

Protozoan Grazing and Nano-Plankton Producer Growth Rates in Varying in Situ Nutrient
Concentrations and Temperature in the West Equatorial Pacific

Gabe Diephuis

gdieph02@uw.edu

University of Washington, Seattle, WA

School of Oceanography

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Abstract:

Protozoa are a diverse group of organisms that impact trophic transfer in marine ecosystems, constituting an important link between producers and higher trophic levels. In this study, I focused on determining how protozoan grazing rates differ in nutrient-rich and poor ecosystems. I used a CTD rosette to collect six seawater samples along the equatorial transect of five degrees south to five degrees north at stations: 5°S, 2°S, 1°S, 0°, 1°N, 4°N, and 5°N along 167°W in January 2024. These samples were filtered to 10 microns and divided into isolated incubation cultures with 0, 25, and 90 percent dilution of 0.2 micron filtered seawater. Change in Chlorophyll was used to calculate the phytoplankton growth rate across the dilution factors. Using a linear model of growth rate by dilution factor, a grazing rate was determined for each sample. Nutrients from the water samples were measured for nitrate, phosphate, and silicate concentrations. A series of linear regression analyses of the protozoan grazing rates by in situ nutrient concentrations revealed negative linear trends with temperature in both growth and grazing rates. The growth rates of phytoplankton ranged from -0.09 day^{-1} to 4.62 day^{-1} . Ambient nitrate, silicate, and phosphate concentrations reached $2.25 \mu\text{M}$, $2.18 \mu\text{M}$, and $0.46 \mu\text{M}$ respectively. Surface temperatures reached 30.46 centigrade, and the grazing rate exhibited a decreasing trend with higher temperatures, eventually reaching zero at 30.3 degrees. As eutrophication events become increasingly common due to climate change and anthropogenic pollution, it is important to determine how protozoan communities respond to changes in dissolved nutrients and temperature.

Plain Language Summary:

Microzooplankton is a classification of plankton that are smaller than 35 microns and are heterotrophic creatures meaning they eat to produce energy. Protozoans are a large subset of microzooplankton which are single-celled eukaryotic organisms, meaning they contain a cell nucleus. The main research goal of this study is to determine how the rate of phytoplankton consumption, or grazing, by protozoans changes due to ambient nutrients, temperature, and environmental conditions. It was found that the greatest control on the grazing rate was the growth rate of the producing organisms, which is consistent with prior knowledge of zooplankton grazing rates (Vaque et al. 1994; Pulsifer and Laws, 2021). However, it was also found that growth and grazing decreased with increased temperature, which is contradictory to previous studies. This could be explained by low nutrient concentrations in the surface waters along the transect, which have been found to decrease thermal tolerance in freshwater-producing species (Bestion et al. 2018). This study confirms that prior knowledge of the relationship between grazing and growth rate applies to microzooplankton in the equatorial Pacific. It also suggests that the anomalously high temperatures and low nutrient conditions at the time of the study have breached the thermal tolerance of the producing organisms.

Introduction:

Protozoans fill an important niche in marine ecosystems, generally linking producers and higher trophic levels. Primary consumer protozoans are diverse in their taxonomy, size, and behaviors (Urban Tillmann, 2005). In the tropical east Pacific Ocean, 98 percent of

microzooplankton, defined as less than 35 μm , were composed of protozoan species (Beers and Stewart, 1971). Additionally, microzooplankton are often the dominant consumers of primary production, especially in subtropical gyres (Tillmann and Hesse 1998). Beers and Stewart examined the population structure and biomass of protozoan grazers and quantified their grazing rates in coastal waters (Beers and Stewart, 1971; Urban Tillmann, 2005). They found that microzooplankton grazing accounted for 70 percent of phytoplankton production in the waters just off the coast of California, with nearly all grazing being protozoans. However, five miles from shore, this number dropped to 23 percent of phytoplankton production, with generous estimates of 59 percent. High microzooplankton grazing rates in tropical latitudes suggest that grazing protozoa form an important link between small primary producers and bigger zooplankton, which in turn cycle carbon to larger organisms (Beers and Stewart, 1971; Louise Schluter 1998; Tsai and Mukhanov 2021). Consequently, these micrograzers are likely to be consumed by higher trophic levels (Urban Tillmann, 2005). Predicting changes in the behavior of these microorganisms is essential to determining the efficiency of trophic transfer, and therefore the carbon cycle in the future.

