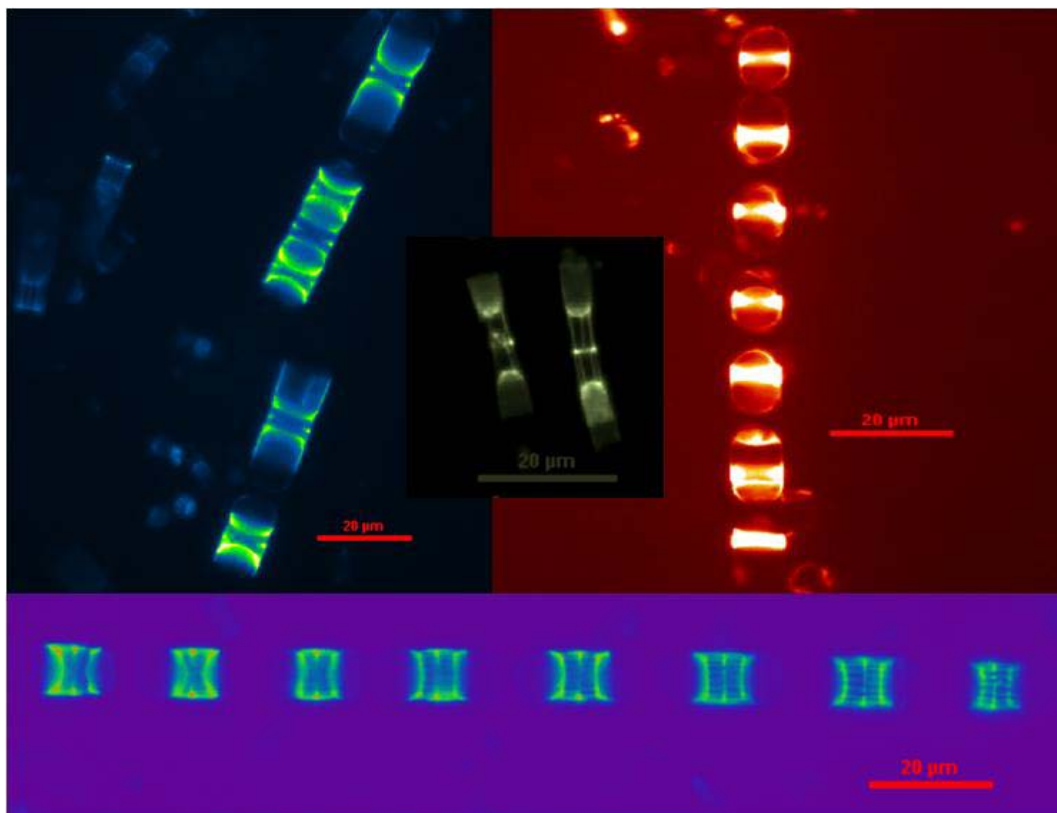


Detecting silica deposition changes in diatoms due to variable nutrient concentrations using a fluorescent silica tracer in the waters of Barkley Sound, Canada



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May 28, 2010

Abstract

Diatoms play a major role in the oceanic silicon and global carbon cycles and require distinct nutrient ratios to maximize their growth and photosynthetic ability. If a required nutrient becomes limiting the cells can become unhealthy and ultimately impact the silicon and carbon cycles. The objective of this study was to understand silica deposition of diatom communities as it is affected by variable nutrient concentrations, in Barkley Sound, British Columbia, Canada. The Barkley Sound system is comprised of a number of channels and inlets. The combination of marine water from the Pacific Ocean and freshwater from numerous rivers provides the sound with unique nutrient concentrations. For this study, data was collected during 20-21 March 2010 aboard the *RV Alta* and *Barkley Star* along a transect through Trevor Channel and Effingham Inlet. Nutrient concentrations were analyzed at each of the 6 sampling stations. The percentage of actively growing cells was calculated using light-microscopy counts for total population size as well as fluorescent-microscopy counts using the 2-(4-pyridyl)-5-[4-(2-dimethylaminoethyl-aminocarbonyl)-methoxy]phenyl}oxazole (PDMPO) staining technique. The number of actively growing cells is similar between Trevor Channel and Effingham Inlet. Effingham Inlet has a higher number of cells undergoing 'maintenance' growth. The percentage growing cells is correlated to the nitrate (NO_3) concentration in Trevor Channel, and to the concentration of ammonia (NH_4) in Effingham Inlet. Trevor Channel has a higher percentage of growing cells due to higher nutrient concentrations and ratios closer to the Redfield ratio. The higher number of maintaining cells in Effingham Inlet is because lower nutrient concentrations make this growth type more efficient.

