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EXAMINATION OF THE DIATOM-BOUND
NITROGEN ISOTOPE PROXY:
NITRATE CONSUMPTION IN THE POLAR OCEAN

BY
MATTHEW G. S. HORN

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DOCTOR OF PHILOSOPHY DISSERTATION

OF

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ABSTRACT

The ratio of stable nitrogen isotopes ($\delta^{15}\text{N}$) is used to infer nitrogen utilization in nutrient replete surface waters through time. Typically, the isotopic composition of bulk sedimentary nitrogen ($\delta^{15}\text{N}_{\text{bulk}}$) is measured. However the isotopic composition of this fraction is known to shift due to alteration during sinking and early burial. More recently, the nitrogen bound within silica microfossils ($\delta^{15}\text{N}_{\text{DB}}$) has been targeted. This fraction is hypothesized to be protected from alteration during sinking and early burial by the biominerals encapsulating it, thus preserving the surface nitrate utilization signal through time. The goal of this project was two-fold, first determining the relationships and reliability of both the bulk and diatom-bound nitrogen isotope proxies over varying nutrient utilization and second, to investigate nitrate consumption in the Southern Ocean to better understand Earth's history since the Last Glacial Maximum (LGM). Several polar diatoms species were cultured to determine the relationship between the isotopic composition of the seawater's nitrate substrate, the bulk organic matter, and the diatom-bound fraction over varying degrees of nutrient utilization. A Rayleigh-type relationship was found to exist for all species, whereby $\delta^{15}\text{NO}_3^- > \delta^{15}\text{N}_{\text{bulk}} > \delta^{15}\text{N}_{\text{DB}}$. The isotopic enrichment factor (ϵ) and the offset between the bulk and diatom-bound fraction ($\delta^{15}\text{N}_{\text{DBoffset}}$) were found to be relatively consistent within replicates, but varied greatly between species. This implies that species assemblage shifts through time may contribute to variations in the sedimentary $\delta^{15}\text{N}$ signal. These relationships were then applied to downcore sedimentary records from the Southern Ocean at core TN057-13PC4, along with stable silicon isotopes ($\delta^{30}\text{Si}$) to investigate large-scale hydrographic changes between the LGM and the Holocene,

today. Coupled N and Si measurements alongside opal accumulation rates provided a quasi-quantitative estimate of nutrient supply, or upwelling. Results suggest that large changes in water column stratification took place during the deglaciation, a highly variable period, which likely resulted in rising atmospheric $p\text{CO}_2$ and caused a transient peak in nutrient supply to the low latitudes. Finally, bulk and diatom-bound $\delta^{15}\text{N}$ values were investigated in five cores (TN057-13PC4, ODP 1094, PA9802-9PC, E17-9 and E23-14) from the Southern Ocean to determine the viability and variability within and between each proxy. Several factors including changes in species assemblage, ϵ , opal flux, nutrient dynamics, and alteration of the bulk and diatom-bound fraction may all contribute to the variability in measured $\delta^{15}\text{N}$ downcore. Species assemblage composition clearly impacts downcore records of sedimentary $\delta^{15}\text{N}$. Experimental results from the first manuscript show that given assemblage changes, our culture data, for relevant species, do not suggest that the observed glacial/interglacial $\delta^{15}\text{N}$ change in Antarctic sediment cores can be explained by bulk-to-diatom-bound isotopic differences. In fact, large changes in species assemblage at TN057-13PC4 greatly affected the $\delta^{15}\text{N}$ of sedimentary material and resulted in the removal of all $\delta^{15}\text{N}$ data prior to 20 kya from the analysis of the deglaciation in the second manuscript. Discrepancies between bulk and diatom-bound $\delta^{15}\text{N}$ were investigated in the third manuscript to determine the reliability of each proxy. This suggests that the diatom-bound material is not as protected as it was once thought to be and is likely subject to alteration.

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PREFACE

The focus of this dissertation is an analysis of the diatom-bound nitrogen isotope proxy for nutrient consumption through the study of diatom cultures and downcore sediment samples. The work covers a wide spectrum of temporal scales from present day cultures that evolve on the time-scale of hours to sedimentary materials that provide insight into our earth's history, here, extending back 250 kyr. Differences between two N isotope proxies available to paleoceanographers working in the Southern Ocean, bulk and diatom-bound N isotopes, are examined to improve our ability to reconstruct earth's biogeochemical history. This dissertation is written in manuscript format and consists of three manuscripts.

High Latitude Control of Glacial / Interglacial CO₂

The Southern Ocean controls a large portion of atmospheric $p\text{CO}_2$ through the net effects of removal from and addition to the atmosphere by biological productivity and deep-water ventilation, respectively. At present, the Southern Ocean is a source of CO_2 to the atmosphere, but this was likely different in the past [Knox and McElroy, 1984; Marinov *et al.*, 2006; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984]. The major nutrients, nitrate and phosphate, are not completely utilized in the modern Southern Ocean as a result of iron and light limitation of phytoplankton [Boyd *et al.*, 1999; Dugdale *et al.*, 1995; Martin *et al.*, 1990a; Martin *et al.*, 1990b; Nelson and Smith, 1991; Sedwick *et al.*, 1997; Sedwick *et al.*, 1999]. It has been hypothesized that more complete drawdown of nutrients in Southern Ocean surface waters during glacial times regulated the decreases in atmospheric $p\text{CO}_2$ observed in ice core records

[*Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984*]. Variations in nutrient utilization and lower $p\text{CO}_2$ may result from changes in the strength of the biological pump and/or changes in deep-water ventilation and the resulting supply of nutrient and CO_2 rich deep waters to the surface [*Broecker, 1982; Francois et al., 1997; Kohfeld et al., 2005; Toggweiler, 1999; Watson and Garabato, 2006*]. Initial hypotheses suggested that increased eolian iron fluxes could potentially enhance the biological pump by relieving iron limitation [*Broecker, 1982; De Baar et al., 1995; Kumar et al., 1995; Martin et al., 1990a; Moore et al., 2000*]. However, unrealistically large inputs of iron may be required, in excess of 100 times the present day value, to stimulate enough production to induce the entire 100 ppmv reduction in atmospheric CO_2 [*Lefevre and Watson, 1999*]. A second set of hypotheses grew from the observation that export productivity was lower in the Antarctic Zone of the Southern Ocean (the region south of Polar Front) during glacial episodes [*Francois et al., 1997*]. Polar ocean stratification, due to either a colder climate which reduced the role of temperature in the density structure of the ocean, and/or an equatorward shift in the westerlies, may have reduced the resupply of nutrients and CO_2 to the surface ocean-atmosphere system [*Sigman et al., 2004; Toggweiler et al., 2006*]. A reduction in overturning circulation during glacial periods is consistent with decreased production [*Charles et al., 1991; Haug et al., 1999; Shemesh et al., 1993*].

Nitrogen isotope proxy records have played a significant role in advancing our understanding of the Southern Oceans role in glacial-interglacial CO_2 [*Brunelle et al., 2007; Brunelle et al., 2010; Francois et al., 1997; Robinson et al., 2004; Robinson et al., 2005; Robinson and Sigman, 2008; Sigman et al., 1999*]. Under nitrate-replete

conditions, the relative degree of nitrate consumption is recorded in the nitrogen isotopic composition of the phytoplankton, as a result of the kinetic fractionation that occurs during the reduction of nitrate to nitrite, by the enzyme nitrate reductase [Needoba and Harrison, 2004; Shearer et al., 1992]. ^{14}N is preferentially incorporated into organic matter relative to ^{15}N [Altabet et al., 1991; Cifuentes et al., 1989; Horrigan et al., 1990; Montoya et al., 1991; Wada and Hattori, 1978; Wada, 1980]. The result is an offset, whereby the $^{15}\text{N}/^{14}\text{N}$ (as $\delta^{15}\text{N} = [(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1] * 1000$, where atmospheric N_2 is the universal reference) of phytoplankton is lower than that of the substrate nitrate in seawater. During progressive consumption, the $\delta^{15}\text{N}$ of both the nitrate substrate ($\delta^{15}\text{NO}_3^-$) and bulk organic matter ($\delta^{15}\text{N}_{\text{bulk}}$) increases, as the concentration of nitrate decreases. Going back in time, relative changes in sedimentary $\delta^{15}\text{N}$ are thought to reflect changes in the degree of nutrient consumption in the surface ocean [Altabet and Francois, 1994].

Overall, N isotope records from bulk sedimentary and diatom-bound N materials suggest that that nutrient consumption was enhanced in the glacial Southern Ocean [Crosta and Shemesh, 2002; Francois et al., 1997; Robinson et al., 2004; Robinson et al., 2005; Sigman et al., 1999], despite the decreased export productivity that is also observed south of the APF during glacial times [Franck et al., 2000; Francois et al., 1997; Kumar et al., 1995; Mortlock et al., 1991]. Enhanced stratification may have reduced supply of nutrients to the surface while the expansion of the marginal ice zone potentially increased biological demand for the major nutrients [Crosta and Shemesh, 2002; Kohfeld et al., 2005; Robinson et al., 2005; Robinson and Sigman, 2008; Toggweiler, 1999]. The melting of sea ice has the dual potential to stimulate blooms

by stabilizing the water column and adding Fe directly to this well lit surface layer [Robinson and Sigman, 2008]. The observed decrease in productivity and increase in utilization imply a bio-physical mechanism for lowering atmospheric CO₂ during glacial episodes [Brzezinski *et al.*, 2002; Matsumoto *et al.*, 2002; Robinson *et al.*, 2004; Robinson *et al.*, 2005; Robinson and Sigman, 2008; Takeda, 1998].

Statement of the Problem

The relationship between nitrate consumption and diatom-bound $\delta^{15}\text{N}$ in culture or *in situ* was never addressed due to sample size limitations. Despite this lack of groundtruthing, diatom-bound $\delta^{15}\text{N}$ was used in several studies [Brunelle *et al.*, 2007; Brunelle *et al.*, 2010; Robinson *et al.*, 2004; Robinson *et al.*, 2005; Robinson and Sigman, 2008; Sigman *et al.*, 1999]. The published Southern Ocean data present a spatially complex picture of nutrient dynamics over the last several glacial cycles [Combes *et al.*, 2008; Crosta and Shemesh, 2002; Robinson *et al.*, 2004; Robinson *et al.*, 2005; Robinson and Sigman, 2008; Sigman *et al.*, 1999] that may stem not only from heterogeneous nutrient dynamics but from potential shortcomings in the proxy itself. The goals of this dissertation are to 1) validate the proxy in cultures, 2) compare culture and sedimentary $\delta^{15}\text{N}$ data, and 3) improve our ability to use sedimentary N isotopes in paleoceanographic reconstructions. The relationships between the $\delta^{15}\text{N}$ of the seawater substrate nitrate ($\delta^{15}\text{NO}_3^-$), the bulk organic matter ($\delta^{15}\text{N}_{\text{bulk}}$), and the diatom-bound fraction ($\delta^{15}\text{N}_{\text{DB}}$) of this organic matter are explored with respect to the progressive consumption of nitrate in seawater. Subsequently, the culture results are used to inform interpretation of downcore measurements, as part of a

paleoceanographic study examining past changes in Southern Ocean circulation and nutrient dynamics. Finally, the differences in bulk and diatom-bound nitrogen isotope values in downcore profiles are investigated. This final component aims to determine the magnitude of overprinted signals on top of the known variations induced by changing nutrient utilization.

Manuscript Descriptions

This doctoral dissertation is broken into three chapters written in the manuscript format. The first chapter, *Nitrogen isotopic relationship between diatom-bound and bulk organic matter of cultured polar diatoms*, investigates the relationship between the isotopic composition of the seawater nitrate ($\delta^{15}\text{NO}_3^-$), the bulk organic material ($\delta^{15}\text{N}_{\text{bulk}}$), and the diatom-bound fraction ($\delta^{15}\text{N}_{\text{DB}}$). The goal was to test, in culture, the notion that diatom-bound $\delta^{15}\text{N}$ records the partial consumption of dissolved nitrate, as it has been assumed for downcore reconstructions of nutrient utilization. Before the culture work could proceed, cleaning techniques to clean freshly cultured material and diatom microfossils had to be developed. A robust cleaning protocol, with a post-cleaning analytical precision of $\pm 0.21 \mu\text{mol N} / \text{g opal}$ for N content and $\pm 0.45\text{‰}$ for $\delta^{15}\text{N}_{\text{DB}}$, that could be used effectively on both cultures and sediments, with only slight modification, resulted. The cleaning techniques were compared across laboratories between the Robinson Lab at URI and the Sigman laboratory at Princeton University for further validation.

In each experiment, the $\delta^{15}\text{N}$ of the nitrate was greater than the bulk, which was in turn greater than the diatom-bound fraction. Each fraction increased with

consumption, as predicted, with a Rayleigh type relationship. The offset between the bulk and the diatom-bound fraction ($\delta^{15}\text{N}_{\text{DBoffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) was positive in culture, something that was not predicted from the sedimentary data. $\delta^{15}\text{N}_{\text{DB}} > \delta^{15}\text{N}_{\text{bulk}}$ is more common, so that the $\delta^{15}\text{N}_{\text{DBoffset}}$ is negative. The difference in the sign of the offsets is difficult to explain and it indicates the need to rethink the assumption that diatom-bound N is protected from alteration. This is addressed in detail in chapter three. The isotope effect (ϵ) was measured in culture and is due to fractionation during nitrate assimilation. ϵ and $\delta^{15}\text{N}_{\text{DBoffset}}$ both vary between species and are linearly related across all species. Differences between species imply a need to know how species assemblages vary through time and indicate that more species-specific work needs to be done. Finally, variations in ϵ , primarily attributed to changes in light availability, may overprint the diatom-bound signal, to a small degree.

The material from the first manuscript was presented under the same title at the American Geophysical Union (AGU) fall meeting in December of 2009, where it won an Outstanding Student Paper Award. The manuscript was submitted to *Paleoceanography* and accepted for publication in January of 2011. It is currently in the final stage of revision.

In the second manuscript, *Southern Ocean nitrogen and silicon dynamics during the last deglaciation*, coupled N and Si isotope measurements are used alongside opal fluxes, to determine a quasi-quantitative estimate of nutrient supply, or upwelling. The goal was to investigate nutrient utilization and changes in the degree of stratification during the deglaciation using the high-resolution (~500 year) core TN057-13PC4. A large change in $\delta^{15}\text{N}_{\text{DB}}$ (and not in $\delta^{15}\text{N}_{\text{bulk}}$) at approximately 18 kya

is synchronous with a large shift in species assemblage. The culture work indicated that large changes in species may impart a shift in $\delta^{15}\text{N}$ and more specifically, the observed shift in assemblage composition was consistent with the increase in $\delta^{15}\text{N}_{\text{DB}}$. Removing all of the $\delta^{15}\text{N}$ data prior to 20 kya from analysis provided a more straightforward interpretation of nutrient utilization. The deglaciation was extremely dynamic in the Southern Ocean, with two large peaks in opal flux that were synchronous with drops in $\delta^{15}\text{N}_{\text{DB}}$. The data imply that nutrient supply was greater than the elevated biological demand (opal). This is attributed to the reinvigoration of overturning circulation during the deglaciation in the Southern Ocean, and implies a significant relocation of CO_2 from the deep ocean to the surface ocean-atmosphere system. This event also appears to be linked to an observed transient peak in nutrient supply to the low latitudes [Pena *et al.*, 2008; Robinson *et al.*, 2009; Spero and Lea, 2002]. This material was presented under the title *High Latitude Controls on Tropical Biogeochemistry* at the International Conference on Paleoceanography's ICP 10 meeting in San Diego California in August of 2010. This manuscript was submitted to *Earth and Planetary Science Letters* in January of 2011 and is currently in review.

The third and final chapter, *Comparing bulk and diatom-bound nitrogen isotopes in Southern Ocean downcore profiles*, examines both the bulk and diatom-bound $\delta^{15}\text{N}$ proxies in detail in light of the culture results presented in Chapter one. Records of bulk and diatom-bound $\delta^{15}\text{N}$ from five Southern Ocean cores are presented. Again, the diatom-bound offsets observed in culture are significantly different from what is observed in sediments and cannot be explained through alteration of the $\delta^{15}\text{N}_{\text{bulk}}$. A mass balance calculation, based upon expectations from

culture and observations from sediments indicate that an isotopically light fraction present in fresh culture material is preferentially lost from the diatom-bound fraction in sediments. Chitin, an important component of the diatom frustule is tentatively targeted as the “missing N” because it is chemically unstable, degrading with time. These findings suggest that the diatom-bound material is not completely protected. However, the impact of losing this low $\delta^{15}\text{N}$ fraction does not appear to erase or significantly overprint nutrient utilization changes, suggesting that its removal may be rapid and complete and that the remaining fraction adequately reflects surface water conditions. This assertion is of critical importance to the future of the proxy and needs to be tested. This manuscript is currently in preparation for submission to *Paleoceanography*.

Complications in the nitrogen isotope proxy have been investigated in this dissertation. While studies have shown that numerous variables may influence the $\delta^{15}\text{N}$ of live culture and sedimentary material, the information here provides tools to tease out changes in past nutrient utilization. Future work needs to investigate various growth conditions in culture and their influence on $\delta^{15}\text{N}$. Additionally, analysis of a single species or a more constrained assemblage, through gravitational SPLITT fractionation or potentially flow cytometry, will improve our ability to isolate the utilization signal. Inter-laboratory standardizations are necessary and inter-laboratory comparisons are recommended, as there is no standardized method for isolating the organic matter in the diatom-bound fraction. In summary, both bulk $\delta^{15}\text{N}$ and diatom-bound $\delta^{15}\text{N}$ adequately record significant changes in nutrient consumption in the past. The addition of common and accessible ancillary data including species assemblage,

opal flux, and stable silicon isotope measurements provide additional constraints to more accurately interpret reconstructions.

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**Nitrogen isotopic relationship between diatom-bound and bulk organic matter of
cultured polar diatoms**

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Abstract

Using batch cultures, the $^{15}\text{N}/^{14}\text{N}$ (hereafter $\delta^{15}\text{N}$) of diatom-bound organic matter was measured and compared to the $\delta^{15}\text{N}$ of bulk diatom biomass during the progressive consumption of a nitrate pool in four polar diatom species (*Fragilariopsis cylindrus*, *Fragilariopsis kerguelensis*, *Pseudo-nitzschia seriata*, and *Thalassiosira nordenskiöldii*) and one temperate species (*Thalassiosira aestivalis*). In general, the $\delta^{15}\text{N}$ of the dissolved nitrate in seawater was greater than that of the bulk, which was greater than that of the diatom-bound N. Rayleigh-type relationships were observed, allowing for estimation of the isotope effect (ϵ) for each species, with a range from 1.0 to 14.0‰ across all species. For all cultured strains, the $\delta^{15}\text{N}$ of the diatom-bound ($\delta^{15}\text{N}_{\text{DB}}$) was lower than that of the bulk diatom biomass ($\delta^{15}\text{N}_{\text{bulk}}$). The isotopic offset between the bulk and diatom-bound N ($\delta^{15}\text{N}_{\text{DBoffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) was relatively constant along the growth curve for each individual species but varied among species, with a range of 1.9-11.2‰. Weak relationships were determined when ϵ and the $\delta^{15}\text{N}_{\text{DBoffset}}$ were compared to cellular size and surface area:volume ratio. More significantly, with the exception of *Pseudo-nitzschia seriata*, a strong positive relationship was found between ϵ and $\delta^{15}\text{N}_{\text{DBoffset}}$. While our culture data indicate a positive $\delta^{15}\text{N}_{\text{DBoffset}}$ across all studied diatom species, surface sediment data suggest a negative $\delta^{15}\text{N}_{\text{DBoffset}}$ for sedimentary assemblages. This indicates that either (1) the growth conditions of our cultures had some effect on $\delta^{15}\text{N}_{\text{DBoffset}}$, or (2) a low- $\delta^{15}\text{N}$ component of the N that we measure as diatom frustule-bound is lost during early diagenesis. Given documented assemblage changes, our culture data for relevant species do not suggest that the higher $\delta^{15}\text{N}_{\text{DB}}$ observed in the Antarctic during ice ages

can be explained by changes in the sedimentary bulk-to-diatom-bound isotopic difference. Future work on the diatom bound material in cultured diatoms grown under *in situ* nutrient conditions, analysis of sediment trap and net tow material, and frustule dissolution experiments will more completely assess this paleoproxy.

1. Introduction

Primary productivity in the Southern Ocean is mainly limited by the availability of iron, and to a lesser degree light and silicic acid [Boyd *et al.*, 1999; Dugdale *et al.*, 1995; Martin *et al.*, 1990a; Martin *et al.*, 1990b; Nelson and Smith, 1991; Sedwick *et al.*, 1997; Sedwick *et al.*, 1999], leaving an excess of nitrate, an essential nutrient, in the surface ocean. Under these nitrate-replete conditions, the relative degree of uptake and assimilation of nitrate is recorded in the nitrogen isotopic composition of the resulting organic matter, where fractionation occurs during the reduction of nitrate to nitrite, by the enzyme nitrate reductase [Needoba *et al.*, 2004; Shearer *et al.*, 1992]. ^{14}N is preferentially incorporated into organic matter relative to ^{15}N [Altabet *et al.*, 1991; Cifuentes *et al.*, 1989; Horrigan *et al.*, 1990; Montoya *et al.*, 1991; Wada and Hattori, 1978; Wada, 1980]. The result is an offset, whereby the $^{15}\text{N}/^{14}\text{N}$ (as $\delta^{15}\text{N} = [(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1] * 1000$, where atmospheric N_2 is the universal reference) of phytoplankton is lower than that of the substrate nitrate in seawater. During progressive consumption, the $\delta^{15}\text{N}$ of both the nitrate substrate ($\delta^{15}\text{NO}_3^-$) and bulk organic matter ($\delta^{15}\text{N}_{\text{bulk}}$) increases, as the concentration of nitrate decreases. Going back in time, relative changes in sedimentary $\delta^{15}\text{N}$ are thought to reflect changes in the degree of nutrient consumption in the surface ocean [Altabet and Francois, 1994].

Nitrogen dynamics in the paleo surface-ocean have been interpreted using bulk sedimentary nitrogen in sediments, which may undergo diagenetic alteration in sediments with poor organic matter preservation and possible isotopic changes during sinking and early burial [Altabet and Francois, 1994; Lourey *et al.*, 2003; Shemesh *et*

al., 1993; *Sigman et al.*, 1999a]. In contrast, the organic fraction encased in the silica shell, known as the diatom-bound N (technically frustule-bound), is thought to be protected from alteration and is hypothesized to be a much more robust archive of historical changes in surface N [*Robinson et al.*, 2004; *Shemesh et al.*, 1993; *Sigman et al.*, 1999a]. Recently, diatom-bound N isotopes have been used to investigate changes in nutrient drawdown in the nutrient-rich polar ocean [*Brunelle et al.*, 2007; *Brunelle et al.*, 2010; *Crosta and Shemesh*, 2002; *Robinson et al.*, 2004; *Robinson et al.*, 2005; *Robinson and Sigman*, 2008; *Shemesh et al.*, 2002; *Sigman et al.*, 1999b].

Uncertainties associated with the sedimentary $\delta^{15}\text{N}$ proxies make consistent interpretations difficult. Offsets in the $\delta^{15}\text{N}$ of total sedimentary nitrogen ($\delta^{15}\text{N}_{\text{bulk}}$) are elevated by 3-5‰ relative to sinking particulate nitrogen (PN) captured in sediment traps [*Altabet and Francois*, 1994]. Differences of up to 3‰ can also occur in the $\delta^{15}\text{N}$ of sinking PN ($\delta^{15}\text{N}_{\text{PN}}$) between shallow and deep traps with $\delta^{15}\text{N}_{\text{PN}}$ ranging between -1‰ to 5‰ [*Altabet and Francois*, 2001; *Lourey et al.*, 2003]. Summertime Southern Ocean $\delta^{15}\text{N}_{\text{PN}}$ values are low, between -0.1‰ and 1.7‰ during this period of peak sedimentation. However, wintertime $\delta^{15}\text{N}_{\text{PN}}$ is much higher during low productivity winter months when nutrient consumption is lowest, diverging from expectations based on isotope fractionation during nitrate assimilation. It was hypothesized that these differences are due to a reduction in the isotope effect during nitrate assimilation, or possibly more extensive isotopic alteration of sinking material during low-flux periods [*Lourey et al.*, 2003]. These observations suggest that the assumption of a constant offset between biomass and bulk sediment $\delta^{15}\text{N}$ is likely incorrect.

The diatom-bound proxy has been applied in the Southern Ocean, where diatoms

are the dominant photosynthetic organisms and are responsible for the majority of export production, producing an opal belt around Antarctica [*Bathmann et al.*, 1997; *Hart*, 1942; *Hasle*, 1969; *Lancelot et al.*, 2000]. Diatom-bound organic compounds are required for silica deposition during periods of growth, as cells reproduce and form a new frustule [*Martin-Jezequel et al.*, 2000; *Sumper and Kroger*, 1994; *Sumper et al.*, 2005; *Sumper and Brunner*, 2008]. Encased in silica, the organic fraction of diatoms' fossil remains makes a promising archive of surface ocean conditions. Diatom-bound $\delta^{15}\text{N}$ includes only organic N bound within the silica frustule and thus avoids uncertainties related to alteration. In addition to removing the effects of alteration during sinking and burial, targeting the diatom-bound fraction eliminates any additional isotopic enrichment related to the incorporation of organic material from multiple trophic levels.

