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The diversity behind the glass curtain:
What grows in Hilo Bay, HI, when diatoms are inhibited?

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Abstract

Diatoms are very prevalent in coastal waters with high nutrient levels. They contribute 60% of all primary production in the oceans, and previous studies have shown that diatoms grow dominantly over other phytoplankton species if silica is available. Hilo Bay, Hawaii, is a good example of such environment. This project aims to determine what other phytoplankton species grow when diatoms are inhibited. The objectives were to identify other species in a diatom-inhibited culture using light and SEM microscopy and to compare the growth rate of an inhibited and non-inhibited culture using cytometer for cell count. Samples were collected from Hilo Bay and enriched with Nitrogen and Phosphorus, and half of the samples were further added Germanium Dioxide (GeO_2) to inhibit diatom growth. Samples were collected every 24 h for fluorometer and flow cytometry. The composition of phytoplankton changed significantly. The treated sample exhibited an overall decrease in biovolume, as diatoms were inhibited and analysis on the SEM revealed a large diversity of flagellates and dinoflagellates together with a high density of detritus and dead cells. The non-treated sample exhibited an increase in biovolume by 2600% as the diatoms reacted very strongly to nutrient enrichment. Both cultures shifted towards a composition of larger phytoplankton: The treated samples due a dinoflagellate, *Gymnodinium*, growing, and the non-treated samples exhibited a shift from smaller *Chaetoceros* diatoms to larger diatoms. This project illustrates there is a high diversity of phytoplankton in Hilo Bay, which is not always visible in water samples where diatoms are extremely dominating. Future experiments should have a larger sample size and lower concentrations of GeO_2 to avoid large impacts on non-diatom species.

Introduction

Phytoplankton are small unicellular organisms accounting for main primary production in the oceans. The community structure of phytoplankton in an ecosystem can regulate the role of the primary production. It affects the secondary trophic level as well as the biogeochemical cycles, such as regulating silica levels or contributing to carbon uptake (Harrison 2000, Cloern & Dufford 2005). The most common group of phytoplankton is diatoms, however, a large diversity of phytoplankton exists and thrives in these same environments and are all competing for resources. The dynamics of the community structure is complex, and dependent on biotic as well as abiotic factors, such as temperature, nutrients, light, mixing and predation (Reynolds 2006).

Diatoms dominate a variety of aquatic and marine habitats with high nutrient environments, given that silica is not a limiting nutrient (Reynolds 2006, Winder et al. 2009). The difference between diatoms and other phytoplankton species lays mainly in the composition of its frustule or shell. While the majority of phytoplankton has an organic calcite shell, diatoms have an inorganic silica frustule. The production of a silica frustule is estimated to require only 8 % of the energy required for organic calcite shell (Yool & Tyrrell 2003). However, the silica frustule is dense, making the diatoms prone to sinking in still waters. Additionally, diatoms are dependent on silica as an extra nutrient compared to calcifying phytoplankton (Reynolds 2006). The community competition revolves around the availability of silica in many ecosystems. Because diatoms have rapid growth responses, germanium dioxide can be added to aquaculture facilities to limit or prevent the growth of contaminating diatoms. Germanium and silica have similar chemical properties, and germanium is favorably taken up by diatoms, and thus inhibiting the formation of the silica frustules (Tribovillard 2013, Durak et al. 2016).

This study aims to investigate the phytoplankton diversity, which is important for the ecosystem in Hilo Bay, HI. Hilo Bay has a high concentration of silica and is therefore mainly dominated by diatoms. This study aims to determine what other species live in the shadow of diatoms. The objectives of this study were to i) identify phytoplankton through light - and Scanning Electron Microscope (SEM) in diatom-inhibited cultures, and ii) compare growth rates and size composition of inhibited and non-inhibited samples with flow cytometry.

Methods and Materials

Study site and sampling:

The study site was Hilo Bay, Hawaii; a semi-enclosed bay with high nutrients that is strongly dominated by diatoms. One sampling effort was completed by kayak close to the University of Hawaii's water quality buoy in the middle of the bay. Approximately 15 L was collected and later divided into cultures.