Grazers fill an important ecosystem function, having a top-down control on phytoplankton populations. Because they are single-celled and relatively small, Protozoan grazers have a fast generation time, as low as a day and a half. This allows their populations to adapt to environmental changes quickly. In grazing organisms, faster generation times allow for quicker responses to environmental changes. Shorter generation times mean faster population growth and genotype shift towards more advantageous phenotypes for new conditions. Because of their quick generation times, they respond to environmental changes faster than larger notable grazing species such as calanoids and euphausiids (Beers and Stewart, 1971; Urban Tillman, 2005; Regaudie-de-Gioux et al, 2015). The fast response time of microzooplankton can remain proportional to rapid increases in algal biomass such as during a bloom. The high number of protozoan species after blooms suggests a strong impact on the decline of blooms, and often a prevention of bloom development (Urban Tillman, 2005). Rapid and large algal blooms are usually excellent sinks for carbon in the ocean, but, if uncontrolled, can trigger the build-up of harmful toxins higher in the food chain (Burford et al., 2012; Pujari et al., 2021). Harmful algal blooms can cause widespread health disparities and economic problems, especially in less developed countries (Burford et al., 2012). Therefore, it could be presumed that grazing protozoans have a beneficial top-down effect on primary producers, which may be important for preventing some harmful algal blooms.

Several studies have outlined microzooplankton responses to increased temperature, acidity, and other climate change responses (Caron and Hutchins, 2012). For example, higher dissolved carbon levels in seawater cause phototrophic growth to be more efficient for mixotrophic species. Mixotrophy is often seen in protozoans, a form of energy production that utilizes multiple sources of energy, in this case, the sun and other living organisms. This may lead to an adaptation in protozoan populations away from grazing and towards autotrophy (Caron and Hutchins, 2012). Additionally, increased temperatures are causing many zooplankton to move poleward, thus changing grazing population compositions at higher latitudes (Caron and Hutchins, 2012). The grazing rate of bacteria from flagellates and zooplankton populations has

shown a positive correlation with temperature. However, this effect may be compounded by the limitation of grazing by the growth of prey organisms, which is also positively correlated with temperature. (Vaque et al. 1994; Pulsifer and Laws 2021). These shifts are directly affecting the grazing behavior of microzooplankton (Caron and Hutchins, 2012). However, research on these organisms is vastly behind research in other fields such as primary production. There is currently a gap in knowledge around this important trophic link (Caron and Hutchins, 2012).

It is important to understand how the behavior of protozoans responds to changes in their environment. The equatorial West Pacific is often characterized by oligotrophic waters that are enriched in cyanobacteria and dinoflagellates. These species experience population growth at higher temperatures than other producing species like diatoms (Pujari et al., 2021; Caron and Hutchins, 2012; Chen, 2015). Many species of Cyanobacteria such as *Microcystis aeruginosa*, a freshwater species, are poor food sources for many larger grazing species due to low fatty acid levels (Park et al., 2003). However, microzooplankton are key to maintaining a viable nutrient composition in ecosystems dominated by unpalatable producers because they metabolize low-nutrient cyanobacteria into higher fatty acid concentrations (Caron and Hutchins, 2012; Park et al., 2003). A shift towards this less nutritionally valuable phytoplankton could be caused by a lack of nutrients, as cyanobacteria demonstrate excellent tolerance to higher temperatures and lower nutrient levels (Caron and Hutchins, 2012; Chen, 2015). However, a study by Bestion et al. (2018) found that low phosphate conditions caused the thermal tolerance of freshwater picoplankton to decrease by 50 percent, and this effect could translate to other nutrients. Consequently, in anomalously high temperatures and low nutrient conditions, growth, and therefore grazing rate could be negatively impacted. Therefore, protozoans may play an increasingly important role in nutrient cycling in the absence of larger phytoplankton. Furthermore, it is important to determine how protozoans respond to different in situ concentrations of nitrate, phosphate, and silicate.

