Isotope-based reconstruction of the biogeochemical Si cycle

Implications for climate change and human perturbation

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Abstract

The global silicon (Si) cycle is of fundamental importance for the global carbon cycle. Diatom growth in the oceans is a major sequestration pathway for carbon on a global scale (often referred to as the biological pump). Patterns of diatoms preserved in marine sediment records can reveal both natural and anthropogenic driven environmental change, which can be used to understand silicon dynamics and climate change. Si isotopes have been shown to have great potential in order to understand the Si cycle by revealing both past and present patterns of dissolved Si (DSi) utilization, primarily when diatoms form their siliceous frustules (noted as biogenic silica, BSi). However, studies using Si isotopes are still scarce and only a few studies exist where stable Si isotopes are used to investigate the biogeochemical Si cycle in aquatic systems. Therefore, this thesis focuses on developing analytical methods for studying BSi and DSi and also provides tools to understand the observed Si isotope distribution, which may help to understand impacts of climate change and human perturbations on marine ecosystems. The Baltic Sea, one of the biggest estuarine systems in the world, was chosen as the study site. BSi samples from a sediment core in Bothnian Bay, the most northern tip of the Baltic Sea, and diatom samples from the Oder River, draining into the southern Baltic Sea were measured and reported in Paper II and III, after establishing a method for Si isotope measurements (Paper I). Si isotope fractionation during diatom production and dissolution was also investigated in a laboratory-controlled experiment (Paper IV) to validate the observations from the field. The major result is that Si isotope signatures in BSi can be used as an historical archive for diatom growth and also related to changes in climate variables. There is isotopic evidence that the Si cycle has been significantly altered in the Baltic Sea catchment by human activities.

Keywords: diatoms, biogenic silica (BSi), dissolved Si (DSi), Si isotope fractionation, the Baltic Sea
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This doctoral thesis consists of a summary and four papers, referred to by the Roman numerals. Below is the list of the four papers.

Paper I:


Paper II:


Paper III:


Paper IV:


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All the work in this thesis has been carried out by the author with the exception for Si isotope measurements before 2005 (Land, M.) in Paper I, sampling of the sediment core and measurement of BSi content (Conley, D.) and calculation of mixed layer depth (Gustafsson, B.) in Paper II, sampling of diatoms and concentration measurements (Pastuszak, M.) in Paper III, diatom cultivation (Olofsson, M.) in Paper IV. All of the co-authors contributed to the four papers by important scientific discussion and text revision.

Xiaole Sun

Stockholm, August 2012
To my grandpa, Yao, I know you would have been proud of what I have achieved today.
1. Introduction

The aquatic ecosystems on Earth have been tremendously influenced by human activities (Carpenter et al., 1992). Ecosystem response depends mainly on changes in the hydrological cycle and variations in environmental cycles of carbon and other nutrients like N, P and Si. Perturbations of these cycles, natural or anthropogenic, have been given considerable interests in the past decades (Chahine, 1992; Falkowski et al., 2000; Gruber and Galloway, 2008; Laruelle et al., 2009). However, until now, most of these studies have focused on carbon budgets due to carbon ubiquity in the biosphere and nitrogen and phosphorus because of eutrophication as a result of human activities. Silicon (Si) is to a lesser extend investigated in the study of nutrient cycling in ecosystems.

The importance of studying the biogeochemical Si cycle arises from its close relation to the global carbon cycle (Berner, 2004). About three quarters of the primary production in coastal and nutrient rich areas of the world oceans is carried out by diatoms, a phytoplankton group that essentially dissolved Si (DSi) to form their unique siliceous frustule, noted as biogenic silica (BSi). In low nutrient areas diatoms still contribute to about 30% of the marine primary production (Nelson et al., 1995). Diatoms transport carbon from atmosphere to deep oceans by sedimentation, a process also known as the "biological pump" (Buesseler, 1998; Goldman, 1988; Nelson et al., 1995). Moreover, diatoms also form the foundation of the shortest desirable food chains, (Cushing, 1989; Ryther, 1969). Diatoms are sensitive to environmental change because of their direct response to many physical, chemical, and biological changes. When DSi concentrations becomes low, it could severely limit diatom biomass build up, leading to ecosystem shifts from production of diatom to non-siliceous algae (Officer and Ryther, 1980), possibly leading to harmful algae blooms and decreased Si export to the open ocean (Dugdale et al., 1995). After diatoms die, preservation of BSi in sediments allows for investigating diatom production over time (Conley and Schelske, 2002).

Silicon is the second most abundant element in the continental crust after oxygen, 28.8 wt% (Wedepohl, 1995), and weathering of silicate minerals are the main source for DSi and silicate containing particles in aquatic systems. From weathering sources to the ocean, DSi transported by rivers serves diatoms as an indispensable nutrient in many biogeochemical processes (Ragueneau et al., 2006; Ragueneau et al., 2000). This makes BSi and DSi potential proxies for investigating environmental change.

Biogenic silica in sediments have been successfully used for reconstructing past environmental changes including climatic variations, for example relating diatoms to temperature (Rosén et al. 2000, Blass et al. 2007, McKay et al. 2008) and to pH and changes in nutrient concentrations (Andren et al., 1999, Braak and Dame 1989, Bennion et al. 1996, Weckström 2006, Olli et al. 2008). However, these studies are associated with large uncertainty because of the difficulty to discriminate between biological and physical processes as driving forces for the observed variations in BSi records. Here this thesis will show that Si isotope signatures in DSi and BSi can be of good complementary use for constraining those uncertainties.

Si isotope fractionation by diatoms

Silicon has three stable isotopes $^{28}$Si, $^{29}$Si, $^{30}$Si with natural abundances of 92.22%, 4.69% and 3.09%, respectively (De Laeter et al., 2003) and one naturally radioactive isotope, $^{32}$Si with a half-life of 178±10 years (Nijampurkar et al., 1998).

It has been shown that diatoms preferentially take up $^{28}$Si to produce BSi, resulting in an isotopic fractionation ($e_{30/28}$ value) of approximately -1.1‰ (De La Rocha et al., 1997); Later, the $e$-value was shown to be -1.08‰ in freshwater (Alleman et al., 2005; Hughes et al., 2011) whereas as a range of -0.6‰ to -2.2‰ for in situ measurements in oceanic water was indicated (Cardinal et al., 2005; Cardinal et al., 2007; De La Rocha et al., 1997; Varela et al., 2004). The documented $\delta^{30}$Si values in BSi of diatoms ranges from -0.3‰ to +2.6‰ (Alleman et al., 2005; Cardinal et al., 2007; De La Rocha et al., 1998; Varela et al., 2004), which is slightly higher values compared to BSi observed in phytoliths produced in terrestrial systems by vascular plants, ranging from -1.7‰ to +2.5‰ (Douthitt, 1982; Ziegler et al., 2005). During the formation of BSI, BSI (residual) is enriched in heavier Si isotopes. In river waters, the measured $\delta^{30}$Si values has been shown to range from +0.4‰ to +3.4‰ (De La Rocha et al., 2000; Ding et al., 2004; Ding et al., 2011; Engström et al., 2010; Georg et al., 2007; Hughes et al., 2011). DSi in a soil solution shows $\delta^{30}$Si values ranging from -0.8‰ to +1.7‰ (Ziegler et al., 2005) and a similar range of -0.2‰ to +1.3‰ is found in groundwater (Georg et al., 2009; Opfergelt et al., 2011). In seawater, the $\delta^{30}$Si values in DSi range from +0.5‰ to +3.2‰ (Beucher et al., 2008; Cardinal et al., 2005; De La Rocha et al., 2000; Reynolds et al., 2006; Varela et al., 2004). Demarest et al. (2009) showed a Si isotope fractionation of -0.55‰ in enriching residual BSI with heavier Si isotopes observed during BSI dissolution of diatoms sampled during
from the Southern Ocean if > 20% of BSi was dissolved.

The range of observed Si isotope values imply that tracing enriched or depleted BSi caused by diatom-inferred environmental changes and evolvement with time in geological records is possible.

**Quantitative relationship between the Si isotopic compositions of BSi and DSi**

Because of the discrimination against heavier Si isotopes during diatom production, \( \delta^{30}\text{Si} \) values of DSi and BSi during diatom production follows a Rayleigh distillation behaviour (Fig. 1), which describes the partitioning of Si isotopes in a closed system between two Si reservoirs: DSi and BSi. Comparing a closed and open system, as the fraction of DSi remaining in water \( f \) decreases i.e. diatom production, the \( \delta^{30}\text{Si} \) values of DSi and the produced BSi increases. At the same time, \( \delta^{30}\text{Si} \) values of accumulated BSi in both systems also increase as DSi concentrations decreases, but only up to the initial \( \delta^{30}\text{Si} \) value of DSi. This means that the accumulated BSi can never reach a \( \delta^{30}\text{Si} \) value higher compared to the initial DSi. \( \delta^{30}\text{Si} \) values of instantly produced BSi in a closed system differs always by -1.1‰ (\( \varepsilon \)-value) compared to remaining DSi, if assuming a fractionation factor (\( \alpha \)) of 0.9989 (De La Rocha et al., 1997). In contrast for an open system, the difference between \( \delta^{30}\text{Si} \) values of the whole pools of DSi and BSi is always 1.1‰.

**2. Thesis objectives**

So far Si isotope data in the literature are still scarce, and only a few studies have attempted to use stable Si isotopes as tracers for understanding the biogeochemical Si cycle and its variations in aquatic systems. Therefore, the aim of this thesis is to provide

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**Fig. 1 Expected Si isotope variations in a closed and an open system.**

**Fig. 2 Flow chart of the four papers in the thesis and their specific objectives**

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1. **Paper I** to develop an analytical technique for precisely and accurately determine the stable Si isotopes using MC-ICP-MS.
2. **Paper II** to investigate the variations in time of diatom production vs. climate change.
3. **Paper III** to examine the enrichment of heavier Si isotopes following seasonal diatom production patterns.
4. **Paper IV** to investigate the Si isotope fractionation factor during diatom production and dissolution.
3. Site description

The Baltic Sea is located in Northern Europe, from 53°N to 66°N latitude and from 20°E to 26°E longitude and is usually separated into seven basins: Bothnian Bay, Bothnian Sea, Gulf of Finland, Baltic Proper, Gulf of Riga, Arkona and Kattegat (Fig. 3).

The Baltic Sea is a semi-closed brackish water body, but also one of the many aquatic ecosystems in the world that show long-term decreasing DSi concentrations in the water column, especially from 1970 to 1990 (Conley et al., 1993; Sandén et al., 1991). Due to the decrease in DSi concentration the biogeochemical cycle of Si in the Baltic Sea has changed within the 20th century. One of the reasons is reduction of DSi in riverine transport due to retention of Si in dams (Conley, 2002; Humborg et al., 2000). Changes in water flow paths due to river regulations leading to less weathering, is also a possible explanation for reduction in Si concentrations (Humborg et al., 2002; Humborg et al., 2004). Another reason is eutrophication (Conley et al., 1993; Schelske et al., 1983), which stimulate diatom production and simultaneously increases the accumulation of BSi in sediments of the Baltic Sea (Conley et al., 2008).

The diatom spring bloom in the Baltic Sea is sometimes terminated by the depletion of DSi (Smetacek, 1985), especially in the Gulf of Riga. DSi limitation may occur during certain periods of the year (Olli et al., 2008). Studies of different aspects of the biogeochemical Si cycle in the Baltic Sea including riverine Si fluxes, estuarine Si fluxes, and concluded that the decreasing DSi concentrations is an on going problem. This decrease might ultimately lead to Si limitation in the entire Baltic Sea. However, recent reports show the decreasing DSi concentrations are levelling out instead of declining further (Papush and Danielsson, 2006).

In all, the Baltic Sea is a good site for studying the interactions between diatoms and Si under human perturbation. In this thesis, two specific sites have been chosen for more detailed studies: one is the northern part of the Baltic Sea, Bothnian Bay and the other is the Oder River draining into the southern Baltic Sea (Fig. 3).

Bothnian Bay

Bothnian Bay (235 499 km²) is the northern portion of the Baltic Sea (Fig. 3). The area has been subjected to recurring glaciations. The last permanent ice melted from the area about 9300 years ago. The average depth of the bay is 43 m and maximum depth is 147 m. The catchment area is sparsely populated and consists mainly of forest, wetland and only a small amount of agriculture land (~1%). The total water volume of the bay is about 1500 km³, which is 7% of the total water volume of the Baltic Sea. The total water discharge into the Bothnian Bay is 97 km³ yr⁻¹ (Humborg et al., 2008) and 60% of the water is derived from rivers regulated by dams. River regulation with the purpose of generating electricity started in the beginning of the 20th century and continued until ca. 1970, with most of the dam constructions between 1940 and 1970, e.g. Kemijoki and Luleå River (Humborg et al., 2006). A consequence is decreased fluxes of weathering related elements such as base cations and Si (Humborg et al., 2002; Humborg et al., 2000), due to changes in water pathways through the catchments and particle trapping of BSi behind dams (Humborg et al., 2006). This has led to a decreased DSi load in the northern Baltic Sea (Humborg et al., 2007).

Temperature variations in the water column are > 15 °C annually. Formation of a winter ice cover in Bothnian Bay starts from October to the end of November. The entire bay is generally covered by ice, even during the mildest winters, and the ice cover often remains for more than 120 days per year. During the years 1961-1990, the summer (ice-free period) average maximum surface temperature in the open sea is around 16 °C (Dannenberger et al., 1997).

A surface thermocline in Bothnian Bay develops about a month earlier than in the other sub-basins of the Baltic Sea and lasts for about three months. Mean DSi concentrations in Bothnian Bay observed between 1970-2001 are 31 µM in winters and decrease to an average concentration of 23 µM in summers (Danielsson et al., 2008). Residence time for DSi is approximate 3.3 years (Papush et al., 2009).

The Oder River

The Oder River is one of the largest rivers in the catchment basin of the Baltic Sea. For the first 112 km from its source, it passes through the Czech Republic, but its middle reach (a distance of 186 km) constitutes the boundary between Poland and Germany before reaching the Baltic Sea via the Szczecin lagoon (Fig. 3). The total length of the Oder River is 854 km, of which 738 km lies in Poland. The total watershed area has been calculated to 119 000 km², of which about 90% is in Polish territory. The annual discharge of the Oder River is 17 km³ (Radziejewska and Schernewski, 2008). 62% of the river basin is in agricultural use, of which 54% is arable land, 8% grassland and 32% of the area is covered by forest.
Fig. 3 Map of the Baltic Sea area (left) and two maps of study sites, Bothnian Bay (upper right) and the Oder River (lower right, data from source of USGS and HELCOM). BB=Bothnian Bay, BS=Bothnian Sea, GF=Gulf of Finland, GR=Gulf of Riga, BP=Baltic Proper, AR=Arkona, KA=Kattegat.
Increased anthropogenic influence has accelerated eutrophication in the Oder River over the past 50 years. Intensive algal blooms, low water transparency, oxygen depletion in some parts, and fish mortality have become common. The present state of the Oder Lagoon is described as eutrophic, hypertrophic or polytrophic, depending on the trophic system (Radziejewska and Schernewski, 2008).

4. Materials and methods

Silicon isotope analysis using MC-ICP-MS (Multi Collectors Inductively Coupled Plasma Mass Spectrometry) requires a relatively free-of-matrix solution for obtaining precise and accurate results, therefore, a series of sample preparation steps are adopted in this thesis, and these methods can be possibly applied to various types of Si samples (Fig. 4).

Diatom extraction

The method for sediment diatom extraction in Paper II is a procedure modified from Morley et al. (2004). Organic and inorganic carbon was removed by mixing samples with 15% H2O2 and 10% HCl. After washing the samples (centrifuging between each wash) each sample was sieved through a 10 μm sieve cloth and an 80 μm steel mesh to recover the 10-80 μm fraction (which contains most of the diatoms) and to remove silt and clay. The sieved samples were collected in centrifuge tubes containing sodium polytungstate (SPT). The density of the SPT was adjusted to be between 1.8 g cm⁻³ and 2.3 g cm⁻³ to separate diatoms from the remaining residuals. This was followed by drying diatom samples at 40 °C.

Diatom alkaline digestion

Clean diatom samples derived from sediments in Paper II and from filtrated river water in Paper III were fused by using solid sodium hydroxide (NaOH) in Ag crucibles at 730°C in a muffle furnace and thereafter washed with MQ-e water and collected in a 0.12 M HCl solution (Georg et al., 2006b).

Another wet digestion method was used in Paper IV, which was adopted from Raguenue et al. (2005). A 0.25 mol L⁻¹ NaOH solution was added into tubes which contained freeze-dried diatoms collected on polyethersulfone filters. HCl was used to stop the digestion by neutralising the pH. After centrifugation, the supernatant was diluted for further treatment.

DSi extraction from seawater

Dissolved Si in the Baltic Sea water samples in Paper IV was extracted by a magnesium two-step co-

precipitation technique adopted from Reynolds et al. (2006). A 1 mol L⁻¹ NaOH solution was added into seawater samples and this was repeated twice. After each addition, samples were shaken and left for 1 hour for precipitating Mg(OH)₂ with Si co-precipitated, and thereafter samples were centrifuged to collect the precipitates. The precipitates were dissolved in 4 mol L⁻¹ HCl. The Si concentration of the removed supernatant was checked to make sure that the Si removal was complete.

Chromatographic purification

The final solutions derived from the methods contain a cationic matrix. Therefore a chromatographic separation method is required to purify the Si solution prior to Si isotope analysis. The used purification process was modified from Georg et al. (2006b). Cation exchange resin AG 50W-X12 (100-200mesh) in H⁺ form was filled in chromatography columns. The samples were loaded on the column, in form of H₄SiO₄, which passed through the resin whereas the resin retains the cations.

