The Boron Geochemistry of Biogenic Silica: Insights from Marine Sponges and Diatoms

Andrea de Leon

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Declaration

The work presented in this thesis is my own except where otherwise stated.

Andrea de Leon
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Abstract

This thesis investigates whether the boron geochemistry of biogenic silica is linked to seawater pH or other environmental variables, and assesses its potential as a tool for palaeoceanographic reconstructions. Biogenic silica is an especially important palaeoceanographic archive in the Southern Ocean, where carbonate preservation is poor, however to date there is little published data on the boron geochemistry of biogenic silica. This study presents new datasets of boron concentration (B/Si) and isotope composition ($\delta^{11}$B) of siliceous sponges, and the B/Si ratio values of diatoms.

Sponge B/Si ratio values range from 2.12 to 5.63 ($\pm 0.23, 2\sigma$) mmol/mol, and do not show any significant correlation with seawater pH or other environmental variables. The sponge-seawater B/Si partition coefficients ($K_D$) range from $1.22 \times 10^{-5}$ to $1.13 \times 10^{-3}$. The relatively narrow range in sponge B/Si ratio values, and the lack of correlation with environmental conditions, indicate boron uptake is closely regulated by sponges.

A new method for sample preparation and analysis of boron isotopes by positive ion thermal ionisation mass spectrometry (PTIMS) was developed in lieu of any published procedures for $^{11}$B/$^{10}$B measurement in biogenic silica. The analytical precision of NIST SRM 951 boric acid was $4.0515 \pm 0.0007$ (2se, n=10), and for SRM 951 standards processed through the entire chemical procedure it was $4.0527 \pm 0.0017$ (2se, n=24), or 0.42‰. The precision for the sponge standard SP150 was $\pm 0.0022$ (2se, n=18), or 0.53‰, and the external reproducibility was 2.2‰ (2σ).

Sponge $\delta^{11}$B values span a relatively large range compared to other marine boron reservoirs, from +5.8 to +24.5‰ ($\pm 2.2, 2\sigma$). $\delta^{11}$B values do not correlate with seawater pH, but correlate well with seawater Si(OH)$_4$ concentrations ($R^2 = 0.71, p<0.0001$). This indicates that boron isotope fractionation is related to silica uptake during spicule
formation. A model for boron uptake and incorporation is proposed that accounts for fractionation during transport from seawater to the sclerocyte, and subsequent Raleigh fractionation during silica polymerisation.

Diatom B/Si ratio values, determined from experimental cultures of *Thalassiosira pseudonana*, display a negative, nonlinear correlation with [Si(OH)₄], with B/Si values ranging from 0.32 to 8.64 (±0.02, 2σ mean). This correlation likely arises because boron uptake by diatoms is relatively constant, therefore B/Si reflects silica uptake and deposition rate, which is a function of substrate (Si(OH)₄) concentration. Diatom B/Si ratios therefore demonstrate promising potential as a palaeoceanographic proxy for seawater [Si(OH)₄]. These findings were used to interpret qualitative variations in seawater Si(OH)₄ concentrations from the B/Si ratios in diatom sediment from a marine sediment core from the Southern Ocean (E33-22), which ranged from 0.33 to 0.69 (±0.2, 2se) mmol/mol. The B/Si record indicates an increase in seawater silica concentration during the last glaciation, consistent with previous palaeoceanographic reconstructions.
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Chapter 1

Introduction

1.1 Overview

The boron geochemistry of marine carbonates is a powerful palaeoceanographic proxy for seawater $p$CO$_2$ and pH. Using boron concentration and isotopic composition of the carbonate remains of foraminifera and corals, the carbon chemistry of the ocean has been reconstructed over recent geological history, providing valuable insight into secular changes in ocean chemistry over glacial-interglacial cycles and the links between the ocean and atmospheric CO$_2$.

However, such palaeoceanographic reconstructions have largely been restricted to low-latitude regions where foraminifera and corals are well-preserved, allowing for high-resolution temporal and spatial records. This is significant because there is a growing appreciation of, and need to understand, the role that the high-latitude oceans play in determining levels of CO$_2$ in the atmosphere. In the Southern Ocean, in particular, poor carbonate preservation has precluded $p$CO$_2$ and pH reconstructions. The Southern Ocean plays a crucial role in air-sea carbon exchange, and is a conduit for ventilation of the deep ocean, such that it is thought to have important links to atmospheric CO$_2$ concentrations (e.g. Sarmiento and Toggweiler 1984; Knox and McElroy 1984; Petit et al 1999; Anderson et al 2002; Kohfeld et al 2005; Marinov et al 2006; Fischer et al 2010).

The development of palaeoceanographic proxies using biogenic silica, which is ubiquitous and well-preserved in the Southern Ocean, has been of increasing interest in recent years (e.g. Mortlock et al 1991; Ragueneau et al 2000; De La Rocha et al 1998; Chase et al 2003; Anderson et al 2009). Wind-driven upwelling in the Southern Ocean draws nutrient-rich
deep water to the surface, which both releases CO$_2$ into the atmosphere and stimulates new primary production in surface waters. However, the relative influence of these two broad processes – CO$_2$ ventilation and biological consumption of CO$_2$ - over atmospheric pCO$_2$ is not fully understood and is the subject to some conjecture (e.g. Sigman and Boyle 2000; Anderson et al 2002; Fischer et al 2010). Given the lack of well-preserved, continuous carbonate records, it is hoped that biogenic silica records might preserve evidence of past changes in seawater carbonate chemistry or nutrient concentrations, thereby providing new tools for understanding how the Southern Ocean influences global climate.

The boron geochemistry of biogenic silica is identified as having significant potential to provide a new palaeoceanographic proxy for the Southern Ocean. Furst (1981) revealed that siliceous sponge spicules contain very high boron concentrations – on the order of hundreds of parts per million (ppm) by weight. Furthermore, Furst (1981) showed the boron concentrations of spicules extracted from a sediment core from the North Atlantic Ocean undergo a marked change between the Holocene and Last Glacial Maximum (LGM) (Figure 1.1). This indicates a likely link between boron uptake by sponges and changes in glacial-interglacial oceanographic conditions. However, little further work on the of boron geochemistry in sponge spicules, or biogenic silica more broadly, has been reported in the peer-reviewed literature since Furst’s (1981) study.
In light of the subsequent development of the B/Ca proxy for carbonate system equilibria and boron isotope proxy for seawater pH, the study by Furst (1981) raises some intriguing questions that this thesis aims to address. Firstly, do boron concentration and/or isotope ratios in biogenic silica vary with seawater pH? If so, is it a viable palaeo-pH proxy, and specifically, can the boron records in biogenic silica contribute to palaeoceanographic records of the Southern Ocean? These questions are investigated using both siliceous sponges and diatoms, with a view to assessing their potential as palaeoceanographic proxies for pH or other oceanographic parameters of benthic and surface waters respectively.

This thesis seeks to expand the current knowledge of boron geochemistry in biogenic silica, by:

1. generating a new, comprehensive dataset of boron concentrations and isotopic ratios in biogenic silica. Current data are limited a handful of diatom analyses (Ishikawa and Nakamura 1993; Mejía et al. 2013) and to Furst's (1981) sponge spicule study; and
investigating whether the boron geochemistry of biogenic silica is determined or influenced by seawater pH or carbonate chemistry, with a view to assessing whether the boron geochemistry in biogenic silica is useful as a palaeoceanographic proxy.

The thesis is structured as follows: Chapter 1 introduces the thesis and states the broad aims of the project. It summarises of the current literature on three topics central to this thesis: (1) boron geochemistry and its application as a palaeoceanographic proxy, (2) siliceous marine organisms and their use in palaeoceanographic reconstruction; and (3) the biogeochemistry and circulation of the Southern Ocean. Chapter 2 characterises boron concentrations of modern siliceous sponges from the Southern Ocean, revisits the work of Furst (1981) and examines the possible mechanisms of boron uptake and incorporation by sponges. Chapter 3 describes the analytical methods used and developed in this project to measure boron isotopes in biogenic silica using positive ion thermal ionisation mass spectrometry (PTIMS). Chapter 4 presents boron isotope compositions of marine sponges from the Southern Ocean, and explores models of isotope fractionation during boron uptake and incorporation within the framework of what is known of silicon uptake by sponges. Chapter 5 presents the boron concentrations of marine diatoms grown in cultures and from a deep-sea sedimentary core taken from the Southern Ocean, and discusses the potential of B/Si ratios in diatoms as a palaeoceanographic proxy. Finally, Chapter 6 synthesises the conclusions of this thesis and suggests directions for future work.

1.2 Literature Review

The following review examines the current literature on several topics that are central to this thesis. First is the boron geochemistry of seawater and its application a proxy for seawater pH using marine foraminifera and corals, which provides some context for
pursuing boron biogeochemistry in silica as palaeoceanographic proxy. The second is a brief description of the biology and biomineralisation of siliceous sponges and marine diatoms, and their use as a palaeoceanographic archive. Understanding the processes by which these organisms take up and deposit silica is crucial to interpreting the boron geochemistry, particularly deconvolving possible biological and environmental influences over trace element and isotope compositions. Finally, this chapter reviews the physical and biological dynamics of the Southern Ocean that make it such an important region for determining global atmospheric CO$_2$, and highlights the gaps in current knowledge that underscore the need for new palaeoceanographic proxies in light of the lack of well-preserved biogenic carbonate.

1.2.1 Boron isotope ratios in marine carbonates: a proxy for seawater pH

The basis for the boron isotope proxy for seawater pH lies in the aqueous speciation and isotope partitioning of boron in seawater (e.g. Hemming and Hanson 1992; Sanyal et al 1996), and the selective uptake of the borate species (c.f. boric acid) from seawater by calcifying organisms. Differences between theoretical and measured boron isotope values have given rise to some controversy over the application of the proxy (e.g. Pagani et al 2005; Hönisch et al 2007). The following section examines the theory behind the boron isotope pH proxy and highlights some of the challenges that have arisen in its development.

1.2.1.1 Boron in seawater

Boron (B) is conservative in seawater; its concentration is 416 µmol kg$^{-1}$ at S = 35 psu and the isotopic composition, expressed as $\delta^{11}$B, is $+39.5\%$ ± 0.1 (2σ, Spivack and Edmond 1987). Boron isotope ratios are expressed using delta (δ) notation relative to NIST standard NBS 951:
\[
\delta^{11}B = \left[ \left( \frac{^{11}B/^{10}B_{\text{sample}}}{^{11}B/^{10}B_{\text{std}}} \right) - 1 \right] \times 1000
\] 

(1.1)

The major sources and sinks of boron, listed in Table 1.1, are approximately equal, meaning that the modern marine boron content and isotopic composition are in or near to steady-state (Lemarchand et al 2002a). Although the fluxes of boron to and from the ocean are not well quantified, modelling studies of the long-term secular variation of seawater \(\delta^{11}B\) have determined the boron residence time to be on the order of 10 Myr (Simon et al 2006) to 14 Myr (Lemarchand et al 2002a). The uniformity of boron concentration and isotope ratios throughout the ocean underpins its use as a proxy for seawater pH and carbonate system equilibrium, as the empirical calibration of this proxy assumes a constant, known value of \(\delta^{11}B_{\text{sw}}\) (see equation 1.5). Both Lemarchand et al (2002a) and Simon et al (2006) conclude that despite approximate steady-state conditions in the modern ocean, fluctuations in secular \(\delta^{11}B\) would occur in response to changes in continental erosion or mid-ocean ridge spreading rates. Lemarchand et al (2002a) calculated the rate of change of \(\delta^{11}B\) to be 0.1‰/Myr, meaning a constant \(\delta^{11}B\) can only be extended with confidence over a few million years before present, taking into account analytical errors in \(\delta^{11}B\) measurements. Simon et al (2006) calculated large changes in \(\delta^{11}B_{\text{sw}}\) (+30‰ to +50‰) in response to changes in mid-ocean ridge spreading rates and ocean crust permeability, and the presence of hydrothermal veins. Both studies, however, rely on a relatively small database of boron source and sink measurements from only a handful of studies, and caution against the extrapolation of modern ocean \(\delta^{11}B\) beyond several million years until the oceanic boron cycle is better constrained.
Table 1.1 Major sources and sinks of boron

<table>
<thead>
<tr>
<th>Sources</th>
<th>$B$ flux ($10^{15}$ g/yr)</th>
<th>$\delta^{11}B$ (‰)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>River discharge (weathering)</td>
<td>38</td>
<td>+10</td>
<td>(a)</td>
</tr>
<tr>
<td>Hydrothermal fluids</td>
<td>1</td>
<td></td>
<td>(b)</td>
</tr>
<tr>
<td>Fluids from accretionary prisms</td>
<td>2</td>
<td></td>
<td>(c)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>41</strong></td>
<td></td>
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</tbody>
</table>

| Sinks                           |                          |                     |           |
| Low T crust alteration          | 26                       | +3.7                | (d)       |
| Clay adsorption                 | 13                       | +15 ±1              | (b), (e)  |
| Coprecipitation in carbonates   | 6                        | +20 ± 5             | (f), (g)  |
| Coprecipitation in silicates    | 1.3                      |                     | (h)       |
| Evaporites                      | ***                      |                     | (i)       |
| **TOTAL**                       | **46**                   |                     |           |

References: (a) Lemarchand et al (2002a); (b) Spivack and Edmond (1987); (c) You et al (1993); (d) Smith et al (1995); (e) Spivack et al (1987); (f) Vengosh et al (1991); (g) Hemming and Hanson (1992); (h) Ishikawa and Nakamura (1992); (i) Smith and Medrano (1996)

Boron exists as two mononuclear species in seawater: boric acid [$B(OH)_3$], and borate [B$\text{OH}^-_4$]. The dissociation of boric acid is given by the equilibria:

$$B(OH)_3 + H_2O \rightleftharpoons H^+ + B(OH)_4^-$$

(K$_B^*$ = $\frac{[B(OH)_4^-][H^+]}{[B(OH)_3]}$)

where the dissociation constant of boric acid (pK$_B^*$) is 8.60 at $T = 25^\circ$C and $S = 35$ psu (DOE 1994) (equation 1.3). The relative concentrations of boron species is dependent on solution pH, with almost all boron occurring as $B(OH)_3$ at low pH or as $B(OH)_4^-$ at high pH.

There are two stable isotopes of boron, $^{11}$B and $^{10}$B, which fractionate between the two boron species according to the exchange reaction:

$$^{10}B(OH)_3 + ^{11}B(OH)_4^- \leftrightarrow ^{11}B(OH)_3 + ^{10}B(OH)_4^-$$
Equations (1.2) and (1.4) can therefore be solved to determine the relative concentrations of $B(OH)_3$ and $B(OH)_4^-$ and the isotopic composition of each species (Figure 1.2) as a function of pH.

![Figure 1.2](image)

Figure 1.2 Concentration (a) and isotopic composition (b) of boric acid ($B(OH)_3$) and borate ($B(OH)_4^-$) in seawater, as a function of pH (at $T=25^\circ C$ and $S=35$ psu). Concentrations are calculated using $pK_{a}^* = 8.60$ (DOE 1994) and isotopic fractionation is calculated using two different fractionation factors ($\alpha$): 1.0194 (Kakihana et al 1977) (solid lines); and 1.0272 (Klochko et al 2006) (dashed lines).

Differences in B-O vibrational frequencies cause $B(OH)_3$ to be enriched in $^{11}$B relative to $B(OH)_4^-$, giving an isotopic equilibrium factor ($^{11-10}$K) greater than 1. The value of the fractionation factor ($\alpha = 1/^{11-10}$K) has been the subject of some debate between the commonly-used theoretical value calculated by Kakihana et al (1977) of 1.0194 at 25$^\circ$C, and an experimentally-determined value by Klochko et al (2006) of 1.0272 ± 0.0006. Numerous other values have also been published (Liu and Tossell 2005; Pagani et al 2005; Zeebe 2005; Byrne et al 2006). The debate as to which $\alpha$ value should be applied when using $\delta^{11}$B to reconstruct pH remains unresolved, with studies promoting the use of the Klochko et al (2006) value arguing it most closely fits the empirically-derived curves measured from biogenic carbonates (Foster 2008; Yu et al 2010). The need for an experimentally-derived value was also supported by Zeebe (2005), who argued that accurate determination of $\alpha$ requires experimental assessment of the vibrational
frequencies of B(OH)$_3$ and B(OH)$_4$, and the theoretical methods used to calculate the molecular forces.

1.2.1.2  

*Boron isotopes in marine carbonates*

The relationship between pH and the B isotope fractionation between B(OH)$_3$ and B(OH)$_4$ has been exploited to develop a proxy for seawater pH using the $\delta^{11}$B of marine carbonate shells (principally foraminifera) and skeletons (corals). The proxy is based on the assumption that only the charged, tetrahedral B(OH)$_4^-$ species is incorporated into the growing shell, and that little or no fractionation occurs during incorporation (Hemming and Hanson 1992). Therefore, the $\delta^{11}$B of the carbonate shell will correspond with that of B(OH)$_4^-$ in seawater, which can be used to calculate the ambient pH of the seawater in which the organism grew, according to the equation:

$$
\text{pH} = pK_B^* - \log \left( \frac{\delta^{11}B_{\text{sw}} - \delta^{11}B_{\text{CaCO}_3}}{\delta^{11}B_{\text{sw}} - \alpha_B \delta^{11}B_{\text{CaCO}_3} - 1000(\alpha_B - 1)} \right)
$$

(1.5)

where $pK_B^* = 8.60$ (at $T = 25^\circ$C and $S = 35$; DOE (1994)), $\delta^{11}B_{\text{sw}} = +39.5\%$ and $\alpha_B = 1.0194$ (Kakihana et al 1977) or 1.0272 (Klochko et al 2006), as previously discussed.

The assumption that only borate is taken up by carbonates is based on observations that (1) the range of $\delta^{11}$B measured in carbonates is similar to that of B(OH)$_4^-$ in the typical seawater pH range (i.e. +19 to +25\%) and varies with seawater pH (e.g. Hönisch et al 2004), whereas B(OH)$_3$ has a substantially heavier $\delta^{11}$B (around +40\%) (Vengosh et al 1991; Hemming and Hanson 1992), and (2) B/Ca ratios in foraminifera shells appear to vary with seawater pH, indicating B incorporation is controlled by the availability of B(OH)$_4^-$ (Yu et al 2007; Ni et al 2007).
Vengosh et al (1991) and Hemming and Hanson (1992) first suggested that the $\delta^{11}$B of foraminifera and other carbonates can linked to seawater pH. Both studies included analyses of modern, naturally-occurring carbonate samples. Vengosh et al (1991) analysed different biogenic carbonates including foraminifera, coral and gastropods, and found that $\delta^{11}$B ranged from +14.2 to +32.2‰, and boron concentrations ranged from 1 - 80 ppm. Hemming and Hanson (1992) also analysed a range of carbonates including calcite, aragonite and high-Mg calcite, but found a narrower range in $\delta^{11}$B values (+19 to +25‰) and boron concentrations (10.9 to 71.4 ppm). Hemming and Hanson (1992) postulated that the tetrahedral borate species was preferentially incorporated into the carbonate site during growth, and therefore that the $\delta^{11}$B of carbonates could be correlated with seawater pH.

The $\delta^{11}$B - pH proxy has been subsequently tested and calibrated by studies using inorganic carbonate precipitation experiments (Hemming et al 1995; Sanyal et al 2000), foraminifera and coral culture experiments (Sanyal et al 2001; Hönsch et al 2003; Reynaud et al 2004; Hönsch et al 2004; Rollion-Bard and Erez 2010) and core-top sediment samples from the deep sea (Hönisch and Hemming 2004; Hönsch et al 2008; Foster 2008; Rae et al 2011; Henehen et al 2013) (Figure 1.3). Both the Hemming et al (1995) and Sanyal et al (2000) studies support the hypothesis that B(OH)$_4^-$ is coprecipitated with calcium carbonate. Hemming et al (1995) found that in aragonite, calcite and high-Mg calcite $\delta^{11}$B is identical to that of B(OH)$_4^-$ at pH $\approx$ 8. Sanyal et al (2000) precipitated calcite over a range of pH (7.9 - 8.6) and found $\delta^{11}$B increases with increasing pH, such that the $\delta^{11}$B vs. pH curve of calcite matched closely that of the theoretical curve of Kakihana et al (1977) for B(OH)$_4^-$, albeit with a small negative offset (2-3‰).
Figure 1.3. $\delta^{11}B$ vs seawater pH of cultured marine carbonates selected from the literature. Dashed lines represent the $\delta^{11}B$ of $B(OH)_4^-$ in seawater: the theoretical $\alpha$ value from Kakihana et al (1997) (black) and the experimentally-derived $\alpha$ value from Klochko et al (2006) (grey). pH values have been adjusted to total pH scale.

Culture experiments have demonstrated that the $\delta^{11}B$ of foraminifera varies principally as a function of seawater pH. Foraminifera species *Orbulina universa* (Sanyal et al 1996), *Globigerinoides sacculifer* (Sanyal et al 2001), *Amphistegina lobefera* (Rollion-Bard and Erez 2010) and *Globigerinoides ruber* (white) (Henehan et al 2013) grown at different pH produce $\delta^{11}B$ vs pH curves that follow the theoretical $B(OH)_4^-$ curve of Kakihana et al (1977). These studies, as well as that by Hönsich et al (2003), in which *O. universa* were grown at varied light intensities in order to determine the influence of symbiont photosynthesis on $\delta^{11}B$, show that foraminiferal $\delta^{11}B$ is systematically lighter than the theoretical seawater $B(OH)_4^-$ value. This negative offset has been attributed to ‘biological effects’, namely alteration of the pH in the foraminifera microenvironment by respiration, calcification and symbiont photosynthesis (Hönisch et al 2003; Sanyal et al 2001). These
authors suggest differences in the magnitude of photosynthesis by symbionts relative to respiration by the foraminifera produce localised changes in $pCO_2$ and therefore pH. Importantly, this effect does not preclude the use of foraminiferal $\delta^{11}B$ as a pH proxy, but rather requires that pH reconstructions are made using an empirical calibration using modern samples of the species in question (Hönisch et al 2003).

Alteration of the microenvironment pH by respiration or calcification was also identified as the cause of an offset between foraminifera and theoretical $\delta^{11}B$ by Rollion-Bard and Erez (2010), who cultured the symbiont-bearing species *A. lobefera* at a pH range 7.90 - 8.45 and measured $\delta^{11}B$ in situ, and report intra-shell $\delta^{11}B$ variations recording a pH range from ambient seawater pH up to ~9. This variation was attributed to the different pH of seawater vacuoles delivered to the site of calcification, which may have elevated pH in order to increase CaCO$_3$ saturation to facilitate calcification. Similar calibration experiments have been conducted with corals. As with foraminifera, Reynaud *et al* (2004) and Hönisch *et al* (2004) found that $\delta^{11}B$ of coral increases with increasing pH, and the $\delta^{11}B$ vs pH curve is parallel to that of B(OH)$_4^-$. Reynaud *et al* (2004) also found no significant difference between specimens grown at different temperatures.

Culture experiments by Hönisch *et al* (2003) found that symbiont photosynthesis produces foraminiferal $\delta^{11}B$ values ~1.5‰ higher than specimens grown in darkness (i.e. no photosynthesis). This corresponds to ~0.2 pH units. However, while the difference between specimens grown in high light cycles and those in complete darkness was significant, the difference in $\delta^{11}B$ between the theoretical $\delta^{11}B_{\text{borate}}$ and observed foraminiferal values are not as pronounced as was previously predicted by previous measurements of microenvironment pH made by Rink *et al* (1998). Furthermore, the difference is constant and species-specific, therefore the $\delta^{11}B$ - pH proxy is valid so long as
empirical calibrations are carried out using modern specimens (Hönisch et al. 2003). These conclusions were consistent with modelling by Zeebe et al. (2003).

The relationship between B/Ca ratios of coccolithophorids and seawater pH was examined by Stoll et al. (2012). Unlike foraminifera and corals, which precipitate calcium carbonate using endocytosis of seawater, coccolithophorids do not use a seawater reservoir to produce calcite, but rather produce coccoliths in a specialised vesicle containing a calcifying fluid that is closely regulated by the organism (Stoll et al. 2012, Young and Henriksen 2003). Therefore, the links between the external seawater pH and in the intracellular processes of calcification, and boron incorporation, are different to those of other marine calcifying organisms. Stoll et al. (2012) observed different responses in coccoliths B/Ca to variations in seawater pH between the two coccolithophore species cultured (*Coccolithus braarudii* braarudii and *Emiliania huxleyi*) and between the three different strains of *E. huxleyi*. The results were interpreted using model of boron uptake by coccolithophorids that infers passive uptake of the uncharged boric acid species from seawater into the coccolith vesicle, and subsequent borate incorporation into the coccolith calcite. Therefore, the boron content of the vesicle is determined by seawater pH (i.e. the availability of boric acid), whereas the fraction of boron available as borate is controlled by the vesicle pH, and by concentration of dissolved inorganic carbon (DIC).

