## SILICANICS 2 Biogeochemistry and genomics of silicification and silicifiers

## 6 - 8 June 2018 University of Victoria Victoria, BC, Canada

www.silicamics2.sciencesconf.org

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### Territorial Acknowledgement – University of Victoria

We acknowledge with respect the Lkwungen-speaking peoples on whose traditional territory the university stands and the Songhees, Esquimalt and <u>W</u>SÁNEĆ peoples whose historical relationships with the land continue to this day.



UVic Office of Indigenous Academic and Community Engagement "New Beginning" © Charles Elliott

### Introduction

The first SILICAMICS conference held in l'Aber Wrac'h (near Brest, France) in September 2015 provided an extraordinary opportunity to develop interdisciplinary connections between researchers with expertise and interest in silicification and silicifiers and to learn about the latest advances in the silicon world. This second conference aims to develop an integrative approach that includes chemistry, biogeochemistry, biochemistry, physiology and genomics to better understand biosilicification and silicifiers in past, contemporary and future oceans.

Silicifiers are among the most important organisms on planet Earth. They are able to take advantage of the abundance of silicon (the second-most-abundant element in the Earth's crust) to build silica structures, which can help for protection against predators, for motility, or for facilitating the penetration of light and nutrients. At the same time, silicifiers have a paramount impact on the cycling of silicon and other nutrients in marine waters.

This transdisciplinary conference focuses on the marine realm, for which numerous unknowns still remain regarding the global silica cycle. Marine diatoms have dominated over siliceous sponges and radiolarians over the last 150 M years. Today diatoms play a key role in the trophic networks of the most productive coastal and open-ocean ecosystems, as well as in the biology-mediated transfer of CO<sub>2</sub> from the surface to the ocean interior (the so-called biological carbon pump). The physiology and biochemistry of biosilicification have been studied in diatoms and other silicifiers but many gaps remain regarding mechanisms, evolutionary significance, variations in response to environmental change and the impact of these processes on marine biogeochemistry. Moreover, benthic diatoms and their role in coastal ecosystems have been largely overlooked despite significant contributions to coastal primary production. Along the same vein, the roles of other siliceous organisms, such as benthic sponges, radiolarians and silicoflagellates in the silica cycle need to be quantified at a global scale. Accumulation of silica in non-siliceous picocyanobacteria has also been shown but the reasons and mechanisms behind such process are still unknown.

In the last 25 years, the genomes of several diatom species have been sequenced. Scientific programs (such as Tara Oceans and the Gordon and Betty Moore Foundation's projects) have provided additional DNA sequence information from diatoms as well as from other silicifying organisms. Genomics data can now be exploited to address fundamental research questions about the role of different silicifiers in coastal and open-open ecosystems, and their controls on C, N, P, and Si biogeochemical cycles. Additional knowledge is also being acquired about interactions between silicifiers and other organisms at different spatial and temporal scales, and their impact on nutrient cycling and ecosystem functioning are beginning to be addressed. It is an exciting time for seeking new opportunities to study the biology of ocean silicification processes. This conference will facilitate the exchange of information between scientists from different 'silicon' disciplines and expertise with the aim of moving forward in our understanding of the impact of silicifiers on Earth.

### **Organizing Committee**

#### **Conference Chair**

Diana Varela, University of Victoria, British Columbia, Canada

#### Scientific Committee

Aude Leynaert, CNRS, Institut Universitaire Européen de la Mer, Brest, France Paul Tréguer, UBO, Institut Universitaire Européen de la Mer, Brest, France Brivaela Moriceau, CNRS, Institut Universitaire Européen de la Mer, Brest, France Jill Sutton, UBO, Institut Universitaire Européen de la Mer, Brest, France Kate Hendry, University of Bristol, Bristol, UK Manuel Maldonado, Centro de Estudios Avanzados de Blanes, Blanes, Spain Chris Bowler, CNRS, Ecole Normale Supérieure, Paris, France Conference Support Shea Wyatt, University of Victoria, British Columbia, Canada Karina Giesbrecht, University of Victoria, British Columbia, Canada Lucianne Marshall, University of Victoria, British Columbia, Canada Brandy Biggar, University of Victoria, British Columbia, Canada

### Supporting Institutions

SILICAMICS 2 has been supported by the following institutions:



University of Victoria

University of Victoria Department of Biology School of Earth and Ocean Sciences



University of British Columbia Faculty of Medicine



Université de Bretagne Occidentale



Institut Universitaire Européen De La Mer



Centre National de la Recherche Scientifique

#### **Useful Information**

#### **Conference Room**

Presentations and discussions are held in Medical Sciences Building (MSB) room 160, on the University of Victoria Campus. A campus map is available on the last page.

#### **Additional Rooms**

Additional space for working groups, smaller meetings, and quiet work space is available in MSB 157 & 159, across the hallway from MSB 160.

#### **Internet Access**

Wi-Fi is accessible through:

- 1. UVicStart users will be directed to a login page, and will have to log in every 2 hours.
- 2. Eduroam users can log in with their own institutional credentials (email and password).

#### **Meal Locations**

Breakfast, lunch, and afternoon breaks – MSB160 foyer. June 6 reception – The University Club of Victoria, on the UVic campus. June 7 dinner – Oak Bay Marina restaurant – 1327 Beach Drive, Victoria

#### Victoria Transit

Victoria Taxi: +1 (250) 383-7111 Yellow Cab: +1 (250) 381-2222 Public Transit: <u>https://bit.ly/2H0Pzpt</u>

#### **Contact Information**

For further information or queries, please contact any of the members below through mobile phone or email.

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#### Scientific Program

#### Wednesday June 6, 2018

#### 8:45 - 9:45 Breakfast & pick up conference package

#### Introduction

Chair – Diana Varela (University of Victoria, Canada)

- 9:45 10:00 Diana Varela (University of Victoria, Canada) Welcome remarks
- 10:00 10:50 Mark Brzezinski **Invited Speaker** (University of California – Santa Barbara, USA) *Introduction to SILICAMICS 2*

Session 1: Key players and processes in the silica cycle

- 10:50 11:40 Paul Tréguer **Invited Speaker** (Université de Bretagne Occidentale, IUEM, France) *Revisiting advances in the marine silicon cycle*
- 11:40 12:00 Kristina Brown (Fisheries and Oceans Canada, Canada) Silicate sources and sinks in the Kitikmeot Sea: An analog system to gain insight into the Arctic Ocean silicate cycle
- 12:00 13:30 Lunch

Chair – Aude Leynaert (LEMAR, Brest, France)

13:30 - 13:50 Jeff Krause (Dauphin Island Sea Lab, Alabama, USA) Does silicon terminate diatom blooms in the Atlantic sector of the Arctic?

