

Impact of Silicate Limitation on Diatom Mortality in the Equatorial Pacific Ocean

Kristine Prado-Casillas

University of Washington, Seattle, WA

School of Oceanography

[kprado02@uw.edu](mailto:kprado02@uw.edu)

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## Abstract

I explored how *Thalassiosira oceanica* cells responded when an infectious agent from the Equatorial Pacific Ocean entered its cells under silicate limited conditions. *T. oceanica* is an open ocean phytoplankton, specifically a diatom, which creates its cell walls out of silicate. *T. oceanica* is a model organism because of its small yet fully sequenced genome and fast growth rate. Research in host-virus dynamics in diatoms is new, especially in open ocean environments. Viruses play a significant role in the biogeochemical cycle, the movement of nutrients and elements through biotic and abiotic factors, by adding organic matter of diatoms to the microbial loop. They do so through a reproductive process called the lytic cycle when enough viruses replicate within a cell and cause it to burst or lyse. In the Equatorial Pacific Ocean, it is known that iron is the limiting factor inhibiting phytoplankton growth. However, it is hypothesized that silicate is the limiting factor instead of iron in this region. A recent study has found that silicate limited regions increase diatom virus infection due to thin cell walls, allowing for viruses to enter easily. However, research on marine viruses in open ocean environments like the Equatorial Pacific Ocean has yet to be conducted. I hypothesize that a silicate limited environment will increase diatom virus infection in the Equatorial Pacific Ocean. The results from growing infected *T. oceanica* in well plates display that 29.7% of dying and uncertain wells in silicate limited conditions died more than in replete conditions, representing only 1.6%. Higher mortality rates of *T. oceanica* can impact the silicate cycle and carbon export rates in the surface oceans to decrease over time while increasing the viral shunt.

## **Plain Language Summary**

I explored how diatom cells will respond when an infectious agent from the Pacific Ocean enters its cells under silicate limited conditions. Diatoms are a type of phytoplankton that photosynthesize and use silicate to build their cell structure. Research in diatom-virus relationships is relatively new, especially in the Pacific Ocean. Viruses play an important role in the movement of nutrients and elements within multiple cycles of the surface oceans. They do so through a reproductive process called the lytic cycle when enough viruses replicate within a cell and cause cell mortality. In the Pacific Ocean, it is known that iron is the limiting factor inhibiting phytoplankton growth. However, it is hypothesized that silicate is the limiting factor in this region rather than iron. A recent study has found that silicate limited regions increase diatom virus infection. However, research on marine viruses in open ocean environments like the Pacific Ocean has yet to be conducted. I hypothesize that a silicate limited environment will increase diatom virus infection in the Equatorial Pacific Ocean. The results from growing infected diatom cells in well plates display that 29.7% of dying and uncertain wells in silicate limited conditions died more than in replete conditions, representing only 1.6%. Higher diatom mortality rates can impact important processes in the oceans and lead to the reduction of carbon fixation which may impact climate change in the future.

## **Introduction**

Phytoplankton are marine algae that undergo photosynthesis and are responsible for primary productivity in the Earth's Ocean which produces around 50% of the Earth's oxygen present in the atmosphere (Winder & Sommer, 2012). These phytoplankton are primary producers because of their ability to photosynthesize, which converts inorganic matter (carbon dioxide and water) to organic matter (glucose) using sunlight which is known as carbon fixation.

These important organisms are microscopic yet highly abundant in the ocean and contribute to carbon fluxing. This is when dissolved inorganic carbon is converted to dissolved organic carbon that then sinks out of the euphotic zone and into the deep ocean (Suttle, 2007). This is known as the biological pump, and phytoplankton plays an important role in carbon fixation. Diatom cell walls called frustules are made out of silicate, and they control the silicon cycle in the surface ocean by rapidly taking up silicate.

