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RAISE YOUR GLASS: A CULTURE EVALUATION OF DIATOMS AS ARCHIVES OF PAST NUTRIENT CONSUMPTION

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RAISE YOUR GLASS:
A CULTURE EVALUATION OF DIATOMS AS
ARCHIVES OF PAST NUTRIENT CONSUMPTION
BY
COLIN ANTHONY JONES

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
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DOCTOR OF PHILOSOPHY DISSERTATION
OF
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ABSTRACT

Nitrate is the most common limiting nutrient in the ocean and plays a critical role in the extent and intensity of marine primary production, and therefore the global ocean's biological pump. Characterization of the supply and demand of nitrate constrains how ocean biology may regulate climate, so understanding the degree of nitrate consumption in the past is a fundamental step towards understanding controls on past climate. The nitrogen isotopic composition (as $\delta^{15}\text{N}$) of phytoplankton biomass can be used to infer the degree of nitrate consumption in nitrate-replete surface waters such as the Southern Ocean. This signal is recorded in the underlying sediment and can be used to construct a history of nitrate utilization. However, $\delta^{15}\text{N}$ values of phytoplankton biomass are subject to alteration during sinking and sedimentation, leading to uncertainty in estimations. The nitrogen isotopic composition of nitrogen within the shells of diatoms ($\delta^{15}\text{N}_{\text{DB}}$), a photosynthetic microorganism, is protected from alteration and potentially a more robust tracer of past nitrate dynamics. However, this assumption may be complicated by species-specific isotope effects and the high variation in Southern Ocean diatom assemblages through climate transitions. The goals of this dissertation are twofold: first, to investigate the impact of different Southern Ocean diatom communities (Chapter 1) and individual species (Chapter 2) on the $\delta^{15}\text{N}_{\text{DB}}$ proxy and second, to use $\delta^{15}\text{N}_{\text{DB}}$ to examine paleo-nutrient utilization and oceanographic conditions of the coastal West Antarctic Peninsula (WAP), a region of high seasonal productivity and carbon drawdown (Chapter 3).

Two distinct Southern Ocean surface ocean diatom communities were grown in triplicate cultures to determine the impact of diatom community composition on

$\delta^{15}\text{N}_{\text{DB}}$. We found that although the community growouts had distinct diatom assemblages, the ϵ_{DB} (= biomass $\delta^{15}\text{N}$ - $\delta^{15}\text{N}_{\text{DB}}$) was indistinguishable between the two growouts at $-4.8 \pm 0.8\text{‰}$. This suggests that species composition is not the primary control on $\delta^{15}\text{N}_{\text{DB}}$ in the Southern Ocean. Furthermore, our measured average ϵ_{DB} was more than 10‰ different from the average value of previous single-species measurements, but consistent with Southern Ocean and North Pacific surface ocean observations. Therefore, if $\delta^{15}\text{N}_{\text{DB}}$ is not altered during sinking and sedimentation, then sedimentary $\delta^{15}\text{N}_{\text{DB}}$ is a robust tool for examining changes to nitrate supply and demand over time.

Single-species cultures of diatoms isolated from the Southern Ocean were grown in triplicate to assess individual species ϵ_{DB} . We show that the average ϵ_{DB} is $-2.2 \pm 1.0\text{‰}$, consistent with the Southern Ocean community data and surface ocean observations. We observe a positive linear relationship between ϵ_{DB} and the Si:N of diatom uptake, implying that silicification plays a role in setting $\delta^{15}\text{N}_{\text{DB}}$. This relationship suggests that heavily silicified diatoms could bias sedimentary $\delta^{15}\text{N}_{\text{DB}}$ toward lower values. However, five of the six diatoms species had indistinguishable ϵ_{DB} from one another, indicating that the impact may be minimal in many cases.

The coastal WAP hosts intense productivity fueled by the delivery of warm, nutrient rich Circumpolar Deep Water (CDW) and seasonal stratification related to sea-ice melting. Stronger Southern Hemisphere westerlies are thought to enhance CDW intrusion onto the WAP shelf and have increased in strength over at least the last four decades. Therefore, it is key to determine the historical variations in CDW, productivity, and westerly winds. These variations are especially relevant in the mid-

Holocene, when CDW intrusion likely varied substantially, yet there is disagreement about the timing of CDW influence in paleoproxy records. We use $\delta^{15}\text{N}_{\text{DB}}$ to study the WAP by unambiguously linking past proxy descriptions of warm, sea-ice free intervals with the nutrient characteristics of CDW. We find that contrary to prior hypotheses, variations in the relationship between $\delta^{15}\text{N}_{\text{DB}}$ and bulk sedimentary $\delta^{15}\text{N}$ cannot be explained by changes to diatom assemblages. We also find a dynamic CDW in the mid-Holocene that is synchronous with atmospheric warming associated with stronger westerlies. As westerly strength increases in the future, we predict that CDW intrusion will play an increasingly dominant role in WAP oceanography, supplying enhanced nutrients and potentially contributing to glacial melting. Altogether, the results presented in this dissertation enhance our ability to quantify past fluctuations in nitrate utilization, which plays a vital role in assessing the biological pump.

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PREFACE

This dissertation is written in manuscript form and consists of three manuscripts.

Motivation

“[A]n examination of how the climate system has responded to...climate forcing in the past is useful in assessing how the same climate system might respond to the large anticipated forcing changes in the future” (IPCC Report, AR4)

“The marine nitrogen cycle is perhaps the most complex and therefore the most fascinating among all biogeochemical cycles in the sea” (Gruber, 2008)

Paleoceanography is a field dedicated not just to understanding what happened at a particular time and place in the ocean, but to understanding the fundamental processes that govern marine systems. Not only is that information interesting for its own sake, but such systematic synthesis underpins our current understanding of future climate change. Indeed, paleoclimate observations provide primary constraints on climate models used to predict future scenarios (Crowley & North, 1991; Jansen et al., 2007; Masson-Delmotte et al., 2013).

A primary goal of paleoceanographic research over the last several decades has been to clarify the mechanisms underlying glacial/interglacial CO₂ changes. The Southern Ocean is a region where upwelling brings nutrient- and carbon-rich deep water to the surface, releasing CO₂ directly to the atmosphere while simultaneously stimulating the biological pump, which stores carbon in the deep ocean. The balance

between the release of CO₂ and the strength of the biological pump plays an important role in setting glacial atmospheric CO₂ (Francois et al., 1997; Sigman et al., 2010). To determine exactly how these processes can contribute to CO₂ change, it is key to know the efficiency of the biological pump. Nitrate, the limiting nutrient for much of the ocean (Moore et al., 2013), is supplied via upwelling and taken up during biological productivity, and in the Southern Ocean its supply and utilization contain key information about the drawdown of carbon. Thus, quantifying the patterns of past nitrate delivery and consumption is an essential step towards determining the fundamental controls on the biological pump and the processes controlling climate change.

Nitrogen isotopes

The primary method used to quantify past patterns of nitrate utilization is sedimentary nitrogen isotope ratios ($\delta^{15}\text{N}$, the ratio of ^{15}N to ^{14}N). The $\delta^{15}\text{N}$ of nitrate in the surface of the Southern Ocean is primarily set by the relative consumption of nitrate by biological nitrate uptake, which isotopically favors the uptake of ^{14}N . Because of this, the $\delta^{15}\text{N}$ of organic material is inversely related to the surface nitrate concentration in nutrient-replete regions of the modern ocean (Wada & Hattori, 1976). Coretop records, i.e. modern sediments, reflect this pattern (Altabet & Francois, 1994). Downcore, sedimentary $\delta^{15}\text{N}$ is a proxy for nitrate supply and demand of the past ocean. Although it has seen widespread adoption, biases related to isotopic alteration of bulk sedimentary N, particularly in the deep ocean, quickly came to light (Altabet & Francois, 1994; Farrell et al., 1995; Robinson et al., 2012). To combat this problem,

measurement of the nitrogen bound in the silica frustules of diatoms, which should protect nitrogen encased in the frustule from alteration, was proposed (Shemesh et al., 1995; Sigman et al., 1999; Robinson et al., 2004, 2005). This is an especially useful proxy for the Southern Ocean, where diatoms dominate surface ocean productivity and sediments (Armand et al., 2005; Crosta et al., 2005). However, a laboratory-based study of cultured diatom material suggested that individual diatom species may have distinct isotopic fractionations (ϵ_{DB}) between their biomass and the nitrogen bound within the frustule (Horn et al., 2011a). This is concerning because sedimentary diatom assemblages in the Southern Ocean can vary dramatically downcore, enough to potentially change the interpretation of diatom-bound $\delta^{15}\text{N}$ records (Horn et al., 2011b).

Statement of the problem

The goal of this dissertation is to further evaluate the influence of diatom species on the diatom-bound $\delta^{15}\text{N}$ proxy in order to improve our ability to reconstruct surface nitrate conditions in the past (Figure 1). Even as proxy calibration and groundtruthing efforts are still underway (Horn et al., 2011a, this dissertation, Robinson et al., 2020 - in revision), diatom-bound $\delta^{15}\text{N}$ continues to be used to assess nitrogen cycling in the Southern Ocean (Robinson et al., 2005, 2014; Robinson & Sigman, 2008; Horn et al., 2011b; Studer et al., 2015), North Pacific (Brunelle et al., 2007; Studer et al., 2012, 2013; Ren et al., 2015), and other environments (Kalansky et al., 2011). It is therefore imperative to have a robust understanding of the potential complications associated with diatom-bound $\delta^{15}\text{N}$. Here, we use shipboard community cultures from the

Southern Ocean (Chapter 1) and further monospecific diatom cultures (Chapter 2) to elucidate the role of individual species and community composition in setting diatom-bound $\delta^{15}\text{N}$. We also generate a record of diatom-bound $\delta^{15}\text{N}$ from the coastal Southern Ocean to 1) examine oceanographic changes on sub-millennial timescales and 2) determine if diatom assemblage changes impacted the diatom-bound $\delta^{15}\text{N}$ proxy (Chapter 3).

Manuscript Descriptions

Chapter 1: Diatom-bound $\delta^{15}\text{N}$ robustly tracks surface ocean nitrate $\delta^{15}\text{N}$: evidence from Southern Ocean community cultures

Diatom communities from two Southern Ocean sites were collected from surface ocean water and grown in shipboard cultures to determine the impact of diatom community composition on diatom-bound $\delta^{15}\text{N}$. Although the two diatom communities were distinct, their $\delta^{15}\text{N}$ values were indistinguishable in nitrate $\delta^{15}\text{N}$, total biomass $\delta^{15}\text{N}$, and diatom-bound $\delta^{15}\text{N}$. The offset between biomass and diatom-bound $\delta^{15}\text{N}$ was $-4.8 \pm 0.8\text{‰}$. This result was surprising because the prior monospecific growouts had shown a positive offset of about $5.3 \pm 4.4\text{‰}$. However, these community values are within the range of offsets found in Southern Ocean and North Pacific surface waters. These results suggest that species composition does not primarily set diatom-bound $\delta^{15}\text{N}$ in the Southern Ocean, and that if it remains unaltered during sinking and sedimentation, diatom-bound $\delta^{15}\text{N}$ is a robust archive of past nutrient dynamics.

Chapter 2: Potential impact of varying silicification on diatom-bound nitrogen isotope ratios: evidence from monospecific cultures

To assess the impact of individual diatom species on the diatom-bound $\delta^{15}\text{N}$ proxy, we grew cultures of six species of diatoms isolated from the Southern Ocean. We found that five of the six species had isotopic offsets similar to those observed in community cultures and the surface ocean. However, the final species was the most heavily silicified with the highest Si:N uptakes, which suggests a potential relationship between Si:N of a diatom and its $\delta^{15}\text{N}$ through silicification. Additional work is required for a robust assessment of the relationship between silicification and diatom-bound $\delta^{15}\text{N}$, but most diatom species were isotopically indistinguishable, which suggests that diatom-bound $\delta^{15}\text{N}$ primarily records surface ocean nutrient dynamics.

Chapter 3: Nitrogen isotopic constraints on mid-Holocene Circumpolar Deep Water intrusion on the West Antarctic Peninsula

Under modern climate change, the Southern Hemisphere westerly winds have strengthened and will continue to strengthen. This affects the oceanography of the West Antarctic Peninsula, which has intense seasonal productivity related to nutrient input from warm, nutrient-rich Circumpolar Deep Water (CDW) and sea-ice driven stratification. To understand future impacts on this region it is important to determine the history of CDW input on sub-millennial timescales. Downcore records of bulk sedimentary and diatom-bound $\delta^{15}\text{N}$ were generated for comparison to available proxy records of sea-surface temperature, sea-ice presence, and westerly wind strength as well as diatom assemblages. We found that variations between bulk and diatom-bound

$\delta^{15}\text{N}$ cannot be explained by diatom assemblage shifts, and that diatom-bound $\delta^{15}\text{N}$ tracks enhanced CDW input during intervals of stronger westerly winds. This demonstrates the utility of the diatom-bound $\delta^{15}\text{N}$ proxy for deciphering past oceanographic shifts and is further evidence that diatom-bound $\delta^{15}\text{N}$ is a robust archive of past surface ocean nutrient conditions.

What we have learned and next steps

Through this dissertation we have learned that diatoms are indeed robust archives of past nutrient consumption. The new measurements of diatoms in monospecific and community cultures show that in both settings the isotopic composition of diatom-bound N conforms to what we would expect based on what we observe in the surface ocean (Chapter 1 and Chapter 2). This contrasts with prior monospecific growouts, which were opposite of surface ocean observations. That's not to say that interpreting $\delta^{15}\text{N}_{\text{DB}}$ is simple – there appears to be a significant relationship to Si:N that will require more research (Chapter 2). However, given what we know now, a mass balance suggests the overall impact of variable diatom Si:N on $\delta^{15}\text{N}_{\text{DB}}$ may be fairly minor (Chapter 2). The downcore measurements of $\delta^{15}\text{N}_{\text{DB}}$ on the mid-Holocene West Antarctic Peninsula have solidified the interpretation of variable CDW intrusion during that interval and demonstrated the utility of $\delta^{15}\text{N}_{\text{DB}}$ as a tracer of oceanographic conditions in this setting, which could be expanded spatially and temporally (Chapter 3). We have also learned that even in locations where we might expect $\delta^{15}\text{N}_{\text{bulk}}$ to be unaltered, $\delta^{15}\text{N}_{\text{DB}}$ was better able to inform us about past nutrient conditions (Chapter 3). The primary impacts of this dissertation are that we

resolved the discrepancy between field and culture records of $\delta^{15}\text{N}_{\text{DB}}$, downcore records of $\delta^{15}\text{N}_{\text{DB}}$ will be interpreted with more confidence, and we have taken another step towards a quantitative understanding of past nutrient utilization.

Of course, the work presented here does not solve all the questions about $\delta^{15}\text{N}_{\text{DB}}$ and has brought several more! The most important diatom species in Southern Ocean sediments are yet to be measured in monospecific growouts. Growouts of *Fragilariopsis kerguelensis*, *Thalassiosira antarctica*, and *Eucampia antarctica* would give us specific information about some of the most prominent species in the sedimentary record. However, given that current monospecific growouts can explain surface ocean and, to some degree, sediment observations, this is a less pressing question than previously thought. In that vein, it would be interesting to look at currently measured ϵ_{DB} in surface ocean particles as a function of their diatom assemblage – do the relationships we observed in monospecific growouts of heavily silicified diatoms with relatively low $\delta^{15}\text{N}_{\text{DB}}$ hold true in the surface ocean? One currently understudied area is the potential for alteration of $\delta^{15}\text{N}_{\text{DB}}$ within the sediment. It is well understood that only a small fraction of the biogenic opal exported from the surface ocean is stored long-term in the sediments. Is there an isotopic fractionation associated with this loss of opal in the water column or sediment? Recently, the nitrogen bound in other microfossil shells has been measured, particularly in foraminifera. A downcore comparison of $\delta^{15}\text{N}_{\text{DB}}$ and foraminiferal-bound $\delta^{15}\text{N}$ would be a useful exercise to ground-truth both proxies. Also of interest is a longer West Antarctic Peninsula $\delta^{15}\text{N}_{\text{DB}}$ record at ODP Site 1098 – what does $\delta^{15}\text{N}_{\text{DB}}$ imply about CDW during dramatic changes like the expansion of sea-ice

around 4 ka or in the early Holocene when CDW is thought to dominate? It would be interesting to measure $\delta^{15}\text{N}_{\text{DB}}$ and track CDW at other WAP sites – we would expect a coherent signal since we hypothesize an atmospheric forcing, but this has not been clearly demonstrated. Finally, the Holocene in East Antarctica is poorly studied compared to the WAP, but the coastal region is also influenced by CDW (e.g. Dove et al., 2020). Are oceanographic conditions similar across all coastal Antarctica?

In summary, the results presented in this dissertation validate the utility of diatoms as archives of past nutrient dynamics and enhance our ability to quantify past fluctuations in nitrate utilization. They also demonstrate where further research can solidify how we understand this proxy and through it, fundamental oceanographic processes in the past.

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Preface Figures

Preface Figure 1. Schematic of the relationship between the major nitrogen isotope measurements described in this dissertation.

Nitrate $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{NO}_3}$) is taken up by surface ocean plankton biomass $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{biomass}}$) with a variable (though well-quantified) fractionation of ϵ_U . Some portion of that biomass is stored in diatom frustules as diatom-bound $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{DB}}$), with a potentially variable and not well-constrained fractionation of ϵ_{DB} . While biomass sinks to bulk sedimentary $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$), it is altered, sometimes dramatically. Since the N in $\delta^{15}\text{N}_{\text{DB}}$ is theoretically protected from alteration, sedimentary $\delta^{15}\text{N}_{\text{DB}}$ should reflect surface ocean $\delta^{15}\text{N}_{\text{DB}}$ with no alteration. The goal of any sedimentary nitrogen isotope proxy is to constrain $\delta^{15}\text{N}_{\text{NO}_3}$, so the better the fractionations can be characterized, the more robustly the proxy can be interpreted.