However, the proxy presents uncertainties of its own. Large changes in diatom $\delta^{15}\text{N}$ within paleoceanographic records and differences between sites have been attributed to a spatially and temporally heterogeneous environment. An alternative explanation for these rapid, high-amplitude changes involves variation in the isotopic relationship between the bulk biomass of diatoms and the N that they incorporate into their frustules. Each diatom species makes their own suite of long chain polyamines (LCPA) or polypeptide silaffins, large N-rich molecules that serve as biomineralization compounds, forming a template for silica frustules [*Kroger and Wetherbee*, 2000; *Kroger et al.*, 2002; *Sumper et al.*, 2005; *Sumper and Brunner*, 2008]. Other nitrogen-containing compounds in the frustule include mycosporine-like amino acids [*Ingalls et al.*, 2010], chitin [*Brunner et al.*, 2010; *Durkin et al.*, 2009],

and probably others that have yet to be characterized. The specific biochemical and biophysical mechanisms of isotope fractionation during diatom frustule-bound N synthesis (other than the fractionation by nitrate reductase) are unknown, as the specific processes and compounds involved in frustule formation are still being studied. Individual compounds may have distinct isotopic signatures. Since these compounds are found in different combinations and relative contributions, diatom species may vary in the $\delta^{15}\text{N}$ relationship between their diatom-bound N and the rest of their biomass.

To examine species-specific variation in the $\delta^{15}\text{N}$ of incorporated N in both the diatom-bound and bulk organic material, we used large batch cultures to quantify the relationships between the growth media ($\delta^{15}\text{NO}_3^-$), bulk biomass N ($\delta^{15}\text{N}_{\text{bulk}}$), and diatom-bound N ($\delta^{15}\text{N}_{\text{DB}}$). Five diatom species encompassing a range of morphology, physiology, and taxonomy were selected. All but one are environmentally relevant species to Southern Ocean studies. Our results suggest that interspecies variations in offsets between the $\delta^{15}\text{N}$ of diatom biomass and the diatom-bound material are significant. Based on these observed species-related differences, we re-evaluate changes in diatom-bound $\delta^{15}\text{N}$ in a Southern Ocean sediment core.

2. Samples and Methods

2.1. Diatom Species and Culturing

Taxonomic diversity was included in this investigation to determine if these differences produce any systematic variability in the isotopic composition of diatom-bound N. These diatom species ranged in reported size from approximately 3 to 75

µm, included fast and slow growing species with varying degrees of silicification, and included several chain-formers. Experiments focused on five diatom species, including *Fragilariopsis kerguelensis*, the dominant pennate diatom species in the Southern Ocean and largest in this study (~25 µm) (supplied by P. Assmy, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany), *Fragilariopsis cylindrus* (Van Heurck) Hasle (Provasoli-Guillard National Center for the Culture of Marine Phytoplankton (CCMP), strain 1102), a pennate ice-edge indicator (~6 µm), *Thalassiosira nordenskioeldii* Cleve (CCMP997), a centric cold-water chain-forming diatom (~13 µm), and *Pseudo-nitzschia seriata* (Cleve) Peragallo (CCMP1309), a pennate domoic-acid producing diatom and the smallest in this study (~5 µm). The temperate centric *Thalassiosira aestivalis* (GSO100), isolated from the eastern North Pacific, was also used (~15 µm).

Semi-continuous batch cultures were maintained in 20 ml sterile filtered F/2 media [Guillard, 1975] by frequently transferring small volumes into fresh media during exponential growth, well before the stationary phase was reached. After multiple generations under constant conditions, the specific growth rate for each species was determined in serial, triplicate cultures. Fluorescence was monitored daily using a model 10-AU fluorometer (Turner Designs). Specific growth rates were determined using the relationship between *in vivo* chlorophyll-*a* fluorescence and the exponential increase in cell numbers [Brand *et al.*, 1981]. The chlorophyll-*a* proxy for growth was verified by volumetric cell counts for each species using a Multisizer II (Coulter, Inc.) with a 140 µm aperture drawing 500 µL per sample. Cell counts were also conducted at 250x using an SZX16 microscope (Olympus) equipped with phase

contrast optics.

Glassware was acid washed and autoclaved. Whatman glass fiber filters (GFF's) were pre-combusted (450°C) in a muffle oven. Medium for experiments was prepared using 0.2 µm sterile-filtered Narragansett Bay seawater and 0.2 µm sterile filtered-nutrient stock solutions [Guillard and Ryther, 1962; Guillard, 1975]. To prevent precipitation of nutrients, F/2 medium was not autoclaved after filtering. Because specific nitrate reductase activity in light was observed to be five times higher than in the dark [Eppley *et al.*, 1970], the cultures were maintained under continuous light at 100 µmol photons m⁻²s⁻¹. Additionally, this light level has been shown to saturate the photochemical apparatus of Antarctic diatoms and would be found at this level or higher throughout the entire summer mixed layer [Knox, 2007; Sakshaug and Holm-Hansen, 1986]. *Thalassiosira aestivalis* was maintained at 18°C, while all other diatom cultures were kept at 2°C.

Experiments began when each 20 L carboy, filled with 20 L of sterile-filtered F/2, was inoculated with ~200 ml of an exponentially-growing culture. To ensure near-complete nitrate consumption during the experiment, 150-250 µM NO₃⁻ was added, equaling only 15-30% of the amount prescribed for F/2 media. Carboys were agitated to prevent settling of diatoms using a magnetic stir shaft with a 7.5 cm bladed propeller spinning at approximately 250 rpm. Tygon® tubing was threaded through the lid to approximately 1 cm above the propeller, fastened and sealed in place. This siphon tube was used to draw samples during the experiment and was otherwise sealed to prevent contamination. Carboy experiments were run in duplicate or triplicate for each species.

Experiments were sampled daily over the course of two to five weeks, along the growth curve, until the stationary phase was reached. Daily measurements included fluorescence, nitrate concentration of the seawater, $\delta^{15}\text{NO}_3^-$, and the $\delta^{15}\text{N}_{\text{bulk}}$. The $\delta^{15}\text{N}_{\text{DB}}$ and nitrogen content of diatom frustules was measured with less frequency due to the nitrogen requirement for each sample. Between four and ten discrete diatom-bound samples were gathered for each culture along the growth curve.

Each fluorescence sample was preserved using 2% acid Lugols solution [Thronsdon, 1978] for cell counts. Cell counts were made using a Multisizer II Coulter Counter on these preserved samples and the relationship of each culture's cell number versus fluorescence was then determined. Cell size was also measured using the Coulter counter and verified with 25 measurements per experiment on an inverted epifluorescent Nikon Diaphot 300 microscope, equipped with phase contrast at 400x. Cell volumes from the Coulter counter were not used as they assumed a spherical diatom. Instead, measurements of each axis of the diatom allowed for the calculation of cell surface area and volume. Water samples (~100 ml) collected for seawater nitrate, phosphate, and silicate concentration and the $\delta^{15}\text{NO}_3^-$ were gently (~50 mm Hg vacuum) filtered through pre-combusted Whatman GFF's. Approximately 25 ml of filtrate was used to rinse the container and the remaining 75 ml aliquots were frozen for chemical analysis. The GFF was rinsed delicately with filtered seawater and frozen separately, to provide the $\delta^{15}\text{N}_{\text{bulk}}$. For diatom-bound sampling, between 1-5 L of water (depending on biomass present) was collected from the carboy, spun down in a centrifuge to collect the diatoms, and the remaining water was poured off. This provided a more rapid method for concentrating the sample and reduced biomass loss

on filters. All samples were stored at -20°C for later analysis as a group.

2.2. Diatom-bound Cleaning

The high organic matter content of freshly harvested cells, relative to typical deep ocean sediments, required alteration of the existing methodology for chemically cleaning frustules [Brunelle *et al.*, 2007; Robinson *et al.*, 2004; Robinson *et al.*, 2005; Sigman *et al.*, 1999a]. Frozen diatom-bound samples were thawed, rinsed with Milli-Q water and cleaned using (1) 35 ml of 2% by weight sodium lauryl sulfate at room temperature for ~15 min, (2) a preliminary oxidation using ~15 ml saturated potassium permanganate and ~15 ml saturated oxalic acid, after 5 ml of 18 M sulfuric acid addition [Hasle and Fryxell, 1970], and (3) a final oxidation using 72% perchloric acid in a 100°C water bath for one hour. Samples were rinsed thoroughly with Milli-Q water between each consecutive treatment. An ultrasonic bath and vortex mixer were used to ensure complete reaction/rinse and prevent any clumping of sample material during each step. Diatom-bound samples were rinsed thoroughly with Milli-Q and then dried in a 60°C oven overnight prior to the oxidation of 2-20 mg of cleaned frustule by 1.5 ml of a 0.22 M potassium persulfate and 1.5 M sodium hydroxide solution in an autoclave at 121°C for 20 minutes [Bronk *et al.*, 2000; Robinson *et al.*, 2004].

2.3. Chemical Analysis

Nitrate concentrations of both the growth media and the persulfate solutions containing oxidized diatom-bound N were measured by chemiluminescence after

reduction to NO using a Teledyne Instruments (Model 200E) chemiluminescence NO/NO_x analyzer and potassium nitrate reference standards [Braman and Hendrix, 1989]. Orthophosphate (USEPA method 365.5) [Grasshoff, 1976; Murphy and Riley, 1962] and silicate [Grasshoff, 1976; Parsons *et al.*, 1984] concentrations were determined using a Lachat Instruments QuickChem 8000 flow injection analyzer.

The $\delta^{15}\text{NO}_3^-$ and $\delta^{15}\text{N}_{\text{DB}}$, were measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) on a Thermo Delta V Advantage IRMS. Nitrate was converted to N₂O for injection following the denitrifier method [Sigman *et al.*, 2001]. The potassium nitrate reference material IAEA-N3 (4.7‰) was used to standardize diatom-bound and substrate $\delta^{15}\text{N}$ results [Bohlke and Coplen, 1995]. The $\delta^{15}\text{N}_{\text{bulk}}$ was measured by combustion of the frozen, oven dried (60°C) material. The glass fiber filters were packed into a tin cup for measurement by elemental analyzer coupled to the IRMS (EA-IRMS). Ammonium sulfate reference materials IAEA-N1 (0.4‰) and IAEA-N2 (20.3‰), and an in-house standard, amino caproic acid, were used to standardize EA-IRMS results [Bohlke and Coplen, 1995; Crosta and Shemesh, 2002; Revesz *et al.*, 1997]. Precision was determined by multiple measurements of standards within each run and replicate measurements of samples. All NO₃⁻ concentrations and 50% of the $\delta^{15}\text{NO}_3^-$ samples were run in duplicate to within $\pm 1.1\%$ for concentration and $\pm 0.3\%$ for isotopic values. Approximately 50% of bulk samples were run in duplicate with $\delta^{15}\text{N}_{\text{bulk}}$ values within $\pm 0.4\%$. Only 15% of the diatom-bound samples were run in duplicate, due to small sample sizes, with average $\delta^{15}\text{N}_{\text{DB}}$ precision of $\pm 0.8\%$. Blank contributions and the associated errors in $\delta^{15}\text{N}_{\text{DB}}$ measurement were significant due to the blank associated with the alkaline persulfate reagent, typically 2-

3 μM . Because of the small sample sizes, in particular early in the growth experiments, the blank accounted for up to 40% of the total N. Direct measurement of the persulfate reagent $\delta^{15}\text{N}$ contribution is not consistently possible. The denitrifiers often die when the necessarily large volume of blank is added to the bacteria vial. However, estimates of the blank $\delta^{15}\text{N}$ were made by measuring the $\delta^{15}\text{N}$ of several dilution series of amino acids with known isotopic compositions. Based on these experiments, a conservative range of 0 to -5‰ was assigned to the $\delta^{15}\text{N}_{\text{blank}}$. The actual $\delta^{15}\text{N}_{\text{DB}}$ was then calculated by mass balance using the measured value and this range. The error bars for each diatom-bound point are based on these error estimates, in addition to replicate measurements made when possible. Statistical significance was determined using a two-tailed, type-3 T-test to provide p-values for variables conservatively determined to be unpaired and independent with unequal variances.

3. Results and Discussion

3.1. Diatom Growth

In replicate carboy experiments, exponential growth rates within each diatom species were comparable, with an average coefficient of variation of 14%, ranging from 1-27%. Specific growth rates between species ranged from 0.41-1.34 day^{-1} , or 18-59 hours doubling time (Table 1.1). Centric species grew significantly faster than pennate species ($p=0.008$). Growth rate has been shown to affect silicification, with slower growths resulting in greater silicification, as more time is spent in the cell wall synthesis phase [Martin-Jezequel *et al.*, 2000]. However, differences in growth rate do not influence the isotopic fractionation of N during NO_3^- assimilation [Needoba and

Harrison, 2004].

3.2. Nutrient Concentrations and $\delta^{15}\text{NO}_3^-$

The average initial concentration of nitrate was $212 \pm 38 \mu\text{M}$ with an initial $\delta^{15}\text{NO}_3^-$ of $8.9 \pm 0.3\text{‰}$ for all experiments. Phosphate and silicate were provided in excess of the expected demand to ensure that NO_3^- was the limiting nutrient (Table 1.1). Average initial concentrations were $33.5 \pm 1.1 \mu\text{M}$ phosphate and $121.5 \pm 3.4 \mu\text{M}$ silicate. In an attempt to account for their increased silica demands, carboys containing *Fragilariopsis kerguelensis* were spiked with $162.6 \pm 3.4 \mu\text{M}$ silicate. On average, the fraction of initial nitrate remaining (f) at the end of experiments was 0.27 ± 0.16 , indicating significant utilization of nitrate. The wide range in final silicate concentrations demonstrates the differences in silica requirement for each species (Table 1.1). Final silicate concentrations of $1.1 \mu\text{M}$ and $2.3 \mu\text{M}$ were observed in *Fragilariopsis kerguelensis* cultures, indicating that they may have become silica limited nearing the end of each experiment. All other cultures had significantly more silica present in their final sample ($4.4\mu\text{M}$ to $75.5\mu\text{M}$), reducing the likelihood of silica limitation during growth. At no point did the phosphate concentration fall below $\sim 15 \mu\text{M}$ in any experiment.

The NO_3^- concentration decreased in each closed system carboy experiment during growth while the $\delta^{15}\text{NO}_3^-$ increased in a logarithmic manner (Figure 1.1). The Rayleigh equation (eq. 1) defines this fractional distillation of the NO_3^- pool as a function of the isotope effect (ϵ), and the degree of nitrate utilization, or f .

$$\delta^{15}\text{NO}_3^- = \delta^{15}\text{NO}_3^-_{t=0} - \epsilon \ln f \quad \text{eq. 1}$$

The fraction of initial nitrate remaining at the time of measurement, f , is defined as $f = [\text{NO}_3^-]_{\text{measured}} / [\text{NO}_3^-]_{\text{initial}}$. Defined as the ratio of assimilation rates for each isotope $[\epsilon = ({}^{14}k / {}^{15}k - 1) \times 1000]$, where ${}^{14}k$ and ${}^{15}k$ are the rate coefficients of the reaction for ${}^{14}\text{N}$ - and ${}^{15}\text{N}$ -containing reactant, respectively, ϵ reflects the preferential incorporation of ${}^{14}\text{N}$ into biomass and the resulting isotopic offset between the organic matter and the nitrate pool at any instant. Therefore, as nitrate utilization increases (decreasing f), both the nitrate pool and the bulk biomass are progressively enriched in ${}^{15}\text{N}$ (Figure 1.2a). ϵ was estimated from the slope of the $\delta^{15}\text{NO}_3 - f$ curves for each species and culture based on equation 1 (Figure 2b-f).

Most species exhibited relatively consistent ϵ over multiple experiments (Table 1.1). The two species with variable ϵ were *Pseudo-nitzschia seriata* and *Fragilariopsis cylindrus*. There was a wide range in estimated ϵ , with observed values between 1-14‰. The overall mean ϵ was $5.03 \pm 3.42\text{‰}$, which encompassed all species and experiments, except *Pseudo-nitzschia seriata* T35, within a 95% CI. The high degree of variability between all species was observed in other culturing experiments and has been attributed to a variety of factors including aeration, mechanical agitation, light levels, and nutrient concentrations [Montoya and McCarthy, 1995; Needoba and Harrison, 2004; Wada, 1980].

Fragilariopsis kerguelensis and *Thalassiosira aestivalis* experiment T34 did not follow the predicted relationship as closely as other species, with an r^2 of 0.50 for both (Figure 1.1). *Fragilariopsis kerguelensis* showed very little change with f , such that the almost negligible slope produced the weak r^2 . *Thalassiosira aestivalis* T34 started with an exceedingly high concentration of NO_3^- (850.6 μM), which was not utilized as

completely as other experiments. The low r^2 here is attributed to fitting a curve to such a limited observed portion of f . However, even with a condensed $\delta^{15}\text{NO}_3^-$ curve, with f ranging only from 1.0 to 0.78, the calculated ϵ still fell, within error, alongside the other ϵ values from replicate *Thalassiosira aestivalis* experiments.

Overall, pennate diatoms were observed to have a much higher degree of variability in ϵ (Table 1.1). This was seen within replicate experiments of the same species as well as between different pennate species. Centric diatoms had more consistent ϵ values throughout. Despite the observed variability within and between species, the average ϵ for pennate ($5.50 \pm 4.37\text{‰}$) and centric ($4.29 \pm 0.71\text{‰}$) diatoms were statistically indistinguishable ($p=0.465$). These results are similar to enrichment factors that have been observed in other phytoplankton species with values typically ranging between 4-9‰, with some as low as 0.9 and others over 12‰, [Altabet and McCarthy, 1985; Altabet et al., 1991; Cifuentes et al., 1989; DiFiore et al., 2009; Goering et al., 1990; Granger et al., 2004; Granger et al., 2008; Horrigan et al., 1990; Montoya et al., 1991; Montoya and McCarthy, 1995; Needoba et al., 2003; Sigman et al., 1999b; Wada, 1980].

3.3. Bulk $\delta^{15}\text{N}$

$\delta^{15}\text{N}_{\text{bulk}}$ values were consistently lower than $\delta^{15}\text{NO}_3^-$ values over the entire growth curve and increased logarithmically with decreasing f (Figure 1.2). Overall, measured $\delta^{15}\text{N}_{\text{bulk}}$ fit expectations based on the accumulated product equation of Mariotti et al. [1981].

$$\delta^{15}N_{bulk} = \delta^{15}NO_3^-_{t=0} - \epsilon \left(\frac{f \ln f}{1-f} \right) \quad \text{eq. 2}$$

Using a $\delta^{15}NO_3^-_{t=0}$ of 8.9‰ and the calculated ϵ 's from the $NO_3^-/\ln f$ curves, we estimated bulk $\delta^{15}N$ for each specific species and carboy. The measured bulk $\delta^{15}N$ values lie along the Rayleigh predictions (Figure 1.2), indicating that the cultures reflect a closed system, where the nitrate was assimilated into biomass with little or no recycling. While Rayleigh predictions were close for each species, several subtleties were present in specific cultures. *Thalassiosira nordenskioeldii* demonstrated negligible trends in $\delta^{15}N_{bulk}$ with observed increases of only 1‰ (Figure 1.2b). Predictions indicated that at least a 2‰ change should have been observed. Additionally, the $\delta^{15}N_{bulk}$ measurements for *Thalassiosira nordenskioeldii* were ~1‰ lower than the predicted values. The bulk values for *Pseudo-nitzschia seriata* were approximately ~1‰ higher than the predicted (Figure 1.2d). However, based upon the predicted versus measured differences in $\delta^{15}N_{bulk}$, no cultures deviated significantly in either direction from the predicted result. There were no statistically significant differences between pennate and centric diatoms.

3.4. Diatom-bound $\delta^{15}N$

$\delta^{15}N_{DB}$ values were consistently lower than $\delta^{15}N_{bulk}$ values over the entire growth curve. Overall, $\delta^{15}N_{DB}$ values tended to increase in much the same way as the $\delta^{15}N_{bulk}$, with decreasing f (Figure 1.2). However, the $\delta^{15}N_{DB}$ measurements were significantly more variable than the substrate or the bulk $\delta^{15}N$ measurements. The

variability could be attributed to several factors including (1) small sample size, (2) a large persulfate blank (relative to the sample), and (3) excessive cleaning and alteration. An average of ~3 mg/sample of cleaned opal was available for diatom-bound measurements, compared to 7-12 mg/sample typically cleaned from fossil cores. This was a larger problem for diatom-bound samples taken near the beginning of each experiment, before any significant growth had taken place and where up to 5 L of water was collected to obtain the required amount of diatoms for cleaning. As a result, error tended to decrease with decreasing f due to increased biomass recovery and a proportional reduction in blank contribution as the experiment progressed (Figure 1.2). In addition to the large blank contributions, cleaning fresh diatom material is difficult. Fresh diatom opal is more soluble and reagent sensitive than fossil diatom opal. Fractionation can occur due to “over-cleaning” and possible frustule dissolution prior to the alkaline persulfate step. We developed several different cleaning protocols and tested them on cultured diatom material. The method used to clean the cultured material was chosen for its high degree of precision in both N content ($\pm 0.21 \mu\text{mol N/g opal}$) and $\delta^{15}\text{N}_{\text{DB}}$ ($\pm 0.45\text{‰}$). It is unlikely that this cleaning protocol fractionated samples, although we cannot completely discount it, given the range of size and morphology of the diatoms cultured. The N-content in cleaned material for diatom-bound analysis of *Pseudo-nitzschia seriata* T35 and *Fragilariopsis cylindrus* T33 was significantly lower ($p=0.001$) than the other experiments, potentially a result of over-cleaning and alteration. However, the generally greater variability in these cultured diatom-bound measurements relative to our previous sedimentary $\delta^{15}\text{N}_{\text{DB}}$ is attributable to the small sample sizes and blank

contamination. The nitrogen content of cleaned diatom-bound material is within the range found in other studies [Robinson *et al.*, 2004; Robinson *et al.*, 2005; Sigman *et al.*, 1999a].

For each individual carboy experiment, the diatom-bound $\delta^{15}\text{N}$ values appeared to be offset from the bulk by nearly constant amounts ($\delta^{15}\text{N}_{\text{DBoffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) over the course of the growth curve. The $\delta^{15}\text{N}_{\text{DBoffset}}$ ranged from approximately 2-10‰, but was relatively consistent for each species with an overall average of 5.3‰ (Table 1.1). Much like the $\delta^{15}\text{N}_{\text{DB}}$ values, inter-species and replicate experiment variability in $\delta^{15}\text{N}_{\text{DBoffset}}$ and the associated measurement error decreased as f decreased and sample size increased. Given no clear trends, we assume that the ϵ for each specific species was constant, and a $\delta^{15}\text{N}_{\text{DB}}$ relationship could be described by:

$$\delta^{15}\text{N}_{\text{DB}} = \delta^{15}\text{NO}_3^-_{t=0} - \epsilon \left(\frac{f \ln f}{1-f} \right) - \delta^{15}\text{N}_{\text{DBoffset}} \quad \text{eq. 3}$$

Six $\delta^{15}\text{N}_{\text{DB}}$ points were higher than their corresponding $\delta^{15}\text{N}_{\text{bulk}}$ measurement (negative values of $\delta^{15}\text{N}_{\text{DBoffset}}$) and most of them occurred early in growth (i.e. high f). The average value of $\delta^{15}\text{N}_{\text{DBoffset}}$ did not change systematically with f . At high f , the range in $\delta^{15}\text{N}_{\text{DBoffset}}$ measurements was large, tapering in towards more consistent values as more NO_3^- was consumed. The initial scatter at high values of f is attributed to random analytical error associated with the aforementioned small sample sizes and blank. *Pseudo-nitzschia seriata* T35 was the single exception to the $\delta^{15}\text{N}_{\text{DBoffset}}$ relationship, whereby diatom-bound points were consistently higher than the bulk

(Table 1.1). *Pseudo-nitzschia seriata*'s negative $\delta^{15}\text{N}_{\text{DBoffset}}$ may be attributed to its small size. Alternatively, the low N-content of cleaned material for *Pseudo-nitzschia seriata* T35 raises the possibility of alteration of the diatom-bound $\delta^{15}\text{N}$ during the chemical cleaning.

In an early study by Wada and Hattori [1978], N isotope fractionation during diatom nitrate assimilation was observed to be inversely related to growth rate. However, subsequent work has not supported this relationship [Granger *et al.*, 2004; Granger *et al.*, 2008; Montoya and McCarthy, 1995; Needoba and Harrison, 2004; Needoba *et al.*, 2004]. Consistent with the latter results, no trend was found to exist between growth rate and ϵ (Figure 1.3a). A slight positive trend was observed when *Pseudo-nitzschia seriata* was removed ($r^2=0.29$), with higher growth rates corresponding with higher ϵ . However, this is counter to the expectation for weakly expressed fractionation under higher growth rates. The $\delta^{15}\text{N}_{\text{DBoffset}}$ had no relationship when compared to growth rate (not shown), indicating that factors other than growth drive fractionation during both uptake and frustule formation.