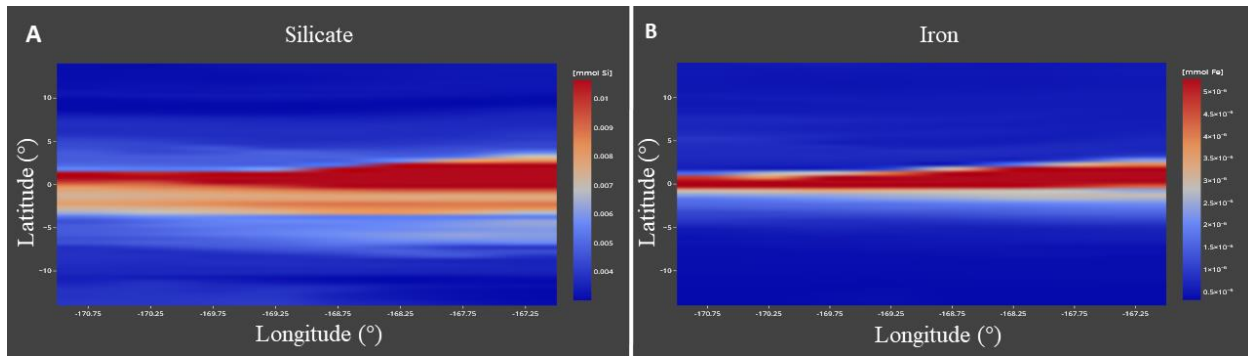
The specific diatom that this study will focus on is *T. oceanica*. This widespread diatom can be found in both freshwater and open oceans around the world. This species has adapted to exist in iron limited regions, specifically in low nutrient conditions (Chappell et al., 2013). *T. oceanica* serves as a model diatom due to the advancement of whole genome sequencing that has increased genomic information available for this species (Lommer et al., 2012). This allows elaborate studies to be conducted to better understand diatom physiology as a whole. Within the phytoplankton community, diatoms play a unique role in controlling and recycling silicate in the surface ocean. They do so by taking up dissolved silicic acid and biosilica to form their frustule (Reyes-Prieto et al., 2009). Changes in nutrient availability can impact diatom species composition, cell division rates, and carbon export (Marchetti et al., 2010). While diatoms play an important role in nutrient cycling and carbon export, other organisms like viruses can impact these processes as well.

Viruses are organisms with varying structures and replication types and can only infect specific hosts. The main structure for viruses include nucleic acids protected by a protein membrane known as a capsid. These organisms are highly abundant in oceans but low in biomass due to their small size (Arsenieff et al., 2022; Suttle, 2007). Due to a virus's inability to swim, they need to be very abundant to find a host. Once the virus meets the specific host, it can

replicate within the diatom genome, through the lytic or lysogenic cycle. It does so by implementing self-replicating genes within the diatom genome that then codes for the production of virus proteins and then viruses itself (Arsenieff et al., 2022). To tell apart which cycle they are using in my experiment, viruses that continuously lyse cells are using the lytic cycle which produce clear water in wells. Producing clear water reduces chlorophyll available, which is a proxy for diatom biomass, and is evidence of diatom mortality. However, it is important to note that the lysogenic cycle is commonly found in temperate regions (Paul, 2008). Regardless, viruses play an important role in the biogeochemical cycle in the surface waters through a process known as the viral shunt. The viral shunt is when nutrients and organic matter are released into the water and added to the microbial loop through lysis of cells due to the lytic cycle (Wilhelm & Suttle, 1999). Research in this field has grown tremendously since the isolation of the first diatom virus in 2004 but much is yet to be uncovered about diatom virus species as only a handful of them have been isolated and studied. This study aims to find how diatom-virus infection changes in the Equatorial Pacific Ocean with varying nutrient concentrations, specifically focused on silicate.

In the Equatorial Pacific surface ocean, phytoplankton take up nitrate due to upwelling waters replenishing nutrients in this region (Dugdale & Wilkerson, 1998). Yet there is a phenomenon in this region where high levels of nitrate and low levels of chlorophyll (HNLC) are found. This was surprising to discover since nitrate is an essential nutrient needed for phytoplankton growth. This led scientists to discover that iron was another essential nutrient for phytoplankton to grow and was found in low concentrations, explaining the HNLC occurrence (Gordon et al., 1997). *T. oceanica* is adapted to live in low iron environments which can explain the low chlorophyll levels found in the Equatorial Pacific. However, it is hypothesized that