- 13:50 14:10 Brivaela Moriceau (LEMAR, Brest, France) The Si cycle in the Baffin Bay
- 14:10 14:30 Lucianne Marshall (University of Victoria, Canada) Diatom contribution to primary production under persistent oligotrophic conditions in the coastal Canadian Arctic
- 14:30 14:50 Karina Giesbrecht (University of Victoria, Canada) Silicon utilization and Si:N ratios in the Pacific Arctic region from 2006 – 2016
- 15:00 15:30 Afternoon break
- Chair Jill Sutton (Université de Bretagne Occidentale, France)

15:30 - 15:50	Diana Varela (University of Victoria, Canada)
	Biogenic silica production across temperate, sub-Arctic and Arctic
	marine ecosystems

- 15:50 16:10 John Berges (University of Wisconsin Milwaukee, USA) Silicon on the rise: Mysteries surrounding use and cycling of silica in a large lake
- 16:10 16:30Erica Young (University of Wisconsin Milwaukee, USA)Defining the role of Si in structuring freshwater communities
  - 17:30 Reception at The University Club of Victoria

#### Thursday June 7, 2018

8:45 - 9:50 Breakfast

Session 2: Silicification in diatoms: Ecophysiology and genomics/proteomics

Chair – Connie Lovejoy (Université Laval – Canada)

- 9:50 10:40 Kim Thamatrakoln **Invited Speaker** (Rutgers University, USA) 'Omic-based approaches to understanding diatom silicification: Insights and implications for silicon cycling
- 10:40 11:00 Chana Kranzler (Rutgers University, USA) The interplay between diatom host-virus dynamics and silicon cycling
- 11:00 11:20 Michael Maniscalco (University of California Santa Barbara, USA) The stoichiometry of staying skinny: Increased Si:N uptake without changes in frustule silica content in an iron stressed diatom assemblage
- 11:20 11:40 Lydia Köhler (TU Dresden, Germany) The incorporation of aluminum in diatom biosilica, biological consequences and possible applications

12:00 - 13:30 Lunch

Session 3: Isotope geochemistry as a tool for understanding the silica cycle

Chair – Brivaela Moriceau (LEMAR, Brest, France)

13:30 – 14:20 Greg de Souza – **Invited Speaker** (ETH Zürich, Switzerland) *The stable isotope composition of silicon as a tracer of the marine biogeochemical Si cycle* 

- 14:20 14:40 Jill Sutton (Université de Bretagne Occidentale, France) Making the link between stable isotope chemistry and environmental change
- 14:40 15:00 Patricia Grasse (GEOMAR, Kiel, Germany) The evolution of silicon isotopes during a mesocosm experiment off Peru
- 15:00 15:30 Afternoon break
- 15:30 15:50 Ivia Closset (University of California Santa Barbara, USA) The forensics of diatoms: Exploring different modelling approaches to reconstruct Si cycling from deep sediment trap data
- 15:50 16:10 Lucie Cassarino (University of Bristol, UK) Are silicic acid transporters responsible for Si isotopic fractionation during diatom uptake?

#### **A Learning Initiative**

- 16:10 16:30 Jill Sutton (Université de Bretagne Occidentale, France) Silica School
  - 19:30 Dinner at Oak Bay Marina Restaurant

### Friday June 8, 2018

#### 8:45 - 9:50 Breakfast

#### Session 4: Silicification in other silicifiers

Chair – Kristina Brown (Fisheries and Oceans Canada – Canada)

9:50 - 10:40	Sally Leys – <b>Invited Speaker</b> (University of Alberta, Canada) <i>The evolution, cell biology and ecology of silicification in sponges</i>
10:40 - 11:00	Lily Burke (Fisheries and Oceans Canada, Canada) Ecological role of glass sponge reefs
11:00 - 11:20	Katsuhiko Shimizu (Tottori University, Japan) Silicatein and glassin: Mining proteins to understand their roles in sponge silicification
11:20 - 11:40	Aude Leynaert (LEMAR, Brest, France) Rhizaria: An unexpected role in the carbon biological pump, and in the silica cycle
11:40 - 12:00	Stephen Baines (Stony Brook University, NY, USA) Silicification in Synechococcus: Observations and open questions
12:00 - 13:30	Lunch

#### Session 5: Open for discussion and networking

- 13:30 15:00 Discussion/networking, Early Career Scientist Workshop
- 15:00 15:30 Afternoon break
- 15:30 16:30 Discussion/networking, Final Remarks



In order of presentation

### Session 1: Key players and processes in the silica cycle

### Introduction to SILICAMICS 2

#### Mark Brzezinski \*\*1

<sup>1</sup> University of California, Santa Barbara – United States

Welcome to SILICAMICS 2. This will be a wide-ranging meeting that addresses topics and scales ranging from silicon metabolism within individual plankton cells, to the relationship between Si metabolism and food web dynamics and onto global silicon biogeochemistry. We will learn about the ever expanding toolkit used to interrogate silicification and the marine silicon cycle from advances in silicon biochemistry to various omic and molecular approaches to novel isotopic methods. The meeting will take a comprehensive view and examine our growing understanding of the role of silicifiers beyond diatoms in the sea. In this introductory talk I will provide the intellectual framework for the meeting providing examples of the exciting areas that each session will explore illustrated by discoveries made by various laboratories.

### Revisiting advances in the marine silicon cycle

#### Paul Tréguer \*\*1

<sup>1</sup> Institut Universitaire Européen de la Mer (IUEM), Université de Bretagne Occidentale (UBO) – France

During the last 5 years there has been significant advancement in our understanding of the marine silicon (Si) cycle. Studies have highlighted whether the Si cycle can be considered in steady state, and the potential mechanisms for the "missing silica sink" such as the burial of silica by siliceous sponges and the contribution of reverse weathering in high sedimentation rate continental margins. Rates of Si inputs to the ocean have been revisited through the delivery of reactive amorphous silica or of silicic acid by rivers, subglacial lakes, submarine groundwater discharge, hydrothermal activities; recent studies suggest the release of silicic acid from solid material deposited at the land-ocean interface and low temperature basalt dissolution on continental margins and in the deep sea. In parallel, the important roles non-diatom groups (e.g., sponges, Rhizarians, picocyanobacteria) play in biogenic silica production, dissolution, export, and burial have been revisited.