The equator provides an ideal location for this study, due to a wide range of surface nutrient concentrations. Based on the Mercator-Pisces biogeochemistry forecasts, the concentrations of nitrate, phosphate, and silicate decrease with higher latitudes north and south of the equator (Simons CMAP, 2023). There is also a random spatial distribution of these nutrients based on currents and productivity in the region (Simons CMAP, 2023). Therefore, sampling at a variety of latitudes from five degrees south to five degrees north will give a range of concentrations for each of our measured nutrients. The variability in this region will be vital in determining protozoan grazing response to nutrient concentrations.

However, isolating the grazing impacts of microbes in a complex trophic web community is difficult. This study will utilize a method pioneered by Landry and Hassett (1982). This method assumes two things: that phytoplankton growth is not affected by the presence of other phytoplankton, and that the probability of predatory interactions is based on prey density (Landry and Hassett, 1982). Using the dilution method, a net growth rate can be determined using the following equation:

$$\text{Net growth rate} = \mu - dg$$

Where μ is the bulk phytoplankton growth rate, d is the fraction of 0.2 micron filtered seawater to the total volume of the incubation, and g is the microzooplankton grazing rate (Fig. 1) (Taniguchi et al., 2012). Using this dilution method, I can experimentally gather the microzooplankton grazing rate without direct observation.

This study aimed to answer several questions aboard R/V Thompson in January of 2024; How do heterotrophic protozoan grazing rates differ in higher and lower in-situ nutrient concentrations near the equator? How do surface temperature and other seawater characteristics affect protozoan grazing rate? Additionally, how does nano-plankton growth rate respond to surface temperature, nutrient concentrations, and other seawater characteristics? Microzooplankton dominates the grazing of phytoplankton in the generally nutrient-poor subtropical gyres. However, a positive correlation between phytoplankton productivity and microzooplankton abundance suggests more microzooplankton will be found in nutrient-rich environments (Beers and Stewart, 1971; Tillmann and Hesse, 1998). However, high carbon dioxide and nutrient levels in upwelled water closer to the equator may cause protozoans to favor autotrophy (Caron and Hutchins, 2012). Temperature is believed to have a positive correlation with growth and grazing rate in bulk plankton populations (Vaque et al. 1994; Pulsifer and Laws, 2021). Therefore, I hypothesize that the protozoans will exhibit higher grazing rates in lower nutrient concentrations and that protozoan grazing rates will show similar positive correlations with temperature. I hypothesize that growth rates will increase with higher nitrate, phosphate, and silicate concentrations, and growth will follow a positive trend with temperature. Other environmental factors such as dissolved oxygen, salinity, and pH may also cause changes in growth and grazing rates. This gives the null hypothesis predicting no change in growth or grazing rate due to variation in nutrient concentrations, temperature, or other environmental factors. I expect that nutrient depletion will make autotrophy for these communities less favorable and that this will cause a shift towards grazing on the phytoplankton communities.

Methods:

Water samples were collected across a north-south transect of the equator in the west Pacific Ocean from five degrees south to five degrees north along a 167 degrees West transect. The sites at 5°S, 2°S, 1°S, 0°, 1°N, 4°N, and 5°N were sampled at 10 m for this experiment. Available sunlight, dissolved oxygen, and carbon dioxide make 10 m ideal for getting samples with grazers and phytoplankton. These sites were chosen to cover a range of latitude values and to have samples north and south of the equator. Concentrations of nitrate, phosphate, and silicate generally decrease when getting further from the equator in this region, so having a range of latitudes is ideal for getting a gradient of nutrient levels. The samples were collected aboard the R/V Thomas G. Thompson in January 2024.

The samples were collected via a CTD rosette device. This instrument uses individual Niskin bottles, which are fired via a remote control at 10 m and collect five liters of seawater each. Once collected, each Niskin sample was separated into three different dilution levels, each of which was given three replicates. However, the first three stations did not have replicates. Incubation bottles of one liter were used for the experiment. The collected seawater samples were filtered through a 10-micron Millipore filter and added to the one-liter bottle, then diluted