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CHAPTER I: Diatom-bound $\delta^{15}\text{N}$ robustly tracks surface ocean nitrate $\delta^{15}\text{N}$: evidence from Southern Ocean community cultures

By

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Key Points

- Two distinct, natural, diatom-dominated communities from the Southern Ocean produced indistinguishable $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ when grown on the same nitrate pool
- The difference between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ was the same in community growouts and surface ocean particles
- In natural community growouts, $\delta^{15}\text{N}_{\text{DB}}$ values are offset above $\delta^{15}\text{N}_{\text{biomass}}$ values – opposite of prior monospecific diatom growouts

Abstract

Nitrate is a key marine nutrient and knowing how its supply and demand changed in the past could help to constrain the role of biological productivity over past climate shifts. The nitrogen isotopic composition (as $\delta^{15}\text{N}$) of marine sediments is a proxy for surface ocean nitrate supply and demand over time but may be subject to alteration during sinking and sedimentation. The isotopic composition of nitrogen contained in the shells of diatoms ($\delta^{15}\text{N}_{\text{DB}}$) is protected and is therefore a potentially more robust tracer of nitrate in the past. Here we show $\delta^{15}\text{N}_{\text{DB}}$ in Southern Ocean community cultures does not depend on species composition and is statistically indistinguishable from surface ocean observations. We found the ϵ_{DB} ($= \text{biomass } \delta^{15}\text{N} - \delta^{15}\text{N}_{\text{DB}}$) of the community growouts was $-4.8 \pm 0.8\text{‰}$, more than 10‰ different from previous monospecific growouts but statistically indistinguishable from previous Southern Ocean and North Pacific surface ocean observations. Furthermore, we found the two community growouts had distinct community compositions ($p = 0.1$) but indistinguishable ϵ_{DB} , suggesting species composition does not primarily set $\delta^{15}\text{N}_{\text{DB}}$ in the Southern Ocean. Our results demonstrate that in nitrate replete regions, $\delta^{15}\text{N}_{\text{DB}}$ sinking from the surface ocean robustly tracks surface ocean nitrate $\delta^{15}\text{N}$ and therefore nitrate supply and demand. If $\delta^{15}\text{N}_{\text{DB}}$ is not altered during sinking and sedimentation, sedimentary $\delta^{15}\text{N}_{\text{DB}}$ is a robust tool for examining changes to nitrate supply and demand over time.

Introduction

Nitrate is a crucial nutrient that supports and limits marine life, now and through the past (Falkowski, 1997; Moore et al., 2013). The biological pump, whereby carbon and nutrients are exported from the surface to the deep ocean by biological processes, is closely linked to atmospheric CO₂ concentrations and global climate. The Southern Ocean has the world's largest pool of surface nitrate, which, if it were fully consumed, would take up CO₂ equivalent to more than 40 ppm in the atmosphere (Sarmiento & Orr, 1991). It is thought a more efficient biological pump in the glacial Southern Ocean may be partially responsible for the CO₂ change during glacial intervals (Sigman & Boyle, 2000). Changes to the efficiency of the biological pump in the Southern Ocean would change the relative supply and demand of nitrate, which can be assessed in the sediment record using nitrogen isotope records to determine how it varied in the past (Altabet & Francois, 1994; Francois et al., 1997; Robinson et al., 2004, 2005; Horn et al., 2011b; Martínez-García et al., 2014; Studer et al., 2015).

The nitrogen isotopic composition (as $\delta^{15}\text{N}$) of any nitrogen pool is set by the source $\delta^{15}\text{N}$ and any transformations within or between nitrogen pools. Organic material, e.g. from surface ocean plankton, records this signature and exports it to the sediment. Because nitrogen fractionation pathways for different processes are distinct, sedimentary $\delta^{15}\text{N}$ has been used to track processes such as nitrogen fixation (e.g. Straub et al., 2013), denitrification (e.g. Galbraith et al., 2013), and nitrate supply and consumption (e.g. Robinson & Sigman, 2008) occurring in the ocean.

In surface ocean phytoplankton, fractionation of bio-available nitrate occurs when phytoplankton incorporate ^{14}N more rapidly than ^{15}N into their organic material. As

more nitrate is consumed, the $\delta^{15}\text{N}$ of both nitrate and organic matter increases. Therefore, in nutrient replete environments like the Southern Ocean, sedimentary $\delta^{15}\text{N}$ can be a paleoceanographic proxy for relative surface ocean nutrient utilization (Altabet & Francois, 1994; Francois et al., 1997). Existing data support the hypothesis that the Southern Ocean biological pump, perhaps stimulated by iron or stratification changes, helped to lower glacial atmospheric CO_2 (Francois et al., 1997; Robinson et al., 2004, 2005; Robinson & Sigman, 2008; Martínez-García et al., 2014; Studer et al., 2015). Of late, this inference has been based largely on microfossil bound $\delta^{15}\text{N}$ records in an attempt to avoid the isotopic alteration associated with bulk sediment $\delta^{15}\text{N}$ records (Altabet & Francois, 1994; Robinson et al., 2012).

Diatom-bound $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{DB}}$) provides a more robust measurement of past surface ocean $\delta^{15}\text{N}$ than $\delta^{15}\text{N}_{\text{bulk}}$ because the measured organic nitrogen is contained in the diatom's silica shell, which may protect it from alteration (Sigman et al., 1999; Robinson et al., 2004). This proxy has been used to refine estimates of relative nitrate consumption in the glacial Southern Ocean (Robinson et al., 2004, 2005; Horn et al., 2011b; Studer et al., 2015) and other places like the North Pacific (Brunelle et al., 2007; Studer et al., 2013). However, groundtruthing data to date are limited for this proxy.

One potentially significant complicating factor impacting $\delta^{15}\text{N}_{\text{DB}}$ is the observation that laboratory-cultured diatoms further fractionate nitrogen during its incorporation into their silica shell (Horn et al., 2011a). The isotope effect of this fractionation between the total cultured biomass $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{biomass}}$) and $\delta^{15}\text{N}_{\text{DB}}$ is quantified as $\epsilon_{\text{DB}} = \delta^{15}\text{N}_{\text{biomass}} - \delta^{15}\text{N}_{\text{DB}}$ and varies by up to 5‰ between species (Horn

et al., 2011a). The observation that different diatom species have different ϵ_{DB} values could be critical for interpreting downcore records (Horn et al., 2011b). If species-specific differences in ϵ_{DB} are significant in the surface ocean, then the recorded sedimentary $\delta^{15}\text{N}_{\text{DB}}$ values could be impacted by factors such as preferential preservation of more heavily silicified diatoms, faster sinking species, dissolution of silica in the water column and sediment, or a change in community composition at one site over time. In one example, variation in the abundance of the diatom species *Fragilariopsis kerguelensis*, assuming species-specific ϵ_{DB} , changed the interpreted $\delta^{15}\text{N}_{\text{DB}}$ downcore by about 3‰ – equal to the entire recorded glacial-interglacial change (Horn et al., 2011b).

To add to the complexities, the isotopic difference between $\delta^{15}\text{N}_{\text{DB}}$ and bulk or biomass $\delta^{15}\text{N}$ pools diverge between culture data, particles, and sediments. We use ϵ_{DB} to notate this isotopic difference in culture because the isotopic difference between biomass $\delta^{15}\text{N}$ and $\delta^{15}\text{N}_{\text{DB}}$ is the result of diatoms' internal fractionation effects. However, in a water column particle-bound or sedimentary setting we notate the difference between bulk $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$) and their co-measured $\delta^{15}\text{N}_{\text{DB}}$ as $\Delta\delta^{15}\text{N}$ (quantified as $\Delta\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) rather than ϵ_{DB} because other factors like diagenesis and non-diatom nitrogen could impact $\delta^{15}\text{N}_{\text{bulk}}$.

Since the absolute values of measured $\delta^{15}\text{N}_{\text{DB}}$ are the sum of initial $\delta^{15}\text{N}_{\text{NO}_3}$ values, any uptake fractionation, and additional fractionation within the diatoms (ϵ_{DB}), the absolute values of $\delta^{15}\text{N}_{\text{DB}}$ can vary widely. This is especially true of $\delta^{15}\text{N}_{\text{DB}}$ from surface ocean particles, which have variable starting $\delta^{15}\text{N}_{\text{NO}_3}$ through space and time. This makes direct comparison of $\delta^{15}\text{N}_{\text{DB}}$ values a matter of initial conditions, which

does not help to validate the proxy. To that end, the most direct way to compare $\delta^{15}\text{N}_{\text{DB}}$ in different pools is through $\Delta\delta^{15}\text{N}$ and ϵ_{DB} .

In monospecific cultures, ϵ_{DB} varied in degree but was positive (i.e. $\delta^{15}\text{N}_{\text{biomass}} > \delta^{15}\text{N}_{\text{DB}}$) (Horn et al., 2011a); in a diatom-dominated Arctic net tow and surface ocean particles $>10\text{ }\mu\text{m}$ from the Southern Ocean, $\Delta\delta^{15}\text{N}$ was negative (i.e. particle $\delta^{15}\text{N}_{\text{bulk}} < \text{particle } \delta^{15}\text{N}_{\text{DB}}$) (Morales et al., 2014, Robinson et al., 2020 - in revision); in sediment, $\Delta\delta^{15}\text{N}$ varied and was not consistently positive or negative (Robinson et al., 2004, Robinson et al., 2020 - in revision). While the factors contributing to ϵ_{DB} and $\Delta\delta^{15}\text{N}$ are not well-understood, the results from monospecific growouts stand out because they record a positive ϵ_{DB} not observed in any other modern samples. Growth in culture does not replicate surface ocean growth conditions, but the change in sign between monospecific culture results and surface ocean observations suggests either some major unknown factor or a potential artifact in the monospecific growout results. Proxy validation must evaluate whether the $\delta^{15}\text{N}_{\text{DB}}$ signal measured in sedimentary diatoms faithfully records nitrate delivery and utilization patterns in the surface ocean and assess the impact of community composition on $\delta^{15}\text{N}_{\text{DB}}$.

In this paper we focus on evaluating the $\delta^{15}\text{N}_{\text{DB}}$ proxy under environmentally relevant conditions by comparing $\delta^{15}\text{N}_{\text{DB}}$ and ϵ_{DB} in shipboard diatom community growouts to $\delta^{15}\text{N}_{\text{DB}}$ and $\Delta\delta^{15}\text{N}$ measured in surface ocean particles (Robinson et al., 2020 – in revision) and monospecific growout (Horn et al., 2011a) $\delta^{15}\text{N}_{\text{DB}}$ and ϵ_{DB} values. Community composition plays no role in setting ϵ_{DB} or $\delta^{15}\text{N}_{\text{DB}}$ values in our community growouts. ϵ_{DB} is negative, opposite of Horn et al's (2011) monospecific growouts but consistent with surface ocean particles. While we observed differences

in the absolute values of $\delta^{15}\text{N}_{\text{DB}}$ measured in surface ocean particles and community growouts, the ϵ_{DB} and $\Delta\delta^{15}\text{N}$ were very similar. Finally, we assess the implications of our findings with regards to how $\delta^{15}\text{N}_{\text{DB}}$ is recorded in the sediment through time.

Methods

Large growth experiments were conducted at two sites along 170°W at ~66°S and ~61°S during NBP17-02 aboard the Nathaniel B. Palmer in late January and February 2017. In each experiment, the *in-situ* community was stimulated with the addition of nutrients and allowed to draw down those nutrients by about 50%. About 30 L of surface water with the naturally occurring phytoplankton community was split into three replicate 20 L carboys and amended with deep water from the site (~1500 m). In order to kickstart growth without limiting nutrients, ocean water was supplemented with nitrate (130 μM), phosphate, trace metals, and silicic acid (350 μM) to make an “enhanced deep-water medium” based on modified f/2 (Guillard, 1975). The carboys were kept at 4°C in light levels approximately equal to the daytime surface light at time of collection. The carboys were continuously stirred and bubbled with filtered air to maintain acidity balance and ambient CO_2 concentrations. We tracked growth with daily measurements of nitrate concentration and fluorescence during the exponential phase of growth. Additional filtered water samples (20 – 50 mL) were stored frozen for dissolved $\delta^{15}\text{N}_{\text{NO}_3}$ measurements. The carboy experiments were stopped after about 50% of the initial nitrate was consumed.

For diatom assemblage counts, duplicate slides were prepared by resuspending 5 and 10 mL from each carboy in 125 mL of filtered seawater to allow for even

dispersal and adding 3 drops of acid Lugol's solution. This slurry was filtered through a 0.45 μm gridded cellulose ester membrane filter, oven-dried at 35°C, and oil-mounted to a glass microscope slide for light microscopy at x1000 at the University of Otago. Diatom community compositions in replicate carboys were within a few percent of each other and were therefore combined to determine an average community composition at each site. A rank sum analysis of similarities (ANOSIM) test on Bray-Curtis distances (adonis2 in R, version 3.6.) was performed to determine whether the communities at the two sites were significantly different (Oksanen et al., 2019).

Biomass from about 100 mL of the culture was filtered onto a GF/F filter for $\delta^{15}\text{N}_{\text{biomass}}$ measurements. $\delta^{15}\text{N}_{\text{biomass}}$ data were obtained by combusting filters packed into tin cups in an elemental analyzer (Costech 4010 Elemental Analyzer) coupled to a mass spectrometer (Thermo Delta V Advantage IRMS). Ammonium sulfate (IAEA N1 and IAEA N2) and an in-house aminocaproic acid standard were used for calibration and precision based on multiple standards measured within each run was better than 0.2‰.

The remaining carboy volume was filtered through 5 μm pore size polycarbonate filters and immediately frozen. Diatom-bound N samples from the community growouts were prepared for analysis following the method for diatom culture cleaning (Morales et al., 2013). First, frozen filters were thawed and rinsed with 2% SDS and gently sonicated. Organic material was oxidized with potassium permanganate, followed by mild (~20%) and harsh (~70%) perchloric acid treatments. The cleaned samples were dissolved and measured following the persulfate-denitrifier method

(Sigman et al., 2001; Robinson et al., 2004). The persulfate blank was under 1 μM , accounting for less than 2% of the measured nitrogen. Two potassium nitrate reference materials (IAEA N3 and USGS 34) along a broad range of isotope values in addition to an in-house seawater standard were used for normalization. Precision was 0.4‰ based on a pooled standard deviation of replicate $\delta^{15}\text{N}_{\text{DB}}$ measurements.

Nitrate, nitrite, ammonia, phosphate, and silica measurements were performed on a Lachat QuikChem 8500 flow injection nutrient analyzer at the University of Rhode Island's Marine Science Research Facility analytical laboratories (<https://web.uri.edu/marinefacility/analytical-laboratories/>). Frozen, GF/F filtered samples were thawed and diluted 6x or 3x with Milli-Q water for initial and final nutrient concentration measurements respectively, and Milli-Q was measured for a dilution blank.

Results and Discussion

Growout diatom community and $\delta^{15}\text{N}$ characteristics

The community growouts could potentially record different ϵ_{DB} values if they have significantly different diatom communities due to species-specific impacts on $\delta^{15}\text{N}_{\text{DB}}$ and ϵ_{DB} (Horn et al., 2011a). Therefore, it was important to determine species composition in each community growout. *Fragilariopsis cylindrus* was the most common species at both sites, making up $84.4 \pm 2.0\%$ of the counts at 66°S and $31.6 \pm 0.3\%$ at 61°S (Figure 1). Other important *Fragilariopsis* species were *F. kerguelensis*, *F. curta*, and *F. pseudonana*, and when taken together *Fragilariopsis* species made up $93.6 \pm 1.8\%$ of the counts at 66°S and $72 \pm 5\%$ of the counts at 61°S. A variety of

Chaetoceros species made up about 4% of the community at both sites. The remainder was largely *Thalassiosira* spp., *Nitzschia longissima*, *Pseudonitzschia turgidula*, and *P. subcurvata*. Diatom communities from the two sites are significantly different at the 90% confidence level ($R^2 = 0.96$ $p = 0.1$). It is not surprising that the p-value was above the typically accepted value of 0.05 given the very small six sample dataset. The samples group by site in both a non-metric multidimensional scaling (NMDS) plot (Figure S1a) and by a Bray-Curtis distance heatmap (Figure S1b), so we consider the community difference robust.

The community growouts demonstrated progressive nitrate consumption during growth and a typical Rayleigh distillation response in $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{biomass}}$ throughout the experiment (Figure 2). Initial nitrate concentrations averaged 133 ± 3 μM and decreased to between 66.2-44.7 μM over 12-14 days (Table 1). $\delta^{15}\text{N}_{\text{NO}_3}$ values increased from an initial value of 2.1-2.9‰ to the final value between 4.9-5.8‰. We calculated the isotope enrichment effect of net uptake (ϵ_u), the degree to which diatoms in the growout preferentially utilize $^{14}\text{NO}_3$ over $^{15}\text{NO}_3$, using a simple Rayleigh distillation equation formulated for an instantaneous product (Mariotti et al., 1981). The estimated slope indicates the two growouts had ϵ_u values of 3.6 ± 0.6 ‰ at 66°S and 3.2 ± 0.9 ‰ at 61°S. The estimated ϵ_u predicts the relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{biomass}}$ in a closed system, with the $\delta^{15}\text{N}_{\text{biomass}}$ value offset below a given $\delta^{15}\text{N}_{\text{NO}_3}$ value by $\sim\epsilon_u$. The measured $\delta^{15}\text{N}_{\text{biomass}}$ values fit expectations for the accumulated product based on closed system nitrate consumption with an ϵ_u of 3.4‰ (solid line, Figure 2).

The $\delta^{15}\text{N}_{\text{DB}}$ data from the growouts presented here are the first data from a natural community and their measured ϵ_{DB} had a different sign than previous culture results. For each community growout we calculated ϵ_{DB} by subtracting the final $\delta^{15}\text{N}_{\text{DB}}$ from $\delta^{15}\text{N}_{\text{biomass}}$. The overall average ϵ_{DB} was $-4.8 \pm 0.8\text{‰}$ and no statistical difference was observed between the ϵ_{DB} of the two sites ($-4.6 \pm 1.2\text{‰}$ at 66°S , $-4.9 \pm 0.4\text{‰}$ at 61°S). This was a surprising result since previous laboratory culture work indicated $\delta^{15}\text{N}_{\text{DB}}$ was systematically depleted in ^{14}N relative to biomass (positive ϵ_{DB} , $5.3 \pm 4.4\text{‰}$), opposite of our observations. Potential explanations for this are explored in a later section. In addition, previous results suggested that since the diatom community composition was significantly different, the species-specific ϵ_{DB} may have differed between the sites as well.