Nitrate is imported across the plasmalemma by active transport mechanisms [Granger *et al.*, 2004; Granger *et al.*, 2008; Tischner, 2000]. Active transport increases the internal concentration of NO_3^- relative to the external environment [e.g. C in Amoroso *et al.*, 1998; N in Needoba *et al.*, 2004], which then allows for nitrate efflux by passive mechanisms. ϵ is related to the ratio of efflux to influx. High relative efflux rates result in larger ϵ , and vice versa [Needoba *et al.*, 2004]. Previous studies have demonstrated that C-isotope fractionation is inversely proportional to cell size [Burkhardt *et al.*, 1999; Laws *et al.*, 1995; Popp *et al.*, 1998], the explanation of which

was related to efflux/influx dynamic described above for isotope fractionation during nitrate assimilation. We found that ϵ had a negative linear correlation to cell size (Figure 1.3b), with larger cells having decreased fractionation. However, cells are not perfect spheres and the path length and exchange interface can be highly variable. The combination of shape and size may be approximated as the ratio of cellular surface-area to volume (SA:V). In the case of elongated cells (high SA:V), there may be the potential for significant expression of isotopic fractionation due to the increased surface area for NO_3^- efflux relative to cell size [Popp *et al.*, 1998]. All else held constant, a high SA:V ratio may lead to a lower ratio of nitrate reduction relative to efflux, thereby exchanging nitrate with the growth medium more readily, leading to less $\delta^{15}\text{N}$ elevation of internal nitrate and more complete expression of the nitrate reduction isotope effect (i.e. higher ϵ) in the external medium. Observed increases in ϵ corresponded with increased SA:V, with a stronger relationship ($r^2=0.70$) when pennate species were investigated alone (Figure 1.3c). Of course, it is unlikely that nitrate reduction scales simply as a function of cell volume, without any regulation of reduction (or uptake) rate by the organism.

An average $\delta^{15}\text{N}_{\text{DBoffset}}$ of $\sim 5.4\text{‰}$ was observed, with no systematic relationships observed when diatom-bound offsets were compared to size and SA:V (Figure 1.3d, 1.3e). A strong linear relationship between ϵ and diatom-bound offset ($\delta^{15}\text{N}_{\text{DBoffset}} = 1.56\epsilon + 0.05$) was observed when *Pseudo-nitzschia seriata* was removed from the analysis (Figure 1.2f). This species had a high degree of error, as well as a wide range in reported $\delta^{15}\text{N}_{\text{DBoffset}}$ values. It is not known if small size and physiology plays a role in this variability, or if the alteration of small cells during cleaning resulted in

analytical error. In any event, it is difficult to explain the observed correlation of the diatom-bound offset (the sum of frustule formation processes) to the ϵ of nitrate assimilation. After nitrate is acquired, fractionation occurs during the reduction of nitrate to nitrite, by the enzyme nitrate reductase [Mariotti *et al.*, 1982; Needoba *et al.*, 2004; Shearer *et al.*, 1992]. This step is the first in a sequence that forms an internal pool of bio-available nitrogen that is isotopically distinct and lighter than the external nitrate pool. Other enzymes piece together long-chain polyamines (LCPA) or biomineralization proteins, such as silaffins, which make up the diatom-bound organic matter. It is likely that each of these enzymes imposes a fractionation as well. It is possible that the allocation of organic N in the cell to either frustule-bound silaffins and LCPAs, or N forms not related to the frustule could produce differences in the diatom-bound offset that are somehow related to size and thus to ϵ (Figure 1.3f). Larger diatoms, with a smaller SA:V, have proportionally more cytoplasm than a smaller diatom. However, the lack of relationship between diatom-offset and size (or between diatom-offset and SA:V) does not support this. Given how little we know about the isotopic fractionation associated with frustule formation, we cannot yet give a satisfying explanation for the observed variation in $\delta^{15}\text{N}_{\text{DBoffset}}$, let alone its correlation with ϵ .

While our single-species cultures in filtered seawater are useful for examining diatom-specific $\delta^{15}\text{N}_{\text{DBoffset}}$, the conditions of the cultures are far from those found in nature. Southern Ocean diatom assemblages are heterogeneous mixtures of different diatom species that vary across time and space, closely connected to frontal systems, water masses, and sea ice distribution [Burckle *et al.*, 1987; Eynaud *et al.*, 1999;

Fenner et al., 1976; *Zielinski and Gersonde*, 1997]. Additionally, present day nitrate concentrations in the summertime Antarctic mixed layer average approximately 25 μM , much less than the nitrate concentrations in excess of 200 μM that apply to the starting conditions of our batch cultures. We observed significant variability in our cultured ϵ and diatom-bound offsets and high variability in ϵ is typical of culture work [*Needoba et al.*, 2003; *Needoba and Harrison*, 2004; *Needoba et al.*, 2004; *Wada and Hattori*, 1978]. Far less variability is observed in ϵ from field studies [*Sigman et al.*, 1999b; *Wu et al.*, 1997]. In this vein, it is possible that diatom-bound offsets in the ocean are also significantly less variable than our culture-based observations suggest. With this caveat in mind, we will discuss the potential implications of our results on paleoceanographic reconstructions.

3.5. Culture Based Comparisons with Cores

Most of the cultured species show changes in $\delta^{15}\text{N}_{\text{DB}}$ with increasing nutrient consumption, suggesting that a sedimentary diatom-bound $\delta^{15}\text{N}$ record at least in part reflects the nutrient status of the surface ocean. However, there is an obvious discrepancy between our culture data and the $\delta^{15}\text{N}_{\text{DB}}$ measured in sediments. Much of the published sedimentary diatom-bound work indicates that, on average, the $\delta^{15}\text{N}$ of bulk sediment is similar to, and often less than $\delta^{15}\text{N}_{\text{DB}}$ [*Brunelle et al.*, 2007; *Robinson et al.*, 2005; *Robinson and Sigman*, 2008; *Sigman et al.*, 1999a]. Bulk sedimentary N in the open ocean has a $\delta^{15}\text{N}$ higher than that of the sinking flux or, in turn, the phytoplankton biomass from which the sinking flux is ultimately derived. Thus, surface sediment $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ values suggest that sedimentary $\delta^{15}\text{N}_{\text{DB}}$ is higher

than the biomass of the diatoms that produced the frustules in the sediment, opposite to our measurements of cultured diatoms.

Higher $\delta^{15}\text{N}_{\text{DB}}$ relative to $\delta^{15}\text{N}_{\text{bulk}}$ in sediments may be the result of post-depositional changes on either the bulk or the diatom-bound $\delta^{15}\text{N}$. The rapid loss of a low $\delta^{15}\text{N}$ fraction could cause the systematic enrichment of sedimentary diatom-bound material. There is no systematic reduction in the diatom-bound N content of cleaned opal with depth that would suggest loss of a portion of the frustule and fractionation of the bound N [Horn and Robinson, in prep 2011; Robinson *et al.*, 2004]. However, we cannot rule out the loss of such a low- $\delta^{15}\text{N}$ fraction before the diatom opal reaches the seabed.

Alternatively, the discrepancy may derive from physiological differences between cultures and open ocean diatoms. Possible differences include iron availability [Hutchins and Bruland, 1998; Leynaert *et al.*, 2004; Takeda, 1998], light limitation, high nutrient contents, bottle effects, and more. More work will need to be done to test this.

On top of this discrepancy in the sign of the diatom-bound offsets, paleo-reconstructions of nutrient utilization must also come to grips with inter-species differences in diatom-bound $\delta^{15}\text{N}$. Sediments contain several different diatom species at any one time and the proportion of each species varies downcore. The relative contribution of each species' characteristic $\delta^{15}\text{N}_{\text{DBoffset}}$ could significantly impact a record of $\delta^{15}\text{N}_{\text{DB}}$. If we work from the measured values of diatom-bound offset, we can make some preliminary estimates of how assemblage changes might impact nutrient status reconstructions.

A mass balance, based upon diatom assemblage data and a range of $\delta^{15}\text{N}_{\text{DBoffset}}$ at core TN057-13PC4 provides us with a simplified estimate of assemblage-related (i.e. not utilization-related) changes between the LGM and the Holocene south of the APF (Figure 1.4) [Horn *et al.*, *in review* 2011; Nielsen, 2004]. On the one hand, *Fragilariopsis kerguelensis* presently makes up the majority (60-90%) of polar Southern Ocean sediments in open ocean areas. During the LGM, its abundance dropped to >30% of the sediment [Crosta *et al.*, 1998; Nielsen, 2004; Zielinski and Gersonde, 1997]. On the other hand, *Fragilariopsis cylindrus* makes up between 1-2% of sediments today and peaked at approximately 8% during the LGM [Nielsen, 2004; Zielinski and Gersonde, 1997]. *Eucampia antarctica* makes up a large portion of the imbalance between these two species with a peak abundance of 30% during the LGM and Holocene values around 3% [Nielsen, 2004]. *Chaetoceros* resting spore abundance also changes substantially, making 35% during the LGM but only 5-10% during the Holocene [Nielsen, 2004]. To simplify, we assume the remainder of the population, *Eucampia antarctica*, and *Chaetoceros* resting spores have a $\delta^{15}\text{N}_{\text{DBoffset}}$ of 6‰. This is both the average value of cultured species, excluding *Fragilariopsis kerguelensis* (Table 1.1), and the average centric value that is assumed for *Eucampia antarctica*, whose species-specific $\delta^{15}\text{N}_{\text{DBoffset}}$ is unknown. From these values, we calculate a community wide $\delta^{15}\text{N}_{\text{DBoffset}}$ value for the Holocene of ~2.4‰ and the LGM of ~4.9‰. This would correspond to a 2.5‰ increase in $\delta^{15}\text{N}_{\text{DB}}$ from LGM to Holocene based on assemblage changes, assuming no change in nutrient utilization. In contrast, decreases of 0-4‰ in $\delta^{15}\text{N}_{\text{DB}}$ from the LGM into the Holocene are observed at sites located across the Southern Ocean [Robinson *et al.*, 2004; Robinson *et al.*,

2005; *Robinson and Sigman, 2008*]. This calculation thus does support the assertion that the downcore $\delta^{15}\text{N}_{\text{DB}}$ changes were driven by assemblage alone. Of course, our understanding of $\delta^{15}\text{N}_{\text{DBoffset}}$ is incomplete, and the above calculation is extremely simplistic. Adding to variations in $\delta^{15}\text{N}_{\text{DB}}$, ϵ may also have changed due to variations in light limitation between the LGM and the Holocene [*DiFiore et al., 2010*]. If the observed ϵ -diatom-bound relationship holds in the field, this would impact the diatom-bound record through its effect on both bulk diatom biomass $\delta^{15}\text{N}$ and the bulk-to-diatom-bound $\delta^{15}\text{N}$ offset.

4. Conclusions

In all but one culture, it was observed that the $\delta^{15}\text{NO}_3^- > \delta^{15}\text{N}_{\text{bulk}} > \delta^{15}\text{N}_{\text{DB}}$. An average bulk-diatom-bound offset ($\delta^{15}\text{N}_{\text{DBoffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) of $5.3 \pm 4.4\text{‰}$ was observed, with an average isotope effect for nitrate assimilation (ϵ) of $5.03 \pm 3.42\text{‰}$. With few exceptions, specific values for $\delta^{15}\text{N}_{\text{DBoffset}}$ and ϵ are relatively consistent within species, but differ greatly between them. Differences between pennate and centric ϵ and $\delta^{15}\text{N}_{\text{DBoffset}}$ were statistically insignificant. ϵ and $\delta^{15}\text{N}_{\text{DBoffset}}$ were observed to be weakly negatively correlated with cell size (that is, decreased with increasing cell size). In addition, a weak positive correlation between ϵ and SA:V was observed for all diatoms; this relationship strengthened when pennate species are investigated alone. $\delta^{15}\text{N}_{\text{DBoffset}}$ is positively correlated with ϵ . However, open ocean testing is required, as ϵ 's correlation and apparent control on $\delta^{15}\text{N}_{\text{DBoffset}}$ is not yet mechanistically understood. Our data indicate a positive $\delta^{15}\text{N}_{\text{DBoffset}}$ across all studied diatom species, whereas sedimentary data suggest a negative $\delta^{15}\text{N}_{\text{DBoffset}}$ between bulk

and diatom bound $\delta^{15}\text{N}$.

Future work will need to investigate whether the growth conditions of our cultures had some effect on $\delta^{15}\text{N}_{\text{DBoffset}}$, or if a low- $\delta^{15}\text{N}$ component of the diatom-bound-associated N is lost during early diagenesis. Further culture studies using a chemostat maintained at environmentally relevant concentrations may also clarify the observed trends. Net-tows and sediment trap data from the Southern Ocean may provide insight into the diagenetic processes that may affect $\delta^{15}\text{N}_{\text{DB}}$ from the time of formation to early sedimentation, and time series studies of fresh, organic-rich material buried in sediments for varying times may provide a first look at the processes in sediments that may affect $\delta^{15}\text{N}$ of bulk and diatom-bound material. Additionally, gravitational sediment separation (SPLITT) could be used to sort diatoms, potentially isolating single species or providing a more consistent assemblage for downcore analysis. Finally, inter-laboratory comparisons are recommended, as there is no standardized method for isolating the organic matter in the diatom-bound fraction. Given assemblage changes, our culture data, for relevant species, do not suggest that the observed glacial/interglacial $\delta^{15}\text{N}$ change in Antarctic sediment cores can be explained by assemblage change alone.

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Table 1.1: Growth rates and measured N isotope parameters for pennate diatoms (*Pseudo-nitzschia seriata*, *Fragilariopsis cylindrus*, and *Fragilariopsis kerguelensis*) and centric diatoms (*Thalassiosira nordenskiöldii* and *Thalassiosira aestivalis*)

Species	μ (day ⁻¹)	Initial [NO ₃] (μ M)	Final <i>f</i>	Final [Si] / [PO ₄] (μ M)	ϵ (‰)	$\delta^{15}\text{N}_{\text{DB offset}}$ (‰)
<i>Pseudo-nitzschia seriata</i> T26 ^a	0.80	258.3	0.21	8.6 / 14.6	8.81	11.2
<i>Pseudo-nitzschia seriata</i> T27	0.72	244.1	0.33	25.7 / 17.2	7.07	2.9
<i>Pseudo-nitzschia seriata</i> T35	0.72	162.3	0.30	26.1 / 21.3	13.97	-1.5 ^d
Average:	0.75				9.95	4.2
	± 0.04^b				± 3.59	± 6.5
<i>Fragilariopsis cylindrus</i> T27	0.66	230.2	0.40	30.3 / 17.6	4.64	9.2
<i>Fragilariopsis cylindrus</i> T28	0.59	228.3	0.57	75.5 / 23.3	4.77	9.0
<i>Fragilariopsis cylindrus</i> T33	0.41	147.0	0.01	7.3 / 14.5	2.41	3.1 ^d
Average:	0.55				3.94	7.1
	± 0.13				± 1.33	± 3.5
<i>Fragilariopsis kerguelensis</i> T4	0.57	174.3	0.32	2.3 / 18.3	1.32	2.0
<i>Fragilariopsis kerguelensis</i> T5	0.66	176.1	0.33	1.1 / 21.2	1.02	1.9
Average:	0.59				1.17	2.0
	± 0.02				± 0.21	± 0.0
Pennate Averages:	0.64	202.6	0.31	22.1 / 18.5	5.50	4.7
	± 0.12	± 42.2	± 0.16	± 24.4 / ± 3.2	± 4.37	± 6.0
<i>Thalassiosira nordenskiöldii</i> T36	1.32	237.4	0.20	52.1 / 18.5	4.73	7.9
<i>Thalassiosira nordenskiöldii</i> T37	1.30	222.1	0.13	61.7 / 17.4	4.26	6.4
Average:	1.31				4.50	7.1
	± 0.01				± 0.33	± 1.1
<i>Thalassiosira aestivalis</i> T34	1.34	850.6	0.78	NA / NA ^c	4.12	5.9
<i>Thalassiosira aestivalis</i> T45	0.81	251.9	0.37	8.9 / 19.9	3.22	5.2
<i>Thalassiosira aestivalis</i> T46	0.94	217.7	0.16	4.4 / 16.4	5.11	5.2
Average:	1.03				4.15	5.4
	± 0.28				± 0.95	± 0.4
Centric Averages:	1.14	232.3	0.22	31.8 / 18.0	4.29	6.1
	± 0.25	± 15.6^c	± 0.11^c	± 29.3 / ± 1.5	± 0.71	± 0.8
Overall Mean:	0.83	212	0.27	25.3 / 18.3	5.03	5.3
	± 0.31	± 38^c	± 0.16^c	± 25.2 / ± 2.7	± 3.42	± 4.4

^aT# refers to transfer number, whereby *Pseudo-nitzschia seriata* T26 is the 26th transfer (batch) of the semi-continuous batch culture used for that specific experiment.

^b pooled standard deviations are reported.

^c*Thalassiosira aestivalis* T34 initial [NO₃⁻] and *f* are removed from mean calculations.

^dN-content of diatom-bound samples were significantly lower, on average, than other experiments indicating that alteration may have occurred. However, corresponding ϵ 's (which do not depend upon cleaning) also differed, necessitating that both remain in the analysis.

^e[Si] / [PO₄] were not measured for this experiment.

Figure 1.1: $\delta^{15}\text{NO}_3^-$ values over the course of each experiment, as the natural logarithm of f (fraction of initial nitrate remaining) decreased to highlight the linearity. The equation for the theoretical change in substrate $\delta^{15}\text{N}$ [Mariotti *et al.*, 1981] was used to determine ϵ for each experiment (T#) with the corresponding r^2 in parentheses. The average ϵ for each species is plotted as a solid line. All of the $\delta^{15}\text{NO}_3^-$ values were included to create a generalized ϵ for all diatom species (dashed pink line). However, species-specific substrate curves were more appropriate, due to large differences in ϵ between species.

Figure 1.2: **(a)** The theoretical relationship between nitrate utilization and the $\delta^{15}\text{N}$ of the different N pools is plotted as a generalized Rayleigh fractionation using the eq. 1-3 [Mariotti *et al.*, 1981]. Note that the $\delta^{15}\text{N}_{\text{DB}}$ is not included in this earlier model. An initial $\delta^{15}\text{NO}_3^-$ of 5‰ and ϵ of 5‰ were used in this example. **(b – f)** The $\delta^{15}\text{NO}_3^-$, $\delta^{15}\text{N}_{\text{bulk}}$, and $\delta^{15}\text{N}_{\text{DB}}$ values for each carboy experiment are presented with f . Error bars for substrate and bulk samples have been included for each specific data point, based upon the standard error of replicate samples. Note that the precision of $\delta^{15}\text{NO}_3^-$ and $\delta^{15}\text{N}_{\text{bulk}}$ is substantially greater than that of the $\delta^{15}\text{N}_{\text{DB}}$, and are small enough to be covered by each data point. Predicted values for substrate and bulk were modeled for each species, based upon the calculated ϵ for each specific group of species, and are displayed as the two upper solid lines. The lowest solid line is the predicted diatom-bound curve based on the overall mean diatom-bound offset for all experiments (Table 1.1). Centric and pennate diatom species are presented on the top and bottom row, respectively. *Thalassisira aestivalis* T34 is not presented for clarity, as all values fall between 1.0 and a final f of 0.78.

Figure 1.3: Relationships between growth rate (μ), cell size, ϵ , and $\delta^{15}\text{N}_{\text{DBoffset}}$. Panel **(a)** represents the relationship between growth rate and ϵ . No relationship ($r^2=0.01$) is apparent, however when *Pseudo-nitzschia seriata* is removed from this analysis, a weak positive linear relationship ($r^2=0.29$) is determined. Panel **(b)** represents the relationship between cell size and ϵ . The value of ϵ , as well as its variability, decreases with increasing cell size. Panel **(c)** represents the relationship between ϵ and the surface area to volume ratio (SA:V) of each species. Panel **(d)** represents the relationship between cell size and $\delta^{15}\text{N}_{\text{DBoffset}}$. Excluding the three low points improves the relationship ($r^2=0.95$), however the higher variability seen at small cell sizes does not allow any specific points to be excluded. Panel **(e)** shows the lack of relationship between SA:V and $\delta^{15}\text{N}_{\text{DBoffset}}$, showing that $\delta^{15}\text{N}_{\text{DBoffset}}$ is relatively consistent over SA:V. Once again smaller cells (those with higher SA:V ratios) have the highest degree of variability. Panel **(f)** represents the relationship between ϵ and $\delta^{15}\text{N}_{\text{DBoffset}}$. No relationship is found ($r^2=0.08$) with all species present. However, when *Pseudo-nitzschia seriata* is removed, a positive linear relationship is observed. Note that the two centric diatoms (*Thalassiosira*) cluster tightly throughout.

Figure 1.4: A mass balance calculation of the average ecosystem $\delta^{15}\text{N}_{\text{DBOffset}}$ based upon observed variations in species assemblage [Nielsen, 2004] during the Holocene and Glacial at a core site south of the APF. Assuming a fixed $\delta^{15}\text{NO}_3^-$ of source waters, no change in utilization, and a constant ϵ , one may expect $\sim 2.5\text{‰}$ difference in $\delta^{15}\text{N}_{\text{DBOffset}}$ based upon assemblage changes alone.

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**Southern Ocean nitrogen and silicon dynamics
during the last deglaciation**

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Abstract

The reinvigoration of overturning in the Southern Ocean is hypothesized to have returned CO₂ from the deep ocean to the atmosphere at the end of the last ice age. Large peaks in opal accumulation have been put forward as evidence for an increase in wind driven upwelling between 10 and 15 ka (Anderson et al., 2009). Here, we use coupled nitrogen and silicon isotope records alongside opal accumulation rates to provide quasi-quantitative estimates of Southern Ocean nutrient supply, by upwelling, and nutrient utilization across this interval. Significant changes in the consumption of N and Si across the two opal accumulation peaks indicate major changes in both upwelling and nutrient demand. We find N and Si consumption to be relatively incomplete during peak opal accumulation at the onset of the deglaciation. Nutrient supply must have been significantly enhanced. The second deglacial peak in opal accumulation is associated with more complete Si consumption and variable N consumption. We suggest that this peak represents strong upwelling and more complete utilization of the available silicic acid pool. Differences between the Si and N responses during opal peaks may stem from decreasing iron availability across the glacial termination. The reinvigoration of overturning circulation during the deglaciation is hypothesized to cause a transient peak in nutrient supply to the low latitudes (Pena et al., 2008; Robinson et al., 2009; Spero and Lea, 2002). This is supported by our data, which indicate that relatively high macronutrient concentrations were maintained in the Southern Ocean surface waters despite high demand.

1. Introduction

It is posited that a reduction in the strength of overturning circulation during the last ice age led to the lowering of atmospheric CO₂ (Francois et al., 1997; Sigman and Boyle, 2000; Sigman et al., 2010; Sigman et al., 2004; Toggweiler and Lea, 2010; Toggweiler et al., 2006). In turn, the resumption of overturning during the last deglaciation, Termination 1 (T1), would then be related to the rapid release of CO₂ and the return of nutrients to the surface ocean-atmosphere system (Anderson et al., 2009; Bradtmiller et al., 2006, 2007; Denton et al., 2010; Robinson et al., 2004; Robinson et al., 2005; Spero and Lea, 2002). However, opal fluxes, although interpreted as an indicator of upwelling by Anderson et al. (2009), are not a direct proxy for upwelling. The increase in opal export may reflect the enhanced supply of nutrients, either through upwelling or an increase in the nutrient content of the water upwelled, greater fractional utilization of nutrients (in the case of Si excess), or improved preservation of sinking opal.

Coupled measurements of nitrogen and silicon stable isotopes of diatoms allow us to better constrain changes in nutrient supply across the deglaciation at core site TN057-13PC4 (Figure 2.1), the same site analyzed by Anderson et al. (2009) and the best-resolved record of increased opal export in the Southern Ocean. Under the nutrient replete conditions of the Southern Ocean, the relative degree of uptake and assimilation of nitrate and silicic acid is recorded in the stable isotopic composition of the resulting organic matter or diatom frustule, respectively. Preferential incorporation of the lighter isotope (¹⁴N or ²⁸Si) into biomaterials leads to an increase in δ¹⁵N and δ³⁰Si of the nutrient substrate and product during progressive consumption of the

nutrient pools (Altabet and Francois, 1994; De La Rocha et al., 1997). Given an estimate of export production, or nutrient demand, we can estimate changes in supply with the relation: $\Delta\text{supply} = \Delta\text{demand} / \Delta\text{utilization}$, approximated as $\Delta\text{opal} / \Delta\delta^{30}\text{Si}$ (or $\delta^{15}\text{N}$).

In using N and Si isotopes together, we can investigate two hypothesized causes of the increase in opal accumulation upon deglaciation: (1) The resumption of overturning enhanced the delivery of nutrients to the surface and (2) The deglacial decrease in iron supply caused a shift in the phytoplankton assemblage toward larger, more heavily silicified organisms that were more readily exported (Boyle, 1998; Hutchins and Bruland, 1998; Smetacek et al., 2002; Takeda, 1998). If the opal accumulation record is the product of purely physical changes in the overturning circulation, then the two proxy records of nutrient consumption should shift in parallel. Dissimilar behavior between the two nutrients likely stems from biogeochemical changes, such as iron availability, which alters Si:N uptake dynamics (Brzezinski et al., 2005; Hutchins and Bruland, 1998; Takeda, 1998).