Silicon isotope analyses

Silicon isotope analyses are described in detail in Paper I using an IsoProbe MC-ICP-MS equipped with a hexapole collision cell at the Laboratory for Isotope Geology, Swedish Museum of Natural History. Due to instrument limitation of only having low-resolution mode (m/Δm ~ 450), measurement of δ²⁸Si values is not possible with the current set up. The instrumental mass discrimination caused by a number of processes in the plasma and in the transmission through the ion optical system (Becker, 2007) was corrected by using the standard-sample-standard bracketing technique. The sensitivity for δ²⁸Si is ~12 V/ 36 µmol L⁻¹ Si (1 mg L⁻¹), with an uptake rate of ~70 µl min⁻¹ via a Cetac® Aridus desolvating nebulizer. The Si isotope ratio of each sample is calculated and is given in δ²⁸Si values (‰) relative to the accepted reference material for silicon isotopes, NBS 28 according to Eq.1. All of the δ³⁰Si values in this thesis are calculated from the relationship δ³⁰Si = δ²⁸Si×1.96, assuming mass-dependent fractionation (Reynolds et al., 2007).

\[
\delta^{28}Si = \left(\frac{^{28}Si}{^{29}Si}\right)_{sample}^{\frac{1}{2}} - \left(\frac{^{28}Si}{^{29}Si}\right)_{standard} \cdot 1000
\]  

Eq.1
Fig. 4 Sample preparation steps prior to Si isotope analysis using MC-ICP-MS.
5. Summary of results

The results of the four papers are briefly described below.

**Paper I: Stable silicon isotope analysis on nanomole quantities using MC-ICP-MS with a hexapole gas-collision cell**

Paper I reports a method developed for precisely and accurately measuring the $\delta^{29}$Si value (2S.D., 0.2‰) using MC-ICP-MS with a total Si consumption of less than 14 nmol (390 ng Si). Data was collected during a four-year period (2005-2009) and the average $\delta^{29}$Si value of IRMM-018 relative to NBS-28 was measured to -0.95‰ (n=23, 2S.D. 0.16‰), with a 95% confidence interval of ±0.028‰. The mean $\delta^{29}$Si value of the Big-Batch standard was found to be -5.50‰ (n=6, 2S.D. 0.26‰). This demonstrates that the used methods give long-term reproducibility with respect to both precision and accuracy. However, it was also shown that the presence of cations, such as Mg, Na, Al etc., can cause severe matrix effects on the Si isotope measurements.

**Paper II: Climate dependent diatom production is preserved in biogenic silica isotope signatures**

Paper II is the first case study using the method developed in Paper I. The obtained Si isotope values in diatoms are used to reconstruct diatom production in Bothnian Bay. The data are based on down-core analysis of Si isotope distribution in BSi covering the period 1820 to 2000. The sediment core is dated using $^{210}$Pb. Results show a linear $^{210}$Pb decay relation with depth in the sediment core ($r^2 = 0.97$). This linearity suggests minimal bioturbation, indicating an average sedimentation rate in this Bothnian Bay core of approximate 2.1mm yr$^{-1}$. The $\delta^{30}$Si values of BSI ranges between -0.18‰ and +0.58‰. By assuming Rayleigh distillation, diatom production can be calculated as fraction of remaining DSI (f values) inferred from measured Si isotope values. This reveals that the sediment core record can be divided into two periods, an unperturbed period from 1820 to 1950 and another period affected by human activities from 1950 to 2000 (Fig. 5). The shift in Si isotope values toward higher values after 1950 is most likely caused by large scale damming of rivers.

The production is shown to be correlated with air and water temperature, which in turn were correlated with the mixed layer depth (ML). The deeper ML depth observed in colder years resulted in lower diatom production. These findings corroborated by pelagic investigations in the 1990’s, have clearly shown that the ML depth controls diatom production in the Baltic Sea (Wasmund et al., 1998). Especially after cold winters and deep water mixing, diatom production was low and DSI concentrations were not depleted in the water column after the spring bloom.

![Graph](https://via.placeholder.com/150)

**Fig. 5** Fraction of the remaining DSI (f values) in the Bothnian Bay water column and f values calculated from observations during the summers 1980 to 2000, plotted together with average summer air temperature through years. The inferior and superior error bars on f values are the first quartile and third quartile for each f value derived from the Monte Carlo simulations (Paper II).
Paper III: Silicon isotope enrichment in diatoms during nutrient-limited blooms in a eutrophied river system

The Oder River is a eutrophied river entering the southern Baltic Sea. The river concentrations in DSI, DIN and DIP display similar variations, i.e. decreasing throughout the spring and summer and increasing in the autumn. In contrast to the dissolved nutrients, BSI shows increasing concentration, which indicates a period of rapid diatom growth in spring and summer, followed by decreasing concentration in late summer and autumn. The analysis of Si:N:P ratios indicates that the diatom production in the Oder River is limited by different nutrients throughout the studied period.

The $\delta^{30}$Si values increased from a minimum value of $+0.75\%o$ in the spring to a maximum of $+3.05\%o$ at the beginning of August, which are some of the highest values ever recorded in freshwater diatoms. The $\delta^{30}$Si values started to decrease in late August, and in September the $\delta^{30}$Si values reached $+1.09\%o$, similar to observed values in April. We suggest that dissolution of BSI becomes more important later in the summer when phosphate limits the diatom production. The Rayleigh model used here to predict $\delta^{30}$Si values of DSI suggests that the initial value is close to $+2\%o$ (before the start of the diatom bloom). This means that there is also a biologic control of the Si entering the river and this is probably caused by Si isotope fractionation during uptake of Si in for example phytoliths.

Paper IV: Effect of diatom growth and dissolution on silicon isotope fractionation in an estuarine system

Previous studies imply that knowledge about Si isotope fractionation is required for a better understanding of the biogeochemical cycle of Si, in estuarine and coastal areas where primary production stands for some 30-50% of the global marine production. Paper IV reports systematical measurements of variations in Si isotope values of DSI and BSI following diatom growth and dissolution of BSI to investigate Si isotope fractionation patterns during these processes. Two species of diatoms from the Baltic Sea, were selected, *Thalassiosira baltica* (TBTV1) and *Skeletonema marinoi* (SMTV1). The Chl a (chlorophyll a) concentrations of the two species increased exponentially, while DSI concentrations decreased during the initial growth period, reaching a level of 200 $\mu$g L$^{-1}$ for TBTV1 and 530 $\mu$g L$^{-1}$ for SMTV1. The DSI concentrations during growth days decreased progressively and the experiment was terminated when DSI concentrations were below 0.4 $\mu$mol L$^{-1}$. The Si isotope values measured in the two species varied by more than $0.7\%o$ for $\delta^{29}$Si and $1.3\%o$ for $\delta^{30}$Si, ranging from $+0.6\%o$ up to $+1.3\%o$ for $\delta^{29}$Si, and from $+1.2\%o$ up to $+2.6\%o$ for $\delta^{30}$Si. The measured $\delta^{29}$Si values in seawater samples increased from $+1.35\%o$ to $+2.58\%o$, $\delta^{30}$Si values from $+2.64\%o$ to $+5.07\%o$, calculated by multiplying $\delta^{29}$Si values with 1.96 (Reynolds et al., 2007). The two species fractionate the Si isotopes during their growth identically (Fig. 6); $^{29}\alpha$ fractionation factors were $0.9993 \pm 0.0004$ ($2\sigma$, TBTV1) and $0.9992 \pm 0.0004$ ($2\sigma$, SMTV1). The average value for $^{29}\alpha$ for all diatom sample measurements was $0.99925 \pm 0.0003$ ($2\sigma$). The calculated average value for $^{30}\alpha$ was $0.9984 \pm 0.0004$ ($2\sigma$).

![Graph](Fig. 6 Plot of Si isotope compositions of diatoms and DSI following diatom growth as a function of f values (fraction of remaining DSI in water). Lines are expected change for Rayleigh fractionation behaviour in a closed system. Error bars of Si isotope values are $2\sigma$ (Paper IV).)
During the dissolution experiment, the Chl a concentrations of SMTV1 gradually decreased to 267 µg L⁻¹, while the DSI concentration increased steadily for 20 days to 50 µmol L⁻¹, and was constant (50-60 µmol L⁻¹) for another 20 days, indicating dissolution of diatom frustules. During the first 25 days in the dark, the siliceous frustules were linearly dissolved (r²=0.9) and 75% of the Si was released back to the medium. Thereafter, during the last 10 days, 90% of the initial BSi was dissolved and released back to the medium. The Si isotope values of SMTV1 and DSI seemed constant during dissolution. Calculation gives the Si isotope fractionation factor during dissolution for ²⁹Si and ³⁰Si as 0.9999 ± 0.0002 and 0.99974 ± 0.00022 (2σ). This is in contrast to findings from an open ocean species, where Si isotope fractionation during dissolution was observed (Demarest et al. 2009).

6. Discussion
Reconstruction of DSI utilization by δ³⁰Si values
To understand the biogeochemical Si cycle and its link with climate, Si isotope signatures of BSi have been used to trace diatom production by reconstructing DSI utilization in oceans e.g. Reynolds et al. (2006), Beucher et al. (2008), De la Rocha et al. (1998), van den Boorn et al. (2010) and in lakes, e.g. Street-Perrott et al. (2008), Opfergelt et al. (2011). However, to date, no studies have reconstructed DSI utilization by diatoms in estuaries or in coastal areas. Estuaries, with gradients in water turnover times, salinity, nutrients and primary production, are also considered to be of significance for the global biogeochemical nutrient and carbon cycles (Fry, 2002; Voss et al., 2000). Paper II attempts to reconstruct DSI utilization during the past 200 years using a sedimentary record of δ³⁰Si values in preserved BSI in Bothnian Bay.

Important to all of these applications is the uncertainty in isotope fractionation during Si uptake by diatoms used in the Rayleigh model (Fig. 1), no matter which aquatic ecosystems diatoms live in. A few studies have investigated the variability in the Si isotope fractionation factor during diatom growth among different species (De La Rocha et al., 1997), in the oceans (Street-Perrott et al., 2008; Varela et al., 2004) and in lakes (Alleman et al., 2005). A study by Demarest et al. (2009) also looked into the Si isotope fractionation during diatom dissolution and showed that a Si isotope fractionation factor of -0.55‰ during dissolution may complicate this Si isotope-based reconstruction of DSI utilization. Again, there are no such studies in estuaries. Paper IV examines Si isotope fractionation patterns during BSI production and dissolution using diatoms isolated from the Baltic Sea. The two species of diatoms examined exhibit an identical Si isotope fractionation factor during growth. This is in agreement with the Si isotope fractionation factors in the oceans and in freshwater. However, no Si isotope fractionation during dissolution was observed, even after 90% of the diatoms were dissolved. This may be attributed to the smaller size of the diatoms living in estuarine systems with lower salinity compared to the open ocean. This suggests that information about the original environmental conditions in estuarine and even coastal systems is directly retained. However, in terms of the complexity of the mechanism behind those biological/physical/chemical processes, these kinds of studies are important but are still scarce.

Our knowledge of the current diatom production in water and the historical diatom production derived from sedimentary records is largely a result of applying the Rayleigh model. The Si isotope fractionation factor is the most important parameter in the model. Higher Si isotope fractionation factor can cause overestimates of DSI utilization; likewise lower Si isotope fractionation factor can result in underestimates of DSI utilization. The problem to estimate DSI utilization can lead to erroneous conclusions about effects from for example climate change. The variability in Si isotope fractionation should be studied in detail in order to constrain the uncertainty in Si isotope data.

Si isotope-inferred climate variations
One important reason to trace diatom dynamics is the sensitivity of diatoms to changes in environmental conditions, for example temperature. Historical records of air and water temperature are required to be able to understand the causes and mechanisms behind current climate change and impact from human activities. Especially high-latitude systems are predicted to experience the greatest impact from climate fluctuations (Carpenter et al., 1992; Douglas et al., 1994; Smol et al., 2005). Annual average surface water temperature in the Baltic Sea has increased by 1.4 °C during the last 100 years, which is higher compared to 63 other studied large marine ecosystems (Mackenzie and Schiedek, 2007). The widely used method for reconstructing temperature is by analyzing oxygen isotope compositions of diatoms, because of a dependence of oxygen isotope fractionation between BSI and water (Swann and Leng, 2009). Variation in BSI content in sediments is also used to reconstruct air temperature (Blass et al., 2007; McKay et al., 2008).

Isotope-based reconstruction of the biogeochemical Si cycle
A major result from this thesis is to provide a possible way of connecting $\delta^{30}\text{Si}$ values and temperature. This is done by sedimentary $\delta^{30}\text{Si}$ BSI values that can be used as an archive for estimating the fraction of DSi utilization (Paper II). This fraction is a function of water turbulence and light condition, which in turn is a function of temperature. The correlation implies that higher temperature causes higher diatom production due to a shallower mixed layer depth, i.e. more diatoms can float to water surface to obtain enough light. Lower temperature leads to lower diatom production due to a deeper mixed layer depth without enough light penetration.

Temperature is predicted to increase in the future (IPCC, 2007), which in general stimulates diatom production. A model developed by Omstedt et al. (2009) indicates that the Baltic Sea acts as a source for CO$_2$ before 1950, but after 1950, the increased primary production enhances the uptake of CO$_2$ due to eutrophication, which makes the Baltic Sea work as both a sink and source of CO$_2$. This suggests that temperature-induced increased diatom production in the future may continue the enhancement of CO$_2$ uptake and possibly shift the Baltic Sea to be a sink for CO$_2$.

Although the sediment records could reveal the past climate and models could help the understanding of the ecosystem’s future, the effect of climate change on ecosystems are difficult to predict. Climate-induced increased water flux also increases nutrient transport to the Baltic Sea, which in turn stimulates diatom growth. It is clear that increased biological processes will have a major influence on the conditions for biota in aquatic systems. This will also affect species composition, distributions, and interactions in ways that are only partly understood at the present time.

**Si isotope-inferred human perturbation**

Recently, the biogeochemical Si cycle has also been perturbed by human activities. Eutrophication and hydrological alterations have been shown to cause DSi depletion and diatom growth in the Baltic Sea (Conley et al., 2008; Conley et al., 1993; Humberg et al., 2006; Humberg et al., 2008). This thesis shows that both eutrophication and hydrological alterations have most likely resulted in increasing $\delta^{30}\text{Si}$ values in the Baltic Sea.

The heavily eutrophied Oder River studied in Paper III is subject to nutrient-limited diatom growth. Depletion of DSi during the excessive diatom bloom leads to very high $\delta^{30}\text{Si}$ values in produced BSi, indicating that the Oder River is a strong source for Si with high $\delta^{30}\text{Si}$ values, which can probably be traced in the Baltic Sea.

Paper II suggests that there is a shift in $\delta^{30}\text{Si}$ values from +1.1‰ measured in a pristine Kalix river (Engström et al., 2010) to +1.4‰ (assumed in Paper II by fitting data into field measurements). This is most likely caused by river damming. Increased BSi behind dams decreases DSi concentrations, leading to increasing $\delta^{30}\text{Si}$ values in both BSi and DSi in river water.

By examining Si isotope fractionation during diatom production and dissolution (Paper IV), different Si isotope patterns in the Baltic Sea basins can be suggested. Si-limited diatom production is observed in Gulf of Riga (Olli et al., 2008), which predicts $\delta^{30}\text{Si}$ values for BSi of +0.85‰ resulting in the $\delta^{30}\text{Si}$ value of residual DSi of +3.64‰. Paper IV also shows that the entire Baltic Sea may face higher $\delta^{30}\text{Si}$ values in DSi although a Si-limited diatom production has not been observed in other basins yet. This forecast is supported by Conley et al (2008), where it is suggested that DSi will be a limiting nutrient in the Baltic Sea in the near future.

Enhanced diatom production can possibly induce oxygen depletion in the bottom water by exporting organic matter into bottom water, accelerating development of hypoxia in the Baltic Sea. This can lead to changes in biogeochemical cycles and habitat loss for fish and other biota (Conley et al., 2011; Conley and Johnstone, 1995) since sufficient oxygen is essential to support healthy biological communities (Vaquer-Sunyer and Duarte, 2009). Kabel et al. (2012) concluded that increasing temperature can substantially deteriorate bottom water conditions in the Baltic Sea by stimulating cyanobacteria blooms. Si isotope values of BSI can be used to estimate the order of magnitude of diatom blooms from a given year by analysing sediment samples. By comparing the results with cyanobacteria blooms, the findings may help us to understand what is most important for the hypoxia in the Baltic Sea: the diatom spring bloom or the summer cyanobacteria bloom. However, to what degree the hypoxic areas influences nutrient biogeochemical cycles are not yet fully understood. More studies with multiple approaches and isotope-based techniques are therefore required.

**Implications for control of $\delta^{30}\text{Si}$ values of Si inputs to the oceans**

About 85% of the DSi delivered to the oceans is carried by rivers, which constitute the major Si source for diatom production in the ocean. Rivers may exhibit large variability in concentration and fluxes of DSi and Si isotope signatures. This variability can drive a shift in Si isotopic compositions of BSI in surface waters of the ocean by changing the relative degree of DSi utilization (and therefore the Rayleigh
Isotope-based reconstruction of the biogeochemical Si cycle

model). This will finally change the Si isotope values of BSi preserved in sediments and thus influence the sedimentary records. Therefore, river inputs with variable Si flux and $\delta^{30}\text{Si}$ values carry important information for the interpretations of the oceanic Si cycles.