Community ϵ_{DB} is consistent with surface ocean particle $\Delta\delta^{15}\text{N}$

While they differ from previous culture results, the community growout estimates of ϵ_{DB} are consistent with surface ocean particle data estimates of $\Delta\delta^{15}\text{N}$ from the Antarctic zone of the Southern Ocean ($-3.5 \pm 2.9\text{‰}$, Figure 3) where the waters were collected (Robinson et al., 2020 – in revision). Although the community growouts were performed in closed systems with high nutrient and light conditions while the particles are the combination of cells, including some non-diatoms, that vary through time and space as a result of mixing and advection, the ϵ_{DB} and $\Delta\delta^{15}\text{N}$ are remarkably similar. The overall similarity between community growouts and the surface ocean particles, which developed under more environmentally relevant nutrient conditions,

indicates that the growouts are replicating surface ocean conditions for incorporation of nitrogen into the diatom frustule.

The ϵ_{DB} from the community growouts and $\Delta\delta^{15}N$ of surface ocean particles at these two sites are similar to the $\Delta\delta^{15}N$ from previously published particle data ($-3.5 \pm 3.9\%$), which was collected from the mixed layer in the Bering Sea (Morales et al., 2014). This implies a relatively consistent ϵ_{DB} and $\Delta\delta^{15}N$ in the mixed layer of diatom-rich polar regions, where the sedimentary $\delta^{15}N_{DB}$ proxy is most often used. If $\Delta\delta^{15}N$ during the nutrient-rich growth season is as consistent as the combined particle and community growout data suggest, the interpretation of downcore records of $\delta^{15}N_{DB}$ may be simplified because $\delta^{15}N_{DB}$ would robustly track surface ocean $\delta^{15}N_{NO_3}$ in diatom-dominated surface waters. However, the effects of nutrient recycling and water column and sedimentary alteration are complex, and more research is needed on this topic (Robinson et al., 2020 – in revision).

Explaining the opposite sign of ϵ_{DB} compared to monospecific growouts

Potentially the most surprising result of this study was the opposite sign of ϵ_{DB} between the community growouts and previous monospecific growouts. Because the isotopic signature of the diatom community should reflect the weighted average signature of the individual species within the community in terms of ϵ_{DB} , we expected to measure a positive ϵ_{DB} (i.e. $\delta^{15}N_{DB}$ values $<$ $\delta^{15}N_{biomass}$ values) since several species in the community growouts had previously been measured in monospecific growouts with positive ϵ_{DB} (Horn et al., 2011a). Nevertheless, the community growout results are consistent with measured ϵ_{DB} from a commercially grown monospecific diatom

sample targeted for aquaculture (Morales et al., 2013) and the aforementioned particle-based $\delta^{15}\text{N}_{\text{DB}}$ measurements and estimates of $\Delta\delta^{15}\text{N}$ in the surface ocean. In this section, we explore several potential causes of the different observed ϵ_{DB} values. One broad category of explanations is culture artifacts. While growth conditions were mostly the same, the prior monospecific growouts had a few differences from the shipboard cultures which could account for some of this discrepancy.

Differences in nutrient conditions should not be significant

The nitrogen pool measured in $\delta^{15}\text{N}_{\text{DB}}$ may consist of structural or scaffolding proteins which are encased when the frustule is being built (Kroger et al., 2000; Sumper et al., 2005). One could imagine a mechanism whereby low silicic acid availability changed the proteins used and therefore the $\delta^{15}\text{N}_{\text{DB}}$. To assess the role of silicic acid limitation on setting ϵ_{DB} , we examined differences in silicic acid between the community growouts, monospecific growouts, and surface ocean conditions and how they impacted ϵ_{DB} and $\Delta\delta^{15}\text{N}$. The final silicic acid concentrations were much lower in the monospecific cultures compared to the community growouts. The end point of the monospecific growouts averaged 25.3 μM silicic acid, with half of them below 10 μM , while all community growouts had final silicic acid concentrations above 187 μM (Table 1). Although silica limitation was not specifically measured in any of the growouts, past studies of silicic acid limitation using ^{32}Si in the high-silicic acid Southern Ocean demonstrated silicic acid limitation in nearly all sites with silicic acid concentrations below 10 μM , at several sites with concentrations between 10 – 40 μM , and only very rarely above 40 μM (Nelson et al., 2001). This indicates that silicic

acid limitation was possible during the monospecific growouts. However, while silicic acid limitation could conceivably alter the incorporation of N into the frustule, only about half of the monospecific cultures ended with silicic acid lower than 10 μM and there is no apparent link between silicic acid concentration and ϵ_{DB} either within or between species (Horn et al., 2011a). Another key finding in this work is ϵ_{DB} was relatively consistent throughout the growth of the monospecific cultures – even when silicic acid concentrations were much higher during early growth. This is evidence that silicic acid limitation does not play a consistent role in setting ϵ_{DB} in culture. In addition, silicic acid concentrations north of 62°S were about 1 μM during NBP17-02, well within the range of silicic acid limitation, and those particles in the mixed layer had a negative $\Delta\delta^{15}\text{N}$ which was indistinguishable from ϵ_{DB} of the silicic acid replete community growouts (Figure 3, Robinson et al., 2020 – in revision). Therefore, it is not likely silica limitation plays a role in setting ϵ_{DB} .

Diatoms in the Southern Ocean are thought to be iron limited (e.g. Martin, 1991; Moore et al., 2013), and appear to have physiological adaptations (Muggli et al., 1996). The monospecific growouts used f/2 base medium, with starting concentrations of trace metals and vitamins somewhat iron enriched relative to the shipboard “enhanced deep-water medium”. Based on iron content in f/2 medium and Redfield uptake ratios, trace metals and vitamins were not likely to be limiting in either experimental design. For typical f/2 medium, the final concentration of dissolved Fe is 11.7 μM , four orders of magnitude above typical surface ocean values around 0.001 μM , and we added ~80 μmol Fe to our ~20 L community growouts for a final concentration of ~4 μM (Guillard, 1975; Johnson et al., 1997). In an extended

Redfield ratio, the proportion of nitrogen to iron uptake is 16:0.00047 (Parekh et al., 2005). For a nitrate uptake that averaged 1200 μmols the iron requirements for the growouts should be about 0.04 μmols . Therefore, our community growouts likely had 2000x more iron than what should be required based on stoichiometry. It is likely the same principles hold true for the rest of the trace metals and vitamins added because we reduced the initial nitrate by 85% compared to f/2 but initial trace metals and vitamins by only 66%. Because the monospecific growouts had even higher concentrations of trace metals and vitamins than community growouts, we do not think trace metal or vitamin limitation likely drove the observed differences in ϵ_{DB} . In addition, surface ocean iron is limiting in much of the Southern Ocean (Moore et al., 2013), yet the surface ocean particle $\Delta\delta^{15}\text{N}$ was negative (Figure 3, Robinson et al., 2020 – in revision). Given the evidence for a negative $\Delta\delta^{15}\text{N}$ in natural particles and ϵ_{DB} in the community growouts, despite differences in iron availability, we assert iron availability does not play a significant role in setting ϵ_{DB} and does not account for the difference between monospecific and community growouts.

Non-diatom impact should be minor

A main difference between the two types of culture experiments is that shipboard growouts contained an entire upper water column community rather than a single species grown in nominally axenic medium. Because the community growouts took unfiltered water from a water depth of 10 m, they contained not only diatoms but also microbes, zooplankton, and other phytoplankton, potentially impacting the relationship between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ in the community growouts compared to

monospecific growouts. Through visual analysis we found most of the biomass was diatoms, as expected for the Southern Ocean. We estimated that the Si:N of the biomass in our cultures was 0.98 ± 0.12 by averaging the ratio of silicic acid to nitrate removed from the dissolved nutrient pools during growth. This is consistent with the ratio of a phytoplankton community dominated by diatom of 1.12 ± 0.33 (Brzezinski, 1985), suggesting that non-diatom organisms likely represent a small proportion of the total biomass.

The introduction of other microbes in the community cultures compared to the axenic monospecific growouts allows for potential recycling and production of ammonium, which, in an open ocean setting, can alter $\delta^{15}\text{N}_{\text{biomass}}$. Average ammonium measurements were indistinguishable between initial ($2.0 \pm 0.6 \mu\text{M}$) and final timepoints in community cultures ($1.5 \pm 0.4 \mu\text{M}$), suggesting microbial recycling did not build up an isotopically distinct pool of N. However, it is possible that nitrification was occurring and lowered ϵ_u and/or $\delta^{15}\text{N}_{\text{biomass}}$. The growouts were performed in a closed system, so any production of ammonium by these other microbes should have been immediately re-integrated into new biomass, and we would therefore not expect to observe significant isotopic impacts simply from a mass-balance standpoint.

Microbial recycling and non-diatoms do not appear to have significantly altered the isotopic relationship between nitrate and diatom biomass. Community growout ϵ_u ($3.4 \pm 0.6\text{‰}$) is consistent with the ϵ_u demonstrated in a monospecific growout of *F. cylindrus* ($3.9 \pm 1.3\text{‰}$), the most prevalent diatom species in the community (Horn et al., 2011a). This is evidence that non-diatoms did not significantly alter ϵ_u in the community growouts. Recent field-based estimates of Southern Ocean community ϵ_u

in the Antarctic Zone are $5.5 \pm 0.6\text{‰}$ (Fripiat et al., 2019), about 2‰ higher than the community growouts. While this is a potentially interesting discrepancy, it is most likely due to difference between open ocean and culture conditions and cannot explain the ~10‰ difference between monospecific and community culture ϵ_{DB} .

Luxury nitrate uptake cannot explain opposite ϵ_{DB}

One observation from prior monospecific growouts is ϵ_u and ϵ_{DB} were significantly correlated (Horn et al., 2011a). A proposed explanation for this relationship was the possibility of luxury nitrate uptake. This luxury uptake could have occurred in either the monospecific growouts, where nitrate concentrations averaged $212 \pm 38 \mu\text{M}$, or community growouts, which averaged $133 \pm 3 \mu\text{M}$. The expressed ϵ_u is driven by a strong fractionation associated with nitrate reductase enzyme followed by the efflux of heavy $^{15}\text{NO}_3$ from the diatom cell (Needoba et al., 2004). If the diatoms in the monospecific growouts had differing capacities to store excess nitrate in their cells, this could impact the relative proportion of heavy $^{15}\text{NO}_3$ lost from the diatom cell and might have varied the ϵ_u . This process may be demonstrated by *F. kerguelensis* from the monospecific growouts, which had the lowest ϵ_u of any of the monospecific growouts (Horn et al., 2011a). Because ϵ_{DB} is calculated based on $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{DB}$, a change to $\delta^{15}\text{N}_{\text{biomass}}$ through ϵ_u could alter ϵ_{DB} . If luxury uptake played a role in the community growouts, we would expect a community growout with significant numbers of *F. kerguelensis* to have a relatively low ϵ_u and ϵ_{DB} as demonstrated in monospecific cultures. However, *F. kerguelensis* comprises 14% of the growout counts at 61°S and under 1% at 66°S (Figure 1) and neither ϵ_u or

ϵ_{DB} from 61°S are significantly lower than those from 66°S as we would expect (Figure 2, Table 1). Although *F. kerguelensis* might engage in luxury uptake in some circumstances, it was likely not during the community growouts. While luxury uptake could theoretically have impacted some of the monospecific growouts and led to the difference in ϵ_{DB} , the average ϵ_u from monospecific growouts was 5‰, close to the measured global average, arguing against it. In any case, it is unclear how this luxury uptake would have led to a low $\delta^{15}N_{DB}$ and a positive ϵ_{DB} in the monospecific growouts since not all diatom species are thought to engage in luxury uptake of nitrate, but every diatom species measured in the monospecific growouts had positive ϵ_{DB} .

Sample size and blanks may have affected monospecific data

Another potential cause of the discrepancy is the very small sample sizes and large blank in the previous monospecific growouts (Horn et al., 2011a). The sample sizes were small enough that the blank, which was generally 2-3 μ M, accounted for up to 40% of the total measured N for $\delta^{15}N_{DB}$. If the $\delta^{15}N$ value of the blank, associated with the alkaline persulfate reagent, was extremely low, this could have pulled down the measured $\delta^{15}N_{DB}$ value below the corresponding $\delta^{15}N_{biomass}$ value. This is unlikely because the relative proportion of the blank would have varied between each sample, which would have led to a variable ϵ_{DB} . However, ϵ_{DB} was relatively constant not only between each sample, but also between different diatom species (Horn et al., 2011a). A blank that had a constant $\delta^{15}N$ could not have accounted for the observed consistent positive ϵ_{DB} . The very low blank $\delta^{15}N$ values required by a mass balance, $< -10\text{‰}$, are inconsistent with a conservative estimate of the blank $\delta^{15}N$ of 0-5‰ (Horn et al.,

2011a). More importantly, the blank associated with the community growout $\delta^{15}\text{N}_{\text{DB}}$ measurements presented here was consistently under 1 μM , and due to the much larger samples sizes accounted for at most 2% of total measured N, meaning the blank has been eliminated as a possible issue for the community growouts.

None of these explanations for the discrepancy are entirely satisfying, but the weight of evidence from the community growouts presented here, in other fresh diatom material (Morales et al., 2013, Chapter 2), and marine particle data (Morales et al., 2014, Robinson et al., 2020 - in revision) suggests there is an issue with the previous monospecific growout data and the fractionation associated with frustule formation results in the inclusion of nitrogen that is isotopically heavier than the bulk biomass. Future monospecific diatom growouts may prove to be the key to understanding the discrepancy between the positive ϵ_{DB} of prior monospecific growouts and the negative ϵ_{DB} of community growouts and marine particulates.

Implications for sedimentary $\delta^{15}\text{N}_{\text{DB}}$

The community growouts demonstrate that although the species compositions were distinct, the $\delta^{15}\text{N}$ from each of the measured pools and the corresponding fractionation effects were indistinguishable between the two community growouts. This is in contrast to conclusions from prior monospecific growouts (Horn et al., 2011a) and sediments (e.g. Des Combes et al., 2008; Studer et al., 2015) which suggested diatom species or class (e.g. pennate or centric) influenced the nitrogen isotopic relationship between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$. While our interpretation is only based on two diatom communities in culture, it appears $\delta^{15}\text{N}_{\text{DB}}$ and ϵ_{DB} are not

primarily driven by species composition. This implies differences seen between, for example, centric and pennate diatom sedimentary $\delta^{15}\text{N}_{\text{DB}}$ (Studer et al., 2015) may be a result of different growth conditions (e.g. seasonality) for a given diatom species or class than intrinsic differences in ϵ_{DB} . For the observation that centric diatoms had higher $\delta^{15}\text{N}_{\text{DB}}$ than pennate diatoms in the Antarctic Zone over the last two glacial cycles, consider a scenario where the bulk of centric diatom growth occurred late in the season, when $\delta^{15}\text{N}_{\text{NO}_3}$ would be expected to be higher: they would have higher $\delta^{15}\text{N}_{\text{DB}}$ because of changes in growth conditions, not because species themselves had fundamentally different ϵ_{DB} . While this could end up biasing the data if the only diatoms preserved were large and heavily silicified late-season diatoms with high $\delta^{15}\text{N}_{\text{DB}}$, there is potentially more environmental information to be gained about changes to growth conditions and seasonal nitrate supply and demand over time. In that case, sedimentary diatom assemblage information would be highly useful complementary data but not required to interpret $\delta^{15}\text{N}_{\text{DB}}$ downcore. While the available data are not able to fully assess this question, future monospecific diatom growouts may prove to be the key to understanding the importance of species in setting $\delta^{15}\text{N}_{\text{DB}}$.

Our interpretation of the apparently robust relationship between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ regardless of diatom community is surface ocean $\delta^{15}\text{N}_{\text{DB}}$ faithfully records surface ocean $\delta^{15}\text{N}_{\text{biomass}}$ and therefore nitrate utilization changes in systems dominated by nutrient uptake and diatom growth. Our data cannot assess whether this is the case in systems where nutrient recycling dominates, or in places where denitrification or nitrogen fixation play a significant role. Because the bulk of

Southern Ocean particle flux occurs during the heart of the growing season when nitrate is in high supply (Honjo et al., 2000), the $\delta^{15}\text{N}_{\text{DB}}$ proxy is likely robust in sinking material in the Southern Ocean. If $\delta^{15}\text{N}_{\text{DB}}$ is protected from alteration during sinking and sedimentation as originally posited (Altabet & Francois, 1994; Farrell et al., 1995; Sigman et al., 1999), then it follows that the sedimentary $\delta^{15}\text{N}_{\text{DB}}$ signal is robust at least when surface conditions are nutrient-replete. However, further research would be needed to determine if this holds where recycling-impacted particles are an important part of the annual sinking flux, when diatoms do not dominate the surface ocean community, or under nitrate limited conditions.

Conclusions

Growouts of distinct diatom communities from two sites in the Southern Ocean produced indistinguishable $\delta^{15}\text{N}_{\text{DB}}$ in growouts that reflected the $\Delta\delta^{15}\text{N}$ of surface ocean particles and was opposite in sign to prior monospecific growouts. Because ϵ_{DB} was unchanged between community growouts and surface ocean $\Delta\delta^{15}\text{N}$ data, we conclude the community growouts approximately replicated surface ocean conditions and there is a consistent relationship between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ in nutrient-replete systems. Based on an assessment of different growth conditions, we conclude the opposite sign from previously published monospecific growouts may be the result of an artifact in the monospecific growouts (Horn et al., 2011a). Distinct diatom communities in the two community growouts did not result in significantly different ϵ_{DB} , which implies species composition is not primarily responsible for ϵ_{DB} . Taken together, we conclude the $\delta^{15}\text{N}_{\text{DB}}$ sinking from the surface ocean robustly tracks

$\delta^{15}\text{N}_{\text{biomass}}$ and therefore surface ocean nitrate supply and consumption in systems where the nitrogen cycle is dominated by uptake. Furthermore, if $\delta^{15}\text{N}_{\text{DB}}$ is not altered during sinking and sedimentation, the implication is sedimentary $\delta^{15}\text{N}_{\text{DB}}$ robustly tracks Southern Ocean surface $\delta^{15}\text{N}_{\text{NO}_3}$ and is a useful tool for examining changes to the biological pump over time.

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Figures and Tables

Figure I-1. Diatom species abundance by count from community growouts at 66°S and 61°S.