Non-technical Summary

Diatoms, a type of phytoplankton, are microscopic organisms that live in the world's oceans where sufficient sunlight and nutrients for photosynthesis is available. They help drive the oceanic silicon and global carbon cycles. Distinct nutrient ratios, in the form of silicic acid (Si(OH)_4), nitrate (NO_3), and phosphate (PO_4), are required to maximize their growth and photosynthetic ability. If this ratio changes and limits the availability of the nutrient, the cells can become unhealthy and ultimately impact the silicon and carbon cycles. The purpose of this study was to investigate the impact of varying nutrient ratios on growing diatoms. The data was collected during 20-21 March 2010 aboard the *RV Alta* and *Barkley Star* in Trevor Channel and Effingham Inlet, located in Barkley Sound, Vancouver Island, British Columbia, Canada. Nutrient samples were collected at each of the 6 sampling stations. The percentage of actively growing cells was calculated from the total diatom population size, counted using a light microscope, and the number of actively growing cells, counted on a fluorescent microscope using a stain that causes newly deposited silica in the cell wall to fluoresce. The number of actively growing cells is similar between Trevor Channel and Effingham Inlet. Effingham Inlet has a higher number of cells undergoing 'maintenance' growth. The percentage growing cells is correlated to the nitrate (NO_3) concentration in Trevor Channel, and to the concentration of ammonia (NH_4) in Effingham Inlet. Trevor Channel has a higher percentage of growing cells due to higher nutrient concentrations and ratios closer to the Redfield ratio. The higher number of maintaining cells in Effingham Inlet is because lower nutrient concentrations make this growth type more efficient.

Acknowledgements

I would like to acknowledge the University of Washington – School of Oceanography for financial support. Additional support and guidance was provided courtesy of the Armbrust Lab at the Center for Environmental Genomics, University of Washington. I would also like to thank professors: R. Kiel, R. Morris, D. Kelley, E. D’Asaro. I would also like to give a big “Thank you” to Colleen Durkin for providing incredible help and expertise. I wish to credit Justin Box for providing writing support. Lastly, a word goes out to Diana Haring for providing a map of Barkley Sound.

Introduction

Diatoms play a major role in the global carbon and oceanic silicon cycles. About 40 percent of the total primary production in the ocean is carried out by diatoms via fixation of atmospheric inorganic carbon, making them the base of marine food webs (Sarhou et al. 2005). The diatom cell wall is composed of silicon, taken up in the form of silicic acid (Armbrust 2009). Diatoms undergo two components of growth: (1) division, where one cell divides into two new cells thereby forming two new valves, and (2) 'maintenance' growth, where the cell deposits new bands where the two valves connect, forming a seal between the two valves (Round et al. 1990). The building of new valves and girdle bands makes diatoms the driver of the oceanic silicon cycle; on average a single molecule of silicon is cycled through diatom cell walls 39 times before it reaches the seafloor (Tréguer et al. 1995). Silicon is also a major limiting nutrient to diatoms, and is considered a controlling factor in the growth of the cells (Martin-Jézéquel et al. 2000). In order to maximize their growth and photosynthetic ability, diatoms require distinct nutrient ratios, known as the Redfield ratio: 15 Silicon (Si) : 16 Nitrogen (N) : 1 Phosphorous (P) (Brzezinski 1985). This ratio is an averaged proportion of the nutrients required for growth. However, there are species-specific variations as shown by Brzezinski (1985) that can assist in the determination of species present in different nutrient environments. When diatoms are limited by nutrients, cells become unhealthy, ultimately impacting the carbon and silicon cycles.

The concentration of nutrients present in diatom-inhabited waters is influenced by a number of water properties, including, but not limited to, up- and downwelling events and continental runoff. Barkley Sound, Vancouver Island, British Columbia, Canada, the location of this study, is a distinctive fjord system that includes exposure to the offshore environment (i.e. Pacific Ocean), large tidal impacts, and terrestrial runoff; potentially laden with chemicals

released during deforestation (Taylor and Haigh 1996). These processes impact the major nutrients (nitrogen, phosphorous, and silicon) required for diatom growth. Barkley Sound is comprised of a number of channels, including Trevor Channel and Effingham Inlet (Fig. 1). Trevor Channel is influenced by two types of oceanic processes: upwelling of deep nutrient-rich water at its interface with the Pacific, and heavy freshwater input from Alberni Inlet, at the opposite end (Hay et al. 2003).

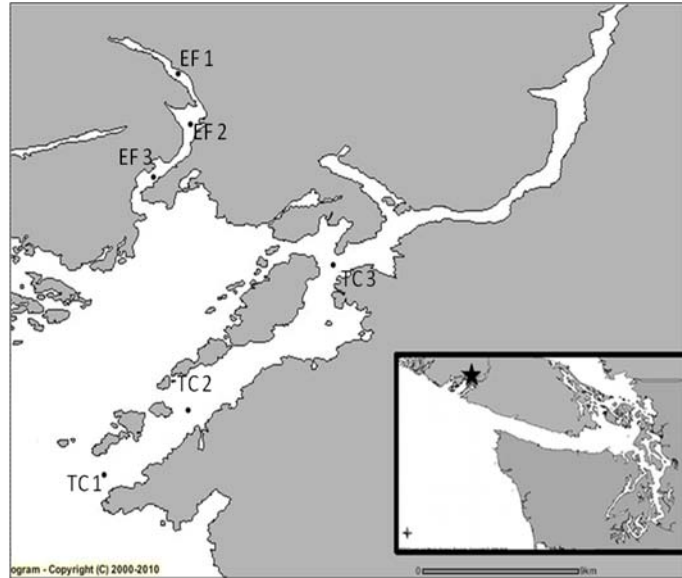


Figure 1: Map of Barkley Sound. Sampling stations are indicated by black circles along with the station identification. Courtesy of Diana Haring.

The channel is provided with both fresh and marine based nutrients from these processes. Generally the surface water is nitrate depleted, but contains enough silica and phosphate to sustain diatom growth (Taylor and Haigh 1996). Community structure analysis performed by Taylor and Haigh (1996) revealed populations of *Skeletonema costatum*, *Pseudo-nitzschia* spp., *Rhizosolenia* spp., *Chaetoceros* spp., and *Thalassiosira* spp..