\*Speaker

<sup>&</sup>lt;sup>†</sup>Corresponding author: paul.treguer@univ-brest.fr

## Silicate sources and sinks in the Kitikmeot Sea: An analog system to gain insight into the Arctic Ocean silicate cycle

Kristina Brown \*<sup>†1</sup>, Sarah Zimmermann <sup>1</sup>, Eddy Carmack <sup>1</sup>, Bill Williams <sup>1</sup>

<sup>1</sup> Fisheries and Oceans Canada – Canada

The Arctic Ocean is considered a net exporter of silicate to the North Atlantic, contributing to the dissolved silica cycle of the global ocean. Although the main source of this exported silicate is Pacific water entering the Arctic via Bering Strait, an imbalance in the Arctic Ocean silicate budget indicates that other external inputs are also important, specifically riverine sources of terrestrial dissolved silica. Thus a more comprehensive understanding these two major silicate sources will not only shed light on the present day Arctic Ocean silicate cycle, but also contribute to predicting the trajectory of future change as the climate continues to warm. In this study we explore the dissolved silica cycle of the Kitikmeot Sea, a semi-enclosed Arctic marine system in the southern Canadian Arctic Archipelago. This region is unique in the Pan-Arctic due to its shallow bounding sills, which limit inflow of deeper Pacificorigin water, and massive freshwater input, which maintains strong summertime stratification that limits vertical mixing across the pycnocline. These physical constraints have two main consequences for the silicate cycle by (1) limiting the availability of nutrient rich, high-silicate Pacific water to the photic zone, and (2) increasing the potential for enhanced terrestrial influence on the semi-enclosed sea as the regional climate warms. The Kitikmeot Sea is characterized by silicate concentrations as high as 40 mmol m<sup>-3</sup>, higher than expected from saline source waters at its two oceanic gateways. Inflow of high nutrient Pacific waters to the Kitikmeot Sea are estimated to deliver 5.4 x 10<sup>9</sup> mol of silicate annually, however riverine inputs could contribute as much as 1.3 x10<sup>9</sup> mol of terrestrially sourced dissolved silica to the system, in addition to an unknown quantity of particulate silica associated with suspended sediments. We discuss the importance of these two sources of dissolved silica to the Kitikmeot Sea and explore the role of sinking diatoms as a mechanism to generate the high silicate concentrations observed in bottom waters.

## Does silicon terminate diatom blooms in the Atlantic sector of the Arctic?

Jeffrey Krause \*<sup>†1,2</sup>, Carlos Duarte <sup>3,4</sup>, Susana Agustí <sup>3</sup>

<sup>1</sup> Dauphin Island Sea Lab – AL, United States

<sup>2</sup> University of South Alabama – United States

<sup>3</sup> King Abdullah University of Science and Technology – Saudi Arabia

<sup>4</sup> Aarhus University – Denmark

Diatoms' contribution to productivity and organic matter export are significant in high-latitude marine ecosystems. Waters in the Atlantic sector of the Arctic (e.g. coastal Greenland, Faroe Islands, Barents Sea, Svalbard) have lower silicic acid concentrations than nitrate. Since diatom consumption of Si and N are typically near unity, silicic acid availability may play an important role in the temporal evolution of phytoplankton blooms in these sectors. Recent publications have reported declining silicic acid concentrations between  $^{60} - 75^{\circ}$ N latitude in the Atlantic. Despite the clear changes in Si availability, there is a paucity of data on the magnitude of diatom silica production and whether it is limited by the ambient silicic acid concentration. Here we report data from the Svalbard archipelago and West Greenland. In Svalbard, diatom contribution to primary production was between 50 - 100% at most stations; however, in post-bloom or pre-bloom conditions, diatom contribution was minor (e.g. < 10%). In both systems, the ambient silicic acid limited the rate of silicon uptake by diatoms, with some evidence for growth limitation. Despite recognition over the last 15 years that declining silicic acid relative to inorganic nitrogen may limit diatom productivity, this is the first direct evidence that silicon availability plays an important role in regional diatom bloom phenology.

### The Si cycle in the Baffin Bay

#### Brivaela Moriceau \*\*1

<sup>1</sup> Laboratoire des Sciences de l'Énvironnement Marin (LEMAR), Université de Bretagne Occidentale – France

The Arctic Ocean is an ecosystem strongly impacted by climate change, particularly considering the retreat of the ice edge. At the basis of this ecosystem, diatoms dominate the primary production, with an early bloom at the bottom of the sea ice, and a pelagic bloom when the ice melt. During two Green Edge expeditions in the Baffin Bay, we monitored temporal change of the Si production, export and stocks of dissolved (dSi) and particulate (bSiO<sub>2</sub>) in the ice and water column to improve our understanding of the development of the diatom bloom in spring. In 2015, we observed a strong bloom in the ice, up to a punctual 20 mmol of bSiO<sub>2</sub> per m<sup>2</sup>. By comparison, the pelagic diatom bloom integrated over 20 m depth reached 38 mmol  $m^{-2}$  at the end of the sampling period highlighting the importance of ice diatoms for the Arctic ecosystem in the Baffin Bay. Enrichment experiments didn't show any nutrient limitation in the ice suggesting that light controlled the production of ice diatoms. While in the water column under the ice we also observed light limitation, diatom production was controlled by nutrient availability despite a global dSi concentration around 5.5 µmol L<sup>-1</sup>. Diatoms growing in the ice and in the water are adapted to different environmental conditions. The ice bloom may have been sustained by a diffusive flux from the surface layer to the ice, as dissolution rate and dSi stocks were low in the ice.  $bSiO_2$  export flux at 2 m and 25 m depth were low during the all sampling season allowing bSiO<sub>2</sub> to progressively accumulate in the water.