Several additional factors are recognised to complicate the use of $\delta^{11}$B\textsubscript{carbonate} as a pH proxy. Klochko et al. (2009) found that both trigonally- and tetrahedrally-coordinated boron exist in coral aragonite and foraminiferal calcite, rather than only borate as has previously been assumed. However, it is unlikely that both species are taken up directly from seawater and incorporated during calcification; the high proportion of B(\text{OH}$_3$)\textsubscript{2} observed (36 - 46%) would produce a much larger $\delta^{11}$B value - closer to that of seawater. Rather, the presence of both boron species suggests that boron uptake and incorporation
might involve a re-equilibration of $^{11}$B and $^{10}$B between the two species caused by localised pH changes, which could produce an enrichment in $^{11}$B in carbonates, accounting for the heavier $\delta^{11}$B values of carbonates relative to the Klochko et al (2006) $\delta^{11}$B value of B(OH)$_4$ (Klochko et al 2009) (Figure 1.3). Although the $\delta^{11}$B values of both inorganic and natural carbonates indicate B(OH)$_4^-$ is the boron species taken up by carbonates, the findings of Klochko et al (2009) point to more complex processes involved in the incorporation of boron during biomineralisation.

An ongoing controversy surrounds the correct value of the equilibrium isotope fractionation factor ($\alpha$). The theoretical value calculated by Kakihana et al (1977) of 1.0194 has been widely applied (e.g. Sanyal et al 2000, 2001; Hönsch et al 2003, 2004, 2008; Pearson and Palmer 2000). Most carbonate $\delta^{11}$B values tend to be lighter than the Kakihana et al (1977) B(OH)$_4^-$ values, producing $\delta^{11}$B-pH curves that are lighter than, but parallel to, the borate curve (Hönisch et al 2008). Recent studies have demonstrated the fractionation factor is larger, notably the experimentally-derived $\alpha$ value of 1.0272 by Klochko et al (2006), which is in closer agreement with other theoretical and experimentally-determined values (Oi 2000; Liu and Tossell 2005; Tossell 2006; Byrne et al 2006). This larger fractionation factor has been adopted by numerous subsequent studies (e.g. Foster et al 2010; Yu et al 2010; Rollion-Bard and Erez 2010; Henahan et al 2013). Although this issue is not yet definitively resolved, there is growing consensus that the larger fractionation value of Klochko et al (2006) more closely reflects the fractionation recorded in carbonates than that of Kakihana et al (1977). Rollion-Bard and Erez (2010) point out that it is difficult to explain the lighter $\delta^{11}$B offset of carbonates from the Kakihana et al (1977) B(OH)$_4^-$ curve, as modification of the microenvironment pH should produce heavier, not lighter, $\delta^{11}$B. Alternatively, any uptake of B(OH)$_3$ rather than exclusive B(OH)$_4^-$ uptake, would also produce heavier $\delta^{11}$B values. Hönsch et al (2008) suggest that the smaller fractionation factor might be more accurate given that, despite the
offset from $\delta^{11}\text{B}_{\text{carbonate}}$, a fractionation factor of $\sim 1.020$ best describes the shape and inflection point of the empirical carbonate curves, and therefore fractionation can be determined using $\alpha \approx 1.020$ and an empirically-derived species-specific offset constant.

Another challenge to the application of the $\delta^{11}\text{B}$-pH proxy is the accuracy and precision of boron isotope analysis. Palaeoceanographic applications require accuracy and precision of better than 1‰, or $\sim 0.1$ pH units (Foster et al 2006) and although most laboratories report precision better than 0.5‰ (e.g. Lemarchand et al 2002b, Hemming et al 1998; Hönisch et al 2004; Ni et al 2010) the interlaboratory variability is much greater. Foster et al (2013) report an interlaboratory variability of $\pm 1.43\%$ (2sd pooled) for carbonate samples measured by four different laboratories, each using different analytical techniques. This is in contrast to the variability of boric acid and seawater samples that were also analysed by these laboratories: the pooled 2sd of three boric acid samples measured was $\pm 0.39\%$, and the mean seawater $\delta^{11}\text{B}$ was $+39.65 \pm 0.41\%$ (2sd) (Foster et al 2013). The cause of the large interlaboratory variability in carbonate analyses was inconclusive, but is broadly attributed to blank contamination during sample processing, and/or matrix effects during analysis (Foster et al 2013). Although the source of interlaboratory bias is yet to be constrained, Foster et al (2013) conclude that this bias does not preclude palaeoceanographic applications of the $\delta^{11}\text{B}$-pH proxy, as relative variations in $\delta^{11}\text{B}$ can still be resolved by a single laboratory or technique.

1.2.1.3 Boron/calcium ratios in carbonates: a proxy for carbonate ion concentration

The theoretical basis for the $\delta^{11}\text{B}$-pH proxy also rings true for boron concentrations in marine carbonates (expressed as boron/calcium ratios, B/Ca). As seawater pH increases, so too does the proportion of $\text{B(OH)}_4^-$, and assuming only borate is incorporated during calcification, the B/Ca ratio is expected to increase with pH.
This relationship has been observed in several studies of planktonic foraminifera, in which B/Ca had a positive correlation with seawater pH (Sanyal et al 2001, 2012; Allen et al 2011; Henehan et al 2013). However, the relationship between carbonate B/Ca and pH is more complex than the theoretical basis. In benthic foraminifera, B/Ca correlates with carbonate ion concentration ([CO$_3^{2-}$]) rather than pH (Yu and Elderfield 2007; Yu et al 2010; Rae et al 2011). In some coccolithophorids B/Ca decreases, rather than increases, with seawater pH (Stoll et al 2012), and in corals correlation between B/Ca and pH has not been observed (Sinclair 2005; Allison and Finch 2010).

Conjecture remains around the application of B/Ca ratios as a proxy for pH or CO$_3^{2-}$ concentration, particularly with respect to the use of partition coefficients (K$_D$) to reconstruct seawater parameters (Tripati et al 2011; Allen and Hönsch 2012). There is also evidence that B/Ca varies with other environmental parameters, including temperature (Yu et al 2007) phosphate concentration (Henehan et al 2015) and growth rate (Uchikawa et al 2015), although the influence of these parameters was not observed in culture experiments conducted by Allen et al (2016). Despite the incomplete understanding of the relationship between B/Ca in marine carbonates and environmental conditions, B/Ca does respond to pH and [CO$_3^{2-}$] in planktonic and benthic foraminifera and therefore is still a potentially useful proxy for seawater chemistry, albeit there is a need to better constrain the influences of environmental parameters (Allen and Hönsch 2012).

1.2.2 Biogenic silica: diatoms and siliceous sponges

Like marine carbonates, biogenic silica – silica produced by diatoms and sponges – is an established archive of palaeoceanographic conditions. It is an especially useful tool in the Southern Ocean, where accumulation of biogenic silica in deep sea sediment makes for continuous, high-resolution records. This thesis will examine whether the boron content
of this biogenic silica can also provide insights into changes in ocean chemistry. However, in order to quantify the relationship between boron elemental and isotopic signatures in biogenic silica and the surrounding seawater environment, it is important to understand how trace elements are incorporated and preserved in the siliceous skeletons of diatoms and sponges, and therefore how these organisms produce silica.

1.2.2.1 Diatoms

Diatoms are photosynthesising unicellular algae that form siliceous cell walls, called frustules. Thousands of species have been identified and are classified primarily according to their frustule morphology, which is often ornate and complex. Diatoms can be divided into two broad categories according to frustule symmetry: centric diatoms have radial symmetry and pennate diatoms show bilateral symmetry. The frustule, illustrated in Figure 1.4, comprises two halves that overlap, the epitheca and the hypotheca, and are connected by structures called girdle bands. Frustule formation occurs in a specialised compartment of the cell called the silica deposition vesicle (SDV), into which silicic acid is transported from seawater, to be polymerised and deposited as biogenic silica. The SDV is bound by a membrane called the silicalemma, and it is thought that once frustule formation is complete the entire SDV is exocytosed, with the silicalemma becoming part of the cell wall (Martin-Jézéquel et al 2000).
1. Introduction

Figure 1.4. Diatom cell wall structure. The siliceous cell wall (frustule) comprises two overlapping sections, the epitheca and hypotheca. Girdle bands of each theca run around the cell. The frustule encloses the protoplasm, including the silica deposition vesicle (SDV) in which silica is polymerised. Adapted from Kröger and Sumper (2004).

Silica transport through the cell to the SDV is not well-understood. Some authors have attributed transport to specialised transport vesicles, although such vesicles have not been specifically identified (Martin-Jézéquel et al 2000), whereas others advocate that transport is mediated either by silicic acid transporters (SITs) or by ionophore-mediated diffusion (Bhattacharyya and Volcani 1980, Martin-Jézéquel et al 2000). The silicon concentration maintained in the SDV is high, up to 340 mM in some species (Martin-Jézéquel et al 2000), making the SDV highly supersaturated with respect to silica. A possible explanation as to how diatoms can maintain such high concentrations of soluble silica is that silica is bound to silicon-binding organic complexes (Sullivan 1979; Perry and Keeling-Tucker 2000). Moreover, the extent of silicon complexation might control the rates of silicon uptake and efflux, with excess unbound silica inducing efflux and reducing uptake (Martin-Jézéquel et al 2000).

Silica polymerisation is initiated with the formation of nanospheres of silica, similar to that observed in sponge spicule formation (Aizenburg et al 2005). The condensation of silica to form these nanospheres is catalysed by peptides identified in the cell wall called silaffins (Kröger et al 1999). In the presence of silaffins, silica polymerises in seconds, much faster than autopolycondensation reactions in supersaturated aqueous
environments (Perry and Keeling-Tucker 2000). The polymerisation and aggregation of silica is also facilitated by the low pH maintained in the SDV (Vrieling et al. 1999); for pH between 2 and 7, silica polymerisation is rapid in aqueous environments, and dissolution of silica is reduced (Vrieling et al. 1999). The arrangement of these nanospheres to form the frustule is thought to be controlled by an organic matrix, possible lining the inside of the silicalemma (Perry and Keeling-Tucker 2000).

1.2.2.2 Sponges

Sponges (Phylum Porifera) are divided into four classes, Hexactinellida, Demospongiae, Homoscleromorpha and Calcarea. The first two of these groups, the hexactinellids and demosponges, generally secrete skeletons made up of a framework of needle-like siliceous spicules (although some demosponges are proteinaceous rather than siliceous). Homoscleromorphs are encrusting sponges with few or no spicules. Calcarea produce calcium carbonate skeletons, and are thought to be more closely related to other metazoa than to the siliceous sponges (Eerkes-Medrano and Leys 2006). The spicule skeleton is surrounded by the soft body of the sponge, a tissue-like layer called the mesohyl. The mesohyl composition varies between species, but generally comprises cells of different types, and proteins including collagen fibres and spongin (Simpson 1984). The mesohyl is bound by an epithelial layer of flagellated cells called choanocytes, and a layer of cells called pinacocytes. It is the choanocytes that drive the movement of water into the sponge through pores in the sponge body, and out through the opening at the top of the sponge, the osculum (Bergquist 1978; Figure 1.5).
Spicules are highly diverse in size and morphology, and are classified as either megascleres or microscleres based on their size and, more importantly, their structural function (Uriz et al. 2003a). Megascleres form the main skeletal framework of a sponge, while the role of microscleres is less obvious, possibly providing protection against predators or helping to form the skeletal structure by joining megascleres (Uriz et al. 2003b). Each spicule is made up of concentric layers of amorphous silica, with a central core of proteinaceous material called the axial filament. The axial filament is composed of silicatein, a protein that catalyses the polymerisation of silica (Shimizu et al. 1998), and so it is thought that the axial filament acts as a template for silica precipitation (Cha et al. 1999). Galactin and collagen, both proteins found in association with silicatein in the axial filament and in the growing spicule and, are also thought to play a role in the orientation and morphology of the silica layers (Schröder et al. 2006).
Spicule growth occurs in two stages: intracellular and extracellular (Müller et al 2005) (Figure 1.6). Studies that have elucidated this process have used primmorphs (dissociated single cells) from demospongiae species (Uriz et al 2000; Custódio et al 2002; Müller et al 2005, Schröder et al 2005). The initial formation of the axial filament occurs within specialised cells called sclerocytes. Silica is transported into the sclerocyte via \( \text{Na}^+/\text{HCO}_3^- [\text{Si(OH)}_4] \) transporters (Schröder et al 2004) and is polymerised to form the first layers of silica around the axial filament. The spicule is then extruded from the sclerocyte into the mesohyl (Uriz et al 2000, Wang et al 2012), where growth continues appositionally (Müller et al 2005). The spicule is surrounded by sclerocytes that produce vesicles containing silicic acid, known as silicasomes (Schröder et al 2007). Silicasomes are released from the sclerocyte to a hollow chamber composed of silicatein and galactein, which surrounds the spicule and in which the silicic acid polymerises and is deposited in concentric layers (Schröder et al 2007). The shaping of different spicule types occurs extracellularly, and is thought to be facilitated by collagen (Wang et al 2012).

![Figure 1.6](image-url). Intracellular and extracellular formation of a spicule. The axial filament (af) is formed inside the sclerocyte, around which the first concentric layers of silica are deposited. The spicule is then released into the mesohyl, where it is surrounded by sclerocytes that release silicasomes containing silicic acid and silicatein. Adapted from Wang et al (2009).
1.2.2.3 *The use of biogenic silica as a palaeoceanographic archive*

The interest in biogenic opal as a palaeoceanographic tool has been growing in recent years, particularly in relation to Southern Ocean oceanography and palaeoceanography where carbonate preservation is poor. Opal accumulation rates, corrected for lateral sediment redistribution using $^{230}$Th, have been used to reconstruct export productivity (Chase *et al* 2003) and, in turn, nutrient supply by nutrient upwelling to surface waters (Anderson *et al* 2009). Further constraints on diatom productivity can be provided by $^{231}$Pa/$^{230}$Th and $^{106}$Ba/$^{230}$Th ratios, which preserve accumulation rates without the effects of opal dissolution (Chase *et al* 2003). Given diatoms are the dominant group of phytoplankton in the Southern Ocean, opal accumulation rates provide a good indicator of export productivity.

Trace element concentrations have also provided valuable insight into environmental conditions and nutrient availability. The germanium/silicon ratio in opal records have been shown to reflect silica availability in surface waters (Froelich *et al* 1989; Shemesh *et al* 1989; Mortlock *et al* 1991). Similarly, Ge/Si of sponge spicules has been used to reconstruct deep water Si concentrations (Ellwood *et al* 2006). Zinc (Ellwood and Hunter 1999; Hendry and Rickaby 2008) and other trace metal concentrations (Lal *et al* 2006) have also been investigated as oceanographic proxies, as have Zn isotope ratios (Andersen *et al* 2011; Hendry and Andersen 2013).

There has been increasing interest in using silicon isotope ratios in biogenic silica as a proxy for Si utilisation by diatoms (De La Rocha *et al* 1997, 1998; Varela *et al* 2004, Brzezinski *et al* 2002, Fripiat *et al* 2011, Egan *et al* 2012), and thereby constrain changes in nutrient availability and export in the Southern Ocean. Several studies of Si isotope ratios in sponge spicules have demonstrated the relationship between Si isotope fractionation and ambient silicic acid concentration (De La Rocha 2003; Wille *et al* 2010;
Hendry et al. 2010; Hendry and Robinson 2012). Pairing reconstructions of surface Si utilisation with silicic acid concentrations of upwelling waters makes the use of Si isotope signatures in diatoms and sponges a potentially powerful tool for understanding past silica production and ocean cycling (Hendry and Brzezinski 2014).

1.2.2.4 Boron in biogenic silica

Studies of the boron geochemistry of biogenic silica are scant. The work of Furst (1981) represents the first, and only, published study of boron in siliceous sponges. Furst (1981) measured the boron concentrations of sponge spicules from both live-collected and fossil samples using in situ radiographic analysis. The author compared the boron concentrations of the modern samples with a range of environmental variables, including temperature, productivity (defined as the number or mass of planktonic organisms per unit volume of near-surface water), salinity and silicon concentration. Boron concentration was found to have a positive correlation with salinity. The causal link with salinity was not suggested, and the author noted that it was difficult to interrogate the relationship between boron concentration and environmental variables with the available data. A broad correlation between boron concentration and temperature was observed, however it was noted that the correlation is not simple, and may instead reflect covariance in water pollution at these sites. The samples from high silicon waters were observed to have the highest boron concentrations, but a correlation between the two variables was not observed in other locations, and Furst (1981) attributes this lack of correlation to the organism’s discrimination between silicon and boron uptake.

Spicules were taken from four marine sediment cores, V19-29, RC12-249, RC13-263 and V23-42. The latter core, V23-42, was taken from North Atlantic (62.183°N, 27.560°W), and a clear distinction in boron concentrations between glacial (9000 - 13,000 years) and
interglacial (1000 - 9000 years) is observed. Similar shifts in boron concentration over time are not recorded in the other cores sampled.

The boron geochemistry of sponge spicules has not been addressed in the literature since Furst's (1981) study. However, the boron content of diatom frustules was presented by Mejía et al (2013), who found a positive correlation between diatom boron content and pH in experimental cultures. This correlation was suggested to reflect co-transport of B(OH)$_4^-$ and HCO$_3^-$, meaning that with increased pH the demand for HCO$_3^-$ increases, and therefore B(OH)$_4^-$ uptake also increases. This would result in increased boron uptake at higher pH.

1.2.3 The Southern Ocean and its role in atmospheric CO$_2$ variability

It has long been recognised that the Southern Ocean plays a vital role in regulating global $p$CO$_2$ variations over glacial-interglacial cycles (e.g. Sarmiento and Toggweiler 1984; Knox and McElroy 1984; Caldeira and Duffy 2000; Sigman and Boyle 2000; Marinov et al 2008; Anderson et al 2009). The correlation between changes in atmospheric CO$_2$ concentration and late Quaternary glacial-interglacial climate shifts recorded in Antarctic ice cores (Fischer et al 1999; Petit et al 1999; Indermühle et al 2000; Monnin et al 2001; Siegenthaler et al 2005; Lüthi et al 2008; Ahn and Brook 2008) points to the Southern Ocean as playing a key role in producing CO$_2$ variations (Wolff et al 2006; Lüthi et al 2008). Although there is broad agreement on the importance of the Southern Ocean in changes in atmospheric CO$_2$, the causal links between CO$_2$ concentrations and the various biological and oceanographic processes remain contentious (e.g. Sigman and Boyle 2000; Archer et al 2000; Anderson et al 2002; Fischer et al 2010).

Several processes occur in this region that make it particularly significant for air-sea CO$_2$ exchange: (1) the upwelling of deep water masses that are enriched in dissolved inorganic carbon (DIC) and nutrients; (2) carbon fixation and export by phytoplankton, fuelled by
the abundant upwelled nutrients, and; (3) the transport of nutrient-rich surface waters to lower latitudes via mode waters. Although the exact mechanisms by which atmospheric CO₂ fluctuates are not fully understood, it has become increasingly accepted that processes occurring in the Southern Ocean must drive these changes. Moreover, it is likely a combination of these different processes is responsible, and that identifying variations in deep water upwelling, associated nutrient flux and nutrient utilisation is central to understanding atmosphere-ocean CO₂ exchange (e.g. Kohfeld et al (2005); Sigman and Boyle 2000; Fischer et al 2010). Palaeoceanographic archives of CO₂ and nutrient concentrations are therefore a crucial tool in investigating the role of the Southern Ocean in glacial-interglacial carbon cycle variations.

The Southern Ocean is the site of ventilation of CO₂-rich deep water masses that have been isolated from the atmosphere. These deep water masses accumulate DIC at lower latitudes through phytoplankton biomass sinking and decay. These deep water masses are transported southwards to form Circumpolar Deep Water (CDW), which is then brought to the surface by wind-driven upwelling south of the Antarctic Polar Front (APF), releasing CO₂ back into the atmosphere (Wyrtki 1961; Toggweiler and Samuels 1995). Circumpolar westerly winds drive the Ekman transport of a portion of this surface water northward, where it is subducted to form Antarctic Intermediate Water (AAIW) and Subantarctic Mode Water (SAMW) and is subsequently transported to low latitudes at intermediate depths (Figure 1.7). The remaining portion drifts southwards and subducts to form Antarctic Bottom Water (AABW).
The Southern Ocean is also a significant sink for atmospheric CO₂. The direction and magnitude of transfer of CO₂ between the surface ocean and atmosphere depends on the difference in partial pressure of CO₂ (pCO₂) between the two reservoirs (Broecker and Peng 1982; Takahashi et al 2002). Surface water pCO₂ is lowered by the consumption of CO₂ by phytoplankton and the subsequent export of organic matter to the thermocline and deep ocean where it is either buried or dissolved, a process referred to as the ‘biological pump’. This process sequesters CO₂ back into the ocean’s interior, thus lowering the surface pCO₂ and drawing CO₂ down from the atmosphere. As a consequence, the net exchange of CO₂ between atmosphere and surface ocean in the Southern Ocean is determined by the difference in CO₂ outgassed from upwelled deep water and the drawdown of CO₂ from atmosphere to surface waters due to biological sequestering of carbon. There remains some controversy over the strength of the CO₂ sink and/or CO₂ source to the atmosphere in the Southern Ocean (e.g. Gruber et al 2009; Takahashi et al 2009). Although there is general agreement that the region south of 44°S is a CO₂ sink, Gruber et al (2009) concluded that the sink flux is relatively uniform across this area.
whereas Takahashi et al (2009, 2012) found that there is a strong CO₂ uptake in the band between 30 and 50°S, and south of 50°S the magnitude of the flux is seasonally variable. The averaged net annual flux is very small because the summer sink conditions negate the winter source flux, and in the seasonal ice zone (south of 62°S) there is a very strong flux from sea to air per unit area of water, but a very small flux in the ice-covered area, which becomes a strong sink in spring when ice melts and there are large phytoplankton blooms (Takahashi et al 2009).

Changes in the ventilation of the deep ocean through the Southern Ocean on millennial timescales has led to the conclusion that the Southern Ocean is a driver of global glacial-interglacial pCO₂ variations (e.g. Sarmiento and Toggweiler 1984; Sigman and Boyle 2000). The efficiency of the biological pump - i.e. the proportion of available nutrients consumed and exported – determines the nutrient content of the intermediate and deep water masses formed in the region. The efficiency of the biological pump can be described in terms of ‘preformed’ and ‘remineralised’ nutrient inventories: preformed nutrients are those brought to the surface in upwelled waters that remain unutilised by phytoplankton, and so are returned to the ocean interior by intermediate or deep water formation. Remineralised nutrients refer to nutrients consumed at the surface and exported to the deep ocean where they are redissolved as the organic matter decays. The ratio of remineralised to preformed nutrients in subsurface waters indicates the biological pump’ efficiency: a very efficient biological pump transforms high proportions of otherwise preformed nutrients into remineralised nutrients. In the region south of the APF where surface waters subduct, inefficient nutrient utilisation leads to high preformed nutrient concentrations in AABW, indicating inefficient sequestration of CO₂ to depth. In contrast, to the north of the APF productivity determines the preformed nutrient content of AAIW and SAMW, which in turn determines the magnitude of export productivity at low latitudes (Sarmiento et al 2004; Marinov et al 2006).
The global inventory of preformed nutrients held in deep water masses is closely linked to sequestered CO$_2$, making atmospheric $p$CO$_2$ particularly sensitive to productivity in high-latitude regions of deep water formation (Ito and Follows 2005, Archer et al 2000; Marinov et al 2008). There is growing evidence of links between nutrient availability and utilisation in the Southern Ocean and greater CO$_2$ sequestration to the deep ocean during the last glacial period, although the causal mechanisms have not been conclusively established. A decrease in preformed nutrient concentrations in deep waters formed in the Antarctic zone requires a reduction in surface water nutrients due to more efficient export production and/or a reduced nutrient supply (i.e. decreased ventilation) (Sigman and Boyle 2000; Sigman et al 2010). Palaeoceanographic records of surface productivity indicate decreased export production in the Antarctic zone during the last glacial, which has been attributed to reduced upwelling (e.g. Mortlock et al 1991; François et al 1997; Chase et al 2003; Anderson et al 2009). Possible causes of reduced upwelling include surface water stratification (François et al 1997), sea-ice cover inhibiting CO$_2$ ventilation (Stephen and Keeling 2000) and phytoplankton productivity (Chase et al 2003) or shifts in the strength and position of midlatitude westerly winds (Toggweiler et al 2006; Anderson et al 2009).