Fig. 1: Study site, Hilo Bay, HI, and sampling site denoted by the red dot

Culturing and daily measurements

Six samples were cultured in 1L bottles in an aquaculture pond at the Pacific Aquaculture & Coastal Resource Center (PACRC) in Hilo to allow sufficient light and temperature regulations. The samples were added Nitrogen and Phosphorus F/2 growing media (1 ml/L) (Andersen 2005). Additionally 5 ml/L Germanium dioxide was added to three bottles (bottle 4,5 and 6) to inhibit diatom growth (Andersen 2005). The samples were grown for four days. Daily, 2 mL samples were collected from each culture and preserved with glutaldehyde at sampling time, and frozen immediately after in $-80\text{ }^{\circ}\text{C}$ at the Marine Science Department at UH Hilo. These samples were later analyzed with a flow cytometer. On the last day a $\approx 200\text{ml}$ mix of each of the treated and non-treated cultures were preserved in Logul's solution for later identification on light microscope and SEM.



Fig. 2: Left: Culturing bottles on day 3 of sampling. Notice bottle number 1, which stopped growing after day 2 and was taken out of the growth calculations, Right: bottles floating in a pond at PACRC to allow sunlight and regulate water temperatures.

Characterizing and growth measurements

Data was recorded in a flow cytometer in cell count pr. mL, estimated spherical biovolume, and fraction of four cell sizes (pico, nano1, nano2, nano3) on the flow cytometer. Phytoplankton are grouped into picophytoplankton (0.2–2 µm) such as cyanobacteria and coccolithophores, nanophytoplankton (2–20 µm) such as flagellates and small diatoms and microphytoplankton (20–200 µm) such as larger diatoms, larger flagellates, and dinoflagellates (Reynolds 2006).

The dataset consisted of measurements of all six cultures over the four days of growth. Phytoplankton was characterized with light microscope and Scanning Electron Microscope (SEM). Tomas (1997) *Identifying marine phytoplankton* was used for identification. In order to concentrate the samples, the logul's solution with phytoplankton was settled in small, covered columns for 24 h prior to preparing them for the SEM. The SEM samples were prepared with gluteraldehyde and sodium cacodylate, and filtered onto a 0.5µm filter and freeze dried using dry ice and a freeze dryer. The samples were coated with a mix of palladium and gold for the SEM.

Results:

Biovolume and growth rates

An exponential regression was fitted to Estimated Spherical Volume (ESV) and to chlorophyll *a* concentrations, and the doubling rate was calculated (Fig 3, left and right respectively). The flow cytometer data showed an overall decrease in biovolume in the treated sample (from 28.1 to 22.9 µm³/L – a decrease by 18%) but the rate was relatively constant and therefor not fitted to a regression. The non-treated sample had a significant increase in biovolume from 30.6 to 830.2 µm³/L, an increase of 2601%. It was fitted to an exponential regression ($y = 34.967e^{1.176x}$, $R^2 = 0.935$). The growth curve shows a large increase from day 0 to 2, but it lags off on the final day, possibly because the water sample reached maximum carrying capacity (Fig. 3). The doubling rate for the sample was calculated as $T_2 = \frac{\ln(2)}{r}$.

$$T_2 = \frac{\ln(2)}{1.176} = 0.59 \text{ days} = 14.1 \text{ hrs}$$

The chlorophyll *a* concentrations showed slightly different trends in growth rates. The non-treated sample had a doubling rate of 10.9 hours ($y = 0.4734e^{1.533x}$, $R^2 = 0.998$).

The treated sample did exhibit an increase in chlorophyll *a* although the biovolume decreased. The chlorophyll *a* showed a doubling rate of 45.9 hours ($y = 0.3947e^{0.362x}$, $R^2 = 0.834$), indicating a shift in phytoplankton composition towards more chl *a* rich cells.

The differences in starting value in the two models can be due to immediate effect of addition of GeO_2 or possibly to fit the slower growth.

