Impact of an Organic Rich Environment on Mixotrophic Diatom Phototaxis

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Abstract

Mixotrophy, when an organism can perform both autotrophy and heterotrophy, can occur in some species of pennate diatoms. However, processing organic carbon through heterotrophy is energetically expensive, and diatoms are observed to mainly perform it under limiting light. Previous research has investigated mixotrophic diatom growth in light and dark conditions, but which feeding method diatoms use when autotrophy requires investment in the form of phototaxis is unknown. Cultures of *Navicula* and apochlorotic *Nitzschia* were grown in agar plates with F/2 media or with added organic carbon media in a control or limited light condition. Daily measurements of the culture's maximum diameter were taken, and an elliptical ratio was calculated to quantify for any phototaxis. No statistically significant results were found, but there were strong trends and observable results. When in an organic rich environment, *Navicula* did not display phototaxis compared to strong phototaxis in the normal F/2 media. *Nitzschia* did not exhibit any phototactic behavior in either medium, but outcompeted *Navicula* in both media types. In both species, the diatoms had a higher growth rate when grown in an organic rich environment. The trend in results indicate the possibility of underestimating the role that heterotrophy plays in important phytoplankton processes such as vertical migration and carbon cycling.

Introduction

Microalgae, also known as phytoplankton, are unicellular organisms that photosynthesize to convert solar energy into chemical energy. This photosynthetic reaction creates oxygen as a byproduct and is key to contributing a large portion of the atmosphere to sustain life. Photosynthesis by phytoplankton is also essential to the biological carbon pump, which plays a role in energy cycling and sequestering carbon (Falkowski et al., 1998). Investigating the behavior of these organisms in varying environmental conditions are key to deepening our understanding of their contribution to marine food webs and their impact on the biological carbon pump.

Although they are simple unicellular organisms, phytoplankton have the capability to detect light and move towards it to optimize the conditions for photosynthesis to occur (Colley & Nilsson, 2016). Under high light, photosynthesis decreases as photoinhibition occurs due to damage to photosynthetic enzymes (Powles, 1984). In low light, the maximum growth rate is not reached as photosynthetic rate decreases. Pennate diatoms are a group of mobile phytoplankton that have a well-studied phototaxis and vertical migration response. Pennate diatoms differ from other microalgae groups due to the presence of a raphe, a slit that can extrude mucus substances and propel the diatom forward (Serôdio, 2021). They display phototaxis and can move towards or away from a light source by using the light contrast detected by the eyespot and correcting their swimming (Hegemann, 2008). They also contain a large slit called the raphe that excretes mucus which propels them forward (Figure 1). When high irradiance light is detected by the tip of the cell, the cell will quickly change direction and move away from the strong light (S. A. Cohn et al., 1999). To move forward towards or away from a stimulus, such as light, pennate diatoms can vary the time between rotations (Apoya-Horton et al., 2006). This is essential as they experience fluctuating light intensity in the mixed layer (Köhler et al., 2018) and must undergo vertical migration to maximize photosynthetic efficiency and produce enough energy to support themselves.

Some pennate diatoms have the remarkable ability to obtain energy through mixotrophy. Mixotrophy occurs in the presence of both light and organic carbon, where diatoms can receive energy through consuming organic carbon or synthesizing their own organic carbon from photosynthesis (Villanova & Spetea, 2021). Heterotrophy by phytoplankton play a key part in the biogeochemical cycle, with mixotrophy being modelled to increase carbon flux by 35% through boosting transfer of biomass to larger size classes (Ward & Follows, 2016). However, heterotrophy is energetically expensive to do, and diatoms are observed to only perform it when it is necessary for their survival, such as in extreme low light conditions (Tuchman et al., 2006). In extreme cases, 0% of amides were oxidized in normal light conditions, but this increased to 100% in low light conditions (Tuchman et al., 2006). The energetic expense to perform heterotrophy makes it so that mixotrophy is mostly observed in benthic diatoms. There are rare instances of diatoms that purely rely on consuming extracellular carbon, such as the colorless *Nitzschia* which have non photosynthetic chloroplasts and can grow in the dark (Kamikawa et al., 2015). While the mechanisms of obtaining energy from autotrophy and heterotrophy are researched separately, there is very little research focused on the tradeoffs of autotrophy and heterotrophy in complex scenarios, such as scenarios where autotrophy can only occur through phototaxis and movement. It is unknown whether it is more energetically favorable for diatoms to move towards light and perform autotrophy, or process nutrients through heterotrophy.

To understand whether mixotrophic diatoms will perform more phototaxis or heterotrophy under limited light, cultures of pigmented *Navicula* and unpigmented *Nitzschia* will be cultured in agar plates with or without added organic carbon. Limited light will be provided to ensure phototaxis is required for autotrophy to occur. The response of the diatoms to conditions when photosynthesis requires investment to achieve will be measured by quantifying whether there is movement towards light. *Nitzschia* is expected to use heterotrophy only and show no phototaxis, due to having no functional pigments. As light has been observed to have no influence on growth of some mixotrophic phytoplankton (Lie et al., 2017a), we hypothesize that *Navicula* would prefer heterotrophy over

phototaxis when the environment is organic rich. Addressing this gap could help deepen understanding of the sources of organic carbon provided by benthic diatoms, if they are photosynthetic products or if they are extracellular.