Many processes can cause the variability of $\delta^{30}\text{Si}$ values of DSI and BSI in rivers over time, such as river damming (Paper II) and uptake of Si by diatoms (Paper III), phytoliths (Ding et al., 2011) and terrestrial plants (Ding et al., 2008). Another process linked to climate is weathering, which also generates varying $\delta^{30}\text{Si}$ values, which are also linked climate (Georg et al., 2006a). An important process is retention of DSI and BSI in estuaries (Conley and Malone, 1992; Treguer et al., 1995). Estuaries can be effective traps for DSI and BSI and prevent Si transport to the ocean. However, these processes will be difficult to distinguish from each other since Si isotope fractionation is always small, only up to a few permil. Analysis of $\delta^{30}\text{Si}$ values combined with analysis of other indicators, such as Ge:Si and Al:Si ratios may provide additional information about processes controlling isotope composition of Si inputs to the oceans. Investigation of Si isotope signature distribution and behavior in rivers and estuaries will help to reveal internal and external Si source control in estuarine systems over time and also have implications for understanding $\delta^{30}\text{Si}$ records in the ocean.

7. Future perspectives

More studies on historical records of climate and environmental changes are needed to understand the past to improve our possibility to predict the future variations. Factors controlling uptake, storage and recycling of Si in and estuarine and coastal systems must be studied in detail. This is important in order to quantitatively assess the response of ecosystems to changes in Si inputs and climate and what impacts these changes will affect the Si cycle in the ocean. These factors will also help to constrain Si budgets for systems with different geological settings in both perturbed and pristine systems. It is also essential that future-monitoring programs includes DSI and BSI measurements and that the monitoring should be extended to samples taken before and after building of dams, as well as river mouths. This will ensure datasets and time series for evaluation and modeling.

Modeling the Si cycle both on global and regional scale is also required for constraining the uncertainties associated with technical, practical and economic limitations, and for revealing how the resilience of diatom-based ecosystems responding to possible ecosystem changes. The future priorities should be pointed out as well.

The Si isotope fractionation factor should be carefully studied before interpreting Si isotope data in different systems and Si isotope compositions in rivers also need more studies to better understand land-sea fluxes of Si.

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Paper I
PAPER
Sun et al.
Stable silicon isotope analysis on nanomole quantities using MC-ICP-MS with a hexapole gas-collision cell

COMMUNICATION
Günther et al.
Development of direct atmospheric sampling for laser ablation-inductively coupled plasma-mass spectrometry

ASU REVIEW
Atomic spectrometry update. Environmental analysis
Stable silicon isotope analysis on nanomole quantities using MC-ICP-MS with a hexapole gas-collision cell

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We demonstrate in this study that a single focusing multiple collector inductively coupled plasma mass spectrometer (MC-ICP-MS) equipped with a hexapole gas-collision cell (GV-instrument Isoprobe) can precisely determine the $\delta^{28}$Si (2S.D., 0.2‰) using a total Si consumption of less than 14 nmole (390 ng Si). Testing and evaluation of background, rinse time, and major matrix effects have been performed in a systematic way to establish a procedure to measure $\delta^{28}$Si in small quantities. Chemical purification prior to analysis is required to remove potential interferences. For data collected during a four-year period, the average $\delta^{28}$Si value of IRMM-018 relative to NBS-28 was found to be $-0.95_{\text{‰}}$ (n = 23, 2S.D. 0.16‰) with a 95% confidence interval ($-0.95 \pm 0.028_{\text{‰}}$). The mean $\delta^{28}$Si value of the Big-Batch standard was found to be $-5.50_{\text{‰}}$ (n = 6, 2S.D. 0.26‰). Although determination of the $\delta^{30}$Si measurements is not possible, with our current instrument we demonstrate that this system provides a fast and long-term reliable method for the analysis of $\delta^{28}$Si in purified samples with low Si concentration (18 μM Si).

Introduction

Silicon is one of the most abundant elements in the continental crust, 28.8 wt%, and constitutes the backbone of silicate minerals, which are continuously altered by weathering processes. Mass-balance studies of the oceanic Si cycle have demonstrated that the gross input is primarily derived from the continents (84%) as dissolved silicate (DSi). The largest part by far is supplied by river transport, but eolian transport (7.5%) is also important. Submarine weathering of basalt is an additional source primarily at sea floor hydrothermal areas. As an essential element, silicon fertilizes the seas by stimulating the production of diatoms, which are a key phytoplankton group responsible for fuelling of the pelagic and benthic food webs and dominating the biological carbon sequestration in the oceans. Weathering of continental silicates is also suggested to be a long-term regulator of atmospheric CO₂ by sequestration of CO₂ carbonates during weathering and consecutive storage in the ocean and the deep sea sediments. The silicon cycle and its variation through time are crucial for the understanding of marine productivity and carbon sequestration and stable silicon isotopes constitute a tool that can be used to study these processes. Variations in the stable silicon isotope composition ($^{28}$Si, $^{29}$Si, $^{30}$Si with abundances of 92.22%, 4.69% and 3.09%, respectively) have been documented in geological materials since the early 1950s. It has been shown that minerals formed at different temperatures were isotopically lighter (i.e. enriched in $^{28}$Si) as a function of decreasing crystallization temperature. Based on theoretical calculations, Grant predicted a positive correlation between $^{28}$Si enrichment and increasing silicon content in rocks. Another study showed that the enrichment of $^{28}$Si in coexisting minerals in igneous rocks increases in the mineral order of biotite, quartz, feldspar, and that granitic rocks were enriched the most in $^{30}$Si compared to meteorites and mafic rocks. However, like most of the other elements above 20 amu, only small isotopic variations of Si are found in nature (<10‰). Therefore, the analytical methods for determining Si isotope ratios need to have sufficient accuracy and reproducibility to determine silicon isotope ratio variations.

Currently, studies on variations in stable Si isotope ratios are focused on low-temperature processes such as biogenic opal formation, clay formation and chemical weathering processes in groundwater aquifers. Opal formation in the ocean is dominated by diatom assimilation of dissolved silicate from the surrounding aqueous phase to build up their silicified cell wall, a process which occurs in micromole Si concentration environments. The opal formed has an isotope value of about 1‰ lower than the source isotope value of DSi, i.e., the heavier Si isotopes are enriched in surface waters as diatoms preferentially sequester the $^{28}$Si to form opal. When diatom growth becomes Si-limited (DSi < 5 μmol/l) as is often the case in coastal and open ocean marine systems, the isotope fractionation becomes even higher. Thus, heavier Si isotopes in surface waters might indicate a shift in the ecosystem, which has occurred in many coastal waters from siliceous (diatoms) to nonsiliceous phytoplankton species. Therefore, methods addressing stable isotope fractionation during opal formation should be operational on micromole concentrations.

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Until recently, Si isotope data in the literature have been limited, largely due to analytical difficulties. The traditional method of analysing Si isotope ratios is by fluorination of silicon and introducing the resulting SiF$_4$ gas into a gas-source isotope ratio mass spectrometer (IRMS). A new preparation method using the acid decomposition of Cs$_2$SiF$_6$ followed by the high-resolution MC-ICP-MS.

The development of multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has opened up new possibilities for the determination of stable silicon isotopes with a precision comparable with that of IRMS. The major advantages with ICP source mass spectrometry is that the sample preparation is simplified and less hazardous, and it requires shorter analytical time and smaller sample size compared to gas-source IRMS.

Stable Si isotope measurements using MC-ICP-MS has mainly utilised double-focusing mass spectrometers with high mass resolution, i.e. m/Δm > 1000, to overcome the problem with polyatomic interferences derived from the ICP source. Instrumentation used for high-resolution analysis includes the NuPlasma HR, the NuPlasma 1700 from NU instruments and the Neptune from ThermoFisher.

In this study, we have established a technique to analyse stable silicon isotopes using a single-focusing low-resolution (m/Δm ~ 450) MC-ICP-MS, the IsoProbe from GV instruments. This instrument is equipped with a hexapole collision cell, which works as an energy filter. The technique permits higher sensitivity and less consumption of Si compared to the double-focusing high-resolution MC-ICP-MS. Below, we present detailed investigations of matrix effects, polyatomic interferences as well as instrument memory and background. Moreover, we document the long-term performance over four years using international standard materials.

**Experimental**

**Si isotope standards**

It is important that the standard materials used are well documented so that results from different sample measurements can be normalized and properly compared. Therefore, several standard materials with different isotope compositions have been introduced to compile data on silicon isotopes. Previously, the standard to which samples were compared was with the Caltech Rose Quartz followed by the RM8564-NBS28-Silica Sand (National Institute of Standards and Technology); the latter is labelled in the following as NBS-28. NBS-28 is nowadays widely used as an universally accepted zero reference material for silicon isotopes, and has a similar Si isotope composition to that of Caltech Rose Quartz. Other standard materials such as the Big-Batch and diatomite with an isotopic composition different than NBS-28 have been produced and used as secondary reference materials. An additional reference material, IRMM-018, has shown a larger than expected variation in isotopic compositions, which might be due to heterogeneity in this material.

**Sample preparation**

Solid materials obtained as part of the inter-laboratory comparison of Si isotope reference materials published by Reynolds et al. have been prepared and used throughout this study. This included the standard material NBS-28 and IRMM-018, and the isotopically highly enriched (both in 28Si and 30Si) material called Big-Batch (prepared at UCSB, University of California Santa Barbara, and initially originated from a commercial Na-metasilicate).

The solid samples of standards were prepared by fusion with LiBO$_2$ and digested in HCl. About 42.5 mg SiO$_2$ was mixed with 150 mg LiBO$_2$ in a graphite crucible and fused at 1000 °C for 30 minutes. After cooling, the formed glass pearl was digested in 20 ml 2.5 M HCl on a shaking table, resulting in a solution containing 35.4 mM Si. This solution was immediately diluted 50 times with 0.075 M HCl, yielding a stock solution with a concentration of 0.7 mM Si in 0.12 M HCl, which was stored in acid-cleaned high-density polyethylene bottles. It is important to keep the Si concentration in samples below about 2 mM Si to avoid polymerization during storage. After four-year storage, the SI concentration in the stock solution was still 0.7 mM Si, indicating that polymerization did not influence the concentration during this period. Water used for dilution was obtained from a Millipore® Milli-Q water purification system. The stock solution was diluted to 18 μM Si in 0.12 M HCl for Si isotope measurements.

The Big-Batch standard material contains about 1.5 μg/g Mo as a result from the preparation process using a molybdate co-precipitation technique, which may cause potential matrix effects. Screening of our stock solution by ICP-OES showed that there was about 4 μM Mo. Furthermore about 2.6 mM Li and 2.6 mM B was present in the Si solution.

**Instrumentation**

All the isotope measurements were carried out in the Laboratory of Isotope Geology at the Swedish Museum of Natural History using an IsoProbe MC-ICP-MS. The operating conditions of the instrument are summarized in Table 1. The ions generated in the plasma are introduced into the hexapole collision cell, where they collide/react with a collision gas. This reduces the energy spread from about 15 V to less than 1 V, and makes the ions suitable, after acceleration, for direct entry into the mass spectrometer. The collision cell also reduces the presence of ions interfering with the measured Si isotopes by collisions between the ions and the collision gas as described below. The low energy spread of the ions allows the refocused beam width to be less than the width of the collector slits, leading to the flat-topped peaks required for accurate isotope ratio measurements. A large variety of gases and gas mixtures can be used in the collision cell and for isotope ratio measurement noble gases such as Ar are frequently used. For optimal transmission of light isotopes, Helium is selected as a collision gas.

A typical torch material is quartz, but for the Si isotope measurements we used a torch in which the outer tube of the torch is made of sylan, an aluminium–silicon–oxynitride alloy, which has a high wear resistance, good durability and low contamination potential. The inner part of the torch is made of...
pure aluminium, which not only has extremely high-temperature stability and wear resistance, but also helps to provide protective atmospheres at high temperature to eliminate contamination or impurity.

The Si concentrations both in the standard and unknown solution were kept at about 18 μM. The solution was introduced into the plasma using a Cetac® Aridus desolvating nebulizer with a sample uptake rate of approximate 50–60 ml/min. A 28Si signal of 8–10 V was usually obtained, but the 28Si signal could occasionally decrease to ~7 V during measurements. The formation of gaseous SiCl4 can be neglected in water-rich solution.

Interferences

The Si isotope ratio measurement using MC-ICP-MS is usually affected by mass bias, isobaric interferences, and matrix effects. Potential isobaric interferences include 14N2 and 12C16O on the 28Si peak, 14N15N and 12C16O1H on the 29Si peak and 14N16O on the 30Si peak. The mass resolution on our Isoprobe is too low (m/Dm < 450) to fully resolve the polyatomic interferences, but they could be reduced by using the hexapole collision cell. The three Si isotopes are influenced by the interfering ions, but compared to the atomic ions the polyatomic ions are slightly heavier and they mainly affect the peaks on the high-mass side (Fig. 1). By adjusting the measurement position to the low-mass side, a well defined flat-top peak can found for 29Si and 30Si (Fig. 1). However, due to a relatively large isobaric interference from 14N16O on the smallest peak, 30Si, no flat area can be defined and we are thus not able to measure this isotope (Fig. 1).

Keeping the measurement position stable is important and this was tested by determination of the 28Si/29Si ratio at two positions, one on the low mass side and the other on the high mass side, of the flat area for 28Si and 29Si. The 28Si/29Si ratio was measured and it was found that the ratios determined on both sides of the measurement position (marked in Fig. 1) differ from the ratio determined at the measurement position. This demonstrates that it is necessary to reduce the isobars to a minimum and carefully adjust the position of the peak before data collection.

By using the hexapole as a reaction cell we can reduce the isobaric interferences to obtain sufficient peak flatness to measure the 28Si/29Si ratio. This is similar to what Moureau et al. have demonstrated when injecting N2O in a reaction cell to remove the isobaric interference 92Zr for 92Mo efficiently and thus make it possible to accurately measure Mo isotope ratios also by using an Isoprobe.

Measurement procedure

An individual measurement of a standard/sample consisted of 5 blocks and 12 cycles per block, with the integration time of 10 seconds for each cycle. Before each measurement the inlet system was rinsed for 180 seconds with 0.12 M HCl. After the rinsing, there was an uptake period of 180 seconds before data acquisition commenced. In total it took about 13 minutes to measure one standard/sample. With an uptake flow of 50–60 μL/min, the

Table 1 Instrument settings

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<tr>
<th>Multi-collector settings</th>
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<tr>
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* Denoted values of these parameters are kept constant during one entire measurement, but it may vary between different measurements in this given range.

ICP parameters

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Cetac® Aridus settings

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Fig. 1 Mass scans with the signals in volts for the 28Si, 29Si and 30Si peaks and possible isobaric interferences from polyatomic ions using 18 μM Si solutions. The isobaric interferences are given and marked by the shaded area as well as their contribution to the signal in volts. The dashed vertical lines on low and high mass side show the positions for test measurements of Si isotope ratios, and the area between them is the measurement position.

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amount of Si consumed during the measurement was approximately 14 nmol (390 ng).

In order to obtain the relative Si isotope variation, we used the standard-sample-standard bracketing technique. This method is frequently applied for determination of isotope ratio using MC-ICP-MS but requires identical matrix conditions in the sample and standard solutions. Each sample was measured using the standard-sample-standard bracketing technique including nine brackets, i.e., nine individual measurements. Before and after each measurement, a blank solution was introduced for 210 seconds and measured with an integration time of 60 seconds. It took approximately 160 minutes to acquire each reported isotope ratio, which was the average value calculated from nine brackets with two times the standard deviation (2S.D.) or at 95% confidence interval.

**Instrument drift**

When using the Isoprobe for isotope ratio determination, a drift in the ratio during the measurement period is commonly observed. This drift can be both in the upward or downward direction, i.e., increasing or decreasing ratios with time. Typically a systematic variation towards a single direction was observed (Fig. 2). Fig. 2a shows the machine upward drift in an individual measurement, which was the case for most of the analyses, and Fig. 2b illustrates the downward drift. The drift in the $^{29}$Si/$^{28}$Si ratio appeared not only in individual measurements, but was also observed during the entire analysis using the standard-sample-standard bracketing technique (Fig. 3). The systematic drift in the ion beam signal is independent of the integration time. This type of drift in the isotope ratio is frequently observed also for other heavier elements such as S and Pb, but can mostly be effectively corrected using the standard-sample-standard bracketing technique.

Mass discrimination or mass bias is observed in all mass spectrometric techniques and can generally be described as the time dependent deviation between observed and “true value”. In ICP-MS the mass bias is the result of a number of processes in the plasma and in the transmission through the ion optical system. Generally the mass bias decreases with increasing mass and is most pronounced for the light isotopes. The applied standard-sample-standard bracketing technique can be used to compensate for mass bias, matrix interferences and backgrounds. However, this requires that the signals obtained from both the standard and the samples are equally high and that the blank solutions have the same matrix as the analytical solution.

The reported delta values in per mil units were calculated according to Equation 1,

$$\delta^{29}\text{Si} = \left( \frac{^{29}\text{Si}}{^{28}\text{Si}} \text{sample} - 1 \right) \cdot 1000$$ (Equation 1)

**Matrix effects**

Matrix effects are caused by the presence of additional ions, such as Na$^+$, Mg$^{2+}$, Al$^{3+}$, Ca$^{2+}$, K$^+$, in the Si solution. These ions can affect the stability of the ion beam signal and lead to inaccurate results. Presence of Na$^+$ in the analytical solution is known to suppress the ion beam of the analyte, i.e. Si. In this study, we made a detailed study of the effect of increasing concentration of Mg$^{2+}$ in the analytical solution on the $\delta^{29}$Si value. Aluminium,
the most abundant metal in the earth crust, might also be a potential problem causing matrix effects. To examine these possible interferences on the $\delta^{29}\text{Si}$ value, a series of measurements with varying concentrations of Mg and Al were performed by adding these elements to the IRMM-018 standard solution. The experiments were performed in 2005 and repeated using different machine settings during 2008/09.

The presence of Mg shown as an increased Mg/Si ratio (Fig. 4) seems to increase the spread in the $\delta^{29}\text{Si}$ value even though the average value of each measurement was similar. At high Mg/Si ratio, >1, the $\delta^{29}\text{Si}$ seems to decrease towards lower values and the precision also seems to decrease. This might be due to changing conditions in the plasma when the ion strength is increasing.