to either 0,25, or 90 percent with 0.2 micron filtered seawater gathered from the onboard seawater intake. 0.25 liters were filtered from the 10-micron filtered niskin sample through a 0.2-micron filter for initial chlorophyll measurement. 10 μM nitrate, 6.03 μM phosphate, 0.08 μM of iron, and 10 μM silicate were added to each incubation to ensure primary production was not limited by any factors besides grazing. These values were set in the same order of magnitude as Pisces nutrient forecasts during January (Simon CMAP), so the samples are not over-saturated in nutrients. The chlorophyll level of each of these sub-samples was measured and adjusted for dilution using the equation, $M_1 V_1 = M_2 V_2$. To measure chlorophyll, 0.25 liters of each incubation was filtered through a 0.2-micron filter, which was centrifuged in 90 percent acetone. After 24 hours in a freezer and 10 minutes in a centrifuge, a fluorescence measurement of the acetone was taken using a fluorometer. From fluorescence, chlorophyll concentration can be determined. After 24 hours of incubation, each sub-sample was filtered with a 0.2-micron filter, which was centrifuged in acetone and measured for fluorescence using a fluorometer in the same fashion. Using the change in chlorophyll in each sub-sample, a mathematically determined growth rate was found (Fig. 1). Using the equation by Landry and Hassett (1982), the net growth rate in the incubations, and a grazing rate was determined for each location.

$$\text{Net growth rate} = \mu - dg$$

Where μ is the bulk phytoplankton growth rate, d is the fraction of 0.2 micron filtered seawater to the total volume of the incubation, and g is the microzooplankton grazing rate (Fig. 1). The net growth rate at each dilution is calculated by taking the natural log of the chlorophyll concentration post-incubation divided by the initial chlorophyll measurement.

$$\text{Net Growth (day}^{-1}\text{)} = \ln \left(\frac{[\text{Chl}_{final}]}{[\text{Chl}_{initial}]} \right)$$

Then, bulk phytoplankton growth at 100 percent dilution can be solved using the linear model. Ambient nutrient levels for the corresponding sampling locations were determined in the lab at the University of Washington from the Niskin bottles collected by AJ Carothers. These grazing rates were then compared by environmental nutrient levels using linear regression analysis to determine if the difference is statistically significant by determining a p and R^2 value. A series of Pearson correlation tests were set up using the ambient nutrient, temperature, chlorophyll, and various other seawater properties versus grazing rate. A significance threshold of 0.05 was chosen for statistical analysis.

Results:

Growth and Grazing Rates:

Out of the six sampling locations, only 5°S exhibited a negative growth rate over the 24-hour incubation. Growth rates ranged from -0.09 to 4.62 day^{-1} , with the equator exhibiting the highest growth rates (Table 1). Across nearly all incubation series, the growth rate increased, with only one exception (Fig.1). The resulting linear fit of growth rate by dilution resulted in a range of grazing rates. Statistical confidence in the fitted grazing rates stays above 70 percent, excluding at 0 degrees, where the confidence is 48 percent.

<i>Lat</i>	<i>Chl T=0</i>	<i>Chl Final (+24 hour)</i>	<i>Dilution</i>	<i>Net Growth Rate (day⁻¹)</i>	<i>Net Growth Standard Dev</i>	<i>N</i>
-5	3.29	3.29	0	-0.45	---	1
-5	2.58	2.58	25	-0.40	---	1
-5	0.00	0.00	90	0.00	---	1
-2	4.76	9.26	0	0.66	---	1
-2	3.57	6.30	25	0.57	---	1
-2	0.48	0.98	90	0.72	---	1
-1	9.15	8.33	0	-0.09	0.01	2
-1	6.86	8.00	25	1.14	0.02	2
-1	0.91	6.23	90	1.92	0.07	2
0	9.84	21.03	0	0.76	0.66	4
0	7.38	18.69	25	0.89	0.35	4
0	0.98	25.72	90	3.17	0.53	4
4	2.67	21.51	0	2.08	0.77	4
4	2.01	21.23	25	2.36	0.07	4
4	0.27	23.86	90	3.86	0.11	4
5	2.68	19.58	0	1.99	0.03	4
5	2.01	20.33	25	2.31	0.04	4
5	0.27	27.56	90	4.62	0.14	4

Table 1: Chlorophyll measurement data. All Chlorophyll measurements are from the 0.2-micron filter for the incubation sample. Filters were preserved in 0.7 mL 90 percent ethanol for 24 hours before being centrifuged and measured. Chlorophyll values are in nanograms per liter. Initial Chlorophyll measurement indicated with T=0. The number of replicates indicated by column “N”