Methods

Organism collection

Starter cultures of pennate diatoms (pigmented *Navicula* and unpigmented *Nitzschia*) were obtained and cultured in agar F/2 media in room temperature and ambient light conditions prior to the experiment. *Navicula* strain 2046 was obtained from University of Texas Culture Collection of Algae and rendered axenic by Charles J. O'Kelly. *Nitzschia* was isolated from eelgrass at Fourth of July Beach, San Juan Island. Collection, isolation, and axenification of the culture by Charles J. O'Kelly.

Experimental design

Cultures were either grown in F media, or G media, which is media with added organic carbon. 16 plates were prepared with F/2 media by using 250mL commercial F/2 and 3.5g agar. An additional 16 plates were prepared with G media by adding 3.5g agar, 1g glucose, 0.25g sodium glutamate, 32.5μL of proline F/2, and 250mL of Natural Sea Water (NSW). Both media were autoclaved at 121°C for 20 minutes and set in plates overnight. For each plate, approximately 2mm square of agar was taken from the starter cultures and moved onto the center of the plate using sterile inoculating loops. For each media type, 8 plates contained *Navicula* and the other 8 plates contained *Nitzschia* for a total of 32 plates. Each media and species condition had 5 treatment replicates, and the remaining 3 plates were controls (Figure 2A). Treatment plates were wrapped in aluminum foil and a 5mm hole was cut out 10mm from the edge of the plate. Control plates were not wrapped in aluminum foil. Treatment plates and control plates were placed under a white light of 262 μmol m^{-2} s^{-1}, and on top of white shelving paper. The plates were in a room with ambient and artificial lights that varied around 3 μmol m^{-2} s^{-1} each day. Plates were in a room temperature environment and left to grow for 4 days.

For each day of measurements, the hole was marked and aluminum foil removed. One treatment replicate did not have daily measurements to control for interference of external light during measurements, and the foil was left on until the last day. Orthogonal measurements of the culture shape were taken during measurements. The first measurement was taken along the longitudinal axis parallel to the light source, while the second measurement was along the vertical axis and taken 90 degrees from the first (Figure 2B). The aluminum foil was then reattached in the same position, and the mark erased. For *Nitzschia*, the plates were examined under a dissecting microscope and the border of the shape was marked on the plate by marker. Measurements can then be taken using the shape drawn. Each sample was exposed to light for around 1.5 minutes for measurements. Measurements were done at the same time each day for 4 days.

Statistical analysis

A ratio to quantify the ellipse of the culture was calculated by dividing the longitudinal diameter by the vertical diameter. A linear regression was fitted, and Spearman's correlation was used using R to determine the correlation in elliptical ratio over time and comparing them between treatments. A Kruskal-Wallis test was used using R to find any differences in means of elliptical ratio between groups on the last day. Growth rate was also calculated by determining the difference between the first and last longitudinal measurement and dividing this by the number of days of the experiment. Wilcoxon signed rank tests were conducted on R to find differences in the mean growth rate of each species in the two different media types.

Results

All cultures had visible growth during the experiment. There were three cultures that had little growth and had a maximum diameter equal or under 10mm (2 *Navicula* in F and 1 *Nitzschia* in G). All other cultures were healthy and had maximum diameters of 15-50 mm. Only one culture of *Nitzschia* in G media had growth covering the entire agar plate. Two plates of *Navicula* in F media had clear observable phototaxis, with the culture forming an elongated shape (Figure 3A). In G media, all

cultures were circular until day 4, where there was a small peak towards the light source (Figure 3B). In *Nitzchia*, all cultures in both F and G media were mostly completely round throughout all 4 days (Figure 3C; Figure 3D).

From the start of the experiment, all culture treatments had an average elliptical ratio above 1. While the average elliptical ratio decreased in all treatments except *Navicula* F, the average elliptical ratio was still above 1 (Figure 4). *Navicula* grown in F media was the only treatment with an increasing ratio, with a strong positive correlation observed with time (Spearman's rank correlation, ρ= 0.8, N= 16, p= 0.333). All other cultures had a negative correlation with time, with *Navicula* G and *Nitzchia* F both having a slight negative correlation (Spearman's rank correlation, ρ= -0.4, N= 16, p= 0.75). In contrast, *Nitzschia G* had a very strong negative correlation with time (Spearman's rank correlation, ρ= -1, N= 16, p= 0.0833). None of the correlations were statistically significant. On day 4, *Navicula* F had a mean elliptical ratio of 1.30 while the other three treatments had a ratio of 1.0083 to 1.097 (Figure 5). There was no statistically significant difference in mean elliptical ratio between treatments on day 4 (Kruskal-Wallis test, H=6.448, N=20, p=0.0917).