In contrast to the Antarctic zone, export productivity in the Subantarctic zone was higher during the last glacial (Mortlock et al 1991; Kumar et al 1995; Chase et al 2003). A more efficient biological pump in this region would have reduced the volume of preformed nutrients transported to low latitudes (Sarmiento et al 2004), affecting global productivity. The increased removal of CO$_2$ from the surface to the abyssal ocean would increase alkalinity and thus increase CO$_2$ storage capacity of the ocean, therefore lowering atmospheric CO$_2$ (Boyle 1988; Sigman and Boyle 2000). Seawater alkalinity may also have increased due to a shift in the ratio of CaCO$_3$ to organic C (CaCO$_3$/C$_{org}$) that is exported to the deep ocean, known as the ‘rain ratio’, and caused by a change in the population
composition of phytoplankton at lower latitudes. There is growing evidence that iron fertilisation in the Subantarctic zone, caused by dust deposition, increased local export productivity (Martin et al 1990) but caused a decrease in the ratio of silicate to nitrate consumed by diatoms, the dominant phytoplankton group in the Southern Ocean. This resulted in greater proportions of unused silicic acid transported to low latitudes via SAMW and AAIW, thereby increasing diatom productivity at the expense of coccolithophorids and decreasing the CaCO$_3$/C$_{org}$ rain ratio (Brzezinski et al 2002; Matsumoto et al 2002). The increased ocean alkalinity brought about by changes in Subantarctic productivity, coupled with the reduced ventilation in the Antarctic zone, may account for a significant proportion of the lower glacial atmospheric pCO$_2$ (Sigman et al 2010). This theory, referred to as the silicic acid leakage hypothesis (SALH) (Brzezinski et al 2002; Matsumoto et al 2002), thus links silica utilisation in the Southern Ocean to global glacial-interglacial atmospheric CO$_2$ fluctuations.

The geochemical proxy records of Southern Ocean circulation changes and silica distribution over glacial-interglacial periods is, however, conflicting. The SALH predicts changes in silica utilisation in the Southern Ocean caused by greater iron availability, which would lead to a decrease in opal accumulation. However, there is no evidence of decreased silica utilisation in the opal accumulation record in the Atlantic and Indian sectors (Chase et al 2003). Rather, there is clear evidence that the opal belt shifted northward during the last glacial period, but total silica utilisation only decreased in the Pacific sector (Chase et al 2003; Bradtmiller et al 2009).

The evidence of increased silica concentrations in AAIW during glacial periods is also conflicting, and silicon isotope records from a sponge spicule record in a low-latitude region bathed in AAIW indicates silica concentrations of southern-sourced waters
increased in pulses coincided with abrupt deglacial events (Heinrich Stadials 1 and 2 and the Younger Dryas), rather than over glacial periods (Hendry et al 2012).

Hendry and Brzezinski (2014) synthesise these geochemical proxy records, and propose a variation of the SALH: that the leakage of silica-rich southern waters occurred during deglacial events. There has been more recent evidence that the ‘leakage’ of silicic acid to low latitude waters did not occur during glacial periods, as the SALH posits, but rather is linked to the climate change events Heinrich Stadials One and Two, and the Younger Dryas (Hendry and Brzezinski 2014). The Silicic Acid Ventilation Hypothesis suggests that shifts in atmospheric and ocean circulation during these deglaciation events strengthened upwelling in low-latitude regions of the North Atlantic and North Pacific oceans, and caused a southward shift in Southern Ocean Westerlies which increased upwelling and reduced stratification in the Southern Ocean. This increased ventilation caused greater export of high silica waters to low latitudes, which in turn would increase diatom productivity in low latitudes (Hendry and Brzezinski 2014). This development in the understanding of the links between nutrient distribution and export in the Southern Ocean and global cooling highlights the complexity of interpreting the ocean circulation of this region.
Chapter 2

Boron/silicon ratios of modern siliceous sponges from the Southern Ocean

2.1 Introduction

The siliceous skeletons of marine sponges contain high concentrations of boron, on the order of hundreds of parts per million (ppm) (Furst 1981). This is in contrast to marine carbonates (10 – 80 ppm, Vengosh et al. 1991; Hemming and Hanson 1992; Ishikawa and Nakamura 1993) and seawater (4.52 ppm, Spivack and Edmond 1987), and yet despite the unusually high boron content of sponge spicules, and the intense interest that has developed in boron chemistry in marine systems, no studies subsequent to that of Furst (1981) have investigated the boron uptake and incorporation by siliceous sponges. This is especially of interest in light of the association between boron uptake by marine carbonates and seawater pH, and the use of marine carbonates as a pH proxy in palaeo-pH reconstructions (Hemming and Hanson 1992).

This chapter examines the boron content (expressed as the B/Si ratio) of modern siliceous sponges in order to investigate links between B/Si and seawater pH and other environmental variables, with the aim of gaining insight into what controls boron uptake from seawater by sponges. Particular focus is placed on establishing whether a relationship exists between the boron geochemistry of siliceous sponges and seawater pH. This information is integrated with results from recent studies of the mechanisms of spicule formation which provide insight into how biogenic silica polymerisation might affect boron concentration of sponges. While the boron geochemistry of biogenic silica is of general interest, fossil sponge spicules provide a potential useful archive for investigating environmental change, particularly given sponges inhabit such a wide range
of depths, temperature, seawater nutrient concentrations and pH compared to other siliceous organisms (i.e. diatoms and radiolarians). This chapter also revisits the findings of Furst (1981) and compares the data obtained in this study in order to consider whether the present findings confirm Furst’s (1981) conclusions that boron concentrations are linked to seawater salinity.

2.2 Materials and methods

Siliceous sponges were collected from two regions: Tasmania, Australia (147°W, 44°S) and George V Land, Antarctica (144°W, 66.6°S), and encompassed a range of species from both Classes Hexactinellid and Demospongiae. In both cases, sponges were collected in summer (December-January) using benthic sled trawls. Upon collection sponges were frozen immediately and then freeze-dried for long-term storage.

Samples were selected for analysis from as wide a range of depths as possible, in order to sample a large gradient in temperature, pH and nutrient concentrations. Where possible, the same sponge species was sampled at different localities, however this was often not practicable because many species were largely restricted to specific locations or depths. Sponges were cut into small (1-2 cm) pieces and immersed in 30% H₂O₂ (reagent grade) and 1M HCl (reagent grade), and left on a hotplate at approximately 50°C for several hours to oxidise organic matter and to dissolve any trapped carbonate material. The spicules were subsequently rinsed several times with deionised water (MQ, Millipore) to remove H₂O₂ and HCl, and then left to dry overnight. The dried spicules were milled to a fine powder with a tungsten carbide mill, and the powdered samples were heated in a furnace at 800°C overnight in aluminium oxide crucible, in order to oxidise any residual organic material. Sample dissolution for analysis followed the method of Wille et al (2010). Briefly, about 10 mg of sample powder was weighed into Teflon bombs, to which was added 300 mg NaOH (Sigma AR grade) and 1 mL Milli-Q™ (>18 MΩ) water. The samples were
digested at 200°C for 48 hours, then diluted to approximately 30 mL with Milli-Q H₂O, and stored in acid-cleaned 50 mL Nalgene™ polyethylene bottles.

Aliquots of the dissolved samples were diluted so that silicon concentrations were approximately 50 ppm, and beryllium was added as an internal standard to correct for instrument drift during boron analysis. Boron and silicon concentrations were measured by quadrupole ICP-MS (Varian 820) at the Research School of Earth Sciences, ANU. Reagent and procedural blanks were routinely measured alongside each batch of samples that were processed, with total blank levels being <1% for B and 2-3% for Si. To circumvent instrument memory problems encountered with trace level boron measurements by ICP-MS due to the volatility of boron in acidic solution, which results in boron being released from the wetted internal surfaces of the spray chamber (Al-Ammar et al 1999), samples were measured at high pH (9-10) in a dilute (~0.002 M) NaOH matrix, rather than in dilute acidic solution. This procedure results in rapid washout times, and facilitates analysis of standards and samples without significant boron memory effects compromising the measurements. Boron volatility was also reduced by cooling to <4°C using a Peltier-cooled Scott double pass spray chamber. The relative standard deviation of repeated analyses of an in-house sponge spicule standard (SP-150) is ±0.23 mmol/mol (2σ, n = 24 analyses).

Boron and silicon concentrations were also measured in individual spicules by laser ablation ICPMS, using an ANU Helex laser ablation system coupled to a Varian 820 quadrupole ICPMS. For this type of analysis spicules were presented to the laser by simply mounting onto carbon tape fixed to a glass microscope slide. Difficulties were initially encountered with erratic spicule ablation producing highly erratic signal intensities, which precluded accurate and precise B/Si analysis. SEM images and prior experience with poorly absorbing large band-gap materials suggested photomechanical ablation processes were dominating the laser sampling of the spicules. It was found that the heating of sponge
spicules prior to analysis in aluminium oxide crucibles at 800°C overnight to remove organic material and water, resulted in more uniform and controlled ablation behaviour.

2.3 Results

The boron and silicon concentrations measured in Tasmanian and Antarctic sponges are reported as B/Si atomic ratios in Table 2.1. Values fall with a relatively limited range from 2.12 to 5.63 mmol/mol. This equates to 382 to 1014 ppm boron, which agrees well with the values reported for modern siliceous sponge spicules by Furst (1981). There is no significant difference between B/Si ratios of hexactinellid and demosponge samples (p = 0.9081). This is consistent with other trace element ratios in siliceous sponges reported by Sandford (2003), who found there was no significant compositional difference between demosponge and hexactinellid spicules. Nonetheless, inter-species differences within these groups can be quite large. This is illustrated by the analysis of five different species of sponge collected from the same site by benthic trawl, the B/Si values of which span the range of the entire data set (that is, from 2.12 to 5.63 mmol/mol).

Boron/silicon variability within individual sponges was investigated by analysis of different spicules selected from several sponges. Spicules were sampled from the opening of the osculum, the middle of the body and the base of the sponges (labelled (a), (b) and (c) respectively in Table 2.2). Boron concentrations were then measured by LA-ICPMS (Table 2.2). The differences in boron concentration between individual spicules is statistically significant in two sponge samples (SP119 and SP151, p<0.0001) but there is no significant difference between spicules in SP222 (p=0.017).
Table 2.1. Sample locations, classification, B/Si results and environmental variables.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Depth (m)</th>
<th>Longitude (°W)</th>
<th>Latitude (°S)</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>B/Si (mmol/l) ± 2SD</th>
<th>B concentration (ppm)</th>
<th>pH (total)</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>seawater [Si] (μmol/kg)</th>
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<tbody>
<tr>
<td>326-328</td>
<td>100</td>
<td>146.270</td>
<td>43.723</td>
<td>Demo</td>
<td>Dicerca</td>
<td>Dacry</td>
<td>2.55 ± 0.2</td>
<td>453</td>
<td>8.08</td>
<td>12.26</td>
<td>35.07</td>
<td>2.02</td>
</tr>
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<td>100</td>
<td>146.976</td>
<td>43.691</td>
<td>Demo</td>
<td>Hadromer</td>
<td>Spirast</td>
<td>2.95 ± 0.2</td>
<td>532</td>
<td>8.07</td>
<td>12.26</td>
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<td>2.18</td>
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<td>146.311</td>
<td>43.723</td>
<td>Demo</td>
<td>Astrophy</td>
<td>Ancori</td>
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<td>11.05</td>
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<td>43.691</td>
<td>Demo</td>
<td>Hadromer</td>
<td>Subere</td>
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<td>34.99</td>
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<td>Liolitidi</td>
<td>demospongiodae</td>
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<td>Eulectellida</td>
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<td>457</td>
<td>144.309</td>
<td>66.321</td>
<td>Demo</td>
<td>Spirophorida</td>
<td>Tetellida</td>
<td>3.96 ± 0.4</td>
<td>713</td>
<td>8.01</td>
<td>-1.90</td>
<td>34.61</td>
<td>87.30</td>
</tr>
<tr>
<td>SP119</td>
<td>540</td>
<td>145.535</td>
<td>66.750</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.65 ± 0.4</td>
<td>836</td>
<td>8.00</td>
<td>-1.90</td>
<td>34.62</td>
<td>86.98</td>
</tr>
<tr>
<td>SP019</td>
<td>702</td>
<td>143.357</td>
<td>66.333</td>
<td>Demo</td>
<td>Spirophorida</td>
<td>Tetellida</td>
<td>3.37 ± 0.4</td>
<td>600</td>
<td>8.00</td>
<td>-1.90</td>
<td>34.63</td>
<td>88.85</td>
</tr>
<tr>
<td>SP018</td>
<td>702</td>
<td>142.959</td>
<td>66.550</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.62 ± 0.5</td>
<td>651</td>
<td>8.00</td>
<td>-1.89</td>
<td>34.65</td>
<td>88.85</td>
</tr>
<tr>
<td>SP066</td>
<td>867</td>
<td>142.959</td>
<td>66.550</td>
<td>Hexactinellida</td>
<td>-</td>
<td>-</td>
<td>3.25 ± 0.3</td>
<td>585</td>
<td>8.12</td>
<td>-1.89</td>
<td>34.65</td>
<td>91.15</td>
</tr>
<tr>
<td>SP060</td>
<td>867</td>
<td>142.959</td>
<td>66.550</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.35 ± 0.2</td>
<td>423</td>
<td>8.12</td>
<td>-1.89</td>
<td>34.65</td>
<td>91.15</td>
</tr>
<tr>
<td>SP110</td>
<td>1198</td>
<td>145.151</td>
<td>67.047</td>
<td>Demo</td>
<td>Spirophorida</td>
<td>Tetellida</td>
<td>2.87 ± 0.2</td>
<td>516</td>
<td>7.93</td>
<td>-1.89</td>
<td>34.65</td>
<td>96.45</td>
</tr>
</tbody>
</table>

* B concentration calculated assuming spicule comprises dehydrated SiO2.

^ pH, temperature, salinity and seawater silica concentrations from Schlitzer (2000).
Table 2.2. Boron (B) concentrations of individual spicules from three siliceous sponges, measured by LA-ICPMS. Individual spicule samples are labelled (a), (b) and (c). For comparison, B concentration values of whole sponge samples are included where available, measured in solution by ICPMS (from Table 2.1).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Spicule B (ppm)</th>
<th>2se</th>
<th>Whole sponge B (ppm)</th>
<th>2se</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP119</td>
<td>1139</td>
<td>23</td>
<td>835</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>915</td>
<td>23</td>
<td></td>
<td></td>
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<tr>
<td>SP151</td>
<td>708</td>
<td>8</td>
<td>712</td>
<td>57</td>
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<td></td>
<td>845</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>653</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP222</td>
<td>1107</td>
<td>19</td>
<td>not determined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1031</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1059</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3.1 Relationships between sponge B/Si and environmental variables

The correlation between sponge B/Si ratios and a range of environmental variables that were measured for each of the sample sites was determined. B/Si ratios are generally invariant with increasing seawater pH ($R^2 = 0.013, p = 0.95$) (Figure 2.1).

![Figure 2.1](image.png)

Figure 2.1. Diatom B/Si values plotted against seawater pH (total scale), illustrating the poor correlation between the two variables ($R^2 = 0.002, p = 0.99$). Error bars represent 2SD.

No relationship between boron concentration and seawater salinity, as postulated by Furst (1981), is evident in the present data set ($R^2 = 0.002, p = 0.99$). The Pearson
correlation R values, displayed in Table 2.3, indicate there is no evidence for a significant correlation between sponge B/Si and other environmental variables including seawater temperature, silicate concentration or pH.

Table 2.3. Correlation matrix of B/Si with seawater depth (m), temperature (°C), salinity, pH and silica and phosphate (PO$_4^{3-}$) concentration. Seawater data from repeat analyses at SR3 transect sites by Rosenberg (1999).

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>Temp</th>
<th>Salinity</th>
<th>pH</th>
<th>[Si]$_{sw}$</th>
<th>[PO$<em>4^{3-}$]$</em>{sw}$</th>
<th>B/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>-0.31</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Salinity</td>
<td>-0.58</td>
<td>0.68</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.64</td>
<td>0.15</td>
<td>0.69</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Si]$_{sw}$</td>
<td>0.28</td>
<td>-0.98</td>
<td>-0.57</td>
<td>-0.08</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PO$<em>4^{3-}$]$</em>{sw}$</td>
<td>-0.54</td>
<td>0.03</td>
<td>0.11</td>
<td>0.07</td>
<td>-0.06</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B/Si</td>
<td>-0.07</td>
<td>0.10</td>
<td>0.04</td>
<td>-0.11</td>
<td>-0.11</td>
<td>0.34</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3.2 B/Si partition coefficients

Assuming boric acid or borate substitutes for silicic acid (Si(OH)$_4$) during the condensation reactions that form biogenic silica, a possible equation for this reaction, based on the silica oligermisation mechanisms (Harrison and Loton 1995; Perry and Keeling-Tucker 2000), is

$$\text{Si(OH)}_4^{(s)} + \text{B(OH)}_4^{(aq)} \rightarrow \text{(OH)}_3\text{Si-O-B(OH)}_3^{(s)} + \text{H}_2\text{O} \quad (2.1)$$

Sponge spicule B/Si compositions can be expressed in terms of the partitioning of boron relative to silicon between the spicules and seawater. The empirical partition coefficient, $K_B$, is defined as:

$$K_B = \frac{m_B^{(m)} / m_{Si}^{(m)}}{m_B^{(sw)} / m_{Si}^{(sw)}} \quad (2.2)$$

where $m_B$ and $m_{Si}$ are the molar concentrations (mmol/mol) of boron and silicon respectively. $K_B$ values for the samples measured in this study are listed in Table 2.1, and were calculated using seawater $m_{Si}$ values obtained from WOCE datasets, and assuming
seawater $m_B = 416 \mu\text{mol kg}^{-1}$ at $S = 35$ psu (Spivack and Edmond 1987). The calculated $K_D$ values reveal that B/Si partitioning is largely independent of taxon-specific partitioning effects. There is however a significant correlation between $K_D$ and temperature ($R^2 = 0.77$, $p <0.001$) (Figure 2.2a), that indicates B/Si incorporation decreases along with although not necessarily due to increasing temperature. There is also an equally significant positive correlation between $K_D$ and the ambient silicon concentration of seawater ($R^2 = 0.79$, $p<0.001$) (Figure 2.2b). The co-variance of temperature and silicic acid concentrations in the Southern Ocean may explain the correlations observed with both of these variables, and may mean that temperature and/or silicic acid concentrations or other correlated variable(s) may exert control over B/Si partitioning by sponges (see discussion below). The spread of $K_D$ values particularly in the low temperature, high silica (Antarctic) samples indicates that other factors, possibly species or site-specific factors, such as growth rate or nutrient supply may also exert further control over B/Si partitioning from seawater during spicule growth.

![Figure 2.2.](image)

Figure 2.2. Empirical partition coefficient values ($K_D$) plotted against (a) seawater temperature and (b) silica concentration. Circles (●) denote samples from Tasmania, triangles (▲) denote Antarctic samples.
2.4 Discussion

2.4.1 Boron uptake and partitioning between seawater and spicule

The incorporation of boron into sponge spicules is not correlated with seawater pH, and is not obviously linked to other environmental variables assessed by this study, including nutrient concentrations, temperature or salinity. The following discussion examines possible mechanisms of boron uptake by sponges, drawing insight from our understanding of the processes of silica uptake and polymerisation.

The B/Si ratio of sponge spicules are relatively uniform, whereas the B/Si ratio of seawater is highly variable due to variation in silica concentrations, which span almost two orders of magnitude from around 2 to 100 \( \mu \text{mol kg}^{-1} \), and the conservative behaviour of boron which has a concentration of around 416 \( \mu \text{mol kg}^{-1} \) at a salinity of 35 mg kg\(^{-1}\) (Spivack and Edmond 1987). It follows that \( K_0 \) values may largely reflect differences in seawater silica content. Furthermore, a strong discrimination against boron (toward silicon) uptake from seawater by sponge spicules is evident in the small size of the empirical \( K_0 \) values (i.e. \( 3.7 \times 10^{-5} \)). This is consistent with silicon uptake being strongly promoted over that of boron by the organism. It is not known whether boron follows silicon uptake pathways, with \( \text{B(OH)}_4^- \) substituting for \( \text{Si(OH)}_4 \), or is incorporated via alternative, B-specific transporters, or rather is transported and polymerised as a consequence of diffusion from seawater and/or surface adsorption, without facilitation by enzymes.

Experimental cultures of demosponges \( H. \text{Panicea} \) (fam. Halichondriidae; order Halichondria) (Reincke and Barthel 1997) and the genus \( Axinella \) (fam. Axinellidae; order Halichondria) (Maldonado et al 2011) reveal silica deposition follows saturable, Michaelis-Menten kinetics, consistent with enzyme-mediated and substrate concentration dependent uptake (Figure 2.3a). These studies also show that the saturated uptake rate
(v_{max}) and half-saturation constant (K_M) differ substantially between sponge species, which is interesting given the B/Si of sponges is relatively uniform, subject to variability in boron concentration with changes in salinity. Little is known about how silica is transported from seawater to the spicule-producing cells (sclerocytes), which are embedded within the sponge tissue (mesohyl) (Figure 1.5) and thus are not in direct contact with seawater (Schröder et al 2004). It has been suggested that a Na^+/HCO_3^- co-transporter may be involved in silica transport across the cell membrane (Schröder et al 2004), which could account for the observed uptake kinetic behaviour.

Inside the sclerocyte silica polymerises around the axial filament (Schröder et al 2004), which comprises a group of proteins called silicateins (Shimizu et al 1998) (Figure 1.6). Silicatein acts as a template for silica deposition (Schwab and Shore 1971) as well as playing an active role in catalysing silica polymerisation (Shimizu et al 1998; Cha et al 1999). It has been proposed that the enzymatic mechanism involves the condensation of silicon alkoxides (Si–O–C) by the hydroxyl group of serine, followed by hydrolysis to form silanol (Si–OH) and then condensation to produce an amorphous silica network (Cha et al 1999).

It is possible that borate substitutes for silicate and is incorporated into spicules by silicatein. In addition to catalysing and directing the polymerisation of silica, silicatein has the ability to catalyse the polymerisation of other metal and metalloid oxides, including titanium dioxide and gallium oxide (Sumerel et al 2003, Kisailus et al 2005). The incorporation of titanium into siliceous spicules grown in a seawater medium spiked with titanium has also been observed (Natalio et al 2010). These observations have been attributed to silicatein-catalysed incorporation of metals into the silica structure by the same mechanism as occurs for silicic acid, that is by nucleophilic attack on Si (or metal) followed by condensation (Zhou et al 1999; Kisailus et al 2005). The flexibility of
silicatein’s activity with respect to different substrates may well extend to boron, which is also a metalloid. In this case boron incorporation into sponge spicules would follow a similar mechanism to that postulated for titanium incorporation.

If boron does follow the same pathway as for silica, the uptake rate would also conform to Michaelis-Menten kinetics, with maximum uptake rates constrained by substrate (B) concentration and enzyme binding site availability. Given \([B]_{sw}\) is constant, B uptake rate \((V_B)\) would also be constant:

\[
V_B = \frac{v_{max,B} \times [B]_{sw}}{K_{M,B} + [B]_{sw}}
\]  

(2.3)

where \([B]_{sw} = 416 \, \mu \text{mol kg}^{-1}\), and \(v_{max,B}\) and \(K_{M,B}\) are the maximum uptake rate and half-saturation constant for B respectively. Since all of these terms are more or less likely to be constants, \(V_B\) must also be constant. The spicule B/Si is determined by \(V_B/V_{Si}\), i.e. the rates of B and Si incorporation. This relationship is illustrated in Figure 2.3a, where \(V_B/V_{Si}\) is plotted against \([Si]_{sw}\) and the same values for \(v_{max,B}\) and \(K_{M,B}\) are adopted as \(v_{max,SI}\) and \(K_{M,SI}\) respectively, noting that these values are arbitrary in lieu of published data on boron uptake kinetics. The resulting curve is reciprocal to the \(V_{Si}\) Michaelis-Menten curve (Figure 2.3a); \(V_B/V_{Si}\) and therefore B/Si decreases with increasing \([Si]_{sw}\) (Figure 2.3b). This behaviour contrasts with the data obtained in this study, which do not show any increase in B/\(Si_{spicule}\) at low \([Si]_{sw}\).
Figure 2.3. (a) Silica uptake rate \( v_{si} \) follows Michaelis-Menten uptake kinetics as a function of substrate concentration. Values for \( v_{max} \) and \( K_M \) taken from Reinke and Barthel (1997). (b) Curve denotes spicule B/Si values calculated using the same \( v_{max} \) and \( K_M \) values used in Figure 2.3(a) (Reinke and Barthel 1997). \( v_{si} \) is constant, therefore the curve – produced from the \( v_{si}/v_{si} \) ratio – is the inverse of the Michaelis-Menten curve. Black circles \( \bullet \) represent the measured spicule B/Si values.

