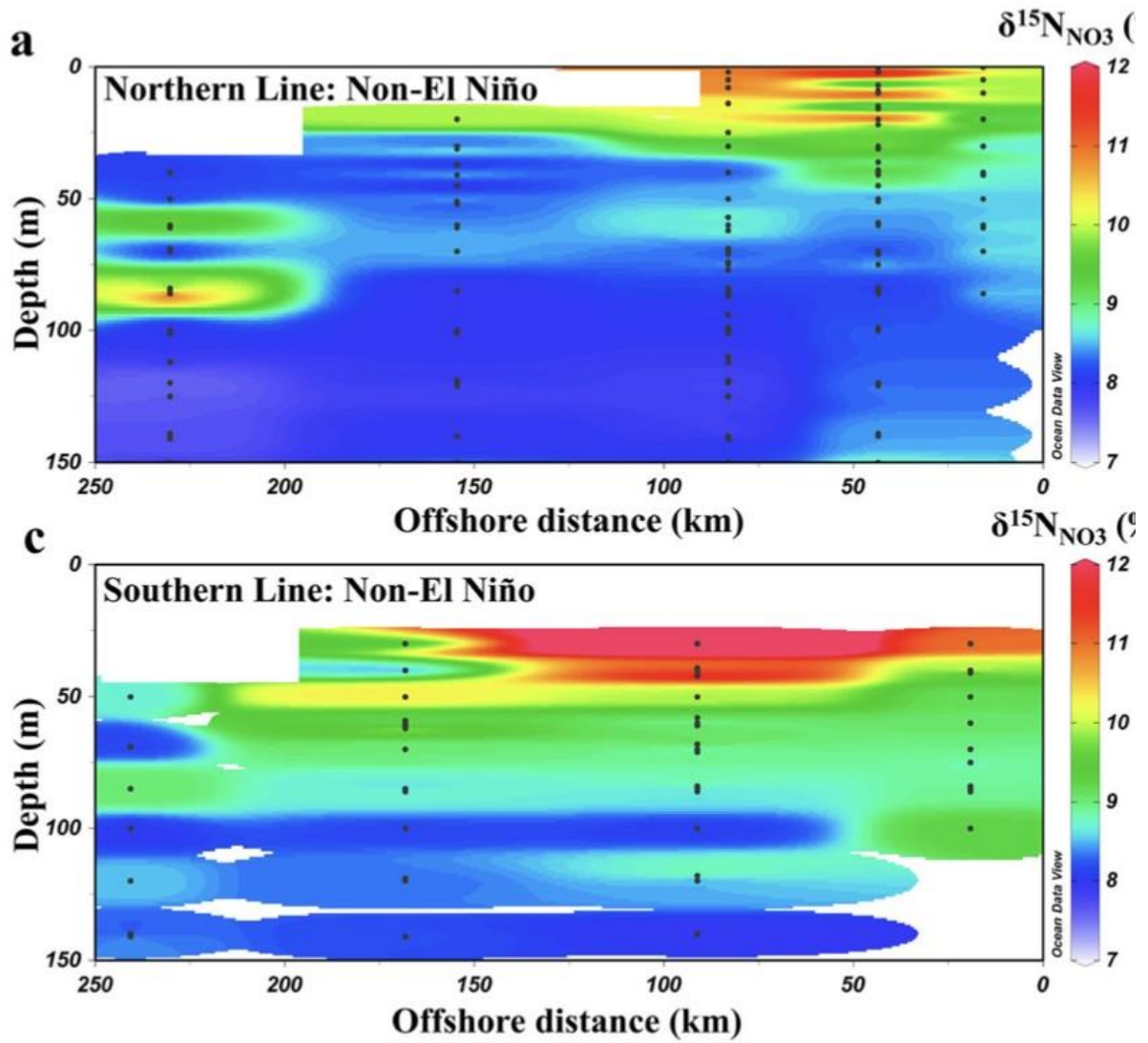


**Figure 13.**  $\delta^{15}\text{N}$  of corals in southern (California Undercurrent) and northern (California Current) locations.  $R^2$  values indicate that variation in depth plays an insignificant role in changing CB- $\delta^{15}\text{N}$ , but average values of each location (17.1 & 16.6 respectively) indicate that  $\delta^{15}\text{N}$  is higher in southern corals.