For both species, the average growth rate along the longitudinal axis was higher in media G compared to media F (Figure 6). However, there was no statistical significance between the mean growth rates of *Navicula* in F and G media (Wilcoxon signed rank test, W= 2, N=8, p=0.1143), and between the mean growth rates of *Nitzschia* in F and G media (Wilcoxon signed rank test, W= 2.5, N=8, p=0.1429). *Nitzschia* in G media had the highest mean growth rate of 5.88 mm/day, and *Navicula* in F media had the lowest of 1.31 mm/day. *Nitzschia* in both media types outperformed *Navicula* in growth rate.

Discussion

While statistical tests were not significant, the observable results and trends in data indicate that strong phototaxis only occurred in *Navicula* grown in F media. When *Navicula* is in G media,

heterotrophy is preferred and little phototaxis was observed. The elliptical ratio of *Navicula* in media F showed a strong positive correlation with time, indicating that it was displaying phototaxis, while all other cultures showed a negative correlation. The error bars are overlapping at the start of the experimentation and grew further apart, indicating that the *Navicula* F began more circular and similar to other cultures, but grew elliptical towards the light. However, the correlations were not statistically significant as indicated by Spearman's correlation. Similarly, there was no statistical significance between the means of elliptical ratios for all treatments on the last day of experimentation shown by the Kruskal-Wallis. *Navicula* in F media had the highest elliptical ratio, and this was maintained until the last day. The lack of statistical significance is likely due to the small number of replicates and duration of experimentation. Two plates in *Navicula* in F media had very strong observable phototaxis (Figure 3A), but the two other plates had little growth and therefore displayed minimal phototaxis. This is likely due to inoculating error, as parts of the starting culture that were unhealthy could have been transferred. The stress of no light could have also been an added stressor and unhealthy diatoms might not have enough energy to move towards the light.

While there aren't other studies looking at the investment in phototaxis under varying nutrient conditions, the trends observed in this study agree with other related studies. A study found that once organic carbon was depleted, changing light intensity did not promote growth of mixotrophic freshwater phytoplankton *Ochromonas* sp. (Lie et al., 2017), aligning with the finding that heterotrophy is preferred to autotrophy when organic carbon is available. Similar to previous studies in ambient light (Gomes et al., 2018; Hansen et al., 2004), there was a trend of mixotrophic phytoplankton growing faster in conditions with available food for heterotrophy compared to unfed cultures. As *Navicula* was observed to grow in G media without moving towards the light, it raises the question of whether diatoms can survive in complete darkness and relying purely on heterotrophy just like *Nitzschia (Blackburn et al., 2009)*. The strain of *Navicula* used in this study has been tested previously to show growth in complete dark conditions when glucose is present, but not with other organic carbon like acetate or lactate (Lewin & Lewin, 1960). This supports the finding that *Navicula*

can grow without responding to light in an organic rich environment. Other diatoms that have been observed to only rely on heterotrophy include diatoms adapted for darkness up to 6 months in Antarctica (Zhang et al., 1998), and it is possible that other strains can do this too for a shorter period of time. As hypothesized, *Nitzschia* was able to grow without any observed phototaxis. Surprisingly, *Nitzschia* was able to grow efficiently in F media with no added organic carbon. The mechanisms in which it can perform heterotrophy so efficiently, faster than *Navicula*, even in media without added organics should be further investigated. Experimentation on *Nitzschia* is lacking and little is known about its use of heterotrophy only. Further testing comparing *Navicula* and *Nitzschia* can reveal the different mechanisms obligate heterotrophs and facultative mixotrophs use, and the specialized adaptations that obligate heterotrophs have that allow *Nitzschia* to be so efficient.

Organic carbon can enter the water column through improper wastewater treatment and other sources of organic pollution (Choi et al., 2024). If the phenomenon of shutting off phototaxis in the presence of an organic rich environment is widespread, we could be underestimating the role of heterotrophy when considering important phytoplankton processes. Future works should focus on understanding the tradeoffs of autotrophy and heterotrophy in mixotrophic phytoplankton, which has possible implications for estimations related to vertical migration and carbon cycling in organic rich environments. Vertical migration is a light driven process where phytoplankton move towards light during the day to maximize photosynthesis. However, if the environment is organically rich through pollutants, heterotrophy may be more important to mixotrophic diatoms than vertical migration as suggested by observed trends. This could potentially impact our estimations of the quantity of phytoplankton that perform vertical migration, which is important for both biodiversity and nutrient input (Wirtz & Smith, 2020). Phytoplankton choosing heterotrophy instead of migrating towards light also has implications for our understanding of food web interactions. Many zooplankton and other organisms use diel migration and move to the surface to graze on phytoplankton at night. Mixotrophic diatoms may be inadvertently avoiding predation from diel migrating organisms due to being able to grow without moving towards light. Lastly, learning more about the tradeoffs between heterotrophy

and autotrophy could have implications for understanding the role of phytoplankton in carbon fixation, which is estimated to be 40% (Falkowski, 1994) and is extremely important for converting inaccessible inorganic carbon into organic carbon for food webs.