An alternative transport pathway could be via a boron-specific transporter. Although no such transporter is known in sponges, a borate transporter (BOR1) has been identified in plant roots (Takano et al. 2002) which belongs to a broader family of bicarbonate transporters found in both plants and animals (Frommer and Von Wirén 2002). The potential uptake pathways of boron and silicon, if such a transporter were to exist in sponges, are illustrated in Figure 2.4. The uptake kinetics in this case are more difficult to predict; however if the process is mediated by boron specific transporters boron uptake is likely to be maintained at a near constant rate, particularly given the relatively uniform availability of boron in seawater. This would produce a B/Si_{sp} vs. [Si]_{sw} curve similar to that in Figure 2.3b, which is the inverse of the \( V_{Si} \) vs. [Si]_{sw} Michaelis-Menten plot. In either instance described above, where boron substitutes for silicon or is incorporated via a specific boron transporter, it is assumed that the total boron in seawater is the available substrate. However, if the transport mechanism specifies a charged molecule (e.g. \( Na^+/HCO_3^- \) co-transporter), \( B(OH)_4^- \) could be the relevant substrate species. Accordingly, the substrate concentration will not be constant throughout the ocean, but instead be
determined by the ambient pH. Boron speciation is closely linked to pH, such that in the case of B(OH)₄⁻ being the preferred species, the available substrate will increase with pH. In the Tasmanian sites sampled for this study, estimated [B(OH)₄⁻] decreases from around 97 µM in shallow (high pH) waters to 66 µM in the deepest (low pH) waters, based on values calculated from averaged pH measurements for these locations and depths (Schlitzer 2000). This should lead to a faster rate of boron uptake by the sponge (i.e. increased \( V_B \)) with increased substrate concentration near the surface, and decreased B/Si\(_{sp} \) with depth. Silicon concentrations also generally increases with depth due to export of diatoms from surface waters and subsequent remineralisation (Millero 2006), in contrast with decreasing [B(OH)₄⁻] with depth. It follows that we might then expect to see high B/Si\(_{sp} \) in sponges from shallow waters (high [B(OH)₄⁻], low [Si]) and low B/Si\(_{sp} \) in sponges from deeper water. However, no relationship is observed between B/Si\(_{sp} \) and pH or depth, indicating B/Si\(_{sp} \) is not simply determined by substrate availability. It must be noted that only the boron speciation in seawater has been considered here, and it is likely that [B(OH)₄⁻] will vary in the mesohyl and sclerocyte in response to changing pH.
Figure 2.4. Cartoon of putative boron and silica transport from seawater to spicule formation. Transport must occur across the epithelial cells into the mesohyl, a collagenous tissue. The mechanism of transport through the mesohyl is unknown, although Uriz (2000) identified areas of high silica concentration in the tissue closest to sclerocytes. Silica transport across the sclerocyte cell membrane is via a NBC-SA transporter. An analogous B transporter has been reported to exist in plants and possibly animal cells (Frommer and Von Wirren 2002). Spicules initial form by silica polymerization inside the sclerocyte, and are then extruded into the mesohyl where growth is thought to continue (Uriz 2000). The sponge cavity (osculum) is lined with specialized cells called choanocytes, which use flagella to move seawater throughout the sponge and extract nutrients.

It is possible that boron uptake and incorporation occurs without the involvement of transporters. The simplest mechanism is by passive diffusion from seawater into the cell, as has been suggested for some plants (Dordas and Brown 2000). The lipid bilayer of cell walls is known to be permeable to boric acid, which is a small molecule with neutral charge, allowing passive diffusion across any activity (and concentration?) gradient. The high boric acid concentration of seawater (160 – 391 µM over a pH range from 8.8 to 7.4) would provide such a concentration gradient. Any diffusion process is unlikely to be simple, however, as the sclerocyte is not in direct contact with seawater. Boron would therefore be transported through epithelial cells and mesohyl to the sclerocyte.

The incorporation of boron into the spicule may also occur by adsorption onto the spicule surface, rather than by catalysis by silicatein. A similar mechanism has been suggested for...
the incorporation of boron into marine carbonate organisms, whereby $\text{B(OH)}_4^-$ adsorbs onto the carbonate surface and then substitutes for $\text{CO}_3^{2-}$ in the carbonate lattice (Hemming and Hanson 1992). The adsorption of boron onto silica gel, which has a hydrated, amorphous silica lattice analogous to biogenic silica (e.g. Loucaides et al 2008), is thought to involve both inner and outer sphere complexes. Inner sphere complexes are formed only by $\text{B(OH)}_4^-$, which reacts to produce $\text{B-O-Si}$ bonds via ligand exchange, whereas trigonal and tetrahedral boron species both form outer-sphere bonds (Kim and Kirkpatrick 2006). As with $\text{CO}_3^{2-}$, it is plausible that tetrahedrally-coordinated $\text{B(OH)}_4^-$ could substitute for Si-O$^-$ in the biogenic silica structure.

2.4.2 The effect of temperature

Temperature can be expected to affect the equilibrium constants and kinetics of boron incorporation during uptake and silica polymerisation, and thereby also influence the empirical $K_0$ value. Indeed, the data show a significant decrease in the empirical $K_0$ with increasing seawater temperature ($R^2 = 0.77$, $p<0.001$, Figure 2.2a). However, as the mechanism/s of boron uptake and incorporation remain unclear and it is likely that boron incorporation involves multiple stages of transport and uptake reactions, it is difficult to predict how temperature might affect boron partitioning between seawater and spicule. Furthermore, $\text{B/Si}_{sp}$ shows no significant influence of temperature, and given temperature and silicic acid concentration co-vary (low T, high Si(OH)$_4$ samples represent Antarctic waters) the observed correlation between $K_0$ and temperature is difficult to deconvolve from any effect of silicic acid concentration.

2.4.3 The effect of polymerisation rate

The correlation between $K_0$ and $[\text{Si(OH)}_4]_{sw}$ might reflect a relationship between boron partitioning and the rate of silica polymerisation. There is a positive correlation between
Chapter 2. Boron/silicon ratios of modern siliceous sponges from the Southern Ocean

$K_D$ and $[\text{Si(OH)}_4]_{\text{sw}}$ (Figure 2.2b), which is consistent with $K_D$ increasing towards unity as the rate of polymerisation increases. A similar correlation between growth rate and trace element partitioning has been observed in abiotically precipitated $\text{CaCO}_3$ (both calcite and aragonite) (Lorens 1981; Morse and Bender (1990); Rimstidt et al 1998; Gaetani and Cohen 2006), and can be broadly attributed to the effects of precipitation rate on mass transport kinetics (e.g. diffusion rates to the mineral surface) or surface reaction kinetics (e.g. adsorption or dehydration reactions).

Despite the observed positive correlation between $K_D$ and $[\text{Si(OH)}_4]_{\text{sw}}$, there is no significant relationship between $B/\text{Si}_{\text{sp}}$ and $[\text{Si(OH)}_4]_{\text{sw}}$. As $K_D$ is an expression of $B/\text{Si}_{\text{sp}}$ with respect to $[\text{Si(OH)}_4]_{\text{sw}}$ and because seawater boron concentration is constant, the observed relationship may be an artefact. $B/\text{Si}_{\text{sp}}$ vs $[\text{Si(OH)}_4]_{\text{sw}}$ may provide a more accurate depiction of the effect of polymerisation rate on $B/\text{Si}$ partitioning. The lack of a significant relationship between $B/\text{Si}_{\text{sp}}$ and $[\text{Si(OH)}_4]_{\text{sw}}$ indicates that silica polymerisation rate does not determine $B/\text{Si}$ partitioning, and perhaps points to the close regulation of both boron and silicon uptake by sponges.

2.4.4 How do these results compare with Furst (1981)?

Furst (1981) reported a weak correlation between boron concentrations of live-collected sponge spicules and salinity, although the effects of other environmental variables that co-vary between sampling sites could not be ruled out for lack of available data. A similar correlation is not observed in the present data (Figure 2.5); however, it is difficult to compare the data of this study to Furst’s (1981) data, as the samples analysed here derive from a more restricted salinity range, and the possibility of much lower $B/\text{Si}$ values in sponges from brackish waters, as reported by Furst (1981), cannot be ruled out.
Several other observations made by Furst (1981) are confirmed by this study: Furst (1981) found that boron concentration does not reflect seawater B/Si, and there is no correlation between spicule boron content and seawater silicon concentrations. Moreover, if seawater B/Si determined spicule boron content, sponges from high silicon waters (e.g. Antarctica) might be expected to have lower boron concentrations, which is not the case. The same is found in the present data, that is, seawater B/Si appears to have no bearing on spicule B/Si. Furst (1981) also compared boron concentrations of individual spicules from the same sponge, and found no significant variations within a single specimen, which is consistent with the laser ablation data collected as part of this study.

Interestingly, Furst (1981) reports a relationship between spicule boron concentration and temperature in the live-collected specimens: the low temperature (<15°C) spicules have a high boron content compared to those in high temperature waters >20°C. However, Furst (1981) points out that there are other environmental variables that differ between the sample sites, including salinity and nutrient runoff from nearby urban areas. The
temperature range between the sample sites in Furst’s (1981) study is much larger than the present study, making it difficult to compare the possible effects of temperature. The boron concentrations measured in this study agree well with the range in boron in low temperature samples reported by Furst (1981); however, samples from tropical locations would need to be analysed for a robust comparison between the two studies. The large contrast in boron concentration between the temperate and tropical sites does raise the question as to whether boron concentrations are influenced by environmental conditions, temperature or other factors, not observed in the present study, which sampled a narrower range of environmental variation. Expanding on the current study to include sponges from tropical sites might shed light on the impact of more extreme differences in seawater conditions like temperature.

Finally, it should be noted that Furst (1981) employed a more restrictive sampling criteria than this study. Spicules were selected for analysis only if they were larger than 20 µm in diameter, and spicules from the root structures of sponges were excluded, as were morphologically-distinct microscleres such as sterraster (small star-like spicules). Boron concentrations were measured in situ using an in-situ radiographic technique (Furst 1981), hence the requirement of large spicules. No such restrictions were placed on spicule selection in this study - spicules of entire sections of a sponge, and in some instances the whole sponge body, were dissolved and analysed as a solution, therefore reported B/Si values represent an average of all spicule types. The LA-ICPMS analyses of individual spicules in this study (Table 2.2) point to variations in boron concentration between spicules from different parts of the sponge body, which is consistent with similar variations in some sponges reported by Furst (1981) although Furst (1981) concluded that if microscleres are excluded from analyses, boron concentrations are generally more uniform. In the present study only megascleres were analysed by LA-ICPMS (due to analytical requirements of sample size). Therefore, given the statistically significant
variation in two of the three sponge samples, these data do not support the conclusions
drawn by Furst (1981) regarding the uniformity of boron concentrations between
microscleres spicules.

2.5 Conclusion

The boron/silicon ratios in modern siliceous sponges from the Southern Ocean span a
range from 2.1 to 5.6 mmol/mol. This equates to B concentrations between 382 and 1014
ppm, which is consistent with the high values previously reported by Furst (1981). Sponge
B/Si ratios show no significant correlation with seawater pH, nor other environmental
variables including temperature, depth or silicic acid concentration. Although boron
uptake and deposition mechanisms in sponges are unknown, the process of silica uptake
and spicule formation sheds light on possible boron uptake mechanisms. Previous work
indicates silica biomineralisation to be largely mediated by enzymatic control by
specialised proteins (specifically various silicateins) which are involved in silica
polymerisation. It is possible that boron transport is also enzyme-mediated, and that
boron incorporation into sponge spicules is driven by competitive interactions between
boron and silicatein. This mechanism of boron uptake and incorporation during sponge
silica biomineralisation contrasts with that observed in biogenic carbonate, whereby
ambient seawater pH or carbonate chemistry appears to control B/Ca ratio values. This
study confirms the high boron concentrations in high latitude sponges reported by Furst
(1981). However, contrary to the findings of Furst (1981), there exists no evidence to
support a role for seawater salinity in determining the boron content of sponges or the
influence on boron concentration during spicule growth.
Chapter 3

A procedure for the separation and PTIMS analysis of boron isotopes in biogenic silica

3.1 Introduction

Several methods for the analysis of boron isotope ratios in marine biogenic carbonates have been published, following the development of the boron isotope palaeo-pH proxy in marine carbonates, including negative ion thermal ionisation mass spectrometry (NTIMS) (Hemming and Hanson 1994; Foster et al. 2006), positive ion thermal ionisation mass spectrometry (PTIMS) (Lemarchand et al. 2002b) and multi-collector inductively-coupled plasma mass spectrometry (MC-ICP-MS) (Foster 2008). These methods, however, cannot be directly applied to the measurement of boron isotope ratios in biogenic silica, due to differences in the sample matrix.

To date there are no reported boron isotope data for live-collected or clay-free biogenic silica samples. The boron isotope composition of biogenic silica - sponges, diatoms and radiolaria - has scarcely been reported in the literature, limited to only a few analyses: the $\delta^{11}\text{B}$ composition of diatomaceous ooze has been estimated to be +4.3‰ (Ishikawa and Nakamura 1993), and radiolaria from sediment cores in the equatorial Pacific Ocean have a reported $\delta^{11}\text{B}$ value of +2.1‰ (Ishikawa and Nakamura 1993).

This chapter presents a method for the separation and purification of boron from biogenic silica, and the boron isotope ratio analysis by PTIMS. This method is adapted from techniques used for boron isotope analysis of silicate rocks and biogenic carbonate, and provides the first known $\delta^{11}\text{B}$ values for siliceous sponge spicules.
3.2 Methods

Boron isotope ratios were measured as Cs$_2$BO$_2^+$ by PTIMS using methods similar to those of Spivack and Edmond (1986), Xiao et al (1988) and Lemarchand et al (2002). Prior to PTIMS analysis, boron needs to be completely separated from the sample matrix in order to avoid the suppression of ionisation associated with residual matrix impurities (Hemming and Hanson 1994). In the case of siliceous sponges, the tissue that binds the spicules together (mesohyl) contributes significantly to the sample matrix and must be removed by a process of oxidation and ion exchange separation. Precision and accuracy of this procedure were monitored using two standard reference materials: NIST SRM 951 boric acid, and an in-house siliceous sponge standard (SP150) prepared as part of this study.

3.2.1 Reagents

All reagents were prepared using ultrapure water (Milli-Q$^\text{TM}$) and acids, and AR grade mannitol and H$_2$O$_2$. Procedural blanks were measured by inductively-coupled plasma mass spectrometry (ICP-MS) and were at levels around $10^{-9}$ g boron, which is insignificant given samples contained around $10^{-6}$ g boron. Also, SRM 951 was routinely processed with each batch of samples processed by column separation chemistry and analysed alongside direct-loaded (unprocessed) SRM 951 to monitor for isotopic fractionation and contamination. The consistency of measured $^{11}$B/$^{10}$B between processed and unprocessed standards (Table 3.2) indicates contamination was not significant.

3.2.2 Removal of organic material

Prior to analysis, the external organic tissue (mesohyl) was oxidised by cutting the sponge samples into small (~2 cm) pieces and immersing them in a 1:1 mix of 30% H$_2$O$_2$ and 1M HCl in a plastic (LDPE) beaker, which was heated on a hotplate at 50°C for 5 hours. The
spicules were then rinsed thoroughly with Milli-Q water and dried. This oxidation process removes the mesohyl tissue but does not remove the protein layers that occur within the silica layers of the spicule. This was evident after dissolving spicules in HF that had been subjected to oxidative cleaning by a brown organic residue that is suspected of interfering with PTIMS analysis (see below). Accordingly, the dried spicules are then milled to a coarse powder in a tungsten carbide mill, and the powder is heated in an alumina crucible at 800°C overnight. The loss of organic matter and water during heating alters the crystalline structure of the amorphous biogenic silica, and carries the risk of losing boron by volatilisation, which would cause isotopic fractionation (Ishikawa and Nakamura 1990). To assess whether heating was causing boron loss, spicules were heated overnight at a range of temperatures (300, 400 and 500°C) and the boron concentrations were measured. Whole spicules were heated in alumina crucibles overnight, and their boron concentrations were measured by laser ablation ICP-MS. Concentrations were measured in situ, rather than in solution, remove uncertainties associated with dissolution and evaporation. No significant difference was found between the different temperature treatments. This suggests boron is structurally-bound within the silica rather than occurring interstitially or adsorbed onto the spicule surface.

Methods for boron isotope analysis reported in the literature have required heating biogenic silica (diatoms) to 500°C, however, in the case of sponge spicules the extent of removal of organics might not be complete at this temperature. Attempts at analysing aliquots of the in-house sponge standard (SP150) heated to 500°C frequently failed because the ion beam was suppressed, which may be caused by the presence of organic matter (Gaillardet et al 2001). It was found that heating the sample powder to 800°C overnight could be consistently analysed by PTIMS. The measured δ$^{11}$B values showed no significant difference between the two temperature treatments ($p = 0.2136$) and so
subsequently all samples were oxidised at 800°C to ensure as complete removal of organic matter as possible.

3.2.3  *Boron separation from silica*

3.2.3.1  *Evaporation of silica as SiF₆*

After the complete oxidation of organic material, biogenic silica, which constitutes approximately 97% of the sponge spicule, is extracted in two steps: first, most is evaporated as SiF₆ after dissolving the milled samples in concentrated HF, and second, the remaining silica and other matrix components are removed by ion exchange separation.

The ANU sponge standard SP150 was prepared from a large siliceous sponge (Class Demospongiae, Craniella leptoderma) that was collected from approximately 450 m depth on the continental shelf of Antarctica. The powdered sample was oxidised in an oven overnight at 70°C, and aliquots of the oxidised powder weighed into a Teflon beaker containing 200 µL of 0.1% mannitol, after which 300 µL of concentrated HF is added to the sample. The beaker was tightly capped, and the sample dissolved by placing the beaker in an ultrasonic bath for 5 minutes and then onto a hotplate at 50°C for 30 minutes. Once completely dissolved, the beakers are left uncapped on a hotplate, under heat lamps, at between 50 and 60°C to facilitate silica evaporation as SiF₆. This and subsequent evaporation steps are performed in a laminar flow hood to eliminate the potential for airborne contamination.

The potential for boron isotope fractionation during both the digestion and evaporation steps is considerable, as boron is volatile in acidic solutions (Feldman 1960; Xiao *et al* 1997; Ishikawa and Nakamura 1990). Evaporation at temperatures below 70°C in the presence of mannitol has been demonstrated to produce no fractionation effect on boron
isotope compositions (Ishikawa and Nakamura 1990). In contrast, fractionation has been shown to occur when samples are left to continue to be heated after reaching complete dryness, which has been attributed to the sublimation of boron (Leeman et al 1991; Xiao et al 1997). Accordingly, samples in this study were not left on the hotplate for longer than 30 minutes after reaching incipient dryness (Ishikawa and Nakamura 1990).

Following previously reported procedures to separate boron from various matrix components (Kiss 1988; Leeman et al 1991; Hemming and Hanson 1994; Lemarchand et al 2002), the boron-specific Amberlite IRA743 resin was initially employed. However, aliquots of SP150 and solutions containing silicon and boron processed this way were difficult to analyse due to poor ionisation of \( \text{Cs}_2\text{BO}_2^+ \), as a result of the matrix not being completely removed. Analysis of column elution steps by ICP-MS indicated silicon was eluted along with boron, likely because samples were loaded onto the IRA743 resin at pH 9-10, at which silica occurs in its anionic form \( \text{Si(OH)}_3^- \) and adsorbs onto the resin.

A method that permits samples to be loaded onto the resin at pH < 7 in 3 N HF was subsequently adapted from Nakamura et al (1992), and is summarised in Table 3.1. After the initial oxidation and HF evaporations steps, samples were redissolved in 1.2 mL of 3 N HF, then capped and heated for about 30 minutes to redissolve the sample. The samples were then loaded onto ion exchange columns containing 0.3 mL of AG 1-X4 100-200 mesh anion exchange resin. The column rinsing, loading and elution procedures closely followed those of Nakamura et al (1992). Columns were first washed with two 2 mL aliquots of 6 M HCl, then conditioned with 0.3 mL 0.03 M HF prior to samples being loaded in one 0.6 mL aliquot. Only half of the 1.2 mL sample solution is loaded onto each column, thus allowing two aliquots of each sample to be processed separately. The resin is washed with two 0.6 mL aliquots of a mixed solution of 0.5 M HF and 2 M HCl, before the sample is eluted with three 0.6 mL aliquots of 6 M HCl. The eluant is collected in Teflon beakers containing 0.1%
mannitol solution, the amount of mannitol being set to be in small excess and give a 1:2 boron-mannitol molar ratio (Lemarchand et al 2002).

Table 3.1. Ion exchange separation procedure, adapted from Nakamura et al (1992). Ion exchange columns contain 0.3 mL AG 1-X4 100-200 mesh resin.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Solution</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash</td>
<td>6 M HCl</td>
<td>2 x 2 mL</td>
</tr>
<tr>
<td>Condition</td>
<td>0.03M HF</td>
<td>1 x 0.3 mL</td>
</tr>
<tr>
<td>Load sample</td>
<td>Dissolved in 3M HF</td>
<td>1 x 0.6 mL</td>
</tr>
<tr>
<td>Wash</td>
<td>0.5M HF + 2M HCl</td>
<td>1 x 0.25 mL</td>
</tr>
<tr>
<td></td>
<td>mixture mixture</td>
<td>2 x 0.6 mL</td>
</tr>
<tr>
<td>Elute</td>
<td>6M HCl</td>
<td>3 x 0.6mL</td>
</tr>
</tbody>
</table>

The eluant is then evaporated on a hotplate under a heatlamp at 50-60°C, but before the solution is completely dry (i.e. a small drop remaining), concentrated (~2600 ppm) CsCl solution is added in an amount that gives a 1:2 B/Cs molar ratio, and the sample is left to continue evaporating. The addition of CsCl is delayed to allow as much fluorine to evaporate before forming Cs$_2$F$^+$, which is subsequently evident as very high (> 1V) signals at mass-to-charge ratio ($m/z$) = 285. Previously published PTIMS methods have added caesium to produce different B/Cs molar ratios, for example, 2:1 (Nakano and Nakamura 1998), 1:2.5 (Tonarini et al 1997) and 3:4 (Lemarchand et al 2002). Given the formation of other caesium compounds, namely Cs$_2$F$^+$ and Cs$_2$Cl$^+$, which are observed during PTIMS analysis, caesium was added in excess (B/Cs = 1:2). Despite concern that excess caesium might affect sample ionisation, repeat measurements of standards with B/Cs at different ratios (1:1, 1:2, 1:4) did not produce significant differences in measured $^{11}$B/$^{10}$B ratio values ($p = 0.6559$) nor in the measured signal intensities.

3.2.3.2 Boron recovery during ion exchange separation

Complete recovery of boron from the ion exchange resin is crucial to ensuring no fractionation occurs during boron purification. To achieve this, aliquots of boric acid (NIST
SRM 951) were mixed with silicic acid to produce synthetic solution equivalents to 10 mg samples of SiO₂ that were then processed through the columns to monitor passage of boron and silica. The loading, washing and eluant solutions were collected separately and boron and silicon concentrations were measured by ICP-MS, using beryllium to monitor instrument drift. The results are shown in Figure 3.1. No boron above detection limits was measured in the loading and washing steps, and the average elution yield of the 2 μg of boron loaded was 102.9%. Approximately 98% of the silicic acid was detected and accounted for in the loading and washing steps, with only trace amounts detected in the elution steps. Furthermore, the mean δ¹¹B values of aliquots of SRM 951 boric acid processed through the full ion exchange and evaporation procedure indicate boron isotopes are not fractionated by the column separation procedure (Table 3.2).