2. Methods:

Diatom $\delta^{15}\text{N}$ and $\delta^{30}\text{Si}$ records were generated from Southern Ocean site TTN057-13PC4 (Figure 2.1) collected from 2850 m of water, approximately 2°N of the average present day winter sea ice extent and south of the Antarctic Polar Front (APF). Diatoms were separated and cleaned to determine the isotopic composition of nitrogen bound within the frustule ($\delta^{15}\text{N}_{\text{DB}}$) (Robinson et al., 2004) and silicon ($\delta^{30}\text{Si}$) (Brzezinski et al., 2006) from the opal fraction. The physical separation, consisting of

sieving ($<150\ \mu\text{m}$), settling, and a density separation with sodium polytungstate ($\rho=2.15\ \text{g/ml}$), prior to cleaning, was adapted from previous studies (Robinson et al., 2004; Sigman et al., 1999).

Physically separated diatom samples were cleaned using (1) a reductive cleaning step using a heated (80°C for 1 hour) sodium dithionite solution to remove adsorbed metals (2) a preliminary oxidation of external organic matter using $\sim 15\ \text{ml}$ saturated potassium permanganate and $\sim 15\ \text{ml}$ saturated oxalic acid, after $5\ \text{ml}$ of $18\ \text{M}$ sulfuric acid (Hasle and Fryxell, 1970), and (3) two final one hour oxidations of organic matter using a weak ($\sim 35\%$) and then strong (72%) solution of perchloric acid in a 100°C water bath. Splits of the clean, dry diatom samples were dissolved and the organic N oxidized to nitrate in a $0.22\ \text{M}$ potassium persulfate and $1.5\ \text{M}$ sodium hydroxide solution in an autoclave at 121°C for 20 minutes (Bronk et al., 2000; Robinson et al., 2004).

Nitrate concentrations of the persulfate oxidized frustule N were measured by chemiluminescence after reduction to NO using a Teledyne Instruments (Model 200E) chemiluminescence NO/NO_x analyzer (Braman and Hendrix, 1989). The $\delta^{15}\text{N}$ was measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) on a Thermo Delta V Advantage IRMS. $20\ \text{nmol}$ of N, as nitrate, was converted to N₂O for injection by the denitrifier method (Sigman et al., 2001). The potassium nitrate reference material IAEA-N3 (4.7‰) was used to standardize $\delta^{15}\text{N}$ results (Gonfiantini et al., 1995; Revesz et al., 1997). Precision was determined by multiple measurements of standards within each run and replicate measurements of samples. Approximately 50% of $\delta^{15}\text{N}$ samples were run in duplicate or triplicate with a standard error of the

mean of 0.5‰.

Splits of the cleaned samples were analyzed for $\delta^{30}\text{Si}$. Aliquots of samples were dissolved in 7.5 M hydrogen fluoride, dissolved silicon was then precipitated as cesium hexafluosilicate by addition of 5M cesium chloride. The precipitate was rinsed with ethanol, dried and introduced in a modified Kiel III (Brzezinski et al., 2006). The cesium complex was decomposed by adding concentrated sulfuric acid to generate silicon tetrafluoride gas which was analyzed on a gas source isotope ratio mass spectrometer (Brzezinski et al., 2006). $\delta^{30}\text{Si}$ samples were run in duplicate with a standard error less than to 0.1‰.

3. Results and Discussion

Both stable isotope records show three distinct stages over the last 30 kyr including a relatively stable glacial period, a dynamic deglaciation composed of three phases, and a stable Holocene. The glacial phase from ~16-30 ka is characterized by low opal accumulation, low $\delta^{30}\text{Si}$, moderate to high $\delta^{15}\text{N}$ from ~16-20 ka, and relatively low $\delta^{15}\text{N}$ prior to 20 ka. This shift from low to high $\delta^{15}\text{N}$ values corresponds to a dramatic shift in diatom assemblage (Figure 2.2h). Culture based evaluation of species-specific offsets between diatom biomass and diatom-bound $\delta^{15}\text{N}$ suggest that diatom-bound $\delta^{15}\text{N}$ in *Fragilariopsis kerguelensis* records a higher diatom-bound $\delta^{15}\text{N}$ than other polar diatoms tested (Horn et al., *in review* 2011). The baseline increase in $\delta^{15}\text{N}$ at 20 ka may result from the transition from *F. kerguelensis* poor to *F. kerguelensis* dominated sediments (Figure 2.2h).

For the purposes of this manuscript, we will only discuss the $\delta^{15}\text{N}$ record between 0-18 ka, when *F. kerguelensis* dominates the record. The observed combination of low silicic acid utilization (Figure 2.2e), moderate nitrate consumption (Figure 2.2d), and low opal accumulation (Figure 2.2c) is consistent with conditions of weak upwelling, diminished demand for silicic acid, and increased consumption of nitrate (Brzezinski et al., 2002; Matsumoto et al., 2002; Robinson et al., 2004; Robinson and Sigman, 2008) during the last ice age. The Holocene, ~10 ka to present, is relatively stable, with high $\delta^{30}\text{Si}$ values indicative of near complete consumption of silicic acid and low $\delta^{15}\text{N}$ indicative of relatively weak nitrate consumption (Figure 2.2d).

In contrast, the deglaciation is dynamic. $\delta^{30}\text{Si}$ increases significantly across the termination, with smaller increases that correspond to the two peaks in opal export (Figure 2.2e – grey shading). Si consumption appears to be increasing steadily, with transient local peaks in consumption during peak opal accumulation episodes. The variation in $\delta^{15}\text{N}$ suggests that nitrate consumption decreases abruptly with the initial increase in opal export and increases as opal export declines during the Antarctic Cold Reversal (ACR ~14 ka). The supply of nitrate during the first opal peak must have far exceeded the demand. $\delta^{15}\text{N}$, and thus nitrate consumption, is more variable during the second opal peak, decreasing, rising significantly, and then dropping once again (Figure 2.2d).

Our first order interpretation of nutrient utilization across the deglaciation is that nutrient supply, i.e. upwelling, changed dramatically in association with the opal export peaks. However, since Si and N utilization did not change in parallel, changes

in opal production/demand and preservation were likely occurring. In order to parse these mechanisms and better quantify nutrient supply changes, we have used the stable isotope data to calculate a metric of silicic acid supply (Figure 2.2g) and Si/N consumption (Figure 2.2f) across this interval.

Change in silicic acid supply associated with changes in upwelling was calculated from the $\delta^{30}\text{Si}$ data based upon opal accumulation (from Anderson et al. (2009)) and the percent silicic acid supply consumed at each time point relative to the present ($\delta^{30}\text{Si} = 1.4\text{‰}$). An open-system (eq. 1) was first applied in conjunction with opal flux data to determine the silicic acid supply (eq. 2).

$$\%Si(OH)_4_{consumed} = 1 - [(\delta^{30}\text{Si} - 1.7) / -1.1] \quad (1)$$

$$Si \text{ Supply} = \frac{F_{opal}^{sample} / F_{opal}^{present}}{\%Si(OH)_4_{consumed}^{sample} / \%Si(OH)_4_{consumed}^{present}} \quad (2)$$

F_{opal} is the flux of opal and $\%Si(OH)_4_{consumed}$ is the percent of the silicic acid supply consumed. -1.7‰ (Cardinal et al., 2005) is the $\delta^{30}\text{Si}$ of upwelled $Si(OH)_4$, and -1.1‰ (De La Rocha et al., 1997) is the isotopic enrichment factor (ϵ) associated with Si uptake and utilization. Summer productivity in the Southern Ocean occurs during periods with enhanced stratification. A closed-system Rayleigh-type equation (eqs. 3 & 4) provides a more conservative estimate of silicic acid supply (eq. 2).

$$\delta^{30}\text{Si}_{sample} = 1 - \delta^{30}\text{Si}_{initial} + (-\epsilon * \ln(\%Si(OH)_4_{consumed})) \quad (3)$$

$$\%Si(OH)_4_{consumed} = 1 - \exp\left[\frac{\delta^{30}Si_{sample} - \delta^{30}Si_{initial}}{\epsilon}\right] \quad (4)$$

In order to account for potential changes in the Si content of the upwelled water, we calculated the index two ways; first, assuming that the deep ocean silicic acid content remained constant with time (eq. 2), and second, with estimates of deep ocean silicic acid concentrations from Hendry et al. (2010) using equation 5 (Figure 2.2g).

$$Si\ Supply = \frac{F_{opal}^{sample} / F_{opal}^{present}}{\%Si(OH)_4_{consumed}^{sample} / \%Si(OH)_4_{consumed}^{present}} * \frac{[Si(OH)_4]_{Deep}^{present}}{[Si(OH)_4]_{Deep}^{sample}} \quad (5)$$

Where $[Si(OH)_4]_{Deep}^{present}$ is the present day deep water concentration of $Si(OH)_4$ and $[Si(OH)_4]_{Deep}^{sample}$ is the reconstructed deep water concentration of $Si(OH)_4$ for a given sample. It is unknown if deep ocean nitrate concentration has changed. Therefore, estimates of Si-consumption relative to N-consumption were made assuming that starting nutrient contents of upwelled waters were static. When the variable silicic acid supply estimate is used, there are two points that deviate significantly from the static approximation and both predict Si/N consumption ratios that fall below the primary estimate (data not shown).

Estimates of silicic acid supply using the linear approximation (eqs. 1&2) suggest an increase on the order of ~8-10 times present day values occurred during the first opal peak ~16 kya (Figure 2.2g). The decrease in opal accumulation and increase in nitrate utilization between the opal peaks likely coincides with a severe reduction in

the silicic acid supply, or upwelling. The second opal peak at ~13 kya corresponds to elevated silicic acid supply, but to a much lesser degree than the initial peak, at ~4-5 times modern values.

Calling at times for ten-fold increases in upwelling appears to be unreasonably high. Reasonable values for Southern Ocean upwelling range from 30-60Sv (Sarmiento and Toggweiler, 1984; Toggweiler, 1999), which requires 300-600Sv of upwelling during the deglaciation. It is likely that the assumption of steady state nutrient supply in eq. 1 is incorrect. The estimates using a Rayleigh-type relationship produces a more reasonable estimate of silicic acid supply, approximately 2-5 times present day values for the deglacial periods of enhanced opal flux (Figure 2.2g). Additionally, this method captures the hypothesized decrease in upwelling during the LGM, with estimated upwelling rates of approximately 55% of present day values.

Comparing opal accumulation and silicic acid supply provides further evidence that using a Rayleigh-type relationship may be more appropriate. If opal accumulation was purely a function of upwelling rates, based upon steady state nutrient supply, then the second opal accumulation peak would be half that of the first. However, opal accumulation during the second peak was >80% of the first. Using the Rayleigh-type relationship, we find that the difference between the first and second peaks was ~20% for both opal accumulation and silicic acid supply. This suggests that the Rayleigh-type relationship may be more appropriate approximation and removes the need to invoke opal preservation differences or species assemblage shifts, which have stabilized at this point (Figure 2.2h).

The essentially inverse relationship in Si and NO_3^- consumption on glacial-interglacial timescales, where silicic acid consumption increases and nitrate consumption decreases, is consistent with previous observations and can be explained by differences in the Si/N uptake ratio of diatoms mediated by Fe supply (Franck et al., 2000; Hutchins and Bruland, 1998; Takeda, 1998). Low Si/N uptake ratios ($\sim 1:1$) are observed under Fe replete conditions, with increases to 4:1 during iron limitation (Hutchins and Bruland, 1998; Takeda, 1998). The observed reduction in dust fluxes/concentrations indicates that Fe becomes progressively more limiting across the termination (Petit et al., 1999) (Figure 2.2b). Our diatom Si/N estimates increase with decreasing Fe supply, suggesting that increasingly more Si is consumed or exported per unit of N across the termination (Figure 2.2f). High and relatively stable Holocene $\delta^{30}\text{Si}$ values occur following the second opal peak when the Fe supply stabilizes. This is also consistent with the diatom assemblage shift toward more heavily silicified diatoms such as *F. kerguelensis* (Figure 2.2h), which may have enhanced the preservation of opal and likely boosted opal accumulation during the second peak. (Smetacek et al., 2002).

The significant increases in nutrient supply implied by the $\delta^{30}\text{Si}$ and $\delta^{15}\text{N}$ records at TN057-13PC4 support the assertion that the intensification of upwelling across the deglaciation excavated nutrients and CO_2 stored in the abyssal ocean during the last ice age and returned them to the surface ocean and atmosphere system (Anderson et al., 2009). Quantification of nutrient concentration changes in the deep glacial ocean is needed to understand how much of this transient increase in supply was driven by accelerated overturning or increased nutrient content of the water being

upwelled in this interval (Hendry et al., 2010). TN057-13PC4 lies south of the Antarctic Polar Front (APF) and slightly north or potentially on the Southern Antarctic Circumpolar Current Front (SACCF), as these two fronts converge in this area of the Southern Ocean. Depending upon where this site sits relative to the SACCF, the surface waters have two potential fates: they may be transported northward and become part of the source for intermediate and mode waters that form in the Subantarctic Zone, or they may be transported southwards toward a region of deep-water formation. This site is north of the winter sea ice edge, and more than likely sits northward of the SACCF at present. Thus, these isotope records are probably representative of northward flowing water and nutrient pools that make up the preformed nutrients that eventually fuel productivity at low latitudes (e.g. Sarmiento et al., 2004). Evidence for enhanced nutrient supply and increased export in the eastern equatorial Pacific, where Subantarctic Mode Water (SAMW) upwells directly to the surface, supports this scenario (Anderson et al., 2009; Brzezinski et al., 2005; Spero and Lea, 2002). At the same time, the large increase in atmospheric $p\text{CO}_2$ at 18 ka supports the incorporation of nutrient-rich waters into Antarctic Bottom Water (AABW) at this time (Ito and Follows, 2005). Similarities between this site and a lower resolution record at RC13-259, located south of the SACCF, suggests that nutrient excess was a regional condition and that the resumption of overturning would have released CO_2 into the atmosphere via the polar Antarctic (Robinson and Sigman, 2008).

4. Conclusions

Coupled nitrogen and silicon isotope records alongside opal accumulation rates provided a quasi-quantitative estimate of Southern Ocean nutrient supply, or upwelling. Significant changes in the consumption of N and Si across the two opal accumulation peaks indicate major changes in both upwelling and nutrient demand. We find N and Si consumption to be relatively incomplete during peak opal accumulation at the onset of the deglaciation, indicative of significantly enhanced nutrient supply, on the order of 5 times present day. The second deglacial peak in opal accumulation is associated with more complete Si consumption and variable N consumption. We suggest that this peak represents strong upwelling and more complete utilization of the available silicic acid pool due to a shift in diatom assemblages or degree of silicification. Differences between the Si and N responses during opal peaks may stem from decreasing iron availability across the glacial termination and may reduce estimates of silicic acid supply during the onset of deglaciation. The reinvigoration of overturning circulation during the deglaciation is hypothesized to cause a transient peak in nutrient supply to the low latitudes (Pena et al., 2008; Robinson et al., 2009; Spero and Lea, 2002). This is supported by our data, which indicate that relatively high macronutrient concentrations were maintained in the Southern Ocean surface waters despite high demand.

Testing the relative importance of overturning on remote biogeochemical changes during the deglaciation depends upon the age control at our study site. With no radiocarbon ages from the deglacial interval, between 15 and 30 ka, it is difficult to

compare the timing of enhanced overturning and nutrient supply between the Southern Ocean and the low latitudes. Such a comparison would also provide additional evidence toward resolving the recent controversy over whether radiocarbon-poor deep water, apparent in the eastern equatorial Pacific and along the North American margin ventilates through the Southern Ocean during deglaciations (Barker et al., 2010; Basak et al., 2010; De Pol-Holz et al., 2010; Marchitto et al., 2007; Rose et al., 2010; Stott, 2010).

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Fig. 2.1. Surface ocean nutrient concentrations and core locations. Maps of nitrate (top) and silicate (bottom) surface concentrations with site locations marked. Southern Ocean site TTN057-13-PC4 (53.2°S, 5.1°E) located in the Atlantic Sector of the Southern Ocean is represented by the white circle. Climate data from Vostok ice core (78.46°S, 106.84°E - diamond) is used for comparison, as well as nutrient utilization data from core RC13-259 (53.88°S, 4.93°W - square). Note that the present day concentration of silicate falls off well before that of nitrate as one moves north from Antarctica.

Fig. 2.2. Southern Ocean nutrient dynamics and diatom abundance with climate variables from the Last Glacial Maximum to present. Southern Ocean export and nutrient dynamics data from TTN057-13PC4 are compared to Antarctic surface temperatures, CO₂ concentrations, (a. δ D, grey line; CO₂, green diamonds) and iron inputs (b. log scale dust, black line with circles) (Petit et al., 1999). Two distinct peaks in opal accumulation rate (c. blue line with squares) (Anderson et al., 2009) are observed during phase 1 and 3 of the deglaciation with corresponding troughs in the frustule bound $\delta^{15}\text{N}$ (d. violet line with circles). Low $\delta^{15}\text{N}$ values older than 18 ka are likely due to assemblage changes and are ignored (d. light violet line with circles). Observed increases in $\delta^{30}\text{Si}$ (e. red line with circles) overprinted on an overall increasing trend correspond to opal accumulation peaks and to increasing silicic acid utilization. This increased utilization is likely driven by enhanced Si uptake by diatoms as the Si/N quota (f. green line with x's) increases as iron limitation becomes progressively greater. Opal accumulation data and the $\delta^{30}\text{Si}$ data were used to calculate silicic acid supply (g), an indication of Southern Ocean upwelling intensity, for a fixed (teal line with diamonds, using equations 1&2) and variable (black points, using equations 1&5) (Hendry et al., 2010) deep ocean silicic acid content for upwelling waters. A Rayleigh-type relationship was also used to calculate silicic acid supply (brown line with inverted triangles, using equations 2-4). The horizontal dotted line represents present day upwelling rates with a value of 1. Finally, the percent abundance of ice-edge indicator species *Fragilariopsis cylindrus* and *Fragilariopsis curta* (h. green line), *Chaetoceros* resting spores (h. orange line), and *Eucampia Antarctica* (h. blue line), are plotted against the more open ocean *Fragilariopsis kerguelensis* (h. black line), which dominates the Southern Ocean today. The vertical dashed line ~18 ka representing the regime shift from predominantly ice edge species to an environment dominated by *F. kerguelensis*. Age constraints (red triangles) were used to generate an age model using a subset of the available AMS-¹⁴C age points from *Neogloboquadrina pachyderma* (Kanfoush et al., 2002) that was selected after personal communication with De Pol-Holz (2010).

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**Comparing bulk and diatom-bound nitrogen isotopes in
Southern Ocean downcore profiles**

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Abstract

The ratio of stable nitrogen isotopes in marine sediments has been used to infer variability in nitrogen utilization of nutrient replete surface waters through time. At the outset, the isotopic composition of bulk sedimentary nitrogen was measured. More recently, the nitrogen bound within microfossils has been targeted. This fraction is hypothesized to be isolated from alteration during sinking and early burial by the biominerals encapsulating it. Recent culture work of Southern Ocean diatom species demonstrated significant variability in the offset between diatom biomass and diatom frustule bound nitrogen isotope values, implying that nutrient utilization may be misinterpreted. Values for bulk sedimentary nitrogen are almost always lower than their complementary diatom-bound measurement, while in culture, biomass $\delta^{15}\text{N}$ values are always higher than the $\delta^{15}\text{N}$ of the diatom-bound fraction. Bulk and diatom-bound nitrogen isotope profiles in five cores (PA9802-9PC, E23-14, TN057-13PC4, ODP 1094, E17-9) from the Southern Ocean were examined for coherent spatial and temporal variability, in an effort to better understand controls on the two sedimentary $\delta^{15}\text{N}$ archives. All sites experience both positive and negative $\delta^{15}\text{N}_{\text{DBoffset}}$ values over time. We hypothesize that some of the differences between measured bulk and diatom-bound profiles may be attributable to (1) variable degrees of fractionation and alteration during sinking and early burial experienced by bulk material (2) changes in diatom species assemblage, (3) changes in the isotope effect (ϵ), and (4) alteration of the diatom-bound fraction. The differences in $\delta^{15}\text{N}_{\text{DBoffset}}$ between our culture and sediment based observations imply that alteration of both fractions takes place during sinking and early burial. The fraction that is lost must have a low $\delta^{15}\text{N}$ value. Despite

differences between bulk and diatom-bound $\delta^{15}\text{N}$ records, both appear to record the significant changes in nutrient utilization through time. Bulk $\delta^{15}\text{N}$ records may be overprinted by alteration during periods with smaller amplitude variation in utilization. Interpretations made with diatom-bound $\delta^{15}\text{N}$ can be further improved with the inclusion of ancillary data, such as opal flux and diatom species assemblage changes, and lead to increased resolution in nutrient utilization.

1. Introduction

Under nutrient replete conditions, the relative degree of uptake and assimilation of nitrate is recorded in the stable isotopic composition of the resulting organic matter, including that bound within a diatom frustule. Preferential incorporation of the lighter isotope (^{14}N) into biomaterials leads to an increase in the isotopic composition of the nutrient substrate and product during progressive consumption of the nutrient pools (Altabet et al., 1991; Cifuentes et al., 1989; Horrigan et al., 1990; Montoya et al., 1991; Wada, 1980; Wada and Hattori, 1978). The result is an offset, whereby the $^{15}\text{N}/^{14}\text{N}$ (as $\delta^{15}\text{N} = [(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1] * 1000$, where atmospheric N_2 is the universal reference) of phytoplankton is lower than that of the substrate nitrate. Going back in time, changes in sedimentary $\delta^{15}\text{N}$ are interpreted as changes in the degree of nutrient consumption in the surface ocean (Altabet and Francois, 1994; Brunelle et al., 2007; Farrell et al., 1995; Francois et al., 1997; Francois et al., 1993; Robinson et al., 2004; Robinson et al., 2005; Sigman et al., 1999b).

Bulk preservation issues and increases in the $\delta^{15}\text{N}$ of bulk material during early sedimentation are well documented in the literature, with alteration signals equal to and at times in excess of the expected variability to be measured (Altabet and Francois, 1994; Lourey et al., 2003). As organic matter falls through the water column it begins to disaggregate and decompose/degrade with progressive ^{15}N enrichment of the particles below the euphotic zone (Altabet, 1988; Saino and Hattori, 1980). Low-flux periods exhibit higher $\delta^{15}\text{N}$ in particulate nitrogen, which may be due to more extensive isotopic alteration of the sinking material from enhanced microbial processing, including heterotrophic scavenging and bacterial remineralization

(Altabet, 1988; Francois et al., 1993; Lourey et al., 2003).

Currently, both bulk sedimentary nitrogen ($\delta^{15}\text{N}_{\text{bulk}}$) and diatom-bound nitrogen ($\delta^{15}\text{N}_{\text{DB}}$) are used to reconstruct past nutrient consumption with varying degrees of confidence. Offsets in the $\delta^{15}\text{N}_{\text{bulk}}$ are elevated by 3-5‰ relative to sinking particulate nitrogen (PN) captured in sediment traps (Altabet and Francois, 1994; Lourey et al., 2003). This may be due to the loss of low $\delta^{15}\text{N}$ ammonium from sinking particles (Altabet, 1988). Finally, it has been speculated that the size composition of sinking particles may affect $\delta^{15}\text{N}_{\text{PN}}$ (Karsh et al., 2003). Despite the evidence for several processes that confound the $\delta^{15}\text{N}$ signal, the spatial variability across a range of sedimentation conditions mirrors the isotopic signature of nutrient consumption, suggesting that even after alteration, the bulk $\delta^{15}\text{N}$ is a useful proxy for surface nutrient consumption (Altabet and Francois, 1994). In addition, the diatom-bound N isotope proxy was developed to avoid the assumption of a constant degree of alteration downcore for work in the Southern Ocean, where alteration is quite significant (Shemesh et al., 1993; Sigman et al., 1999a). An operational definition of diatom-bound nitrogen, technically frustule-bound, is the organic fraction encased in the silica shell that is protected from the chemical cleanings used to remove any external organics prior to analysis. It is assumed that this fraction of the organic N has been isolated from alteration by microbial activity during sinking and burial in the seafloor (Robinson et al., 2004; Shemesh et al., 1993; Sigman et al., 1999a). The fraction analyzed for $\delta^{15}\text{N}$ represents approximately 60% of the total organic N of Southern Ocean sediments (Ingalls et al., 2003).

While both bulk and diatom-bound $\delta^{15}\text{N}$ have been used to infer past nutrient utilization, differences between the two proxies imply that one or both may be recording additional processes. The published sedimentary diatom-bound work for which $\delta^{15}\text{N}_{\text{bulk}}$ is available indicates that the $\delta^{15}\text{N}$ of bulk sediment is similar to, and often less than less than $\delta^{15}\text{N}_{\text{DB}}$ (Brunelle et al., 2007; Robinson and Sigman, 2008; Robinson et al., 2005; Sigman et al., 1999a). On average, this produces a negative diatom-bound offset ($\delta^{15}\text{N}_{\text{DOffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$). This is in stark contrast to the results from culture experiments with multiple diatom species (Horn et al., *in review* 2011b), which demonstrate that fresh biomass is enriched relative to fresh frustule-bound N. Since $\delta^{15}\text{N}_{\text{bulk}}$ values tend to increase with alteration, a positive $\delta^{15}\text{N}_{\text{DOffset}}$ is expected at all times.