For future studies, the proportion of heterotrophy and autotrophy performed could be quantified in *Navicula*, to determine whether autotrophy is completely turned off when there is no phototaxis. Carbon dating and molecular approaches can be used to investigate this (Cecchin et al., 2018; Millette et al., 2023). Replication for this study with higher statistical power is needed to confirm the observable trends. Further studies should focus on replicating results in other strains and species, as well as wild populations to understand how widespread this phenomenon is. Future work will help us better understand the impact of organic carbon on mixotrophic phytoplankton and the possible implications it has on our knowledge of global food webs and biogeochemical cycles.

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Figures

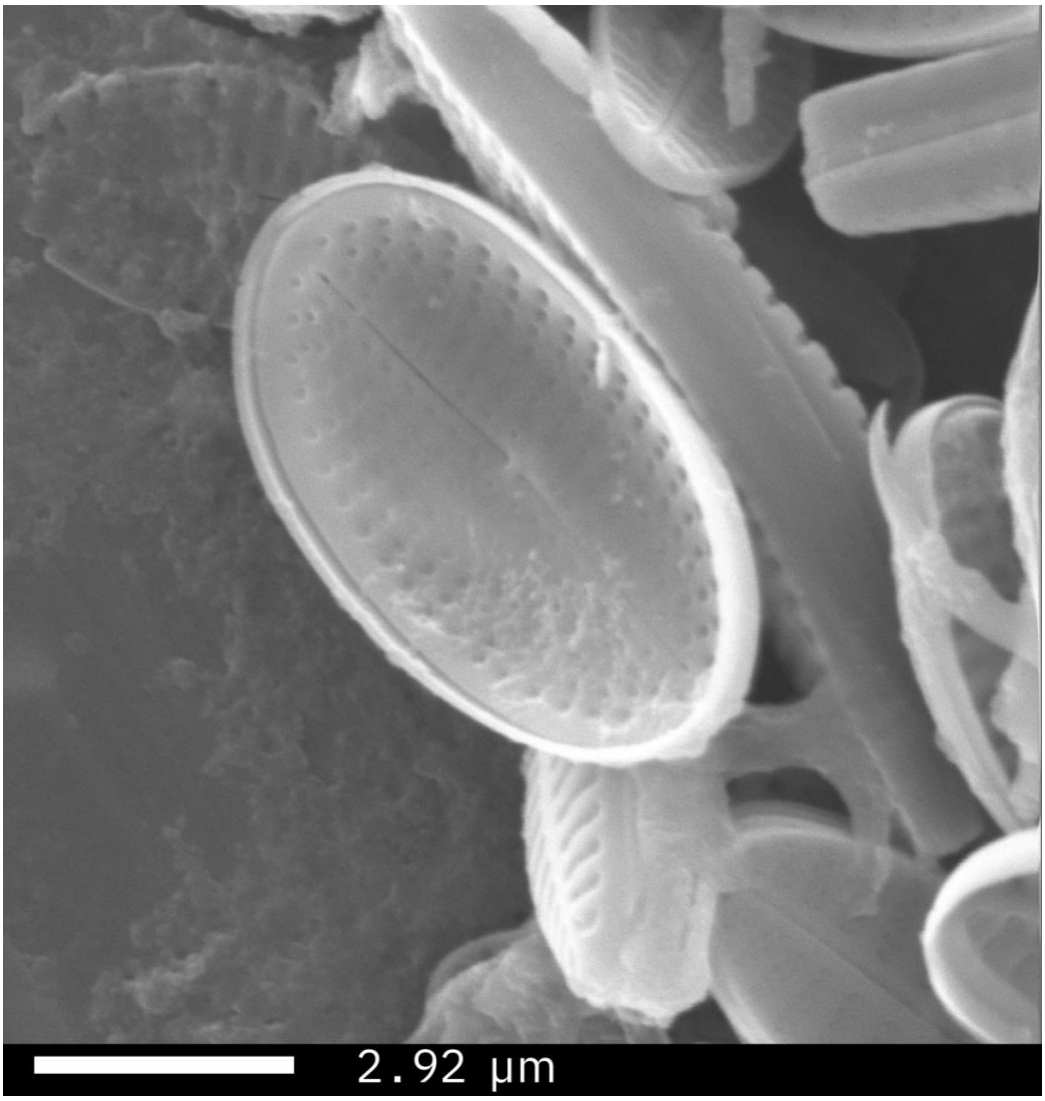

Figure 1: Pennate diatom with raphe pictured in the middle for movement. Scanning electron microscope photo captured by Karina Lai and Charles J. O'Kelly of diatoms from Friday Harbor Laboratories invertebrate tanks.