![Figure 3.1. Elution curves for the ion exchange separation procedure.](image)
Table 3.2. $^{11}$B/$^{10}$B ratio values of NIST SRM 951 direct-loaded and processed, and sponge standard SP150.

<table>
<thead>
<tr>
<th></th>
<th>NIST SRM 951 direct-loaded</th>
<th>NIST SRM 951 processed</th>
<th>SP150 (sponge standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}$B/$^{10}$B</td>
<td>$2\sigma_{\text{mean}}$</td>
<td>$2\sigma_{\text{mean}}$</td>
<td>$2\sigma_{\text{mean}}$</td>
</tr>
<tr>
<td>4.0512</td>
<td>± 0.0005</td>
<td>4.0530 ± 0.0002</td>
<td>4.1507 ± 0.0010</td>
</tr>
<tr>
<td>4.0521</td>
<td>± 0.0003</td>
<td>4.0503 ± 0.0006</td>
<td>4.1496 ± 0.0005</td>
</tr>
<tr>
<td>4.0530</td>
<td>± 0.0002</td>
<td>4.0495 ± 0.0006</td>
<td>4.1560 ± 0.0003</td>
</tr>
<tr>
<td>4.0495</td>
<td>± 0.0006</td>
<td>4.0572 ± 0.0007</td>
<td>4.1576 ± 0.0003</td>
</tr>
<tr>
<td>4.0512</td>
<td>± 0.0005</td>
<td>4.0498 ± 0.0007</td>
<td>4.1578 ± 0.0003</td>
</tr>
<tr>
<td>4.0503</td>
<td>± 0.0006</td>
<td>4.0478 ± 0.0004</td>
<td>4.1566 ± 0.0007</td>
</tr>
<tr>
<td>4.0516</td>
<td>± 0.0005</td>
<td>4.0577 ± 0.0005</td>
<td>4.1538 ± 0.0004</td>
</tr>
<tr>
<td>4.0515</td>
<td>± 0.0009</td>
<td>4.0559 ± 0.0007</td>
<td>4.1550 ± 0.0007</td>
</tr>
<tr>
<td>4.0530</td>
<td>± 0.0003</td>
<td>4.0463 ± 0.0004</td>
<td>4.1511 ± 0.0004</td>
</tr>
<tr>
<td>4.0520</td>
<td>± 0.0003</td>
<td>4.0591 ± 0.0003</td>
<td>4.1457 ± 0.0004</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>4.0516 ± 0.0005</strong></td>
<td>4.0570 ± 0.0003</td>
<td>4.1554 ± 0.0015</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>4.0556 ± 0.0005</td>
<td>4.1582 ± 0.0002</td>
</tr>
<tr>
<td>$2\sigma$</td>
<td>0.0022</td>
<td>4.0504 ± 0.0005</td>
<td>4.1619 ± 0.0002</td>
</tr>
<tr>
<td>$2\sigma_{\text{relative (%)}}$</td>
<td>0.054</td>
<td>4.0513 ± 0.0009</td>
<td>4.1539 ± 0.0002</td>
</tr>
<tr>
<td>$2\sigma_{\text{mean}}$</td>
<td>0.0007</td>
<td>4.0501 ± 0.0010</td>
<td>4.1538 ± 0.0002</td>
</tr>
<tr>
<td>$2\sigma_{\text{mean, relative (%)}}$</td>
<td>0.018</td>
<td>4.0580 ± 0.0004</td>
<td>4.1480 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0473 ± 0.0004</td>
<td>4.1453 ± 0.0008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0482 ± 0.0003</td>
<td>4.1499 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0527 ± 0.0005</td>
<td>4.1533 ± 0.0005</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>4.0569 ± 0.0003</td>
<td>2.189</td>
</tr>
<tr>
<td>$2\sigma$</td>
<td>0.0091</td>
<td>4.0506 ± 0.0003</td>
<td>0.0022</td>
</tr>
<tr>
<td>$2\sigma_{\text{relative (%)}}$</td>
<td>0.20</td>
<td>4.0517 ± 0.0003</td>
<td>0.531</td>
</tr>
<tr>
<td>$2\sigma_{\text{mean}}$</td>
<td>0.0017</td>
<td>4.0546 ± 0.0003</td>
<td>0.04</td>
</tr>
<tr>
<td>$2\sigma_{\text{mean, relative (%)}}$</td>
<td>0.04</td>
<td>4.0472 ± 0.0003</td>
<td></td>
</tr>
</tbody>
</table>
3.2.4 Mass spectrometry

Boron isotope ratios were measured using a Finnigan TRITON thermal ionisation mass spectrometer (TIMS) at the Research School of Earth Sciences, ANU, following the Cs$_2$BO$_2^+$ PTIMS method described previously by Ramakumar et al (1985), Spivack and Edmond (1986) Leeman et al (1991) and Lemarchand et al (2002). Samples were measured using outgassed single rhenium filaments, which were first coated with 1 μL of a graphite-ethanol suspension and dried under a ceramic heat lamp, in order to enhance the ionisation of Cs$_2$BO$_2^+$ (Xiao et al 1988). The dried eluted sample was redissolved in 2 μL of 0.1 M HCl, and a 1 μL aliquot (equivalent to 1 μg B) of this was loaded on top of the graphite coating and dried.

Boron isotope ratios were measured simultaneously as Cs$_2^{10}$BO$_2^+$ and Cs$_2^{11}$BO$_2^+$, $m/z$ = 308 and 309 respectively, using a double Faraday cup collector set to the H3 and H4 positions. Measuring the two mass signals statically, rather than by peak jumping (e.g. Nakamura et al 1992, Lemarchand et al 2002) eliminates any small biases that arise with signal decay and instability (Nakano and Nakamura 1998, Deyhle 2001).

For each analysis, the filament was heated slowly by raising the current at a rate of 50 mA min$^{-1}$ to 800 mA, at which point the mass 309 signal is typically less than 5 mV. The current is then increased in 25 mA increments at 100 mA min$^{-1}$ until the signal reaches 10 mV, at which point the beam is focussed. The filament is then heated further at 25 mA min$^{-1}$ until the $m/z$ 309 signal is greater than 100 mA, usually achieved between 200 and 400 mA, and data collection is commenced. Data is typically collected in one or two blocks comprising 200 three-second measurement cycles. Measured boron isotope ratios are expressed in delta notation, as per mil values relative to the average SRM 951 value, $\delta^{11}$B (see Chapter 1, equation 1.1). Measured $^{11}$B/$^{10}$B ratios were corrected for the contribution of Cs$_2^{18}$B$^{17}$O$^{16}$O$^+$ at mass 309 by subtracting 0.00078 from each 309/308 cycle value (Spivack and Edmond 1987).
3.3 Results: precision and accuracy

The reproducibility of the boron separation and analysis procedure was assessed by repeated processing and measurement of two standard materials, NIST SRM 951 boric acid and the in-house siliceous sponge standard (SP150). The mean values and error are presented in Table 3.2. The mean value of the unprocessed (direct loaded) SRM 951 is $4.0516 \pm 0.0007$ (2se, $n = 10$). Although this value is higher than the certified value of $4.0437 \pm 0.0014$ (Catanzaro et al 1970), it is consistent with other results reported in the literature by Lemarchand et al (2002b) ($4.05174 \pm 0.00032$), Nakano and Nakamura (1998) ($4.0512 \pm 0.0002$) and Deyhle (2001) ($4.0522 \pm 0.0003$) (see Table 3.3). The processed SRM 951 gave a slightly higher mean value of $4.0527 \pm 0.0017$ (2se, $n = 24$). The mean $^{11}\text{B}/^{10}\text{B}$ ratio obtained for the sponge standard SP150 was $4.1533 \pm 0.0022$ (2se, $n = 18$).
Table 3.3. Summary of previous repeated $^{11}$B/$^{10}$B values for NIST SRM 951.

<table>
<thead>
<tr>
<th>Analytical method</th>
<th>Direct-loaded or processed</th>
<th>$^{11}$B/$^{10}$B</th>
<th>$2\sigma$</th>
<th>$2\sigma_{\text{mean}}$</th>
<th>n</th>
<th>sample size ($\mu$g B)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTIMS static</td>
<td>Direct</td>
<td>4.0516 ± 0.0022</td>
<td>0.0007</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>PTIMS</td>
<td>Direct</td>
<td>4.0512 ± 0.0014</td>
<td>0.0004</td>
<td>15</td>
<td>0.5-1.0</td>
<td>(a)</td>
<td></td>
</tr>
<tr>
<td>PTIMS</td>
<td>Direct</td>
<td>4.0529 ± 0.0002</td>
<td>-</td>
<td>22</td>
<td>0.25</td>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>PTIMS static</td>
<td>Direct</td>
<td>4.0528 ± 0.0018</td>
<td>0.0004</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>(c)</td>
</tr>
<tr>
<td>PTIMS static</td>
<td>Direct</td>
<td>4.0528 ± 0.0026</td>
<td>0.0010</td>
<td>7</td>
<td>0.1</td>
<td>(c)</td>
<td></td>
</tr>
<tr>
<td>PTIMS static</td>
<td>Direct</td>
<td>4.0549 ± 0.0037</td>
<td>0.0018</td>
<td>4</td>
<td>0.05</td>
<td>(c)</td>
<td></td>
</tr>
<tr>
<td>PTIMS</td>
<td>Direct</td>
<td>4.0545 ± 0.0008</td>
<td>0.0002</td>
<td>11</td>
<td>0.2-0.6</td>
<td>(e)</td>
<td></td>
</tr>
<tr>
<td>PTIMS static</td>
<td>Direct</td>
<td>4.0530 ± 0.0008</td>
<td>0.0003</td>
<td>7</td>
<td>1</td>
<td>(f)</td>
<td></td>
</tr>
<tr>
<td>PTIMS</td>
<td>Processed</td>
<td>4.0527 ± 0.0082</td>
<td>0.0017</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>PTIMS</td>
<td>Processed</td>
<td>4.0547 ± 0.0012</td>
<td>0.0003</td>
<td>14</td>
<td>0.25</td>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>PTIMS</td>
<td>Processed</td>
<td>4.0474 ± 0.0016</td>
<td>0.0003</td>
<td>26</td>
<td>50</td>
<td>(d)</td>
<td></td>
</tr>
<tr>
<td>PTIMS</td>
<td>Processed</td>
<td>4.0437 ± 0.0017</td>
<td>0.0004</td>
<td>19</td>
<td>0.2-0.6</td>
<td>(e)</td>
<td></td>
</tr>
<tr>
<td>TE-NTIMS</td>
<td>Processed</td>
<td>4.0396 ± 0.0028</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td>NTIMS</td>
<td>Processed</td>
<td>4.0014 ± 0.0002</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td>MC-ICP-MS</td>
<td>Processed</td>
<td>4.0435 ± 0.0006</td>
<td>-</td>
<td>7</td>
<td>0.030</td>
<td>(i)</td>
<td></td>
</tr>
</tbody>
</table>

References: (a) Nakamura et al 1992; (b) Lemarchand et al 2002; (c) Nakano and Nakamura et al 1998; (d) Leeman et al 1991; (e) Tonarini et al 1997; (f) Deyhle 2001; (g) Foster et al 2006; (h) Hemming and Hanson 1994; (i) Foster 2008.

* presented as ±2RSD (%)

3.4 Discussion

3.4.1 Accuracy

The accuracy of the full boron separation procedure and PTIMS analysis can only be determined using replicates of NIST SRM 951, as there is no biogenic silica reference material available for boron isotope analysis. Silicate rock standards were not considered appropriate for this use because they require extra processing steps to remove additional cations present in the matrix, and are therefore unlikely to reflect the true accuracy and reproducibility of the method.

The mean $^{11}$B/$^{10}$B ratio values of replicate analyses of both direct loaded and processed SRM 951 are presented in Table 3.3, alongside values from the literature for comparison. The mean $^{11}$B/$^{10}$B of the processed SRM 951 agrees well with reported mean ratios of SRM 951 measured by PTIMS (Table 3.2). The mean value of the direct loaded (unprocessed)
SRM 951 is lighter than that of the processed standards, but there is no significant difference between the mean values of the two treatments ($p = 0.23$).

### 3.4.2 Precision

The precision obtained using the PTIMS method developed here is poorer than that of similar methods reported in the literature (see Table 3.2). The reason for this remains unclear, but previous studies point to several potential sources of bias in boron isotope analysis. Variability in one or more of these sources of bias might contribute to the poor precision observed in this study.

#### 3.4.2.1 Residual organic matter

The presence of organic matter is thought to interfere with TIMS boron isotope analysis in two ways: by suppressing the ionisation of boron, and by producing isobaric interferences due to the formation of CNO compounds, namely $\text{Cs}_2\text{CNO}^+$ at mass 308 (Xiao et al 1988; Hemming and Hanson 1994, Wei et al 2004). Any organic matter affecting the reproducibility of the analyses reported herein most likely originates from the degradation of the ion exchange resin during separation chemistry rather than from the samples, as the poor precision affects both the processed SRM 951 replicates and the SP150 results. Even so, residual refractory organic matter could persist through the two applied oxidation steps (described above) as its breakdown is only assessed subjectively by visual examination. Previous attempts to oxidise organic matter after sample processing have generally been unsuccessful (e.g. Hemming and Hanson 1994; Lemarchand et al 2002b; Foster et al 2006) although separation by microsublimation rather than oxidation has been shown to separate boron effectively from organic matter (Gaillardet et al 2001; Lemarchand et al 2002b).
Wei et al (2004) have proposed interferences at both m/z 308 and 309 caused by $^{133}$Cs$_2^{12}$C$^{14}$N$^{16}$O$^+$ and $^{133}$Cs$_2^{12}$C$^{15}$N$^{16}$O$^+$ respectively. However, the high abundance of $^{14}$N relative to $^{15}$N means interferences caused by Cs$_2$CNO$^+$ ions should affect m/z 308 to a larger extent and bias results towards low 309/308 values and thus $^{11}$B/$^{10}$B ratio values. Comparison between analyses from this study, and those of other published studies using NTIMS or ICP-MS (Table 3.3), suggests there is no such bias in the data, as the $^{11}$B/$^{10}$B ratios in this study are generally heavier, not lighter, than other studies.

Poor precision has been linked to the suppression of boron ionisation by organic contamination (Foster et al 2006; Ni et al 2010). Foster et al (2006) observed that in standards mixed with a carbonate matrix, poorer reproducibility correlated with higher calcium concentrations, although there was no bias in isotopic fractionation. The poor precision was attributed to organic contamination causing reduced boron ionisation which might cause both isobaric interferences and changes in fractionation due to the increased time the filament is heated to burn off organic compounds (Ni et al 2010). The poorer reproducibility observed by Foster et al (2006) in high calcium standards ($\sim$3‰, 2σ) is similar to that observed for the processed standards in the present study, which might indicate similar fractionation effects take place with silica-based matrices. It is difficult to assess whether this is the case given organic compounds (at m/z 312 and 328) are not monitored during filament heating c.f. Wei et al (2004), Hemming and Hanson (1994), Foster et al (2006). Furthermore, both Foster et al (2006) and Ni et al (2010) measure isotope ratios using the total evaporation NTIMS method, whereby data collection is carried out from the moment the $^{11}$BO$_2^-$ beam intensity is >20 mV until boron ionisation is exhausted (until the $^{10}$BO$_2^-$ signal decays below 5 mV). This approach contrasts with traditional NTIMS and PTIMS boron isotope analysis protocols, including that used here, in which the measured current is increased until the ion beam is stable and above 100 mV. This traditional approach relies on organic compounds having evaporated
from the filament prior to data collection. Ni et al (2010) found that the ionisation of organics during the initial filament heating suppresses the ionisation of light isotopes, resulting in heavier boron isotope ratios. Different extents of organic matter ionisation within and between samples could be producing slight variations in the measured $^{11}$B/$^{10}$B ratio and causing the degraded precision observed in this study.

In summary, contamination by organic matter, whether originating from the samples or from the degradation of the ion exchange resin, could produce shifts towards either heavier or lighter boron isotope compositions. Isobaric interferences are likely to affect $m/z$ 308 more than 309, and bias results towards lower $^{11}$B/$^{10}$B ratios, whereas the suppression of ionisation is likely to result in heavier ratios (Ni et al 2010). These fractionation effects may produce the larger variability in measured values that is observed in this study for the processed standards (SRM 951 and SP150). The lack of systematic bias could be due to varying amounts of organic matter following processing of different sample aliquots.

3.4.2.2 Incomplete recovery during ion exchange separation

Incomplete recovery of boron from the ion exchange resin can produce large fractionations in boron isotopes. It has been demonstrated that during elution from the anion resin IRA 743, the heavier isotope is preferentially removed at the beginning of the elution curve, making the tail of the curve significantly lighter (Lemarchand et al 2002b). The elution curves of the column separation procedure were measured several times ($n = 5$) over a two year period, and routinely produced recoveries between 97 and 102%, with the bulk of the boron being removed from the resin in the first two elution steps (Figure 3.1). However, one elution curve was anomalous and showed a shift in the shape of the curve towards a later elution, with a broader, flatter peak and a significant amount ($\sim 7\%$) of boron being lost to the protracted tail of the curve. As well as incomplete recovery of
boron, this anomalous elution curve would likely produce a heavier $^{11}$B/$^{10}$B ratio. The reason for this later and extended elution curve is unclear. A second elution curve collected and measured in the same process batch indicates the shift in elution volume is not due to different reagent concentrations, contamination by other anions or ambient temperature, all of which can alter resin characteristics (Korkisch 1989). Rather, column-specific conditions may have affected this particular elution curve, such as air bubbles or heterogeneity in the resin bed, which can affect the diffusion rate of both the adsorbing ion and the eluting solution (Korkisch 1989). Such conditions, possibly caused by degradation of the resin over time or by disturbance of the resin during or prior to sample loading and processing, can alter not only the adsorption of boron onto the resin but also the efficiency of ion exchange with the eluting solutions.

It is possible that such variations in the elution curve could cause isotopic fractionation of the boron in the eluant. However, columns were always inspected for air bubbles or decreased height of the resin bed (indicating degradation of the resin) before use, and resin was replaced after ten uses of each column. The mean value of the processed standards agrees well with both unprocessed standards and with values reported in the literature. If incomplete boron recovery is affecting some samples during processing, it is not occurring frequently enough to cause an overall bias in the accuracy of the $^{11}$B/$^{10}$B value.

3.4.2.3 Airborne contamination

Contamination by airborne boron is another potential source of analytical error. Stevie et al (1991) found that airborne boron from borosilicate glass used in HEPA filters produced ambient aerosol boron concentrations of around 240 ng m$^{-3}$. The amount of boron adsorbed onto silicon wafers exposed to this air was demonstrated to increase with exposure time. Foster et al (2006) measure 10 to 40 pg boron blanks in samples loaded
onto filaments in a HEPA filtered environment, to which the authors attributed a -1.5‰ shift in $\delta^{11}$B.

Given that much of the sample processing in the present procedure takes place in HEPA filtered laminar flow hoods, and dissolved samples are exposed for extended periods of time (up to 12 hours) during column separation, evaporation and filament loading, it is possible that some airborne contamination occurs. However, a systematic shift towards lighter $^{11}$B/$^{10}$B is not observed. Moreover, the sample size used here (1 $\mu$g B) is more than a thousand times larger than that used by Foster et al (2006) (400 pg), such that the level of contamination to that reported by Foster et al (2006) would produce only a miniscule shift in the isotopic composition of the samples measured here (i.e. <0.004%). Reagent blanks were routinely found to have boron concentrations below 0.5 ppb, and procedural blanks were typically slightly higher, at around 1.0 ppb.

3.4.2.4 The effects of $\text{Cs}_2\text{F}^+$ on boron ionisation

Initial attempts at analysing $^{11}$B/$^{10}$B ratios in biogenic silica by PTIMS analysis proved largely unsuccessful because ion beams at $m/z = 309$ and 308 could not be raised above 100 mV before rapid signal decay precluded analysis. The cause of this behaviour was thought to be the preferential ionisation of $\text{Cs}_2\text{F}^+$, which produces a persistent ion beam at $m/z = 285$ in both processed samples and standards. The intensity of the $m/z = 285$ signal and the rate of its decay varied between samples, although it was consistently detected before the $m/z = 309$ signal, and decayed at lower currents. It was observed that whenever the $m/z = 285$ signal intensity was higher than at $m/z 309$, it was difficult to produce a strong and stable $m/z = 309$ beam, and the intensity of the latter was only able to be increased in small increments and then decayed before it could be measured. However, if the $m/z = 285$ signal was allowed to decay to an intensity lower than that of $m/z = 309$ early during the filament heating process (at a filament current of around 850
mA), the \( m/z = 309 \) signal was observed to increase at a more rapid rate during subsequent heating, allowing data collection to commence at a lower current. This reduced the amount of signal decay during analysis, and allowed for at least 200 measurement cycles (three seconds each) to be made. The likely reason for this behaviour is that the ionisation of \( \text{Cs}_2 \text{F}^+ \) suppresses the efficient ionisation of \( \text{Cs}_2 \text{BO}_2^+ \) during early filament heating. Although the competing \( m/z = 285 \) signal was linked to reduced intensity of boron ionisation, it is unclear whether this resulted in isotopic fractionation. If isotopic fractionation did occur, it might operate in a way similar to that during the evaporation of organic matter from the filament (as discussed above), that is, with \(^{11}\text{B} \) being preferentially ionised. To avoid any effects of \( \text{Cs}_2 \text{F}^+ \) ionisation on boron analysis, steps were taken to reduce the amount of \( \text{Cs}_2 \text{F}^+ \) formed during sample preparation and filament heating. During sample evaporation after ion exchange separation, mannitol was added to each sample, which was then dried down to a small drop before \( \text{CsCl} \) was added to form \( \text{Cs}_2 \text{BO}_2^+ \). This allowed as much fluoride to evaporate as possible and therefore reduce the amount of \( \text{Cs}_2 \text{F}^+ \) formed. Also, during PTIMS analysis, filament heating was paused at around 850 mA, when the \( m/z = 285 \) ion beam is usually around 40 to 100 mV, and the beam was allowed to decay to below 10 mV before heating was recommenced. This would usually take between 10 and 30 minutes, with the final signal intensity and rate of decay being quite variable between samples. These procedures permitted processed standards and samples to be measured consistently at \( m/z = 309 \) signal intensities of 200 to 300 mV.

3.5 Summary and conclusion

Boron can be successfully separated from its biogenic silica and organic matrix, and purified for PTIMS analysis, allowing for the boron analysis of siliceous sponges and diatoms. Analyses of SRM 951 that were processed through the entire procedure provide a mean \(^{11}\text{B}/^{10}\text{B} \) ratio of 4.0527 ± 0.0017 (2se), which agrees well with the mean standard values reported in the literature (Table 3.2). This value is also consistent with the mean
The external reproducibility of the procedure is relatively poor (±2.2‰, 2σ), compared to the generally high precision attributed to boron isotope analysis by PTIMS (Gonfiantini et al 2003). The reason(s) for the poor precision remains unclear, but is considered most likely due to varying extents of isotopic fractionation caused by the presence of residual organic matter in the sample when loaded onto the filament. Incomplete recovery during ion exchange separation, and the ionisation of Cs₂F⁺ during filament heating, are also possible causes of isotopic fractionation. That measured isotope ratios do not show any bias towards lighter or heavier isotope ratios suggests several factors may be contributing to the poor precision. Nevertheless, it is worth noting that the level of uncertainty obtained using this method is small relative to the large variability of sponge sample δ¹¹B (see Chapter 4), and therefore does not compromise any of the interpretations based on the boron isotope ratios measured in this study.
Chapter 4

The boron isotope composition of marine siliceous sponges

4.1 Introduction

Siliceous sponges (Phylum Porifera, Classes Hexactinella and Demospongiae) have been demonstrated to be proxy recorders of seawater silica (Si(OH)₄) concentrations and utilisation, using the silicon isotope ($^{30}\text{Si}$) and germanium/silicon (Ge/Si) ratios of siliceous spicules (Ellwood et al 2006; Wille et al 2010; Hendry et al 2010). Sponges are sessile, benthic organisms that are widely occurring throughout the ocean. Their ubiquity makes spicules preserved in deep sea sediment useful archives of deep water silica concentrations over time. As such, sponge spicule records complement similar silica concentration and utilisation reconstructions of surface waters using diatom frustule $^{30}\text{Si}$ and Ge/Si records (e.g. Froelich et al 1989; De La Rocha et al 1997; Varela et al 2004; Egan et al 2012).