Negative $\delta^{15}\text{N}_{\text{DOffset}}$ values imply that either: (1) the bulk material is altered in the seafloor, preferentially losing the enrichment imparted in the water column, while the diatom-bound fraction remains protected, or (2) both the bulk and diatom-bound material are altered to varying extents. In the latter case, both fractions witness the loss of isotopically light N, with the diatom-bound material experiencing slightly more fractionation or alteration to a higher degree. Alternatively, one may question the validity of culture-based studies due to their high starting nutrient conditions. However, nitrate reductase, the enzyme responsible for the fractionation associated with the uptake and assimilation of nitrate, is saturated at the nitrate concentrations of the Southern Ocean implying that culture based studies are relevant (Tischner, 2000). Furthermore, the concentration of nitrate within a diatom's internal pool is on the order of milli-molar (Needoba and Harrison, 2004), much higher than the culture

based external concentrations (Horn et al., *in review* 2011b). Therefore, enzymes associated with fractionations after nitrate reductase, such as those associated with the biosynthesis of polyamines and proteins, are likely saturated as well.

Only 15-40% of the biogenic opal produced in surface waters is preserved in the Southern Ocean sediments (DeMaster, 2002; Nelson et al., 1995). This is considered extremely high when compared to a global average for silica preservation of only ~3% (Treguer et al., 1995). Nonetheless, the loss of 60-85% of the total biogenic silica produced suggests that loss of the organic fraction may also occur and any loss has the potential to impart an isotopic alteration. On the other hand, we recover a consistent amount of diatom-bound N/ g of opal despite intense chemical cleanings. This suggests that the diatom-bound N fraction is protected by the remaining opal from chemical attack and is likely native to the opal. Here, we explore the differences between bulk and diatom-bound nitrogen isotopes in sediment cores to determine the extent and variability of alteration in both fractions and to evaluate the robustness of both N isotope proxies for nutrient utilization.

2. Methods

2.1. Core Sites and Age Models

Sediments from five Southern Ocean core locations were measured for $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ (Table 3.1) (Figure 3.1) (Orsi et al., 1995). Core site PA9802-9PC (formerly NBP 9802-9) is located in the Pacific sector on the modern Subantarctic Front (SAF) with sediments mainly composed of clays and some siliceous microfossils. Cores from TN057-13PC4 and ODP Site 1094 (nominally the same site)

were located in the Atlantic sector, approximately 2°N of the present day winter sea-ice extent, at the northernmost extent of the sea ice influence and the southernmost extent of variability of the Antarctic Polar Front (APF) (Moore et al., 1999).

Sediments were taken from a small sedimentary basin near Bouvet Island and were mainly siliceous ooze (Shipboard Scientific Party, 1999). E23-14 is ~2° south of the APF in the Pacific sector and is dominated by clays and siliceous microfossils.

Finally, E17-9 is further south in the Pacific sector ~3° south of the southern boundary of the Antarctic Circumpolar Current (ACC), and is composed of a siliceous ooze.

Age models for each of the cores are based on radiocarbon measurements, where possible, and comparisons to other cores with better age constraints when radiocarbon measurements were not possible. Seven ^{14}C Accelerator Mass Spectrometry (AMS) measurements of *Neogloboquadrina pachyderma* from TN057-13PC4 (Kanfoush et al., 2002) were used as tie-points to generate an age model (pers. comm. De Pol-Holz, 2010). The first six points covered 0-15 kya while a single point stretches the age model to 30kya. The age model for ODP Site 1094 (pers. comm. Jaccard, 2010) was generated using $\delta^{18}\text{O}$ measurements and AMS ^{14}C back to 37 kya (Hodell et al., 2003; Kanfoush et al., 2002). When radiocarbon measurements were not possible, site-specific age models were generated by comparing the abundance of *Eucampia antarctica*, % opal, % carbonate, magnetic susceptibility, and the U/Th/Pa decay series with nearby cores that had better age constraints (Bradt Miller et al., 2009; Chase et al., 2003). Age models for PA9802-9 (Chase et al., 2003) and E23-14 (Bradt Miller et al., 2009) were generated using this comparative approach, while the age model for E17-9 was based on similar comparisons, including unpublished abundance stratigraphies of

Eucampia antarctica from L. Burckle and unpublished data of *Cycladophora davisiana* from J. Hays (Chase et al., 2003). In each core, age models are of coarse resolution, often with large gaps (>15 kya) between tie-points. As a consequence, the onset of the glacial-interglacial transition in TN057-13PC4 and ODP 1094, nominally the same core, differ by 1-2 kya (Shemesh et al., 2002). As we are not particularly interested in timing, this type of error is acceptable for this study.

2.2. Bulk Sedimentary Analysis

Splits of the bulk sedimentary material were freeze-dried and ground with a mortar and pestle. Powdered material was packed into a tin cup for determination of $\delta^{15}\text{N}_{\text{bulk}}$ by combustion in an elemental analyzer coupled to the IRMS (EA-IRMS; Costech 4010-Thermo Delta V Advantage). Ammonium sulfate reference materials IAEA-N1 (0.4‰) and IAEA-N2 (20.3‰), and an in-house amino caproic acid standard were used to standardize EA-IRMS results (Bohlke and Coplen, 1995; Crosta and Shemesh, 2002; Revesz et al., 1997). Precision was determined by multiple measurements of standards within each run and replicate measurements of samples. Approximately 50% of $\delta^{15}\text{N}_{\text{bulk}}$ measurements were made in duplicate or triplicate with a standard deviation of $\pm 0.4\text{‰}$.

2.3. Diatom-bound Sedimentary Analysis

Diatoms were separated and cleaned from the remaining split of bulk sedimentary material for measurement of the diatom-bound N (Robinson et al., 2004). Physical separations to remove any lithogenics included sieving (<150 μm), settling

three times in a sodium hexametaphosphate deflocculant solution (2% by weight), and density separation with sodium polytungstate ($\rho=2.15\text{g/ml}$) (Robinson et al., 2004; Sigman et al., 1999a).

Physically separated sediment samples were cleaned using (1) a reductive cleaning step using a heated (1 hour at 80°C) sodium dithionite solution to remove adsorbed metals (2) a preliminary oxidation of external organic matter using potassium permanganate (Hasle and Fryxell, 1970), and (3) a final one hour oxidation of organic matter using a strong (72%) solution of perchloric acid in a 100°C water bath. Samples from TTN057-13PC4 included an additional weak ($\sim 35\%$) perchloric acid step for one hour prior to the final oxidation. Once clean, the diatom-bound N was oxidized to nitrate in a 0.22M potassium persulfate and 1.5M sodium hydroxide solution at 121°C for 20 minutes (Bronk et al., 2000; Robinson et al., 2004). During this step, the internal nitrogen contained within the mineral portion is released and oxidized to nitrate.

Nitrate concentrations of the persulfate oxidized frustule N were measured by chemiluminescence after reduction to NO on a Teledyne Instruments (Model 200E) chemiluminescence NO/NO_x analyzer and potassium nitrate reference standards (Braman and Hendrix, 1989). The $\delta^{15}\text{N}_{\text{DB}}$ was measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) on a Thermo Delta V Advantage IRMS. Nitrate was converted to N₂O for injection by the denitrifier method (Sigman et al., 2001). The potassium nitrate reference material IAEA-N3 (4.7‰) was used to standardize $\delta^{15}\text{N}_{\text{DB}}$ results (Bohlke and Coplen, 1995). Additionally, the potassium nitrate reference materials USGS 32 (180‰) and USGS 34 (-1.8‰) were used to

standardize diatom-bound samples from all cores, with the exception of TN057-13PC4. Precision was determined by multiple measurements of standards within each run and replicate measurements of samples. The NO_3^- concentration of oxidized diatom-bound samples were measured in duplicate to within $\pm 5\%$ or $\sim 1 \mu\text{M}$. $[\text{NO}_3^-]$ was used to calculate N content of the opal fractions. No content reproducibility was better than $\pm 0.9 \mu\text{mol N} / \text{g opal}$. Approximately 50% of $\delta^{15}\text{N}_{\text{DB}}$ samples were run in duplicate and precision was $\pm 0.3\%$.

Statistical significance was determined using a two-tail, type-3 T-test to provide p-values for variables conservatively determined to be unpaired and independent with unequal variances. Correlations were determined using Pearson product-moment correlation coefficients for two-tailed probabilities.

3. Results and Discussion

3.1 General Observations

In every core, the differences between diatom and bulk $\delta^{15}\text{N}$ varied both in magnitude and in sign. That is to say, both positive and negative values for $\delta^{15}\text{N}_{\text{DBoffset}}$ ($\delta^{15}\text{N}_{\text{DBoffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) were calculated. However, on average, diatom-bound $\delta^{15}\text{N}$ was greater than that of the bulk (Figure 3.2; Table 3.1). Antarctic Zone (AZ) sites, located to the south of the APF, were statistically indistinguishable from one another with respect to the average $\delta^{15}\text{N}_{\text{bulk}}$ and average $\delta^{15}\text{N}_{\text{DB}}$. Located 3.4°N of the APF and on the modern Subantarctic Front (SAF), PA9802-9 had generally higher $\delta^{15}\text{N}_{\text{bulk}}$ ($p < 0.001$) and $\delta^{15}\text{N}_{\text{DB}}$ ($p < 0.001$) values when compared to southern sites. This fits expectations, based on the lower surface nitrate concentrations in the

overlying waters due to progressive nutrient utilization during northward transport away from the core of upwelling (Sigman et al., 1999b). Bulk and diatom-bound $\delta^{15}\text{N}$ values from core MD84-527, located in the Indian Sector of the Southern Ocean in the Subantarctic Zone (SAZ), were also significantly higher ($p < 0.001$) than samples in the AZ (Robinson et al., 2005). Significant differences between average $\delta^{15}\text{N}_{\text{DBoffset}}$ values at PA9802-9 and those at the more southern sites ($p < 0.001$) are also apparent, with larger offsets in the SAZ site. Average $\delta^{15}\text{N}_{\text{DBoffset}}$ in AZ sites are also statistically indistinguishable from one another, indicating no relationship between $\delta^{15}\text{N}_{\text{DBoffset}}$ and distance from the APF. If the $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ were both recording nutrient utilization exclusively, then one would expect a positive relationship between the two variables. However, no statistically significant relationship ($p = 0.28$) was found to exist between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ at any core location (Figure 3.3). Likewise, no relationship was found to exist between bulk N content and diatom-bound N content (Figure 3.4). Taking all sites together, we measure a large range in $\delta^{15}\text{N}_{\text{DBoffset}}$, from -5.6‰ to 2.9‰ (Table 3.1) with positive and negative offsets occurring at each site. The observed changes in the $\delta^{15}\text{N}_{\text{DBoffset}}$ include (1) variable degrees isotopic alteration of the bulk $\delta^{15}\text{N}$ signal during sinking and early burial, (2) changes in species assemblage, (3) changes in the isotope effect of nitrate assimilation (ϵ), and (4) isotopic alteration of the diatom-bound fraction.

3.2 Offset Relationship with Opal Flux

Changes in the total particle export to the seafloor have the potential to affect the isotopic composition of sedimentary nitrogen by impacting the degree of alteration

during sinking and early sedimentation (Altabet and Francois, 1994; Lourey et al., 2003). Changes in export, as exhibited by changes in the total particle accumulation rate, are quite clear throughout the Southern Ocean. Typically, the SAZ experienced greater sediment accumulation rates during the last glacial period relative to the Holocene while the situation is reversed for the AZ. This difference is attributed to the shift in the high productivity PFZ northward, with a corresponding shift in the underlying opal belt (Bareille et al., 1998; Bareille et al., 1991; Charles et al., 1991; Dezileau et al., 2003; Mortlock et al., 1991). Given sediment trap data suggesting that particles sinking during low flux periods (e.g. wintertime) experience greater isotopic enrichment than those during higher flux or bloom conditions, one might hypothesize that the bulk $\delta^{15}\text{N}$ may be affected similarly on glacial-interglacial scales (Lourey et al., 2003).

It follows then that the $\delta^{15}\text{N}$ values of particulate nitrogen during high-flux periods might be lower and closer to actual values of the accumulated product. Assuming that diatom-bound material is a robust archive of surface conditions and not impacted by alteration, one might expect a significant relationship between opal flux and $\delta^{15}\text{N}_{\text{DBoffset}}$. High opal flux periods should be characterized by less alteration of the $\delta^{15}\text{N}_{\text{bulk}}$ values and thus a more positive $\delta^{15}\text{N}_{\text{DBoffset}}$. This simple relationship is not observed between $\delta^{15}\text{N}_{\text{DBoffset}}$ values and opal flux, however a suggestion of better preservation at high opal flux is present (Figure 3.6a). A significant ($p=0.01$) negative correlation was determined to exist between $\delta^{15}\text{N}_{\text{DBoffset}}$ and opal flux with a Pearson correlation coefficient of -0.45. Previous studies at core MD84-527 described differences of up to 4‰ between bulk and diatom-bound $\delta^{15}\text{N}$, with positive

$\delta^{15}\text{N}_{\text{DBoffset}}$ values during low opal flux periods (Robinson et al., 2005). Increasingly negative diatom-bound offsets, from $\sim 2.5\text{‰}$ down to -1.0‰ , were observed as opal flux increased from 0.8 to $1.8 \text{ g/cm}^2\text{ky}$. This was attributed to greater alteration of bulk material during periods of slower sedimentation, but may also be due to species related changes.

Opal flux varies between 0 - $1.5 \text{ g/cm}^2\text{ky}$ for all sites except TN057-13PC4, which experiences opal accumulation rates as high as $6 \text{ g/cm}^2\text{ky}$ (Figure 3.6a). The negative relationship previously observed at MD84-527 (Robinson et al., 2005) appears to hold over the same range in opal flux values, between 0.5 - $2.0 \text{ g/cm}^2\text{ky}$, for these cores ($r^2=0.53$). However, there is significant deviation from the trend at high and low opal fluxes for all of the cores investigated. At low opal flux, values below $\sim 0.5 \text{ g/cm}^2\text{ky}$, $\delta^{15}\text{N}_{\text{DBoffset}}$ values are highly variable, ranging from $+2\text{‰}$ to -6‰ (Figure 3.6a). At high opal flux, values above $\sim 2.0 \text{ g/cm}^2\text{ky}$, $\delta^{15}\text{N}_{\text{DBoffset}}$ values average -1.1‰ . All of these values occurred at site TN057-13PC4 during the deglaciation (~ 9.5 - 17.5kya). While higher flux periods could result in decreased alteration of the bulk, thus reducing $\delta^{15}\text{N}_{\text{DBoffset}}$, it does not explain the negative $\delta^{15}\text{N}_{\text{DBoffset}}$ values.

Deglacial trends in $\delta^{15}\text{N}_{\text{bulk}}$, $\delta^{15}\text{N}_{\text{DB}}$, and even $\delta^{15}\text{N}_{\text{DBoffset}}$ from cores TN057-13PC4, E17-9, and ODP 1094 are similar (Figure 3.2), suggesting that variability is in nutrient utilization and not the product of alteration. E17-9 is not as high as TN057-13PC4 during the deglaciation, and it is possible that species assemblage or epsilon is important, as well as different degree of utilization depending on geography. N

content may also relate to species assemblage and silicification (Ren et al., *in prep.* 2011).

3.3 Species Assemblage Shifts

Large shifts in the assemblages of diatom species present occur downcore in the Southern Ocean as a result of changing growth conditions such as ice presence or absence, nutrient and light availability, temperature fluctuations and more (Crosta et al., 1998; Nielsen, 2004; Zielinski and Gersonde, 1997). Culture-based studies suggest that the $\delta^{15}\text{N}_{\text{DBoffset}}$ may vary as a function of species abundance, particularly if the relative contribution of certain key species varies significantly (Horn et al., *in review* 2011b). A large change in diatom assemblage occurs at 18 kya, near the onset of deglaciation, in core TN057-13PC4 (Figure 3.8e) (Nielsen et al., 2004). This assemblage change corresponds to significant shifts in both the $\delta^{15}\text{N}_{\text{DBoffset}}$ and $\delta^{15}\text{N}_{\text{DB}}$ (Figure 3.8). The shift in $\delta^{15}\text{N}$ values is attributed to the observed increase in the abundance of *Fragilariopsis kerguelensis*. Culture studies, using relevant diatom species, suggest that a $\delta^{15}\text{N}_{\text{DBoffset}}$ of 5‰ should be expected for most species, however much lower values of around 2‰ are expected for *Fragilariopsis kerguelensis* (Horn et al., *in review* 2011b). If one were to correct the diatom-bound $\delta^{15}\text{N}$ data to account for this species change, in essence normalizing to a *Fragilariopsis kerguelensis* offset, the abrupt increase in $\delta^{15}\text{N}_{\text{DB}}$ at 18 kya disappears (Figure 3.8a). The corrected $\delta^{15}\text{N}_{\text{DBoffset}}$ is negative, similar to what is observed for the deglaciation (Horn et al., *in review* 2011a). Corrected, or *Fragilariopsis kerguelensis* normalized $\delta^{15}\text{N}_{\text{DB}}$ was then compared to $\delta^{15}\text{N}_{\text{bulk}}$ and opal flux with a weak positive

and negative trend, respectively (Figure 3.9).

3.4 Changes in ϵ

ϵ varies significantly across the Southern Ocean, with highest values near the SAF (Altabet and Francois, 2001; DiFiore et al., 2010; Lourey et al., 2003; Sigman et al., 2000), with variations attributed to light availability (Needoba et al., 2004). Enhanced light limitation, the result of deeper mixed layer depths, results in higher values for ϵ (DiFiore et al., 2010; Needoba and Harrison, 2004). ϵ is $\sim 5\text{‰}$ in the marginal ice zone and AZ, and increases to 8-9 ‰ in the Subantarctic Zone, where mixed layer depth increase to as much as 500m (DiFiore et al., 2010; Sigman et al., 1999b). If the relationship observed in culture held in the field, then one would expect a smaller $\delta^{15}\text{N}_{\text{DBoffset}}$ in the AZ relative to the SAZ, as observed here. Enhanced stratification at the ice edge and during periods with reduced upwelling could alleviate light limitation. In a temporal sense, one might predict that the $\delta^{15}\text{N}_{\text{DBoffset}}$ in AZ cores would decrease into the glacial, due to expansion of the marginal ice zone and, potentially, stratification of the water column. However, the AZ already witnesses ϵ values of 5 ‰ , making further shifts in ϵ unlikely for most of the sites studied (DiFiore et al., 2010). Looking specifically at SAZ site PA9802-9, where a shift in ϵ is possible, one sees no clear glacial-interglacial transition in the offset. A reduction in ϵ and diatom-bound offset might be expected if frontal structure shifted northwards, however this is not observed.

3.5 Alteration of the Diatom-bound Material

None of the potential impacts on the diatom-bound offset address the most significant difference between culture based observations and those from sedimentary material; negative $\delta^{15}\text{N}_{\text{DBoffset}}$ values during most of each record. The best explanation for this observation is that the diatom-bound N found in cultures is significantly different isotopically from what is preserved in sediments. Here we explore the potential cause of this difference: the loss of isotopically light N from sedimentary diatoms.

The $\delta^{15}\text{N}$ of the N fraction that has been lost may be calculated using observed culture based relationships and the measured sedimentary $\delta^{15}\text{N}$ values (Figure 3.7). Using average Holocene $\delta^{15}\text{N}_{\text{bulk}}$ values from TN057-13PC4 in conjunction with the $\delta^{15}\text{N}_{\text{DBoffset}}$ from culture (Horn et al., *in review* 2011b), we estimate a range of $\delta^{15}\text{N}$ values for the lost fraction that varies with the proportion of N lost from the initial diatom-bound fraction. Values for the $\delta^{15}\text{N}$ of the lost fraction must be negative to correct for the observed negative $\delta^{15}\text{N}_{\text{DBoffset}}$. To account for differences between fresh biomass and bulk sedimentary $\delta^{15}\text{N}$, i.e. isotopic alteration of the bulk, we made end-member estimates, assuming no fractionation and 5‰ enrichment of the bulk. Given observations, the $\delta^{15}\text{N}$ of the missing fraction falls between -80‰ and -5‰. The higher, more reasonable $\delta^{15}\text{N}$ values, occur when significant (>50%) loss of N has occurred. This is possible when we compare the diatom-bound N content data from cultures and from sediments. Diatom-bound N content is approximately 30 $\mu\text{mol N} / \text{g}$ opal in culture and approximately 15 $\mu\text{mol N} / \text{g}$ opal in *Fragilariopsis kerguelensis*

dominated sediments. However, this is difficult to reconcile when other species are investigated with similar N content from cultures and sediments.

The composition of the missing fraction lost from the diatom-bound is unknown. To date, most organic compounds that have been characterized in close association with the frustules are proteins and polyamines, which serve to facilitate the biomineralization of silica (Scheffel et al., 2011; Schröder et al., 2008). Other compounds not involved in biosilicification, such as weakly basic amines (Desclès et al., 2008; Gröger et al., 2008) and mycosporine-like amino acids (MAAs) (Ingalls et al., 2010), may also be incorporated into frustules. Chitin, a relatively unstable organic compound, has been found in great abundance within diatom cell walls, making up a lattice work or scaffold upon which biomineralization may take place (Brunner et al., 2010; Durkin et al., 2009). However, chitin in zooplankton was observed to have very high $\delta^{15}\text{N}$ value (Macko et al., 1990; Uhle et al., 1997; Uhle et al., 1999). A large review of chitin has shown that on average, insect chitin is slightly negative ($\sim -1.7\text{‰}$) and extremely variable (range $>10\text{‰}$) (Gupta, 2011). Fibers within silica-forming protein microrings resemble the nonporous portions of girdle band silica (Scheffel et al., 2011). This insoluble organic matrix exhibits species-specific nanopatterns and appears to facilitate enhanced silicification along girdle bands. Selective preservation of girdle bands in sediments and species-specific compounds reinforces the idea that species assemblage data is required to more appropriately interpret nutrient utilization from $\delta^{15}\text{N}_{\text{DB}}$. Very few studies to date have focused on low-molecular weight organic compounds contained within the frustule (Ingalls et al., 2004) and there is much need for further research to identify the composition and function of bound compounds.

3.6 Investigation of TN057-13PC4

To synthesize this information, we will look in detail at site TN057-13PC4 using N isotope, species abundance, and opal flux data. In order to first account for known species related differences, we will work from the *Fragilariopsis kerguelensis* corrected $\delta^{15}\text{N}$ profile for TN057-13PC4. With this effect essentially removed, we can simplify our expectations. Changes in nitrate utilization should impact both $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$. Variations in opal flux may enhance alteration, likely affecting the $\delta^{15}\text{N}_{\text{bulk}}$ and potentially the $\delta^{15}\text{N}_{\text{DB}}$ and the $\delta^{15}\text{N}_{\text{DBoffset}}$. Increased iron availability has been shown to shift diatom assemblages towards smaller, more dissolution susceptible species, which also may result in/or enhance the alteration of diatom-bound material. (Horn et al., *in review* 2011a). Mesoscale iron enrichment experiments have further demonstrated that silicification changes significantly, with a reduction in frustule thickness (Coale et al., 2004; Hutchins and Bruland, 1998). This affect of iron fertilization is associated with a decrease in the Si:N uptake quota in diatoms (Hutchins and Bruland, 1998; Leynaert et al., 2004; Takeda, 1998). Iron fluxes were greatly enhanced during glacial times (Petit et al., 1999) and this change may have impacted preservation of the diatom-bound signal. With these considerations in mind, we can examine the temporal variability in $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{DBoffset}}$ at TN057-13PC4.

During the glacial, when opal fluxes were lower and Fe availability relatively high, one might expect preservational artifacts in both the bulk and the diatom bound $\delta^{15}\text{N}$ records. However, these two records, once adjusted for the species-specific $\delta^{15}\text{N}_{\text{DBoffset}}$, are very similar, suggestive of enhanced nitrate consumption during the LGM. The diatom-bound offset, again corrected for species variations, is negative.

This suggests that the diatom-bound $\delta^{15}\text{N}$ may have been altered during this high Fe low-flux period.

The deglaciation witnesses a huge increase in opal flux attributed to enhanced upwelling (Anderson et al., 2009) (Figure 3.8). A synchronous drop in $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ is consistent with an increase in nutrient supply, as they indicate reduced nitrate consumption during peak opal accumulation (Horn et al., *in review* 2011a). The diatom-bound offset increases from a minimum during the deglaciation to positive values in the early Holocene, in concert with decreasing iron limitation (Horn et al., *in review* 2011a). This shift occurs as preservation likely increased due to enhanced silicification. At the same time, $\delta^{15}\text{N}_{\text{bulk}}$ may have experienced huge differences in preservation between the high opal accumulation rate peaks and the lower flux period in the early Holocene. While changes in total sediment accumulation rates may have affected the $\delta^{15}\text{N}_{\text{bulk}}$, variations in $\delta^{15}\text{N}_{\text{DBoffset}}$ and silicification suggest that preservation likely varied in the $\delta^{15}\text{N}_{\text{DB}}$ as well. Both proxies, when corrected for species changes, appear to reveal similar changes in nutrient consumption. Two complications include changes in the amplitude of $\delta^{15}\text{N}_{\text{DB}}$ variability that exceeds that of the $\delta^{15}\text{N}_{\text{bulk}}$, and the diatom-bound shows an increase in the late Holocene that is not apparent in the bulk. The latter may be due to species related changes as mentioned above.

A comparison of several Atlantic and Pacific sector cores from the Southern Ocean provide insight into cross-basin similarities (Figure 3.10). Taking age model uncertainties of up to ~2 ky, consistently low $\delta^{15}\text{N}_{\text{DB}}$ values are found during the glacial with elevated and variable values during the deglaciation. Note that a similar

peak in $\delta^{15}\text{N}_{\text{DB}}$ is found at cores south of the polar front in both the Atlantic (RC13-259) and Pacific (E17-9) sectors. $\delta^{15}\text{N}_{\text{DB}}$ values return to intermediate values during the Holocene for each core. While these trends are very general, they do suggest that a relatively consistent and large-scale shift occurred from the LGM to present. It is possible that a similar *Fragilariopsis kerguelensis* correction could be applied to each to determine a more appropriate $\delta^{15}\text{N}_{\text{DB}}$ value to reconstruct nutrient utilization.