In contrast to Mg, the presence of Al causes a substantial effect on the $\delta^{29}\text{Si}$ value and larger errors (Fig. 5). At Al/Si weight ratios as low as 0.1, the $\delta^{29}\text{Si}$ is lowered by >0.7‰. In addition to these detailed investigations of the effects of Mg and Al, we have observed that Si intensity can be greatly reduced in solutions with high concentrations of Na$^+$ and Ca$^{2+}$, which might also cause poor measurement accuracy. However, this effect on the $\delta^{29}\text{Si}$ has not been quantitatively determined.

The formation of $^{25}\text{MgH}_2^+$, $^{27}\text{AlH}^+$ and $^{27}\text{AlH}_2$ are potential problems in that they cause isobaric interferences. However, the formation of metal hydrides is likely to be small and can most likely be omitted. In addition, van den Boorn et al. reported that anionic species, such as $\text{SO}_4^{2-}$, could also cause a substantial matrix effect on the Si isotope measurement.

The Li and B (67.5 µM Li and 65 µM B) in the 18 µM Si solution of the fused standards materials were not removed. However, we cannot exclude the possibility for a matrix effect from those elements on the Si isotope ratio. This was avoided by addition of Li and B to all solutions to keep the Li and B concentrations on the same level and thus affect the plasma in a similar way. We adjusted the amount of Li and B in the samples and measured the elemental concentrations by ICP-OES before Si isotope analysis to assure that the matrix of Li and B were properly adjusted.

Clearly, the presence of alkali metal and alkaline earth metals as well as Al ions in the analytical solution could severely affect the precision and accuracy of the Si isotope ratio measurements. Also other species including both cations and anions could be a potential problem for high precision Si isotope measurements. Therefore it is crucial to remove interferences and isobars by chemical purification prior to the measurements. Alternatively, matrix effects from elements like Li and B, which are hard to quantitatively remove by column chemistry, could be eliminated by determining and adjusting the concentrations in samples.

### Background and rinse time test

Since the variations in $\delta^{29}\text{Si}$ values between samples are likely to be small, even a tiny amount of Si from a previous standard/sample remaining in the nebuliser and the analytical system could potentially affect the measured ratio. The system was rinsed using 0.12 M HCl between each individual measurement but the effectiveness of this cleaning needed to be tested thoroughly. The rinse time was investigated by measuring IRMM-018 relative to NBS-28 and the results show that a rinse time of at least 150 seconds between samples is necessary to remove the previous samples. A rinse time of 180 seconds was applied to ensure that the entire system was clean. We efficiently eliminated the instrument memory effects and could reach an error of $\delta^{29}\text{Si}$ less than 0.2‰ (2S.D.).

Instrumental background is mainly derived from the analysed solutions, but the build-up of impurities on the cones as well as on the transfer optics over time also contributes to the background. We investigated the background contribution by using a 180 s rinse time and conducting blank determinations at the beginning and end of each measurement. The observed background contributed up to approximately 2.5‰ on the $^{28}\text{Si}$ and $^{29}\text{Si}$ intensities. The background was found to be similar before and after the analysis. Part of this background comes from build-up of impurities in the inlet area of the instrument. By careful cleaning it could be reduced to about 0.6‰. However, during a session of measurements the background was generally found to be stable and could be corrected using the standard-sample-standard bracketing technique.

### Results and discussion

#### Long-term reproducibility

To determine our ability to precisely measure and reproduce the $\delta^{29}\text{Si}$ value using a MC-ICP-MS equipped with a collision cell, we...
The results for both the IRMM-018 and the Big-Batch are summarised in Table 2 along with literature data. The average $\delta^{29}\text{Si}$ value for IRMM-018 during the entire four-year period was found to be $-0.95\,\%_{\text{oo}}$ (2S.D. 0.16$\%_{\text{oo}}$) with a 95% confidence interval of $\pm 0.028\,\%_{\text{oo}}$. The results are in agreement with the value of $-0.85\,\%_{\text{oo}}$ (2S.D. 0.14$\%_{\text{oo}}$) obtained in the inter-calibration study by Reynolds et al.$^{30}$ The precision (2S.D.) for a reported $\delta^{29}\text{Si}$ value is typically between 0.1 and 0.2$\%_{\text{oo}}$ although smaller and larger errors (0.061 to 0.27$\%_{\text{oo}}$) occurred occasionally, most probably caused by machine random errors during measurements.

Similar to IRMM-018, we have also analysed the Big-Batch standard and our mean $\delta^{29}\text{Si}$ value was found to be $-5.50\,\%_{\text{oo}}$ (2S.D., 0.26$\%_{\text{oo}}$, Fig. 7). This can be compared to previously published values of $-5.39\,\%_{\text{oo}}$ (2S.D., 0.17$\%_{\text{oo}}$),$^{31}$ $-5.39\,\%_{\text{oo}}$ (2S.D., 0.3$\%_{\text{oo}}$)$^{30}$ and $-5.35\,\%_{\text{oo}}$ (2S.D., 0.3$\%_{\text{oo}}$) reported by Reynolds et al.$^{30}$ in the inter-calibration study (Table 2).

The amount of Si consumed during one measurement was approximately 400 ng per sample. This is comparable with other methods using double-focusing high-resolution MC-ICP-MS consuming between 200 and 500 ng Si,$^{26,30,31}$ but lower than the 3 µg Si typically used by a double-focusing low-resolution MC-ICP-MS.$^{28}$ The sensitivity for $^{28}\text{Si}$ was found to be $\sim 18$ V/36 µM Si (1 mg/L), which is $\times 3$ times higher than 5 ± 0.5 V/36 µM observed by Engström et al.$^{27}$ and slightly higher than $\sim 13$ V/36 µM reported by Georg et al.$^{28}$ using a high-resolution MC-ICP-MS. Clearly, our method yields high signals using small amounts of Si which can produce $\delta^{29}\text{Si}$ values with a precision similar to other methods.

**Conclusion**

This study demonstrates that by using a single focusing low-resolution MC-ICP-MS equipped with a hexapole gas-collision cell, we can precisely and accurately measure the $\delta^{29}\text{Si}$ (2S.D., 0.2%$_{\text{oo}}$) with a total Si consumption of less than 14 nmol (390 ng Si). Analysis of the same standard material performed during 2005 and 2008/09 gave a similar precision and accuracy. Given that the instrument’s hardware and settings have been partly changed during the last four years, the reproducibility in $\delta^{29}\text{Si}$ is found to be stable. For precise measurement of $\delta^{29}\text{Si}$ values it is necessary to use a Si solution with low concentrations of interference ions to avoid matrix effects. Thus, chemical purification to remove these interferences of the Si solution prior to analysis is required. Alternatively, matrix effects from elements like Li and B, which are not easy to remove by using ion-exchange, can be eliminated by determining and adjusting the concentrations in samples and standards to the same level.

**Acknowledgements**

We would like to thank Hans Schöberg and Torsten Persson in the Laboratory for Isotope Geology at the Swedish Museum of Natural History for assisting with sample preparation and providing technical support. We are also grateful for the funding from the Swedish Research Council (VR-2007-4763). We appreciate insightful comments and constructive suggestions from two anonymous reviewers, who greatly improved this manuscript.

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**Table 2** Summary of the results in this study and comparison with other published data

<table>
<thead>
<tr>
<th>Standard</th>
<th>$\delta^{29}\text{Si},%_{\text{oo}}$</th>
<th>2S.D.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRMM-18</td>
<td>-0.95</td>
<td>0.16</td>
<td>21</td>
</tr>
<tr>
<td>IRMM-18$^a$</td>
<td>-0.85</td>
<td>0.14</td>
<td>740</td>
</tr>
<tr>
<td>Big-Batch</td>
<td>-5.51</td>
<td>0.25</td>
<td>6</td>
</tr>
<tr>
<td>Big-Batch$^a$</td>
<td>-5.35</td>
<td>0.3</td>
<td>198</td>
</tr>
<tr>
<td>Big-Batch$^b$</td>
<td>-5.39</td>
<td>0.3</td>
<td>28</td>
</tr>
<tr>
<td>Big-Batch$^c$</td>
<td>-5.39</td>
<td>0.17</td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$ Inter-laboratory calibrated Si isotope values.$^{30}$

$^b$ Measurements from van den Boorn et al.$^{37}$

$^c$ Measurements from Chmeleff et al.$^{31}$
References

Paper II
Climate dependent diatom production is preserved in biogenic Si isotope signatures

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Abstract. The aim of this study was to reconstruct diatom production in the subarctic northern tip of the Baltic Sea, Bothnian Bay, based on down-core analysis of Si isotopes in biogenic silica (BSi). Dating of the sediment showed that the samples covered the period 1820 to 2000. The sediment core record can be divided into two periods, an unperturbed period from 1820 to 1950 and a second period affected by human activities (from 1950 to 2000). This has been observed elsewhere in the Baltic Sea. The shift in the sediment core record after 1950 is likely caused by large scale damming of rivers. Diatom production was inferred from the Si isotope composition which ranged between $\delta^{30}$Si $-0.18 \%$ and $+0.58 \%$ in BSi, and assuming fractionation patterns due to the Raleigh distillation, the production was shown to be correlated with air and water temperature, which in turn were correlated with the mixed layer (ML) depth. The sedimentary record showed that the deeper ML depth observed in colder years resulted in less production of diatoms. Pelagic investigations in the 1990’s have clearly shown that diatom production in the Baltic Sea is controlled by the ML depth. Especially after cold winters and deep water mixing, diatom production was limited and dissolved silicate (DSi) concentrations were not depleted in the water column after the spring bloom. Our method corroborates these findings and offers a new method to estimate diatom production over much longer periods of time in diatom dominated aquatic systems, i.e. a large part of the world’s ocean and coastal seas.

1 Introduction

Dissolved silicate (DSi) is an essential nutrient for diatoms, which play an important role in regulating the uptake and fate of C and N in the world oceans (Smetacek, 1998). Diatom dynamics is an effective measure of environmental change due to its sensitivity to a variety of physical and ecological conditions. In high-latitude ecosystems of the subarctic and arctic region, climate fluctuations on short growing seasons for diatoms may lead to major biological influences (Ding et al., 2011). After diatom death and cell lysis, the siliceous cell walls of diatoms can be well preserved in sediments. This allows for the reconstruction of diatom production by analysis of the preservation of biogenic silica (BSi) over time (Conley and Schelske, 2002). BSi has been used previously to track climate-related changes in aquatic production over millennial time scales (Blass et al., 2007; Rosén et al., 2000). Recently, the lacustrine BSi flux was used to reconstruct air temperature with decadal resolution back to 1580 CE in the Swiss Alps (Blass et al., 2007) and also to quantitatively infer summer temperature for the past 2 kyr in South-Central Alaska (McKay et al., 2002).

The discovery of Si isotopic fractionation of about $-1.1 \%$ during formation of diatom cell walls (De La Rocha et al., 1997) has provided a proxy for investigation of diatom-related processes. Many other studies have shown that isotopic fractionation is independent of temperature, interspecies effect and the partial pressure of CO$_2$ (De La Rocha et al., 1997; Milligan et al., 2004; Varela et al., 2004). Hence, variations in the $\delta^{30}$Si values of diatoms are therefore related to DSi utilization efficiency in water during diatom
production and can provide information on the role of the biological pump (the transport of C into deep water body and the regulation of atmospheric concentrations of CO$_2$) in palaeoclimatic and palaeoceanographic studies (Brzezinski et al., 2002; De La Rocha et al., 1998; De la Rocha, 2006; Beucher et al., 2007; van den Boorn et al., 2010), but also ongoing environmental changes (Reynolds et al., 2006; Brzezinski et al., 2001; De La Rocha et al., 2000; Cardinal et al., 2005; Wille et al., 2010). For example, DSI utilization efficiency in the Baltic Sea is also controlled by environmental conditions such as the supply of macronutrients (N, P, Si) and light which are functions of the physical mixing regime of the upper water column (Wasmund et al., 1998). The physical mixing regime is a function of local wind and temperature conditions and overall climate. The Si isotope signature of sedimentary BSI could therefore be a potential tracer for climate variation.

In this study we report an application of Si isotope analyses of diatoms to reconstruct diatom production variability from 1820 to 2000 in the subarctic Bothnian Bay. The studied period can be divided into an unperturbed period from 1820 to 1950 and a perturbed period from 1950 to 2000.

3 Materials and methods

3.1 Sediment core

A short sediment core (~38 cm) taken at a depth of 112 m in Bothnian Bay, Station 263870 (64°33.581 N and 21°54.769 E, Fig. 1) was sliced into 1 cm sections and freeze-dried. This core was dated and sediment accumulation rates were determined by analysing $^{210}$Pb (46.51 keV), $^{214}$Pb (351.99 keV) and $^{137}$Cs (661.63 keV) on an EG&G ORTEC® co-axial low energy photo spectrometer (LEPS) with a high-purity germanium crystal. Each sediment section was measured for 2 to 3 days after having been left standing for 2 weeks to achieve radionuclide re-equilibrium of decay products. Thereafter, the externally calibrated standard (pitchblende, Stackebo, Sweden) was added to 5 of the already measured samples at different depths to determine the relatively efficiency of the gamma detector system. The sedimentation rate was calculated by Eq. (1):

$$^{210}\text{Pb}_x = ^{210}\text{Pb}_0 \cdot e^{-\lambda t}$$

where $^{210}\text{Pb}_x$ is the activity of $^{210}$Pb per mass weight of sample at depth x, $^{210}\text{Pb}_0$ is the activity of $^{210}$Pb at the surface ($x = 0$), $\lambda$ is the decay constant of $^{210}$Pb (0.0311 yr$^{-1}$), and t is the age of the sediment sample. The BSi content in sediments was determined using the method described by Conley and Schelske (2002).

3.2 Diatom extraction

The method used for extraction of diatoms from the sediment was modified from Morley et al. (2004). Organic carbon was removed from each sediment slice by repeated mixing with 20 ml 15% H$_2$O$_2$ followed by incubation for 12 h and then heating to 90°C until no bubbles were evident after subsequent addition of H$_2$O$_2$. Addition of 10 ml 10% HCl and allowing the reaction to proceed for several hours removed inorganic carbon. There was relatively little inorganic carbon, <0.1%, in most of the samples. The samples were washed 3 times at each step using MilliQ-e water and centrifuged between each wash. Thereafter each sample was sieved through a 10 µm sieve cloth and an 80 µm steel mesh to recover the 10–80 µm fraction and to remove silt and clay. The 10–80 µm fraction contained most of the diatoms. Purity
was evaluated with an optical microscope and diatoms were found to constitute about 95% of the phytoplankton. The sieved samples were collected in centrifuge tubes containing sodium polytungstate (SPT, $3\text{Na}_2\text{WO}_4\cdot9\text{WO}_3\cdot\text{H}_2\text{O}$). The density of the SPT was adjusted to be between 1.8 g cm$^{-3}$ and 2.3 g cm$^{-3}$ in order to separate diatoms from the remaining clay and mineral materials. Centrifugation was at 4200 rpm for 5 min and repeated until clean diatoms were retrieved. Subsequently, all diatom samples from each sediment slice were carefully rinsed with MilliQ-e water and sieved at 5 µm to remove all traces of SPT. Finally, the diatom samples were dried at 40°C for 24 h.

### 3.3 Diatom fusion and chromatographic purification

About 5 mg of each extracted diatom sample were mixed with ca. 180 mg solid NaOH and fused in Ag crucibles at 730°C for 10 min in a muffle furnace. This was followed by washing with MilliQ-e water into a 0.12 M HCl solution in order to neutralize the NaOH (Georg et al., 2006). The final stock solution was diluted to 200 ml to yield a Si concentration of $\sim$10 mg l$^{-1}$ and stored in pre-cleaned HDPE bottles to minimize Si polymerization.

Interfering elements remaining after diatom dissolution were removed prior to Si isotope analyses by using cation exchange columns. A protonated 1 ml BioRad cation exchange resin AG 50W-X12 (100–200 mesh) was placed in 2 ml BioRad columns. The resin rinsing and diatom sample load processes are reported in Table 1, and the recovery shown in Fig. 2.

### 3.4 Si isotope analysis

The $\delta^{29}\text{Si}$ value of each sediment slice was measured using a single focus multiple-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) equipped with a hexapole gas-collision cell. Analytical precision for replicate samples was better than 0.2‰ (2σ) with a total Si consumption of less than 14 nmol (390 ng) and the internal reproducibility was tested by measuring IRMM-18 and Big Batch (Sun et al., 2010). The $^{30}\text{Si}$ could not be measured directly due to the instrumental limitation of using low mass resolution ($m/\Delta m$ $\sim$450) which does not fully resolve the isobars. However, the $\delta^{30}\text{Si}$ values were calculated from the mass ratio relationship $\delta^{30}\text{Si} = \delta^{29}\text{Si} \times 1.96$ which assumes mass-dependent fractionation (Reynolds et al., 2007). $\delta^{x}\text{Si}$‰ was expressed as relative to the standard material NBS 28 (Eq.2):

$$\delta^{x}\text{Si} = \left(\frac{\left[\frac{x}{28}\text{Si}\right]_{\text{sample}}}{\left[\frac{x}{28}\text{Si}\right]_{\text{NBS 28}}} - 1\right) \cdot 1000$$

where $x = 29, 30$.

### 4 Results and discussion

#### 4.1 Silicon recovery of cation-exchange columns

The quality of the chemical purification is of vital importance to obtain good precision and accuracy of the Si isotope analysis. Figure 2 shows the recovery of Si when using the cation-exchange column. Predominant Si species after NaOH fusion and HCl dissolution exhibit no affinity for the resin and thus pass directly through the resin. More than
Table 1. The purification process used in this study for dissolved diatom samples from Georg et al. (2006).