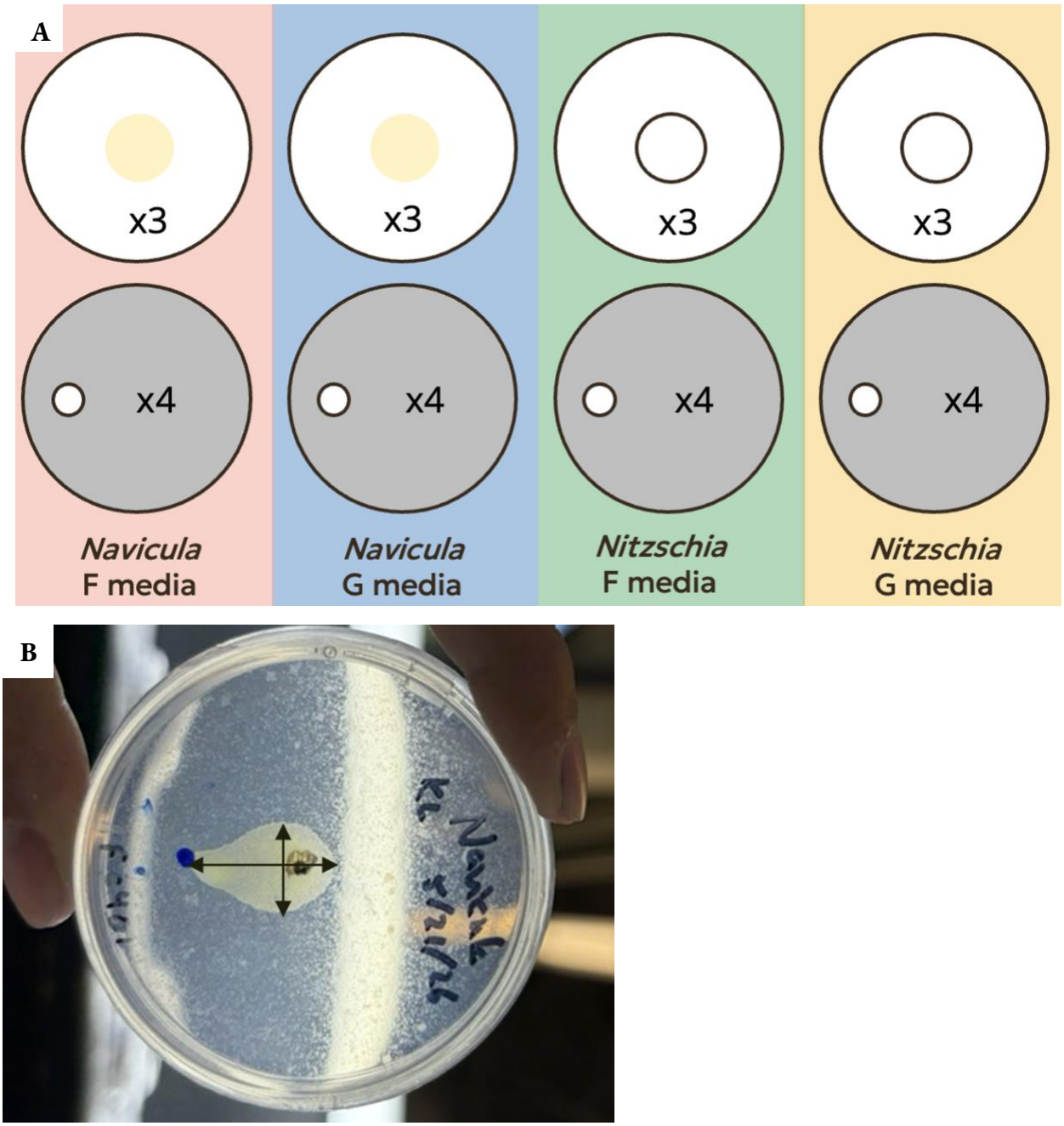

Figure 2A: Experimental design with top row representing controls and bottom row representing treatments wrapped in foil, with a small hole for light source. The 4 treatment groups are outlined in color and replicates shown. **Figure 2B**: Diagram representing the measurements taken for each culture, with the blue dot representing the hole in the foil where light enters. The longitudinal axis is parallel to the light, and the vertical axis which is perpendicular is represented.