Latitude	Carboy	Initial Values			Final Values							
		[NO ₃] (μM)	δ ¹⁵ NO ₃ (‰)	[Si] (μM)	[NO ₃] (μM)	δ ¹⁵ NO ₃ (‰)	[Si] (μM)	Si:N Uptake	δ ¹⁵ N _{biomass} (‰)	δ ¹⁵ N _{DB} (‰)	ε _u (‰)	ε _{DB} (‰)
66°S	1	134.1	2.1	342	58.4	5.7	266.1	1	-0.3	5.7	4.2	-6
66°S	2	135.5	2.5	342	66.2	4.9	284	0.8	0.5	4.5	3.4	-4
66°S	3	127.7	2.7	324.9	44.7	5.8	251.4	0.9	0.6	4.4	3.1	-3.8
61°S	4	136.7	2.7	291.6	48.4	4.9	187.7	1.2	-0.4	4.8	2.2	-5.2
61°S	5	129.8	2.5	310.3	61.7	5.1	236.5	1.1	0	4.5	3.5	-4.5
61°S	6	135.7	2.9	301.3	60.7	5.6	233	0.9	-0.8	4.4	3.9	-5.1

Table I-1. Initial and final nutrient concentrations and δ¹⁵N_{NO3}, and final Si:N uptake, δ¹⁵N_{DB}, δ¹⁵N_{biomass}, ε_u, and ε_{DB} for each community growout.

Figure I-2. $\delta^{15}\text{N}$ vs nitrate consumption for two community growouts.

Oranges are from 66°S and blues are from 61°S. Circles are $\delta^{15}\text{N}_{\text{NO}_3}$, triangles are $\delta^{15}\text{N}_{\text{biomass}}$, and diamonds are $\delta^{15}\text{N}_{\text{DB}}$. Theoretical $\delta^{15}\text{N}$ for NO_3^- and biomass pools were calculated assuming Rayleigh systematics using the calculated ϵ_u of 3.4 (Mariotti et al., 1981).

Figure I-3. Vertical profiles from 66°S and 61°S of $\delta^{15}\text{N}_{\text{bulk}}$ from particle pumps, $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ from sediment, and $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ from natural community growouts.

Supplemental Figures

Supplemental Figure I-1. Growout community statistics.

A. Multidimensional scaling (NMDS) plot of assemblages from the two community growouts. B. Bray-Curtis Distance heatmap for the six individual growouts. Darker maroon indicates closer Bray-Curtis distance/similarity, lighter yellow indicates dissimilarity. In both visualizations the individual incubations group by Site.

CHAPTER II: Potential impact of varying silicification on diatom-bound nitrogen isotope ratios: evidence from monospecific cultures

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Key Findings

- There is a significant positive linear relationship between Si:N uptake and $\delta^{15}\text{N}_{\text{DB}}$
- $\delta^{15}\text{N}_{\text{DB}}$ is on average offset above $\delta^{15}\text{N}_{\text{biomass}}$ by $2.0 \pm 0.7\text{‰}$ in monospecific cultures of six Southern Ocean diatom species
- The offset between $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{biomass}}$ in monospecific cultures is close to the range found in previously published Southern Ocean community cultures ($\sim 4.8\text{‰}$) and North Pacific and Southern Ocean particles ($\sim 3.5\text{‰}$)

Abstract

Nitrate is an important nutrient in marine systems. Tracking the supply and demand of nitrate in the past ocean can help to constrain the role of biology and circulation in regulating climate. The nitrogen isotopic composition ($\delta^{15}\text{N}_{\text{biomass}}$) of marine phytoplankton follows nitrate dynamics but may not be well preserved in the sediments. The isotopic composition of nitrogen contained within the frustules of diatoms ($\delta^{15}\text{N}_{\text{DB}}$) is protected from alteration and is therefore a potentially more robust tracer of nitrate in the past. Here we document the relationship between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ from several Southern Ocean monospecific diatom growouts. $\delta^{15}\text{N}_{\text{biomass}}$ values are on average $2.0 \pm 0.7\text{‰}$ lower than $\delta^{15}\text{N}_{\text{DB}}$ values. Our results are consistent with ϵ_{DB} values ($\delta^{15}\text{N}_{\text{biomass}} - \delta^{15}\text{N}_{\text{DB}}$) from Southern Ocean phytoplankton community growouts ($-4.8 \pm 0.8\text{‰}$) and previous Southern Ocean and North Pacific surface ocean observations (-3.5‰), but are more than 7‰ different from, and the opposite sign as, previous monospecific diatom cultures ($5.3 \pm 4.4\text{‰}$). In this paper we explore possible explanations for and implications of these results. Across species there is a significant positive linear relationship between ϵ_{DB} and the Si:N of diatom biomass and a significant negative relationship between ϵ_{DB} and the Si:N within cleaned frustules, suggesting a role for N allocation relative to Si in setting $\delta^{15}\text{N}_{\text{DB}}$. ϵ_{DB} is highest in species that are relatively rich in N relative to Si, but that produce low N frustules. Such species may decrease the value of the sedimentary $\delta^{15}\text{N}_{\text{DB}}$ signal, although this result is largely based on one species. However, most of the diatom species had ϵ_{DB} values indistinguishable from one another, which suggests that sedimentary $\delta^{15}\text{N}_{\text{DB}}$

values largely reflect surface ocean nitrate $\delta^{15}\text{N}$ and therefore nitrate supply and demand.

Introduction

Reconstructing past nitrogen dynamics – including supply through upwelling or advection, biological consumption, and transformations between nitrogen pools – is critical to understanding the role of ocean biology in regulating atmospheric CO₂ and global climate. The Southern Ocean is the world's largest high-nutrient, low-chlorophyll region, where phytoplankton growth appears to be limited by light and iron (Martin, 1991; Moore et al., 2013). Large scale upwelling brings nutrient and C rich deep water to the surface where biological productivity and export removes a fraction from the atmosphere to the deep ocean. Through this process, large-scale productivity changes in the Southern Ocean, regulated by both vertical supply of nutrients and the stimulation of productivity by iron delivered by wind-blown dust, likely impacted atmospheric CO₂ on glacial-interglacial timescales (Francois et al., 1997; Kohfeld et al., 2005; Blain et al., 2007; Anderson et al., 2009; Sigman et al., 2010; Martínez-García et al., 2014). In parts of the Southern Ocean, macronutrient delivery changes via fluctuations in upwelling or stratification also play a role in determining biological productivity (Francois et al., 1997; Shevenell et al., 2011; Etourneau et al., 2013; Peck et al., 2015). Because nutrient delivery and utilization are integral to the biological pump, a robust proxy for nutrient supply and demand is critical for understanding the Southern Ocean in the past.

The nitrogen (N) isotopic composition (as $\delta^{15}\text{N}$) of any pool is set by the source $\delta^{15}\text{N}$ and any transformations within or between pools. Because the nitrogen fractionation pathways are distinct, sedimentary nitrogen isotopes have been used to track processes such as nitrogen fixation (e.g. Straub et al., 2013), denitrification (e.g.

Galbraith et al., 2013), and nitrate supply and consumption (e.g. Altabet & Francois, 1994). In macronutrient-rich environments like the Southern Ocean, sedimentary $\delta^{15}\text{N}$ values are a paleoceanographic proxy for relative surface ocean nitrate utilization since nitrate is the most abundance form of bioavailable nitrogen. Existing sedimentary $\delta^{15}\text{N}$ data suggest that during glacial periods circulation changes and a more efficient biological pump, perhaps stimulated by iron or stratification changes, helped to lower atmospheric CO_2 (Altabet & Francois, 1994; Francois et al., 1997; Robinson et al., 2004, 2005; Robinson & Sigman, 2008; Martínez-García et al., 2014; Studer et al., 2015). Fractionation of nitrate occurs when phytoplankton incorporate ^{14}N more rapidly than ^{15}N into their organic material, quantified as the isotope effect of utilization (ϵ_u) (Mariotti et al., 1981). As proportionally more nitrate is consumed, the $\delta^{15}\text{N}$ of both nitrate and organic matter increases. In the underlying sediment, the relative change in $\delta^{15}\text{N}$ values reflects changes in degree of nitrate delivery and consumption through time. However, bulk sedimentary $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}_{\text{bulk}}$) are subject to a wide range of potentially biasing factors including preferential sinking of some surface ocean species, diagenetic effects, and nitrogen from other sources such as terrigenous materials and nitrogen from higher trophic levels (Kienast et al., 2005; Brunelle et al., 2007; Beucher et al., 2008; Horn et al., 2011b; Studer et al., 2013). Relatively small changes in $\delta^{15}\text{N}$ can lead to vastly different interpretations of nutrient dynamics and it is therefore necessary to reduce uncertainty.

Nitrogen isotopes within the silica frustules of diatoms ($\delta^{15}\text{N}_{\text{DB}}$) provide a more robust measurement of past surface ocean nitrogen isotopic compositions than $\delta^{15}\text{N}_{\text{bulk}}$ because the organic nitrogen is contained in the diatom's silica shell, which protects it

from alteration (Sigman et al., 1999; Robinson et al., 2004). The $\delta^{15}\text{N}_{\text{DB}}$ proxy helps to reduce biases associated with preferential sinking, diagenetic effects, and variable terrigenous sources, but may add another problem: species-specific impacts on $\delta^{15}\text{N}$ during incorporation of organic material into the silica frustule (Horn et al., 2011a). Isotopic offsets between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ observed in culture indicate that diatoms further fractionate nitrogen during its incorporation into their cell walls (quantified as $\epsilon_{\text{DB}} = \delta^{15}\text{N}_{\text{biomass}} - \delta^{15}\text{N}_{\text{DB}}$) and that this incorporation factor is not consistent between species (Horn et al., 2011a). In addition, ϵ_{DB} varies among cultured species, net tow samples, and in the sediment, as estimated by comparing $\delta^{15}\text{N}_{\text{bulk/biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$. In monospecific cultures, ϵ_{DB} varied in absolute value but was consistently positive (i.e. $\delta^{15}\text{N}_{\text{biomass}} > \delta^{15}\text{N}_{\text{DB}}$) (Horn et al., 2011a). In contrast, in a sub-Arctic net tow and Southern Ocean particles, the relationship, presented in a related, non-culture notation $\Delta\delta^{15}\text{N}$ ($= \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) was negative (i.e. total particulate $\delta^{15}\text{N} < \delta^{15}\text{N}_{\text{DB}}$) (Morales et al., 2014, Robinson et al., 2020 - in revision) and in sediment, $\Delta\delta^{15}\text{N}$ is negative or close to zero (i.e. $\delta^{15}\text{N}_{\text{bulk}} \leq \delta^{15}\text{N}_{\text{DB}}$) (Horn, 2011; Robinson et al., 2019). Sound application of the proxy for paleoceanographic reconstructions requires resolving the discrepancy between culture and field results.

The driving question for the validation of the $\delta^{15}\text{N}_{\text{DB}}$ proxy is whether the $\delta^{15}\text{N}_{\text{DB}}$ signal measured in sedimentary diatoms faithfully records nitrate delivery and utilization patterns in the surface ocean. Any improvement to the proxy would lead to better estimates of the role of the biological pump in drawing down CO_2 across glacial cycles. Several diatoms that are important in the sedimentary record have already been assessed for their species-specific contribution to $\delta^{15}\text{N}_{\text{DB}}$ (Horn et al., 2011a), but

uncertainty remains as to the cause of the significant offset between water column particles and culture studies. In this paper, we aim to assess whether the previously reported positive ϵ_{DB} in monospecific batch cultures is robust and to determine what factors, if any, contribute to ϵ_{DB} . If ϵ_{DB} varies between individual diatom species, we want to know the relative importance of any individual species or diatom characteristic to predict the ϵ_{DB} of a community from its assemblage. If ϵ_{DB} of a community can be predicted from its assemblage and the $\delta^{15}N_{DB}$ is not significantly altered during sinking and sedimentation, then sedimentary $\delta^{15}N_{DB}$ with a correction for assemblage is a potentially robust proxy for past nutrient supply and demand. In this paper we present data from new nutrient replete monospecific cultures of six diatom species with measurements of cellular and frustule silica and nitrogen content, $\delta^{15}N_{NO_3}$, ϵ_u , $\delta^{15}N_{biomass}$, $\delta^{15}N_{DB}$, and ϵ_{DB} . We find that the $\delta^{15}N_{DB}$ was greater than $\delta^{15}N_{biomass}$ in five out of six species measured (average $\epsilon_{DB} = -2.0 \pm 0.7\text{‰}$) and that ϵ_{DB} varies with both whole cell and frustule Si:N.

Methods

Diatoms Studied

We chose diatom species that covered a range of diatom families from the Southern Ocean, including species that were previously measured (Horn et al., 2011a) and important species from prior community cultures (Chapter 1). Each of the diatom strains used were isolated on relatively recent Southern Ocean cruises by our group or provided by T. Rynearson and B. Jenkins (Table 1). In each case, single cells were

isolated at sea and maintained in 1.5 mL batch cultures in sterile filtered F/2 medium at 2°C at the Marine Science Research Facility at the University of Rhode Island.

Culture Conditions

The growth conditions chosen were based on those used in prior community (Chapter 1) and monospecific growouts (Horn et al., 2011a). We grew individual diatom species in 20 L of modified F/2 in order to have sufficient material for $\delta^{15}\text{N}_{\text{DB}}$ measurements. To set up the cultures, about 20 L of Narragansett Bay water was sterile filtered (0.2 μm) into each of three 20 L carboys. The carboys sat overnight at 4°C to ensure water temperature had stabilized. Initial nutrients were added to make a modified F/2 medium. Phosphate, trace metals, and vitamins were at F/2 concentrations in the carboys, while nitrate was decreased, and silicic acid increased. We aimed for initial nitrate of about 120-150 μM so diatoms could draw down >50% of the nitrate without suffering from self-shading or crowding. Average diatom Si:N uptake is about 1:1 (Brzezinski, 1985) and we did not want silicic acid limitation to occur, so we aimed for silica concentrations of about 2x that of nitrate, about 250 – 300 μM . Diatom cultures were initiated in well plates and grown in subsequently higher volumes, up to 200 mL, before being added to 20 L carboys for the final phase of the growth experiments. To start off the growout, 200 mL of rapidly growing diatom inoculant was added to each carboy. Because the 200 mL of diatoms were grown in F/2 medium before inoculation and the media was added to the 20 L carboys as well, initial nutrient conditions in the 20 L carboys varied.

After inoculation, diatom cultures were stirred with a magnetic stir rod at 180-200 rpm. Two 1/8" ID PVC tubes were inserted through holes drilled in the carboy caps to control airflow and venting. One tube was attached to the stir rod about 2/3rds down from the cap and was constantly bubbling filtered air from an aquarium pump. The other tube was inserted just inside the cap and served as a vent. For sampling, after vigorous shaking, a pump was attached to the vent tube and sample was collected out of the bubbling tube. Because of the constant positive pressure and sampling technique, potential contamination was minimized. Samples were measured for fluorescence to track growth every few days until exponential growth phase was reached, then approximately daily. Because the initial lag phase length, growth rates, and inoculant diatom density varied greatly, cultures grew for between 5 and 48 days before harvesting.

After initial nitrate in the cultures was drawn down more than 60%, cultures were harvested for subsequent measurements. This level of drawdown ensured sufficient diatom material for several replicate $\delta^{15}\text{N}_{\text{DB}}$ measurements. 100 mL from each culture was filtered through a precombusted GF/F filter. The filtrate was immediately frozen for final nutrient concentration and nitrate isotope measurements. The filter was immediately frozen for diatom biomass isotope measurements. The remainder of the diatom material was filtered onto several 142 mm diameter, 1.2 μm pore size polycarbonate filters and immediately frozen for later analysis.

Nutrient Measurements

Phosphate, silicic acid, nitrate, and ammonium were measured for both the initial and final timepoints of the culture experiments. In each case, GF/F filtered water was frozen after collection until analysis. Phosphate and ammonium measurements were performed on a Lachat QuikChem 8500 flow injection nutrient analyzer at the University of Rhode Island's Marine Science Research Facility analytical laboratories. Nitrate was measured by chemiluminescent NO detection after conversion with a heated vanadium reagent (Braman & Hendrix, 1989). Silicic acid was measured following the molybdate blue colorimetric method after allowing samples to thaw for at least 24 hours (Mortlock & Froelich, 1989).

Diatom-bound Cleaning and Measurements

Samples for $\delta^{15}\text{N}_{\text{DB}}$ from the community growouts were prepared for analysis following the method for diatom culture cleaning (Morales et al., 2013), though most samples required repeated cleaning step, likely because of their relatively large volumes. First, frozen filters were thawed and rinsed several times with 2% SDS and gently sonicated. Some diatom material clumped and was gently broken apart with a glass stir rod. Organic material was oxidized with potassium permanganate in 10 mL of 9 M sulfuric acid, then reacted with oxalic acid (Hasle & Fryxell, 1970). This step was followed by mild (~20%, 20 minutes) and harsh (~70%, 60 minutes) perchloric acid treatments at 100°C. Because of large sample sizes additional cleaning was often required. In these cases, the potassium permanganate step was repeated in order to minimize potential frustule dissolution in the hot perchloric acid step. Finally, about 10-15 mg of the diatom samples, measured to within 0.1 mg precision, was dissolved

and nitrogen was converted to nitrate with 0.22 M potassium persulfate and 1.5 M sodium hydroxide in a pressure cooker for 30 minutes (Robinson et al., 2004). Samples were subsequently acidified to about pH 4 with 6 N HCl. Nitrogen content of the samples was measured using the chemiluminescent method described above, and $\mu\text{mol N:g Si}$ was calculated. Samples were considered clean when additional cleaning did not alter $\delta^{15}\text{N}_{\text{DB}}$ of diatom material. The persulfate and acidification blank was under 0.5 μM , accounting for less than 5% of the measured nitrogen.

The resulting solution was measured using the denitrifier method (Sigman et al., 2001). 20 nmols of N from each sample was added to *Pseudomonas chlororaphis*, a denitrifying bacterium, in a gas-tight vial to be measured as N_2O . The gas in the gas-tight vial was sampled into a custom gas chromatograph (GC) front end coupled to a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) for quantitative measurement. Two potassium nitrate reference materials (IAEA N3 and USGS 34) in addition to an in-house seawater standard were used for calibration and assessment. Internal precision was 0.4‰ based on a pooled standard deviation of replicate $\delta^{15}\text{N}_{\text{DB}}$ measurements.