silicate may be the limiting factor for diatoms instead of iron which can provide an alternative explanation for the HNLC phenomenon (Dugdale & Wilkerson, 1998). Additionally, silicate is at low concentrations during an El Niño event in 2015 (Fig 1). An El Niño event can be described as weakened trade winds that typically blow west in the equator but now blow east. Warm water that usually moves in the direction of trade winds then moves back towards the east, reducing the upwelling of cold water (Kug et al., 2009). Decreasing upwelling waters that replenish nutrients affects silicate and iron concentrations where they are both at low concentrations (Fig 1A; 1B). This produces silicate and iron limiting regions, but most importantly affects diatom's ability to create their silicate cell wall due to low silicate concentrations. As a consequence of low silicate concentrations, a recent study (Kranzler et al., 2019) found that diatom virus infection rates increased in silicate limited regions. However, further research found that iron limited regions did facilitate viral infection (Kranzler et al., 2021). While it remains unclear exactly why diatom virus infection rates increase in silicate limited regions, it could be due to the thinning of diatom cell walls, meaning that less silicate is used, and provides better entry for viruses. The consequence of increased diatom virus infection rate would increase the efficiency of the viral shunt and therefore decrease the rate of the biological pump in silicate limited regions (Kranzler et al., 2019). Research in diatom virus infection in the Equatorial Pacific Ocean, specifically near American Samoa, has not been conducted in this region before and allows for a research opportunity to contribute to the growing diatom virus field. In this study, I will test how an infected *T. oceanica* responds to silicate limited conditions in the Equatorial Pacific Ocean. Understanding virus-host dynamics in the surface waters of the Equatorial Pacific Ocean can clarify viruses' importance in the biogeochemical cycle and silicon chemistry under silicate limited conditions.



**Figure 1.** Heat map displaying modeled silicate and iron concentrations in 2015 during an El Niño event. Graphs A and B show both low concentrations of silicate and iron during this event. Plots generated by Simons CMAP.

## **Methods**

### **In the lab**

To determine the sensitivity of infected *T. oceanica* to silicate limitation, *T. oceanica* filtered from the Puget Sound was grown in 12 small flasks. *T. oceanica* was first grown in a 24°C incubator with a light set to 8 hours on and 16 hours off. The *T. oceanica* stock was then counted using the Guava flow cytometer to calculate abundance in order to keep cell counts consistent in flask replicates. Once cell counts were determined to be around  $6 \times 10^6$  cells/ml in the *T. oceanica* stock and target abundance for each small flask was determined to be around  $1 \times 10^6$  cells/ml, 10 ml of *T. oceanica* cells were then added into the 12 small flasks. To grow *T. oceanica* cells in varying silicate conditions, 10 ml of FSW + F/2 media was added into each 12 flasks. Of those 12 flasks, 6 flasks contained silicate replete media and the other 6 flasks contained silicate limited media. In the silicate replete media, 3 flasks served as a control for virus infection and the other 3 flasks contained a supposed virus but now known as a bacteriophage isolated from Hawaii. The other 6 flasks that contained silicate limited media

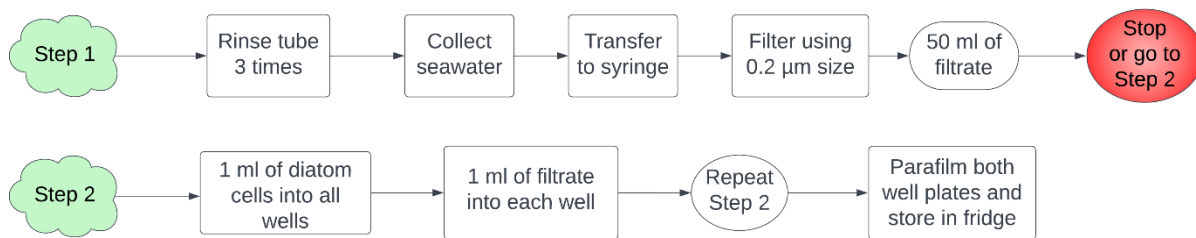
followed the same format as the silicate replete flasks, 3 flasks as control and 3 flasks for infection. The nutrient concentration for the silicate replete flasks contained  $8.82 \times 10^{-4}$  M of nitrate,  $3.62 \times 10^{-5}$  M phosphate, and  $1.06 \times 10^{-4}$  M of silicate. The silicate replete flask also contained a trace metal solution and vitamin solution all with different components and at varying concentrations. The nutrient concentration for the silicate limited flask contained the same levels of nitrate, phosphate, trace metal solution, and vitamin solution previously listed. Since this is a silicate limited experiment, silicate was not added in the silicate limited flasks. Once nutrients were added, 100  $\mu$ l's of the isolated bacteriophage were added into 6 flasks for infection. All 12 flasks were then stored in incubator for 7 days of growth before counting cell abundance using the Guava flow cytometer by sampling 150  $\mu$ l's from all flasks. Data collected from this experiment was then used to help determine my experiment on the boat (Fig 3).