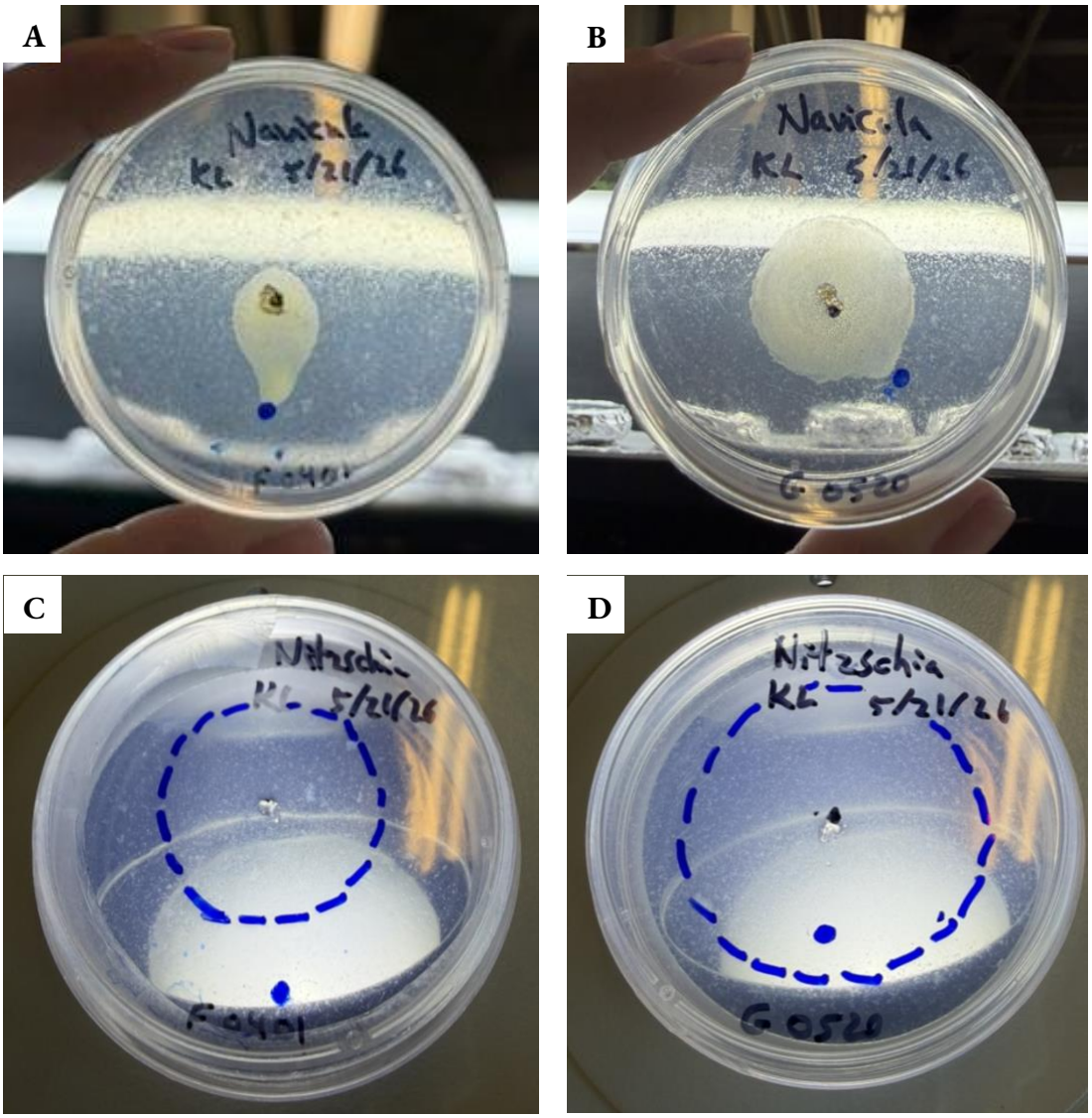

Figure 3: Treatment cultures on day 4 of experimentation. The blue dot represents the hole in the foil where light enters. *Navicula* in F media **(3A)**, *Navicula* in G media **(3B)**, *Nitzschia* in F media **(3C)**, and *Nitzschia* in G media **(3D)** are shown. Dotted blue line in *Nitzschia* plates represent outline of culture drawn through observing culture in a dissecting microscope.