This chapter seeks to investigate the relationship between seawater pH and the boron isotope geochemistry of siliceous sponges. The boron concentration of sponge spicules has previously been shown to be very high – on the order of hundreds of parts per million (ppm) – compared to marine carbonates and clays (Furst 1981; see Chapter 2, section 2.3). The boron concentrations of spicules extracted from a sediment core from the North Atlantic by Furst (1981) further revealed a marked difference in average boron concentrations of siliceous sponges between the higher concentrations in the last glacial period and the lower concentrations in the Holocene. Herein, I will test a working hypothesis that this difference might reflect a change in boron speciation, specifically
increased borate relative to boric acid, due to higher seawater pH during glacial conditions in this region of the deep North Atlantic. This hypothesis is founded on the possible preferential substitution of the tetrahedral borate (B(OH)$_4^-$) species for SiO$_4^{2-}$ in the silica lattice or adsorption onto the growing surfaces of a sponge spicule. The speciation of boron in seawater is closely linked to seawater pH, with B(OH)$_4^-$ concentration increasing with pH (see Figure 1.2). The dissociation constant of boric acid (pK$_b^+$) is 8.60 at $T$ = 25°C and $S$ = 35 psu (DOE 1994).

The boron isotope fractionation between dissolved borate and boric acid is near-constant and well-constrained, meaning the boron isotope composition of borate and boric acid can be calculated for any given seawater pH (see Figure 1.2). The boron isotope composition of marine carbonates (namely foraminifera and corals) has been employed on a similar basis as a proxy for seawater pH, and is used to reconstruct past seawater pH and pCO$_2$ (Hemming and Hanson 1992; Sanyal et al 1996; Hönisch et al 2003; Hönisch et al 2004).

Understanding the processes by which boron is incorporated into spicules is important to interpreting the boron isotope composition of siliceous sponge spicules from ambient seawater, and any isotopic fractionation that may occur during boron uptake and incorporation. It is unknown whether boron has a specific biochemical role in siliceous sponges or whether is actively taken up and/or regulated by sponges. Boron has been demonstrated to be an important nutrient in plants (e.g. Blevins and Lukaszewsk 1998), animals (Hunt 2003, Park et al 2004), bacteria and algae (Lewin 1966; Lewin and Chen 1976), and while the role of boron in animals and algae (including diatoms) is still poorly understood, boron transporters have been identified in both animals and plants that might provide some insight into boron uptake by sponges.
Whether the boron content and isotope composition of siliceous sponge spicules reflects ambient seawater pH as is the case with biogenic carbonates has not previously been addressed in the literature. This study examines whether a relationship exists between the boron isotope composition of siliceous sponges and seawater pH, with the view to testing the hypothesis that $\text{B(OH)}_4^-$ concentration may be preferentially incorporated into spicules from seawater, and understanding the mechanisms that might influence boron isotope fractionation between sponges and seawater. This is done using modern, live-collected siliceous sponges from the Southern Ocean, the compositions of which are compared with hydrographic parameters measured at the sample sites. This analysis leads to the suggestion of possible mechanisms that would account for the observed boron isotope fractionation between siliceous sponges and seawater.

4.2 Materials and Methods

4.2.1 Sampling

Siliceous sponges were collected by beam trawl from George V Land, Antarctica (144°W, 66°S) and near Tasmania, Australia (147°W, 44°S). Samples were frozen immediately after collection, and the Antarctic samples were subsequently dried at low temperature (40°C) for storage.

Sponge samples were selected for analysis from a range of depths in order to sample a large range of seawater pH and nutrient concentrations. In preparation for analysis, each sample was first submerged in a 1:1 mix of $\text{H}_2\text{O}_2$ (reagent grade, Sigma) and 1 M HCl (reagent grade, Sigma) and heated on a hotplate at 50°C for 5-12 hours to remove all external organic material. The spicules were then rinsed several times in deionised water (MQ, Millipore) and left overnight to dry. Dried spicules were milled to a coarse powder in
a tungsten carbide ring mill and heated in alumina crucibles at 550°C for 16 hours to oxidise the remaining organic material.

4.2.2 **Boron isotope analysis**

Boron isotope ratios were measured by positive ion thermal ionisation mass spectrometry (PTIMS) following the Cs$_2$BO$_2^+$ method (Spivack and Edmond 1986; Lemarchand and et al 2002b). The sample preparation and analytical procedure is described in detail in Chapter 3, and is therefore only briefly outlined here. The milled sample powder is first dissolved in concentrated HF (AR grade, quartz distilled) and most of the silica is removed by evaporation as SiF$_6$ in the presence of mannitol, which is added to prevent the volatilisation of boron (Ishikawa and Nakamura 1990; Xiao et al 1997). The remaining matrix components are removed by ion exchange separation, using anion exchange resin Dowex 1-X4, 100-200 mesh, following the column separation procedure of Nakamura et al (1992). After ion exchange separation, CsCl solution is added to the purified boron sample to form Cs$_2$BO$_2^+$, and samples are loaded onto single rhenium filaments. Boron isotope ratios are measured statically using a Finnigan TRITON thermal ionisation mass spectrometer as m/z = 309 and 308, Cs$_2^{11}$BO$_2^+$ and Cs$_2^{10}$BO$_2^+$ respectively (Nakano and Nakamura 1998; Deyhle 2001). Isotope ratios are expressed using δ notation relative to NIST boric acid standard SRM 951. All measured 309/308 ratios are corrected for oxygen isotopes by subtracting 0.00078 (Spivack and Edmond 1986).

4.3 **Results**

The measured boron isotope values (δ$^{11}$B) of modern siliceous sponges from the Southern Ocean (Antarctica and Tasmania) are presented in Table 4.1. Seawater silicic acid concentration data ([Si]$_{sw}$) presented here are the average of data collected from nearby
sites over multiple sampling voyages along the WOCE SR-3 transect, including seawater sampled at the Antarctic trawl sites.

The sponge $\delta^{11} \text{B}$ compositions span a very large range, from +2.4 to +24.5‰ (±2.2‰, 2σ). The Tasmanian sponges record lower values than those from Antarctica, with the lightest values in the Tasmanian samples being similar to the values for diatomaceous ooze (+4.5‰) and radiolaria (+2.1‰) reported by Ishikawa and Nakamura (1993). The highest $\delta^{11} \text{B}$ value, +24.5‰, is similar to the value of $\text{B(OH)}_4^-$ in seawater, and the compositions that are recorded in marine carbonates.
Table 4.1. Sponge $\delta^{11}$B results, sample localities, taxonomic classification and seawater silica concentration.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>depth (m)</th>
<th>Longitude (°W)</th>
<th>Latitude (°S)</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>$\delta^{11}$B (%)</th>
<th>± 2σd (%)</th>
<th>$[\text{Si}]_{sw}$ (umol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>326-328</td>
<td>100</td>
<td>146.270</td>
<td>43.723</td>
<td>Demospongiae</td>
<td>Dictyoceratida</td>
<td>Dysideidae</td>
<td>7.1</td>
<td>0.9</td>
<td>2.0</td>
</tr>
<tr>
<td>326-393</td>
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<td>146.311</td>
<td>43.723</td>
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<td>Astrophorida</td>
<td>Ancorinidae</td>
<td>5.8</td>
<td>1.7</td>
<td>2.6</td>
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<td>326-092</td>
<td>200</td>
<td>146.967</td>
<td>43.691</td>
<td>Demospongiae</td>
<td>Hadromerida</td>
<td>Suberitidae</td>
<td>6.2</td>
<td>0.9</td>
<td>2.5</td>
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<td>326-250</td>
<td>200</td>
<td>146.329</td>
<td>44.000</td>
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<td>Poecilosclerida</td>
<td>Podospongiida</td>
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<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
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<td>44.000</td>
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<td>Hadromerida</td>
<td>Suberitidae</td>
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<td>0.6</td>
<td>2.6</td>
</tr>
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<td>326-465</td>
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<td>Halichondridae</td>
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<td>44.253</td>
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<td>Amphidiscosida</td>
<td>-</td>
<td>15.1</td>
<td>0.9</td>
<td>57.1</td>
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<td>326-179</td>
<td>1100</td>
<td>147.179</td>
<td>44.326</td>
<td>Hexactinellida</td>
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<td>Euplectellida</td>
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</tr>
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<td>144.686</td>
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<td>Demospongiae</td>
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<td>Hexactinellida</td>
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<tr>
<td>SP092</td>
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<td>144.686</td>
<td>66.939</td>
<td>Demospongiae</td>
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<td>-</td>
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<td>Tetillidae</td>
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<td>0.8</td>
<td>83.8</td>
</tr>
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<td>Demospongiae</td>
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<td>-</td>
<td>14.1</td>
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<td>66.324</td>
<td>Demospongiae</td>
<td>Poecilosclerida</td>
<td>Isodictyida</td>
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<td>144.309</td>
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<td>Spirophorida</td>
<td>Tetillidae</td>
<td>24.5</td>
<td>0.5</td>
<td>87.3</td>
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<tr>
<td>SP151</td>
<td>457</td>
<td>144.309</td>
<td>66.321</td>
<td>Demospongiae</td>
<td>Spirophorida</td>
<td>Tetillidae</td>
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<td>87.3</td>
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<td>540</td>
<td>145.535</td>
<td>66.750</td>
<td>Demospongiae</td>
<td>-</td>
<td>-</td>
<td>17.8</td>
<td>0.9</td>
<td>87.0</td>
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<td>SP019</td>
<td>702</td>
<td>143.357</td>
<td>66.333</td>
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<td>Spirophorida</td>
<td>Tetillidae</td>
<td>22.3</td>
<td>1.2</td>
<td>87.0</td>
</tr>
<tr>
<td>SP018</td>
<td>702</td>
<td>142.959</td>
<td>66.550</td>
<td>Demospongiae</td>
<td>-</td>
<td>-</td>
<td>13.5</td>
<td>3.1</td>
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<td>66.550</td>
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<td>-</td>
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<td>1.2</td>
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<td>67.047</td>
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<td>Tetillidae</td>
<td>23.6</td>
<td>1.3</td>
<td>98.5</td>
</tr>
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</table>

Dash (-) denotes samples that were not taxonomically classified below Class. 2σd (%) represents the analytical error of the PTIMS analysis, n=200.
$\delta^{11}\text{B}$ in sponges does not correlate with ambient seawater pH ($r^2 = 0.01$, $p = 0.96$) (Figure 4.1), unlike marine carbonates. However, there is a significant correlation between $\delta^{11}\text{B}$ and seawater silicic acid concentration ($r^2 = 0.71$, $p < 0.00001$), with heavier isotope values occurring in sponges from higher [$\text{Si}$_sw] waters (Figure 4.2a). This relationship is also clear in the Tasmanian samples, which span a large silica concentration gradient, from 2 to 57 µM.

![Figure 4.1. Boron isotope values ($\delta^{11}\text{B}$) of sponges plotted against seawater pH (total scale). Circles (○) represent Tasmanian samples, triangles (▲) represent Antarctic samples. Error bars represent 2sd. $r^2=0.01$, $p = 0.96$.](image)
The correlation between sponge δ¹¹B and other environmental parameters is provided in Table 4.2, which shows the poor correlation with depth, salinity and phosphate concentration. However, the correlation with between δ¹¹B and temperature is significant (R = 0.84, p<0.00001) (Figure 4.2(b)), and discussed further below (section 4.1.1).

Table 4.2 Correlation matrix of δ¹¹B with seawater depth (m), temperature (°C), salinity, pH, and silica and phosphate (PO₄³⁻) concentration. Seawater data from CTD measurements taken along the WOCE SR3 transect by Rosenberg (1999).

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>Temp</th>
<th>Salinity</th>
<th>pH</th>
<th>[Si]sw</th>
<th>[PO₄³⁻]sw</th>
<th>δ¹¹B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>-0.31</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity</td>
<td>-0.58</td>
<td>0.68</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.64</td>
<td>0.15</td>
<td>0.69</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Si]sw</td>
<td>0.28</td>
<td>-0.98</td>
<td>-0.57</td>
<td>-0.08</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PO₄³⁻]sw</td>
<td>-0.54</td>
<td>0.03</td>
<td>0.11</td>
<td>0.07</td>
<td>-0.06</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>δ¹¹B</td>
<td>0.28</td>
<td>-0.84</td>
<td>-0.54</td>
<td>-0.10</td>
<td>0.84</td>
<td>-0.14</td>
<td>1</td>
</tr>
</tbody>
</table>

Large variation in boron isotope composition between sponges of different family groups occurs in the samples from the same sites. At two sites, samples were collected in a single benthic trawl sample and therefore from the same environmental conditions and seawater pH, temperature and silicate compositions (Table 4.3; see also Figure 4.2). The sponges...
sampled from this site represent six different species, including five demosponges and one hexactinellid. The range in $\delta^{11}$B recorded in these sponges exceeds 10‰, which spans the total variability observed at a given [Si]$_{sw}$ of the whole sample set more broadly.

Table 4.3 Sponge $\delta^{11}$B for samples from the same site localities.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>$\delta^{11}$B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>144.68°W, 66.94°S, 348 m</td>
<td>Demospongiae</td>
<td>Hadromerida</td>
<td>Suberitidae</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Demospongiae</td>
<td>Poecilosclerida</td>
<td>Tedaniidae</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>Demospongiae</td>
<td>Spirophorida</td>
<td>Tetillidae</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td>Hexactinellida</td>
<td>Lyssacinosida</td>
<td>Rossellidae</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>Demospongiae</td>
<td>-</td>
<td>-</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>Demospongiae</td>
<td>-</td>
<td>-</td>
<td>14.1</td>
</tr>
<tr>
<td>146.32°W, 44.00°S, 200 m</td>
<td>Demospongiae</td>
<td>Poecilosclerida</td>
<td>Podospongiida</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Demospongiae</td>
<td>Hadromerida</td>
<td>Suberitidae</td>
<td>7.2</td>
</tr>
</tbody>
</table>

4.4 Discussion

The correlation between $\delta^{11}$B and [Si]$_{sw}$, and the lack of correlation with seawater pH, points to a mechanism for boron uptake and incorporation in sponges that differs significantly from that in marine carbonates. The following discussion addresses the factors that might influence boron isotope fractionation, and proposes a simple model for boron incorporation into spicules that might account for the observed range in $\delta^{11}$B values and their relationship with dissolved silica concentrations. This model draws upon existing knowledge of the sponge silification process, which unfortunately remains limited and requires a number of assumptions to be made regarding how boron uptake might be linked to silification. Nonetheless, despite the limitations and assumptions of the proposed model, it provides a framework for interpreting the boron isotope composition of sponge spicules that accounts for the different cellular processes that might be involved.
4.4.1 Environmental factors affecting $\delta^{11}B$

As pointed out in the previous section, while there is a significant correlation between $\delta^{11}B$ and $[\text{Si}]_{sw}$, there is also significant variability of $\delta^{11}B$ values among sponges sampled from sites with the same dissolved silica concentrations, particularly for locations with high silica waters. This indicates that factors additional to $[\text{Si}]_{sw}$ influence boron isotope fractionation in siliceous sponges. A number of other environmental parameters display poor correlations with $\delta^{11}B$ (Table 4.2), and so can be discounted as having a significant influence over boron isotope fractionation. The correlation with temperature, however, is significant ($r = -0.84$, $p < 0.00001$), with fractionation decreasing as temperature increases. This is similar to the correlation with $[\text{Si}]_{sw}$ ($r=0.84$, $p<0.00001$), and it is likely this simply reflects the co-variation of seawater temperature and silica concentration, rather than a causal link between temperature and boron isotope fractionation. It is unlikely the wide range in sponge $\delta^{11}B$ can be attributed to temperature variations of the scale experienced by these sponge samples. Increased temperature results in decreased $pK_B$ (the dissociation constant of B(OH)$_3$) (Manov et al. 1944), which affects boron speciation and would therefore affect the $\delta^{11}B$ of B(OH)$_3$ and B(OH)$_4^-$ (see Chapter 1, Section 1.2.1.1). Assuming the preferential uptake of B(OH)$_4^-$ into the spicule, the $\delta^{11}B$ of the spicule would increase with increasing temperature. However, even a $\sim 10^\circ C$ rise in temperature would only produce an increase in $\delta^{11}B$ on the order of 1‰ at pH 8.2, meaning $pK_B$ variations are unlikely to account for the correlation between temperature and sponge $\delta^{11}B$, which range from around +2 to +25‰ over a temperature range of around 2 to 12°C. Similarly, temperature also affects the isotope fractionation factor ($\alpha$), with the value of $\alpha$ decreasing with increasing temperature (Kakihana et al. 1977; Zeebe and Wolf-Gladrow 2001). That is, isotope fractionation between B(OH)$_3$ and B(OH)$_4^-$ increases with increasing temperature, which would manifest as increased $\delta^{11}B$ of the boron pool in the sclerocyte. Again, the increase in $\delta^{11}B$ is only of the order of 1‰ or less.
for a rise in temperature of 10°C, which is small relative to the observed variations in sponge δ¹¹B. Therefore, although temperature likely affects boron isotope fractionation by sponges, it is unlikely that temperature variations account for the wide range in observed sponge δ¹¹B. Similarly, the correlation with salinity (r=-0.54, p = 0.01) is expected given that salinity co-varies with silica concentration and temperature, but the large range in sponge δ¹¹B values is unlikely to be attributable to the relatively small variations in salinity.

4.4.2 Species-specific fractionation

The large differences in δ¹¹B values of the different sponge species collected from the same location (Table 4.3) indicate boron isotope fractionation may vary between sponge taxa. Considering that these sponges were growing under identical conditions, these observations rule out environmental parameters such as temperature or salinity as the cause of variable boron isotope fractionation at any given silicon concentration. Instead, it is clear that biological factors associated with the different sponge species, and not just environmental, factors influence isotope fractionation. This is not surprising, given that spicules are produced in specialised cells (sclerocytes) located within the sponge tissue. The growing spicule is not in direct contact with seawater but rather the content of the sclerocyte is mediated by transporters across the cell wall (see Chapter 1, section 1.3.2), and is therefore subject to biological regulation.

It follows that it might be possible the variations in δ¹¹B observed amongst the sponges sampled for this study are simply a reflection of the different sponge species occurrences at different locations, rather than the seawater silica concentration. It is difficult to assess this with the available data, because sponges of the same family were rarely recovered from different seawater conditions. There were five samples analysed from the same family (Class Demospongiae, Order Spirophorida, Family Tetillidae) that were sampled
from different locations and may provide some insight, albeit these sites all had relatively high silica concentrations and therefore it is difficult to deconvolve the effects of silica concentration (Table 4.4). Boron isotope values range from +22.3 to +24.5‰, which is a small variability relative to that observed between different species from the same site. This suggests that boron isotope fractionation does not vary significantly between individuals of the sponge species that have grown under similar conditions. It follows that given the large range in $\delta^{11}\text{B}$ between different sponge species from the same locality, it is likely that individuals from the same taxonomic group, sampled over a range of $[\text{Si}]_{\text{sw}}$, may more closely reflect the relationship between sponge $\delta^{11}\text{B}$ and $[\text{Si}]_{\text{sw}}$. Intra-species $\delta^{11}\text{B}$ variability could be more thoroughly evaluated by the analysis of samples from a range of $[\text{Si}]_{\text{sw}}$ in order to deconvolve sponge species-related and $[\text{Si}]_{\text{sw}}$ effects on boron isotope fractionation.

Table 4.4. Seawater silica concentrations and $\delta^{11}\text{B}$ values for individual sponge samples belonging to Class Demospongiae, Order Spirophorida, Family Tetillidae.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>$[\text{Si}]_{\text{sw}}$ (μmol L$^{-1}$)</th>
<th>$\delta^{11}\text{B}$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP093</td>
<td>83.84</td>
<td>+22.3</td>
</tr>
<tr>
<td>SP150</td>
<td>87.30</td>
<td>+24.5</td>
</tr>
<tr>
<td>SP151</td>
<td>87.30</td>
<td>+24.2</td>
</tr>
<tr>
<td>SP019</td>
<td>86.85</td>
<td>+22.3</td>
</tr>
<tr>
<td>SP110</td>
<td>98.45</td>
<td>+23.6</td>
</tr>
</tbody>
</table>

4.4.3 The relationship between $\delta^{11}\text{B}$ and dissolved silica in seawater

The lack of correlation between $\delta^{11}\text{B}$ and seawater pH indicates boron speciation does not determine the isotopic composition in sponges, whereas the relationship between $\delta^{11}\text{B}$ and $[\text{Si}]_{\text{sw}}$ suggests boron isotope fractionation could be controlled by the rate of silica deposition during spicule growth, which is known to vary as a function of $[\text{Si}]_{\text{sw}}$ (Reincke and Barthel 1997; Frölich and Barthel 1997; see also Chapter 2). It is useful to consider the potential mechanisms of boron isotope fractionation within this framework by examining the processes that are involved in silica uptake and deposition, namely the transport of silica from seawater into the sclerocyte (the specialised cell in which spicules
are formed), silica polymerisation during spicule growth, and the efflux of residual silica. The proceeding discussion suggests a model for boron isotope fractionation into the spicules that links fractionation to silica deposition rate, with isotope fractionation occurring during uptake of boron from seawater into the sclerocyte, and subsequently within the sclerocyte by Raleigh fractionation during incorporation into the growing spicule.

4.4.3.1 Boron transport from seawater into the sclerocyte

The isotopic composition of the intracellular boron pool in the sponge sclerocyte is likely determined by the speciation of boron during transport across the cell membrane. Dordas et al. (2000) proposed that boron entered plant cells via facilitated (passive) diffusion of the uncharged B(OH)$_3$ species across the cell membrane. Passive uptake of boron by sponges is possible, particularly given the high concentration of boron in seawater which could create a large concentration gradient from seawater to sclerocyte, although the concentration gradient is difficult to assess because the intracellular boron concentration is unknown. Passive uptake of B(OH)$_3$ would result in the isotopic composition of the intracellular boron pool of +44.0‰ at an ambient seawater pH of 8.0 and pK$_B$ of 8.7, appropriate for typical deep ocean conditions (T = 2°C, S = 34.7 and P = 3000 dbar, Rae et al. 2011), assuming the fractionation factor between boric acid and borate ($\alpha_{B_4O_3}$) is 1.0272 (Klochko et al. 2006).

Alternatively, the identification of boron transporters in plants (Takano et al. 2001) and a homologous transporter in animals (Park et al. 2004), as well as a boron-binding siderophore in bacteria that might function as a boron transporter (Amin et al. 2007), indicates that active transport mechanisms may also be involved in many biological systems, and may extend to sponges. Active transmembrane transport of boron would most likely take place in its tetrahedral B(OH)$_4$ form. This speciation has been observed in
the Na\(^+\)/HCO\(_3\)^\(-\) – [B(OH)\(_4\)]\(^-\) transporters identified by Park \textit{et al} (2004) and Takano \textit{et al} (2002), and B(OH)\(_4\) was also the observed species of boron in the dinoflagellate siderophore vibroferrin (Amin \textit{et al} 2007, Harris \textit{et al} 2007). Alternatively, boron uptake might occur via a silica transporter, such as the Na\(^+\)/HCO\(_3\)^\(-\) – [Si(OH)\(_4\)] transporter identified by Schröder \textit{et al} (2004). In this case, borate might be unintentionally transported in place of Si(OH)\(_4\), given the similarity in their coordination and chemistry, and given the substantially higher boron concentration in seawater compared to silicon. The preferential transport of borate over boric acid would dictate the isotopic composition of the intracellular boron pool, such that if borate is the only species transported into the cell, the \(\delta^{11}\)B of the pool would match the \(\delta^{11}\)B of B(OH)\(_4\) in the surrounding seawater, which would produce a sclerocyte pool value of \(\delta^{11}\)B = +16.8‰.

It is possible that boron uptake occurs by a combination of passive and active uptake, or by a mechanism not explored here, that entails uptake of both boric acid and borate. Accordingly, this discussion considers several intracellular boron isotope values based on the uptake of different proportions of each boron species (Table 4.5).