<table>
<thead>
<tr>
<th>Step</th>
<th>Solution</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-cleaning</td>
<td>4 M HCl</td>
<td>3</td>
</tr>
<tr>
<td>Pre-cleaning</td>
<td>8 M HCl</td>
<td>3</td>
</tr>
<tr>
<td>Pre-cleaning</td>
<td>4 M HCl</td>
<td>3</td>
</tr>
<tr>
<td>Conditioning</td>
<td>MQ-e water</td>
<td>6</td>
</tr>
<tr>
<td>Sample load</td>
<td>Acidified diatom samples</td>
<td>2</td>
</tr>
<tr>
<td>Elution</td>
<td>MQ-e water</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 2. Recovery of Si using a cation-exchange resin (AG 50W-X12, 100–200 mesh) in a 1 ml resin bed.

60% of the loaded Si is recovered from the initial elution of the 2 ml sample aliquot through the resin. The remaining Si in the resin is eluted using 4 ml Milli-Q-e water. No breakthrough of ambient cations is observed if only Milli-Q-e water is added. The cations start to elute when adding 4 M HCl (Fig. 2). Si is quantitatively recovered using this chromatographic purification with recovery efficiencies greater than 99%.

4.2 210Pb dating

The sediment core is dated using 210Pb as shown in Fig. 3. Figure 3a exhibits a gradually decreasing trend with increasing depth. Fig. 3b shows the linear 210Pb decay relation with depths in the sediment core (r² = 0.97). The linearity suggests minimal bioturbation and the slope indicates an average sedimentation rate in this Bothnian Bay core of approximate 2.1 mm yr⁻¹ which is consistent with previous measurements from this area (Grasshoff et al., 1983).

4.3 BSi vs. Si isotopes in BSi

The depth profiles of BSi contents and δ³⁰Si values are plotted in Fig. 4 with the corresponding time scale obtained from the 210Pb dating. The BSi content of the sediment core (Fig. 4a) is approximately 3.5% below 10 cm depth which is material deposited before the 1950s. It increases to a maximum of 7.8% in the surface sediments. This is due to increased diatom production, probably caused by anthropogenic nutrient emissions, especially N and P, to the Baltic Sea (Conley et al., 2008). Four periods of decreases in BSi contents were observed around 1825, 1850, 1880 and 1912. These dates are extrapolated from the sedimentation rate from the 210Pb dating. The range of δ³⁰Si values are displayed in Fig. 4b and vary between −0.18‰ and 0.58‰. Before the 1950s the average δ³⁰Si is about 0.16‰ with a small negative peak of −0.18‰ at 17 cm, i.e. 1910s, followed by a gradual increase to ~0.5‰ toward the surface. In general, increased BSi contents result in higher δ³⁰Si values.

It should be noted that the sedimentary BSi is not simply a measure of the entire Bothnian Bay primary production, but is a balance between diatom production and dissolution. The nearly linear sedimentation rate (Fig. 3) suggests that the variation in the sedimentation rate does not drive the variability of the BSi contents. During diatom production, there is Si isotopic fractionation of −1.1‰ (De La Rocha et al., 1997). However, dissolution of diatoms also exhibits Si isotopic fractionation of −0.55‰ if the dissolution is more than 20% of the total amount of BSi (Demarest et al., 2009). This means that the δ³⁰Si values of the preserved BSi can potentially increase, i.e. 28Si is preferentially released during diatom dissolution. Although no diatom dissolution rate estimates exist for Bothnian Bay, the excellent preservation of diatoms and the low mineralization rate in the bottom water combined with a shallow water depth of 112 m and rapid sedimentation rates imply that dissolution of BSi is probably low. This means that the potential shift of Si isotope values in BSi caused by dissolution may be ignored in this study.
4.4 Quantitatively reconstructing diatom production

The amount of DSi remaining in the water column is controlled by diatom production which occurs between the middle of May and September in Bothnian Bay. Due to the relatively long residence time of DSi compared to a short diatom growing season, Bothnian Bay can be assumed to be a closed system, i.e. a steady state with respect to the physical input of nutrients and its biological removal, to which Rayleigh distillation equations (Eq. 3) can be applied (Hoefs, 2009). The fraction of remaining DSi \( f \), is a factor between 0 and 1. A number close to 1 indicates that very little DSi is taken up by BSi in the water. The \( f \)-values can be calculated using \( \delta^{30}\text{Si} \) values according to Eq. (3):

\[
1 - f^\alpha = \frac{\delta^{30}\text{Si}_{\text{BSi}} + 1000}{\delta^{30}\text{Si}_{\text{river input}} + 1000} \tag{3}
\]

where \( \alpha = 0.9989 \) and denotes the Si isotope fractionation factor during diatom production (De La Rocha et al., 1997); \( \delta^{30}\text{Si}_{\text{BSi}} \) and \( \delta^{30}\text{Si}_{\text{river input}} \) represent \( \delta^{30}\text{Si} \) of BSi and of the river input to Bothnian Bay, respectively. \( \delta^{30}\text{Si}_{\text{BSi}} \) is the values shown in Fig. 4 and the \( \delta^{30}\text{Si}_{\text{river input}} \) value is taken to be +1.1‰ which is derived from measurements of an unregulated boreal river, the Kalix River (Opfergelt et al., 2011). This represents unperturbed river inflow.

Each point in Fig. 5 represents the calculated 5 yr average \( f \) value using Eq. (3) (each 1 cm section of sediments corresponds to approximately five years). The error of the calculation due to the uncertainty of the measured \( \delta^{30}\text{Si}_{\text{BSi}} \) values was examined by Monte Carlo analysis. 10 000 random \( \delta^{30}\text{Si}_{\text{BSi}} \) values were generated assuming a normal distribution around a mean value and the standard deviation of that mean. Here the average of measured \( \delta^{30}\text{Si}_{\text{BSi}} \) values and its standard deviation is used to produce 10 000 \( f \)-values. The first quartile and third quartile for each \( f \) value were calculated and are shown in Fig. 5 as error bars, which means that 50% of the \( f \)-values fall in this range. It should also be noted that the calculated variations in \( f \)-values are independent of the \( \alpha \)-value, i.e. the absolute values of \( f \) will change with the \( \alpha \)-value, but the variation will be unchanged.

The air temperature data displayed in Fig. 5 are the observed average temperatures between May and September of each year from 1824 to 2000 with a 5 yr moving average. By plotting air temperature and the calculated fraction of the remaining DSi \( f \) from Si isotope measurements a clear pattern emerges where cold periods are associated with high \( f \)-values, i.e. low diatom production, and warm periods show relatively lower \( f \)-values, i.e. high diatom production.

The temperature history and correlation with the isotopically derived \( f \)-values stand out particularly for several peaks. For example, the last period of cooling associated with the Little Ice Age corresponds to the late 19th century (Bradley and Jonest, 1993). Another cold period in the early 19th century is possibly caused by the Dalton Minimum, a period with low solar activity (Büntgen et al., 2006). The largest amount of remaining DSi, \( f = 0.98 \), is observed around 1910 which corresponds to a period with very cold summers. More recently, another period with high \( f \)-values is observed around 1983, which also was a period with cold summers.

Wasmund et al. (1998) showed that diatom blooms in the southern Baltic Sea were triggered by the reduction in depth of the mixed layer (ML) improving light conditions
Figure 6. Exponential regression fit of the fraction of remaining DSi ($f$) vs. the 5 yr moving average of air temperature for each depth interval.

Figure 7. Comparison of air temperature and water temperature between 1948 and 1963. (A) Plot of daily air temperature vs. daily water temperature. (B) Plot of average summer air temperature vs. average summer water temperature.

for phytoplankton growth. A possible explanation for the Si isotope-based diatom-temperature relationship observed in Bothnian Bay is that temperature-induced variations of the ML depth control diatom production. This would imply that air temperature regulates the water temperature which is correlated with the ML depth. In fact, Fig. 7a shows daily air temperature plotted vs. daily water temperature between 1948 and 1963 giving a correlation coefficient of $R^2 = 0.78$. The correlation is improved if average summer temperatures are used, $R^2 = 0.93$ (Fig. 7b). To further support our hypothesis that diatom growth and air temperature are linked via the depth of the ML, an estimate of the correlation is calculated between stratification strength and surface water temperature for each month from May to August (Appendix A, Fig. 8). Generally, the depth of the ML represented by the pycnocline depth is negatively correlated with summer surface temperature. The steepest slope appears in June and July (Fig. 8b and 8c), indicating a strong correlation between the pycnocline depth and the surface temperature. Figure 8a shows a gentle slope possibly due to the ice cover in May causing low diatom production. In August, the slope is less steep than those in June and July. This indicates that summer stratification isolates the growing diatoms from the deep nutrient reservoir, leading to phosphorus-limited diatom production (Humborg et al., 2003). Therefore, diatom production in June and July represents classical spring growth conditions and contributes most to the diatoms found preserved in sediments. In summary, diatom production in Bothnian Bay is controlled by depth of the ML, which is negatively correlated with the air temperature.

Figure 5 shows that the fraction of the remaining DSi ($f$)-values can be divided into two periods, before and after 1950. Before 1950, Bothnian Bay is likely to have been less disturbed due to anthropogenic eutrophication and river regulations (Humborg et al., 2006) and may be used here to infer the general relationship between diatom production and air temperature in Bothnian Bay. Earlier than 1950, the isotopic fractionation factor ($\alpha$) is relatively constant, indicating that a closed system can be applied, i.e. the assumption of a closed system, in our
case, much longer DSi residence time than diatom growth periods in Bothnian Bay.

After ca. 1950, the $f$-values exhibit a decrease (Fig. 5). This is not consistent with calculated $f$-values using field observations from 1980 and onwards. To get a good fit between observed and calculated data the $\delta^{30}\text{Si}_{\text{river input}}$ value used for calculations in Eq.(3) has to be shifted to $+1.4\%_{\text{ec}}$. The shift coincides in time with a period of large scale hydroelectric and flood control projects in the major rivers draining into Bothnian Bay (Humborg et al., 2002, 2006), leaving only a few rivers unregulated today. There is no change in bedrock and vegetation in the catchment which suggests that the isotope composition of source Si is constant. The shift in the fractionation factor is thus most likely to occur during the transport of DSi into the Bothnian Bay. Damming of rivers increases diatom production in the reservoirs behind the dams (Humborg et al., 2006) and enriches the remaining DSi in the river water with the heavier isotopes, ultimately leading to increased $\delta^{30}\text{Si}_{\text{river input}}$ values. To test this hypothesis, additional analysis of samples from major rivers draining into Bothnian Bay and behind dams as well as diatoms should be made. However, assuming that the $\delta^{30}\text{Si}_{\text{river input}}$ value of $+1.4\%_{\text{ec}}$ is correct, the remaining fraction of DSi ($f$) can be calculated for the summers between 1980 and 2000 and can be compared with $f$-values calculated with Eq.(4) using the observed air temperatures for the same period. A paired t-test shows that there is no significant difference between the observed and calculated $f$-values ($p \leq 0.003$).

5 Conclusions

Three conclusions can be drawn from this study in the subarctic area of the Baltic Sea, Bothnian Bay as follows:

1. stable Si isotope signature in sedimentary BSi can be used to study variations in time of diatom production;
2. air and water temperatures can be used as a proxy for the mixed layer depth (which controls diatom production) and as shown also correlates well with diatom production;
3. large scale anthropogenic activities such as changing the hydrological regimes in rivers by damming are likely to be imprinted on the sedimentary Si isotopic record.

Appendix A

Estimations of the relation between stratification strength and surface temperature

A1 Estimation of stratification strength from observations

A convenient way of analysing continuous stratification is to use the integrated properties profile potential energy and integrated buoyancy, i.e. steric height. This is analogous to the applications in various systems of the world (e.g. Gustafsson, 1999; Björk et al., 2001; Andersson and Stigebrandt, 2005). The profile potential energy is defined as:

$$P = g \frac{\rho_0}{\rho} \int_0^D (\rho_0 - \rho) z dz$$

(A1)

$$B = g \frac{\rho_0}{\rho} \int_0^D (\rho_0 - \rho) dz$$

(A2)

where $g$ is the acceleration of gravity, $\rho_0$ is the density at depth $D$ and $\rho$ is the density profile.

The two layer equivalents for these are

$$P = g \frac{(\rho_0 - \rho_1)}{2\rho_0} h^2$$

(A3)

$$B = g \frac{(\rho_0 - \rho_1)}{\rho_0} h$$

(A4)

where $\rho_1$ is a surface layer density and $h$ the depth of the surface layer. From Eqs. (A3–A4) we can derive:

$$h = \frac{2P}{B}$$

(A5)

$$\frac{g (\rho_0 - \rho_1)}{\rho_0} = \frac{B^2}{2P}$$

(A6)
Thus, by integrating Eqs. (A1–2) from measured profiles, we can estimate an equivalent homogeneous surface layer using Eqs (A5–6). In the present application, the equivalent depth \( h \) gives us a rough estimate on the depth of the surface layer.

A2 Application to Bothnian Bay

606 profiles were extracted from the BED data base. A criterion for selection was that the vertical resolution was at least 5 m in the upper 20 m and at least 10 m down to the reference depth \( D \), which was chosen to 50 m. Each profile was interpolated to 1 m resolution using a cubic spline, with normal conditions at the surface and at a maximal depth of the analysis. \( h \) was calculated for each profile using eq.s (A1–2 and 5).

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References


Silicon isotope enrichment in diatoms during nutrient-limited blooms in a eutrophied river system

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Abstract

We examined the Si isotope fractionation in diatoms by following a massive nutrient limited diatom bloom from a eutrophied natural system. We hypothesized that the Si isotope fractionation should be larger in comparison to observations in less nutrient rich environments. The Oder River, which is a eutrophied river draining the western half of Poland and entering the southern Baltic Sea, shows that a diatom bloom may cause extreme Si isotope fractionation. The rapid nutrient depletion and fast biogenic silica (BSi) increase observed during the spring bloom suggests a Rayleigh behavior for a closed system for dissolved Si (DSi) and BSi in the river at certain time scales. An enrichment factor (\(\varepsilon\)) of up to -1.6‰ is found based on observations between April and June, 2004. A very high \(\delta^{30}\text{Si}\) value of up to +3.05‰ is measured in diatoms. This is about 2 times higher than previously recorded \(\delta^{30}\text{Si}\) in freshwater diatoms. The Rayleigh model used to predict the \(\delta^{30}\text{Si}\) values of DSi suggests that the initial value before the start of the diatom bloom is close to +2‰. This indicates that there is a biological control of the Si isotope compositions entering the river, probably caused by Si isotope fractionation during uptake of Si in phytoliths. Clearly, eutrophied rivers with enhanced diatom blooms deliver \(^{30}\text{Si}\)-enriched DSi and BSi to the coastal ocean, which can be used to trace the biogeochemistry of DSi/BSi in estuaries.

Keywords: biogenic silica, Si isotopes, a eutrophied river system, nutrient limitation

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1. Introduction
Dissolved Si (DSi) is an essential nutrient in aquatic systems for the growth of siliceous organisms such as diatoms, radiolarians, and sponges, which play an important role in carbon sequestration (Goldman, 1988; Nelson et al. 1995). These organisms use Si to produce structural components made of opal, i.e. biogenic silica (BSi). The interactions between Si and aquatic ecosystems have been of wide interest since Schelske and Stoermer (1971) introduced the hypothesis that DSi depletion caused by excessive growth of diatoms was due to increased fluxes of phosphorus in Lake Michigan, USA. The same phenomenon has also been described for the other Great Lakes in North America (Schelske et al. 1983; Conley et al. 1993). Exhaustion of DSi has also been reported in Chesapeake Bay (Fisher et al. 1988; Conley and Malone 1992) and in the Mississippi River plume, where the DSi concentration decreased to < 0.93 µmol L⁻¹ (Nelson and Dortch 1996). Around 90% depletion of DSi has been reported in High Nutrient Low Chlorophyll (HNLC) coastal ocean water around Peru (Dugdale et al. 1995), while approximately 77% utilization of DSi was recorded in the Southern Ocean (Brzezinski et al. 2001; Leynaert et al. 2001). This indicates that some coastal areas are approaching Si limitation and therefore understanding the interaction between siliceous organisms and DSi is important.

The total algal growth in an aquatic ecosystem is primarily regulated by the availability of N and P. Availability of Si relative to N and P, i.e. Si:N and Si:P ratios, can influence the composition of the coastal phytoplankton community. A decreasing Si:N ratio may exacerbate eutrophication by reducing the potential for diatom growth, in favor of noxious flagellates (Officer and Ryther 1980; Conley et al. 1993; Humborg et al. 1997). Non-diatom species are known to be less available to higher trophic levels and some non-diatom based food webs are economically undesirable. Therefore, the proportion of diatoms in the phytoplankton community is of primary importance globally for many fisheries (Struyf et al. 2009).

Silicon isotopes have been recognized as an important proxy, not only for paleoclimatic and paleoceanographic studies (e.g. De La Rocha et al. 1998; Brzezinski et al. 2002; Van Den Boorn et al. 2010), but also for tracing present environmental and ecological changes (e.g. Cardinal et al. 2005; Engström et al. 2010; Wille et al. 2010). The principle behind these studies is that diatoms preferentially take up $^{28}$Si during cell wall formation, resulting in a Si isotope fractionation factor of approximately -1.1‰ (De La Rocha et al. 1997). This selective utilization of DSi leads to lower Si isotope values in diatom opal shells, leaving behind non-utilized DSi enriched in heavier Si isotopes with time (higher Si isotope values). Thus, measuring Si isotopes in DSi in aquatic systems and knowing the range of isotope fractionation that diatom blooms produce will allow calculating the magnitude of diatom blooms. The magnitude of Si isotope fractionation is apparently neither affected by CO₂ levels (Milligan et al. 2004), nor temperature in three species of diatoms during their growth (De La Rocha et al., 1997), and it is similar for diatoms both in freshwater and in marine systems (Alleman et al. 2005). Therefore, the change in Si isotopic composition generally depends on the availability of DSi, i.e. input and output of Si to the system, and whether the system is closed or open. The Si isotope composition in a closed and open system can be calculated by assuming a Rayleigh distillation behavior (Fig. 1). In this way, Si isotopes determined in DSi may be used to estimate both the magnitude of diatom blooms and other related fluxes, e.g. the downward flux of carbon.
The Si isotope fractionation caused by diatom production is also considered to be responsible for an enrichment of the heavier Si isotopes in river waters in comparison to primary minerals in igneous rocks in river catchments (De La Rocha et al. 2000; Ding et al. 2004; Georg et al. 2006a). Silicon taken up by vegetation (vascular plants) in the watershed is another possible explanation for the positive shift to higher $\delta^{29}\text{Si}$ and $\delta^{30}\text{Si}$ values in river water (Opfergelt et al. 2006). Furthermore, the production rate of diatoms is rather high (Conley and Malone 1992), which suggests that effects on the DSi concentration and Si isotope values may occur on very short time scales. A recent study with monthly data on Si isotopes in DSi and BSi in the Congo River has shown seasonal variations explained by diatom production, but the seasonality effect from diatom growth in a tropical river is limited (Hughes et al. 2011).