$\delta^{15}\text{N}$ values consistently differ between the bulk and diatom-bound fraction in opal-rich sediments (Figure 3.3). It has been hypothesized that bulk measurements may be contaminated by an additional N pool (likely atmospheric) as N_2 is trapped within or adsorbed onto the bulk combustion samples (Robinson et al., 2004). Therefore, we assert that the diatom-bound proxy is likely a more robust proxy for nutrient utilization, assuming one is able to account for changes in species abundance and correct $\delta^{15}\text{N}_{\text{DB}}$ values.

4. Conclusions

There are caveats to the use of $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$ as a proxy for surface nitrogen utilization. An analysis of five cores in the Southern Ocean demonstrates that the $\delta^{15}\text{N}_{\text{DBOffset}}$, the difference between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{DB}}$, varies significantly over time in each core. Each core experiences both positive to negative values of $\delta^{15}\text{N}_{\text{DBOffset}}$ over the course of our investigation. The cause of this variability appears to be largely due to species assemblage changes with time and shifts in the degree of silicification associated with Fe availability. However, changes in nutrient consumption do not appear to be overprinted entirely once species assemblage

changes are considered. During periods with lower utilization or smaller amplitude variation in utilization, changes in alteration from silicification and opal flux may be important. Regardless, there is a loss of an isotopically light fraction of in sedimentary diatom-bound material that needs to be identified.

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Table 3.1: Core locations and isotopic data relative to the APF.

Site	Latitude	Longitude	Water Depth (m)	Relative APF ^a (°)	Average $\delta^{15}\text{N}_{\text{bulk}}$ (‰)	Average $\delta^{15}\text{N}_{\text{DB}}$ (‰)	Average $\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Range $\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)
PA9802-9	56.94°S	170.23°W		3.4°	5.6 ± 0.9^b	7.4 ± 1.3	-1.8 ± 1.6	6.6
E23-14	63.82°S	108.85°W	4957	-2.1°	3.0 ± 1.3	4.1 ± 1.9	-0.5 ± 2.2	8.5
ODP 1094	53.18°S	5.13°E	2818	-3.5°	4.0 ± 0.7	4.4 ± 0.7	-0.5 ± 0.9	4.3
TN057-13PC4	53.2°S	5.1°E	2850	-3.5°	3.8 ± 0.5	4.0 ± 0.9	-0.3 ± 1.0	4.2
E17-9	63.08°S	135.08°W	4849	-4.5°	3.8 ± 1.0	4.3 ± 1.0	-0.4 ± 1.2	5.4
Overall Average ^c					3.9 ± 1.1	4.6 ± 1.4	-0.6 ± 1.3	

^aRelative APF reflects position of cores expressed as degrees north of the APF as defined by *Orsi et al.* (1995)

^bReported variability in core data is the standard deviation of all measurements

^cReported overall averages and variability include weighted averages and standard deviations.

Figure 3.1: Core locations are plotted on a map of surface ocean NO_3^- from WOD09 (Schlitzer, 2010). The modern Subantarctic Front (SAF – outer dashed line), Antarctic Polar Front (APF – middle solid line), and the southern boundary of the Antarctic Circumpolar Current (ACC – inner dashed line) are plotted for reference (Orsi et al., 1995). Core MD84-527 from the Sub Antarctic Zone (SAZ) is included for comparison (Robinson et al., 2005).

Figure 3.2: Bulk and diatom-bound $\delta^{15}\text{N}$ are plotted for each core location along the top axes, in addition to the corresponding diatom-bound offset ($\delta^{15}\text{N}_{\text{DBOffset}} = \delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) along the bottom axes. High sampling resolution in TN057-13PC4 allows one to see an anti-correlated trend between the diatom-bound offset and the isotopic composition of bulk and diatom-bound material.

Figure 3.3: The isotopic composition of diatom-bound samples for each core is plotted against their corresponding bulk values for each core. No statistically significant relationships were found to exist.

Figure 3.4: The nitrogen contents of bulk and diatom-bound samples are plotted against one another from each core. No statistically significant relationships were found to exist.

Figure 3.5: The nitrogen contents of the bulk (green) and diatom-bound (blue) fractions are plotted against $\delta^{15}\text{N}_{\text{DBOffset}}$ for core. A weak positive relationship was found to exist between the bulk N content and the $\delta^{15}\text{N}_{\text{DBOffset}}$ at core PA9802-9 with an $r^2=0.20$. However, all other r^2 values for bulk and diatom-bound N content fell below 0.12 implying that there is no statistically significant relationship between N content and $\delta^{15}\text{N}_{\text{DBOffset}}$.

Figure 3.6: The $\delta^{15}\text{N}_{\text{DBOffset}}$ for each core was linearly interpolated down to the resolution of opal flux data and compared between 0-40kya. Opal flux data from TN057-13PC4 (Anderson et al., 2009), E23-14 (Bradtmitter et al., 2009), E17-9, and PA9802-9 (Chase et al., 2003) are used for this comparison, as well as core MD84-527 from the Indian sector of the SAZ (Robinson et al., 2005). **(a)** $\delta^{15}\text{N}_{\text{DBOffset}}$ has a large range during periods of low opal flux, but converges to a value of approximately -1.1‰ during periods of enhanced opal flux. Weak linear relationships ($r^2 \approx 0.10$) are depicted with the grey lines to draw attention to a general pattern. There is the caveat that opal fluxes in excess of 2.0 g/cm²ky were only observed in this study at TN057-13PC4 during the deglaciation. **(b)** No relationship was found to exist between $\delta^{15}\text{N}_{\text{DBOffset}}$ and age. However, only positive values are found between 0 to 5 kya.

Figure 3.7: The $\delta^{15}\text{N}$ of the lost fraction is calculated for two hypothetical cases of bulk alteration. An average Holocene $\delta^{15}\text{N}_{\text{bulk}}$ of 4‰ is observed at TN057-13PC4 with an expected $\delta^{15}\text{N}_{\text{DBoffset}}$ of 5‰ from culture data (Horn et al., *in press* 2011). The difference between these two values would produce the expected $\delta^{15}\text{N}_{\text{DB}}$, a value of -1‰. In this first example, it is assumed that there is no bulk alteration (0‰). The $\delta^{15}\text{N}$ of the lost fraction may then be calculated using the following equation:

$$\text{Lost Fraction } \delta^{15}\text{N} = \text{Expected } \delta^{15}\text{N}_{\text{DB}} - \text{Observed } \delta^{15}\text{N}_{\text{DB}} * \frac{(1-F)}{F} \quad \text{eq.1}$$

The calculated $\delta^{15}\text{N}$ is plotted as a function of F, whereby F represent fraction of the material lost. As the expected alteration of the bulk increases, the $\delta^{15}\text{N}$ of the lost fraction must become increasingly negative to maintain the observed Holocene $\delta^{15}\text{N}_{\text{DB}}$ value of 4‰. In all cases, the $\delta^{15}\text{N}$ of the lost fraction is negative to correct for the negative $\delta^{15}\text{N}_{\text{DBoffset}}$ observed downcore.

Figure 3.8: Southern Ocean export and nutrient dynamics data are compared to species abundance. All data presented are from core TTN057-13PC4 using the same updated AMS- ^{14}C age points of *Neogloboquadrina pachyderma* (pers. comm. De Pol-Holz, 2010; Kanfoush et al., 2002). **(a)** The $\delta^{15}\text{N}_{\text{DBoffset}}$ ($\delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{DB}}$) (dark green diamonds) varies between positive and negative values from the LGM to present. Glacial $\delta^{15}\text{N}_{\text{DBoffset}}$ values have been normalized to account for variations in *Fragilariopsis kerguelensis* and *Fragilariopsis cylindrus* (gray squares) based upon previous work investigating the effects of species composition between the LGM and the Holocene (Horn et al., *in review* 2011b). Corresponding bulk (red circles) and diatom-bound (blue squares) **(b)** $\delta^{15}\text{N}$ and **(c)** N content are included. Note that scales and range are different between diatom-bound and bulk. Again, $\delta^{15}\text{N}_{\text{DB}}$ values were corrected using the normalized $\delta^{15}\text{N}_{\text{DBoffset}}$ from species assemblage changes (light violet triangles) **(d)** Periods with enhanced upwelling typically resulted in higher opal flux values (green circles) (Anderson et al., 2009). **(e)** Finally, the percent abundance of ice-edge indicator species *Fragilariopsis cylindrus* and *Fragilariopsis curta* (dashed green line), *Chaetoceros* resting spores (solid orange line), and *Eucampia Antarctica* (dotted blue line), are plotted against the more open ocean *Fragilariopsis kerguelensis* (solid black line), which dominates the Southern Ocean today (Nielsen, 2004). There was a regime shift from predominantly ice edge species to an environment dominated by *Fragilariopsis kerguelensis* at approximately 18kya. Dark grey bars correspond to periods with peaks in $\delta^{15}\text{N}_{\text{DBoffset}}$, opal flux, and likely upwelling during the deglaciation. Lighter grey bars correspond to “baseline” periods that result in negative $\delta^{15}\text{N}_{\text{DBoffset}}$.

Figure 3.9: The *Fragilariopsis kerguelensis* normalized $\delta^{15}\text{N}_{\text{DB}}$ from TN057-13PC4 is compared to corresponding $\delta^{15}\text{N}_{\text{bulk}}$ (left) and opal flux data (right). A weak positive relationship exists between the normalized $\delta^{15}\text{N}_{\text{DB}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ ($r^2 = 0.20$). A weak negative relationship exists between normalized $\delta^{15}\text{N}_{\text{DB}}$ and opal flux ($r^2 = 0.19$). Normalized $\delta^{15}\text{N}_{\text{DB}}$ was interpolated down to the resolution of opal flux data.

Figure 3.10: A similar pattern in diatom-bound $\delta^{15}\text{N}$ is observed at several Southern Ocean core locations over the last 40 kya. Pacific sector cores E17-9 and PA9802-9, as well as Atlantic sector cores TN057-13PC4 and RC13-259 are compared.

APPENDIX A

MANUSCRIPT 1 – DATA TABLES

Culture Data

The following tables represent data collected from each 20L carboy culture. The species in italics (e.g. *Thalassiosira aestivalis*) is followed by the species culture code (e.g. GSO100) and replicate or transfer number (e.g. T34). A description of the methods and instruments used to collect these data may be found in Manuscript 1. A summary table is also provided with relevant averages for each culture experiment.

Nutrient Data

Initial and final PO_4^{3-} and Si concentrations are presented. Data was collected by Amy Van Keuren of the Nixon Lab (GSO).

Core Data

Relevant N isotope data from core TN057-13PC4 is provided. In addition, average species abundance for relevant species is presented for the Holocene and LGM. Species data was interpreted from Nielsen [2004].

Data Requests

A digital copy of the data may be acquired by contacting Professor Rebecca Robinson at GSO (rebeccar@gso.uri.edu), or Matthew Horn (horn@gso.uri.edu).

Appendix A: Culture Data

Thalassiosira aestivalis (GSO100-T34)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
12/2/08 14:45	0.00	0.15	850.56	0.00	1.00				8.78
12/3/08 10:35	19.83	0.37			1.00				8.87
12/4/08 10:45	44.00	1.09			1.00				8.93
12/5/08 10:45	68.00	2.93	728.25	0.14	0.86				8.71
12/6/08 13:20	94.58	6.69	734.11	0.14	0.86				8.98
12/7/08 13:10	118.42	14.81	709.02	0.17	0.83				8.55
12/8/08 14:15	143.50	24.15	693.24	0.18	0.82	6.57	-5.32	9.05	9.69
12/9/08 16:20	169.58	31.18	687.04	0.19	0.81	5.71	-0.53	10.71	9.74
12/10/08 15:30	192.75	39.82	682.23	0.20	0.80	5.72	-1.31	7.66	9.11
12/11/08 13:25	214.67	47.22	675.92	0.21	0.79	5.97	2.84	7.76	10.01
12/12/08 14:40	239.92	40.25	667.59	0.22	0.78	5.43	2.12	8.92	10.03
12/13/08 12:25	261.67	33.18	663.93	0.22	0.78	5.35	-2.69	7.89	9.98
12/14/08 13:35	286.83	27.42	655.01	0.23	0.77	5.40	-5.40	7.77	10.11
12/15/08 10:15	307.50	28.19	660.94	0.22	0.78	5.58	3.32	8.59	9.64

Appendix A: Culture Data

Thalassiosira aestivalis (GSO100-T45)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
1/22/09 12:01	0.00	0.17	248.46	0.01	0.99				8.86
1/23/09 10:10	22.15	0.29	251.87	0.01	0.99	2.40			8.54
1/24/09 14:45	50.73	0.65	248.67	0.01	0.99	3.15			9.18
1/25/09 10:30	70.48	0.85	244.63	0.03	0.97	3.09			9.09
1/26/09 10:30	94.48	1.45	244.34	0.03	0.97	3.22	0.63	34.07	8.86
1/27/09 9:35	117.57	2.70	238.39	0.05	0.95	4.60			9.20
1/28/09 12:45	144.73	4.78	223.79	0.11	0.89	4.82	-0.29	13.36	9.72
1/29/09 8:40	164.65	8.88	212.47	0.16	0.84	6.13	-2.16	11.61	8.72
1/30/09 13:55	193.90	16.32	176.37	0.30	0.70	6.66	-0.30	9.84	8.85
1/31/09 11:20	215.32	27.34	150.38	0.40	0.60	6.98	0.40	10.39	9.97
2/1/09 14:40	242.65	41.11	122.95	0.51	0.49	7.09	-0.61	11.00	10.92
2/2/09 10:50	262.82	36.38	116.42	0.54	0.46	6.90		8.83	11.15
2/3/09 10:00	285.98	36.41	104.35	0.59	0.41	7.20	1.17	7.89	11.60
2/4/09 10:45	310.73	35.91	94.24	0.63	0.37	7.41	5.40	10.06	12.60

Appendix A: Culture Data

***Thalassiosira aestivalis* (GSO100-T46)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
1/22/09 12:01	0.00	0.22	217.68	0.00	1.00				8.88
1/23/09 10:10	22.15	0.25	213.91	0.02	0.98	5.80			8.79
1/24/09 14:45	50.73	0.59	213.24	0.02	0.98	0.82			9.31
1/25/09 10:30	70.48	0.99	210.69	0.03	0.97	0.14			8.93
1/26/09 10:30	94.48	1.89	208.03	0.04	0.96	2.60	-1.14	38.13	8.74
1/27/09 9:35	117.57	3.53	201.39	0.07	0.93	3.63			9.45
1/28/09 12:45	144.73	7.65	185.76	0.15	0.85	4.39	-2.96	13.57	8.92
1/29/09 8:40	164.65	12.71	163.14	0.25	0.75	4.85	-2.53	12.88	9.29
1/30/09 13:55	193.90	27.68	116.69	0.46	0.54	6.03	-0.89	8.77	10.50
1/31/09 11:20	215.32	42.54	86.05	0.60	0.40	5.81	0.64	8.58	12.11
2/1/09 14:40	242.65	55.35	64.27	0.70	0.30	6.44	-0.03	8.80	14.66
2/2/09 10:50	262.82	59.05	51.77	0.76	0.24	6.58	1.80	8.19	16.41
2/3/09 10:00	285.98	64.15	41.75	0.81	0.19	6.54	3.17	10.53	17.34
2/4/09 10:45	310.73	62.51	34.72	0.84	0.16	6.74	2.68	9.66	18.19

Appendix A: Culture Data

Thalassiosira nordenskioldii (CCMP997-T36)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
2/14/09 11:30	0.00	0.48	237.42	0.00	1.00				8.84
2/15/09 11:00	23.50	1.70	228.47	0.04	0.96	5.88			8.79
2/16/09 8:45	45.25	3.54	224.28	0.06	0.94	4.72			9.08
2/17/09 10:45	71.25	9.48	203.33	0.14	0.86	5.05		119.09	9.27
2/18/09 10:45	95.25	19.18	165.59	0.30	0.70	4.60	-1.52	18.93	10.87
2/19/09 10:30	119.00	21.95	159.63	0.33	0.67	3.82	-3.25	14.53	11.95
2/20/09 14:30	147.00	21.28	96.64	0.59	0.41	4.83	-4.54	11.27	12.97
2/21/09 11:30	168.00	37.64	77.75	0.67	0.33	4.95	-5.76	11.61	13.34
2/22/09 12:15	192.75	42.12	70.49	0.70	0.30	4.82	2.50	16.42	14.18
2/23/09 9:30	214.00	60.35	61.58	0.74	0.26	5.64	-2.73	11.76	14.79
2/24/09 10:15	238.75	79.55	49.35	0.79	0.21	4.89	-4.97	10.30	14.88
2/25/09 11:20	263.83	83.19	47.21	0.80	0.20	5.53	-2.26	8.08	16.16
2/27/09 9:45	310.25	53.63	48.49	0.80	0.20	5.72	1.87	18.15	19.19

Appendix A: Culture Data

Thalassiosira nordenskioldii (CCMP997-T37)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
2/14/09 11:30	0.00	0.49	222.15	0.00	1.00				8.72
2/15/09 11:00	23.50	1.63	219.75	0.01	0.99				9.11
2/16/09 8:45	45.25	3.20	211.63	0.05	0.95	6.61			9.06
2/17/09 10:45	71.25	8.32	197.64	0.11	0.89	4.85			9.00
2/18/09 10:45	95.25	19.21	167.04	0.25	0.75	4.91	6.56	70.10	10.68
2/19/09 10:30	119.00	25.85	122.10	0.45	0.55	4.80	0.13	14.63	11.45
2/20/09 14:30	147.00	35.48	81.07	0.64	0.36	5.56	-1.29	11.81	12.61
2/21/09 11:30	168.00	50.38	61.83	0.72	0.28	5.46	-4.80	15.74	13.55
2/22/09 12:15	192.75	55.98	54.92	0.75	0.25	4.72	-5.46	13.06	14.28
2/23/09 9:30	214.00	73.85	56.79	0.74	0.26	5.58	-2.86	12.80	17.10
2/24/09 10:15	238.75	94.02	39.06	0.82	0.18	5.65	2.58	7.07	16.21
2/25/09 11:20	263.83	95.79	35.13	0.84	0.16	6.01	-5.30	15.23	16.52
2/27/09 9:45	310.25	102.83	29.11	0.87	0.13	5.85	-1.58	8.69	17.40

Appendix A: Culture Data

Pseudo-nitzschia seriata (CCMP1309-T26)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
3/17/09 11:05	0.00	-0.02	258.34	0.00	1.00				8.76
3/18/09 11:00	23.92	-0.05							
3/19/09 10:00	46.92	-0.05							
3/21/09 9:50	94.75	-0.02							
3/23/09 10:45	143.67	0.07	241.74	0.06	0.94				8.89
3/24/09 10:15	167.17	0.17	258.14	0.00	1.00				9.41
3/25/09 14:25	195.33	0.47	255.14	0.01	0.99	6.59			8.36
3/26/09 15:00	219.92	0.86				3.91			8.61
3/27/09 12:45	241.67	1.21	247.70	0.04	0.96				8.41
3/30/09 14:00	314.92	6.62	230.32	0.11	0.89	3.86	-0.34	18.52	9.29
3/31/09 11:30	336.42	11.35	222.80	0.14	0.86	2.91	-9.44	42.37	9.62
4/1/09 15:50	364.75	18.41	185.66	0.28	0.72	1.77	-10.48	15.64	10.97
4/2/09 10:15	383.17	26.01	166.51	0.36	0.64	2.27	-7.13	15.40	12.07
4/3/09 10:00	406.92	38.05	145.43	0.44	0.56	3.31		9.90	13.85
4/5/09 19:45	464.67	43.43	87.28	0.66	0.34	2.90	-5.71	7.23	18.02
4/7/09 11:15	504.17	51.91	70.04	0.73	0.27	5.50	-2.32	9.85	20.27
4/9/09 14:00	554.92	47.30	54.29	0.79	0.21	5.22	-0.17	8.39	22.00

Appendix A: Culture Data

Pseudo-nitzschia seriata (CCMP1309-T27)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N DB	δ ¹⁵ N μmol N / g Opal Substrate	δ ¹⁵ N
3/17/09 11:05	0.00	-0.02	244.15	0.00	1.00			8.78
3/18/09 11:00	23.92	-0.04						
3/19/09 10:00	46.92	-0.05						
3/21/09 9:50	94.75	-0.05						
3/23/09 10:45	143.67	0.00	243.41	0.00	1.00			8.93
3/24/09 10:15	167.17	0.04	242.95	0.00	1.00			9.10
3/25/09 14:25	195.33	0.16	240.01	0.02	0.98	11.96		9.09
3/26/09 15:00	219.92	0.34	238.67	0.02	0.98			9.06
3/27/09 12:45	241.67	0.44	239.82	0.02	0.98	2.66		8.90
3/30/09 14:00	314.92	2.07	236.97	0.03	0.97	2.61		9.65
3/31/09 11:30	336.42	3.36	224.16	0.08	0.92	4.34		8.72
4/1/09 15:50	364.75	4.73	224.33	0.08	0.92	2.99	3.83	9.72
4/2/09 10:15	383.17	7.05	207.54	0.15	0.85	3.51	50.78	9.96
4/3/09 10:00	406.92	13.01	176.65	0.28	0.72	1.47	35.04	10.13
4/5/09 19:45	464.67	28.90	139.91	0.43	0.57	2.98	25.12	12.11
4/7/09 11:15	504.17	43.61	120.54	0.51	0.49	-1.26	9.00	14.55
4/9/09 14:00	554.92	52.93	80.67	0.67	0.33	4.76	6.51	16.79

Appendix A: Culture Data

Pseudo-nitzschia seriata (CCMP1309-T35)

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk $\delta^{15}\text{N}$ DB	$\delta^{15}\text{N}$ μmol N / g Opal Substrate	$\delta^{15}\text{N}$
7/15/09 14:30	0.00	0.19	168.90	-0.04	1.00			9.32
7/16/09 11:00	20.50	0.26						
7/17/09 14:50	48.33	1.24						
7/20/09 13:35	119.08	14.07	162.27	0.00	0.96	-2.17	3.22	10.67
7/21/09 16:00	145.50	32.66	137.16	0.15	0.81	-2.37	0.12	13.12
7/22/09 10:45	164.25	50.23	115.70	0.29	0.69	-1.70	0.30	15.70
7/23/09 11:00	188.50	60.28						
7/28/09 14:35	312.08	57.26	52.29	0.68	0.31	2.14	2.45	25.83
7/29/09 11:15	332.75	54.29	48.44	0.70	0.30	2.47	2.19	27.15

Appendix A: Culture Data

***Fragilariopsis cylindrus* (CCMP1102-T27)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
4/21/09 12:15	0.00	0.09	230.16	0.00	1.00				9.35
4/23/09 10:10	45.92	0.04	227.75	0.01	0.99				8.95
4/24/09 10:15	70.00	0.09							
4/26/09 11:45	119.50	0.23	227.15	0.01	0.99				9.17
4/27/09 10:45	142.50	0.35	221.76	0.04	0.96				8.89
4/28/09 14:45	170.50	0.47							8.93
4/30/09 13:45	217.50	1.63	221.71	0.04	0.96				8.52
5/1/09 10:10	237.92	2.33	220.17	0.04	0.96	6.44			9.20
5/3/09 10:50	286.58	6.09	215.71	0.06	0.94	7.68			9.20
5/4/09 13:10	312.92	9.00	212.53	0.08	0.92	8.44			9.33
5/5/09 9:00	332.75	13.26	209.68	0.09	0.91	7.46	-0.12	57.48	9.04
5/6/09 9:45	357.50	19.06				7.15	0.83	16.08	10.22
5/7/09 10:00	381.75	28.78	186.80	0.19	0.81	7.96	2.59	22.74	10.10
5/8/09 10:20	406.08	42.55	172.16	0.25	0.75	7.15	3.05	87.08	10.86
5/9/09 11:00	430.75	59.30	152.53	0.34	0.66	7.72	3.03	69.31	11.36
5/11/09 10:20	478.08	93.02	110.48	0.52	0.48	6.92	2.74	9.29	12.07
5/12/09 9:45	501.50	95.61	91.86	0.60	0.40	7.56	5.58	7.95	13.11

Appendix A: Culture Data

***Fragilariopsis cylindrus* (CCMP1102-T28)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N DB	δ ¹⁵ N μmol N / g Opal	Substrate δ ¹⁵ N
4/21/09 12:15	0.00	0.06	228.29	0.00	1.00			9.18
4/23/09 10:10	45.92	0.03	226.47	0.01	0.99			9.28
4/24/09 10:15	70.00	0.09						
4/26/09 11:45	119.50	0.20	222.18	0.03	0.97			9.30
4/27/09 10:45	142.50	0.29						9.22
4/28/09 14:45	170.50	0.35	221.47	0.03	0.97			
4/30/09 13:45	217.50	1.08	221.68	0.03	0.97			9.51
5/1/09 10:10	237.92	1.40	218.88	0.04	0.96	7.86		8.90
5/3/09 10:50	286.58	3.27	216.88	0.05	0.95	7.63		8.99
5/4/09 13:10	312.92	4.95	213.74	0.06	0.94	8.13		9.35
5/5/09 9:00	332.75	6.67	210.77	0.08	0.92	7.19		8.99
5/6/09 9:45	357.50	9.90	203.80	0.11	0.89	8.87	23.76	9.14
5/7/09 10:00	381.75	13.28	199.92	0.12	0.88	8.46	12.84	9.47
5/8/09 10:20	406.08	20.95	197.27	0.14	0.86	8.08	8.48	9.64
5/9/09 11:00	430.75	29.86	186.10	0.18	0.82	8.79	53.18	9.62
5/11/09 10:20	478.08	55.22	151.42	0.34	0.66	7.94	9.29	11.31
5/12/09 9:45	501.50	62.48	130.27	0.43	0.57	7.86	7.95	11.59