However, this explanation of source nitrate determining CB-  $\delta^{15}\text{N}$  does not explain the ~9 offset observed between suspended PON  $\delta^{15}\text{N}$  and CB- $\delta^{15}\text{N}$  in our data. If suspended PON is not determining CB- $\delta^{15}\text{N}$ , and neither is sinking PON, which has even lower  $\delta^{15}\text{N}$  than suspended PON, then zooplankton and other higher trophic organisms such as nekton larvae are the only explanation remaining (Mueller et al., 2014; Lim, 2020). Using process of elimination, we are then redirected to Hypothesis (d) of the original study—that  $\delta^{15}\text{N}$  is being processed through multiple trophic levels before being absorbed by corals.



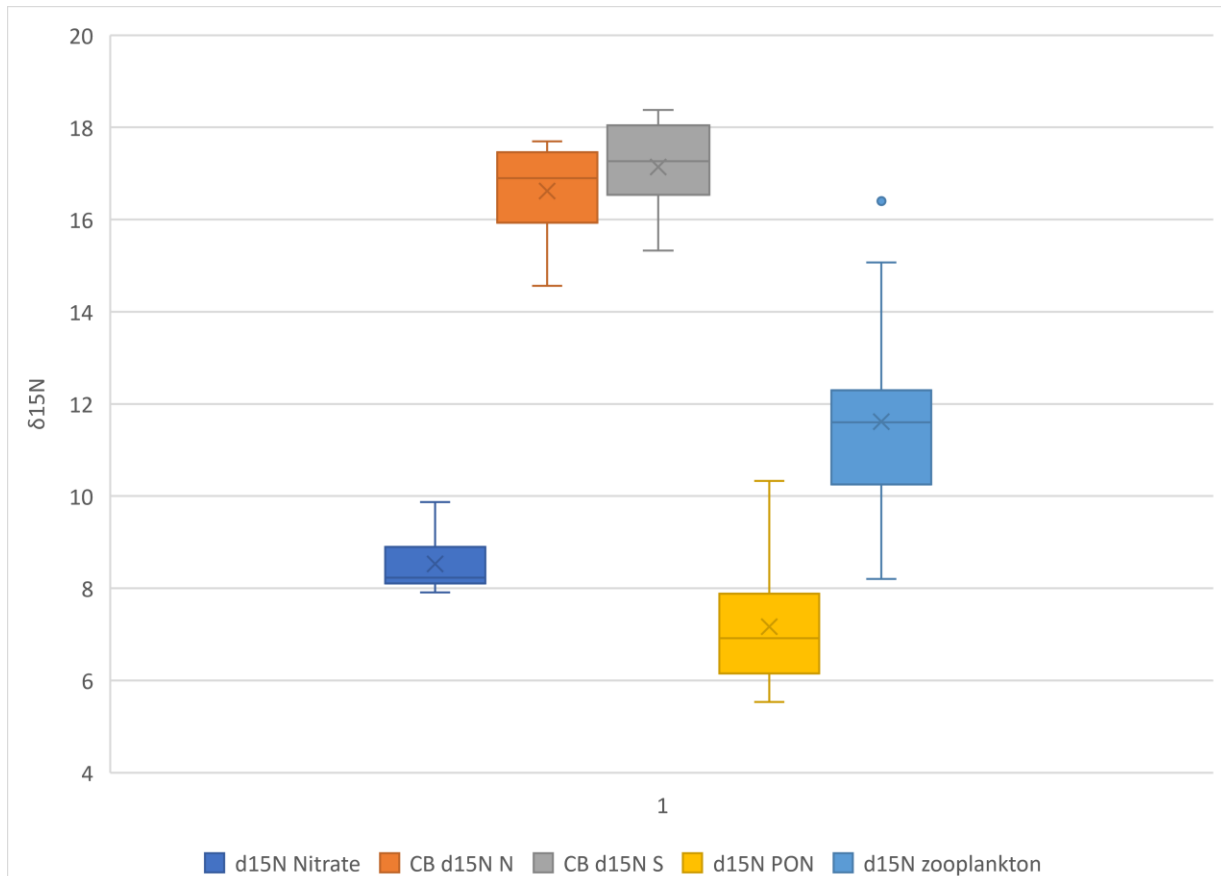
**Figure 14.**  $\delta^{15}\text{N}$  of northern nitrate (top) and southern nitrate (bottom), from White et al., (2022).



**Figure 15.**  $\delta^{15}\text{N}$  of northern nitrate (top) and southern nitrate (bottom), from White et al., (2022).