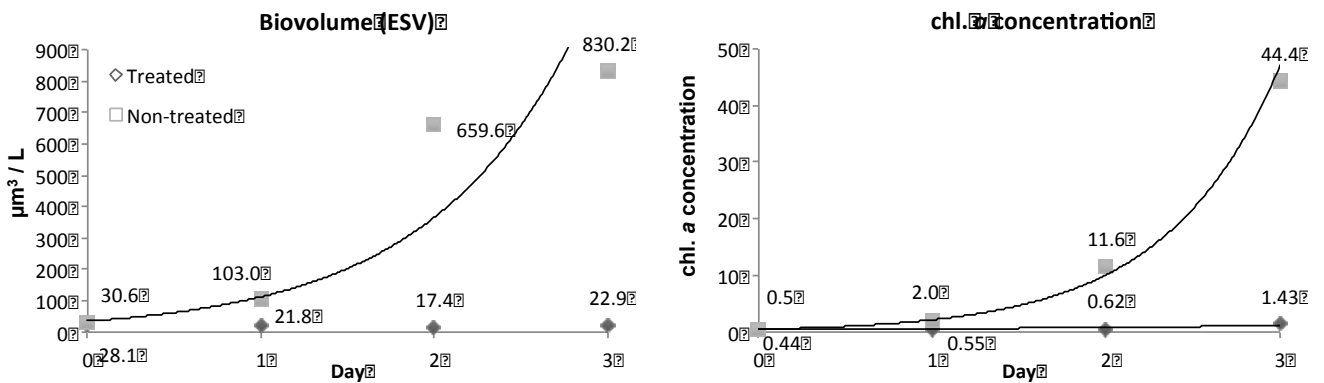


Fig. 3: Left: Biovolume ($\mu\text{m}^3/\text{L}$) of the treated vs. non-treated sample, right: chlorophyll *a* concentration of the treated and non-treated samples with exponential fit.

Size distribution

The individual growth rates of pico and nano phytoplankton in the treated and non-treated samples illustrate significant differences (Fig. 4). Figure 4 illustrates the cell counts pr. mL of sample of pico and nano phytoplankton. The treated samples experienced a significant decrease in pico phytoplankton over the duration of the experiment, however there was a doubling of the nanophytoplankton, which corresponds with the size classes of dinoflagellates observed through the SEM, described later. The non-treated sample grew in both size classes. The picophytoplankton experienced a decrease on day 3, but the nanophytoplankton increased at a rate close to exponential (Fig 1). Diatoms exhibit a range of size classes and it can be suggested that the increase in both nano and picophytoplankton are diatoms.

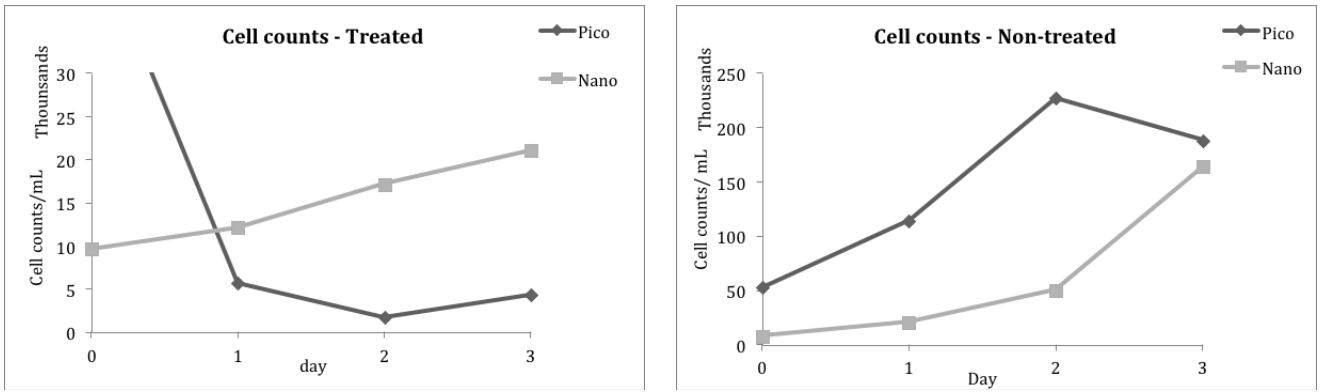


Fig. 5: cell counts pr mL of pico and nano phytoplankton size classes in treated and non-treated samples.