### In the field

*T. oceanica* was used as a model diatom and grown in two large plastic culture flasks. Each flask contained 100 ml of FSW + F/2 media in silicate replete and silicate limited conditions along with 15 ml of *T. oceanica*. Each flask had a total of 115 ml. The nutrient concentration for the silicate replete and limited flasks contained the same concentrations as described in the methods conducted in the lab. Once *T. oceanica* cells have grown in their media, I transferred *T. oceanica* cells into their respective silicate conditions.

Samples were collected on the senior thesis cruise aboard the R/V Thomas G. Thompson on December 28<sup>th</sup> 2023 - January 11<sup>th</sup> 2024. Figure 2 highlights the workflow of my methods done on the research vessel. At each station, a 50 ml Falcon Tube was rinsed three times before collection to ensure whatever is left inside the reusable Falcon tube is diluted (Fig 2). Once

rinsing was done, 50 ml of seawater was collected into the reusable 50 ml Falcon tube and then transferred into a syringe with a 0.2  $\mu\text{m}$  filter attached and filtered it into a new Falcon tube (Fig 2). After filtering, experiment was either continued or the 50 ml of filtrate was stored in the 20°C fridge for later use. Then, a 24-well plate (6 rows x 4 columns) was used to fill 20 out of the 24 wells with 1 ml of *T. oceanica* cells in each well. Additionally, each of those same wells was filled with 1 ml of seawater filtrate. The remaining four wells served as a negative control and contained 1 ml of *T. oceanica* cells and 1 ml filtered seawater (FSW) from the Puget Sound in each well. This process was repeated twice to create duplicate plates for each station (Fig 2). The duplicate 24-well plates were grown under a white grow light for 12 hours on and 12 hours off in a 20°C fridge. To determine if diatom mortality occurs after a week of growth, a qualitative analysis was conducted based on the color of each well. Clear wells could mean that an infectious agent had lysed the diatoms and brown wells means diatom cells are still growing.

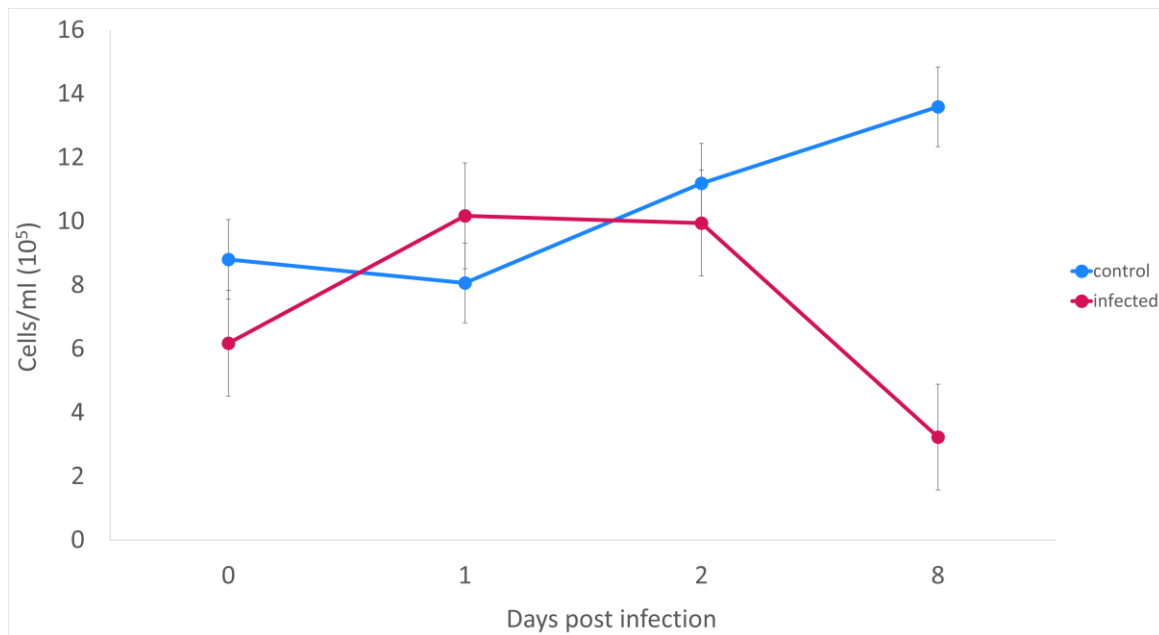


**Figure 2.** Experimental workflow done on the ship. Two main steps with a stopping point in case infection step did not happen right away. Output of experimental workflow for one water sample is two well plates.