Nitrogen Isotopes in Biomass and Nitrate

Nitrate isotopes were also measured by the denitrifier method (Sigman et al., 2001) and standardized identically. Filters for biomass measurements were dried overnight at 60°C, then cut with ethanol-cleaned stainless-steel scissors before being folded into a 9x10 mm tin. Filters were measured in a Costech 4010 Elemental Analyzer coupled to the IRMS. IAEA N1 and IAEA N2 ammonium sulfate standards

and an in-house aminocaproic acid standard were used for calibration. Precision based on a pooled standard deviation of replicate $\delta^{15}\text{N}_{\text{biomass}}$ measurements was 0.7‰.

Results and Discussion

Nutrient contents and uptake parameters

Initial nitrate varied between 102 and 160 μM , with an average of $139 \pm 15 \mu\text{M}$ (Table 1). Initial $\delta^{15}\text{N}_{\text{NO}_3}$ varied between -0.6 and 3.0‰, with an average of $0.9 \pm 0.8\text{‰}$ (Table 1). The variability in nitrate and $\delta^{15}\text{N}_{\text{NO}_3}$ was expected given that cultures were inoculated with 200 mL of fast-growing diatoms in F/2 medium with potentially different nitrate and $\delta^{15}\text{N}_{\text{NO}_3}$ and the stock nitrate was remade several times, but this should not impact our comparisons. Final nitrate varied between 0.1 – 53 μM , and final $\delta^{15}\text{N}_{\text{NO}_3}$ ranged from 3.6 – 8.4‰ (Table 1). In most culture experiments, intermediate samples were measured for nitrate and $\delta^{15}\text{N}_{\text{NO}_3}$.

Final silicic acid concentrations were generally above 100 μM , and the species with the lowest final silicic acid, *F. rhombica*, ended with an average of 18 μM (Supplemental Table 1). This suggests that our monospecific cultures were not silicon limited during growth. Cell Si:N ratios were estimated with the difference between initial and final silicic acid and nitrate. Si:N averaged across species was 0.6 ± 0.5 with a range from 0.05 – 1.67, somewhat lower than expectations (Brzezinski, 1985) (Table 1). Other than the modified nitrate and silicic acid concentrations the culture media followed the F/2 formula (Guillard, 1975), so we expect trace metals and vitamins were present in excess. Measured final phosphate was above 5 μM for all species (Supplemental Table 1) and was therefore assumed to not be limiting.

The isotopic enrichment effect associated with nitrate uptake (ϵ_u) was calculated using the Rayleigh distillation equation assuming a closed system (Mariotti et al., 1981). In cases where nitrate was fully drawn down, intermediate samples still allowed for the calculation of ϵ_u except for *P. glacialis*, which did not have any intermediate samples saved. Average ϵ_u in these monospecific growouts was $2.5 \pm 0.5\text{‰}$ (Table 2). This is lower and less variable than the $5.0 \pm 3.4\text{‰}$ observed in prior monospecific growouts (Horn et al., 2011a) and lower than the $5.2 \pm 0.1\text{‰}$ observed in Antarctic surface water (Fripiat et al., 2019). Prior examinations of the isotope effect of N assimilation in diatoms and other phytoplankton concluded that culture effects were a primary control on ϵ_u , though exactly how these factors influence ϵ_u remains highly unconstrained (e.g. Granger et al., 2010; Granger et al., 2004; Rohde et al., 2015).

Biomass $\delta^{15}\text{N}$

$\delta^{15}\text{N}_{\text{biomass}}$ averaged $-0.7 \pm 0.8\text{‰}$ (Table 1) across species and was somewhat lower than expectations based on the accumulated product equation (Mariotti et al., 1981). Expected $\delta^{15}\text{N}_{\text{biomass}}$, calculated based on initial $\delta^{15}\text{N}_{\text{NO}_3}$, calculated ϵ_u , and f , the fraction of nitrate consumed, was on average about 0.9‰ higher than measured. We measured relatively high ammonium ($>5 \mu\text{M}$, Supplemental Table 1) at the end of several growouts, which suggests that organic material remineralization occurred. However, we also measured starting ammonium that was on average $4 \pm 3 \mu\text{M}$, indicating the persistent presence of ammonium in initial filtered seawater. In either case, ammonium was available in the carboys. The difference between calculated and

measured $\delta^{15}\text{N}_{\text{biomass}}$ is relatively small given the uncertainty associated with $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{biomass}}$ measurements, however, it could be due to preferential incorporation of isotopically light ammonium during diatom growth.

Diatom-bound $\delta^{15}\text{N}$ and ϵ_{DB}

Measured $\delta^{15}\text{N}_{\text{DB}}$ values were higher than $\delta^{15}\text{N}_{\text{biomass}}$ values for all species with the exception of *F. rhombica* (Table 2, Figure 1). *F. rhombica* had significantly different (Two-tailed *t*-test, $p = 0.004$) ϵ_{DB} values than the other five species (*F. rhombica*: $0.5 \pm 0.4\text{‰}$; other species: $-2.0 \pm 0.7\text{‰}$; all species: $-1.6 \pm 1.2\text{‰}$). Because *F. rhombica* was significantly different than all other species, it is not included in the reported average. The ϵ_{DB} values from replicate cultures of the same species varied by up to 2‰, suggesting high natural or measurement variability. The mean values of two triplicate growouts with different strains of the same species (*C. neogracilis*) were indistinguishable, which we interpret as a constant species effect.

We compared ϵ_{DB} to ϵ_{u} , Si:N content, and $\mu\text{mol N:g Si}$ of cleaned frustules and found a strong significant positive linear relationships between ϵ_{DB} and Si:N uptake (Figure 3, $p = 0.0002$) and a somewhat weaker but still significant negative relationship between ϵ_{DB} and $\mu\text{mol N:g Si}$ in cleaned frustules (Figure 4, $p = 0.011$). ϵ_{DB} is highest in species that are relatively rich in N relative to Si overall, but that produce low N frustules. Such species may decrease the value of the sedimentary $\delta^{15}\text{N}_{\text{DB}}$ signal, although this result is largely driven by the high ϵ_{DB} , Si:N, and $\mu\text{mol N:g Si}$ of cleaned frustules in *F. rhombica* (Table 1, Figure 3, Figure 4), so care must be taken when extrapolating to other species or communities. Several cleaning

replicates of $\delta^{15}\text{N}_{\text{DB}}$ for *F. rhombica* are within measurement precision of each other and demonstrate that the result is robust and not a cleaning artifact (Appendix B).

The relationships between ϵ_{DB} and Si:N elemental ratios in cells and frustules is likely related to interspecific variations in cell physiology. To make their frustules, diatoms construct long-chain polyamines (LCPAs) and proteins such as silaffins (Kroger et al., 1999; Hildebrand & Lerch, 2015) and silacidins (Wenzl et al., 2008), which can precipitate silica. The nitrogen measured for $\delta^{15}\text{N}_{\text{DB}}$ most likely reflects nitrogen from these molecules. Variability in $\delta^{15}\text{N}_{\text{DB}}$ and ϵ_{DB} can arise as different diatom species and classes use additional proteins or different proportions of proteins and LCPAs (Kroger et al., 2000; Bridoux et al., 2012). Given known differences in structural molecules between species and structural molecules being the likely nitrogen pool measured for $\delta^{15}\text{N}_{\text{DB}}$, we find the correlation between ϵ_{DB} and Si:N uptake a compelling suggestion that differences in silicification or structural molecule selection between diatom species play a role in setting $\delta^{15}\text{N}_{\text{DB}}$. This is also supported by the correlation between ϵ_{DB} and $\mu\text{mol N:g Si}$ in cleaned diatoms (Figure 4). If diatoms have relatively high ϵ_{DB} and $\mu\text{mol N:g Si}$, then it may mean that they have either more structural molecules per g of silica in their frustule or utilize more nitrogen-rich molecules in their frustule. Either of these options relate to silicification and could directly impact $\delta^{15}\text{N}_{\text{DB}}$ if the molecules had different isotopic compositions. However, sedimentary data have a wide range of $\mu\text{mol N:g Si}$ (under 10 to over 40 $\mu\text{mol N:g Si}$), and $\Delta\delta^{15}\text{N}_{\text{DB}}$ in sediments does not appear to be related to $\mu\text{mol N:g Si}$ (Robinson et al., 2019). The mechanism(s) behind the difference in the culture versus field results remain to be investigated.

Comparison to prior results

The average ϵ_{DB} for the species examined here (-2.0 ± 0.7) is more than 7‰ different than that reported in previous culture studies (5.3 ± 4.4 ‰, Figure 2) and has the opposite sign (Horn et al., 2011a). Growth conditions were similar between the two experiments, and since both were experiments with monospecific cultures, differences due to culture artifact should be minimal. One difference between our measured species and those previously measured is that a majority of the prior cultures had been grown in a lab for more than 30 years (Horn et al., 2011a). Our cultures were relatively recently collected (within 1-5 years), and therefore potentially less impacted by evolutionary or morphological changes in culture that could impact ϵ_{DB} . A species which has grown in high nutrient and light condition for decades could become adapted to these conditions, and that could impact the nitrogen isotope pathways. One piece of evidence that goes against this theory is that a monospecific culture of *Thalassiosira weissflogii* isolated in 1985 was used to develop the $\delta^{15}\text{N}_{\text{DB}}$ cleaning methods 30 years later and had a ϵ_{DB} of -3‰ (Morales et al., 2013).

Our average ϵ_{DB} value of -2.0‰ roughly match those found in the analysis of commercially available *T. weissflogii* (CCMP1051), which had an ϵ_{DB} of -3‰ (Morales et al., 2013). They are also close to surface ocean particle ϵ_{DB} of -3.5‰ reported from the North Pacific and Southern Ocean (Morales et al., 2014, Robinson et al., 2020 - in revision) and a Southern Ocean community growout, which was -4.8‰ (Chapter 1, Figure 2). Differences between monospecific culture and

community growouts and/or water column samples that incorporate non-diatom biomass and a heterogeneous consortium of diatoms is not surprising.

What is surprising is that, with the exception of *F. rhombica*, the species examined had an opposite ϵ_{DB} from previous monospecific growouts (Horn et al., 2011a). The closest comparison to *F. rhombica* from the Horn et al., (2011a) experiments is *F. kerguelensis*, which interestingly was an outlier in that dataset. While *F. rhombica* is a sea-ice associated diatom and *F. kerguelensis* is an open-ocean species, they are similar in their degree of silicification and, in particular, their Si:N uptake. They are also similar in that cleaned samples of both species have relatively high $\mu\text{mol N:g Si}$. For *F. rhombica* the average was 10 $\mu\text{mol N:g Si}$ compared to 5 $\mu\text{mol N:g Si}$ on average for the remaining species. In the prior monospecific experiments, *F. kerguelensis* had 35 $\mu\text{mol N:g Si}$ while the average of the other species was 7 $\mu\text{mol N:g Si}$ (Horn, 2011). The relationship between Si:N uptake and $\mu\text{mol N:g Si}$ of cleaned frustule suggests that perhaps the heavy silicification is supported by a less nitrogen-rich framework within the frustule. In that case, these heavily silicified species may be using a different selection of proteins with different internal offsets than less heavily silicified diatoms, which could explain their different ϵ_{DB} values. It is important to measure more monospecific growouts of heavily silicified diatoms to test this theory, especially since the more heavily silicified species are more likely to be preserved when sinking through the water column or within the sediment. Sedimentary evidence for difference between groups has found that centric diatoms were isotopically heavier than a pennate diatom community dominated by the

heavily silicified *F. kerguelensis*, which is what would expect from our culture observations (Studer et al., 2015).

Contrary to prior monospecific growouts (Horn et al., 2011a), we do not find a significant relationship between ϵ_u and ϵ_{DB} (Figure 5). This relationship in prior monospecific cultures had been difficult to explain and may have been a culture artifact.

Implications for sedimentary $\delta^{15}N_{DB}$

Most of the diatom species measured in this study had negative ϵ_{DB} that were within 1‰ of each other (Table 1). This suggests that many diatom species have similar ϵ_{DB} and that species effects may not have a large impact on sedimentary $\delta^{15}N_{DB}$. One potential difficulty for interpreting sedimentary $\delta^{15}N_{DB}$ records comes from the relationships between Si:N, $\mu\text{mol N:g Si}$, and ϵ_{DB} . Diatoms that sink through the water column and are preserved in the sediment are more likely to be large, heavily silicified diatoms. If more heavily silicified diatoms have low $\delta^{15}N_{DB}$ (high $\Delta\delta^{15}N$), it could bias sedimentary $\delta^{15}N_{DB}$ low, dependent on species composition in the sediment. However, more work on heavily silicified diatom species is needed to ensure that this relationship is robust.

If we assume that the relationship between Si:N uptake and ϵ_{DB} holds true for all Southern Ocean diatom species and there is no change to $\delta^{15}N_{DB}$ during sinking and sedimentation, we can calculate the possible impact on sedimentary $\delta^{15}N_{DB}$. Most diatoms fall within the range of 0.5 – 2 Si:N (Brzezinski, 1985). The variation of ϵ_{DB} in this range based on the linear fit ($\epsilon_{DB} = 1.8\text{‰} \cdot \text{Si:N} - 2.5\text{‰}$, Figure 3) is 2.7‰. This

suggests that if an assemblage were to go from having high Si:N requirements (Si:N = 2) such as being composed of 100% *F. rhombica* to those with low Si:N requirements (Si:N = 0.5), like *C. neogracilis*, and no other environmental parameters changed, $\delta^{15}\text{N}_{\text{DB}}$ would increase by 2.7‰.

To examine a more realistic scenario (Figure 6), we follow the lead of Horn et al (2011a), who examined the difference between Holocene and glacial diatom assemblages from TN057-PC13, a core site south of the Antarctic Polar Front with a full diatom assemblage record (Nielsen, 2004). As in the previous mass balance, we assume that source $\delta^{15}\text{N}_{\text{NO}_3}$ and ϵ_u have not changed. Since diatom sizes vary dramatically in the sediment, it is more appropriate to use area contribution rather than count contribution to the assemblage to assess the impact of species on $\delta^{15}\text{N}_{\text{DB}}$ (e.g. Redmond, 2017). Volume would be the most accurate biometric to use, however area is more widely measured, and volume typically assumes the frustule height. Areas for several diatom species have been measured in sediment at this site (Shukla et al., 2013, 2016), in Southern Ocean surface sediments (Allen, 2014), and in the modern ocean (Cefarelli et al., 2010). Because *Chaetecoros spp.* resting spore morphometry is not available at this site and is highly variable depending on the originating species, we estimated an average area based on visual examination of several published SEM images by multiplying the width and height of several resting spores (Crosta et al., 1997; Oku & Kamatani, 1997; Rynearson et al., 2013). We also require Si:N uptake for each contributing species (Oku & Kamatani, 1997; Hoffmann et al., 2007; Meyerink et al., 2017) – for species where that data was not available we used average diatom Si:N of 1 (Brzezinski, 1985).

First, we calculate an ϵ_{DB} for each species based on literature Si:N uptake values. Next, we weight the assemblage by area using published and estimated area measurements. Finally, we sum the ϵ_{DB} from each species weighted by area contribution for Holocene and Glacial intervals. We find that estimated ϵ_{DB} is 0.2‰ during the glacial and -0.3‰ during the Holocene. This indicates an expected assemblage contribution to the changing sedimentary $\delta^{15}\text{N}_{\text{DB}}$ of only -0.5‰ across transition, well within our measurement error and far lower than the observed change of ~3‰. Mass balance calculations in prior monospecific growouts had concluded that species-specific change could account for the entire ~2-3‰ decrease observed from the LGM to the Holocene (Horn et al., 2011a), but our results suggest that this is not the case. This analysis is an estimate, and many parameters such as Si:N uptake of different diatoms in the past, the robustness of the Si:N to ϵ_{DB} relationship, and potential effects of other nutrient such as iron on Si:N requirements are not addressed (Hoffmann et al., 2007). However, if this analysis proves robust and broadly applicable, it indicates that the impact of assemblage changes may be minimal in the Southern Ocean. Using counts instead of areas, we find that estimated ϵ_{DB} is 0.5‰ during the glacial and 0.8‰ during the Holocene. While the absolute values change, the conclusion that changes to diatom assemblage likely do not have a significant impact on measured $\delta^{15}\text{N}_{\text{DB}}$ holds in either calculation.

Chaetoceros spp. resting spores are an interesting target for future work. The *Chaetoceros* species in this study had uniformly negative ϵ_{DB} , but resting spores are much more heavily silicified than vegetative cells and may have a different $\delta^{15}\text{N}_{\text{DB}}$ signature. In addition, *C. socialis* has been demonstrated to continue taking up Si after

N has been depleted taking its Si:N from 0.5 to 2 (Kudo, 2003). Extra Si uptake may be involved in resting spore formation and might reduce $\delta^{15}\text{N}_{\text{DB}}$, in opposition to the offset potentially due to a depleted nitrate pool.

Surface sediment context

Our results shed some light on the observation in meridional transects of the Southern Ocean that $\delta^{15}\text{N}_{\text{DB}}$ was mostly higher than $\delta^{15}\text{N}_{\text{bulk}}$, except for the opal belt, the region of most intense biogenic opal deposition (Robinson & Sigman, 2008; Robinson et al., 2019). The heavily silicified diatom *F. kerguelensis*, which we estimate as likely having a near-zero ϵ_{DB} , dominates sediment in the opal belt (Crosta et al., 2005), often making up >75% of the diatom community by count. Other diatoms that are potentially less heavily silicified have greater contributions outside of the opal belt, and we would hypothesize that those diatoms have on average a more negative ϵ_{DB} . This may be especially true in the more silicic acid-limited sub-Antarctic. This change in silicification and diatom assemblage may go some way towards explaining the observed patterns of $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ in surface sediments, but likely also require processes such as alteration of $\delta^{15}\text{N}_{\text{bulk}}$ during sinking and sedimentation to fully explain the relationship.

Conclusions

Monospecific growouts of six Southern Ocean diatom species bear $\delta^{15}\text{N}_{\text{DB}}$ values that are typically greater than $\delta^{15}\text{N}_{\text{biomass}}$, and therefore produced an average ϵ_{DB} that was negative, in line with Southern Ocean community growouts and surface ocean

particles. This is opposite from past monospecific batch cultures. We find a significant relationship between Si:N uptake and ϵ_{DB} , suggesting that the process of silicification could impact $\delta^{15}\text{N}_{\text{DB}}$ and that heavily silicified diatoms may bias $\delta^{15}\text{N}_{\text{DB}}$ lower. However, based on a mass balance using area-weighted Si:N-derived ϵ_{DB} , we conclude that the impact on sedimentary $\delta^{15}\text{N}_{\text{DB}}$ is likely small across glacial/interglacial cycles.