In the Baltic Sea, especially in the Gulf of Riga and the Gulf of Finland, DSi-limited diatom production has been reported (Conley et al. 2008; Danielsson et al. 2008). In rivers such as the Oder River, draining into the southwestern Baltic Sea, there is evidence for DSi-limited diatom production. The BSi fraction has in some cases been reported to comprise 99.8% of the total Si pool during spring and summer, but in winter, the fraction of BSi diminishes to only 0.8% (Humborg et al. 2006; Pastuszak et al. 2008). Thus, BSi from the Oder River can be considered as an important source for Si in the Baltic Sea (Humborg et al. 2006). Since this river system is eutrophic and has high diatom production with frequently occurring Si limitation, it is a potentially good site to evaluate seasonal changes in Si isotopes in river water, and may even show the maximum possible range of Si fractionation in a natural aquatic system.

2. Materials and methods

2.1 Nutrient and water flow data sources

The biweekly water sampling for nutrients (nitrogen, phosphorus, and silicon) was performed at the lowermost Oder River monitoring station (Krajnik Dolny) (Fig. 2) by the Sea Fisheries Institute (SFI) Branch in Świnoujście (Poland) during the period January 2003 - June 2005 within the EU SIBER project. Nutrient analyses were done by the SFI according to standard methods as recommended by Grasshoff (1983) and UNESCO (1983). The nutrient data have been published in Pastuszak et al. (2008) and Pastuszak and Witek (2009a,b). The biweekly measurements of BSi at the same sampling site were performed from April 2003 to June 2005. The BSi samples were prepared in duplicates; 200 ml to 400 ml of water was filtered using polycarbonate 0.6 micron Ø 47 mm filters and Sartorius polycarbonate holders for vacuum sterile filtration. Filters with the samples were dried at room temperature and stored in microbiologically sterilized Ø 47 mm Millipore® petri dishes. BSi analyses from a portion of the filter samples were performed according to the method by DeMaster (1981) (Pastuszak et al. 2008), while the remaining fraction was subjected to diatom analyses performed at Stockholm University. The monthly discharge in the Oder River during 2004 was obtained from IMWM (2004) (Fig. 3).

![Fig.2 Map of the lower Oder River indicating the sampling site in Krajnik Dolny.](image)

![Fig.3 Water discharge in the Oder river during 2004 (source: IMWM, 2004).](image)

2.2 Sample preparation
Diatom samples from April to September 2004 were selected in this study due to availability of samples and BSi contents although DSI samples were not available since the DSI concentrations were very low and therefore could not be measured. Each diatom sample deposited on the filter was mixed with 4 mL dichloromethane in a polypropylene centrifugation tube. The filter dissolved rapidly and left diatoms and insoluble organic and mineral materials residue. After centrifugation, the top clear solution was removed and new dichloromethane was added. This was repeated several times to ensure that the entire polycarbonate filter was dissolved and removed. After this procedure the samples were dried at 40 °C to evaporate all of the dichloromethane.

In order to remove the organic carbon the dried diatom samples were treated with 15% H2O2 and heated until all visible bubbles disappeared. This was followed by treatment with SPT heavy liquid (3Na2WO4·9WO3·H2O) to separate the diatom fraction from the remaining inorganic material. The SPT density was adjusted to a range between 1.9 g cm⁻3 and 2.3 g cm⁻3 (Morley et al. 2004). Thereafter, all diatom samples were carefully rinsed with MilliQ-e water to remove all the SPT, and dried at 40 °C for 24 h. The samples were examined under an optical microscope to ensure a pure diatom fraction. The dried diatoms were fused with NaOH and heated until all visible bubbles disappeared. This was followed by treatment with SPT heavy liquid (3Na2WO4·9WO3·H2O) to separate the diatom fraction from the remaining inorganic material. The SPT density was adjusted to a range between 1.9 g cm⁻3 and 2.3 g cm⁻3 (Morley et al. 2004). Thereafter, all diatom samples were carefully rinsed with MilliQ-e water to remove all the SPT, and dried at 40 °C for 24 h. The samples were examined under an optical microscope to ensure a pure diatom fraction. The dried diatoms were fused with NaOH and ensured to evaporate all of the dichloromethane.

Because of the instrumental limitation of measuring in low mass resolution (m/Δm ~ 450), the δ30Si was not measured in this study. Therefore, the δ29Si was calculated using the formula \( \delta^{29}\text{Si} = \delta^{28}\text{Si} \times 1.96 \) i.e. assuming a mass-dependent fractionation of Si isotopes (Reynolds et al., 2007). This relationship has been demonstrated by others and it has been shown that long-term reproducibility are within an error of less than 0.15‰ for \( \delta^{29}\text{Si} \) and 0.24‰ for \( \delta^{30}\text{Si} \) (Engström et al. 2006; Reynolds et al. 2007; Chmieleff et al. 2008).

### 3. Results and discussion

#### 3.1 Possible range of Si isotope values in diatoms

The average concentrations of DSI, BSI, DIN (dissolved inorganic nitrogen) and DIP (dissolved inorganic phosphate), calculated for the period April to September 2004, are 37.2 µmol L⁻¹, 58.5 µmol L⁻¹, 47.9 µmol L⁻¹, and 0.5 µmol L⁻¹, respectively in the river water (Table 1). The concentrations of DSI, DIN and DIP display similar variations, i.e. decreasing throughout the spring and summer and increasing in the autumn (Fig. 4). In contrast to the nutrients, BSI shows increasing concentration, which indicates a period of rapid diatom growth in spring and summer, and decreasing concentration in late summer and autumn (Fig. 4). The Oder River shows peak discharge during snowmelt and early spring (Fig. 3) and during peak flow the fast flowing water carries more suspended matter (soil particles) compared to the slowly flowing water during the other seasons, leading to the increased water turbidity and reduced light condition, which is a crucial parameter in diatom development. Despite abundant nutrients at this time of the year, diatoms show low production. After the fast transition from the snowmelt to late spring, soil particles settle out of the water column.

There are a number of shortcomings in using N:P:Si ratios to evaluate the limiting role of nutrients in phytoplankton growth. These ratios might be inaccurate when reaching very low concentrations close to the detection limit, and calculated ratios may be affected by large errors. Nonetheless, nutrient ratios have still been widely used (Howarth 1988; Fisher et al. 1992). It is shown that diatoms in freshwater having sufficient nutrients produce biomass with a C:N:Si:P ratio of 106:16:84:1 (Conley et al. 1989; Goldman 1988) and that variations in the stoichiometry of dissolved inorganic nutrients can provide evidence for which nutrient is most likely to limit the biomass production.
Silicon isotope enrichment in diatoms during nutrient-limited blooms in a eutrophied river system

Table 1 Temporal nutrient and BSi concentrations, as well as Si isotopic compositions of BSi expressed by \( \delta^{30}Si \) in the Oder River. The uncertainty for \( \delta^{30}Si \) is reported by 95% confidence interval (C.I.).

<table>
<thead>
<tr>
<th>Sampling date 2004</th>
<th>DSI (µM)</th>
<th>BSI (µM)</th>
<th>DIN (µM)</th>
<th>DIP (µM)</th>
<th>DIN:DIP</th>
<th>DSI:DIN</th>
<th>DSI:DIP</th>
<th>( \delta^{30}Si ) (%)</th>
<th>( \delta^{30}Si ) (%)</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 April</td>
<td>158.3</td>
<td>21.3</td>
<td>186.4</td>
<td>0.9</td>
<td>200.4</td>
<td>0.8</td>
<td>170.2</td>
<td>0.38</td>
<td>0.75</td>
<td>0.24</td>
</tr>
<tr>
<td>21 April</td>
<td>78.8</td>
<td>33.5</td>
<td>168.0</td>
<td>0.7</td>
<td>258.4</td>
<td>0.5</td>
<td>121.2</td>
<td>0.58</td>
<td>1.13</td>
<td>0.19</td>
</tr>
<tr>
<td>10 May</td>
<td>12.7</td>
<td>68.5</td>
<td>111.4</td>
<td>0.9</td>
<td>131.1</td>
<td>0.1</td>
<td>15.0</td>
<td>0.76</td>
<td>1.49</td>
<td>0.24</td>
</tr>
<tr>
<td>24 May</td>
<td>7.2</td>
<td>78.7</td>
<td>74.2</td>
<td>0.3</td>
<td>239.4</td>
<td>0.1</td>
<td>23.3</td>
<td>0.61</td>
<td>1.20</td>
<td>0.22</td>
</tr>
<tr>
<td>07 June</td>
<td>0.2</td>
<td>81.3</td>
<td>14.4</td>
<td>0.4</td>
<td>36.1</td>
<td>0.0</td>
<td>0.4</td>
<td>1.12</td>
<td>2.12</td>
<td>0.18</td>
</tr>
<tr>
<td>21 June</td>
<td>1.7</td>
<td>94.6</td>
<td>8.4</td>
<td>0.4</td>
<td>22.6</td>
<td>0.2</td>
<td>4.7</td>
<td>1.25</td>
<td>2.35</td>
<td>0.14</td>
</tr>
<tr>
<td>08 July</td>
<td>2.0</td>
<td>100.2</td>
<td>1.0</td>
<td>0.1</td>
<td>6.9</td>
<td>4.0</td>
<td>27.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23 July</td>
<td>5.8</td>
<td>89.7</td>
<td>0.2</td>
<td>0.1</td>
<td>2.9</td>
<td>34.2</td>
<td>98.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 August</td>
<td>23.0</td>
<td>76.7</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>48.5</td>
<td>49.9</td>
<td>1.56</td>
<td>3.05</td>
<td>0.21</td>
</tr>
<tr>
<td>24 August</td>
<td>32.9</td>
<td>36.2</td>
<td>1.5</td>
<td>0.4</td>
<td>3.7</td>
<td>21.6</td>
<td>80.0</td>
<td>1.29</td>
<td>2.53</td>
<td>0.21</td>
</tr>
<tr>
<td>07 Sept.</td>
<td>46.3</td>
<td>14.4</td>
<td>0.6</td>
<td>0.2</td>
<td>2.8</td>
<td>73.4</td>
<td>202.2</td>
<td>0.60</td>
<td>1.18</td>
<td>0.24</td>
</tr>
<tr>
<td>21 Sept.</td>
<td>73.9</td>
<td>10.4</td>
<td>7.8</td>
<td>0.6</td>
<td>12.1</td>
<td>9.5</td>
<td>114.9</td>
<td>0.56</td>
<td>1.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Average</td>
<td>37.2</td>
<td>58.5</td>
<td>47.9</td>
<td>0.5</td>
<td>76.4</td>
<td>16.1</td>
<td>75.7</td>
<td>0.87</td>
<td>1.71</td>
<td>0.092</td>
</tr>
</tbody>
</table>

*Measured by the SFI, Gdynia, Poland and published by Pastuszak et al. (2008) and Pastuszak and Witek (2009)
*Calculated by \( \delta^{30}Si = \delta^{29}Si \times 1.96 \) (Reynolds et al., 2007)
*Sample not available

The DIP concentration decreased to about 0.5 µmol L\(^{-1}\) during spring blooms (Fig. 4) and thus seems to be the primary limiting nutrient for the diatom growth. The DSI:DIP ratio reached the highest value, with a maximum of about 170 on 5 April 2004 (Table 1). In June there is a sharp decrease in the DSI concentrations from 1.7 to 0.2 µmol L\(^{-1}\) (Pastuszak et al. 2008), which is below the level of approximately 2 µM reported as a threshold concentration for diatom growth (Egge and Aksnes 1992). The DSI:DIP ratio decreased to very low values, 0.4 to 4.7, and thus the limiting nutrient shifted from DIP to DSI. The \( \delta^{30}Si \) values in diatoms increased from +0.75‰ in April to values more than +2.46‰ in June. The period from July to September was characterized by a sharp decrease in the DIN concentrations (DIN=NO\(_3\)-N+NO\(_2\)-N+N\(_2\)O-NH\(_4\)-N), from 7.8 to 0.2 µmol L\(^{-1}\) (Pastuszak and Witek 2009a; Pastuszak and Witek 2009b) simultaneously with the diatom biomass peak of 100.2 µmol L\(^{-1}\) in July, which is followed by a decrease due to the exhausted nutrient supply. The very low DIN concentration during summer was a result of the low water flux, which contributes to lower N emissions to the river basin. Nitrogen mainly originates from diffuse sources, with agricultural activity playing a key role (Pastuszak et al. 2011). The DSI:DIP ratio reaches 48.5 whereas the DIN:DIP decreased to as low as 1.0 in August 2004 and the limiting nutrient was thereafter shifted to nitrogen (Table 1).

The BSI shows positive \( \delta^{29}Si \) values throughout the sampling period with an average of +0.87‰, and a calculated \( \delta^{29}Si \) value of +1.7‰ (Fig. 5). This is consistent with previously reported \( \delta^{29}Si \) values in natural diatoms ranging from -0.3 to +2.6‰ (De La Rocha et al., 1998; Varela et al., 2004). However, the Si isotope compositions in the diatoms show large variations during spring and summer. The \( \delta^{30}Si \) values increase from a minimum value of +0.75‰ in the spring to a maximum in excess of +3‰ at the beginning of August, which is higher than previously recorded values in freshwater diatoms, although there is a small drop in May (Fig. 5). The \( \delta^{30}Si \) values start to decrease in late August, and in September the \( \delta^{30}Si \) values reach around +1.09‰ similar to that observed in April, which is the starting value of the spring bloom. These changing ratios during the growth

---

Fig. 4 DIN, DIP, DSI and BSI concentrations in the Oder River during April to September 2004. The inset shows a magnified scale for the June and July DIN, DIP and DSI data (source: Pastuszak et al., 2008; Pastuszak and Witek, 2009).
Fig. 5 The Si isotopic composition of BSi reported as δ30Si, and DSI and BSI concentrations in the Oder River during April to September 2004. The δ30Si error bars represent the 95% confidence interval. DSI and BSI values from Pastuszak et al., 2008.

season indicate that the Oder River is facing an alternating nutrient limitation of the diatom production, which is simultaneously leading to the very high δ30Si values in the diatoms.

3.2 Si isotope fractionation during spring blooms

The relationship between the variations of the δ30Si values and DSI and BSI concentrations between April and June, 2004 (Fig. 5) suggests that the diatom production dominates, as demonstrated by the rapid and sharp decrease in the DSI concentration. It is checked under a light microscope that BSI mainly originates from the diatom bloom and not from phytoliths or weathering of silicate minerals delivered from the terrestrial environment. Fig. 6 shows that δ30Si values between April and June are in agreement with the curve of accumulated BSI in a closed system. Therefore, we hypothesize that the river system is a closed Si reservoir at the timescale of diatom blooms, i.e. the production rate of diatoms is considerably faster than replenishment of new DSI to the system, especially during the DIP and DSI dramatically decreasing period. In contrast to the observed concentrations, a completely open system would show very small changes in the DSI concentrations.

The Rayleigh model for a closed system is introduced here to constrain the isotope variations. Fig. 6 shows that the measured data from April to June, 2004 are plotted together with the Rayleigh model for the accumulated BSI (Eq. 2) (Hoefs 2009). The reason why this is not extended to September will be discussed later. Apparently, the measured data do not fit for the Rayleigh model for an open system. The fractionation factor (α) of 0.9984 as well as initial δ30Si of riverine input is derived from the model for a closed system expressed by Eq.3 (R²=0.66), which is a transformation of Eq.2 (Fig. 6).

In addition, a curve of the δ30Si in the remaining DSI fraction is constructed using the Rayleigh equation for the depletion of remaining DSI (Eq.4):

\[
1 - f = \frac{\delta^{30}\text{Si}_{\text{BSi}} + 1000}{\delta^{30}\text{Si}_{\text{river input}} + 1000}
\]

where \(\alpha\) is the Si isotope fractionation factor during diatom production; \(\delta^{30}\text{Si}_{\text{BSi}}\), \(\delta^{30}\text{Si}_{\text{DSI}}\), and \(\delta^{30}\text{Si}_{\text{river input}}\) represent δ30Si in BSI, remaining DSI and the river input, respectively.

The Si isotope fractionation factor (\(\alpha_{30/28}\)) during diatom production in the Oder River reached 0.9984 corresponding to an enrichment factor \(e_{\text{DSI-BSI}}\) of -1.6‰. The error of the calculation due to the uncertainty of the measured δ30Si values was examined by Monte Carlo analysis. 5000 random δ30Si values were generated assuming a normal distribution around a mean value and the standard deviation of that mean. Each of measured δ30Si values and its standard deviation is used to produce 5000 \(e_{\text{DSI-BSI}}\) values, giving an average \(e_{\text{DSI-BSI}}\) of -1.6 ± 0.04‰ (95% confidence interval). However, the fractionation factor is still lower than -1.1‰ reported for diatom culturing laboratory experiments (De La Rocha et al. 1997) and -1.1% ± 0.4% found in Lake Tanganyika (Alleman et al. 2005).