## Results

In the experiment conducted in the lab, infected cells decreased in cell count after 2 days post infection (Fig. 3). The control cells continued to grow 2 days post infection (Fig. 3).

Infected cell counts at day zero were around  $6 \times 10^5$  cells/ml and at day 8 cells were around  $3 \times 10^5$  cells/ml (Fig. 3). Control cell counts at day zero were around  $8 \times 10^5$  cells/ml whereas at day 8 cells were around  $13 \times 10^5$  cells/ml (Fig. 3). Based on this preliminary experiment, I expected these results to be reflected in the experiment done on the research vessel.



**Figure 3.** Growth experiment results with changes in cell abundance after start of the experiment. Cells grown in Puget Sound filtered seawater media. The blue line represents uninfected diatom cells, and the red line represents infected cells from bacteriophage isolate. The error bars account for replicates of three small flasks.

Wells were separated into 3 categories: growing, dying, and uncertain (Table 1). This was determined by the color of the wells. A well that had a brown or green color to it, it was considered growing. A well that had no brown or green color to it and displayed a transparent color, was considered dying. A well that had a light brown or light green color was categorized as uncertain (Table 1).

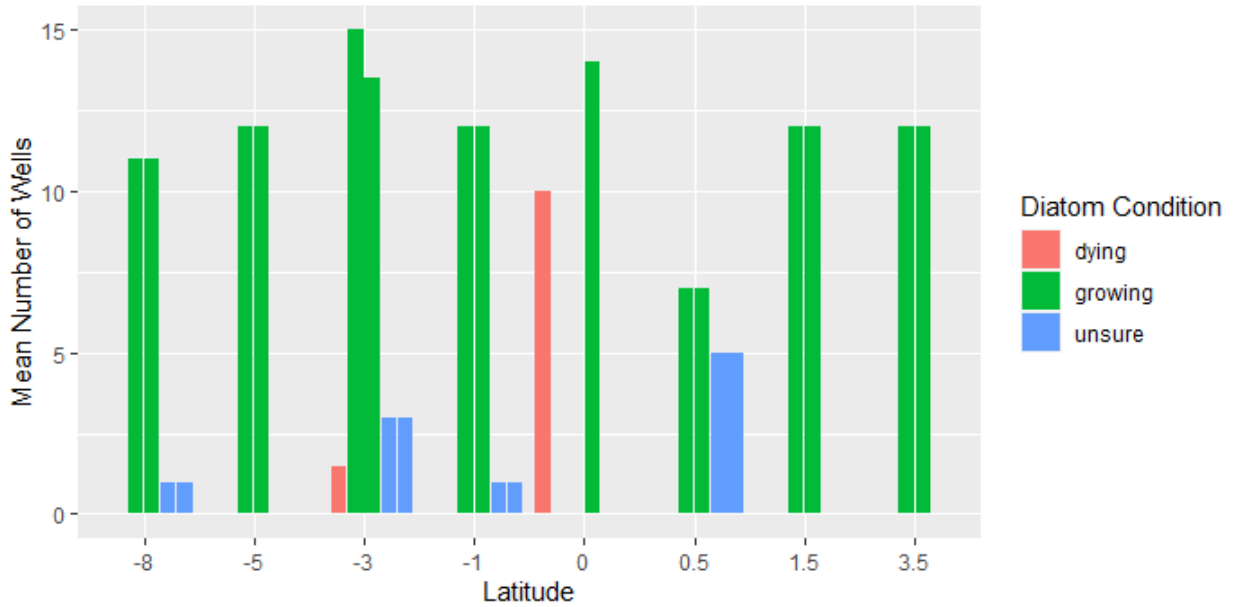
In the replete row, 1.6% of the wells died while 98.4% of wells continued to grow (Table 1). In the silicate limited row, 29.7% of the dying and unsure wells were dying out of the

70.3% wells that were growing (Table. 1). In total, 15.6% of the wells were dying in addition to the uncertain wells while 84.4% of the total wells were growing (Table. 1). Between the replete and limited dying wells, there was a higher percentage of wells dying in the silicate limited row, representing 29.7% of dying wells (Table. 1).