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Figures

Diatom Species	Lab Code	Cruise Isolated	Origin	Year	Latitude (°S)	Longitude (°W)
Chaetoceros neogracile	LH4B4	NBP1608	WAP	2016	*	*
Chaetoceros neogracile	1410WAP6A8B	NBP1410	Flanders Bay, WAP	2014	65.05	63.32
Chaetoceros socialis	1410WA11A8	NBP1410	Gerlache Strait, WAP	2014	64.90	63.68
Porosira glacialis	IB P. glacialis	NBP1701	Grandidier Channel, WAP	2017	66.45	66.84
Thalassiosira tumida	IB T. tumida	NBP1701	Grandidier Channel, WAP	2017	66.45	66.84
Fragilariopsis cylindrus	NZ97	NBP1702	Pacific Sector, Antarctic Zone	2017	64.20	170.08
Fragilariopsis rhombica	170163C8	NBP1701	WAP	2017	*	*

Table II-1. Diatom species and isolation record.

		Initial		Final								
Species	Code	[NO ₃]	δ ¹⁵ N _{NO₃}	[NO ₃]	δ ¹⁵ N _{NO₃}	ln(<i>f</i>)	ε _N	δ ¹⁵ N _{biomass}	δ ¹⁵ N _{DB}	ε _{DB}	μmolN:g Si	Si:N Uptake
F. cylindrus	NZ97 C4	130	1.6	41.6	4.7	-1.1	2.59	-0.7	1.1	-1.9	6.97	1.00
	NZ97 C5	145	1.3	53.2	3.9	-1.0	2.58	0.2	0.5	-0.3	6.51	1.02
	NZ97 C6	160	1.0	39.3	4.9	-1.4	2.80	-0.3	1.2	-1.4	6.36	0.92
F. rhombica	170163C8 C1	132	0.9	2.1		-4.1	3.18	0.1	-1.0	1.1	15.45	1.56
	170163C8 C2	155	1.0	2.6		-4.1	2.93	0.3	-1.0	1.3	6.81	1.67
	170163C8 C3	145	1.2	1.7		-4.4	1.72	-0.1	-0.2	0.1	9.49	1.65
T. tumida	IB T. tumida C1	141	0.5	22.1	6.7	-1.9	3.34	-0.7	2.3	-3.0	4.20	0.13
	IB T. tumida C2	138	0.5	18.2	6.2	-2.0	2.81	-0.3	2.4	-2.7	4.85	0.28
	IB T. tumida C3	129	0.7	8.8	8.4	-2.7	2.85	0.0	2.2	-2.2	5.14	0.17
P. glacialis	IB P. Glacialis C1	139	-0.6	0.4		-5.8		-0.3	0.9	-1.2	5.33	0.05
	IB P. Glacialis C2	137	-0.3	0.1				-0.9	1.9	-2.8	5.39	0.24
	IB P. Glacialis C3	150	-0.1	0.2		-6.7		-0.4	1.7	-2.1	5.10	0.19
C. socialis	1410WA11A8 C1		1.7									
	1410WA11A8 C2	155	0.7	28.4	5.4	-1.7	2.75	-1.1	1.9	-3.0	5.87	0.31
	1410WA11A8 C3	153	0.0	20.8	7.1	-2.0	2.85	-0.5	2.2	-2.7	4.04	0.62
C. neogracilis	1410WAP6A8B C1	142	0.6	19.9	5.6	-2.0	2.58	-2.8	-1.3	-1.5	2.36	0.26
	1410WAP6A8B C2	147	0.5	8.6	7.2	-2.8	2.37	-2.1	-1.1	-0.9	4.42	0.14
	1410WAP6A8B C3	140	1.1	10.5	6.5	-2.6	2.10	-2.4	0.4	-2.9	6.09	0.24
C. neogracilis	LH4B4 C1	128	1.7	34.0	3.7	-1.3	1.44	-0.5	0.8	-1.4	4.13	0.30
	LH4B4 C2	102	2.2	48.6	3.6	-0.7	1.67	-0.4	0.1	-0.5	2.98	0.23
	LH4B4 C3	103	3.0	42.4	4.2	-0.5	2.63	-0.4	1.8	-2.1	4.86	0.44

Table II-2. Growout nutrient and isotope characteristics.

Figure II-1. Growout $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$.

Monospecific growout $\delta^{15}\text{N}_{\text{biomass}}$ (blue) and $\delta^{15}\text{N}_{\text{DB}}$ (red). Open markers are individual growout measurements, with replicate standard deviations when applicable. Closed markers are averages of individual measurements, with standard deviation of that average.

Figure II-2. Comparison to prior culture studies.

Monospecific growout $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ (colors, species as colorbar) in the context of past monospecific growouts (red) and community cultures from Chapter 1 (brown). 1:1 lines denote ϵ_{DB} as labeled. Variation along the lines reflects starting nutrient and uptake isotope effects, not ϵ_{DB} .

Figure II-3. Si:N Uptake vs ϵ_{DB} .

Figure II-4. $\mu\text{mol N:g Si}$ vs ϵ_{DB} .

Figure II-5. ϵ_u vs ϵ_{DB} .

Figure II-6. Mass balance calculation comparing potential Holocene vs Glacial diatom assemblage impact on $\delta^{15}\text{N}_{DB}$.

Species	Code	Initial				Final			
		[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]
F. cylindrus	NZ97 C4	130.5	448.3			41.6	360.2	4.1	2.4
	NZ97 C5	144.8	481.2			53.2	387.7	5.7	4.1
	NZ97 C6	161.9	452.3			39.3	340.7	4.2	3.3
F. rhombica	170163C8 C1	132.5	234.7	23.0	14.1	2.1	31.7	8.0	1.1
	170163C8 C2	155.3	263.6	28.9	2.9	2.6	9.3	7.1	4.2
	170163C8 C3	145.1	243.1	31.6	4.1	1.7	6.8	6.3	1.3
T. tumida	IB T. tumida C1	140.9	261.5	30.1	1.6	22.1	245.6	19.2	2.5
	IB T. tumida C2	137.8	274.7	32.9	2.7	18.2	241.0	17.4	2.4
	IB T. tumida C3	129.3	269.0	31.1	2.5	8.8	248.1	16.2	1.7
P. glacialis	IB P. Glacialis C1	138.7	172.7	28.1	1.7	0.4	166.3	9.5	4.9
	IB P. Glacialis C2	137.4	158.8	23.7	2.4	0.1	125.5	7.0	0.9
	IB P. Glacialis C3	150.0	170.8	26.0	1.0	0.2	142.9	5.5	1.1
C. socialis	1410WA11A8 C1	158.5	344.6	32.6	5.8				
	1410WA11A8 C2	155.3	332.4	31.6	4.9	28.4	292.7	20.7	8.2
	1410WA11A8 C3	153.5	334.7	32.1	4.4	20.8	252.6	15.6	7.9
C. neogracilis	1410WAP6A8B C1	142.0	234.0	32.4	6.1	19.9	201.7	14.2	9.5
	1410WAP6A8B C2	146.8	247.1	30.5	5.8	8.6	227.4	14.4	13.4
	1410WAP6A8B C3	139.7	263.0	30.6		10.5	232.1	15.6	11.7
C. neogracilis	LH4B4 C1	128.2	257.6	31.9	4.0	1.9	229.3	16.7	1.9
	LH4B4 C2	101.7	223.9	29.4	2.5	1.5	211.5	17.8	1.5
	LH4B4 C3	102.9	238.0	28.3	1.9	1.7	219.0	18.9	1.7

Supplemental Figure II-1. Growout initial and final nutrients.

CHAPTER III: Nitrogen isotopic constraints on mid-Holocene Circumpolar Deep Water intrusion on the West Antarctic Peninsula

By

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Key Findings

- $\delta^{15}\text{N}_{\text{DB}}$ tracks CDW intrusion onto the WAP shelf
- Westerly wind shifts forced CDW intrusion on sub-millennial timescales during the mid-Holocene
- No significant relationships between any individual diatom species and $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ is recording a different signal than $\delta^{15}\text{N}_{\text{DB}}$

Abstract

Intense seasonal productivity and carbon drawdown on the West Antarctic Peninsula (WAP) is driven by upwelling of nutrient-rich Circumpolar Deep Water (CDW) and subsequent stratification. CDW influence on the WAP is thought to have varied dramatically during the mid-Holocene, 5-7 ka. Here, we use diatom-bound nitrogen isotopes ($\delta^{15}\text{N}_{\text{DB}}$), a nutrient utilization proxy, from ODP Site 1098 in Palmer Deep to study variations in the presence of CDW on sub-millennial timescales in the mid-Holocene. CDW intrusion is synchronous with atmospheric warming over Antarctica, suggesting that stronger and/or more southerly Southern Hemisphere westerlies enhanced CDW intrusion onto the WAP shelf. Our results also suggest that bulk sedimentary nitrogen isotopes ($\delta^{15}\text{N}_{\text{bulk}}$) do not track the same processes as $\delta^{15}\text{N}_{\text{DB}}$ at this site and that this cannot be explained by changes in diatom assemblages.

1 Introduction

1.1 West Antarctic Peninsula oceanography during the mid-Holocene

Warming of the atmosphere and ocean likely influences the evolution of sea ice and biology on the West Antarctic Peninsula (WAP). The WAP is particularly sensitive to modern climate change because it extends northwards from the Antarctic continent and is less isolated from broader temperature changes (e.g. Cook, 2005). The input of warm and nutrient rich circumpolar deep water (CDW) onto the shelf (e.g. Henley et al., 2017; Moffat et al., 2009) is one of the strongest controls on WAP biogeochemistry and ice dynamics. Enhanced Southern Hemisphere westerlies have forced more CDW onto the WAP shelf over the last five decades (e.g. Thompson & Solomon, 2002; Swart & Fyfe, 2012), concurrent with glacial retreat across the peninsula (Cook, 2005). CDW that episodically spills onto the shelf provides nutrients and heat that stimulate primary production and melt sea-ice when mixed to the surface. The absence of CDW allows for the expansion of sea-ice and seasonal water column stratification. How CDW's influence on the WAP has changed in the past is unclear. Documentation of its past variability will lead to a better understanding of CDW's role in driving climate responses in this system.

Here, we use the mid-Holocene, from 5-7 ka, as a case study for examining CDW variations. This is a transition from the early Holocene peak warmth (centered on ~10 ka) when CDW likely dominated the WAP (Taylor & Sjunneskog, 2002; Shevenell et al., 2011; Etourneau et al., 2013; Peck et al., 2015) toward cooler conditions with expanded sea-ice (Bentley et al., 2009; Etourneau et al., 2013). Currently available paleo-proxy records give inconclusive, often conflicting evidence for the timing and

extent of CDW intrusion during the mid-Holocene (e.g. Ishman & Sperling, 2002; Shevenell & Kennett, 2002; Taylor & Sjunneskog, 2002; Shevenell et al., 2011; Etourneau et al., 2013; Peck et al., 2015). We use a record of nutrient utilization to help elucidate the role of changing CDW upwelling across the mid-Holocene and link sea surface temperature (SST) and sea-ice variability to water mass changes. Because CDW delivers nutrients to the shelf, greater CDW intrusion should be reflected by higher nutrient supply. Conversely, less CDW should lead to enhanced sea-ice and surface stratification, which allows for relatively high productivity with near complete nutrient consumption.

1.2 Nitrogen isotope ratios as past surface nutrient tracers

In nutrient replete systems, nitrogen isotope ratios are an effective proxy for nutrient supply and demand in the past when paired with records of export productivity. As proportionally more nitrate is consumed by phytoplankton, the nitrogen isotopic composition (as $\delta^{15}\text{N}$) of both nitrate and organic matter increases. In the underlying sediment, the relative change in $\delta^{15}\text{N}$ values reflects changes in the degree of nitrate consumption through time, which depends upon both supply and demand for nitrate. However, nitrogen isotope measurements of sedimentary organic matter are susceptible to syn- and post-depositional changes which could change the paleoceanographic interpretation (Altabet & Francois, 1994; Robinson et al., 2012).

Diatom-bound nitrogen isotopes ($\delta^{15}\text{N}_{\text{DB}}$) provide a more robust measurement of past surface ocean nitrogen isotopic compositions than bulk sedimentary $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$) because the organic nitrogen contained in the diatom's silica frustule is

protected from alteration (Sigman et al., 1999a; Robinson et al., 2004). Laboratory-based culture work indicates that diatoms fractionate nitrogen during incorporation of nitrogen into their frustules (quantified as $\epsilon_{\text{DB}} = \delta^{15}\text{N}_{\text{biomass}} - \delta^{15}\text{N}_{\text{DB}}$) and that this incorporation factor may not be consistent between species (Horn et al., 2011a), which could cause significant issues for interpretation of sedimentary $\delta^{15}\text{N}_{\text{DB}}$ (Horn et al., 2011b). Therefore, it is important to critically assess potential impacts of diatom species change on sedimentary $\delta^{15}\text{N}_{\text{DB}}$ records.

Site 1098 in Palmer Deep on the WAP has a high-resolution diatom assemblage record (Sjunneskog & Taylor, 2002), a well-constrained age model (Domack et al., 2001), biogenic silica-rich sediment (Shipboard Scientific Party, 1999), and existing SST (Shevenell et al., 2011) and sea-ice (Etourneau et al., 2013) records. This allows us to address two problems simultaneously, one proxy related and one paleoceanographic: 1) Do diatom assemblage changes have a significant impact on $\delta^{15}\text{N}_{\text{DB}}$? 2) Does $\delta^{15}\text{N}_{\text{DB}}$ confirm previous records of CDW intrusion onto the WAP shelf? In this paper we present new records of $\delta^{15}\text{N}_{\text{DB}}$, $\delta^{15}\text{N}_{\text{bulk}}$, total organic carbon (TOC), total nitrogen (TN), and biogenic silica. We find that there is no significant relationship between any individual diatom species and the isotopic offset between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$, that $\delta^{15}\text{N}_{\text{bulk}}$ records a different signal than $\delta^{15}\text{N}_{\text{DB}}$, and that $\delta^{15}\text{N}_{\text{DB}}$ tracks enhanced CDW intrusion.

2 Methods

2.1 Site Description and Age Model

ODP Site 1098 is located in Palmer Deep at 64°51'S, 64°12' W at a depth of 1010 m, in a glacially deepened basin on the Pacific margin of the WAP (Figure 1, Shipboard Scientific Party, 1999). Mid-Holocene aged sediments at this site consist of rapidly accumulating (>2 m/ky) laminated, muddy diatom oozes. Domack et al. (2001) constrained sediment ages with 54 radiocarbon analyses over the last 13 ka and used a reservoir age of 1230 years. 5 of those radiocarbon dates fall between 4.9-7.1 ka for a mid-Holocene age resolution of ~400 years (Domack et al., 2001). Our samples (n = 37) span 4.9-7.1 ka with an age resolution of ~60 years. A re-analysis of the age model in this interval using magnetic susceptibility to reassess the mcd scale for Hole B and updating the ^{14}C ages using Calib 7.1 and the Marine13 timescale did not result in significant changes, so we use the Domack et al. (2001) age model for ease of comparison to previously published data (Shipboard Scientific Party, 1999; Reimer et al., 2013; Stuiver et al., 2020).

2.2 Proxy Measurements

Biogenic silica measurements were made using a molybdate blue colorimetric method adapted from Mortlock & Froelich (1989). About 25 mg of dried ground sediment was digested in 0.5 M sodium hydroxide at 80°C for 4 hours. Precision based on a pooled standard deviation of replicate samples (n = 48) was $\pm 2.6\%$.

Whole sediment samples were measured for TN and $\delta^{15}\text{N}_{\text{bulk}}$ on a Costech 4010 Elemental Analyzer (EA) coupled to a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS). Samples for TOC were first digested with 6% sulfurous acid at room temperature to remove inorganic carbon, then measured on the EA (Verardo et

al., 1990). Concentrations and isotope values were standardized with ammonium sulfate (TN only, IAEA N1 and IAEA N2) and an in-house aminocaproic acid standard (TN and TOC) were used, and precision based on a pooled standard deviation was $\pm 0.014\%$ for TOC ($n = 47$), $\pm 0.013\%$ for TN ($n = 95$), and $\pm 0.4\%$ for $\delta^{15}\text{N}_{\text{bulk}}$ ($n = 53$).

Samples for $\delta^{15}\text{N}_{\text{DB}}$ were prepared following the methods outlined in Horn et al (2011) and measured with the denitrifier method (Sigman et al., 2001). $\delta^{15}\text{N}$ values were standardized using two potassium nitrate standards (IAEA N3 (4.7‰) and USGS 34 (-1.8‰)). Methodological precision for diatom-bound measurements, based on pooled standard deviation ($n = 95$), was 0.5‰.

3 Results

At Site 1098, $\delta^{15}\text{N}_{\text{DB}}$ values rise from 5.8‰ at 6.9 ka to a maximum value of 9.4‰ around 6.1 ka (Figure 2). This was followed by a decrease to the minimum observed value of 5.7‰ over the next 600 years, and finally a more muted rise to 8.3‰ by 5 ka. The low, centered around 5.4 ka, is interrupted by a one-point positive excursion to 8.7‰ around 5.3 ka. In comparison, the $\delta^{15}\text{N}_{\text{bulk}}$ record is stable throughout the entire record, with a slight downward trend from 5.7 ka to the end of the record.

Biogenic silica varies between 30-50% over the record. TOC content is relatively high, with values between 0.9-1.3%. Biogenic silica and TOC records from Site 1098 (Figure 2) show parallel trends with overall higher frequency fluctuations than the

$\delta^{15}\text{N}_{\text{DB}}$ or $\delta^{15}\text{N}_{\text{bulk}}$ records. There are no apparent millennial-scale or longer trends in either parameter during the mid-Holocene.

4 Discussion

4.1 Factors influencing $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$

Nitrogen isotope data were generated to identify CDW intrusions on the WAP shelf based on the premise that variations in $\delta^{15}\text{N}$ values signal changes in relative supply and demand for nitrate, and that these changes are related to nutrient rich CDW intrusion. Before interpreting our results, we must first address the assumptions that go into the use of this proxy including the following: 1) $\delta^{15}\text{N}_{\text{bulk}}$ is not significantly altered in this high accumulation rate setting, 2) $\delta^{15}\text{N}_{\text{DB}}$ reflects surface nitrate $\delta^{15}\text{N}$ variations, not species related fractionations, and 3) the $\delta^{15}\text{N}$ value of source nitrate is relatively constant. We will begin by addressing 1 and 2 together.