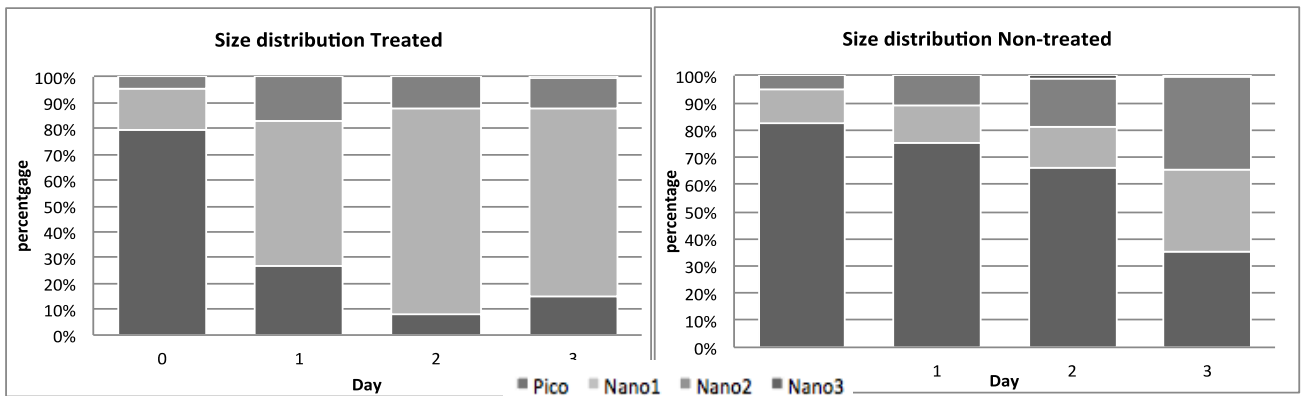


Fig. 4: Size distribution of phytoplankton in the treated and non-treated samples. Both cultures are dominated by picophytoplankton on day 0. This decreases in the treated sample, which is dominated by nanophytoplankton on day 3. The non-treated cultures shift to an even distribution of pico, nano1 and nano2 by day 3.

Identification of phytoplankton

Observations on the SEM revealed that the dominating phytoplankton in the treated culture is a *Gymnodinium*: A class of ‘naked’ dinoflagellates that do not form thecal plates. This class corresponds with the growing nano size class observed on the cytometer (Fig. 7). The culture still had some diatoms growing despite the added GeO₂, and large quantities of detritus and dead cells. The non-treated culture was comprised mainly of *Chaetoceros*, a pico-sized diatom. However many larger diatoms species were found as well (Fig. 6).

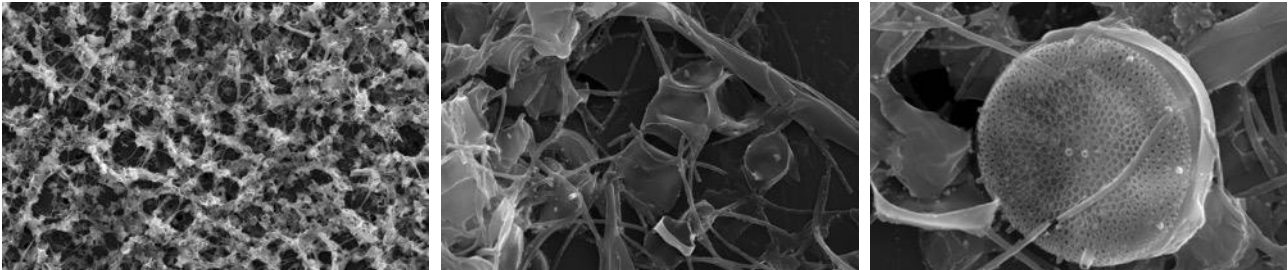


Fig. 6: Results from the non-treated, diatom-dominated culture. Left: a 100µm scale for overview of a very dense sample of mainly *Chaetoceros*, Middle: the dominating species of genus *Chaetoceros* (scale bar 10µm) Right: a small centric diatom.