**Table 1.** Diatom condition under varying silicate conditions after 8 days of growth

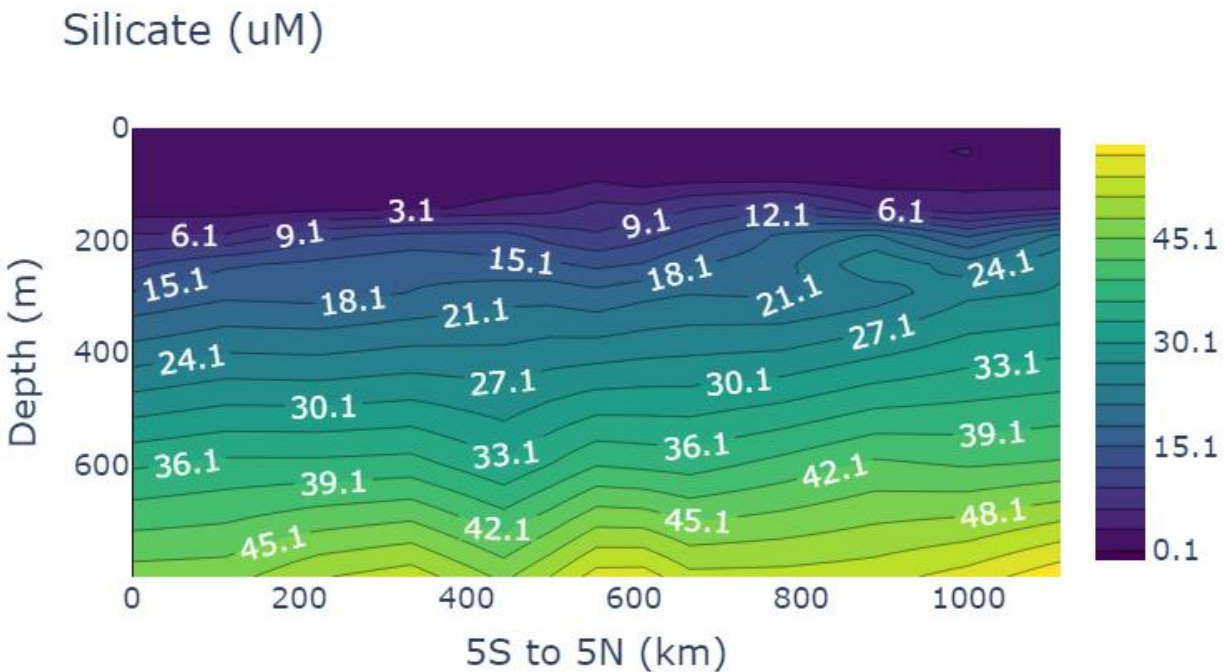
Silicate condition	Growing wells	Dying wells	Uncertain wells
Replete	189	3	0
Limited	135	20	38
<b>Total</b>	<b>323</b>	<b>23</b>	<b>38</b>

All latitudes had the highest number of wells present in growing wells, most notably at 3 south with 15 wells growing (Fig. 4). Dying wells were only present at latitudes 3 south and at the equator (Fig. 4). Latitudes 3 south only had ~3 wells dying and the equator had ~10 wells dying (Fig. 4). Unsure wells were present at latitudes 8 south, 3 south, 1 south, and 0.5 south, with the highest number of wells being 5 unsure wells (Fig. 4).



**Figure 4.** Diatom conditions based on latitude of sample locations. Each latitude represents samples taken from the Pacific Ocean. Each latitude has 4 bars, representing replicate wells in varying silicate conditions.