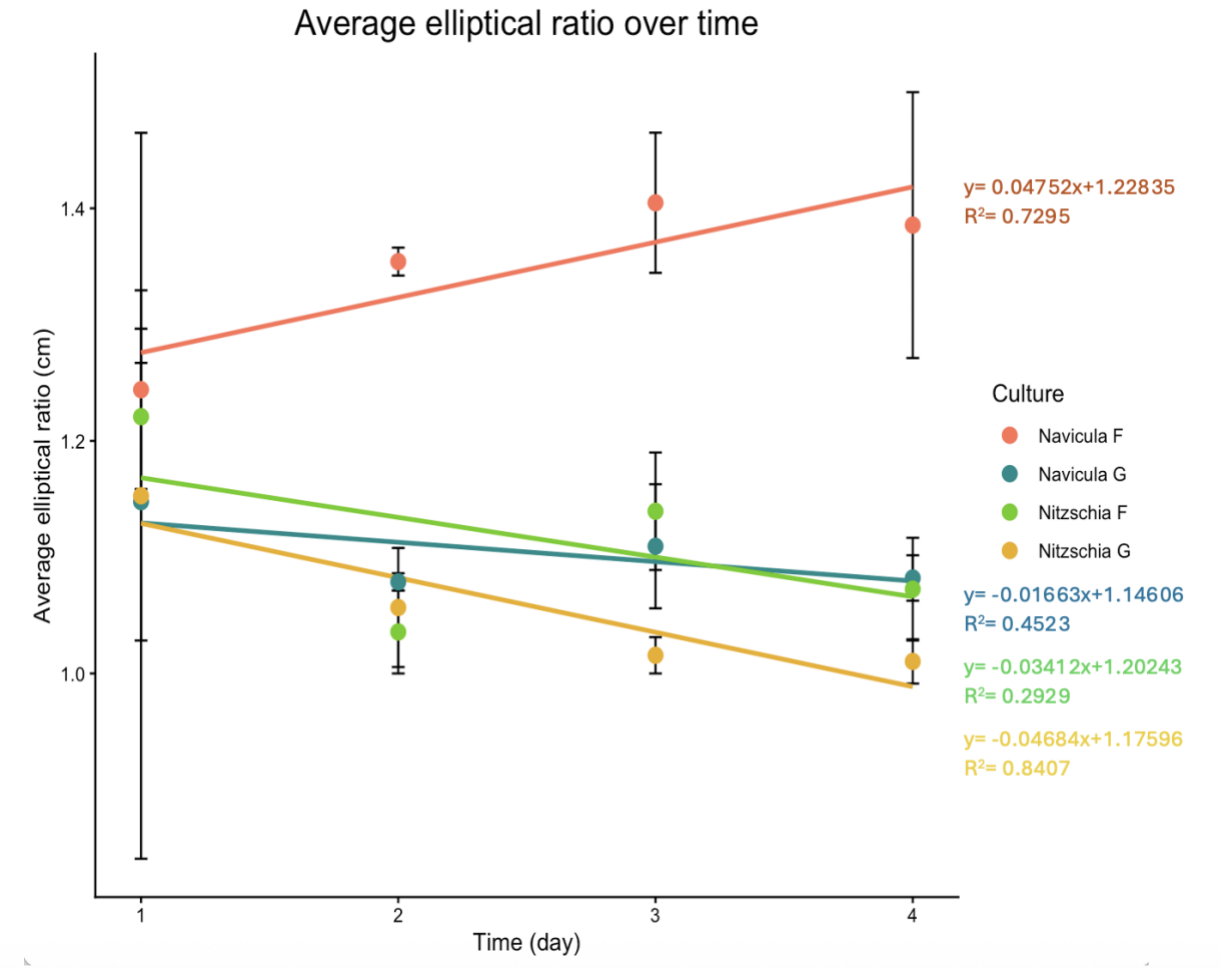

Figure 4: Mean elliptical ratio (longitudinal/vertical measurement) of *Navicula* and *Nitzschia* in F and G media (organic rich media) over the 4 days of experimentation. Error bars represent standard error.