Appendix A: Culture Data

***Fragilariopsis cylindrus* (CCMP1102-T33)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
6/29/09 12:00	0.00	0.01	146.68	0.00	1.00				8.55
6/30/09 10:45	22.75	0.02							
7/1/09 9:00	45.00	0.02							
7/3/09 10:00	94.00	0.31	147.05	0.00	1.00				8.82
7/5/09 22:45	154.75	0.58							
7/6/09 11:15	167.25	0.78							
7/7/09 11:10	191.17	1.24							
7/8/09 14:35	218.58	1.84	140.61	0.04	0.96	1.35			9.10
7/10/09 13:45	265.75	2.79							
7/13/09 13:25	337.42	10.97	124.76	0.15	0.85	4.95			9.51
7/14/09 10:50	358.83	10.48	119.04	0.19	0.81	5.28	3.20	8.54	9.79
7/15/09 11:10	383.17	11.65							
7/16/09 11:00	407.00	19.76							
7/17/09 14:50	434.83	20.89	88.25	0.40	0.60	6.60	5.40	2.47	10.28
7/20/09 13:35	505.58	38.07	53.73	0.63	0.37	8.08		4.73	11.34
7/21/09 16:00	532.00	57.09	36.64	0.75	0.25		2.78		13.11
7/22/09 10:45	550.75	65.63							
7/23/09 11:00	575.00	75.55	6.44	0.96	0.04	9.51	3.57	3.36	16.22
7/28/09 14:35	698.58	40.59	1.26	0.99	0.01	10.12	6.86	3.79	

Appendix A: Culture Data

***Fragilariopsis kerguelensis* (PAB1-T4)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
9/15/09 16:20	0.00	0.02	172.99	0.01	0.99				
9/16/09 10:25	18.08	0.01							8.95
9/18/09 9:30	65.17	0.01	174.30	0.00	1.00				8.78
9/21/09 14:10	141.83	0.85	167.77	0.04	0.96	3.08			
9/22/09 13:20	165.00	2.37							
9/23/09 11:15	186.92	3.66							
9/24/09 14:15	213.92	4.52	164.56	0.06	0.94	4.96			
9/25/09 14:25	238.08	6.27	160.79	0.08	0.92	5.21			8.67
9/26/09 13:45	261.42	8.59	144.29	0.17	0.83	6.26			9.71
9/27/09 11:45	283.42	12.14	144.63	0.17	0.83	8.02	6.99	33.71	9.76
9/29/09 10:15	329.92	19.41	125.84	0.28	0.72	8.85	9.09	33.70	9.38
10/1/09 14:45	382.42	29.42	94.77	0.46	0.54	9.57	6.60	26.84	8.96
10/2/09 10:00	401.67	28.57	81.89	0.53	0.47		8.21	32.03	10.29
10/9/09 14:15	573.92	25.72	56.10	0.68	0.32	9.85	8.90	35.09	10.53

Appendix A: Culture Data

***Fragilariopsis kerguelensis* (PAB1-T5)**

Date / Time	Hour	Fluorescence	Nitrate Concentration (μM)	% Utilization	f	Bulk δ ¹⁵ N	DB δ ¹⁵ N	μmol N / g Opal	Substrate δ ¹⁵ N
9/15/09 16:20	0.00	0.05	170.46	0.03	0.97				9.64
9/16/09 10:25	18.08	0.02							
9/18/09 9:30	65.17	0.04	174.06	0.01	0.99				8.53
9/21/09 14:10	141.83	0.67	176.14	0.00	1.00	3.74			9.32
9/22/09 13:20	165.00	2.37							
9/23/09 11:15	186.92	2.68							
9/24/09 14:15	213.92	4.18	168.66	0.04	0.96	5.79			9.87
9/25/09 14:25	238.08	5.87	161.98	0.08	0.92	7.67			9.62
9/26/09 13:45	261.42	8.64	159.46	0.09	0.91	9.70			10.40
9/27/09 11:45	283.42	10.70	155.64	0.12	0.88	6.97	7.70	33.22	9.54
9/29/09 10:15	329.92	18.05	132.52	0.25	0.75	8.48	5.24	33.63	9.58
10/1/09 14:45	382.42	28.62	98.42	0.44	0.56	9.24	7.52	34.26	10.35
10/2/09 10:00	401.67	30.80	85.88	0.51	0.49	9.90	5.58	32.28	10.09
10/9/09 14:15	573.92	32.12	57.67	0.67	0.33	9.79	8.60	36.24	10.54

Appendix A: Culture Summary Table

Average values for each experiment

Culture	f	Doubling Time	μ	Initial [NO ₃]	Initial Sub $\delta^{15}\text{N}$	DB Error	$\delta^{15}\text{N}_{\text{offset}}$	$\delta^{15}\text{N}_{\text{offset}}$	stdev	ϵ
CCMP997-T36	0.20	18.21	0.05	237.42	8.84	0.86	7.88	2.83		4.73
CCMP997-T37	0.13	18.46	0.05	222.15	8.72	0.89	6.37	5.00		4.26
CCMP1102-T27	0.40	36.15	0.03	230.16	9.35	0.45	9.24	0.62		4.64
CCMP1102-T28	0.57	41.00	0.02	228.29	9.18	0.33	9.03	0.57		4.77
CCMP1102-T33	0.01	58.58	0.02	147.05	8.55	0.89	3.12	2.06		2.41
CCMP1309-T26	0.21	29.97	0.03	258.34	8.76	1.50	11.23	5.28		8.81
CCMP1309-T27	0.33	33.15	0.03	244.15	8.78	1.91	2.88	0.30		7.07
CCMP1309-T35	0.30	33.15	0.03	162.27	9.32	0.73	-1.47	0.96		13.97
GSO100-T34	0.78	17.86	0.06	850.56	8.78	0.50	5.88	3.54		4.12
GSO100-T45	0.37	29.56	0.03	251.87	8.86	0.63	5.21	2.72		3.22
GSO100-T46	0.16	25.44	0.04	217.68	8.88	0.47	5.24	1.77		5.11
PAB1-T4	0.32	41.93	0.02	174.30	8.95	0.69	1.96	1.33		1.32
PAB1-T5	0.33	39.77	0.03	176.14	8.53	0.69	1.95	1.94		1.02
avg:	0.28	32.56	0.03	212.49	8.88	0.81	5.27	2.23		5.03
stdev:	0.15	11.51	0.01	37.59	0.26	0.44	4.44	1.61		3.42
min:	0.01	17.86	0.02	147.05	8.53	0.33	-1.47	0.30		1.02
max:	0.78	58.58	0.06	850.56	9.35	1.91	11.23	5.28		13.97

Appendix A: Initial and final phosphorus and silica concentrations

Culture	Initial PO ₄ μ M	Initial Si μ M
CCMP 997- T36 2/14/09	34.35	126.04
CCMP 997- T37 2/14/09	34.20	128.02
CCMP 1102- T27 4/21/09	31.85	121.37
CCMP 1102-T28 4/21/09	33.91	123.98
CCMP 1102- T33 6/29/09	31.75	130.55
CCMP 1309- T26 3/17/09	34.25	123.01
CCMP 1309- T27 3/17/09	35.08	125.04
CCMP 1309 T35 7/15/09	18.72	94.88
GSO 100 T45 1/21/09	33.03	119.37
GSO 100 T46 1/21/09	34.64	122.71
PABI T4 9/15/09	32.54	160.19
PABI T5 9/15/09	33.32	165.02

Culture	Final PO ₄ μ M	Final Si μ M
CCMP 997-T36 2/27/09	18.45	52.07
CCMP 997-T37 2/27/09	17.37	61.66
CCMP 1102-T27 5/12/09	17.58	30.25
CCMP 1102-T28 5/12/09	23.32	75.51
CCMP 1102- T33 7/28/09	14.45	7.33
CCMP 1309- T26 4/9/09	14.61	8.62
CCMP 1309- T26 4/7/09	13.58	13.00
CCMP 1309- T27 4/9/09	17.17	25.69
CCMP 1309- T35 7-29-09	21.27	26.08
GSO 100- T45 2/4/09	19.92	8.94
GSO 100- T46 2/4/09	16.40	4.38
PABI- T4 10-9-09	18.25	2.28
PABI- T5 10/9/09	21.17	1.14

Appendix A: $\delta^{15}\text{N}$ data from core TN057-13PC4

Depth (cm)	Age (ky)	%N	Bulk N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)
0	0.00	0.05	37.46	3.82
26	0.78	0.04	31.39	3.64
50	1.50	0.04	32.04	3.82
75	2.13	0.05	32.49	4.41
97	2.70	0.04	31.98	4.12
125	3.44	0.04	30.88	4.06
152	4.16	0.05	33.57	4.20
173	4.72	0.05	34.36	4.71
202	5.50	0.05	34.34	4.15
225	6.12	0.06	40.96	3.24
252	6.84	0.05	38.66	3.42
276	7.34	0.06	44.38	3.41
305	7.59	0.06	40.59	3.27
329	7.92	0.06	40.59	3.76
344	8.12	0.05	36.30	4.25
372	8.49	0.05	36.81	4.04
398	8.84	0.05	34.62	3.14
424	9.19	0.05	36.71	2.90
448	9.51	0.05	33.67	3.63
471	9.77	0.05	35.20	3.40
494	9.70	0.04	26.28	3.68
512	9.78	0.04	29.43	3.30
533	10.27	0.05	35.22	2.85
552	10.48	0.04	27.62	3.42
572	10.96	0.04	28.92	3.28
590	11.34	0.03	23.32	3.22
608	11.68	0.03	23.98	3.34
628	12.06	0.04	26.70	3.42
648	12.44	0.04	27.75	3.63
668	12.85	0.05	34.00	3.30
691	13.27	0.05	32.79	3.33
711	13.69	0.05	34.38	3.66
721	13.84	0.05	37.19	4.13
730	14.01	0.05	35.33	3.65
743	14.26	0.05	34.76	3.70
748	14.81	0.05	34.05	3.37
754	15.32	0.06	39.73	3.28
759	15.77	0.06	40.48	3.36
763	16.12	0.06	39.96	4.12
770	16.71	0.06	44.44	4.68
775	17.15	0.06	46.37	4.32
780	17.58	0.07	48.37	4.85
785	18.01	0.07	50.66	4.45
789	18.35	0.08	54.79	4.35
794	18.78	0.09	60.98	4.01
802	19.39	0.08	54.65	4.42
812	20.33	0.06	42.84	4.70
824	21.41	0.06	40.60	4.28
835	22.32	0.06	40.95	3.54
846	23.26	0.05	35.21	4.10
855	24.04	0.05	38.44	3.58
865	24.90	0.05	38.89	4.12
875	25.77	0.06	40.85	3.50
886	26.71	0.06	42.87	3.69
895	27.49	0.07	46.83	3.31
904	28.26	0.06	45.77	2.74

Appendix A: $\delta^{15}\text{N}$ data from core TN057-13PC4

Depth (cm)	Age (ky)	DB N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)
0	0.00	12.21	4.34	-0.52
26	0.78	13.00	3.21	0.43
50	1.50	12.76	3.91	-0.09
75	2.13	12.94	3.91	0.49
97	2.70	12.80	3.90	0.23
125	3.44	10.76	3.72	0.34
152	4.16	12.06	3.96	0.25
173	4.72	13.60	4.01	0.70
202	5.50	13.10	4.40	-0.25
225	6.12	16.18	3.90	-0.66
252	6.84	10.45	4.15	-0.73
276	7.34	11.59	3.71	-0.30
305	7.59	12.50	3.82	-0.54
329	7.92	12.72	4.17	-0.41
344	8.12	11.34	3.93	0.32
372	8.49	11.58	4.00	0.04
398	8.84	12.73	4.50	-1.36
424	9.19	13.72	3.67	-0.77
448	9.51	14.81	3.44	0.18
471	9.77	12.94	4.12	-0.72
494	9.70	13.60	4.58	-0.90
512	9.78	14.24	3.97	-0.68
533	10.27		4.24	-1.39
552	10.48	10.37	4.13	-0.71
572	10.96	15.53	4.38	-1.11
590	11.34	12.29	5.59	-2.37
608	11.68	13.76	5.25	-1.92
628	12.06	14.46	4.11	-0.68
648	12.44	17.83	4.23	-0.60
668	12.85	17.45	5.25	-1.95
691	13.27	17.41	5.55	-2.21
711	13.69	16.01	4.34	-0.68
721	13.84	16.98	5.90	-1.77
730	14.01	15.66	4.31	-0.66
743	14.26	18.65	4.03	-0.32
748	14.81	19.09	4.27	-0.90
754	15.32	18.46	4.06	-0.78
759	15.77	20.09	4.33	-0.96
763	16.12	19.08	5.27	-1.15
770	16.71	14.89	6.73	-2.05
775	17.15	16.12	4.69	-0.37
780	17.58	14.54	4.50	0.35
785	18.01	13.67	4.57	-0.13
789	18.35	14.05	4.67	-0.31
794	18.78	14.89	3.99	0.02
802	19.39	13.97	3.71	0.71
812	20.33	12.82	2.98	1.72
824	21.41	11.71	2.96	1.32
835	22.32	12.75	2.58	0.95
846	23.26	13.31	2.28	1.81
855	24.04	14.00	2.65	0.93
865	24.90	14.42	2.68	1.44
875	25.77	14.99	3.08	0.43
886	26.71	13.79	2.72	0.97
895	27.49	15.41	1.95	1.36
904	28.26	17.80	1.99	0.75

Appendix A: Dominant diatom species at TN057-13PC4 during the Holocene and LGM. Data interpreted by M. Horn from the doctoral dissertation of Simon Harder Holm Nielsen [Nielsen, 2004]

Dominant Species	Relative Abundances (%)	
	Holocene	LGM
Sea Ice Species		
<i>Eucampia antarctica</i>	4	20
<i>Fragilariopsis cylindrus</i>	2	5
<i>Fragilariopsis curta</i>	6	7
Water Stratification Species		
<i>Chaetoceros</i> Resting Spore	5	30
<i>Rhizosolenia antennata</i> var. <i>semispina</i>	1	3
Polar Open Ocean Species		
<i>Thalassiosira gracilis</i>	10	4
<i>Fragilariopsis kerguelensis</i>	55	25
<i>Thalassiosira lentiginosa</i>	13	8
<i>Fragilariopsis separanda</i>	4	0
<i>Thalassiothrix antarctica</i>	3	2
	103	104

APPENDIX B

MANUSCRIPT 2 – DATA TABLES

Core Data

The following tables represent data collected from core TN057-13PC4, including $\delta^{15}\text{N}$, $\delta^{30}\text{Si}$, utilization, Si/N uptake ratio, and the calculated upwelling index from the coupled N and Si measurements. A description of the methods and instruments used to collect this data may be found in Manuscript 2.

Data Requests

A digital copy of the data may be acquired by contacting Professor Rebecca Robinson at GSO (rebeccar@gso.uri.edu), or Matthew Horn (horn@gso.uri.edu).

Appendix B: $\delta^{15}\text{N}$ data from core TN057-13PC4

Depth (cm)	Age (ky)	%N	Bulk N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)
0	0.00	0.05	37.46	3.82
26	0.78	0.04	31.39	3.64
50	1.50	0.04	32.04	3.82
75	2.13	0.05	32.49	4.41
97	2.70	0.04	31.98	4.12
125	3.44	0.04	30.88	4.06
152	4.16	0.05	33.57	4.20
173	4.72	0.05	34.36	4.71
202	5.50	0.05	34.34	4.15
225	6.12	0.06	40.96	3.24
252	6.84	0.05	38.66	3.42
276	7.34	0.06	44.38	3.41
305	7.59	0.06	40.59	3.27
329	7.92	0.06	40.59	3.76
344	8.12	0.05	36.30	4.25
372	8.49	0.05	36.81	4.04
398	8.84	0.05	34.62	3.14
424	9.19	0.05	36.71	2.90
448	9.51	0.05	33.67	3.63
471	9.77	0.05	35.20	3.40
494	9.70	0.04	26.28	3.68
512	9.78	0.04	29.43	3.30
533	10.27	0.05	35.22	2.85
552	10.48	0.04	27.62	3.42
572	10.96	0.04	28.92	3.28
590	11.34	0.03	23.32	3.22
608	11.68	0.03	23.98	3.34
628	12.06	0.04	26.70	3.42
648	12.44	0.04	27.75	3.63
668	12.85	0.05	34.00	3.30
691	13.27	0.05	32.79	3.33
711	13.69	0.05	34.38	3.66
721	13.84	0.05	37.19	4.13
730	14.01	0.05	35.33	3.65
743	14.26	0.05	34.76	3.70
748	14.81	0.05	34.05	3.37
754	15.32	0.06	39.73	3.28
759	15.77	0.06	40.48	3.36
763	16.12	0.06	39.96	4.12
770	16.71	0.06	44.44	4.68
775	17.15	0.06	46.37	4.32
780	17.58	0.07	48.37	4.85
785	18.01	0.07	50.66	4.45
789	18.35	0.08	54.79	4.35
794	18.78	0.09	60.98	4.01
802	19.39	0.08	54.65	4.42
812	20.33	0.06	42.84	4.70
824	21.41	0.06	40.60	4.28
835	22.32	0.06	40.95	3.54
846	23.26	0.05	35.21	4.10
855	24.04	0.05	38.44	3.58
865	24.90	0.05	38.89	4.12
875	25.77	0.06	40.85	3.50
886	26.71	0.06	42.87	3.69
895	27.49	0.07	46.83	3.31
904	28.26	0.06	45.77	2.74

Appendix B: $\delta^{15}\text{N}$ data from core TN057-13PC4

Depth (cm)	Age (ky)	DB N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)
0	0.00	12.21	4.34	-0.52
26	0.78	13.00	3.21	0.43
50	1.50	12.76	3.91	-0.09
75	2.13	12.94	3.91	0.49
97	2.70	12.80	3.90	0.23
125	3.44	10.76	3.72	0.34
152	4.16	12.06	3.96	0.25
173	4.72	13.60	4.01	0.70
202	5.50	13.10	4.40	-0.25
225	6.12	16.18	3.90	-0.66
252	6.84	10.45	4.15	-0.73
276	7.34	11.59	3.71	-0.30
305	7.59	12.50	3.82	-0.54
329	7.92	12.72	4.17	-0.41
344	8.12	11.34	3.93	0.32
372	8.49	11.58	4.00	0.04
398	8.84	12.73	4.50	-1.36
424	9.19	13.72	3.67	-0.77
448	9.51	14.81	3.44	0.18
471	9.77	12.94	4.12	-0.72
494	9.70	13.60	4.58	-0.90
512	9.78	14.24	3.97	-0.68
533	10.27		4.24	-1.39
552	10.48	10.37	4.13	-0.71
572	10.96	15.53	4.38	-1.11
590	11.34	12.29	5.59	-2.37
608	11.68	13.76	5.25	-1.92
628	12.06	14.46	4.11	-0.68
648	12.44	17.83	4.23	-0.60
668	12.85	17.45	5.25	-1.95
691	13.27	17.41	5.55	-2.21
711	13.69	16.01	4.34	-0.68
721	13.84	16.98	5.90	-1.77
730	14.01	15.66	4.31	-0.66
743	14.26	18.65	4.03	-0.32
748	14.81	19.09	4.27	-0.90
754	15.32	18.46	4.06	-0.78
759	15.77	20.09	4.33	-0.96
763	16.12	19.08	5.27	-1.15
770	16.71	14.89	6.73	-2.05
775	17.15	16.12	4.69	-0.37
780	17.58	14.54	4.50	0.35
785	18.01	13.67	4.57	-0.13
789	18.35	14.05	4.67	-0.31
794	18.78	14.89	3.99	0.02
802	19.39	13.97	3.71	0.71
812	20.33	12.82	2.98	1.72
824	21.41	11.71	2.96	1.32
835	22.32	12.75	2.58	0.95
846	23.26	13.31	2.28	1.81
855	24.04	14.00	2.65	0.93
865	24.90	14.42	2.68	1.44
875	25.77	14.99	3.08	0.43
886	26.71	13.79	2.72	0.97
895	27.49	15.41	1.95	1.36
904	28.26	17.80	1.99	0.75

Appendix B: $\delta^{30}\text{Si}$ data from core TN057-13PC4

Age (ky)	$\delta^{30}\text{Si}$ (‰)	Si utilization (%)	$\mu\text{mol Si}$ (90 μM upwells)	$\delta^{15}\text{N}$ (‰)	N utilization (%)	$\mu\text{mol N}$ (30 μM upwells)	Si/N uptake ratio
0	1.44	0.76	68.4	4.34	0.73	8.0	8.5
0.78	1.34	0.67	60.3	3.21	0.96	1.3	47.9
1.5	1.32	0.65	58.5	3.91	0.82	5.5	10.7
2.13	1.47	0.79	71.1	3.91	0.82	5.5	13.0
2.7	1.41	0.74	66.6	3.90	0.82	5.4	12.3
3.44	1.45	0.77	69.3	3.72	0.86	4.3	16.0
4	1.30	0.64	57.6	3.96	0.81	5.8	10.0
4.16	1.39	0.72	64.8	4.01	0.80	6.1	10.0
4.72	1.30	0.64	57.6	4.40	0.72	8.4	10.7
5.5	1.38	0.71	63.9	3.90	0.82	5.4	6.9
6.12	1.25	0.59	53.1	4.15	0.77	6.9	11.8
6.84	1.17	0.52	46.8	3.71	0.86	4.3	7.7
7.34	1.22	0.56	50.4	3.82	0.84	4.9	11.0
7.59	1.24	0.58	52.2	4.17	0.77	7.0	10.2
7.92	1.08	0.44	39.6	3.93	0.81	5.6	7.4
8.12	0.95	0.32	28.8	4.00	0.80	6.0	7.1
8.49	1.07	0.43	38.7	4.50	0.70	9.0	4.8
8.84	0.94	0.31	27.9	3.67	0.87	4.0	4.3
9.19	1.33	0.66	59.4	3.44	0.91	2.6	6.9
9.51	1.24	0.58	52.2	4.58	0.68	9.5	22.5
9.7	1.23	0.57	51.3	4.12	0.78	6.7	5.5
9.77	1.15	0.5	45.0	3.97	0.81	5.8	7.6
9.78	1.42	0.74	66.6	4.24	0.75	7.4	7.7
10.27	1.18	0.52	46.8	4.45	0.71	8.7	9.0
10.48	1.36	0.69	62.1	4.38	0.72	8.3	5.4
10.96	1.26	0.6	54.0	5.59	0.48	15.5	7.5
11.34	1.18	0.52	46.8	5.25	0.55	13.5	3.5
11.68	0.99	0.35	31.5	4.11	0.78	6.7	3.5
12.06	1.23	0.57	51.3	4.23	0.75	7.4	4.7
12.44	1.08	0.44	39.6	5.25	0.55	13.5	7.0
13.27	0.93	0.3	27.0	5.55	0.49	15.3	2.9
13.69	0.87	0.24	21.6	4.34	0.73	8.0	1.8
13.84	1.05	0.41	36.9	5.90	0.42	17.4	2.7
14.01	1.03	0.39	35.1	4.31	0.74	7.9	2.1
14.26	0.92	0.29	26.1	4.03	0.79	6.2	4.5
14.81	0.91	0.28	25.2	4.27	0.75	7.6	4.2
15.32	1.01	0.37	33.3	4.06	0.79	6.4	3.3
15.77	0.84	0.22	19.8	4.33	0.73	8.0	5.2
16.12	0.68	0.07	6.3	5.27	0.55	13.6	2.5
16.71	0.82	0.2	18.0	6.73	0.25	22.4	0.5
17.15	0.86	0.24	21.6	4.69	0.66	10.1	0.8
18.01	0.67	0.06	5.4	4.57	0.69	9.4	0.6
18.35	0.79	0.17	15.3	4.67	0.67	10.0	1.5
18.78	0.71	0.1	9.0	3.99	0.80	5.9	1.5
19.39	0.61	0.01	0.9	3.71	0.86	4.3	0.2
20.33	0.71	0.1	9.0	2.98	1.00	-0.1	-75.0
21.41	0.64	0.03	2.7	2.96	1.01	-0.2	-11.3
22.32	0.68	0.07	6.3	2.58	1.08	-2.5	-2.5
23.26	0.82	0.2	18.0	2.58	1.08	-2.5	-7.1
24.04	0.86	0.24	21.6	2.65	1.07	-2.1	-10.3
24.9	0.96	0.33	29.7	2.68	1.06	-1.9	-15.5
26.71	0.80	0.18	16.2	3.63	0.87	3.8	4.3
27.49	0.89	0.26	23.4	1.95	1.21	-6.3	-3.7
28.26	0.90	0.27	24.3	1.99	1.20	-6.1	-4.0

Appendix B: Upwelling Index for core TN057-13PC4. The first column assumes a constant 90 μM Si upwelling source from depth. The second column allows the deep ocean Si concentration to vary, based on sponge spicule data from Hendry et al. [2010].