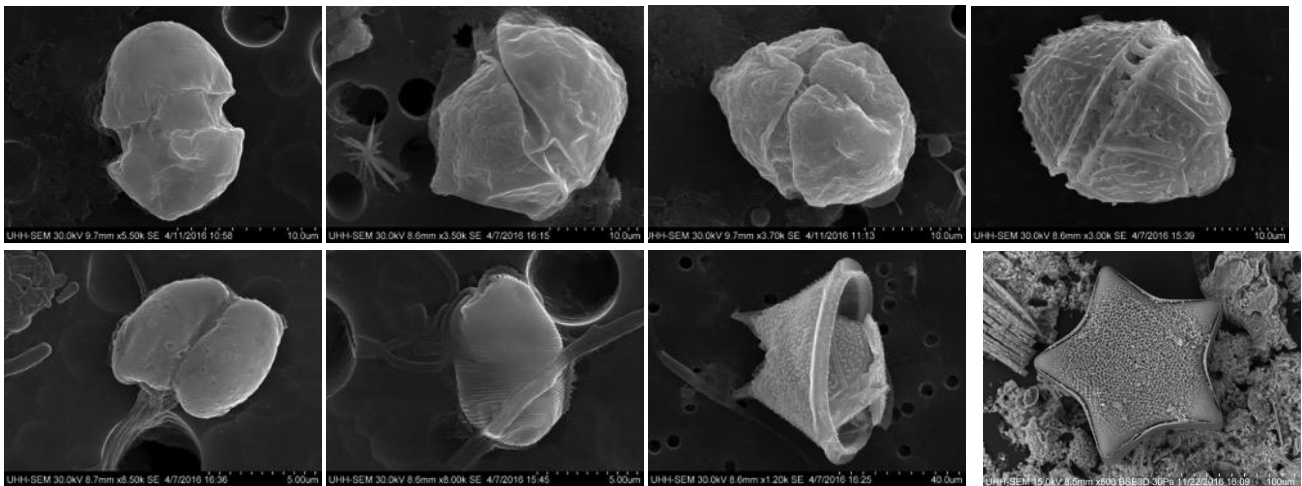


Fig. 7: Results from treated culture with larger diversity. From left to right, Top: Three ‘naked’ *Gymnodinium* species that dominated the treated culture, *Lingulodinium*. Bottom from left to right: Small unidentified flagellate, *Euglenoid*, *Protoperidinium* and lastly *Triceratium formosum*

Discussion

Results show a significant difference in community composition in the treated vs. non-treated culture. Overall, the cell density and biovolume of the treated culture decreased throughout the duration of the experiment. This indicates that there is some component, other than nutrients, lacking or inhibiting growth. Both cultures had the same conditions for temperature, light and nutrients. A possible explanation is the amount of Germanium dioxide or silica availability. Germanium dioxide (GeO_2), is generally argued to be a diatom-specific inhibitor, however studies show that it may have an inhibiting effect on calcification in other phytoplankton species. Durak et al. (2016) found that GeO_2 affected the calcification in coccolithophores as the samples with high Ge:Si ratios experienced malformations and defects in the calcification and that their growth response was lower. They found that it was the ratio of Ge:Si that determines the inhibitory efficiency rather than the concentration. However in a study Shea & Chopin (2007) it was determined that concentrations of 0.10 - 0.50mL of GeO_2 / L seawater was most efficient, but diatom growth was inhibited even at concentrations of 0.02 mL/L. For this experiment, a concentration of 5 mL/L was used (Andersen 2005), which is much higher than suggested by Shea & Chopin (2007). Andersen (2005) also states that GeO_2 may have inhibitory affects on the photosynthesis of phytoplankton. The concentrations used in this experiment may have been too high and can explain the decrease in biomass, if photosynthesis decreased. The main species growing in the treated sample was *Gymnodinium*, a 'naked' dinoflagellate, which does not produce the thick thecal plates. Although these thecal plates are made of cellulose, and no studies were found to have investigated implications of germanium dioxide on cellulose plate production, it could be a possible explanation of why *Gymnodinium* are dominating. The high concentration of GeO_2 likely explains the rapid drop in biomass caused by a decrease in diatoms. The ratio of pico:nano phytoplankton decreased significantly in both the samples, however for different reasons. For the non-treated sample, it was due to a rapid growth in nano-sized phytoplankton, whereas the treated sample changed mainly because the picophytoplankton decrease to less than 10% of the culture.