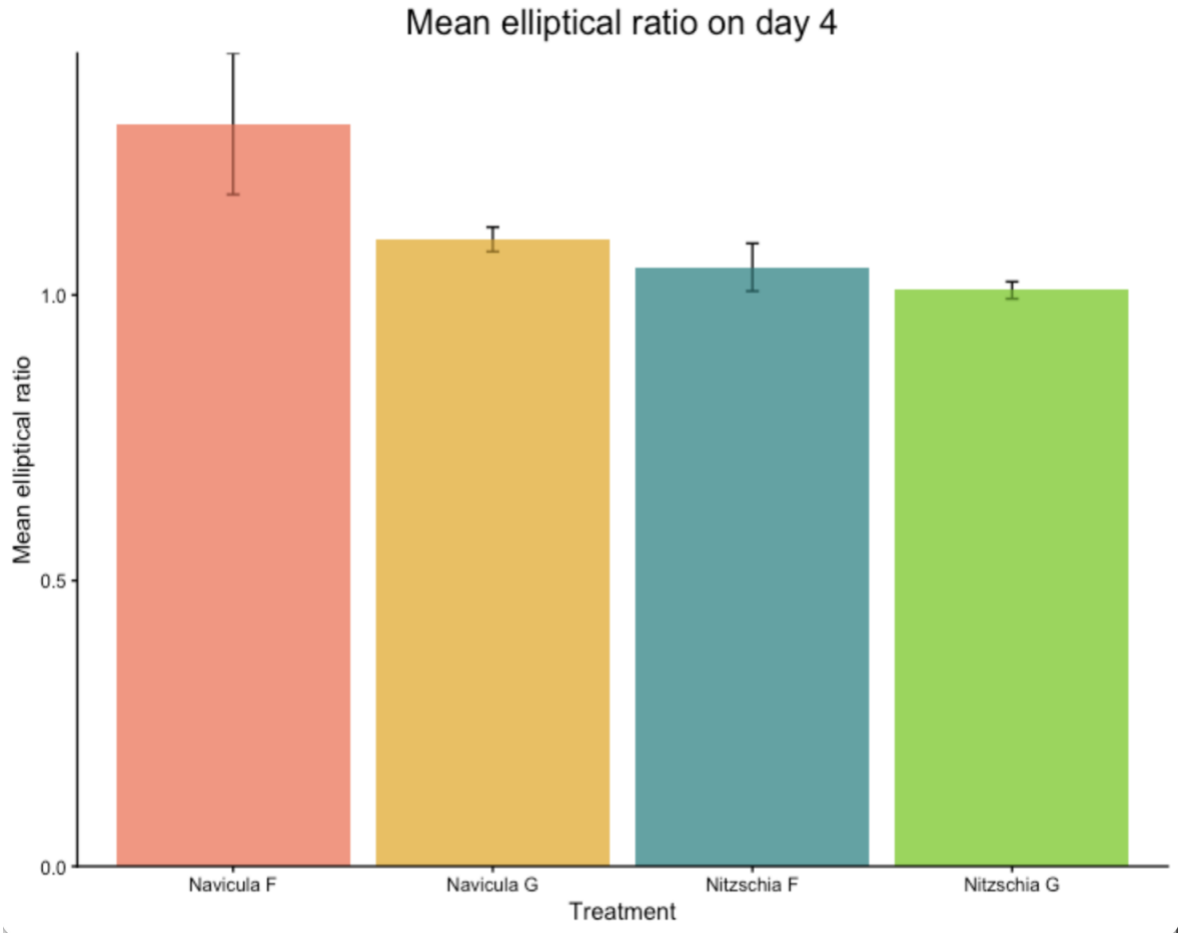

Figure 5: Mean elliptical ratio (longitudinal/vertical measurement) of *Navicula* and *Nitzschia* in F and G media (organic rich media) on day 4 of experimentation. Error bars represent standard error.

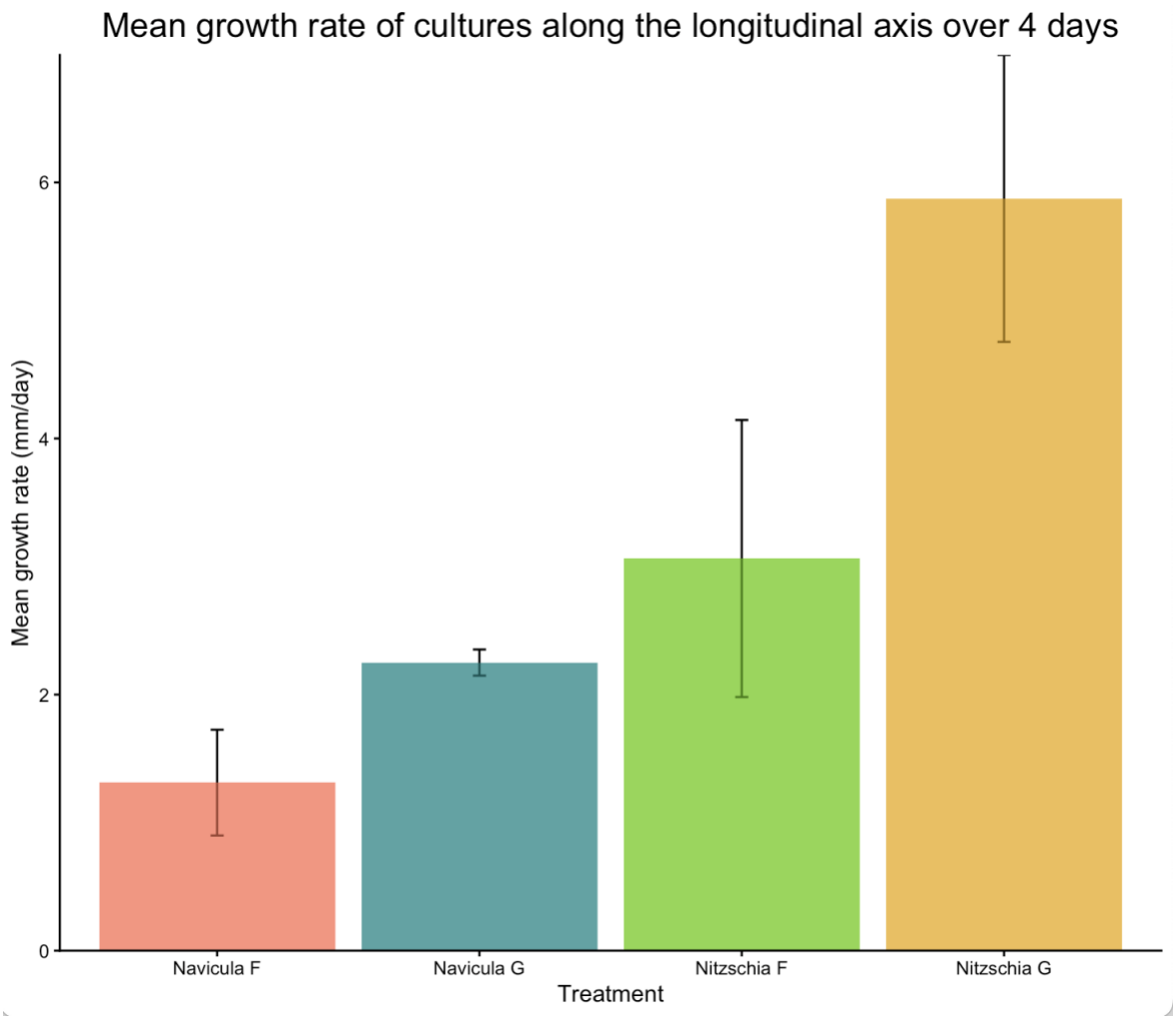

Figure 6: Mean growth rate (mm/day) of *Navicula* and *Nitzschia* in F and G media (organic rich media) over the 4 days of experimentation. Mean growth rate was quantified by the change in longitudinal measurements over time. Error bars represent standard error.