Effingham Inlet is comprised of three basins (head (EF 1), middle (EF 2), entrance (EF 3)) each separated by a sill. The inner basin is fed solely by the Effingham River. The outer basin is open to Imperial Eagle Channel, which connects to the Pacific Ocean. Due to the low discharge volume of Effingham River, the inlet has been characterized as a low runoff fjord (Pickard 1963). The low freshwater input from Effingham River at the surface produces a weak estuarine circulation pattern which results in depletion of surface nutrients required for diatom

growth (Hay et al.2009). The diatom community is similar to that of Trevor Channel but the abundances are lower (Hay et al. 2009).

Limited and nonexistent biological studies of Trevor channel and Effingham Inlet respectively have left much to be desired by the scientific community. Thus far, studies have focused on diatom community structures (Taylor and Haigh 1996) in Trevor Channel and sedimentation in Effingham Inlet (Hay et al. 2003). There has been an absence of studies on factors influencing diatom communities i.e. variability of nutrients, and no studies have quantified actively growing cells in this area. The objective of this study is to improve our understanding of diatom communities and how the unique physical and chemical features of the fjords affect the nutrient concentrations and ultimately silica deposition in these communities. This paper addresses the following: What percentage of the diatom community is actively growing and how does this percentage relate to the nutrient concentrations? The percentage of actively growing cells is predicted to be higher in waters where silicon concentrations are greatly exceeding the concentrations of nitrogen and phosphorous, because the excess of silicon would not be the limiting factor to their growth. Alternatively, if the concentration of silicon is lower relative to the concentrations of nitrogen and phosphorous, then the percentage of actively growing cells would be low, because the deficiency of silicon will have limited their growth and prevent diatoms from outcompeting other phytoplankton. The health of the diatom communities, depending on nutrient concentrations, throughout Barkley Sound can have a positive or negative effect on the carbon and silicon cycles in the region.

Methods

This study was conducted aboard the *RV Alta* and *RV Barkley Star* during 20-21 March 2010 in Trevor Channel and Effingham Inlet (Fig. 1). Three stations were sampled in each region

ranging from the seaward entrance, landward. These areas have a high potential for widely differing nutrient concentrations due to many different physical and chemical processes present on a local scale.

Water was collected 5 meters beneath the surface. Chlorophyll maximums were not sampled, because it was likely that nutrient ratios would be very close to the Redfield ratios. However, this project was aimed to find how well cells grow when conditions e.g. nutrient ratios were not optimal. The water samples were obtained using a SeaBird Electronics SBE-9 CTD with attached 3-liter Niskin bottles in conjunction with a 3-liter handheld Niskin bottle. A 50 milliliter water sample was analyzed for nutrients (nitrogen, phosphorous, and silicic acid). The nutrient samples were filtered through a 0.20 micron syringe filter and frozen at -20°C to be processed following the World Ocean Circulation Experiment (WOCE) Hydrographic Program nutrient analysis protocol (Gordon et al. 1992) using a Technicon AAI system. This analysis occurred at the University of Washington Marine Chemistry Laboratory by Kathy Kroglund. The data collected was used to calculate nutrient ratios and concentrations.

Due to the possibility that the diatom populations were too dilute, 2 liters of water collected from the Niskin were concentrated to 400 milliliters using a peristaltic pump with a 45 millimeter, 3 micron white polycarbonate filter. Total and actively growing cell quantities were obtained using this concentrated sample. A 200 milliliter sample was treated with 8 milliliters of formalin (Welsh 1948). From this, 10 and 25 milliliter sub-samples were settled over 24 hours using an Utermohl Chamber. Total diatom enumeration was determined via light microscopy, by counting 15 fields at a magnification level of 40x and extrapolated to cells L^{-1} using the objective field and slide areas. All counts were duplicated.

The total number of actively growing diatom cells was determined by the use of fluorescence-microscopy. Growing cells were quantified using a 2-(4-pyridyl)-5-[4-(2-dimethylaminoethyl-aminocarbonyl)-methoxy]phenyl}oxazole (PDMPO) (LysoSensor™ Yellow/Blue DND-160, L7545) stain. This particular stain was used to trace the incorporation of silicon into cell walls of actively growing diatoms (Shimizu *et al.* 2001, LeBlanc and Hutchins 2005). The stain fluoresced under ultraviolet (UV) illumination, which made it possible to

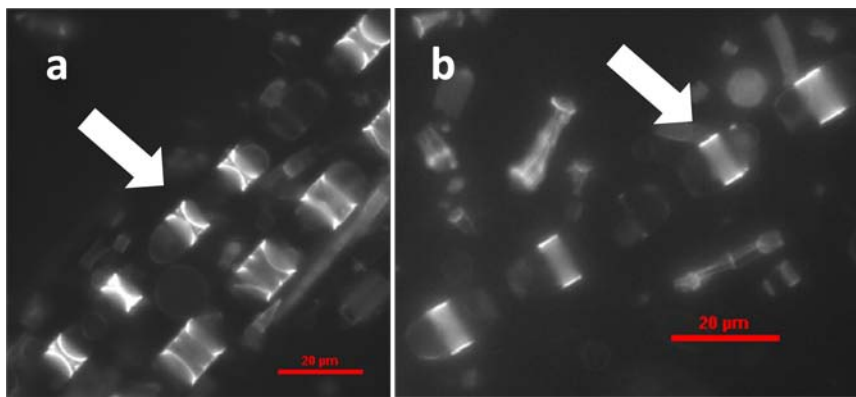


Figure 2: Types of growth in diatoms, active growth (a) and girdle band formation (b) as seen in the PDMPO stained samples. Photos taken by Franziska Lutz.

quantify the amount of silica deposition and the number of cells depositing silica.