Site 1098 has relatively organic-rich and rapidly accumulating sediments, which should lead to a $\delta^{15}\text{N}_{\text{bulk}}$ signal that is isotopically unaltered (Altabet et al., 1999; Shipboard Scientific Party, 1999; Robinson et al., 2012). Its location on the WAP also precludes significant terrestrial organic matter inputs. If $\delta^{15}\text{N}_{\text{bulk}}$ is a good reflection of sinking biomass $\delta^{15}\text{N}$ and $\delta^{15}\text{N}_{\text{DB}}$ primarily records sinking biomass $\delta^{15}\text{N}$ with some constant offset, we expect to see a constant $\Delta\delta^{15}\text{N}$ ($= \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$). However, we observe that $\Delta\delta^{15}\text{N}$ varies throughout the record (Figure 2) and given the relatively unchanging $\delta^{15}\text{N}_{\text{bulk}}$ values, this variability must be driven by variability in $\delta^{15}\text{N}_{\text{DB}}$. Several possibilities could explain this observation. Individual diatom species may impart substantially different offsets between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ that are recorded

in sediment. $\delta^{15}\text{N}_{\text{bulk}}$ may record a different signal than $\delta^{15}\text{N}_{\text{DB}}$ because it incorporates nitrogen from organisms other than diatoms which may be susceptible to trophic influences or different seasonality, it was altered, or some undetermined factor. $\delta^{15}\text{N}_{\text{DB}}$ could reflect factors not apparent in $\delta^{15}\text{N}_{\text{bulk}}$, species variation, or undergo alteration itself. Some potential explanations for the observed variability in $\Delta\delta^{15}\text{N}$ are explored below.

It has been hypothesized that individual diatom species or class may control the offset between $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ (Horn et al., 2011a; Studer et al., 2013). If individual diatom species imparted consistent, different offsets between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ (e.g. ϵ_{DB}) during growth, and their relative contributions changed significantly through time, this species effect should be recorded in the $\Delta\delta^{15}\text{N}$ values. *Chaetoceros* spp. resting spores form the bulk of diatom counts in this sediment and some evidence has pointed to resting spore formation primarily when nitrate was low (French III & Hargraves, 1985), which would lead to a $\Delta\delta^{15}\text{N}$ biased high. We observe no significant relationships ($p > 0.05$, Figure 3) between the most prominent species/groups at this site (*Fragilariopsis kerguelensis*, *Thalassiosira* spp., centrics:pennates, and *Chaetoceros* spp. resting spores) and $\Delta\delta^{15}\text{N}$, which suggests that species variations do not play a primary role in setting $\Delta\delta^{15}\text{N}$ or $\delta^{15}\text{N}_{\text{DB}}$ at this site. The lack of relationship between *Chaetoceros* spp. resting spores and $\Delta\delta^{15}\text{N}$ is consistent with evidence from the North Atlantic where resting spore formation before nitrate depletion was observed (Rynewson et al., 2013). *F. kerguelensis*, which had a distinct ϵ_{DB} in culture (Horn et al., 2011a) and is the most common open-ocean diatom species in Southern Ocean sediments (Crosta et al., 2005), has a weak positive

relationship with $\Delta\delta^{15}\text{N}$ that is close to significant ($p = 0.07$, $R^2 = 0.10$), suggesting that it may be coupled to low $\delta^{15}\text{N}_{\text{DB}}$. Although the counts of *F. kerguelensis* reach a maximum of 2.7%, their volumetric contribution is greater since the dominant species by count, *Chaetoceros* spp. resting spores, are much smaller than *F. kerguelensis*. However, diatom assemblages and $\delta^{15}\text{N}_{\text{DB}}$ are both expected to change as oceanographic conditions change. Open-ocean diatom species like *F. kerguelensis* are associated with CDW so this weak positive relationship may be driven by climatic shifts rather than species' impact on $\Delta\delta^{15}\text{N}$. Centric diatoms are thought to typically grow later in the season and thus the centric:pennate diatom ratio has been interpreted as recording relative seasonality of growth (Studer et al., 2015). In our data, centric:pennate has a weak positive relationship with $\Delta\delta^{15}\text{N}$, opposite of what we would expect if seasonality were driving $\delta^{15}\text{N}_{\text{DB}}$.

Although we asserted that the $\delta^{15}\text{N}_{\text{bulk}}$ is not altered, the variation in $\Delta\delta^{15}\text{N}$ does not appear to be species related, which suggests that $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ do not record the same signal in the sediment at ODP 1098. Alteration of $\delta^{15}\text{N}_{\text{bulk}}$ may lead to variable $\Delta\delta^{15}\text{N}$ but it is not what we would expect in this environment where high sedimentation rates and shallow depths should enhance organic matter preservation (Hartnett et al., 1998). That said, Palmer Deep is unique in that there is oxygen to ~20 m depth in the sediments, suggesting that there could be significant oxic respiration occurring in the mid-Holocene aged sediments that span about 13-20 m depth (Shipboard Scientific Party, 1999; Domack, 2002). The laminations are due to high deposition rates (Leventer et al., 2002), not anoxia in the bottom waters.

It is possible that $\delta^{15}\text{N}_{\text{bulk}}$ is recording an environmentally distinct signal from $\delta^{15}\text{N}_{\text{DB}}$. $\delta^{15}\text{N}_{\text{DB}}$ comes from strictly from diatoms while $\delta^{15}\text{N}_{\text{bulk}}$ is a composite of multiple groups and trophic levels, and therefore may not be tightly coupled with surface nutrient $\delta^{15}\text{N}$. Overall, TOC:N and biogenic silica:TOC were relatively consistent through the record (Figure 2), suggesting that the overlying ecology probably remained dominated by diatoms. Changes to seasonality of growth between diatoms and non-diatoms may play some role during the mid-Holocene. Sea-ice data suggest variations in sea-ice seasonality during the mid-Holocene (Etourneau et al., 2013), which influences diatom assemblage and may have shifted the ecological succession of Palmer Deep and thereby changed $\delta^{15}\text{N}_{\text{bulk}}$ and $\Delta\delta^{15}\text{N}$. Changes to nitrogen cycling could also play a role since the recycling of nitrogen in the water column, which could lower the $\delta^{15}\text{N}$ of nitrate, has been observed in the WAP (Henley et al., 2017). Using our data, we cannot say with certainty whether $\delta^{15}\text{N}_{\text{bulk}}$ is altered or what particular signal it may be tracking.

We must also address the possibility that $\delta^{15}\text{N}_{\text{DB}}$ does not track surface ocean nutrients. Part of the utility of the $\delta^{15}\text{N}_{\text{DB}}$ proxy is that the nitrogen is thought to be protected from alteration (Sigman et al., 1999a; Robinson et al., 2004). While it is possible that alteration of $\delta^{15}\text{N}_{\text{DB}}$ occurs, the significant linear relationship between $\delta^{15}\text{N}_{\text{DB}}$ and other geochemical proxies such as SST (Figure S1) and sea-ice implies that it instead tracks broader oceanographic processes. The most parsimonious interpretation is that environmental factors rather than species effects or alteration drive the observed changes in $\Delta\delta^{15}\text{N}$. Records of $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ differ at this site and given known deficiencies of $\delta^{15}\text{N}_{\text{bulk}}$ (e.g. Robinson et al., 2012), we choose to use

$\delta^{15}\text{N}_{\text{DB}}$ as a nitrate supply and demand proxy to assess past nutrient supply and demand. This choice is supported by culture work which has shown that $\delta^{15}\text{N}_{\text{DB}}$ effectively tracks nitrate uptake (Horn et al., 2011a), the significant relationship between $\delta^{15}\text{N}_{\text{DB}}$ and other geochemical proxies, and the lack of evidence for systemic changes to $\delta^{15}\text{N}_{\text{DB}}$ by species variability or alteration.

4.2 Mid-Holocene Circumpolar Deep Water intrusion

Mid-Holocene CDW intrusion onto the WAP shelf was previously inferred from SST and sea-ice records (Shevenell et al., 2011; Etourneau et al., 2013). One weakness with using SST and sea-ice is that enhanced CDW upwelling is not the only potential cause of warmer SSTs and lower sea-ice – for example, atmospherically forced warm upper ocean temperatures could readily force a similar proxy response. This $\delta^{15}\text{N}_{\text{DB}}$ record links the nutrient characteristics of the water mass to the temperature changes and cannot be the result of only surface temperature changes. $\delta^{15}\text{N}_{\text{DB}}$ values are significantly negatively correlated with a TEX_{86} SST record from Palmer Deep (Shevenell et al., 2011) ($p = 0.04$, points within 50 years, Figure S1). During intervals of enhanced CDW influence, warm and nutrient rich CDW upwelled to increase SST and enhance surface nutrient supply (Figure 4). During intervals where CDW was deeper or further off the shelf, shelf waters may have been cooler and/or sea-ice driven stratification may have isolated surface waters from CDW influence (Figure 4). Together, the SST and $\delta^{15}\text{N}_{\text{DB}}$ data are consistent with variable intrusion of CDW on the mid-Holocene WAP. Our interpretation of variable CDW intrusion is supported by the highly branched isoprenoid (HBI) diene:triene proxy for sea-ice presence

(Etourneau et al., 2013). This record is largely consistent with $\delta^{15}\text{N}_{\text{DB}}$ and SST records from Site 1098 and indicates low sea-ice during warm, nutrient-rich intervals.

The highest TOC and biogenic silica values are coeval with the highest $\delta^{15}\text{N}_{\text{DB}}$ values, suggesting that stratification enhanced total productivity (Figure 2). This interpretation would benefit from biogenic silica and TOC mass accumulation rate data, however, given the ~400 year age model resolution and unevenly laminated sediment, we cannot resolve mass accumulation rates on the scale of CDW changes (Shipboard Scientific Party, 1999; Domack et al., 2001).

We can use $\delta^{15}\text{N}_{\text{DB}}$ to quantitatively assess nitrate supply and demand during the mid-Holocene by calculating relative nitrate consumption in the primarily sea-ice vs CDW influenced scenarios that we propose. We assume that the nitrate concentration and $\delta^{15}\text{N}_{\text{NO}_3}$ in CDW did not change significantly over the Holocene (Sigman et al., 1999b; Galbraith et al., 2013). We therefore use modern CDW nitrate values – 33 μM and 5‰ in our calculations (Henley et al., 2017; Fripiat et al., 2019). Although estimates of the average isotope effect of nitrate uptake (ϵ_u) vary, we use the modern Southern Ocean surface average of 5.2‰ (Fripiat et al., 2019). To estimate $\delta^{15}\text{N}_{\text{biomass}}$ from $\delta^{15}\text{N}_{\text{DB}}$ we use observed differences between $\delta^{15}\text{N}_{\text{biomass}}$ and $\delta^{15}\text{N}_{\text{DB}}$ in surface ocean particles (-3.5‰) (Morales et al., 2014, Robinson et al., 2020 - in revision) and Southern Ocean community culture experiments (-4.7‰) (Jones et al., unpublished) as upper and lower bounds.

Given these assumptions we use closed Rayleigh systematics to calculate the drawdown required for our high (9.4‰) and low (5.7‰) sedimentary $\delta^{15}\text{N}_{\text{DB}}$ values. The highest observed $\delta^{15}\text{N}_{\text{DB}}$ value requires drawdown of 98 - >100% of the supplied

nitrate (0-1 μM remaining), while a drawdown of about 40 - 65% (11.5 – 20 μM remaining) was enough to obtain the low, CDW influenced $\delta^{15}\text{N}_{\text{DB}}$ values. In the modern WAP, summertime nitrate varies spatially and year to year, but in years with low surface salinity (e.g. sea-ice melt conditions) nitrate is $<5 \mu\text{M}$ while in more CDW-influenced years nitrate is $\sim 15 \mu\text{M}$ (Henley et al., 2017). Our calculations fit these observations well, supporting our interpretation of high $\delta^{15}\text{N}_{\text{DB}}$ intervals as stratified, with low CDW supply and near-complete surface nitrate drawdown. On the other hand, 40 - 65% drawdown in the shoaled CDW, high nutrient supply interval implies significant export of nutrient-rich waters from the coastal region.

We now can construct a history of CDW upwelling at Palmer Deep during the mid-Holocene. CDW was shoaled around 6.8-7 ka, depressed around 6-6.1 ka, then shoaled again around 5.3 ka. Our results point to CDW supply to the surface ocean as the proximal cause for the observed oceanographic shifts in the mid-Holocene WAP.

Comparison of our data to an ice core δD record from Taylor Dome (Steig et al., 1998), which is west of the Ross Sea in East Antarctica, suggests a broader-scale forcing mechanism behind the oceanographic changes. δD largely records atmospheric temperature change and has been used as a proxy for shifts in the position of the westerly wind belts. More negative δD values are associated with equatorward westerlies and more positive values are associated with poleward westerlies. There is a very clear correspondence between δD , SST, and $\delta^{15}\text{N}_{\text{DB}}$ with poleward westerlies linked to warmer SSTs and lower $\delta^{15}\text{N}_{\text{DB}}$ interpreted as high nutrient supply (Figure 4). Equatorward westerly wind position appears to have led to nutrient enrichment of CDW during parts of the mid-Holocene (Struve et al., 2020). We show that near-

complete nutrient consumption occurs when the westerlies are equatorward such that any slight CDW enrichment signal in $\delta^{15}\text{N}_{\text{DB}}$ would be overprinted. Our results support westerly wind position controlling oceanographic conditions on the WAP during the mid-Holocene.

An important forcing during the Holocene is the reduction in both annual mean and summer insolation at 65°S since about 10 ka. However, the millennial- and shorter timescale $\delta^{15}\text{N}_{\text{DB}}$ changes observed during the mid-Holocene cannot be explained by the smooth insolation decrease across the mid-Holocene (Berger & Loutre, 1991; Bentley et al., 2009). Likewise, CO_2 constantly increases during this interval and does not appear to be related to CDW changes (Indermöhle et al., 1999), indicating that changes to WAP oceanographic dynamics are insufficient to alter the global carbon system.

5 Conclusions

Our new $\delta^{15}\text{N}_{\text{DB}}$ record from Site 1098 at Palmer Deep paints a picture of variable nutrient supply and demand across the mid-Holocene. We find a significant negative correlation between Palmer Deep SST (Shevenell et al., 2011) and $\delta^{15}\text{N}_{\text{DB}}$ histories that suggest variable CDW input to the coastal WAP on millennial and centennial timescales, consistent with other geochemical proxies of surface ocean conditions at Palmer Deep (Etourneau et al., 2013). Antarctic ice core evidence for concurrent shifts in surface air temperature related to the strength and/or position of the westerly wind belts indicate that westerly wind shifts drove changes to CDW upwelling on the WAP (Steig et al., 1998; Shevenell et al., 2011).

Our data also demonstrate that $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ do not vary in tandem at Palmer Deep as we would expect if both robustly tracked surface ocean nitrate supply. By comparison to a diatom assemblage record (Sjunneskog & Taylor, 2002), we find that diatom species variation cannot explain how $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ differ as has been suggested in the past (Horn et al., 2011a; Studer et al., 2013). Since $\delta^{15}\text{N}_{\text{DB}}$ closely tracks with other geochemical proxies and $\delta^{15}\text{N}_{\text{bulk}}$ appears wholly unrelated, we conclude that $\delta^{15}\text{N}_{\text{bulk}}$ is tracking some other process or is potentially altered at this site.

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Figures

Figure III-1. Map and temperature section for Palmer Deep.

A. Bathymetric map of the WAP region (Antarctica inset, GeoMapApp). Approximate Circumpolar Deep Water location and heading are in solid black lines, with dashed black lines denoting shelf intrusion (adapted from Bentley, 2009). Palmer Deep is located with a red star. The oceanographic section in panel B is located with a dashed orange line. **B.** Temperature section from Palmer Station LTER, cruise LMG01-01 (<https://oceaninformatics.ucsd.edu/datazoo/catalogs/pallter/sources>) demonstrating warm CDW intrusion onto the shelf.

Figure III-2. New proxy records from Site 1098

Nitrogen isotope records include $\delta^{15}\text{N}_{\text{DB}}$ (red) and $\delta^{15}\text{N}_{\text{bulk}}$ (orange), and $\Delta\delta^{15}\text{N}$ (brown). Replicate measurements are denoted with open markers. Productivity related proxies biogenic silica (dark blue) and TOC (dark green) are presented with TOC:TN (light green) and biogenic silica:TOC (light blue).

Figure III-3. Diatom species/class vs $\Delta\delta^{15}\text{N}$

Diatom species/class vs $\Delta\delta^{15}\text{N}$, with diatom assemblage data from Taylor & Sjunneskog (2002). **A.** sum of *Thalassiosira* species **B.** *Fragilariopsis kerguelensis* **C.** ratio of centric diatoms to pennate diatoms, not including *Chaetoceros* spp. resting spores. **D.** *Chaetoceros* spp. resting spores, including counts noted as *Chaetoceros* “empty ovals”

Figure III-4. Comparison to other records.

HBI (grey cross; dark grey line is a 5-point smooth, Etourneau et al., 2013), Taylor Dome δD (light green; dark green is a 10-point smooth, Steig et al., 1998).. SST (black, Shevenell et al., 2011), $\delta^{15}\text{N}_{\text{DB}}$ (red, inverted axis). Blue arrows indicate proposed CDW shifts and are coeval between atmospheric and oceanographic parameters.