A limitation of this study was the small sample size that did not allow for statistical analysis. Only 6 cultures were grown, and one of three non-treated cultures were excluded from the study, as the sample seemed to be dead or gone after day 2 (Fig 2). Possible explanations include grazers or zooplankton, or some form of toxic contamination, however it was not determined why this culture was so significantly different from the two other non-treated.

For future studies it is suggested to use much smaller concentrations of GeO_2 as diatom inhibitor. Ideally, this study could be replicated with different concentrations of GeO_2 in order to determine the lowest possible concentration that can inhibit diatoms and reduce the impact on other species. Lastly, water samples vary seasonally. This sample was collected in February which, according to Gettings et al. (2014), is when the diatoms start blooming the most. A sample in the late spring or summer may have yielded different results with larger concentrations of dinoflagellates (Gettings et al. 2014). Chl. *a* data was also collected and may be used as a measure of photosynthetic potential in a specific composition of species, and it illustrated that the treated sample exhibited a shift towards species with higher concentrations of chl. *a*, whereas the non-treated sample had a larger growth in biovolume than in chl. *a*, respectively, indicating larger growth of cells with lower chl. *a* density.

Conclusion

By inhibiting diatoms in water samples from Hilo Bay the community composition of phytoplankton changed significantly. Although there was a decrease in biovolume in the treated culture (18% decrease), there was a growth of *Gymnodinium*, a ‘naked’ dinoflagellate. The non-treated culture increased by 2600% with the diatoms reacting very strongly to nutrient input. One explanation to the lacking growth in the treated sample can be that concentrations of germanium dioxide were too high, which can have inhibitory effects on non-diatom species. However this project shows that phytoplankton communities are sensitive and react strongly to differences in nutrients, light, and competition. The microscope analysis on the treated cultures illustrated there was a high diversity of phytoplankton, which is sometimes masked by diatoms in water samples from Hilo Bay where diatoms are extremely dominating. Future experiments should have a larger sample sizes and smaller concentrations of GeO_2 to avoid large impacts on non-diatom species

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References

- Andersen RA (2005) *Algal Culturing Techniques*, 1st Ed. Elsevier Academic Press
- Cloern JE, Dufford R (2005) Phytoplankton community ecology: principles applied in San Francisco Bay. *Mar Ecol Prog Ser* 285:11–28
- Durak GM, Taylor AR, Probert I, Vargas C de, Audic S, Schroeder DC, Brownlee C, Wheeler GL (2016) A role for diatom-like silicon transporters in calcifying coccolithophores. *Nat Commun*:in review
- Gettings RM, Townsend DW, Thomas MA, Karp-Boss L (2014) Dynamics of late spring and summer phytoplankton communities on Georges Bank, with emphasis on diatoms, *Alexandrium* spp., and other dinoflagellates. *Deep Res Part II Top Stud Oceanogr* 103:120–138
- Harrison KG (2000) The role of increased silica input on Paleo-CO₂ levels. *Paleoceanography* 15:292–298
- Reynolds C (2006) *Ecology of Phytoplankton*, 1. Ed. Cambridge University Press, New York
- Shea R, Chopin T (2007) Effects of germanium dioxide, an inhibitor of diatom growth, on the microscopic laboratory cultivation stage of the kelp, *Laminaria saccharina*. *J Appl Phycol* 19:27–32
- Tribovillard N (2013) The Ge/Si ratio as a tool to recognize biogenic silica in chert. *Comptes Rendus - Geosci* 345:160–165
- Winder M, Reuter JE, Schladow SG (2009) Lake warming favours small-sized planktonic diatom species. *Proc Biol Sci* 276:427–435
- Yool A, Tyrrell T (2003) The role of diatoms in regulating the ocean's silicate cycle. *Global Biogeochem Cycles* 17:1–24