\*Speaker

<sup>&</sup>lt;sup>†</sup>Corresponding author: moriceau@univ-brest.fr

## Diatom contribution to primary production under persistent oligotrophic conditions in the coastal Canadian Arctic

#### Lucianne Marshall \*<sup>†1</sup>, Shea Wyatt <sup>1</sup>, Diana Varela <sup>1,2</sup>

<sup>1</sup> Department of Biology, University of Victoria – British Columbia, Canada

<sup>2</sup> School of Earth and Ocean Sciences, University of Victoria – British Columbia, Canada

Despite recent work showing spring phytoplankton blooms developing under ice, little is known about pelagic primary production before sea-ice break-up and even less about changes in diatom and nutrient dynamics occurring along the transition between ice-covered to open marine waters in the Arctic. We will show results obtained from a period of sea-ice cover (mid-June) to open waters (early August) in a coastal location (Cambridge Bay) in the Canadian Arctic Archipelago in 2016. Surface sampling and in situ primary production experiments were conducted daily for the first month and every other day until early August. We found persistently low nitrate concentrations, which translated into low phytoplankton activity and low biogenic silica production. Although diatoms accounted for  $49 \pm 17\%$  of total phytoplankton cell abundance during the study period, they did not dominate the assemblages until late July-August, when silicic acid was drawn down and biogenic silica production increased to a maximum of 0.43  $\mu$ mol Si L<sup>-1</sup> d<sup>-1</sup>. On August 1, cells > 5  $\mu$ m contributed greater to carbon utilisation and chlorophyll a biomass than previously in the season, and biogenic silica reached a maximum of 0.8 µmol L<sup>-1</sup>. The silica increase was attributed to the diatom Chaetoceros sp., which was responsible for up to 61% of total phytoplankton abundance and 98% of silica production. The contribution of diatoms to total phytoplankton biomass in Cambridge Bay will be put into context by comparing results from this coastal region to others measured previously from west to east across the Arctic Ocean.

## Silicon utilization and Si:N ratios in the Pacific Arctic Region from 2006 – 2016

#### Karina Giesbrecht \*<sup>+1</sup>, Diana Varela <sup>1,2</sup>, Jozef Wiktor <sup>3</sup>, Jacqueline Grebmeier <sup>4</sup>

<sup>1</sup>School of Earth and Ocean Sciences, University of Victoria – British Columbia, Canada

<sup>2</sup>Department of Biology, University of Victoria – British Columbia, Canada

<sup>3</sup>Department of Marine Ecology, Institute of Oceanology of the Polish Academy of Sciences – Poland

<sup>4</sup>Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science – United States

Diatoms are the largest consumers of dissolved silica in the oceans and the dominant primary producers in surface waters of coastal and shelf regions. The continental shelves of the Arctic Ocean account for ~50% of its total area, yet there is limited knowledge of the cycling of silicon and its relationship to other marine biogeochemical cycles in this rapidly changing region. To address this, we evaluated biogenic silica dynamics and the diatom contribution to primary production (PP) and nitrate (NO<sub>3</sub><sup>-</sup>) uptake in the euphotic zone of the Bering and Chukchi Seas as part of the Distributed Biological Observatory (DBO). We found that diatoms accounted for ~60% of the PP and ~90% of the NO<sub>3</sub><sup>-</sup> uptake in these regions. Taxonomic analysis also showed that diatoms were the dominant taxa everywhere in the Pacific Arctic Region, except in areas influenced by low nutrient waters near the Alaska coast in the Chukchi Sea. These coastal waters were dominated by small flagellates, and had much lower biomass and productivity. While diatoms still accounted for ~80% of the NO<sub>3</sub><sup>-</sup> uptake in these low nutrient waters, they were only responsible for ~20% of the PP. Our results also show that both Si:NO<sub>3</sub><sup>-</sup> utilization ratios and Si:N ratios of suspended particles were close to the expected ratios for nutrient replete diatoms. An exception was at stations north of 70°N, where Si:NO<sub>3</sub><sup>-</sup> uptake ratios were often 3-4x higher than the Si:N ratios of suspended particles. This suggests that diatoms may have been using another, likely regenerated, source of N in addition to NO<sub>3</sub><sup>-</sup>. Diatom utilization of regenerated N could have far reaching effects on biogeochemical models, which typically assume that diatoms mainly use  $NO_3^-$  as an N source.

## Biogenic silica production across temperate, sub-Arctic and Arctic marine ecosystems

Diana Varela \*<sup>+1,2</sup>, Karina Giesbrecht <sup>+2</sup>, Gregory de Souza <sup>3</sup>, Colin Maden <sup>3</sup>

<sup>1</sup> Department of Biology, University of Victoria – British Columbia, Canada

<sup>2</sup> School of Earth and Ocean Sciences, University of Victoria – British Columbia, Canada

<sup>3</sup> Institute of Geochemistry and Petrology, ETH Zürich – Switzerland

Marine diatoms contribute to about 20% of the annual production of organic carbon on Earth, and exert a major control on the silicon (Si) cycle through the formation of biogenic silica (bSiO<sub>2</sub>). Spatial and temporal variations in the rates of bSiO<sub>2</sub> production can be quantified in enrichment experiments using isotopic tracers onboard ships. For a wider spatio-temporal approach, natural variations in Si isotopes  $(\delta^{30}Si)$  can also be used to trace bSiO<sub>2</sub> production since diatoms discriminate against heavy Si isotopes during uptake. We will present results from a 2-year (2014-2015) high-resolution temporal study of bSiO<sub>2</sub> production in a temperate coastal region of the NE Pacific Ocean (Saanich Inlet). We will then provide a broad-scale comparison of mid-summer bSiO<sub>2</sub> production rates in Saanich Inlet with those in sub-Arctic (NE Pacific open waters) and Arctic regions (North Bering and Chukchi Seas, Canadian Arctic Archipelago (CAA) and Baffin Bay) during 2015-2016. The highest bSiO<sub>2</sub> production rates were measured in the Bering and Chukchi Seas, followed by those in offshore North Pacific waters. The lowest midsummer rates were observed in Saanich Inlet, the CAA and Baffin Bay. Biogenic silica production rates in the CAA and Baffin Bay were also found to decrease from west to east. Furthermore, the assessment of  $\delta^{30}$ Si(OH)<sub>4</sub> signals in sub-Arctic and Arctic marine waters revealed that  $\delta^{30}$ Si(OH)<sub>4</sub> can track modified Pacific waters moving from the Bering and Chukchi Seas through the Canada Basin, and can reflect Si utilization in modified Pacific waters as they transit from west to east.