Appendix A: Diatom Counts from Shipboard Cultures

Diatom Species	66°S			61°S		
	C1	C2	C3	C4	C5	C6
<i>Fragilariopsis cylindrus</i>	1970	1543	1989	110	126	113
<i>Fragilariopsis pseudonana</i>	71	112	97	25	32	25
<i>Fragilariopsis rhombica</i>	1	0	1	0	0	0
<i>Fragilariopsis kerguelensis</i>	13	18	13	47	47	44
<i>Fragilariopsis curta</i>	79	74	115	67	108	55
<i>Chaetoceros dicaeta</i>	4	2	1	0	0	0
<i>Chaetoceros atlanticus</i>	12	6	10	0	3	1
<i>Chaetoceros cf socialis/neglectus</i>	83	78	61	8	11	18
<i>Chaetoceros castracanei</i>	1	0	1	0	0	0
<i>Pseudonitzschia subcurvata</i>	59	39	19	0	0	0
<i>Pseudonitzschia turgidula</i>	0	0	0	39	20	16
<i>Rhizosolenia cf simplex</i>	0	1	0	0	0	0
<i>Thalassiosira antarctica</i>	3	0	0	0	1	1
<i>Thalassiosira gracilis</i>	0	0	0	0	0	1
<i>Proboscia inermis</i>	14	5	5	0	0	0
<i>Cylindrothecum cloisterium</i>	0	0	0	10	16	23
<i>Synedropsis</i> spp. (hyperboreoides?)	0	0	0	1	0	1
Centric (girdle view)	4	2	0	5	4	16
<i>Corethron pennatum</i>	0	0	1	1	0	0

Appendix A Table 1. Diatom community counts for shipboard incubation experiments on NBP1702. C1-C3 are replicate carboys from 66°S, and C4-C6 are replicate carboys from 61°S. Counts continued until 300 non-*F. cylindrus* individuals were counted for C1-C3, and until 300 total individuals for C4-C6.

Appendix B: Individual Growout Nutrient and Isotope Parameters

Species name: *Fragilariopsis cylindrus*

Replicate 4

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/4/2018		130.5	448.3	12.9			1.6			
5/10/2018	noon	85.8					3.3			
5/10/2018	6pm	62.7								
5/11/2018		41.6	360.2	4.1	2.4		4.7	-0.7	1.1	-1.9

Species name: *Fragilariopsis cylindrus*

Replicate 5

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/4/2018		144.8	481.2	14.5			1.3			
5/10/2018	noon	53.4					3.8			
5/10/2018	3pm	53.2	387.7	5.7	4.1		3.9	0.2	0.5	-0.3

Species name: *Fragilariopsis cylindrus*

Replicate 6

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/4/2018		161.9	452.3	13.2			1.0			
5/10/2018	noon	80.8					3.6			
5/10/2018	6pm	67.0								
5/11/2018		39.3	340.7	4.2	3.3		4.9	-0.3	1.2	-1.4

Appendix B Table 1. *Fragilariopsis cylindrus* (1702-NZ97) growout nutrient, growth, and isotopic parameters.

Species name: *Chaetoceros socialis*

Replicate 2

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
9/17/2018		155.3	332.4	31.6	4.9	0.2	0.7			
9/28/2018						0.4				
10/2/2018						8.5				
10/3/2018	noon					16.7				
10/4/2018	3pm	28.4	292.7	20.7	8.2	46.9	5.4	-1.1	1.9	-3.0

Species name: *Chaetoceros socialis*

Replicate 3

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
9/17/2018		153.5	334.7	32.1	4.4	0.2	1.4			
9/28/2018						1.1				
10/2/2018		76.5				35.7				
10/3/2018	noon	20.8	252.6	15.6	7.9	51.1	7.1	-0.5	2.2	-2.7

Appendix B Table 2. *Chaetoceros socialis* (1410WA11A8) growout nutrient, growth, and isotopic parameters.

Species name: *Fragilariopsis rhombica*

Replicate 1

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
12/5/2018		132.5	234.7	23.0	14.1	0.3	0.9			
12/11/2018						0.2				
1/8/2019		141.9								
1/17/2019		69.3				13.2	2.9			
1/19/2019						16.5				
1/20/2019	5pm	2.1	31.7	8.0	1.1			0.1	-1.0	1.1

Species name: *Fragilariopsis rhombica*

Replicate 2

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
12/5/2018		155.3	263.6	28.9	2.9	0.3	1.0			
12/11/2018						0.2				
1/8/2019		154.8								
1/17/2019		14.1				24.8	8.0			
1/18/2019	8pm	2.6	9.3	7.1	4.2			0.3	-1.0	1.3

Species name: *Fragilariopsis rhombica*

Replicate 3

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
12/5/2018		145.1	243.1	31.6	4.1	0.3	1.2			
12/11/2018						0.2				
1/8/2019		151.2								
1/17/2019		64.2				15.3	2.6			
1/19/2019						16.9				
1/20/2019	1pm	1.7	6.8	6.3	1.3			-0.1	-0.2	0.1

Appendix B Table 3. *Fragilariopsis rhombica* (170163C8) growout nutrient, growth, and isotopic parameters.Species name: *Chaetoceros neogracilis*

(1410WAP6A8B)

Replicate 1

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
1/25/2019		142.0	234.0	32.4	6.1	1.4	0.6			
1/30/2019	11am					66.2				
1/30/2019	8pm	19.9	201.7	14.2	9.5		5.6	-2.8	-1.3	-1.5

Species name: *Chaetoceros neogracilis*

(1410WAP6A8B)

Replicate 2

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
1/25/2019		146.8	247.1	30.5	5.8	1.5	0.5			
1/30/2019	11am					57.9				
1/30/2019	11pm	8.6	227.4	14.4	13.4		7.2	-2.1	-1.1	-0.9

Species name: *Chaetoceros neogracilis*

(1410WAP6A8B)

Replicate 3

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
1/25/2019		139.7	263.0	30.6		1.2	1.1			
1/30/2019	11am					58.2				
1/30/2019	12pm	10.5	232.1	15.6	11.7		6.5	-2.4	0.4	-2.9

Appendix B Table 4. *Chaetoceros neogracilis* (1410WAP6A8B) growout nutrient, growth, and isotopic parameters.

Species name: *Porosira glacialis*

Replicate 1

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/8/2019		138.7	172.7	28.1	1.7		-0.6			
5/16/2019	6pm	0.4	166.3	9.5	4.9			-0.3	0.9	-1.2

Species name: *Porosira glacialis*

Replicate 2

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/8/2019		138.7	172.7	28.1	1.7		-0.6			
5/16/2019	9:30pm	0.4	166.3	9.5	4.9			-0.3	0.9	-1.2

Species name: *Porosira glacialis*

Replicate 3

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/8/2019		138.7	172.7	28.1	1.7		-0.6			
5/16/2019	1pm	0.4	166.3	9.5	4.9			-0.3	0.9	-1.2

Appendix B Table 5. *Porosira glacialis* (IB *P. glacialis*) growout nutrient, growth, and isotopic parameters.

Species name: *Thalassiosira tumida*

Replicate 1

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/24/2019		140.9	261.5	30.1	1.6	2.5	0.5			
5/30/2019						16.8				
6/1/2019	noon	37.7				34.5				
6/1/2019	4pm	22.1	245.6	19.2	2.5		6.7	-0.7	2.3	-3.0

Species name: *Thalassiosira tumida*

Replicate 2

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/24/2019		137.8	274.7	32.9	2.7	2.0	0.5			
5/30/2019						9.5				
6/1/2019	noon	63.8				17.0				
6/2/2019										
6/3/2019	noon					23.5				
6/3/2019	9pm	18.2	241.0	17.4	2.4		6.2	-0.3	2.4	-2.7

Species name: *Thalassiosira tumida*

Replicate 3

Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{biomass}}$	$\delta^{15}\text{N}_{\text{DB}}$	ϵ_{DB}
5/24/2019		129.3	269.0	31.1	2.5	1.8	0.7			
5/30/2019						8.7				
6/1/2019	noon	65.6				17.7				
6/2/2019						23.5				
6/3/2019	noon					34.5				
6/3/2019	7:30pm	8.8	248.1	16.2	1.7		8.4	0.0	2.2	-2.2

Appendix B Table 6. *Thalassiosira tumida* (IB *T. tumida*) growout nutrient, growth, and isotopic parameters.

Species name: <i>Chaetoceros neogracilis</i> (LH4B4)						Replicate 1				
Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	δ ¹⁵ N _{NO₃}	δ ¹⁵ N _{biomass}	δ ¹⁵ N _{DB}	ε _{DB}
7/22/2019		128.2	257.6	31.9	4.0		1.7			
7/26/2019		115.0				11.3	2.1			
7/27/2019	noon	104.7				18.8				
7/29/2019		34.0	229.3	16.7	1.9		3.7	-0.5	0.8	-1.4

Species name: <i>Chaetoceros neogracilis</i> (LH4B4)						Replicate 2				
Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	δ ¹⁵ N _{NO₃}	δ ¹⁵ N _{biomass}	δ ¹⁵ N _{DB}	ε _{DB}
7/22/2019		101.7	223.9	29.4	2.5		2.2			
7/26/2019		100.1				24.0	2.6			
7/27/2019	noon	57.0				46.5				
7/27/2019	3pm	48.6	211.5	17.8	1.5		3.6	-0.4	0.1	-0.5

Species name: <i>Chaetoceros neogracilis</i> (LH4B4)						Replicate 3				
Date	Time	[NO ₃]	[Si(OH ₄)]	[PO ₄]	[NH ₄]	Fluorescence	δ ¹⁵ N _{NO₃}	δ ¹⁵ N _{biomass}	δ ¹⁵ N _{DB}	ε _{DB}
7/22/2019		102.9	238.0	28.3	1.9		3.0			
7/26/2019		94.7				28.3	2.8			
7/27/2019	noon	51.9				51.2				
7/27/2019	5pm	42.4	219.0	18.9	1.7		4.2	-0.4	1.8	-2.1

Appendix B Table 7. *Chaetoceros neogracilis* (LH4B4) growout nutrient, growth, and isotopic parameters.

Species name: <i>Fragilariopsis rhombica</i>			
	Replicate 1	Replicate 2	Replicate 3
Date	$\delta^{15}\text{N}_{\text{DB}}$	$\delta^{15}\text{N}_{\text{DB}}$	$\delta^{15}\text{N}_{\text{DB}}$
12/12/2020	-1.5		
1/29/2020	-0.7	-0.1	0.2
2/24/2020	-1.0	-1.0	-0.2

Appendix B Table 8. *Fragilariopsis rhombica* (170163C8) $\delta^{15}\text{N}_{\text{DB}}$ cleaning replicates.

Appendix C: Chapter III Supplemental Information

Introduction

The supplemental information is intended to give additional context for the age model (S1) and TEX₈₆ proxy choice given multiple proxy records (S2). Figure S1 shows the relationship between $\delta^{15}\text{N}_{\text{DB}}$ and TEX₈₆ SST from Shevenell et al. (2011).

S1. Age Model

The age model of Domack et al., (2001) relies largely on radiocarbon samples from Hole C and was calibrated using CALIB 98. Our data are from Hole B and the mcd scale has been re-assessed since original publication (e.g. Pike et al., 2013), so we examined the magnetic susceptibility of Hole C and Hole B during the mid-Holocene to determine the fit of the mcd scale (Shipboard Scientific Party, 1999). While there were some differences between Hole A and Holes B and C, Holes B and C were aligned within <5 cm (~25 years) during the mid-Holocene. Updating the age model calibration from CALIB 98 to CALIB 7.1 using the Marine13 ¹⁴C curve changed ages by <50 years (Reimer et al., 2013; Stuiver et al., 2020). Since all changes were minor and smaller than our age resolution of about 60 years, we chose to plot all data on the Domack et al., (2001) age model for ease of comparison to previously published records.

S2. TEX₈₆ SST Proxy Choices

The TEX₈₆ SST proxy is complex to interpret and the Shevenell et al., (2011) record differs somewhat from other WAP estimates of SST. Shevenell et al., (2011) use a global calibration of TEX₈₆ from Kim et al. (2008) with 7 regional coretop data points added for SST calculations to expand the number of high-latitude/cool temperature datapoints in the 2008 calibration. According to the authors, this calibration leads to realistic temperatures at the cold end, but likely overestimates warmer temperatures (Shevenell et al., 2011). However, the trends in temperature are

likely robust even if the absolute values and amplitudes of variation are not, since it remains a linear calibration. A more recent TEX-based temperature record from Etourneau et al. (2013) from a nearby site within Palmer Deep (NBP9903 JPC-10) uses an updated calibration from Kim et al. (2010) that includes more high latitude sites and calibrates to upper water column temperatures (0-200m) rather than SST. The record from JPC-10 was compared to Site 1098 using new measurements from Site 1098 and the same 2010 calibration, but there were significant differences between the SST at the two sites, suggesting potential local differences rather than calibration drives the difference between Site 1098 and JPC-10. We choose to use the record from Site 1098 in order to minimize any local differences and do not interpret absolute SSTs.

Figures

Figure S1. SST (Shevenell et al., 2011) vs $\delta^{15}\text{N}_{\text{DB}}$.

Because data were not measured on the same samples, SST data were interpolated using the nearest-neighbor method with a restriction that samples must have been within 50 years since that is below the scale of most variation in the record.

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Appendix D: Site 1098 Proxy Measurements

Exp	Site	Hole	Core	Core Type	Section	Top Interval (cm)	Bottom Interval (cm)	Depth (mcd)	Age (years)	Opal%	σ	n	TOC%	σ	n	TN%	σ	n	$\delta^{15}\text{N}_{\text{bulk}}$	σ	n	$\delta^{15}\text{N}_{\text{DB}}$	σ	n
178	1098	B	2	H	4	104	105	12.4	4935	36.6		1	1.19	0.010	2	0.16	0.002	3	3.00		1	6.95	0.86	2
178	1098	B	2	H	4	121	122	12.57	4986	42.5	0.8	2	1.28	0.012	2	0.16	0.017	4	3.47	0.40	2	8.39	0.02	2
178	1098	B	2	H	4	141	142	12.77	5045	37.5	3.9	2	0.97		1	0.12	0.012	2	3.76		1	7.72	1.04	2
178	1098	B	2	H	5	21	22	13.07	5135			1	1.09		1	0.15	0.009	3	3.34	0.83	2	6.79	0.31	2
178	1098	B	2	H	5	41	42	13.27	5194	35.1		1	1.03		1	0.14	0.007	3	3.50	0.68	2	6.76	0.28	2
178	1098	B	2	H	5	61	62	13.47	5253	35.0		1	1.09		1	0.14	0.018	2	4.20		1	7.11	0.48	2
178	1098	B	2	H	5	81	82	13.67	5312	32.0		1	0.97		1	0.12	0.015	2	4.01		1	6.38	0.33	2
178	1098	B	2	H	5	101	102	13.87	5371	34.7		1	1.14		1	0.14	0.014	2	3.62		1	8.70	0.80	3
178	1098	B	2	H	5	121	122	14.07	5430	39.2	0.4	2	1.23	0.001	2	0.16	0.019	4	4.34	0.02	2	5.69	0.51	2
178	1098	B	2	H	5	141	142	14.27	5489	42.0		1	1.11		1	0.15	0.021	2	3.94		1	6.11	0.64	6
178	1098	B	2	H	6	21	22	14.57	5576	42.5		1	1.22		1	0.16	0.018	2	3.59		1	6.88	0.80	3
178	1098	B	2	H	6	41	42	14.77	5635	32.2	0.5	2	1.10		1	0.14	0.015	3	4.34	0.58	2	7.25	0.02	2
178	1098	B	2	H	6	61	62	14.97	5693	32.9		1	1.12		1	0.13		1	3.66		1	8.03	0.37	2
178	1098	B	2	H	6	81	82	15.17	5752	32.3		1	1.07		1	0.14	0.003	2	3.41		1	8.64	0.27	3
178	1098	B	2	H	6	101	102	15.37	5810	40.9		1	1.23		1	0.16	0.006	2	3.55		1	8.14	0.10	2
178	1098	B	2	H	6	121	122	15.57	5869	41.9		1	1.19		1	0.15	0.003	2	4.10		1	7.43	0.96	3
178	1098	B	2	H	6	141	142	15.77	5927	34.3		1	1.19		1	0.15	0.001	2	3.78		1	8.38	0.09	2
178	1098	B	2	H	7	21	22	16.07	6015	34.2	1.8	2	1.09	0.016	2	0.14	0.012	3	4.29	0.05	2	6.89	0.08	3
178	1098	B	2	H	7	41	42	16.27	6073	49.3		1	1.33		1	0.18	0.002	4	4.33	0.25	3	9.37	0.37	4
178	1098	B	2	H	CC	11	12	16.47	6132	39.5		1	1.28		1	0.17	0.009	3	3.87	0.28	2	8.99	0.33	3
178	1098	B	3	H	1	21	22	17.23	6356	39.7		1	1.14	0.017	2	0.15	0.004	4	3.83	0.28	2	7.83	0.03	2
178	1098	B	3	H	1	41	42	17.43	6416	32.2	3.4	2	1.16	0.012	2	0.15	0.001	4	4.13	0.23	2	7.52	0.45	3
178	1098	B	3	H	1	61	62	17.63	6476	40.1	1.9	2	1.08		1	0.14	0.013	2	3.99		1	7.14	0.67	2
178	1098	B	3	H	1	81	82	17.83	6535	36.8	1.5	2	1.12	0.007	2	0.15	0.004	4	3.84	0.07	2	7.13	0.48	2
178	1098	B	3	H	1	101	102	18.03	6596	37.1		1	1.07		1	0.13	0.014	2	4.21		1	7.91	0.81	3
178	1098	B	3	H	1	121	122	18.23	6656	32.9		1	1.04		1	0.14	0.003	2	3.91		1	6.85	0.36	3
178	1098	B	3	H	1	141	142	18.43	6717	41.1		1	1.15		1	0.15	0.001	2	4.00		1	7.66	0.31	2
178	1098	B	3	H	2	21	22	18.73	6808	42.5		2	1.18		1	0.15	0.009	3	4.01	0.15	2	6.47	0.70	3
178	1098	B	3	H	2	41	42	18.93	6869	36.5	0.4	2	1.16	0.020	3	0.14	0.001	3	3.96	0.15	2	6.03	0.27	3
178	1098	B	3	H	2	61	62	19.13	6931	35.2		1	1.11		1	0.15	0.003	2	3.92		1	7.52	0.41	2
178	1098	B	3	H	2	81	82	19.33	6993	36.5		1	1.10		1	0.15	0.014	4	4.70	0.18	2	6.99	0.63	3
178	1098	B	3	H	2	101	102	19.53	7056	41.7	1.9	2	1.14		1	0.14		1	3.91		1	7.56	0.23	4
178	1098	B	3	H	2	121	122	19.73	7119	37.2		1	1.04		1	0.14	0.005	2	4.38		1	6.71	0.50	2

Appendix C Table 1. Proxy measurements from ODP Site 1098. Age converted from meters composite depth (mcd) using chronostratigraphy from Domack et al., 2001. For each proxy, the average measurement, standard deviation (σ), and number of samples measured (n) are reported. Horizontal lines separate core sections.