Although the PDMPO stain protocol normally required triplicate samples to allow for

statistical analysis, due to a limited amount of stain, duplicate samples were taken to permit a greater number of stations. Fifty micro liters of PDMPO stain (LeBlanc and Hutchins 2005) were added to 100 milliliters of the concentrated water. The bottles were placed in an incubator located outside, used to model the natural environment, and incubated for 24 hours. Subsequent to incubation, samples of 30 and 70 milliliters were filtered onto two 25 millimeter, 0.2 micron black polycarbonate filters, preserved on slides and frozen at -20°C . Only the 30 milliliter slides were counted, due to the concentration of cells on the 70 milliliter slides. These slides were analyzed via fluorescence-microscopy, where 10 fields were counted and characterized as ‘growing’ (actively dividing) and ‘maintenance growth’ (deposition of new girdle bands) (Fig. 2)

at a magnification level of 40x and extrapolated to cells L^{-1} using the objective field and filter areas. The percentage of actively growing cells was calculated using the light-microscopy counts and the ‘growing’ fluorescence-microscopy counts.

Results

Nutrient Concentrations

Nutrient analyses at the sampled stations in Trevor Channel (Fig. 3a) revealed that at TC 1 the concentration of silicic acid ($Si(OH)_4$) was highest ($26.44 \mu M$), followed by nitrogen, in the form of NO_3 ($13.97 \mu M$) and finally phosphate (PO_4) ($1.31 \mu M$). This gave a nutrient ratio of 20 Si : 11 N : 1 P, which was similar to the true Redfield ratio (15 Si : 16 N

: 1 P). The nutrient

concentrations at TC 2

followed the same pattern as that of TC 1, where the concentrations of $Si(OH)_4$, NO_3 , and PO_4 were $30.38 \mu M$, $11.19 \mu M$, and $1.21 \mu M$, respectively. This established a nutrient ratio of 27 Si :

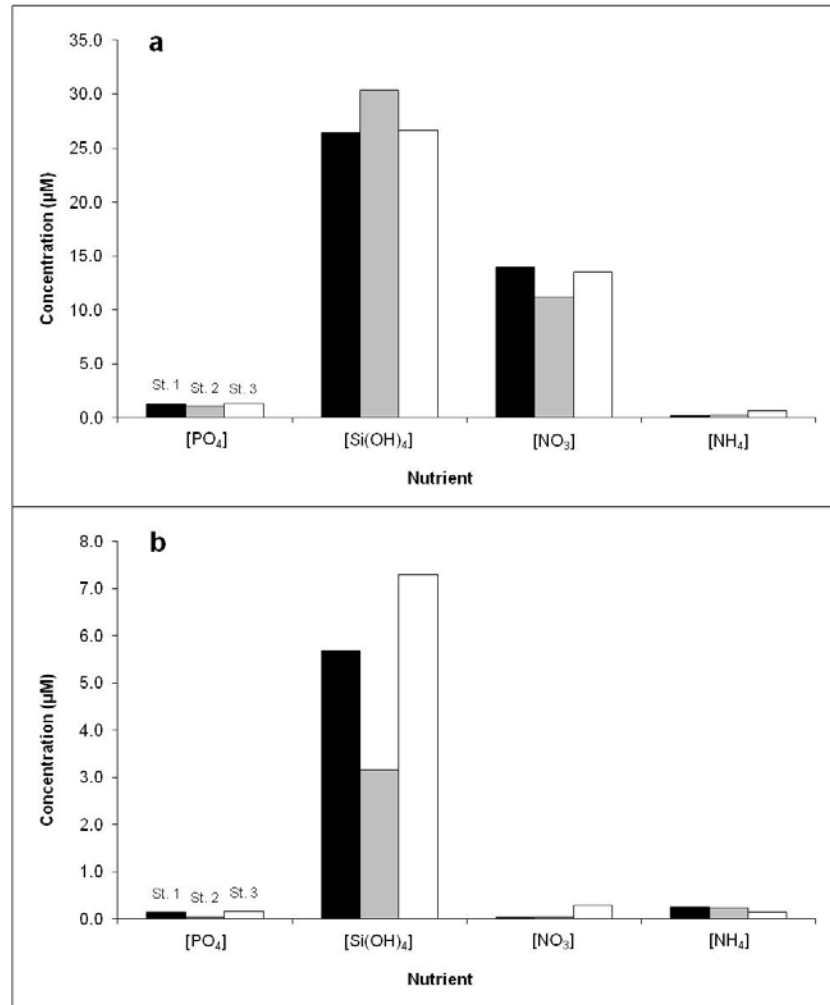


Figure 3: Concentrations of $Si(OH)_4$, NO_3 , NH_4 , and PO_4 at Stations 1 (black), 2 (gray), and 3 (white) sampled at 5m depth in Trevor Channel (a) and Effingham Inlet (b). Note scale of y-axis.