<table>
<thead>
<tr>
<th>Boron species</th>
<th>Intracellular (\delta^{11})B value (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(OH)(_3) only</td>
<td>+16.8</td>
</tr>
<tr>
<td>B(OH)(_4) only</td>
<td>+44.0</td>
</tr>
<tr>
<td>B(_{TOTAL})</td>
<td>+39.5</td>
</tr>
<tr>
<td>B(OH)(_4):B(OH)(_3) ratio</td>
<td></td>
</tr>
<tr>
<td>80:20</td>
<td>+22.3</td>
</tr>
<tr>
<td>50:50</td>
<td>+30.4</td>
</tr>
<tr>
<td>20:80</td>
<td>+38.6</td>
</tr>
</tbody>
</table>

Table 4.5 Possible \(\delta^{11}\)B values of the intracellular (sclerocyte) boron pool, based on uptake of different proportions of the boron species B(OH)\(_3\) and B(OH)\(_4\) including exclusive uptake of either species, uptake of both species without discrimination (B\(_{TOT}\)) and different B(OH)\(_4\):B(OH)\(_3\) ratios.
4.4.3.2 Incorporation of boron during spicule formation: a link between boron isotope fractionation and silica deposition rate?

The correlation between spicule $\delta^{11}$B and seawater silica concentration might arise from a link between boron incorporation from the intracellular pool into the spicule, and the silica deposition rate. Reinke and Barthel (1997) reported that silica deposition rate increases with increasing silica concentration in seawater, following Michaelis-Menten uptake kinetics. The rate of silica polymerisation ($v_p$) is given by the relationship:

$$v_p = \frac{v_{\text{max}} \times [\text{Si}]}{K_m + [\text{Si}]}$$  \hspace{1cm} (4.1)

where $v_{\text{max}}$ is the maximum uptake rate, $K_m$ is the half-saturation constant, and [Si] is the seawater silica concentration. The values of $v_{\text{max}}$ and $K_m$ were reported to be 19.33 μmol h$^{-1}$ g$^{-1}$ and 46.6 μM respectively, for silica uptake rate by the cultured demosponge *Halichondria panicea* (Figure 4.3).

![Figure 4.3. Michaelis-Menten curve, depicting substrate (Si) uptake rate (v) as a function of substrate concentration ([Si]$_{sw}$), using the values of $v_{\text{max}}$=19.33 μmol h$^{-1}$ g$^{-1}$ and $K_m$=46.6 μM (Reinke and Barthel 1997).](image)
If boron isotope fractionation is controlled by silica deposition rate, it follows that for the observed trend in sponge $\delta^{11}\text{B}$ with $[\text{Si}]_{\text{sw}}$ in Figure 4.3 that boron isotope fractionation should decrease as the rate of silica polymerisation increases. This relationship can be described in terms of Rayleigh fractionation, whereby boron is incorporated into the growing spicule in a ‘closed’ system (i.e. no new input), or a ‘quasi-closed’ system where the supply of boron relative to silicon is low compared to the rate of spicule growth. In this system, the lighter boron isotope would be preferentially incorporated into the silica, meaning that as the pool supply is progressively depleted and its isotopic composition becomes increasingly heavy.

The $\delta^{11}\text{B}$ of the deposited spicule and the remaining intracellular pool ($\delta^{11}\text{B}_{\text{spicule}}$ and $\delta^{11}\text{B}_{\text{pool}}$ respectively) are defined by mass balance to be:

\begin{equation}
\delta^{11}\text{B}_{\text{spicule}} = \delta^{11}\text{B}_{\text{initial}} + \varepsilon \cdot \ln \left( \frac{f}{1-f} \right) \tag{4.2}
\end{equation}

\begin{equation}
\delta^{11}\text{B}_{\text{pool}} = \delta^{11}\text{B}_{\text{initial}} - \varepsilon \cdot \ln f \tag{4.3}
\end{equation}

where $f$ is the fraction of the intracellular pool of boron remaining at any point, $\varepsilon$ is the per mille enrichment factor: $\varepsilon = (\alpha-1) \cdot 1000$, $\alpha$ is the $^{11}\text{B}/^{10}\text{B}$ fractionation factor, and $\delta^{11}\text{B}_{\text{initial}}$ is the starting composition of the boron pool (Van Hale and Frew 2010). The $\delta^{11}\text{B}$ value of a sponge spicule can then be calculated as a function of $f$ for a given value of the intracellular boron pool $\delta^{11}\text{B}$ (see Table 4.5). The value of $\alpha$ is calculated assuming the lowest measured sponge value of $\delta^{11}\text{B}$ (i.e. $+2.4\%$, see Table 4.1), represents the fractionation from the assumed value of the initial pool composition:

\begin{equation}
\alpha_{\text{spicule-pool}} = \frac{R_{\text{spicule}}}{R_{\text{pool}}} \tag{4.4}
\end{equation}
where \( R_{spicule} \) and \( R_{pool} \) are the \(^{11}\text{B}/^{10}\text{B} \) ratios, the lowest measured sponge \(^{11}\text{B}/^{10}\text{B} \) ratio and the initial pool composition, respectively.

The calculated curve for the intracellular \( \delta^{11}\text{B} \) value of +16.8‰, representing exclusive uptake of \( \text{B(OH)}_4^- \) from seawater, shows a poor fit to the measured sponge \( \delta^{11}\text{B} \) values (\( R^2 = 0.37 \)). The closest correlation with the measured data is the curve for \( \delta^{11}\text{B} \) of +22.3‰, which corresponds with a \( \text{B(OH)}_4^- : \text{B(OH)}_3 \) ratio of 80:20 (\( R^2 = 0.63 \)) (Figure 4.4).

![Figure 4.4](image.png)

Figure 4.4. Calculated sponge \( \delta^{11}\text{B} \) for different intracellular boron pool values, plotted with measured sponge \( \delta^{11}\text{B} \) (black circles). The \( \delta^{11}\text{B} \) values in the legend correspond with the values for different proportions of \( \text{B(OH)}_4^- \) and \( \text{B(OH)}_3 \) described in Table 4.5.
The model outlined above assumes that $\delta^{11}\text{B}_{\text{spicule}}$ is determined by $f$, the proportion of the intracellular boron pool that is incorporated into the spicule. It follows that $f$ is a function of $v_p$ (the uptake rate) and the amount of boron incorporated increases with $v_p$, then the rate of boron incorporation ($v_{p,B}$) will be proportional to $v_{p,\text{Si}}$. A relationship between the rate of boron incorporation and the drawdown of the internal pool might arise if there is an increase in the total amount of silica precipitated as the seawater silica concentration increases. Although an explicit relationship between the rate of silica formation and the total amount of silica deposited has not been documented in the literature, several studies have observed increased spicule size corresponding with increased silica concentrations in seawater (Simpson 1984; Maldonado et al 1999; Frölich and Barthel 1997; Uriz 2000). The growth of larger spicules might conceivably occur with the consumption of a greater fraction of the intracellular boron pool, as boron incorporation increases proportionally to the amount of silica deposited.

In fitting a Raleigh fractionation curve to the observed sponge $\delta^{11}\text{B}$ data it can be further assumed that the range of $[\text{Si}]_{\text{sw}}$ represented by the sample sites corresponds to a range of $f = 0$ to 1, i.e. from low to almost complete utilisation of the intracellular boron pool. To test this assumption, spicules from a hexactinellid sponge from high Si waters (approximately 170 µM), collected from the NE Pacific Ocean (34.83°N, 123.00°W) at 4100 m, were analysed for boron concentration and isotopic composition. If the Antarctic samples, which come from waters with ~70 µM Si, are not associated with the near complete utilisation from the boron pool, this NE Pacific sponge sample should record $\delta^{11}\text{B}$ even higher than the Antarctic sponges. The $\delta^{11}\text{B}$ of this sample from the NE Pacific is +20.00‰, within the range of the Antarctic samples, indicating the Antarctic sponges can be taken to represent the maximum extent of boron incorporation, corresponding to the highest $\delta^{11}\text{B}$ observed in siliceous sponges. Finally, it should be noted that if the intracellular boron pool is an open system (and the boron supply is not limited), the
spicule $\delta^{11}{\text{B}}$ would be a uniform value determined by $\alpha$ and the $\delta^{11}{\text{B}}$ value of the sclerocyte boron pool.

The model described does not specify the mechanism by which boron is incorporated into the growing spicule. One possibility is that boron is adsorbed onto the silica surface and is subsequently incorporated into the amorphous silica structure, as is thought to occur with clays (Palmer et al 1987) and marine carbonates (Hemming and Hanson 1992). Kim and Kirkpatrick (2006) observed a strong affinity for boron adsorption onto silica gel, which has an amorphous, hydrated silicate structure and therefore may serve as an analogue for biogenic silica. It is possible that the hydroxyl groups of borate or boric acid undergo similar condensation reactions with silica to form $\text{Si-O-B}$ bonds. NMR analysis of boron adsorbed onto silica gel indicates boron forms inner-sphere $\text{B-O-Si}$ bonds, predominantly in its $\text{B}^{4+}$ form (Kim and Kirkpatrick 2006). Boron would then be incorporated into the silica lattice as polymerisation continues, substituting for $\text{Si(OH)}_4$. As yet, no fractionation factor for boron adsorption onto amorphous silica has been reported in the literature. Several attempts to determine the fractionation factor were made as part of this study, using silica gel in experimental conditions similar to Kim and Kirkpatrick (2006) and Liu et al (2009). Silica gel was left to react with known quantities of boric acid standard (SRM 951) in an incubator whilst being constantly rotated to assist with equilibration. The boron isotope composition of both the gel and the remaining solution were measured by PTIMS. Unfortunately, these experiments were unsuccessful because the small amount of boron precipitated from the boric acid solution was insufficient to produce a measurable composition and from there determine an isotopic fractionation factor. A solution with a much lower boric acid concentration is needed to produce a quantifiable fractionation, but this approach would preclude precise analysis by PTIMS, and would require use of a more sensitive analytical technique (e.g. MC-ICPMS or NTIMS).
An alternative mechanism to adsorption of boron is the possibility that boron is incorporated by reaction directly with silicatein, the protein that catalyses and directs silica polymerisation, with borate (or boric acid) substituting for silicic acid. The polymerisation of metals and metalloids, such as titanium dioxide and gallium oxide, by silicatein has been previously reported (Sumerel et al. 2003; Kialilus et al. 2005; Natalio et al. 2010), and the possibility of borate substitution and polymerisation by silicatein has been discussed in Chapter 2 of this thesis.

Another possibility, also raised in Chapter 2, is the existence of a boron-specific transporter, such as a bicarbonate transporter similar to those previously identified in plant roots. This transporter belongs to a group of anion exchangers found in mammals (Takano et al. 2002), which would presumably transport the charged $\text{B(OH)}_4^-$ molecule, rather than $\text{B(OH)}_3^-$. This mechanism would therefore produce an sclerocyte $\delta^{11}$B value of $+16.8\%_\text{o}$ according to the model proposed here, corresponding with exclusive uptake of $\text{B(OH)}_4^-$ (Table 4.5). This model produced a poor correlation with the measured sponge $\delta^{11}$B values, which suggests that if an anion exchanger is a mechanism of boron uptake used by sponges, there might be concurrent uptake of $\text{B(OH)}_3$ by another pathway, such as passive uptake.

### 4.5 Conclusion

This study presents the first reported boron isotope compositions of siliceous sponge spicules. Sponge spicules from the Southern Ocean span a boron isotope composition range that is larger than any other marine boron reservoir, from $+2.43\%_\text{o}$ to $+24.51\%_\text{o}$. Unlike marine carbonates, the $\delta^{11}$B of modern siliceous sponges does not correlate with seawater pH. This negates the hypothesis that borate is incorporated exclusively from seawater into the spicule. Rather, the $\delta^{11}$B compositions of siliceous sponges show a
positive correlation with ambient dissolved silica concentrations ($r = 0.84$). The lowest \( \delta^{11}\text{B} \) values, that is, the most fractionated from seawater, occur in low Si waters, and the highest values in high Si waters. This relationship suggests that boron isotope fractionation is related to silica uptake and deposition during sponge spicule formation.

A simple model for boron uptake and incorporation is proposed that accounts for boron isotope fractionation during transport from seawater into the sclerocyte, and subsequent Raleigh fractionation during silica deposition. This model requires a quasi-closed system, based on the low B/Si ratio of sponges relative to seawater which indicates strong discrimination against boron during uptake. Following a Raleigh fractionation model, the \( \delta^{11}\text{B} \) values of sponge spicules can be calculated with the assumption that the fraction of intracellular boron that is consumed by the growing spicule is proportionate to the rate of silica deposition. The modelled results indicate that if this model is correct, the intracellular pool boron isotope composition is determined by uptake of both boron species, rather than exclusive uptake of either \( \text{B(OH)}_4^- \) (by active transport) or \( \text{B(OH)}_3^- \) (by passive diffusion). However, the observed sponge \( \delta^{11}\text{B} \) values still vary substantially compared to the calculated values, which perhaps reflects differences in the fractionation factors between different sponge species, corresponding to differences in boron and silicon uptake rates. Better insights into the processes of boron uptake by sponges, and the links with silica deposition rate, would elucidate the processes that affect boron isotope fractionation. In particular, experiments using silica gels and sponge spicule cultures could provide such insights and help to distinguish the biological controls over boron isotope fractionation by sponges.
Chapter 5

Boron/silicon ratios in marine diatoms: a proxy for silica concentration in seawater?

5.1 Introduction

Diatoms are an important part of the ocean's biological pump, and the links between atmospheric $pCO_2$ and nutrient utilisation by diatoms is the focus of studies seeking to understand the links between primary productivity and glacial-interglacial climate fluctuation (e.g. Sarmiento and Toggweiler 1984; Sarmiento and Orr 1991; Martin et al 1994; Petit et al 1999; Boyd et al 2000; Caldeira and Duffy 2000, Sigman and Boyle 2000). Silica ($Si(OH)_4$) is an essential nutrient for diatoms, which are responsible for about 40% of surface primary productivity (Nelson et al 1995). Accordingly, silica availability and carbon export are closely linked, and insight into the cycling and distribution of silica is important for understanding the nexus between the biological pump and atmospheric $CO_2$. Numerous geochemical proxies have been applied in efforts to understand the links between past changes in silica cycling and atmospheric $CO_2$ levels, including proxies for opal (biogenic silica) export (Chase et al 2003; Bradtmiller et al 2007) and geochemical proxies for dissolved $Si(OH)_4$ concentration and its utilisation by diatoms based on Si isotope ratios (De La Rocha et al 1997; Varela et al 2004; Wille et al 2010; Hendry et al 2010) or germanium/silicon ratios (Froelich et al 1989; Ellwood et al 2006).

This chapter examines the boron/silicon (B/Si) ratio values of diatoms, specifically investigating whether there is a correlation between B/Si and seawater $Si(OH)_4$ concentration. Previous studies have demonstrated that diatoms, like higher plants, have a nutritional requirement for boron (Lewin 1966; Lewin and Chen 1976; Smyth and Dugger
1981). Lewin (1966) reported growth slowed or ceased in 12 marine diatom species and four freshwater species, when in boron-free culture media. However, subsequent published studies of the boron content of diatoms are limited to a few measurements of boron concentrations in diatom nannofossil and radiolarian oozes (Ishikawa and Nakamura 1993) and a recent study of the correlation between the boron content of diatoms and seawater pH (Mejía et al. 2013). In light of the links between the boron geochemistry of sponge spicules and seawater Si(OH)$_4$ concentrations revealed in this thesis (Chapters 2 and 4), this study will test the hypothesis that B/Si in diatoms decreases with increasing seawater [Si(OH)$_4$] due to competitive incorporation of Si(OH)$_4$, leading to the dilution of boron at high seawater [Si(OH)$_4$]. The hypothesis is first tested experimentally using cultures of the diatom *Thalassiosira pseudonana*. The resulting calibrated relationship is then used to generate a record of Si(OH)$_4$ using a sediment core from the Pacific sector of the Southern Ocean (core E33-22, 54.92°S, 120°W, 2743 m), allowing comparison with previous Si(OH)$_4$ studies of this region. The measurement of B/Si is relatively straightforward, and this study demonstrates the potential for its use as a proxy for the reconstruction of dissolved Si(OH)$_4$ concentrations. If so, it could provide a proxy for Si(OH)$_4$ that is analytically much simpler to measure and apply than either Ge/Si or Si isotopes.

5.2 Methods

5.2.1 Diatom cultures

The coastal diatom species *Thalassiosira pseudonana* was grown in batch cultures in acid-cleaned polycarbonate jars. *T. pseudonana* was chosen because it is fast-growing and its silica uptake and growth characteristics have been well-studied (e.g. Milligan et al. 2004; Thamatrakoln and Hildebrand 2008). Culture media was made to f/2 nutrient concentrations using 0.2 µm filtered open ocean seawater and stock solutions. All cultures
were grown under continuous PAR illumination at approximately 200 µmol photons m$^{-2}$ s$^{-1}$ at 20°C. Stock diatom cultures were maintained in two media formulations: one in f/2 concentrations and one in which all nutrients were in f/2 concentrations except silica, which was at f/8 concentration (i.e. 10 µmol L$^{-1}$). These stock cultures were maintained and cell counts monitored over several generations to acclimate diatoms in high and low Si conditions before experiments were conducted.

Experimental cultures were grown in triplicate at different Si concentrations, ranging from ~10 to ~80 µmol L$^{-1}$, in 250 mL media. Cell concentrations and growth rates were monitored using a FlowCAM (Fluid Imaging Technologies, USA) and by manual counting using a haemocytometer at least twice per day. Each experiment was terminated near the end of the exponential growth phase in order to maximise the amount of biogenic silica produced for ICPMS analysis. At the end of each experiment the cultures were vacuum-filtered through a 1 µm polycarbonate filter, and the filter paper stored in a freezer at -4°C until analysis. The filtered media were stored in acid-cleaned polycarbonate jars at 4°C.

To prepare cultured material for analysis, filters were rinsed repeatedly into an acid-cleaned Teflon beaker with high purity (MilliQ) >18.2 MΩ water. About 5 mL of ~30% H$_2$O$_2$ (AR grade, Sigma Aldrich) was added to the beaker to oxidise organic material, then the beaker was loosely capped, sonicated for 5 minutes to disaggregate the sample, before being placed on a hotplate at 60°C for 5-8 hours. Each sample was then centrifuged and the supernatant discarded. The oxidation step was repeated if organic matter remained, which was observed visually as the presence of brown residues (indicative of organic material) or if samples continued to react to extra additions of H$_2$O$_2$. Once completely oxidised, samples were transferred to acid-cleaned Teflon vials and air-dried in a laminar-flow hood. They were dissolved with 1 mL 0.2 M NaOH (reagent grade, Sigma Aldrich) and beryllium was added as an internal standard for ICPMS analysis. Vials were tightly capped
and heated on a hotplate for approximately 8 hours. Solutions were then diluted with Milli-Q water for ICPMS analysis.

5.2.2 Sediment core sample preparation

Marine sediment was sampled from a deep-water core from the Pacific sector of the Southern Ocean (E33-22: 54.92°S, 120.00°W, 2744 m; Figure 5.1), which is situated in the Subantarctic zone, north of the modern Antarctic Polar Front (APF). Previous work on this core has yielded $\delta^{13}$C records from planktonic foraminifera *Neogloboquadrina pachyderma* (Ninneman and Charles 1997) and opal and lithogenic accumulation rates (Chase et al. 2003). Sampling was limited to intervals with sufficient material for repeat analysis (at least 10 mg), which precluded high-resolution sampling in several sections. Sample preparation followed Shemesh et al. (1988). Briefly, approximately 10 mg of dried core sediment was weighed into a beaker and 100 mL of 1M HCl (reagent grade, Sigma Aldrich) and 100 mL ~10% $\text{H}_2\text{O}_2$ was added to dissolve carbonate and oxidise organic material. The beaker was placed on a hotplate for approximately 5 hours at 50°C and then allowed to settle and cool before supernatant was removed. Approximately 100 mL of 5% sodium hexametaphosphate was then added and boiled for 15 minutes before cooling and removal of supernatant. About 2 mg of cleaned, dried sediment was weighed into an acid-washed Teflon beaker, and 0.5 mL 0.2 M NaOH (reagent grade, Sigma Aldrich) was added. Beakers were capped and left on a hotplate at approximately 60°C for several hours to dissolve the sample. Once dissolved samples were diluted with MilliQ water and analysed by ICPMS (see below).
5.2.3 **Silica concentration analysis of culture media**

Silica concentrations of culture media solutions were determined by silicomolybdate assay using a Varian UV-Vis spectrophotometry (Strickland and Parsons 1968), with a 1 cm$^3$ cell. Adsorbance was measured at 810 nm and samples were calibrated using matrix-matched standards.

5.2.4 **B/Si analysis by ICPMS**

Boron and silicon concentrations were determined by external calibration with internal standardization using a Varian 820 quadrupole ICPMS at the Australian National University. Samples were introduced into a peltier cooled double pass spray chamber with a PFA nebulizer (100 µL/min uptake) in 0.02 M NaOH solution.
Blank and instrument drift were monitored by repeat analyses of standards throughout each analytical session, and measurement of an internal standard (Be). Accuracy was monitored by repeat measurements of matrix-matched standards (filtered seawater) of known boron and silica concentrations (2σ = 0.01) Blanks were also routinely measured and their average boron concentration was 0.29 ±0.63 ppb (2se).

5.3 Results

5.3.1 Cultured diatoms

The B/Si (mmol/mol) of cultured *T. pseudonana* decreased with increasing silica concentration of the culture media (Figure 5.2). Averaged B/Si molar ratios ranged from 2.09 ± 0.01 (2se) in low Si media (9.67 µM) to 0.06 ± 0.01 in high Si media (79.07 µM). The decrease in B/Si\textsubscript{diatom} is nonlinear, and approaches horizontal asymptote at high Si(OH)\textsubscript{4}.

![Figure 5.2. Diatom B/Si (mmol/mol) ratio plotted against initial media Si(OH)\textsubscript{4} concentration (µmol/L). Analytical uncertainties (2sd) are smaller than data points.](image)
The culture media B and Si concentrations measured before and after the experiments (Table 5.1), revealing that more than 90% of the boron remained after the experiment ended, whereas silica consumption ranged from 90 to nearly 100% of total silica available.

Table 5.1. Diatom culture experiment results, including concentrations of Si and B measured in culture media before and after the experiments were conducted.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Initial [Si] (µmol kg⁻¹)</th>
<th>Final [Si]* (µmol kg⁻¹)</th>
<th>% Si* consumed</th>
<th>Initial [B] (µmol kg⁻¹)</th>
<th>Final [B]* (µmol kg⁻¹)</th>
<th>% B* consumed</th>
<th>Diatom B/Si (mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S10</td>
<td>10.95</td>
<td>1.044</td>
<td>90.47</td>
<td>439.0</td>
<td>395.6</td>
<td>9.88</td>
<td>A</td>
</tr>
<tr>
<td>S30</td>
<td>25.42</td>
<td>0.812</td>
<td>96.81</td>
<td>430.5</td>
<td>414.3</td>
<td>3.78</td>
<td>B</td>
</tr>
<tr>
<td>S60</td>
<td>50.71</td>
<td>0.645</td>
<td>98.73</td>
<td>419.9</td>
<td>394.0</td>
<td>6.16</td>
<td>C</td>
</tr>
<tr>
<td>S100</td>
<td>79.07</td>
<td>0.464</td>
<td>99.41</td>
<td>402.2</td>
<td>366.4</td>
<td>8.90</td>
<td>n/a</td>
</tr>
<tr>
<td>Sf8</td>
<td>9.670</td>
<td>0.728</td>
<td>92.47</td>
<td>441.8</td>
<td>407.4</td>
<td>7.78</td>
<td>0.14</td>
</tr>
<tr>
<td>Sf2</td>
<td>29.90</td>
<td>1.208</td>
<td>95.96</td>
<td>411.1</td>
<td>396.8</td>
<td>3.47</td>
<td>1.04</td>
</tr>
</tbody>
</table>

*averaged values, n=3. n/a: not analysed.

5.3.2 Sediment core E33-22

The measured B/Si ratio values of diatoms from sediment core E33-22 are presented in Figure 5.3. The chronology was constructed by Ninneman and Charles (1997) using δ¹⁸O of planktonic foraminifera *N. pachyderma*. Diatom B/Si ratios in this sediment core range from 0.33 to 0.69 ± 0.2 (2σ mean), with low B/Si ratio values occurring in Holocene samples to higher B/Si values during the glacial maximum (18 kya). Small fluctuations in B/Si<sub>diatom</sub> occur with increasing age down core but there is little secular variation, although fluctuations increase in magnitude between 120-180 kyr.