Age (ky)	Δ upwell (Si constant)	Δ upwell (Si changes)
1.9	1	
3.4	1.08	
4.1	1.3	
5.4	1.37	1.37
6.8	2.45	
7.0	1.96	
7.6	2.93	3.4
8.2	4.04	
9.5	2.41	
9.6	5	
10.2	5.46	
10.6	3.38	
11.2	4.16	
11.3	4.16	
11.8	8.41	
12.1	4.49	
12.3	6.5	10.83
13.3	5.4	
13.5	6.26	
13.7	3.74	
14.1	4.34	
14.2	9.06	
14.9	10.19	
15.1	9.78	15.37
15.2	7.95	
16.2	17.34	
16.5	4.02	
16.9	2.86	
17.9	6.01	
18.9	3.28	4.24
20.3	2.41	2.94
23.1	1.87	
24.7	1.33	
26.5	1.68	

APPENDIX C

MANUSCRIPT 3 – DATA TABLES

Core Data

The following tables represent data collected from core TN057-13PC4, including $\delta^{15}\text{N}$, $\delta^{30}\text{Si}$, utilization, Si/N uptake ratio, and the calculated upwelling index from the coupled N and Si measurements. A description of the methods and instruments used to collect this data may be found in Manuscript 2.

Data Requests

A digital copy of the data may be acquired by contacting Professor Rebecca Robinson at GSO (rebeccar@gso.uri.edu), or Matthew Horn (horn@gso.uri.edu).

Appendix C: $\delta^{15}\text{N}$ data from core TN057-13PC4

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBnet}}$ (‰)	Remainder (‰)
0	0.00	37.46	3.82	12.21	4.34	-0.52	3.6
26	0.78	31.39	3.64	13.00	3.21	0.43	3.8
50	1.50	32.04	3.82	12.76	3.91	-0.09	3.8
75	2.13	32.49	4.41	12.94	3.91	0.49	4.6
97	2.70	31.98	4.12	12.80	3.90	0.23	4.2
125	3.44	30.88	4.06	10.76	3.72	0.34	4.2
152	4.16	33.57	4.20	12.06	3.96	0.25	4.3
173	4.72	34.36	4.71	13.60	4.01	0.70	5.0
202	5.50	34.34	4.15	13.10	4.40	-0.25	4.0
225	6.12	40.96	3.24	16.18	3.90	-0.66	2.9
252	6.84	38.66	3.42	10.45	4.15	-0.73	3.2
276	7.34	44.38	3.41	11.59	3.71	-0.30	3.3
305	7.59	40.59	3.27	12.50	3.82	-0.54	3.1
329	7.92	40.59	3.76	12.72	4.17	-0.41	3.6
344	8.12	36.30	4.25	11.34	3.93	0.32	4.4
372	8.49	36.81	4.04	11.58	4.00	0.04	4.1
398	8.84	34.62	3.14	12.73	4.50	-1.36	2.6
424	9.19	36.71	2.90	13.72	3.67	-0.77	2.6
448	9.51	33.67	3.63	14.81	3.44	0.18	3.7
471	9.77	35.20	3.40	12.94	4.12	-0.72	3.1
494	9.70	26.28	3.68	13.60	4.58	-0.90	3.1
512	9.78	29.43	3.30	14.24	3.97	-0.68	2.9
533	10.27	35.22	2.85		4.24	-1.39	
552	10.48	27.62	3.42	10.37	4.13	-0.71	3.1
572	10.96	28.92	3.28	15.53	4.38	-1.11	2.3
590	11.34	23.32	3.22	12.29	5.59	-2.37	1.3
608	11.68	23.98	3.34	13.76	5.25	-1.92	1.6
628	12.06	26.70	3.42	14.46	4.11	-0.68	2.9
648	12.44	27.75	3.63	17.83	4.23	-0.60	2.9
668	12.85	34.00	3.30	17.45	5.25	-1.95	1.9
691	13.27	32.79	3.33	17.41	5.55	-2.21	1.7
711	13.69	34.38	3.66	16.01	4.34	-0.68	3.2
721	13.84	37.19	4.13	16.98	5.90	-1.77	2.9
730	14.01	35.33	3.65	15.66	4.31	-0.66	3.2
743	14.26	34.76	3.70	18.65	4.03	-0.32	3.4
748	14.81	34.05	3.37	19.09	4.27	-0.90	2.5
754	15.32	39.73	3.28	18.46	4.06	-0.78	2.8
759	15.77	40.48	3.36	20.09	4.33	-0.96	2.7
763	16.12	39.96	4.12	19.08	5.27	-1.15	3.4
770	16.71	44.44	4.68	14.89	6.73	-2.05	4.0
775	17.15	46.37	4.32	16.12	4.69	-0.37	4.2
780	17.58	48.37	4.85	14.54	4.50	0.35	4.9
785	18.01	50.66	4.45	13.67	4.57	-0.13	4.4
789	18.35	54.79	4.35	14.05	4.67	-0.31	4.3
794	18.78	60.98	4.01	14.89	3.99	0.02	4.0
802	19.39	54.65	4.42	13.97	3.71	0.71	4.5
812	20.33	42.84	4.70	12.82	2.98	1.72	5.0
824	21.41	40.60	4.28	11.71	2.96	1.32	4.5
835	22.32	40.95	3.54	12.75	2.58	0.95	3.7
846	23.26	35.21	4.10	13.31	2.28	1.81	4.5
855	24.04	38.44	3.58	14.00	2.65	0.93	3.8
865	24.90	38.89	4.12	14.42	2.68	1.44	4.5
875	25.77	40.85	3.50	14.99	3.08	0.43	3.6
886	26.71	42.87	3.69	13.79	2.72	0.97	3.9
895	27.49	46.83	3.31	15.41	1.95	1.36	3.6
904	28.26	45.77	2.74	17.80	1.99	0.75	2.9

Appendix C: $\delta^{15}\text{N}$ data from core E17-9

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Remainder (‰)
35	10.8	33.9	4.0	11.3	5.1	-1.1	3.6
46	12.5	30.3	4.0	10.1	4.4	-0.4	3.8
56	14.0	32.0	4.0	9.6	5.5	-1.5	3.5
66	15.0	28.7	4.5	10.5	6.7	-2.2	3.6
76	15.5	30.5	3.2	9.1	5.5	-2.2	2.6
89	16.0	33.3	3.9	11.1	4.9	-1.0	3.6
100	16.8	41.2	5.8	9.2	6.9	-1.1	5.6
110	17.0	38.1	4.2	10.7	5.6	-1.4	3.9
119	17.8	40.8	3.6	9.6	6.2	-2.7	3.1
132	18.1	33.3	2.7				
139	18.7	33.3	4.9				
148	19.0	38.8	4.7	9.7	4.7	0.0	4.7
158	19.6	40.9	4.3	9.8	5.0	-0.6	4.2
168	20.6	96.9	6.9				
179	21.8	44.8	4.5	12.6	3.7	0.8	4.7
190	22.0	40.7	2.8	11.8	4.7	-1.9	2.2
199	22.8	39.4	4.2	11.9	4.3	-0.1	4.2
209	24.0	43.1	2.9				
218	25.5	39.9	4.6				
228	28.1	35.6	3.6				
238	30.4	33.9	2.3				
249	32.6	36.7	4.4				
258	34.4	36.0	5.6	12.2	4.7	0.9	5.9
268	36.4	37.3	4.3				
278	37.7	41.6	3.9	9.9	4.7	-0.8	3.7
289	39.6			10.3	3.6		
296	41.3	37.6	3.3	10.6	3.3	0.0	3.3
307	44.0	37.0	6.3				
318	46.8	36.3	5.2				
329	49.5	37.2	4.2	10.9	5.5	-1.3	3.9
338	51.8	35.9	3.3				
349	54.5	29.6	5.5	10.9	6.0	-0.5	5.4
358	56.8	31.0	3.9				
368	59.3	34.6	4.0	11.1	4.8	-0.8	3.8
378	61.8	34.1	3.8				
389	64.5	33.7	4.9	10.7	3.3	1.6	5.3
400	67.3	34.4	5.7	9.8	5.3	0.5	5.9
410	69.8	31.3	4.3				
428	74.3	32.4	4.7				
438	76.8	29.0	4.0	9.0	4.0	-0.1	4.0
448	79.3	30.8	4.7	8.7	4.6	0.1	4.8
457	81.5	28.6	3.9	9.7	3.5	0.4	4.0
469	84.5	31.3	4.3				
479	87.0	33.7	5.3	9.5	2.6	2.7	5.9
489	89.5	27.5	3.1				
498	91.8	30.7	2.8	11.8	4.9	-2.1	2.0
509	94.5	37.5	3.5				
519	97.0	33.6	4.5	10.0	4.4	0.0	4.5
529	99.5	36.3	4.2	10.2	4.6	-0.4	4.1
540	102.3	31.5	5.3	10.3	5.0	0.3	5.4
549	104.5						
557	106.5	34.3	4.1	10.7	4.5	-0.4	4.0
567	109.0	31.9	2.7				
579	112.0	29.8	4.1	10.5	1.6	2.6	4.9
589	114.5	37.4	3.6				
597	116.5	26.7	3.4	11.1	4.0	-0.6	3.2
609	119.5	29.3	4.5	12.3	4.6	-0.1	4.5

Appendix C: $\delta^{15}\text{N}$ data from core E17-9

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Remainder (‰)
618	121.8	31.1	3.6	10.8	3.9	-0.3	3.5
629	124.5	24.8	3.6	10.2	2.7	0.8	3.9
638	126.8	33.8	4.8	10.7	4.2	0.6	5.0
649	129.5	26.9	4.5				
659	132.0	26.4	4.2	9.0	1.8	2.4	4.9
669	134.5	24.9	4.5				
679	137.0	53.6	5.5				
689	139.5	25.5	2.2	8.1	2.8	-0.6	2.0
697	141.5	27.4	3.0				
708	144.3	29.0	5.5	8.7	5.9	-0.4	5.4
718	146.8	27.5	3.1				
729	149.5	23.6	2.9				
739	152.0	24.9	3.7	9.3	4.4	-0.7	3.5
749	154.5	25.8	3.0	10.1	4.1	-1.2	2.6
758	156.8	23.3	2.8	8.8	5.0	-2.2	2.1
768	159.3	20.4	2.1	8.3	3.5	-1.4	1.6
777	161.5	19.8	2.5				
788	164.3			8.1	5.2		
799	167.0	22.8	3.1	7.9	4.8	-1.7	2.6
808	169.3			8.4	3.8		
817	171.5			8.0	2.8		
829	174.5	20.7	1.5	8.8	4.2	-2.8	0.4
837	176.5	24.8	2.6				
849	179.5	28.6	2.4				
859	182.0	30.7	4.4	7.4	5.4	-1.0	4.2
869	184.5	25.7	3.1	9.1	4.5	-1.4	2.7
878	186.8	26.4	3.6				
889	189.5	23.4	2.6				
896	191.3	20.9	3.4	8.6	3.7	-0.3	3.2
919	197.0	20.3	3.9				
929	199.5	22.3	3.0	8.8	3.8	-0.8	2.7
939	202.0	21.9	4.9	9.1	3.6	1.4	5.5
948	204.3	23.2	1.9	9.7	3.9	-2.0	1.1
958	206.8	24.5	3.2	10.2	2.9	0.3	3.3
970	209.8	102.2	3.9	11.2	2.0	1.9	4.0
978	211.8	24.6	2.2	10.0	3.6	-1.4	1.7
988	214.3	22.9	3.2				
998	216.8	24.7	2.3		3.6	-1.3	
1017	221.5	25.8	3.2	9.5	4.3	-1.1	2.8
1030	224.8	21.5	4.0				
1048	229.3	23.0	2.7				
1057	231.5	16.3	2.3		4.2	-1.9	
1070	234.8	18.1	2.8				
1080	237.3	26.4	3.9				
1089	239.5	30.9	3.8				
1099	242.0	28.7	4.2				
1110	244.8	35.9	4.0				
1121	247.5	32.4	4.4				
1129	249.5	34.7	5.0	12.5	4.3	0.6	5.1
1139	252.0	37.4	4.6	14.5	3.6	1.0	4.9
1150	254.8	48.9	3.8				
1160	257.3	38.5	3.2				
1169	259.5	41.4	3.5				
1179	262.0	39.6	3.7				
1190	264.8	35.4	4.5	13.7	4.6	-0.1	4.5

Appendix C: $\delta^{15}\text{N}$ data from core E17-9

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Remainder (‰)
1200	267.3	34.8	3.6	11.5	4.2	-0.7	3.4
1209	269.5	35.6	2.6				
1220	272.3	34.1	2.9				
1230	274.8	32.4	2.4				
1240	277.3	34.0	3.7				
1249	279.5	32.1	3.6	10.9	5.5	-1.9	3.1
1259	282.0	35.1	4.1	8.6	4.0	0.2	4.2
1269	284.5	31.5	5.1		4.7	0.4	
1278	286.8	33.4	5.3				
1288	289.3	31.3	4.2				
1298	291.8	30.2	2.5				

Appendix C: $\delta^{15}\text{N}$ data from core E23-14

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N} / \text{g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DDB/DB}}$ (‰)	Remainder (‰)
4	7.0	37.1	5.9				
9	12.0	26.7	3.5				
14	16.0	27.6	6.1	10.5	5.1	1.0	6.2
19	18.5	24.1	2.9				
24	22.0	25.5	3.9				
34	30.7	16.0	4.4	7.3	1.5	2.9	4.6
39	32.3	26.2	4.8	8.1	7.7	-2.9	4.5
44	34.0	22.2	4.4				
49	36.0	19.4	2.0				
54	38.5	17.7	3.3				
59	41.0	18.6	3.8				
64	43.5	17.5	4.8	6.4	6.3	-1.5	4.4
69	45.3	18.0	3.2				
74	46.2	17.0	3.9	5.8	4.2	-0.3	3.9
79	47.0	18.0	3.4	5.8	5.0	-1.6	3.0
84	47.8	16.8	1.4				
94	49.4	14.9	3.0				
98	50.1	17.3	2.1				
104	51.1	16.8	1.3				
109	51.9	16.1	0.7				
114	52.7	14.1	1.3				
119	53.6	15.4	0.7				
124	54.4			6.1	2.4		
129	55.2	18.9	3.7	6.1	5.2	-1.5	3.3
134	56.0	21.1	3.4				
139	56.9	25.0	3.9	6.1	2.1	1.8	4.2
144	57.7	26.1	2.8				
149	58.5	21.9	1.3				
154	59.3	22.8	1.8				
159	60.7	11.2	0.3	5.6	5.9	-5.6	-2.0
164	62.7	6.3	1.8	5.0	5.3	-3.5	-1.2
174	66.8	21.2	3.5				
179	68.9	17.0	2.8				
184	70.9	17.0	3.7	10.6	3.4	0.3	3.8
189	73.0	22.0	2.8				
193	74.6	22.5	3.1	10.6	0.5	2.6	4.0
199	77.1	20.9	2.6				
204	79.1	21.2	3.5				
209	81.2	21.5	3.0	10.4	4.4	-1.4	2.5
215	83.6	22.7	3.0				
219	85.3	27.3	2.3				
224	87.3	19.0	3.1	14.4	2.5	0.6	3.6
229	89.4	20.9	1.3				
239	93.5	25.4	0.9				
244	95.5	26.3	2.6				
249	97.6	27.8	2.4				
254	99.6	21.2	2.5				
259	101.7	25.5	3.2	8.5	5.4		2.7
269	105.8	23.4	2.4	8.5	1.6	0.9	2.7
274	107.8	37.8	6.3	8.2	5.4	1.0	6.5
279	109.9	21.9	4.2				
284	112.0	28.2	3.8				
290	114.1	25.7	4.7	8.1	4.7	0.0	4.7
294	115.3	19.2	2.1	6.7	4.1	-2.0	1.6

Appendix C: $\delta^{15}\text{N}$ data from core ODP1094

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Remainder (‰)
0.1	1.5	21.8	4.7	11.0	5.1	-0.4	4.4
0.4	2.4	19.6	4.2	10.0	4.8	-0.5	3.8
0.7	3.2	21.3	3.9	11.2	5.6	-1.7	2.8
1.0	4.0	23.7	4.0	12.7	4.2	-0.3	3.8
1.3	4.9	22.9	4.0	9.0	4.8	-0.7	3.7
1.6	5.7	22.9	4.0	10.2	4.6	-0.6	3.7
1.9	6.5	24.4	4.3	12.4	4.6	-0.3	4.1
2.2	7.2	21.2	4.1	13.2	4.2	-0.1	4.0
2.5	7.5	19.4	3.5	11.8	4.2	-0.7	2.8
2.8	7.9	23.4	4.2	10.4	5.5	-1.3	3.4
3.1	8.2	13.9	3.6	10.6	4.9	-1.4	1.1
3.4	8.6	18.4	3.2	12.2	4.2	-1.0	1.8
3.7	8.9	21.2	3.6	10.5	5.5	-1.8	2.2
4.0	9.3	20.7	3.6	12.7	5.2	-1.6	2.0
4.3	9.6	18.1	3.5	9.9	4.9	-1.4	2.3
4.6	10.0	19.6	3.4	10.6	4.9	-1.5	2.1
4.9	10.4	21.3	3.7	10.9	5.1	-1.4	2.7
5.2	10.7	15.5	3.5	11.7	5.1	-1.6	1.8
5.5	11.0	23.5	4.3	14.3	4.9	-0.6	3.8
5.8	11.4	22.7	4.3	11.7	5.6	-1.3	3.6
6.1	11.7	18.6	3.9	10.4	5.0	-1.1	3.0
6.4	12.3	18.0	4.6	11.9	4.7	-0.1	4.5
6.7	13.2	22.5	3.8	12.3	5.2	-1.4	2.6
7.0	14.1	24.4	4.3	13.8	4.6	-0.2	4.1
7.3	15.1	22.4	3.9	12.8	5.3	-1.3	2.8
7.6	16.5	25.4	4.3	13.5	4.9	-0.5	3.9
7.9	17.9	26.2	5.1	12.4	5.3	-0.2	5.0
8.2	19.3	20.1	5.0	12.4	4.2	0.8	5.3
8.5	20.7	16.4	4.2	13.6	4.3	-0.2	4.0
8.8	22.1	18.1	3.8	14.8	3.6	0.1	3.8
9.1	23.5	11.9	3.4	12.7	4.9	-1.5	1.5
9.6	28.2	17.0	3.5	13.6	3.3	0.2	3.5
9.9	29.6	24.6	5.1	10.9	3.9	1.1	5.4
10.2	31.0	12.4	2.8	13.0	4.4	-1.7	0.9
10.5	32.5	24.7	5.1	7.9	4.4	0.7	5.2
10.8	33.9	17.7	3.9	12.2	4.0	-0.1	3.9
12.2	43.0	11.7	3.3	13.3	3.6	-0.3	2.9
12.5	44.4	26.2	4.6	12.1	3.0	1.6	5.2
12.8	45.9	26.2	4.5	10.7	4.4	0.1	4.6
13.1	47.3	16.7	4.2	10.4	4.1	0.1	4.2
13.4	48.7	26.6	4.8	9.6	5.7	-0.9	4.5
13.6	49.9	16.7	4.1	10.0	4.3	-0.1	4.1
14.4	57.9	19.2	4.1	10.4	4.2	-0.1	4.1
15.2	58.3	19.8	4.8	9.8	4.2	0.6	5.0
14.7	59.2	12.1	3.0	8.8	4.5	-1.5	2.3
15.5	59.6	13.7	3.4	8.1	4.9	-1.5	2.6
14.9	60.1	27.3	5.1	9.8	4.2	0.9	5.3
15.8	60.9	17.1	4.0	12.9	3.7	0.3	4.4
15.2	61.4	26.3	4.5	11.5	3.0	1.6	5.0
16.1	62.2	21.3	4.7	9.8	4.5	0.2	4.8
15.4	62.3	24.1	4.4	9.4	4.2	0.1	4.4
16.4	63.6	25.5	4.5	8.3	3.7	0.8	4.7
16.7	69.8	23.0	5.0	10.7	4.1	0.9	5.4
17.0	71.7	15.9	3.6	10.2	4.1	-0.5	3.2
17.3	73.6	17.4	4.2	10.3	3.7	0.6	4.7
17.6	75.5	12.9	3.2	8.2	4.6	-1.4	1.8
17.9	77.4	18.8	3.3	11.5	3.3	0.0	3.3

Appendix C: $\delta^{15}\text{N}$ data from core ODP1094

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DBoffset}}$ (‰)	Remainder (‰)
18.2	79.2	15.8	3.1	8.7	3.8	-0.7	2.7
18.5	82.6	11.7	2.8	10.4	2.9	-0.1	2.6
18.6	85.1	17.2	3.6	9.6	5.3	-1.7	2.6
18.9	89.9	16.6	3.7	11.4	4.7	-1.0	2.7
19.2	94.6	9.7	2.4	8.8	4.3	-1.9	-3.3
19.5	99.3	16.2	3.0	12.4	3.3	-0.3	2.5
19.8	104.0	11.3	2.9	10.4	3.7	-0.8	1.0
20.1	108.8	15.0	3.4	8.8	5.1	-1.8	1.7
20.4	113.5	18.8	3.5	9.5	4.2	-0.7	3.0
20.7	116.5	19.8	3.7	11.2	3.9	-0.2	3.6
21.0	118.1	12.4	2.9	9.1	4.7	-1.9	0.0
21.3	119.7	18.5	3.1	11.4	3.7	-0.6	2.7
21.7	124.4	19.4	3.4	10.9	3.8	-0.4	3.1
22.0	125.2	19.1	3.5	12.4	2.7	0.9	4.3
23.0	126.0	17.4	3.1	9.6	4.1	-1.0	2.7
23.3	126.8	18.9	3.2	13.7	4.1	-0.8	1.6
23.9	128.3	17.6	3.0	12.0	5.8	-2.7	-1.3
24.1	129.1	13.7	3.4	11.2	3.5	0.0	3.4
24.4	129.9	19.8	3.6	14.3	5.4	-1.8	0.3
24.7	131.6	23.1	4.2	9.0	4.8	-0.6	3.9
25.0	133.8	22.4	5.3	12.7	4.0	1.2	5.7
25.3	135.8	27.8	5.1	13.4	4.0	1.0	5.4
25.6	137.8	19.0	4.7	11.8	5.0	-0.3	4.5
25.9	139.8	22.0	5.1	10.0	4.9	0.2	5.1
26.2	141.7	18.4	4.3	11.5	5.3	-1.0	3.9
26.5	143.7	26.2	5.6	11.0	4.4	1.2	5.9
26.8	145.7	18.1	4.3	12.5	4.6	-0.3	4.1
27.1	147.7	25.1	5.0	11.2	3.9	1.1	5.3

Appendix C: $\delta^{15}\text{N}$ data from core PA9802-9PC

Depth (cm)	Age (ky)	Bulk N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{bulk}}$ (‰)	DB N content ($\mu\text{mol N / g opal}$)	$\delta^{15}\text{N}_{\text{DB}}$ (‰)	$\delta^{15}\text{N}_{\text{DB/Total}}$ (‰)	Remainder (‰)
0	10.0	34.6	6.6	4.5	9.6	-2.9	6.5
20	14.0	55.0	6.7	7.6	6.6	0.0	6.7
40	18.0	32.4	5.6	5.2	9.6	-4.0	5.4
60	22.0	34.3	4.9	6.2	6.3	-1.4	4.8
80	26.0	30.1	4.5	8.3	7.9	-3.4	3.9
100	30.8	32.3	4.9	9.1	6.9	-2.0	4.6
120	36.5	28.9	4.5	7.5	6.8	-2.2	4.2
140	42.2	48.0	6.1	8.5	7.8	-1.7	5.9
160	47.0	27.1	3.4	7.6	8.5	-5.2	2.7
180	51.1	31.8	5.2	7.1	8.9	-3.8	4.8
200	55.2	28.6	4.5	7.3	5.7	-1.2	4.3
220	60.0	41.9	6.8	7.4	5.3	1.5	6.9
225	62.5	35.7	6.4	8.7	6.1	0.2	6.4
230	65.0	54.2	6.2	7.3	8.5	-2.3	6.1
235	67.5	31.2	4.5	11.2	8.3	-3.7	3.9
240	70.0	40.9	6.1	6.9	6.6	-0.5	6.0
245	72.5	31.7	5.2	6.0	7.1	-1.8	5.1
250	75.0	43.4	6.3	7.1	6.8	-0.4	6.3
255	77.5	53.0	6.2	7.7	6.1	0.1	6.2
260	80.0	35.9	6.6	7.4	6.7	-0.1	6.5
265	82.5	32.9	5.6	8.1	7.2	-1.6	5.5
270	85.0	30.2	6.0	8.4	5.3	0.7	6.1
275	87.5	28.9	6.4	6.3	8.4	-2.0	6.2
280	90.0	36.9	6.5	9.2	8.5	-1.9	6.3
285	92.5	33.6	6.4	8.1	8.1	-1.7	6.2
290	95.0	27.0	5.1	6.9	9.5	-4.3	4.7
295	97.5	28.8	4.8	8.6	6.4	-1.7	4.6
300	100.0	32.7	5.9	5.3	8.7	-2.9	5.7

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