# Silicon on the rise: Mysteries surrounding use and cycling of silica in a large lake

John Berges \*<sup>†1</sup>, Erica Young <sup>†1</sup>, Loretha Jack <sup>2</sup>, Allison Driskill <sup>2</sup>

<sup>1</sup> Department of Biological Sciences and School of Freshwater Sciences, University of Wisconsin-Milwaukee – WI, United States

<sup>2</sup> Department of Biological Sciences, University of Wisconsin-Milwaukee – WI, United States

Silica is a potentially-limiting and structuring element in freshwater ecosystems, yet we understand its cycling in larger water bodies like the Laurentian Great Lakes very poorly. Dissolved silicate (dSi) in Lake Michigan has increased substantially in the past three decades: at an offshore station (100 m depth) average dSi in the upper 45 m of the water column increased from 10  $\mu$ M during 1988-92 to over 30  $\mu$ M in 2007-9, while total phosphorus, particulate carbon and chlorophyll a in > 10  $\mu$ m size fractions declined. Such increases are typically associated with declines in planktonic diatoms related to invasive dressenid mussels, but mechanisms are unclear. We need to evaluate rates of uptake and release of dSi from diatoms but also include other groups like the chrysophytes, cyanobacteria and benthic algal assemblages. We sampled nearshore waters of Lake Michigan near Milwaukee for benthic green macroalgae (Cladophora sp.) and their dense epiphytic diatoms, and measured biogenic silicate (bSi) in the algae and suspended particles using a carbonate digestion method, and dSi in overlying waters. We also used scanning electron microscopy in combination with elemental mapping, and a novel bSi label (PDMPO) to identify specific taxa accumulating Si. dSi in the nearshore was lower and much more variable than offshore, suggesting a nearshore sink for Si. Cladophora with epiphytes averaged a remarkable 166  $\pm$  68 mg bSi/g dry mass, a significant portion of which was accounted for in the green algal tissue. Using estimates of Cladophora coverage from aerial photos, SCUBA-sampled biomass and bSi, we modeled dSi use by benthic algae in the nearshore region. Daily Si demand of Cladophora assemblages represented an impressive 7 - 70% of dissolved Si in the nearshore water column. Variations in nearshore dSi and bSi could be correlated with riverine run-off and deep-water upwelling events. We are developing a partial model/budget for Si for the Milwaukee nearshore region of Lake Michigan. Current culture experiments involving Si limitation, PDMPO labelling and the inhibitor germanium dioxide are aimed at determining Si requirements for diverse freshwater taxa as well as regeneration rates of dSi.

# Defining the role of Si in structuring freshwater communities

#### Erica Young \*1, John Berges 1, Loretha Jack 2, Allison Driskill \*12

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In freshwaters, silicate is usually considered a 'structuring' element rather than a limiting nutrient for communities, but even after decades of research, there are fundamental questions concerning silicate requirements of different phytoplankton taxa. Productive urban ponds provide tractable ecosystems to examine Si and macronutrient dynamics in freshwater phytoplankton communities. Long-term studies of Estabrook Park pond, Wisconsin suggest oscillation between N and P limitation due to urban nutrient runoff, and observed fluctuations in dissolved Si (dSi) during the growing season correlate with appearance of dead cells. In summer 2017, we used bioassay experiments to determine the importance of dSi in phytoplankton community composition. Dissolved Si concentrations were < 5 μM but additions of Si alone did not stimulate net phytoplankton growth. Treatments +N+P and +N+P+Si both showed net growth but resulted in different community composition - flow cytometry identified distinct groups and microscopic analysis revealed more diatoms when 20 µM silicate was added with N and P. These differences in community composition have implications for food web structure. To further investigate Si cycling within the pond, we examined the significance of biogenic Si (bSi) within the dominant submerged macrophyte, Myriophyllum which supports abundant epiphytes. In fall 2017, field sampling and a macrophyte degradation experiment demonstrated sizable stores of bSi in Myriophyllum; the Siincorporation stain PDMPO, appeared in both plant cells and epiphytic diatoms. As Si pools in degrading macrophytes declined, suspended Si increased, but remineralization to dSi was very slow. We are revisiting classical experiments to quantify Si demand in freshwater phytoplankton using lab culture of known Si-demanding species and taxa with ambiguous Si needs. Growth measurements of cultures in media with low and high dissolved Si and/or GeO<sub>2</sub> have confirmed a Si demand, and GeO<sub>2</sub> inhibition of growth in the diatom Cyclotella, lack of Si demand in the green alga Chlorella, but revealed  $GeO_2$ insensitivity of growth in the Chrysophyte Chrysocapsa. We aim to expand this survey of freshwater taxa, evaluating Si incorporation with PDMPO staining and quantifying bSi pools in diverse taxa to improve our understanding of the role of Si in structuring freshwater phytoplankton communities.

## Session 2: Silicification in diatoms: Ecophysiology and genomics/proteomics

## 'Omic-based approaches to understanding diatom silicification: Insights and implications for silicon cycling

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Over the past decade, 'omic-based approaches have revolutionized our understanding of how microbes shape the environment and how the environment shapes microbes. Genome sequencing has expanded our understanding of diversity while transcriptomics has revealed previously unknown biochemical potential. Diatoms form a critical link between the silicon (Si) and carbon (C) cycle, and since the first diatom genome published in 2004 was released, more have followed and our molecular understanding of these ecologically important organisms is growing. The challenge marine microbial oceanographers now face is how to contextualize all of that sequence information into a framework for understanding how this functional capacity shapes ecosystems. In our own work, we have been using metatranscriptomics, coupled to classical measurements of silica production, to better understand the underlying genetic and physiological mechanisms that regulate the observed rates. We are also using metatranscriptomics to explore the impact that diatom-infecting viruses have on silicification and silicon cycling. In this talk, we will explore how an 'omic-based understanding of diatom silicification can be integrated into a biogeochemical context.

# The interplay between diatom host-virus dynamics and silicon cycling

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The role of algal viruses in mediating global biogeochemical cycles is increasingly appreciated, yet surprisingly little is known about the impact of diatom-specific viruses on diatom populations. Historically thought to be immune to viral infection due to the physical protection provided by their silica-based cell wall, the discovery of diatom-infecting viruses is altering our view of these enigmatic host-virus interactions. Using a combination of field- and laboratory-based approaches, we are exploring the interplay between diatom-virus dynamics and silicon (Si) cycling. Using metatranscriptomics to identify cell-associated diatom viruses and in situ kinetic measurements of Si stress, we examined the prevalence of viral infection at stations within and north of Monterey Bay, California. Viral read abundance exhibited a highly significant positive correlation with the degree of kinetic limitation of Si uptake, suggesting that Si stress may impact diatom-virus dynamics. Using the model laboratory diatom host, Chaetoceros tenuissimus, and its associated virus, CtenDNAV, we observed that physiological Silimitation facilitates rapid host lysis compared to nutrient replete cultures suggesting Si stress enhances host susceptibility to viral infection. Using a mathematical model based on these empirical data, alterations in viral adsorption and host lysis parameters emerged as likely drivers of the observed infection dynamics under Si limitation. Together these data explore a heretofore unappreciated role that diatom-infecting viruses play in diatom-mediated carbon and silicon biogeochemical cycling across different oceanic nutrient regimes.