10 N : 1 P. TC 3 had a high concentration of Si(OH)_4 (26.65 μM), followed by NO_3 (13.53 μM),

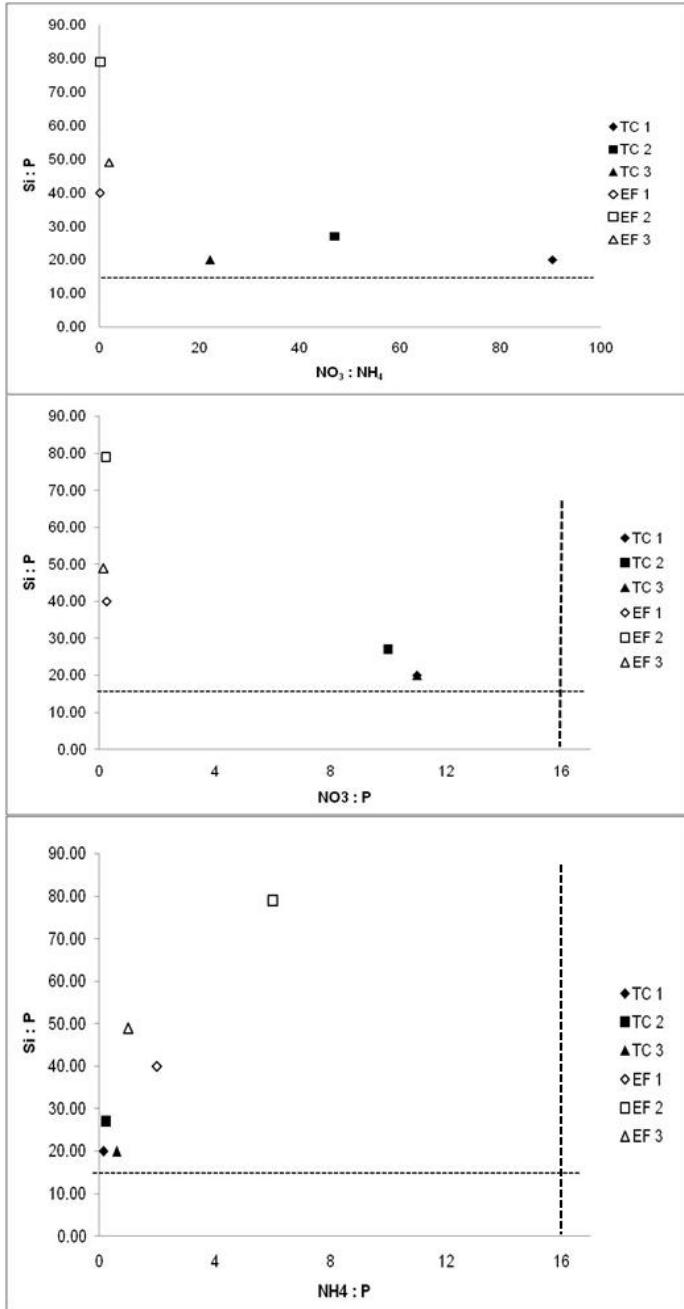


Figure 4: Comparison of Si: P to $\text{NO}_3 : \text{NH}_4$, $\text{NO}_3 : \text{P}$, and $\text{NH}_4 : \text{P}$ in Trevor Channel and Effingham Inlet. Dotted line represents Redfield ratio of 15 Si : 16 N : 1 P.

and finally PO_4 (1.33 μM). The resulting nutrient ratio for this station was 20 Si : 10 N : 1 P.

In Effingham Inlet at EF 1 the concentration of Si(OH)_4 was 5.68 μM , which was higher than the concentrations of NH_4 (0.27 μM) and PO_4 (0.14 μM). The nutrient ratio at this station was equal to 40 Si : 2 N : 1 P. The concentration of Si(OH)_4 at EF 2 was 3.16 μM , which was much higher than the concentrations of NH_4 (0.23 μM) and PO_4 (0.036 μM), and established a nutrient ratio of 79 Si : 6 N : 1 P. Finally at EF 3, the concentration of Si(OH)_4 was 7.30 μM , the concentration of NO_3 was 0.16 μM and the concentration of PO_4 was 0.16 μM . These

concentrations gave a nutrient ratio equal to 49 Si : 1 N : 1 P, which greatly varied from the true Redfield ratio. The nutrient concentrations seen throughout Effingham Inlet are extremely low.

The nutrient ratios in Trevor Channel and Effingham Inlet (Fig. 4) showed an excess of $\text{Si}(\text{OH})_4$ compared to N and P. The ratio of Si : P was much higher in Effingham Inlet than in Trevor Channel, exceeding the Redfield ratio of 15 Si : 1 P. The ratio of NO_3 : NH_4 showed a higher concentration of NH_4 compared to NO_3 throughout Effingham Inlet, which was an indication that the prevalent nitrogen source for diatoms was NH_4 . While the most dominant nitrogen source in Trevor Channel was NO_3 . Even though the sources of nitrogen were different between the two locations, they were the limiting nutrient. Comparing both NO_3 : P and NH_4 : P showed a ratio much lower than the required ratio by Redfield of 16 N : 1 P, which was the indicator of nitrogen being the limiting nutrient.

Population Growth

Table 1: Diatom community data, population size, and growth data for Trevor Channel and Effingham Inlet.