Fig. 6 The δ30Si plotted vs. the fraction of remaining of DSI. Si isotope change under closed- and open-system Rayleigh model for diatom production with \(\alpha = 0.9984\). Open symbols represent measured data from April to September 2004.
Compared to marine diatoms, the $\varepsilon_{\text{DSi-BSi}}$ of -1.6‰ is also lower than the values observed in the Southern Ocean of -0.6‰ (Demarest et al. 2009), but within the range of -1.1‰ to -1.9‰ found in the Antarctic circumpolar current (Varela et al. 2004). The rapid depletion of DSi leads to a variation in the $\delta^{28}\text{Si}$ values in diatoms in excess of +2.3‰ (Fig. 5), which is around 1.5-2 times higher compared to previously reported values in diatoms from freshwater systems. These findings indicate that diatoms are able to fractionate Si isotopes to very high values in a freshwater system limited by nutrient supply. As shown in Fig. 6, if the DSi concentration decreases to a very low level and with a simultaneous increase in the BSi concentration, the Si isotope values in the remaining DSi could in the Oder River be extremely higher than any current recorded values. The regression curve in Fig. 6 indicates that the BSi accumulation in the Oder River follows a Rayleigh behavior for a closed system, which means that Eq.2 could be used to reconstruct diatom production represented by f values if $\alpha_{30/28}$ and the Si isotope values in BSi and DSi are known. It should be noted that clay formation can also lead to Si isotope fractionation, and it remains unclear as to what degree this process is controlling the $\delta^{30}\text{Si}$ values in the Oder River. This question calls for future studies, with a high-resolution time series of water and sediment samples.

The regression curve (Eq.3) also suggests that the $\delta^{30}\text{Si}$ value in DSi of river water ($\delta^{30}\text{Si}_{\text{river input}}$) prior to the spring bloom is around +2‰, i.e. the isotope starting value for diatom-driven Si isotope fractionation. This value is in agreement with the range of BSi in phyoliths documented to be in the range between -1.4 and +2.8‰ (Douthitt 1982) and also in the range of DSi in soil solution, -0.8 to +1.7‰. One possible reason for this relatively high value of +2‰ could be that the DSi contribution to the Oder River preferentially comes from the upper soil layers, where biogeochemical cycling of Si results in more enriched, positive $\delta^{30}\text{Si}$ values, than in the lower soil layers indicating a biological control of $\delta^{30}\text{Si}$ values export to this aquatic system (Derry et al. 2005; Ding et al. 2008). This might be a fundamental process in a variety of geological settings, supporting Si uptake by vegetation as important for Si cycling.

In August 2004, the Si isotope composition in BSi increased to a peak value of +3.05‰, while the DSi concentration increased with decreasing BSi concentration. This does not fit Rayleigh distillation behavior in a closed system (Fig. 6). The high $\delta^{30}\text{Si}$ value is most likely affected by other processes. One reason could be that the diatom dissolution rate is enhanced, whereas the diatom production rate is low due to the nutrient limitations, especially DIN. This dissolution can lead to preferentially releasing $^{30}\text{Si}$, which can exhibit an enrichment factor of -0.55‰, enriching the heavier Si isotope in the remaining BSi, if dissolution is higher than 20% of the total BSi (Demarest et al., 2009). This can potentially shift the $\delta^{30}\text{Si}$ values in the remaining BSi towards even higher values. The true mechanism of dissolution-induced influence on high $\delta^{30}\text{Si}$ value is not clear. It is also possible that increased input of a source with a high $\delta^{30}\text{Si}$ value is coming from upstream eutrophied areas in the watershed area, mostly consisting of agricultural lands and forest (Humborg et al. 2006; Humborg et al. 2008). This would lead to not only enhanced primary production in the river, but also increased uptake of DSi by terrestrial and aquatic vegetation (Ding et al. 2005). This does not seem to be a likely reason because of rather low river flow and very limited diatom production. It is also possible that Si is incorporated into secondary minerals, e.g. kaolinites, and since $^{30}\text{Si}$ is preferentially bonded to secondary mineral phases this can result in an increasing $\delta^{30}\text{Si}$ value of the dissolved phase (De La Rocha et al., 2000; Ziegler et al., 2005). However, this process is kinetically very slow and therefore not very likely.

Another possible reason is that the samples from August is sampled near the surface of water column, so it is actually a mixture of instant BSi and accumulated BSi, as shown in Fig. 6 those data points stand in the middle of instant and accumulated curves. DSi distribution in river is not homogeneous, the sampled diatoms is from a part of water with little DSi, and water samples from another part of river with more DSi. This means in Fig. 6, the data points in August are not associated with real f values, i.e. not real Si utilization during production of this sampled diatom.

Since eutrophied rivers, such as the Oder River, with enhanced diatom production and dissolution are important sources for DSi delivered to the coastal ocean, the enrichment of $^{30}\text{Si}$ in DSi makes it possible to trace the fate and transport of the DSi and BSi in the Baltic Sea and other similar environments.

4. Conclusion

This rapid nutrient depletion and fast diatom production during the spring bloom allows a closed system approximation for the DSi in the river. A Rayleigh distillation model is used to calculate a Si isotope fractionation factor of -1.6‰ which is higher compared to other freshwater systems, -1.1‰ and is
possibly the upper range of Si fractionation in natural aquatic systems. The $\delta^{30}$Si values for the river diatoms are also constructed and similar to the observed data showing a seasonal pattern. The calculated input of the $\delta^{30}$Si value before the onset of the diatom bloom is about +2%. This value coincides with reported data for phytopliths and therefore suggests that there is a biologic control of the Si isotopic composition of the input to the river. A high $\delta^{30}$Si value up to 3.05‰ in diatoms is observed in August with simultaneous rapid and strong depletion of the major nutrients (N, P and Si). Since eutrophied rivers, such as the Oder River, involving many complex processes, the signatures of Si isotopes are helpful to unravel diatom-related processes in the Baltic Sea and other similar environments.

Acknowledgements

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References


Silicon isotope enrichment in diatoms during nutrient-limited blooms in a eutrophied river system

Silicon isotope enrichment in diatoms during nutrient-limited blooms in a eutrophied river system


Paper IV
Effect of diatom growth and dissolution on silicon isotope fractionation in an estuarine system

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Abstract

Si isotopes provide a powerful tool to reveal past and present patterns in diatom production. Most studies have focused on Si fractionation factors during diatom growth in open ocean systems and have found lower Si isotope values in diatom shells (biogenic silica). Recent findings indicate that even the fractionation of Si isotopes during the physicochemical dissolution of diatom shells in the opposite direction produces higher \( \delta_{30}^{29}\text{Si} \) values in the remaining biogenic silica (BSi), allowing for the interpretation of diatom production patterns over geological time scales. However, estuarine and coastal primary production represents approximately 30-50\% of global marine production, and there are hardly any studies on Si isotope fractionation during either diatom growth or dissolution. In this study, Si isotope fractionation during diatom growth and the dissolution of the frustule were measured. Two species of diatoms from the Baltic Sea, one of the largest estuarine systems in the world, were selected for this study. The results show that both species of diatoms during growth yields an identical Si isotope fractionation factor of 0.99925 for \( ^{29}\text{Si} \) and 0.9984 for \( ^{30}\text{Si} \). In contrast to findings from open ocean species, no Si isotope fractionation during dissolution was observed even after 90\% of the diatoms dissolved. Whether there is isotope fractionation during dissolution or not will have profound implications for studies using Si isotopes to interpret the Si cycle in marine and estuarine systems. We propose that the small size of the diatoms living in estuarine systems with low salinity may explain the non-existence of Si isotope fractionation during dissolution. Therefore, we suggest that Si isotopes are an instrumental variable holding information about original environmental conditions of estuarine and even coastal systems. Finally, we tested the Si isotope fractionation patterns gained from the lab experiments on a sediment core, corroborating the observed dissolved Si (DSi) uptake rates in the above water column during diatom growth.
1 Introduction

Land-ocean fluxes from rivers contribute 80% of the dissolved Si (DSi) load to marine systems (Treguer et al., 1995); therefore, estuaries are a crucial link between land and marine systems. One of the most important characteristics of estuaries is that those systems are highly dynamic, resulting in gradients in water turnover times, salinity, nutrients and primary production. Meanwhile, eutrophication, a major worldwide problem caused by human activities, is concentrated around estuaries and shallow coastal waters (Conley et al., 1993; Fisher et al., 1988; Schelske and Stoermer, 1971). The high input of nutrients derived from land can result in high biological activity in estuaries, leading to the removal of DSi from water and a decrease in the DSi flux to open oceans. Hence, estuarine systems in general can be regarded as a sink for soluble silica during low water turnover and high primary productivity (Treguer et al., 1995). Due to the complexity of these systems, their biogeochemical Si cycle is difficult to study and to model conceptually. To better assess DSi land-ocean fluxes, it is essential to identify and quantify various biological processes in estuaries controlling DSi; indeed, the production, retention and dissolution of diatoms are the most important regulators for DSi in these processes. The uncertainty in production, retention and dissolution is mainly attributed to our poor understanding of the distribution and biogeochemical fate of BSi and DSi and also heavily influenced by eutrophication (Conley et al., 2008; Conley et al., 1993) and river damming (Humborg et al., 2000; Humborg et al., 1997; Humborg et al., 2006).

Thus, considerable work is required to assess the estuarine cycling of Si, in particular the role of siliceous algae, such as diatoms, which are a dominating algae species and play an essential role in the biogeochemical cycles of Si (Buesseler, 1998; Nelson et al., 1995).

The recognition that diatoms preferentially take up $^{28}$Si, resulting in an isotope fractionation (δ-value) of approximately -1.1‰ (De La Rocha et al., 1997), has afforded the possibility to estimate past and current diatom production in various systems because diatom production quantitatively corresponds to δ$^{30}$Si values of BSi. Currently, an increasing number of studies on diatoms and Si isotopes have appeared regarding open ocean and terrestrial systems. In oceans, δ$^{30}$Si values of DSi range from +0.5‰ to +3.2‰, corresponding to δ$^{30}$Si values of BSi in diatoms ranging from -0.3‰ to +2.6‰ (Beucher et al., 2008; Cardinal et al., 2005; De La Rocha et al., 2000; Reynolds et al., 2006; Varela et al., 2004); these values are slightly higher than the BSI of phytopliths produced in terrestrial systems, which range from -1.7‰ to +2.5‰ (Douthitt, 1982; Ziegler et al., 2005). In river waters, the measured δ$^{30}$Si values range from +0.4‰ to +3.4‰ (De La Rocha et al., 2000; Ding et al., 2004; Ding et al., 2011; Engström et al., 2010; Georg et al., 2007; Hughes et al., 2011). DSi in a soil solution also shows δ$^{30}$Si values ranging from -0.8‰ to +1.7‰ (Ziegler et al., 2005), and a similar range of values is found in groundwater, -0.2‰ to +1.3‰ (Georg et al., 2009; Opfergelt et al., 2011). Based on all these measured δ$^{30}$Si values, the Si isotope fractionation factor is indicated to be -1.08‰ for freshwater (Alleman et al., 2005; Hughes et al., 2011) and to be in the range of -0.6‰ to -2.2‰ for in situ measurements of oceanic waters (Cardinal et al., 2005; Cardinal et al., 2007; De La Rocha et al., 1997; Varela et al., 2004). However, none of these studies has been performed for estuarine and coastal areas, which are responsible for 30-50% of global marine production.

Using Si isotopes to estimate the role of diatoms in biogeochemical fluxes along the aquatic continuum from land to the open ocean still requires further study, especially in estuarine and coastal systems, and there might be typical ranges of Si isotopes and fractionation factors in the various realms. When interpreting Si isotope signatures in diatoms and DSi, much of our knowledge still comes from assumptions about Si isotope fractionation factors (Reynolds et al., 2006; Sun et al., 2011). Moreover, a recent study by Demarest et al. (2009) shows a Si isotope fractionation value during dissolution of -0.55‰, enriching residual BSi with heavier Si isotopes if > 20% BSI is lost during dissolution. This implies that if Si isotope fractionation is not taken into account, diatom production may be significantly overestimated, i.e. higher dissolution-induced δ$^{30}$Si values of BSi are not equivalent to actual production in water.

Therefore, to study Si isotope fractionation factors and to evaluate the potential of using Si isotopes as environmental traces from land to oceans, two estuarine species of diatoms from the Baltic Sea, one of the largest estuarine systems in the world, were selected for this study. We systematically analysed the dynamics of Si isotope values during diatom growth and dissolution in a laboratory-controlled system. The resulting fractionation factors were tested by a sedimentary record of BSI of the most northern basin of the Baltic Sea, Bothnian Bay.

2 Materials and methods

2.1 Diatom cultures and experimental setup

Two species of diatoms, the solitary *Thalassiosira baltica* (TBTV1) and the chain-forming *Skeletone marinoi* (SMTV1), were isolated from the Gulf of Finland by Dr. Anke Kremp (Finnish Environment Institute-SYKE, Helsinki, Finland), and cultured at the School of Natural Sciences, Linnaeus University, Sweden. The stock cultures were grown in f/2 medium (Guillard, 1975) prepared from 1 µm filtered and autoclaved Baltic seawater (salinity 7 psu). Diatoms were grown at 8-10 °C, under a photon flux of 200-250 µmol photons m$^{-2}$ s$^{-1}$ and a 16/8h light/dark cycle. To measure Si isotope values...
during diatom growth, cells were collected in the exponential phase by filtering the stock culture through 5-10 µm nylon nets. Cells were rinsed four times and resuspended with silicate-free f/2 medium (f/2-Si) to minimise the input of DSI from the stock cultures. Cells were inoculated in duplicate into 5 L PET bottles in f/2-Si Baltic seawater medium at chlorophyll a (Chl a) at concentrations of 10 µg Chl a L⁻¹ for TBTV1 and 7 µg Chl a L⁻¹ for SMTV1. The initial Baltic seawater used for the inoculation of cultures contained approximately 20 µmol L⁻¹ DSI. To ensure a reliable diatom biomass and remaining DSI for Si isotopic analyses, additional DSI was added to the culture medium to reach final DSI concentrations above 60 µmol L⁻¹. The diatom cultures were gently bubbled with air to mix the cells and to keep them in suspension until DSI depletion, and incubated under the same conditions as the stock culture. TBTV1 and SMTV1 cultures were monitored daily for 5 and 7 days for cell counts, Chl a, DSI concentrations and Si isotopic analyses.

After DSI was depleted in the two cultures, the growth experiment was terminated. To measure Si isotope values during the dissolution of the diatom frustule, a fraction of the SMTV1 culture (4 L) was distributed into two 2 L polycarbonate bottles. The bottles were kept in the dark at 22-24 °C for 44 days. The same set of samples used during diatom growth was initially taken daily, but less frequently after the fifth day until 90% of the diatom frustules were dissolved. In addition, the O₂ concentration was measured regularly during dissolution to ensure that no anoxia occurred.

Duplicate aliquots (2 ml) from each replicate were fixed with acidic Lugol’s solution, and cells were counted in an inverted light microscope (Olympus BX50) using a Palmer-Maloney counting chamber. Samples (2 x 5-15 ml) were filtered (Pall AE/E filters) and extracted with 96 % ethanol, overnight at room temperature for chlorophyll a fluorometric analysis (Jespersen and Christoffersen, 1987). To collect diatom cells, 150-200 ml of water samples was filtered daily (alternatively twice a day if DSI decreased rapidly) through 0.2 µm polycethersulphone membrane filters. One hundred millilitres of the filtrate was immediately analysed for DSI concentration according to Valderrama (1995). The remaining filtrates (50 ml) were kept at 4 °C, and diatom cells collected onto the filters were freeze-dried prior to preparations for Si isotope analysis.

2.2 Sample preparation prior to Si isotope analysis

The method used for BSI digestion was modified from Raguenneau et al. (2005). Polycethersulphone filters containing 2-7 mg of freeze-dried diatoms were placed into 15 ml polypropylene (PP) tubes. A 5 ml solution of 0.25 mol L⁻¹ NaOH was added and the samples were heated in a water bath for 1 hour at 90 °C. After cooling, HCl was used to stop the digestion by neutralising pH. Centrifugation was performed at 4200 rpm for 5 minutes. The supernatant was transferred to another PP tube and diluted to 10 ml solution for further treatment.

Dissolved Si in water samples was extracted by a magnesium 2-step co-precipitation technique adopted from Reynolds et al. (2006). Two hundred microlitres of 1 mol L⁻¹ NaOH was added to 10 ml of the water samples. Samples were shaken and left for 1 hour, and thereafter centrifuged at 4200 rpm for 5 minutes. Another 100 µl of 1 mol L⁻¹ NaOH was dropped into the separated supernatant to precipitate more Mg(OH)₂. After samples were shaken and left for another hour, they were centrifuged and the supernatant was removed. The precipitates were then dissolved in 4 mol L⁻¹ HCl. The removed supernatant was analysed to make sure that the Si removal was complete.

The purification of Si from the dissolved diatom solution and water samples was performed using a BioRad AG50W-X12 cation-exchange resin. Five hundred microliters of the samples in 0.2 mol L⁻¹ HCl was loaded on the column with a 1 ml resin bed and eluted with 4 ml Mille-Q water. Si having undergone no charges flowed directly through the resin with Mille-Q water. Detailed information regarding this step can be found in Sun et al. (2011).