## The stoichiometry of staying skinny: Increased Si:N uptake without changes in frustule silica content in an iron stressed diatom assemblage

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The cross-shelf dynamics in the California Coastal Upwelling system create a mosaic of iron (Fe) concentrations across surface waters. Under Fe stress, diatoms can increase the ratio of silicic acid (Si) to nitrate (N) uptake from 1:1 to higher values preferentially consuming Si, resulting in surface waters that are high in N, deplete in Si, and often low in chlorophyll. The possible mechanisms that lead to an increase in Si:N uptake create vastly different scenarios for potential export; increased silica ballast can lead to higher export velocity while lower nitrogen content can lead to diminished export of organic matter from the euphotic zone. The cellular mechanisms involved in this response were investigated during a simulated upwelling deckboard incubation experiment using water collected from the 10°C isotherm off the Big Sur coastline. The high initial N:Fe (26:1) indicated that phytoplankton nutrient utilization would drive the system into physiological Fe stress. Incubation treatments were either unamended controls, or amended with 5 nM dissolved Fe or 200 nM Desferal (DFB), a strong ironbinding siderophore. After 72 hrs, the diatoms, Pseudo-nitzschia and Chaetoceros grew to dominate the phytoplankton assemblages within all treatments. Dissolved Si was preferentially drawn down relative to N only in treatments that presented signs of iron stress. Metatranscriptomic analysis of these same communities revealed that multiple clades of silicon transporters were upregulated under Fe stress, while suites of genes involved in N assimilation and photosynthesis were strongly downregulated consistent with the increase in Si:N uptake. Taxon-specific cellular Si content and silica production rates using the fluorescent probe PDMPO were used to determine that the increase in Si:N within diatoms was primarily due to decrease in N content rather than an increase in the silicon content of cells. As cellular nitrogen content diminished under iron stress, the POC:PON ratio stayed relatively constant leading to lower carbon per cell. These data present a scenario where iron stress had no effect on frustule silica content, and had the potential for a significant decrease in export of organic material from the euphotic zone.

#### \*Speaker

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# The incorporation of aluminum in diatom biosilica, biological consequences and possible applications

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Diatoms exhibit intricately structured, porous cell walls. These so-called frustules consist not only of silica and organic substances, but also of foreign elements. Aluminum plays a special role among these elements due to its chemical similarity to silicon. An increased aluminum uptake and cell wall incorporation may affect the biological, chemical and physical properties of diatoms. This can in turn influence the biogeochemical cycle. The current study focuses on the diatom species Thalassiosira pseudonana and deals with biological and materials aspects of aluminum incorporation. Consequences of a high aluminum concentration in the culture medium were investigated, including effects on growth, cell shape, and chemical composition of the biosilica. Additionally, the influence of illumination and growth phase of the cells were elucidated. From a materials science point of view, the cell walls largely consist of an interesting alumosilicate. After harvesting, frustules were cleaned via lysis buffer and calcination. The resulting biosilica was characterized with infrared and <sup>27</sup>Al Solid State NMR (Nuclear Magnetic Resonance) spectroscopy. Furthermore, a possible application as catalyst was evaluated. Therefore, the obtained biosilica was ion exchanged by stirring in an NH<sub>4</sub>Cl solution and subsequent heating. Samples at different treatment stages were analyzed with respect to porosity and acidity. Both properties are detrimental for catalytic activity. The latter was evaluated with an acid catalyzed test reaction, converting benzene to diphenylmethane in a Friedel-Crafts alkylation.

## Session 3: Isotope geochemistry as a tool for understanding the silica cycle

## The stable isotope composition of silicon as a tracer of the marine biogeochemical Si cycle

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When siliceous marine organisms take up dissolved silicon from seawater, they preferentially incorporate the lighter Si isotopes into their biogenic opal. This isotope fractionation results in a complementary enrichment of the dissolved pool in the heavier isotopes of Si, i.e. an elevated  $\delta^{30}$ Si value. At the largest scale, this is reflected in the fact that the mean ocean  $\delta^{30}$ Si value is ~0.5 higher than the isotopic composition of inputs of Si to the ocean, reflecting a loss of isotopically light Si to sediment. But at the regional to basinal scale, the  $\delta^{30}$ Si value of silicon dissolved in seawater shows a large range of more than 2, increasing upward in the water column, and exhibiting the highest values in the surface mixed layer. This ocean-internal  $\delta^{30}$ Si variation reflects the dynamic cycling of Si by diatoms, which take up and isotopically fractionate Si in the surface ocean, producing an isotopic signal of Si cycling that can be used to trace the marine Si cycle at a range of scales. Taking the large database of marine  $\delta^{30}$ Si observations as my starting point, and presenting a  $\delta^{30}$ Si dataset for the subarctic North Pacific, I will discuss the insights that these data give us into the physical-biological interactions that govern the cycling of Si in the sea, especially when combined with models of ocean biogeochemistry that trace the stable isotope composition of Si.

# Making the link between stable isotope chemistry and environmental change

#### Jill Sutton \*\*1

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Changes to the global silicon cycle are important to understand due to the major role it has in maintaining climatic stability on geological time scales via silicate weathering and because silicon is an important nutrient for many organisms. Over the past 20 years, the steady development of biogeochemical tools (e.g.  $\delta^{30}$ Si in silicifying organisms) has helped to constrain our understanding of the role that environmental change may have on the global silicon cycle, although there are still many questions that remain unanswered. The aim of this presentation is to briefly review the utility of stable isotope chemistry to help untangle our current understanding of the global silicon cycle and to provide examples of the major gaps in knowledge.

## The evolution of silicon isotopes during a mesocosm experiment off Peru

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The Peruvian Upwelling is characterized by extremely high primary productivity and one of the globally largest Oxygen Minimum Zones. Due to upwelling of silicate-rich subsurface waters, the primary productivity in this region is generally dominated by diatoms.

Previous studies have investigated the stable Si isotope distribution in seawater ( $\delta^{30}$ dSi), biogenic material ( $\delta^{30}$ bSi) as well as bSi material from sediments to improve our understanding of the present as well as of the past silicon cycle. However, disentangling processes like utilization and dissolution from water mass mixing exerts difficult in this highly dynamic region. Therefore, we participated in a mesocosm experiment to investigate these processes without the influence of water mass mixing.