	Number of <i>S. costatum</i>	Number of total population	Number of actively growing cells	Girdle band growth of <i>S. costatum</i>	Girdle band growth of other diatoms
TC 1	1.09×10^6	1.12×10^6	1.86×10^6	4.64×10^5	5.34×10^4
TC 2	2.53×10^6	2.92×10^6	1.64×10^6	9.55×10^5	6.68×10^4
TC 3	1.80×10^6	1.92×10^6	2.81×10^6	8.28×10^5	
EF 1	1.98×10^6	2.00×10^6	1.39×10^6	1.36×10^6	5.01×10^4
EF 2	3.70×10^6	3.81×10^6	1.39×10^6	1.97×10^6	2.67×10^4
EF 3	3.97×10^6	4.14×10^6	1.83×10^6	2.65×10^6	

Analysis of the stations within Trevor Channel and Effingham Inlet revealed a dominance of the diatom species *Skeletonema costatum*. The size of the total population (Fig. 5) and the number of *S. costatum* in the population were higher throughout Effingham Inlet than in Trevor Channel (Table 1). The population size was the high at TC 1 (mouth) and 3 (head) and low at TC 2 (middle) in Trevor Channel, while in Effingham Inlet population size increased from the head (EF 1) to the entrance (EF 3). The largest population was seen at EF 3 with 4.14×10^6 cells L^{-1} and the lowest was seen at TC 1 (1.12×10^6 cells L^{-1}). *S. costatum* comprised an average of 97.3% of the population in Effingham Inlet and 92.4% in Trevor Channel.

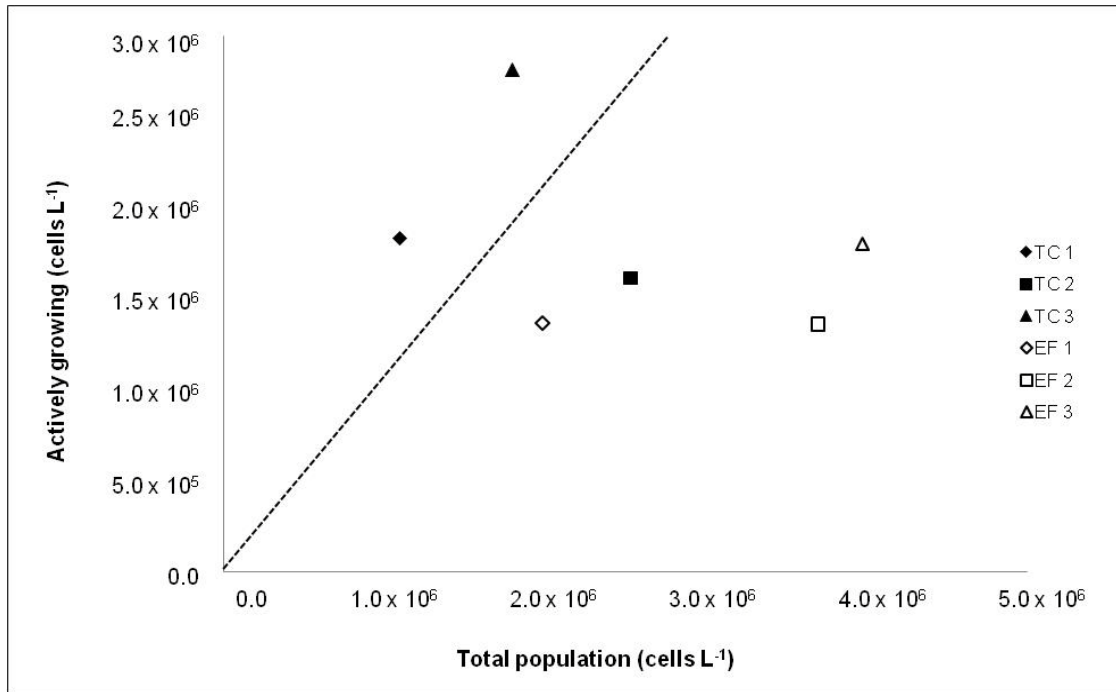


Figure 5: Comparison of the Total diatom population to the number of actively growing cells in Trevor Channel (TC) and Effingham Inlet (EF). Dotted line represents 100% growth of Total population.

S. costatum was the only species displaying active growth, characterized by cellular division, in Trevor Channel and Effingham Inlet (Table 1). With exception of TC 2, which has the largest number of growing cells, the number of actively growing cells throughout Trevor Channel and Effingham Inlet were similar (Fig. 5). The number of growing cells was high at TC 1 (mouth) and 3 (head) and low at TC 2 (middle) in Trevor Channel, while it increased from EF 1 (head) to EF 3 (entrance) in Effingham Inlet. The percentage of the population actively growing (Fig. 5) was higher in Trevor Channel (100% (TC 1), 56% (TC 2), and 100% (TC 3)) than in Effingham Inlet (70% (EF 1), 36% (EF 2), and 44% (EF 3)). It should be noted that two stations in Trevor Channel (TC 1 and 3) revealed percentages greater than 100%, which can be attributed to errors in light microscopy counts for total population size, but for this study they were normalized to 100%.