Silicate concentrations from 0 to 200 meters are  $\sim 9.1 \mu\text{M}$  or less (Fig. 5). The lowest silicate concentrations in the surface layer are  $\sim 0.1 \mu\text{M}$  (Fig. 5). Silicate concentrations from 200-400 meters are  $\sim 20 \mu\text{M}$  (Fig. 5). Silicate concentrations from 400-600 meters are  $\sim 35 \mu\text{M}$  (Fig. 5). Silicate concentrations from 600 to 800 meters are  $\sim 41 \mu\text{M}$  (Fig. 5). Overall silicate concentration ranges from  $0.1 \mu\text{M}$  to  $\sim 50 \mu\text{M}$  (Fig. 5).



**Figure 5.** Depth profile of silicate concentrations collected from latitudes 5 south to 5 north. Data interpolated to create silicate gradient at depth. Samples collected and figure created by AJ Carothers.

## Discussion

Based on the results from Figure 3, cells infected with a bacteriophage were counted and found to have decreased after 2 days, which means infection in the lab does occur. The control cells, however, continued to grow after 3 days, confirming that diatom mortality is due to an infectious agent. Based on these results, I expected diatom mortality to occur out at sea despite silicate concentrations because viruses are highly abundant (Arsenieff et al., 2022). If an infectious agent is present in the seawater filtrate, then I would expect a decline in diatom growth after 2 days and diatom mortality by day 8, based on Figure 3. The results from growing infected *T. oceanica* in well plates based on Table 1 display that 29.7% of dying and uncertain wells in silicate limited conditions died more than in replete conditions, representing only 1.6%. These results can be reflected at certain latitudes and found that dying and unsure wells were located at

latitudes 3 south, 1 south, the equator, and 0.5 south. Silicate limited conditions may have impacted diatom mortality to increase in this region. Silicate concentration maybe have been limited to around 100 meters where most samples were taken at with a concentrations at  $\sim 9.1$   $\mu\text{M}$  or less (Fig. 5).

The implications of this means that if silicate is the limiting nutrient in the Equatorial Pacific, then there may be higher rates of *T. oceanica* mortality in this region. Higher mortality rates of *T. oceanica* can impact the silicate cycle in the surface oceans to decrease over time. Less silicate will be taken up due to low *T. oceanica* abundance and lead to an overall decrease of silicate use. Another impact of higher *T. oceanica* mortality rates is a decrease in carbon export rates. If *T. oceanica* is less abundant in this region, then carbon export rates could be affected and may decrease (Marchetti et al., 2010). If this infectious agent is a virus then the viral shunt in the surface oceans will increase and have cascading effects for organisms around. When viruses lyse their host specific target, they release dissolved organic matter into the environment. This is used as a food source for heterotrophic bacteria and can fuel the mortality rate of microzooplankton and eventually the mortality rate of *T. oceanica* (Wilhelm & Suttle, 1999). This may have the opposite effect on *T. oceanica* cells as the heterotrophic bacteria decrease the abundance of zooplankton and can help *T. oceanica* proliferate.

While silicate limited conditions are shown to increase diatom infection for *T. oceanica* based on the results from Table 1, it is important to note that silicate conditions may not be influencing diatom mortality and it may be due to another factor. It was recently found that diatom mortality was found to have been largely influenced by iron limited conditions rather than silicate limited conditions as previously reported (Kranzler et al., 2021). This can be further supported as silicate concentrations ranged from  $\sim 9.1$   $\mu\text{M}$  or less in American Samoa while

predicted El Niño conditions had around 0.01  $\mu\text{M}$  (Fig. 1; Fig. 5). The Equatorial Pacific in American Samoa could have been iron limited rather than silicate limited and could explain why El Niño did not have an effect on silicate levels. Since my experiment did not take into account iron concentrations, iron may have influenced diatom mortality in my experiment, especially under an El Niño event.

## **Conclusion**

Diatom mortality under silicate limited conditions in the Equatorial Pacific is supported by my results and past literature. Diatom mortality has tremendous effects on the silicate cycle in the surface ocean, carbon export rates, and the viral shunt. The understanding of the diatom-virus relationship has been known for 20 years but the exact details and underlying effects between the two is still unknown. Despite silicate conditions, I expected an infectious agent to infect *T. oceanica*, and found that 29.7% of silicate limited wells were dying. That percentage makes up about 22 wells that I determined were dying and extracted them and sent them to the lab to later reinfect and hopefully isolate them. As a follow up to this study, I want to conduct preliminary experiments in the lab for iron limited conditions rather than silicate to see if infection is exacerbated in these conditions.

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