Eight pelagic mesocosms (~50,000 L) were deployed off Peru between February and April 2017. Over a period of 50 days samples for Si isotope measurements in seawater ( $\delta^{30}$ dSi), biogenic material ( $\delta^{30}$ bSi) as well as bSi material from the attached sediment traps ( $\delta^{30}$ bSised) were collected. The unique setting made it possible to study the evolution of  $\delta^{30}$ Si in the water column and to directly compare diatom  $\delta^{30}$ bSi from the water column to the accumulated bSi in the attached sediment traps. Each mesocosm showed a stratified water column with an oxygenated mixed layer and an anoxic bottom layer, which were sampled separately. At the beginning of the experiment, 30% of deep waters with different N:Si and N:P ratios were added to simulate an upwelling, which influenced the phytoplankton community. DSi concentrations were never completely exhausted during the experiment and only diminished by approximately 50%. Surface waters showed rather high  $\delta^{30}$ dSi values (up to +3.1) compared to the bottom layer with  $\delta^{30}$ dSi values in surface waters, which was associated with the occurrence of silicoflagellates in these samples. Overall we observed unexpected low dSi, bSi concentration as well low abundances of diatoms during the experiment due to the very unusual occurrence of a coastal El Niño.

### The forensics of diatoms: Exploring different modelling approaches to reconstruct Si cycling from deep sediment trap data

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Diatoms are major primary producers and key exporters of organic matter and silica in the Southern Ocean. We used the mass and Si isotopic composition ( $\delta^{30}$ Si) of sinking biogenic silica (BSi) collected in sediment traps in the Antarctic, Polar Front and Sub-Antarctic Zones (AZ, PFZ and SAZ, respectively) to identify and quantify the seasonal evolution of Si fluxes and Si-isotopic balance in the mixed layer (ML) for each zone. The fractionation of Si isotopes during formation of their siliceous frustules closely links the Si isotopic composition of BSi to the degree of silicic acid (DSi) utilization in the ML. South of the subantarctic front (SAF) and during the productive period, the system behaved as a closed isotope system with a fractionation factor  $(^{30}\varepsilon)$  of -1.2 similar to other estimates from the Southern Ocean. North of the SAF, the system behaved more like an open isotope system with a different  $^{30}\varepsilon$  (from -0.42 to -0.89). The  $^{30}\varepsilon$  values and the seasonal variations of particle sinking velocities estimated from the trap data were combined with literature values on seasonal variations in light, silica production and dissolution, and ML depth in a box model to simulate the annual cycle of BSi production and export and  $\delta^{30}$ Si in the AZ and PFZ. The model results indicated that the production of BSi consumed approximately 30% of the winter DSi stock in the ML in both the AZ and PFZ, leaving 8.7  $\mu$ mol L<sup>-1</sup> and 3.9  $\mu$ mol L<sup>-1</sup>, respectively, at the end of summer. The model successfully reproduced the rise in  $\delta^{30}$ Si for exported BSi observed during spring in the traps. Successful simulation of the near constant  $\delta^{30}$ Si in traps during winter required no fractionation of sinking BSi, reinforcing the relevance of using  $\delta^{30}$ Si of BSi from sediments as a tool to investigate Si fluxes in the past ocean.

# Are silicic acid transporters responsible for Si isotopic fractionation during diatom uptake?

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Silicon isotopes are a powerful tool for the study of the present and past silica cycle. It has the power to tracked biological consumption of silicic acid by diatoms since the lighter isotopes of silicon are preferentially taken up. The determination of the isotopic fractionation factor during silicon uptake is vital for understanding modern silicon isotope cycling and for interpreting biogenic silica as a paleoproxy. However, the biomineralisation and the biochemical pathway responsible for the fractionation are unknown.

Diatom cell membrane contain specific silicon transporters (SITs), which are essential for silicon uptake. SIT proteins were microinjected into *Xenopus* laevis oocytes to investigate the potential silicon isotopic fractionation during the transport phase of silicon uptake by diatoms.

Preliminary results show a fractionation factor, from the protein corresponding to the group *Thalassiosira oceanica*, in the same range of previous observations. However, this fractionation factor is likely a reflection of the diffusive uptake of silicon across the cell membrane due to the high silicic acid media concentration used during the experiments. The measurement of silicon isotopes from experiments undertaken at low ambient silicon concentrations, needed to isolate and activate the SIT protein, has required the development of a new analytical method.

For the first time, silicon isotopes measurements from SIT experiments are presented to deconvolve the biochemical step responsible for the silicon isotopic fractionation.

#### A Learning Initiative

### Silica School

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A new graduate level transdisciplinary program, the "Silica School", is being developed by the European Institute for Marine Studies (Institut Universitaire Européen de la Mer - IUEM), and its international collaborators. The Silica School will be comprised of a global consortium of universities and agencies that provide graduate level education and research opportunities (i.e. international student exchanges and internships) and offer an e-learning course; providing academic background on the subject of "Silica: from stardust to the living world". The perspectives of the Silica School, in research and in education at the graduate level (Master's and PhD), are briefly presented.

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#### Session 4: Silicification in other silicifiers

## The evolution, cell biology and ecology of silicification in sponges

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Sponges (Porifera) are the only metazoan phylum to make skeletons of silica. Spicule formation and positioning of the skeleton in the sponge body is species specific and has long been the principal tool for sponge systematics. The main sponge classes diverged over 600 Mya, and yet today the cells that produce larval spicules are cytologically very similar suggesting that the physiology and cell biology of silicification is conserved. Not all sponges are siliceous however, and some siliceous sponges also produce aragonite skeletons. How silicification first arose and whether early sponges had organic, carbonate or silica skeletons or both, is of interest in interpreting the fossil record of the earliest evolving animals and how silicification first arose. How sponges produce the complexity of spicules and localize these to specific regions of the body is a focus of new molecular and cell biological studies. Aging silica in sponges, and understanding the role of sponge grounds, including glass sponge reefs, in sequestering silica are challenges that require innovative approaches. Here I will give an evolutionary, cell biological view on silicification in sponges.

### Ecological role of glass sponge reefs

Anya Dunham<sup>+1</sup>, Stephanie Archer<sup>+1</sup>, Lily Burke <sup>\*+1</sup>

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Glass sponge reefs, built by up to three species of dictyonine hexactinellid sponges, are unique habitats found along the Pacific coast of Canada and the United States. Since 2012, Fisheries and Oceans Canada (DFO) has surveyed 21 sponge reefs from the Strait of Georgia to Chatham Sound, British Columbia. Here we share the research DFO is undertaking to understand variation in live sponge cover and patterns and drivers of biodiversity found on glass sponge reefs. We also discuss implications this research has for glass sponge reef conservation and monitoring.