‘Maintenance’ growth (Table 1) was mainly exhibited by *S. costatum*, but four samples (TC 1 and 2, EF 1 and 2) containing other diatom species showed this growth type as well,

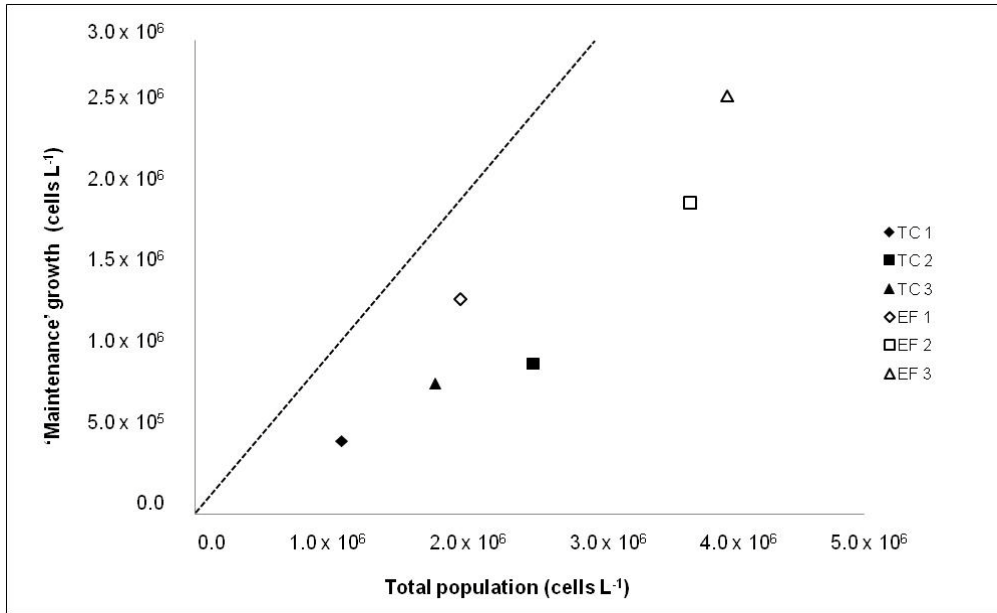


Figure 6: Comparison of the Total diatom population to the number of cells undergoing 'maintenance' growth in Trevor Channel (TC) and Effingham Inlet (EF). Dotted line represents 100% of the total population undergoing 'maintenance' growth.

although in very low concentrations. The number of cells in 'maintenance' growth was higher in Effingham Inlet than in

Trevor Channel (Fig. 6). The number of maintaining cells was highest at TC 2 and lowest at TC 1 in Trevor Channel, while in Effingham Inlet the number increased from EF 1 (head) to EF 3 (entrance). The number of maintaining cells increased proportionally to the increase in population size, which meant that a similar percent of the population undergoes 'maintenance' growth.

Trevor Channel showed no correlation between the percentage of growing cells and the concentration of NH₄ (Fig. 7a). It showed strong correlations to the concentration of NO₃ and the excess of Si(OH)₄. In Effingham Inlet the percentage of actively growing cells showed no correlation to the concentration NO₃ or the excess of Si(OH)₄ (Fig. 7b). It showed a weak correlation to the concentration NH₄, because of the data at EF 2 where the data showed an anomaly compared to EF 1 and EF 3.

Discussion

The percentage of the diatom population actively growing is higher in Trevor Channel than in Effingham Inlet, even though the number of actively growing cells is similar between the two areas. This can be attributed to the fact

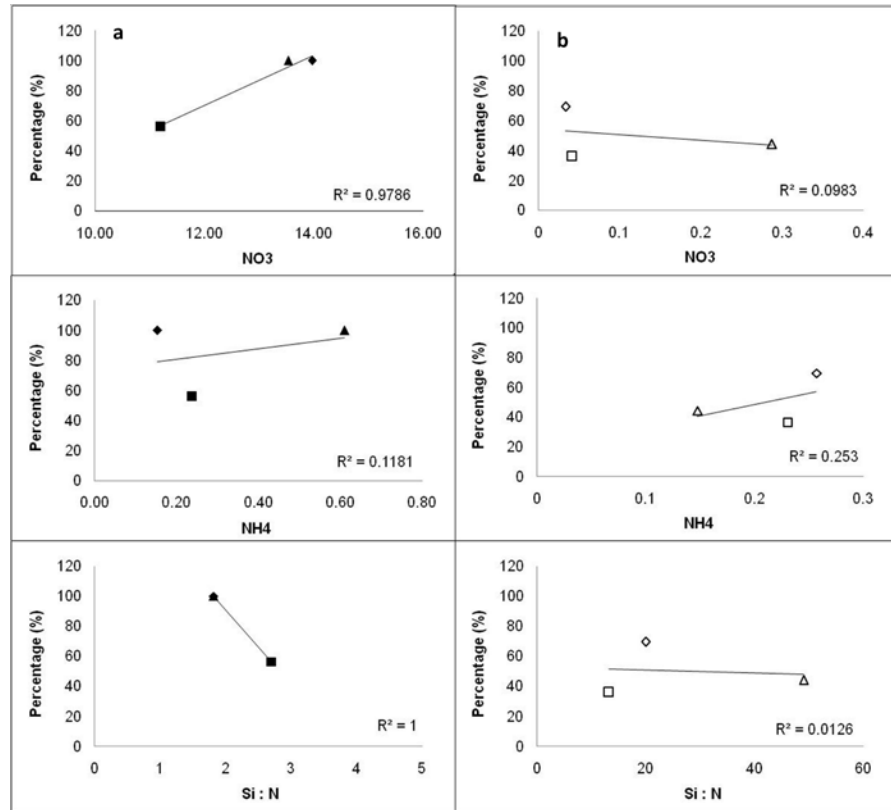


Figure 7: The correlation of the percentage of the population actively growing to the nitrogen concentrations (NO₃ and NH₄) as well as the ratio of Si : N, in Trevor Channel (a) and Effingham Inlet (b).

that the nutrient concentrations seen in Trevor Channel are much higher than those seen in Effingham Inlet. Trevor Channel is much wider and consists of one big basin that receives heavy influxes from the Pacific Ocean and Alberni Inlet, which bring nutrient rich waters to the surface for diatoms to utilize. Pickard (1963) characterized Effingham Inlet as a low runoff fjord with a weak estuarine circulation system, due to the low freshwater input from Effingham River at the surface and low deep marine water input from the Pacific Ocean over the sills.