Paper	$\delta^{15}\text{N}$
Ohman et al., 2011	$\sim 10.5\text{-}14.1$ ‰
Decima et al., 1984	$\sim 11.9 - 15.0$ ‰
Mullin et al., 2013	$\sim 8.2 - 12.5$ ‰
Average	$11.6 \pm 0.6$ ‰

**Table 3.** Zooplankton  $\delta^{15}\text{N}$  in the California Bight calculated from three different source papers (Ohman et al., 2011, Decima et al., 1984; Mullin et al., 2013).



**Figure 16.**  $\delta^{15}\text{N}$  of nitrate, northern corals, southern corals, suspended PON, and zooplankton in the California Bight. Values for nitrate and suspended PON were measured by MP in the California Margin during the summers of 2013 and 2014 at San Pedro Ocean Time series site, 33°33' N, 118°24' W.

Prior studies have been conducted on zooplankton  $\delta^{15}\text{N}$  in the California Bight. Although these studies do not take depth into account, they indicate that  $\delta^{15}\text{N}$  varies widely by species and across time. Based on a compilation of studies, zooplankton  $\delta^{15}\text{N}$  appears most concentrated in the 10.3-12.3 ‰ range, though values may range as low as 8.2 ‰ and as high as 15.1 ‰ (Ohman et al., 2011; Mullin et al., 1984; Décima et al., 2013). Consequently, although more zooplankton data specific to water mass and including vertical profiles is required here, these preliminary indications suggest that although zooplankton has higher  $\delta^{15}\text{N}$  than suspended PON and could be a potential influence on CB- $\delta^{15}\text{N}$ , zooplankton  $\delta^{15}\text{N}$  alone is still not high enough to explain the ~9 ‰ offset. The difference between the average  $\delta^{15}\text{N}$  of corals and that of zooplankton is ~5.6

‰, which is greater than a 3-3.5 ‰ offset from trophic enrichment. Two final potential influences which could resolve this offset is that of nekton larvae, such as oyster gametes (Lim, 2020), as well as the slight offset of ~1-2 ‰ observed between coral tissue and skeleton  $\delta^{15}\text{N}$  (Mottram et al., in prep). Although extensive data has not been conducted on  $\delta^{15}\text{N}$  of nekton larvae in the California Bight, oyster gametes fed to lab-grown corals are reported to be ~9 ‰ higher than phytoplankton, suggesting offsets from zooplankton which could approach 5-6 ‰ (Lim, 2020). In addition, some studies on lab-grown corals have suggested preferential uptake of oyster gametes over food from lower trophic levels (Lim, 2020). Therefore, even in small amounts, nekton larvae could have a substantial influence on the average  $\delta^{15}\text{N}$  of coral diet and subsequently CB- $\delta^{15}\text{N}$ . However, in order for this proposition to have credibility, depth profiles of nekton larvae  $\delta^{15}\text{N}$  in the California Bight are necessary.

Although this thesis is only the first piece of a much greater study, it provides preliminary estimates as to the determining factors in CB- $\delta^{15}\text{N}$ . Source nitrate, zooplankton, and nekton larvae each likely regulate CB- $\delta^{15}\text{N}$  to some degree, though further research is necessary to confirm this theory. Nevertheless, as we can eliminate suspended PON from the pool of potential influencing factors, our analysis here brings us one step closer to using deep-sea corals as proximates of  $\delta^{15}\text{N}$  in surrounding water and as records of paleoceanographic climate conditions.

### Acknowledgements

I would first like to thank my two readers, Maria Prokopenko and Lars Schmitz, for their guidance and support throughout this project. It was an honor to be mentored by Maria Prokopenko and included in her work, especially in this specific project, which has such far-reaching implications for future climate research. Lars Schmitz also provided critical feedback, and consistently helped me plan and execute each aspect of this paper. This thesis would not have been possible without either of their help. Austin Cordova, research assistant of Maria Prokopenko in the Pomona College Geology Department, was also a key contributor to this study, as my time spent observing his work in the lab greatly benefited my understanding of the project's procedure. I would also like to thank the Keck Science Department and the Pomona College Geology Department for making this opportunity possible for me and for providing the resources necessary to execute this study. Funding for this research was provided by the OCE-NSF194984 grant awarded to Maria Prokopenko and Austin Cordova, which enabled sample processing. Lastly, I would like to thank my friends and family for their unwavering encouragement and support throughout this project.

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