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# Silicatein and glassin: Mining proteins to understand their roles in sponge silicification

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Silicified sponges produce silica structures in the form of spicules. In some species, the amounts of spicules are so large as to make these species a uniquely tractable model system for isolating proteins occluded within the biogenic silica, and exploring the mechanisms of silicification in these animals. We have identified two key proteins in sponge silicification, "silicatein" from silica spicules of the demosponge Tethya auratium and "glassin" from the hexactinellid sponge genus Euplectella. Each spicule of T. auratium contains a central protein filament, which is completely covered with silica, and thus can be isolated without organic contamination. The protein filaments can be dissociated to a single protein, silicatein. The amino acid sequence of silicatein is significantly similar to that of cathepsin L, an animal lysosomal protease and a member of the papain-like cysteine protease superfamily. Silicatein is the first protein isolated from the silicon biominerals, and characterized both as a template for silicification and as a catalyst for hydrolysis of silicon alkoxides and polycondensation of silica at neutral pH and room temperature. Then, silicatein and its gene has been identified among a wide range of the class Demospongiae and in some species of the class Hexactinellida. Although spicules of Euplectella contain the central filaments, our attempt to isolate the filaments or silicatein from the silica skeleton was unsuccessful. Instead, we discovered a protein in the water-soluble fraction, named glassin, which is a histidine-rich protein sharing no significant relationship with any proteins including silicatein. Glassin directs acceleration of silica precipitation from silicic acid at neutral pH and room temperature. Discovery of glassin in addition to wide distribution of silicatein in both the classes Demospongiae and Hexactinellida gave us a great deal of information on sponge silicification, however, made the queries how and when sponges acquired silicification more complicated. To solve these issues, further exploration for proteins involved in sponge silicification is required.

# Rhizaria: An unexpected role in the carbon biological pump, and in the silica cycle

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Among silicifiers, diatoms play a key role in the trophic networks of the most productive coastal or openocean ecosystems, as well as in the biology-mediated transfer of  $CO_2$  from the surface to the ocean interior. Studies have focused mainly on pelagic diatoms, yet, the importance of other silicifiers in carbon and silicon cycles is poorly documented. Recently, combination of genomic and biogeochemical data have evidenced that another group of silicifiers, the Rhizaria (and more specifically Radiolaria and Phaeodaria), has been largely underestimated. It would represent 33% of the large zooplankton (> 600  $\mu$ m) in the world. Proportions far above what was imagined! Moreover, the presence of Rhizaria are correlated with the carbon export fluxes at 150 m depth, highlighting an unexpected role of this taxa in the carbon biological pump and thus very likely also in the biogeochemical cycling of Si (Lampitt et al., 2009; Biard et al., 2016; Guidi et al., 2016). Although rhizaria are frequently studied in micropaleontology (as biostratigraphic markers), relatively few biologically oriented studies of the living rhizaria in the plankton have actually been undertaken. For the first time, we determine the silica uptake rates and the elemental composition of different rhizaria species sampled from the Mediterranean Sea. The results that we present here confirm their importance relative to other silicifiers highlighting the necessity to better understand their role in the silica cycle.

# Silicification in *Synechococcus*: Observations and open questions

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Individual Synechococcus cells in the field can accumulate silicon from ambient water to surprising but variable degrees. Cellular Si:P molar ratios measured by Synchrotron X-ray fluorescence (SXRF) microscopy averaged 400:1 at a site in the eastern equatorial Pacific. These ratios approached those in a dominant group of co-occurring diatoms. Among sites in the Atlantic, these ratios were lower, varying from <10:1 to ~100:1. This variation could not be statistically related to environmental differences among sites. In the Sargasso Sea where picocyanobacteria are predominant, the picophytoplankton fraction constituted ~15% of biogenic Si (bSi), but up to half of the production of bSi, suggesting that bSi produced by picocyanobacteria is significant but recycled much more quickly than that produced by diatoms. Cultured Synechococcus exhibited less pronounced uptake of dissolved silicon than did cells in the field. Uptake varied over 10-fold among six strains from distinct clades, and among co-occurring cells within a clone. Uptake of dissolved silicon was negatively impacted by ambient phosphate concentrations and increased with ambient concentration in a roughly bilinear fashion, with a lower slope when silicic acid concentrations were < 50 µmol/L. Half units of silicon transporters (SITs) are encoded in genomes of some Synechococcus strains. However, they are not present in those strains that concentrate Si most. The retention of silica may be allowed by conversion of silicic acid or silicate to a non-diffusing form inside the cell. A substantial portion of the silicon (40-50%) in cells is soluble, and in concentrations high enough to suggest some stabilization by ligands inside the cell to prevent polymerization. X-ray spectroscopy of dried cells suggests that the form of silicon is distinct from that in diatoms, and may exist as a gel in association with Mg or Na ions. There are many remaining uncertainties concerning the mode of uptake and concentration of Si by Synechococcus and the forms of Si within cells. I will discuss several possible physiological models for silicon concentration that are consistent with the data, as well as many points of remaining ambiguity.

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#### University of Victoria Campus Map lan lan lan Ar Ar Ar F7 16 STEWART F8A 3957 GORDO HEAD ROAD Wallace Field F6 ISC 1 F8B нит ү 🔷 нит у 🕈 Commun**i**ty Garden CHILD C F2B Playing Field Artificial F2A F5 E. Artificial Turf Playing Field F3 Artificial 8 **Bus Loop** 8 Bowker Creek $(\mathbf{n})$ JSINESS & **University Club** SINCLAIR 0 FIRST PEOPLES Petch Founte 0 Ŧ( 100 McPHERSO VISUA Alternate Bus Stop Lect Conference <sup>в</sup> е , ( NIVERSITY 6 0 ENGINEERING / COMPU SCIENCE BUILI Cunninghar INIVERSITY OF VICTORIA FINNERTY GARDENS -Mystic Vale / Hobbs Creek Alumni Garry Oak South V Cedar HIII Corner Property 4 **General Pay Parking** Buildings Bus Stop 100 0 200 Metres C **Reserved Parking** Feet Residences 0 Food Service Outlet Parkade PK -(N)-**Electric Vehicle Buildings Under** ₿ Welcome Centre (j) **Charging Station** Construction April 2018