Trevor Channel experiences greater mixing throughout the column due to the great influences of the Pacific Ocean and Alberni Inlet, while Effingham Inlet experiences little to no mixing of the water column. This not only gives a possible explanation for the variably

population sizes but also for the similar number of actively growing cells (Fig. 5). The turbulent mixing in Trevor Channel distributes diatoms throughout the water column, whereas the diatom community remains at a certain depth in Effingham Inlet. Even though both locations have similar numbers of actively growing cells, the percentage of actively growing cells is much higher in Trevor Channel. The diatoms in Trevor Channel have access to greater concentrations of nutrients than those in Effingham Inlet. The nutrient ratios in Trevor Channel are also closer to the Redfield ratio (Fig. 4), which represents the proportion of nutrients required for growth. The percentage of actively growing cells is related to the concentration of NO_3 , and inversely related to the excess of Si(OH)_4 in Trevor Channel (Fig. 7a). An increasing concentration of NO_3 corresponds with an increasing percentage of growing cells, because more nitrogen is available to allow more diatoms in the population to grow. However, Trevor Channel also shows that the percentage of actively growing cells decreases with an increasing Si : N ratio. This shows that as the concentration of N decreases in relation to the concentration of Si the percentage of growing cells also decreases. In Effingham Inlet, the percentage of growing cells is weakly related, due to the anomalous data at EF 2, to the concentration of NH_4 .

Round et al. (2009) suggested that cellular divisions require a greater amount of energy as well as a larger concentration of silicic acid than the addition of girdle bands. The number of cells depositing girdle bands (maintenance growth) in Effingham Inlet is higher than in Trevor Channel, where the concentration of higher Si(OH)_4 is also higher. The concentration of Si(OH)_4 in Effingham Inlet does show an influence on the number of maintaining cells, because a higher concentration responds to a higher number of cells in 'maintenance' growth. With the exception of EF 2 where there seems to be an anomaly to the data compared to EF 1 and 3. Although Si(OH)_4 shows an influence on the number of maintaining cells, by combining Trevor Channel

and Effingham Inlet, it can be seen that the number of cells maintaining themselves is related to the number of cells in the total population (Fig. 6). This indicates that a set percentage of the population is always undergoing 'maintenance' growth, independent of the nutrient concentrations in the surface waters. Much like the percentage of actively growing cells is below 100%, the percentage of maintaining cells is also under 100%. This suggests that diatoms switch between active and 'maintenance' growth depending on the nutrient concentrations. If a population experiences low nutrients, such as Effingham Inlet, it will have a lower percentage of actively dividing cells, but it will have a higher number of cells undergoing 'maintenance' growth.

The differing nutrient concentrations and ratios in both Effingham Inlet and Trevor Channel may also provide an explanation to the dominance of *S. costatum* seen throughout these regions. *S. costatum* is known for its ability to quickly utilize the available nutrients, and multiply quite rapidly (Pratt 1966). In addition to the ability to quickly appropriate available, *S. costatum* prefers an NH_4 based nitrogen source over NO_3 , if available in high concentrations (Eberlein et al. 1983).

The results from Trevor Channel and Effingham Inlet do not support the initial hypothesis. It was believed that the concentration of Si(OH)_4 influences the percentage of actively growing cells, where a lower concentration leads to a lower percentage of growing cells and a higher concentration leads to a higher percentage of growing cells. In Barkley Sound, the percentages of actively growing cells in the populations are influenced by the concentration of nitrogen in the surface waters, as well as the ratio of these nutrients. Higher concentrations and ratios closer to the Redfield Ratio will allow for a greater percentage of actively growing cells in a population.

Conclusion

The study successfully determined the percentage of actively growing diatoms within a population using a new silica detection method (PDMPO stain). It was found that although the number of actively growing cells is similar in both areas, the percentages of the population that is actively growing are quite different. Trevor Channel has a higher percentage of growing cells than Effingham Inlet. The percentages are correlated to the concentration of nitrogen in both Trevor Channel (NO_3) and Effingham Inlet (NH_4), which causes the original hypothesis to be rejected. It has been widely accepted that if diatoms are nutrient limited the cells can become unhealthy and ultimately affect the global carbon and silicon cycles, due to decreased productions. According to the findings of this study, the diatoms in Effingham Inlet are exposed to low nutrient concentrations and ratios, which results in a lower percentage of growing cells. This means that the amount of carbon and silicon cycled through the water in Effingham Inlet would be lower than that in Trevor Channel. In Trevor Channel the higher nutrient concentrations in addition to ratios closer to the Redfield ratio showed a higher percentage of actively growing cells, indicating that the cells are much healthier than those in Effingham Inlet. It also shows that the amount of carbon and silicon cycled through the water column in Trevor Channel is high. In order to determine the true health of the diatom communities in Trevor Channel and Effingham Inlet, a supplementary study investigating the gene regulations in the diatom communities would be ideal.

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