The measured B/Si ratio values in sediment core match values observed in diatoms grown in culture media with high silica (around 80 µM) in the experiments described above.
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Figure 5.3. B/Si ratio values measured in diatoms from Pacific Ocean core E33-22. Numbered marine isotope stages (MIS) are marked in blue and white above the plot. The grey band represents the last glacial maximum (LGM). Error bars are 2σ. The $\delta^{30}$Si record of sponge spicules from E33-22 (Ellwood et al 2010) reflect silica utilisation in benthic waters – heavier isotope signatures correspond with greater silica utilisation. *N. pachydemra* $\delta^{13}$C and $\delta^{18}$O records are from Ninneman and Charles (1997).
5.4 Discussion

The results of the cultured diatom experiment indicate B/Si composition of diatoms is related to external seawater silicon concentrations. Here I explore the uptake and deposition of boron and silica by diatoms to elucidate the processes that effect B/Si ratios. The empirical relationship between B/Si and [Si(OH)₄]sw is then used to interpret B/Si in the sediment core E33-22 in order to assess the potential utility of B/Si as a proxy for seawater silica concentrations.

5.4.1 Impact of pH on boron uptake?

The aqueous speciation of boron is closely linked to pH (see Chapter 1), meaning that if one species is preferentially taken up by diatoms, the boron content of diatoms would be linked to seawater pH. Whether this relationship, which is the basis of the δ¹¹B - pH proxy in marine carbonates (see Chapter 1), exists in diatoms was tested by Mejía et al. (2013), who found a positive correlation between boron content and pH in the cultured diatom species *T. pseudonana* and *T. weissflogii*. The authors suggest that this correlation might be due to B(OH)₄⁻ uptake via HCO₃⁻ transporter protein, as at higher pH, demand for HCO₃⁻ for photosynthesis increases as CO₂ availability decreases. This then leads to increased co-transport of B(OH)₄⁻. It is also suggested that increasing borate concentration with pH would be reflected by increasing B(OH)₄⁻/HCO₃⁻ ratios.

The pH of the seawater culture media was not measured in the present study, but it can be estimated using the Si/C ratios of *T. pseudonana* measured by Mejía et al. (2013). Consumption of DIC in each experimental batch was calculated from the drawdown of silicon, which was measured in each experiment, and the shift in pH was then calculated using CO₂calc (Robbins et al. 2010), using the carbonic acid dissociation constants (K₁ and K₂) from Mehrbach et al. (1973), refitted by Dickson and Millero (1987). The increase in pH as DIC is consumed by the diatoms would range from approximately 0.05 to 0.09 units in
the low Si (~10 μM) media and 0.09 to 0.19 units in high Si (~80 μM) media, from an initial pH of ~8.1. This range is smaller than that measured by Mejía et al (2013), which spanned pH 7.50 to 8.63 with a corresponding boron concentration range of 4.3 to 12.4 ppm. The predicted increases in pH in the cultures in the present study were relatively small in comparison, and would not account for the large B/Si range observed here. It’s therefore likely that the B/Si variations reported here are due to changes in silica availability rather than pH-related boron speciation.

5.4.2 Are boron/silicon ratios in diatoms a function of silica uptake kinetics?

Numerous previous studies describe silica uptake by diatoms as following Michaelis-Menten kinetics (e.g. Paasche 1973; Sullivan 1976; Del Amo and Brzezinski 1999; Milligan et al 2004). The correlation between B/Si_{diatom} and external silica concentration indicates silica uptake kinetics, rather than boron uptake, are controlling the B/Si ratio. It is known that silica uptake by diatoms is an enzyme-mediated, saturable process, and that uptake rate (v) is dependent upon external silica concentration (Sullivan 1976; Conway and Harrison 1977; Del Amo and Brzezinski 1999). This relationship produces a curve in which v approaches a horizontal asymptote as [{Si(OH)}_4]_{sw} increases towards saturation (v_{max}) (Figure 5.4(a)). Figure 5.4(b) illustrates the similarity between the B/Si_{diatom} data and the inverse v vs [{Si(OH)}_4] curve, in which v and B/Si show similar asymptotic behaviour. This similarity can be explained if boron uptake occurs at a constant or near-uniform rate, and that as a result, B/Si variation is driven by relative changes in silica uptake rate.
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Figure 5.4. (a) Silica polymerisation rate \( v_{p,\text{Si}} \) as a function of silica concentration \( ([\text{Si(OH)}_4]) \), according to the Michaelis-Menten equation \( v_{p,\text{Si}} = \frac{v_{\text{max}}[\text{Si(OH)}_4]}{K_m + [\text{Si(OH)}_4]} \), where \( v_{\text{max}} \) is the maximum uptake rate and \( K_m \) is the substrate concentration where \( v = \frac{1}{2}v_{\text{max}} \). (b) The inverse \( (1/v) \) of the Michaelis-Menten curve. Similar asymptotic behaviour is seen in the diatom B/Si vs \([\text{Si(OH)}_4]\) curve (see Figure 5.2).

Thamatrakoln and Hildebrand (2008) have shown that the uptake kinetics of \textit{T. pseudonana} are more complex than simple Michaelis-Menten kinetics, and on short timescales (<30 minutes) display sigmoidal or nonsaturable uptake, depending on the external concentration of \textit{Si(OH)}_4. However, over longer timescales (2-3 hours) all cultures, as well as those of other diatom species, shifted to saturable uptake. This was attributed to equilibration between the capacity of silica binding components inside the cell and the rate at which silica is transported from the binding site to the cell wall and polymerised. Given that all cultures in this study were incubated for 24-48 hours, saturable Michaelis-Menten kinetics should describe observed \textit{Si(OH)}_4 uptake.
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If diatom B/Si compositions reflect silicon uptake rate and therefore external seawater [Si(OH)₄], there is the potential to use B/Si as a proxy for seawater [Si(OH)₄] in palaeoceanographic reconstructions. The long residence time and uniform concentration of boron in seawater (Spivack and Edmond 1987; Lemarchand et al 2002) means modern calibrations should be applicable over at least the last few million years.

5.4.3 Boron partitioning between diatom opal and seawater

A possible mechanism for the incorporation of boron into the opal diatom frustule is by substitution of borate for silicate in the polycondensation reaction described by Harrison and Loton (1995):

\[
\text{Si(OH)}_4^{(s)} + B(OH)_4^{(aq)} \rightarrow (OH)_2\text{Si}^− - O - B(OH)_3^{(s)} + H_2O
\]  

(5.1)

The fractionation of boron between diatom opal and seawater can be expressed as an empirically-calculated partition coefficient, K:\text{D}, defined as:

\[
K_D = \frac{(B/Si)_{\text{diatom}}}{(B/Si)_{sw}}
\]  

(5.2)

Although the diatom cultures in this study were terminated while still undergoing exponential growth, a substantial proportion of the silica was utilised. Therefore, calculation of K:\text{D} must also account for the fractional depletion of silica (f) from the culture media, treating the batch culture as a Rayleigh fractionation process, according to the Raleigh distillation equation:

\[
K_D = \frac{\ln \left( \frac{1 - (B/Si)_{\text{diatom}} \times (1-f)}{(B/Si)_{\text{init sw}}} \right)}{\ln f}
\]  

(5.3)
where \((B/Si)_{\text{init,sw}}\) is the B/Si of culture media at the beginning of the experiment, and \((B/Si)_{\text{diatom}}\) is the B/Si measured in the cultured diatoms and represents the accumulated \(B/Si_{\text{diatom}}\) over the evolution of the culture media \(B/Si\). \(K_0\) was calculated for each experiment and averaged to produce a value of \(7.92 \times 10^{-6} \pm 4.69 \times 10^{-6}\) (2 se). The calculated \(B/Si_{\text{diatom}}\) produced by this \(K_0\) is plotted as a function of \([\text{Si(OH)}_4]_{\text{sw}}\) alongside the measured data for comparison in Figure 5.5.

![Figure 5.5. Calculated diatom B/Si (mmol/mol), with an average opal-seawater \(K_0\) of \(7.92 \times 10^{-6} \pm 4.69 \times 10^{-6}\) (2 se), plotted against the initial \([\text{Si(OH)}_4]_{\text{sw}}\) concentration (μmol/L) of the culture media. Measured diatom B/Si ratios are plotted for comparison.](image)

The \(K_0\) values are empirical, and the calculations make no assumptions about the processes involved in boron and silicon uptake. One factor that will affect \(K_0\) is the effect of seawater pH on boron uptake. As discussed previously, Mejía et al. (2013) demonstrated boron concentrations in diatoms increase with increasing pH. The increase in boron content with pH is small relative to the changes in B/Si observed in this study and it is unlikely that pH changes would obscure the effects of \([\text{Si(OH)}_4]_{\text{sw}}\) on diatom B/Si. The
effect of pH on $K_D$ can be seen by calculating the $K_D$ values of the *T. pseudonana* samples from Mejía *et al* (2013), in which the media $[\text{Si(OH)}_4]$ is 100 $\mu$mol L$^{-1}$. $K_D$ increases with increasing pH, reflecting the positive correlation between boron content and pH (Figure 5.6). The average $K_D$ calculated from the data of Mejía *et al* (2013) is $1.48 \times 10^{-5}$. This can be compared to $K_D$ values calculated from this study using initial culture concentrations (i.e. instantaneous $K_D$ values) which gives a value of $2.35 \times 10^{-5}$. The $K_D$ for the data from Mejía *et al* (2013) is calculated based on the total seawater boron concentration ($B_{tot}$), meaning the pH effect observed here is due to boron speciation, namely the seawater $B(\text{OH})_4^-$ concentration.

Figure 5.6. Empirical opal-seawater $K_D$ calculated from Mejía *et al* (2013), plotted against pH.

Another variable that must be considered is the $\text{Si(OH)}_4$ uptake rate, which varies between diatom species, both in $v_{\text{max}}$ values and the time taken to reach saturation (Thamatrakoln and Hildebrand 2008). Therefore, empirical $K_D$ values are likely species-specific, and would need to be measured for a given species before diatom B/Si calibrations can be applied with confidence to marine sediment records.
5.4.4 Down-core record of B/Si in the Southern Ocean

The results of the culture experiments are now used to interpret B/Si ratios in diatoms samples taken from core E33-22 from the Pacific sector of the Southern Ocean, to investigate whether B/Si is a useful proxy for [Si(OH)₄] reconstruction. I interpret only relative changes in [Si(OH)₄], rather than apply an empirical calibration to calculate absolute values, for reasons that are discussed below.

Boron/silicon ratios in this core record are generally lower during interglacial periods than during glacial periods. The lowest B/Si values occur in the most recent (Holocene) sediment, and correspond with relatively high [Si(OH)₄] values, which is consistent with the culture experiment results. Across the last glacial-interglacial cycle this increase in silica coincides with the termination of the last glacial period, and continues into the Holocene. There is little variation across MIS 3 to MIS 5d, during which time relatively high B/Si ratio values persisted (mean B/Si = 0.61 mmol/mol). A marked change in B/Si also occurs during MIS 5e, the last interglacial period (127-116 ka) (CLIMAP 1984), where a relatively rapid increase in B/Si is observed across the transition from MIS 5e to 5d.

The increase in [Si(OH)₄] during the last deglaciation recorded by B/Si coincides with a rapid increase in the supply of Si to surface waters of the Southern Ocean due to increased upwelling of nutrient-rich deep water, as deduced from opal burial records in cores south of the APF (Anderson et al. 2009). Anderson et al. (2009) reported marked increases in opal production during the deglacial in all three basins of the Southern Ocean, correlating with lighter δ¹³C signatures associated with upwelled deep water. While a rapid increase in [Si(OH)₄] is observed during the ultimate deglaciation, the record does not extend to more recent than ~10 ka, so it is not possible to ascertain whether B/Si subsequently records the decrease in [Si(OH)₄] during the Holocene reported by Anderson et al. (2009).
The low [Si(OH)₄] values that are implied throughout the last glacial period from the B/Si record are inconsistent with previous reconstructions of opal productivity and Si utilisation by diatoms, which indicate that under glacial conditions Si utilisation decreased resulting in higher [Si(OH)₄] in surface waters (De La Rocha et al 1998; Beucher et al 2007). Opal burial records, including that measured in E33-22, indicate diatom productivity decreased in the Antarctic zone but increased in the SAZ during the LGM (Chase et al 2003). These records support the silicic acid leakage hypothesis (SALH), which posits that lower atmospheric CO₂ during glacial periods can be attributed to a decrease in silica uptake by diatoms relative to nitrate (Si:N ≈ 1:1) compared to today (Si:N ≈ 4:1) due to increased iron concentrations in the surface waters (Brzezinski et al 2002; Matsumoto et al 2002). The unused silica in surface waters is transported northwards to low latitude upwelling zones, via subantarctic mode water (SAMW) and Antarctic Intermediate Water (AAIW), causing diatom productivity to outcompete other taxa, principally coccolithophorids, and thereby leading to reduced CaCO₃ export and increased alkalinity (Matsumoto et al 2002). While it is difficult to explain the lower [Si(OH)₄] implied in the E33-22 B/Si record in light of these previously published reconstructions, it should be noted that these other studies have estimated silica utilisation by diatoms and not the concentration of silica in seawater. The SALH implies that the supply of silica does not change over glacial-interglacial periods. The measurement of opal burial and δ³⁰Si does not reveal whether this is the case, but rather only sense changes in the uptake and export of silica by diatoms. Further studies of B/Si in opal from south of the APF, and from the Atlantic and Indian sectors, might therefore provide more direct insight into the changes in silica supply that have occurred over time. It is possible that analysis of B/Si in diatoms from cores in the Atlantic sector of the Southern Ocean might produce more pronounced shifts in [Si(OH)₄] between glacial and interglacial periods than in the Pacific sector, given reconstructions indicate the greatest
opal fluxes during the LGM were in the Atlantic sector, and the smallest in the Pacific (Chase et al. 2003).

Although the culture experiments indicate diatom B/Si compositions are highly sensitive to ambient [Si(OH)$_4$], caution is required with use of these data to reconstruct absolute [Si(OH)$_4$] values from sediment cores. Only a single, coastal species (T. pseudonana) was used here for the modern culture calibration, and if B/Si variability is a function of silica uptake rate, different species are likely to produce different B/Si v [Si(OH)$_4$] curves. Previous studies indicate silica uptake kinetics vary significantly between diatom species, which have very different $K_M$ and $v_{max}$ values (e.g. Paache 1973; Sullivan 1976; Thamatrakoln and Brzezinski 2008). Furthermore, synchrotron-based X-ray fluorescence (SXRF) analysis of individual cells has revealed regional variation in the degree of silicification between diatoms from low silica, high temperature waters (equatorial Pacific) and high silica, low temperature waters (Southern Ocean) (Baines et al. 2010). Cells from the Southern Ocean contained approximately six times more silica than those from the equatorial Pacific, which was attributed to regional variation in the nutrient requirements of the specific diatom community (Baines et al. 2010). Interestingly, Baines et al. (2010) also found the average silica content of diatoms in the Southern Ocean was more than ten times higher than diatoms cultured in nutrient replete conditions. These findings suggest that B/Si calibration studies using endemic diatom species, both cultured and field-collected, would be necessary to accurately predict seawater [Si(OH)$_4$] using B/Si of diatoms recovered from seafloor sediment.

5.4.5 How and why do diatom and sponge B/Si systematics differ?

An interesting finding of this study is that B/Si ratios of diatoms correlate well with ambient Si(OH)$_4$ concentrations, whereas sponge B/Si do not. The B/Si ratios measured in sponges from the Southern Ocean (Chapter 2) range from 2.12 to $5.63 \pm 0.23 (2\sigma)$
mmol/mol over a seawater [Si(OH)_4] of 2.02 to 98.45 ± 0.01 (2σ) μmol. Diatom B/Si ratios spanned a larger composition range, 0.91 to 20.97 mmol/mol, over seawater [Si(OH)_4] ranging from 10.95 to 79.07 μmol. The boron isotope ratios of the sponge samples indicate boron uptake is linked to silica uptake and polymerisation (Chapter 4), and it might therefore be expected that B/Si ratios would display similar systematics to diatom B/Si. However, the biomineralisation processes of diatoms and sponges are very different, and although boron uptake is likely linked to silica uptake and polymerisation in both organisms, trace element incorporation is more complex than a simple first-order relationship, and in the case of sponges is mediated by various enzymes and transmembrane transporters. It is therefore not surprising that diatom and sponge B/Si do not show the same relationship with silica concentrations.

The boron isotope composition of diatoms would likely shed more light on the differences (or similarities) between sponge and diatom boron geochemistry. Unfortunately, attempts to measure boron isotope ratios in diatoms for this study were unsuccessful, although preliminary results suggest diatom δ¹¹B signatures are lighter and cover a smaller range relative to sponges.

5.5 Conclusion

Diatom culture experiments indicate a marked correlation between B/Si ratios in diatom frustules and seawater [Si(OH)_4] for the diatom T. pseudonana. This relationship likely arises because boron uptake by diatoms is relatively constant and as a result B/Si reflects the silica uptake and deposition rate which is a function of substrate (Si(OH)_4) concentration.

The potential for diatom B/Si to be used as a tool for seawater Si(OH)_4 palaeo-reconstruction is demonstrated by applying the empirical relationship established in this
study to diatoms extracted from core E33-22, from the Pacific sector of the Southern Ocean. Distinct shifts in B/Si coincide with changes in silica supply to the surface waters, reported by Anderson et al (2009).

This study reveals the potential utility of diatom B/Si compositions as a palaeoceanographic [Si(OH)₄] proxy. Clearly, further work is required to understand how the B/Si compositions of different diatom species varies as a function of Si(OH)₄. This is particularly important given the uptake mechanism of boron by diatoms remains unknown, and silica utilization is likely to vary between species making reliable reconstructions likely to be reliant upon empirical species specific calibrations.
Chapter 6

Summary and conclusions

6.1 Summary

The broad aim of this thesis was to generate new data on the boron geochemistry of biogenic silica and investigate its potential as a palaeoceanographic archive. This required developing analytical protocols for the analysis of B/Si and $\delta^{11}$B values in biogenic silica using a combination of solution ICPMS, laser ablation ICPMS and positive ion TIMS, and has resulted in new and novel datasets of boron concentration and $\delta^{11}$B values in siliceous sponge spicules and diatom frustules grown in nature and in culture experiments. In turn, these data have been compared to contemporaneous oceanographic data in order to address the specific questions: 1) is the boron geochemistry of biogenic silica related to seawater pH?; 2) is it a viable palaeoceanographic tool?

Twenty-four live-collected sponge samples from the Southern Ocean were analysed. This dataset expands upon existing boron concentration data from Furst (1981) (Chapter2) and provides the first reported $\delta^{11}$B values for sponge spicules (Chapter 4). Diatom B/Si ratios and $\delta^{11}$B values were also measured (Chapter 5) from both cultured and marine sediment samples, adding substantially to the existing published data (Ishikawa and Nakamura 1992; Mejía et al 2013).

The main findings of this thesis can be summarised as follows:

- The high boron concentrations in sponge spicules reported by Furst (1981) are confirmed. Boron/silicon ratios range from 2.12 to 5.63 ± 0.23 (2$\sigma$) mmol/mol,
which is equivalent to 382 to 1014 parts per million (ppm), the highest known values for boron in natural material, other than boron minerals.

- The B/Si ratio values of marine sponge spicules do not correlate with seawater pH, nor with other environmental variables such as salinity, temperature or seawater silica concentrations (Chapter 2);

- Sponge spicule $\delta^{11}$B values range from $+5.8$ to $+24.5 \pm 2.2\%$ (2σ), a much larger range than observed in any other marine boron reservoir. Sponge $\delta^{11}$B values show no relationship with seawater pH, but do have a significant positive correlation with seawater silica concentrations ($R^2=0.71$, $p<0.0001$; Chapter 4). This suggests boron isotope fractionation is linked the rate of silica polymerization, which is a function of seawater silica concentration.

- Diatom B/Si ratio values correlate with seawater silica concentration, indicating a link between boron and silicon uptake (Chapter 5). This relationship for the diatom species *Thalassiosira pseudonana* can be described by an empirical partition coefficient ($K_D$) of $3.05 \pm 0.92 \times 10^{-5}$ (±2σ), that further suggests diatom B/Si may be a useful proxy for seawater [Si(OH)$_4$].

These findings have several implications. The links between boron geochemistry and seawater silica concentrations give rise to the potential for new methods for reconstructing past silica concentrations in both surface and deep waters. The most promising silica proxy is the B/Si compositions of diatom frustules, which are relatively straightforward to analyse and can be calibrated for different diatom species by culture experiments. Similar reconstructions using sponge spicule $\delta^{11}$B are intrinsically more difficult, and will require a better understanding of the variations in boron isotope fractionation between sponge species and during uptake and incorporation from seawater, but appear to have the potential to complement silicon isotope studies of sponge spicules.
6.2 Future work

This study has raised interesting questions about boron geochemistry in sponges and diatoms that warrant further investigation to both probe further into the relationship between boron geochemistry and seawater silica concentrations, and to address some of the limitations of the present study.

More work is required to test the relationship between diatom B/Si and seawater silica concentration, particularly in order to assess whether diatom B/Si can be applied to palaeoceanographic reconstructions, and to extend experimental culture results beyond the single diatom species (*T. pseudonana*) and seawater variable (silica concentration) examined here. Although the results of the current study are promising, a more robust assessment would require testing for the effects of seawater pH variation in order to determine whether boron speciation is linked to B/Si ratios. Culture experiments by Mejía *et al* (2013) found a positive correlation between seawater pH and diatom boron concentrations, suggesting active uptake of $\text{B(OH)}_4^-$ by diatoms. The variation in boron concentration as a function of pH is much smaller than that as a function of silica concentration measured in this study, but the effects of pH cannot be ignored and are crucial to understanding how boron is incorporated into diatom frustules, and whether boron in diatoms may be a useful palaeoceanographic archive. In addition to more experimental testing of the effects of seawater pH and silica concentrations, the use of different diatom species, particularly Southern Ocean species, will be important to calibrate the empirical relationship between diatom B/Si and seawater silica concentrations.

The application of empirical calibrations of diatom B/Si – [Si]$_{sw}$ to marine sediment cores should be extended to core locations where there is a larger variation in silica concentrations over glacial-interglacial periods, such as south of the Antarctic Polar Front,
or from the Atlantic sector of the Southern Ocean, where changes in [Si(OH)₄] are thought to be more pronounced (Chase et al 2003). This would provide a clearer picture of how diatom B/Si varies with silica concentrations, and whether an empirical calibration from cultured samples can be accurately applied to core sediment. Ideally, a sediment core that has previously been used for silica reconstructions with δ³⁰Si should be sought, as this would provide a more rigorous test of diatom B/Si for palaeoceanographic applications.

The interpretation of boron isotope (and elemental ratio) data from live-collected sponge spicules was also somewhat constrained by the availability of samples, which limited the ability to deconvolve species-specific isotope fractionation. Although samples were selected from as broad a depth range as possible in order to sample large variation in environmental conditions, because different environments are inhabited by different species, this sampling strategy meant there could be little control over which species was sampled. This is an important issue to address before this work is extended to spicules extracted from sediment cores as it is usually not possible to identify to which species a spicule belongs once it is no longer bound to the sponge body.

This study provides new insight into a subject matter in biogeochemistry with little precedent, and so was largely exploratory and involved novel applications of existing analytical procedures. Consequently, the most significant limitations in undertaking this project were analytical capability, namely difficulties measuring boron isotopes by PTIMS. The sample separation and purification methods were developed using modern sponge samples which were available in large quantities and had high boron concentrations. However, adapting these procedures to much smaller samples – diatoms – proved difficult, and although there was some success measuring large samples of live-collected phytoplankton and diatomaceous chert, most modern and sediment samples did not yield enough boron to analyse with PTIMS with the required accuracy and precision. These
shortcomings meant the question of whether the boron geochemistry of diatoms is
determined by pH or other environmental variables could only be addressed in part.

Therefore, different analytical methods will likely need to be used to measure $\delta^{11}$B in
diatoms. The analytical capabilities of PTIMS restricted this study to analysis of boron
concentrations only, but the results indicate isotope fractionation might also provide
important insights into how boron is incorporated into diatom frustules, the relationship
between boron and silica uptake, and consequently, whether the $\delta^{11}$B analysis of diatoms
is a useful palaeoceanographic tool. Analysis by multicollector ICPMS would likely yield
better results for these small sample sizes.

This study is one of only a small number to investigate the boron geochemistry of biogenic
silica, and has produced data with new and unexpected results. Hence there remains room
for a broader exploration of the role boron might play in the growth or function of
biogenic silica, and what boron geochemistry can tell us about biosilification and the
ocean's silica cycle.
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