2.3 Si isotope analysis

The Si isotope compositions of both DSI and BSI were analysed in the Laboratory for Isotope Geology at the Swedish Museum of Natural History, Stockholm, Sweden, using an IsoProbe MC-ICP-MS with a hexapole collision cell. Generally, this instrument is capable of precisely and accurately measuring δ²⁸Si (2 S.D., better than 0.2‰), although measuring δ²⁶Si is not possible due to the instrument’s low resolution (m/Δm ~450). The instrumental mass discrimination is corrected by applying the standard-sample bracketing technique. The sensitivity for ²⁸Si is ~12 V/ ³⁶µmol L⁻¹ Si (1 mg L⁻¹), with an uptake rate of ~70 µl min⁻¹ via a Cetac® Aridus desolvating nebuliser. The detailed measurement procedure and the long-term reproducibility shown by IRMM-18 and NBS 28 have been documented in Sun et al. (2010).

The Si isotope ratio of each sample is calculated and expressed in δ²⁸Si values (‰) relative to the standard, NBS 28, and according to Eq.1, δ²⁶Si values are calculated from the relationship δ²⁶Si = δ²⁸Si x 1.96, which assumes mass-dependent fractionation (Reynolds et al., 2007).

\[
\delta^{28}Si = \left( \frac{\Delta^{28}Si_{sample}}{\Delta^{28}Si_{standard}} - 1 \right) \cdot 1000
\]  

(Eq.1)

2.4 Data reduction
A closed system usually means that a substrate is continuously removed to form a product, but neither undergoes any exchange with the outside system. During the diatom experiment, we set up a closed system in 5 L containers without replenishing DSi and BSi. To interpret the Si isotope values of such a system, the Rayleigh distillation equations are used. The relationship between Si isotope values in products and substrates can be expressed by the following equations:

\[
\frac{\delta^{29}\text{Si}_{\text{product}} + 1000}{\delta^{29}\text{Si}_0 + 1000} = f^{(\alpha-1)}
\]

(Eq.2)

\[
\frac{\delta^{29}\text{Si}_{\text{substrate}} + 1000}{\delta^{29}\text{Si}_0 + 1000} = 1 - f^\alpha
\]

(Eq.3)

where \(\delta^{29}\text{Si}_{\text{substrate}}\) and \(\delta^{29}\text{Si}_{\text{product}}\) are Si isotope values of the substrates, products and initial substrate, respectively; \(\alpha\) is the Si isotopic fractionation factor, and \(f\) is the fraction of remaining substrates in water. By rearranging Eq. 2, \(\alpha\) can be solved as shown in Eq. 4:

\[
\alpha = \frac{\ln\left(\frac{\delta^{29}\text{Si}_{\text{substrate}} + 1000}{\delta^{29}\text{Si}_0 + 1000}\right)}{\ln f} + 1
\]

(Eq.4)

Alternatively by rearranging Eq. 3, \(\alpha\) can be solved as shown in Eq. 5:

\[
\alpha = \frac{\ln[1 - \frac{\delta^{29}\text{Si}_{\text{product}} + 1000}{\delta^{29}\text{Si}_0 + 1000}] + (1 - f)\ln f}{\ln f}
\]

(Eq.5)

### 3 Results

Table 1 and Fig. 1 and 2 summarise the BSi and DSi concentrations and the Si isotope values of BSi and DSi during the culture experiment. For TBTV1 and SMTV1 the Chl a concentrations increased exponentially, while the DSI concentrations decreased during the initial the DSI concentrations decreased during the initial growth period (Fig.1). During exponential growth, the maximum growth rate was 0.42 day\(^{-1}\) for TBTV1 and 1.08 day\(^{-1}\) for SMTV1. Chl a levels reached 200 µg L\(^{-1}\) for TBTV1 and 530 µg L\(^{-1}\) for SMTV1 (Figure A1-1 and 1B-1). The DSI concentrations decreased progressively during growth days and the experiment was terminated when the DSI concentrations fell below 0.4 µmol L\(^{-1}\) (Fig. 1A-2 and 1B-2).

Fig.1 also shows that the two species of diatoms have similar Si isotopic compositions and that the \(\delta^{30}\text{Si}\) values increased during diatom growth both in cells and in the remaining DSI. During diatom growth, the Si isotope values in the two species varied by more than 0.7‰ for \(\delta^{29}\text{Si}\) and 1.3‰ for \(\delta^{30}\text{Si}\), ranging from +0.6‰ up to +1.3‰ for \(\delta^{29}\text{Si}\) and from +1.2‰ up to +2.6‰ for \(\delta^{30}\text{Si}\), respectively. The two species fractionate Si isotopes during their growth, producing almost identical \(\alpha\) fractionation factors of 0.9992 ± 0.0003 and 0.9993 ± 0.00038 (2σ), respectively (calculated by Eq. 5). A two-sample t-test, assuming equal variance, shows that there is no significant difference between TBTV1 and SMTV1 (\(p \leq 0.061\)). The average value for \(\alpha\) over all diatom sample measurements was 0.99925 ± 0.00034 (2σ), corresponding to a difference in Si isotopic compositions between DSI and diatoms up to +0.75‰ for \(\delta^{29}\text{Si}\). The measured \(\delta^{29}\text{Si}\) values in seawater samples increased from 1.35‰ to 2.58‰; \(\delta^{30}\text{Si}\) values are calculated by multiplying \(\delta^{29}\text{Si}\) by 1.96 (Reynolds et al., 2007), giving values ranging from +2.64‰ to +5.07‰. The calculated average value of \(\alpha\) is 0.9984 ± 0.0004 (2σ), corresponding to a difference of +1.6‰ for \(\delta^{30}\text{Si}\), i.e. the seawater is continually enriched with the heavier Si isotopes.

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Fig.1 Plot of Si isotope values following diatom growth curves as a function of time. A-1 and A-2 are plots of TBTV1 growth; B-1 and B-2 are plots of SMTV1 growth. Error bars of Si isotope values are 2σ.
Table 1 Summary of Si concentrations and Si isotope values of DSi and BSi during SMTV1 and TBTV1 production and dissolution, as well as the fractionation factor (α) calculated by Eq. 4 and 5. The 2σ of the calculated α is shown below the average values.

<table>
<thead>
<tr>
<th>Days</th>
<th>Chl a (µg/L)</th>
<th>DSi conc. (µM)</th>
<th>f</th>
<th>δ²⁹Si_{BSI}</th>
<th>2σ</th>
<th>δ³⁰Si_{BSI}</th>
<th>δ²⁹Si_{DSi}</th>
<th>2SD</th>
<th>δ³⁰Si_{DSi}</th>
<th>²⁹α by Eq. 5</th>
<th>³⁰α by Eq. 5</th>
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<tr>
<td>0</td>
<td>6.8</td>
<td>64.8</td>
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<td>+1.23</td>
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<td>+2.64</td>
<td>N/A</td>
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<td>+0.69</td>
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<td>+1.36</td>
<td>+1.52</td>
<td>0.13</td>
<td>+2.98</td>
<td>0.99930</td>
<td>0.99864</td>
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<td>+2.58</td>
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Effect of diatom growth and dissolution on silicon isotope fractionation in an estuarine system

4 Discussion

Our findings suggest that i) diatoms from estuarine systems show identical Si isotope fractionation factors of 0.99925 for $^{29}\text{Si}$ and 0.9984 for $^{30}\text{Si}$, which are very similar to those of oceanic species; ii) the fractionation factors during diatom production closely follow the theoretical values calculated by the Rayleigh distillation approach; iii) when applying these fractionation factors to a sediment record, we can reproduce the observed water column data on the fraction of remaining DSi during the growth period; iv) in contrast to previous studies on diatom dissolution, the decrease in BSi in the lab experiments is not associated with Si isotope fractionation (at least up to 90% dissolution), making Si isotopes a powerful tracer for past diatom blooms in estuarine systems.

4.1 Mechanism of Si isotope fractionation

The calculated Si isotope fractionation factors during the Baltic Sea diatom’s growth, $^{29}\alpha$ and $^{30}\alpha$, were calculated to be 0.99925 ± 0.00034 (2σ) and 0.9984 ± 0.00035 (2σ), respectively, which are in the same range as values previously reported for other cultured marine diatoms: Thalassiosira weissflogii and Skeletonema costatum (De La Rocha et al., 1997). Our values are also consistent with the value of 0.99944 for $^{29}\alpha$ found in Lake Tanganyika (Alleman et al., 2005) and 0.9981-0.9989 for $^{30}\alpha$ reported in Antarctic surface water (Varela et al., 2004).

The exact mechanism of Si isotope fractionation during diatom growth is difficult to explain. A plot of δ$^{30}\text{Si}$ values as a function of f values (Fig. 3) shows a very good agreement between the experimental results and the Rayleigh model expressed by Eq. 4 and 5. This behaviour is typical for a kinetic isotope effect in a closed system, i.e., the Si isotope values vary with f values such that the remaining DSi is enriched in the heavier Si isotopes. The fact that diatoms are an effective ‘trap’ for keeping Si in the water column without exchange with the remaining DSi does not indicate an isotope equilibrium effect in this study.

BSi dissolution commences when dissociated hydroxyls from water weaken and eventually break the adjacent Si-O bond (Dove and Crerar, 1990). The open linkage binds with hydroxyl to form Si-OH. This process is repeated until all Si-O bonds are broken, and the surface then assumes the form Si(OH)$_2$. This silica-water interaction tends to be slow and is influenced by many different factors, but it is commonly believed that temperature, pH, the presence of solution species, particle size, etc., can have a great impact on the dissolution of BSi (Fournier et al., 1982; Iler, 1979; Loucaides et al., 2008). The experiments conducted in our study and those in the study by Demarest et al. (2009) were laboratory controlled, and the variations in temperature are similar; thus, they are unlikely to be the reason for the difference in the observed Si isotope fractionation between these two studies. A possible reason could be that cations present in the solution are attracted to the negatively charged surface sites of BSi by electrostatic forces. In Baltic Sea water with much lower salinity (7 PSU measured in this experiment) than that in the Southern Ocean, the surface of BSi is less ‘saturated’ than BSi in ocean water. This allows hydroxyls to easily approach the Si-O bonds on the surface and break them without biasing Si isotopes. Another possible explanation is that the effective surface area increases with a decrease in particle size (e.g., Knauss and Wolery, 1988). Because
the diatom size in low-salinity water with a shallower mixed layer depth is normally smaller than that of diatoms growing in ocean water (Litchman et al., 2009). BSi dissolution in the Baltic Sea could be faster and more effective, which would make the different Si isotopes subject to simultaneous dissolution. Although we try to explain the lack of isotope fractionation during BSi dissolution, we cannot fully rule out possible Si isotope fractionation during BSi dissolution. Perhaps this fractionation is too small to be detected by the MC-ICP-MS employed in this study. One limitation is that only $\delta^{30}$Si can be measured; the mass difference between $^{28}$Si and $^{29}$Si is harder to detect than the difference between $^{28}$Si and $^{30}$Si considering the current analytical error. However, this is unlikely in this case because the deviation between the calculated $\delta^{30}$Si values of remaining BSi after 90% dissolution is still smaller than the documented analytical errors for $\delta^{30}$Si, indicating an undetectable Si isotope fractionation between the $\delta^{30}$Si values of remaining BSi.

4.2 Applications of the Si isotope fractionation factor

It is well known and has been shown that Si isotopes have the potential to reveal both past and present patterns of DSI utilisation mainly by diatoms, which can contribute to both modern and paleoceanographic studies. However, Deramest et al. (2009) suggest that Si isotope fractionation during diatom dissolution may complicate this Si potential of revealing DSI utilisations and cause discrepancies between the measured $\delta^{30}$Si of BSI and the real oceanic conditions inhabited by diatoms. In contrast, our observation of unfractonated diatom dissolution implies that diatoms can still possibly record Si isotope information generated under original environmental conditions. We therefore suggest that $\delta^{30}$Si isotopes of diatoms smaller than oceanic species and alternatively growing in low-salinity can be reliably used as a proxy for estuarine and even coastal studies. In our study, we tested this notion by applying our measured Si isotope fractionation factor to our measured $\delta^{30}$Si values preserved in BSI derived from Bothnian Bay, the most northern part of the Baltic Sea.

The Baltic Sea, located in Northern Europe, has a surface area of 377 000 km² and a water volume of 21 000 km³. As one of the largest estuarine system in the world, it consists of several basins, from north to south: Bothnian Bay, Bothnian Sea, Gulf of Finland, Baltic Proper, Gulf of Riga, Danish Sounds and Kattegat, and finally connects to the North Sea. The shallow sills between the North Sea and the Baltic Sea restrict the water exchange leading to a long water residence time of approximately 20 years (Papush et al., 2009). The residence time of DSI is 11.2 years for the whole Baltic Sea (Wulff and Stigebrandt, 1989), which makes the Baltic Sea resemble a closed system comparable to the experimental set up of our lab experiments.

There is a biological control on Si isotope signatures in the Baltic Sea water due to the large proportion of diatoms in phytoplankton (Klais et al., 2011). Given an $^{29}\alpha$ of 0.99925 and the calculated $^{30}\alpha$ of 0.9984 for diatom production, it is possible to address the potential range of variations in the Si isotope values in the Baltic Sea. Fig. 3 shows the behaviour of diatom production following the Rayleigh model and illustrates the possible Si isotope values in remaining DSI and diatoms determined by the experiments performed in this study. Using a $\delta^{30}$Si value of +1.4‰ in rivers draining into Bothnian Bay (Sun et al., 2011) as $\delta^{30}$Si, an $^{30}\alpha$ of 0.9984 and approximately 30% depletion of DSI (Danielsson et al., 2008), i.e., $f = 0.7$, +2.0‰ of $\delta^{30}$Si of remaining DSI is calculated by Eq. 2. Likewise, +0.15‰ of $\delta^{30}$Si product in accumulated BSI is calculated by Eq.3. Monte Carlo analysis is adopted to estimate the uncertainty of this
calculation derived from the measured Si isotope fractionation factor. Five thousand $^{30}\alpha$ are randomly generated assuming a normal distribution based on the average $^{30}\alpha$ of 0.9984 and its 2σ of 0.00035, giving an average $\delta^{30}\text{Si}$ value of accumulated BSi, +0.15‰ ± 0.30‰ (2σ). This is comparable with our previously reported $\delta^{30}\text{Si}$ value of +0.40‰ ± 0.20‰ (2σ) in sedimentary BSi (Sun et al. 2011) without statistically significant differences between these values. It also implies that there is no measurable dissolution-induced Si isotope fractionation, i.e., BSi preserved in sediments still preserves information concerning the initial water conditions.

We further suggest possible Si isotope patterns in the various basins of the Baltic Sea using the measured Si isotope fractionation factor to be tested in future studies. The Baltic Sea shows a range of DSI uptake rates expressed as the relative amount of DSI depleted (f values) after the growth period that vary between years (Wasmund et al. 1997) but also shows a typical range between the various marine basins. Table 2 shows an estimate of Si isotope values of diatoms and DSI in basins of the Baltic Sea based on measurements and other estimates, not taking into account dissolution effects. The f values of different basins are derived from average DSI concentrations measured before and after diatom blooms between 1970 and 2000 (Danielsson et al., 2008). The $\delta^{30}\text{Si}_0$ value of Baltic Proper before diatom blooms was measured to be +1.6‰ in this study, resulting in $\delta^{30}\text{Si}$ values of +0.53‰ ± 0.25‰ (2σ) and +2.67‰ ± 0.25‰ (2σ) for $\delta^{30}\text{Si}$ in accumulated diatoms and remaining DSI, respectively. The values 2σ is derived from 5000 values of $\delta^{30}\text{Si}$ calculated by randomly generating 5000 $^{30}\alpha$ as $^{30}\alpha$ are randomly generated assuming a normal distribution based on the average $^{30}\alpha$ of 0.9984 and its 2σ of 0.00035, giving an average $\delta^{30}\text{Si}$ value of accumulated BSi, +0.15‰ ± 0.30‰ (2σ). This is comparable with our previously reported $\delta^{30}\text{Si}$ value of +0.40‰ ± 0.20‰ (2σ) in sedimentary BSi (Sun et al. 2011) without statistically significant differences between these values. It also implies that there is no measurable dissolution-induced Si isotope fractionation, i.e., BSi preserved in sediments still preserves information concerning the initial water conditions.

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<th>Basins</th>
<th>Average depletion of DSI (%)</th>
<th>f values</th>
<th>$\delta^{30}\text{Si}$ of input DSI</th>
<th>$\delta^{30}\text{Si}$ of accumulated diatom (‰) ± 2σ</th>
<th>$\delta^{30}\text{Si}$ of remaining DSI (‰) ± 2σ</th>
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<tbody>
<tr>
<td>Bothnian Bay</td>
<td>30%</td>
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<td>+1.4%(^2)</td>
<td>+0.15 ± 0.30</td>
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<td>Gulf of Finland</td>
<td>62%</td>
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<td>+1.6% (assumed)</td>
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<td>+3.10 ± 0.35</td>
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<tr>
<td>Gulf of Riga</td>
<td>73%</td>
<td>0.27</td>
<td>+1.6% (assumed)</td>
<td>+0.85 ± 0.17</td>
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<td>Baltic Proper</td>
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<td>+1.6% (measured)</td>
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<tr>
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<td>+0.75 ± 0.20</td>
<td>+3.32 ± 0.38</td>
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1 Calculated by interannual variations in mean DSI concentrations between 1970 and 2000 (Danielsson et al. 2008)
2 Sun et al. (2011)

5 Conclusion

This study shows that diatom production yields an identical non-species-dependent Si isotope fractionation factor of 0.99925 for $^{29}\text{Si}$ and 0.9984 for $^{30}\text{Si}$ in an estuarine system. In contrast to the results of another study, the Si isotope fractionation during dissolution of diatom frustules is not observed, possibly due to the lower salinity of the surrounding water and the smaller size of the diatoms. This indicates that $\delta^{30}\text{Si}$ signatures preserved in BSi retain direct information about original environmental conditions. These findings may contribute to a better understanding of the biogeochemical cycle of Si in estuarine and coastal environments and hold implications for marine studies using Si isotopes as a proxy to estimate the historical development and magnitude of recent and past diatom blooms.

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