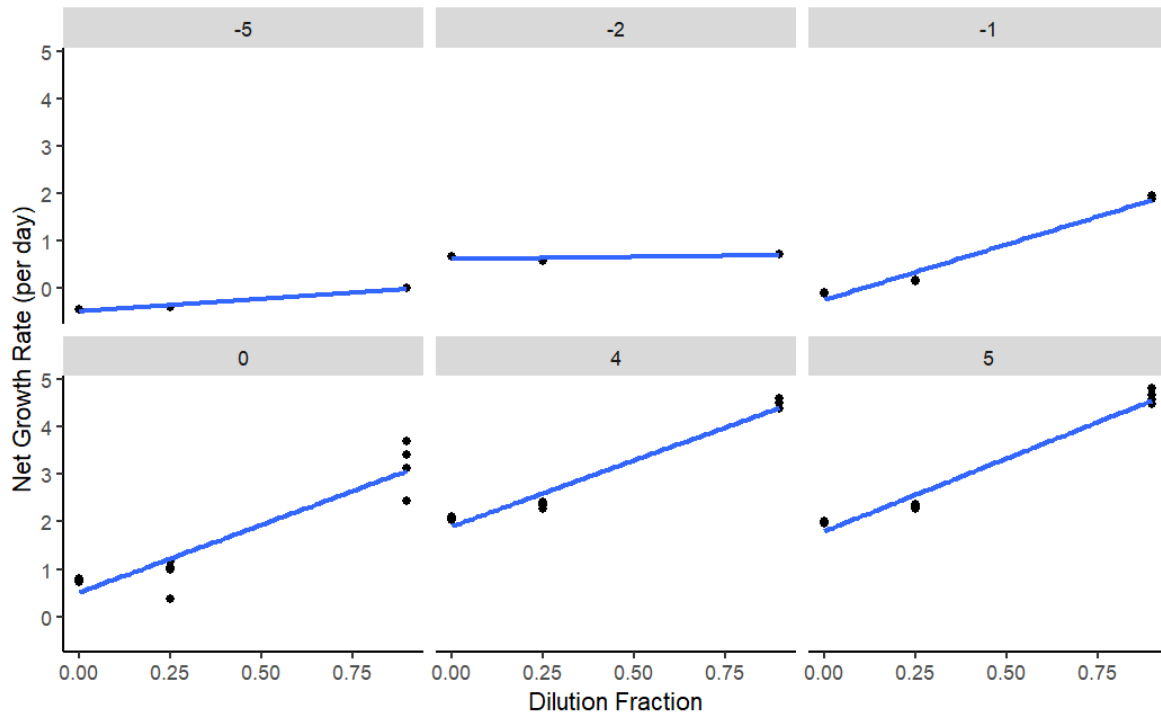


Figure 1: Growth rate by dilution level. The numbers above graphs indicate latitude, the indicated slope of each graph is the average grazing rate at that latitude.

Response to Nutrients:

Ambient nitrate, silicate, and phosphate concentrations reached as high as 2.25 μM , 2.18 μM , and 0.46 μM respectively during the time of the study. Nitrate and phosphate peaked at 0 degrees, whereas silicate peaked at 4 degrees north. The overall highest nutrient concentrations were found at the equator. Nitrate in the southern hemisphere stayed higher than silicate, while in the northern hemisphere, this relationship was reversed (Table 2). A second-degree polynomial correlation test was run for growth and grazing versus the nutrient concentrations. The highest grazing rate values were found at low and high nitrate concentrations, and the lowest values were found around 1.5 μM . However, this relationship is not significant, $p\text{-value} = 0.21$. Similarly, the lowest grazing rate values were concentrated near 0.35 μM of phosphate, while the highest values were found on the low and high end of the concentration gradient. This relationship is also non-significant, $p\text{-value} = 0.28$. Silicate contrasted both nitrate and phosphate, showing the highest grazing at 1.5 μM , and lowest at the high and low end of the concentration gradient (Fig. 2). Grazing rate did not significantly change with silicate concentrations, $p\text{-value} = 0.36$. Both growth and grazing rates followed corresponding trends with the various nutrient concentrations, exhibiting minimums and maximums at the same nutrient concentrations (Fig. 2). Linear regression analysis shows a low relationship between grazing rate and all 3 nutrients (Fig. 2).

Table 2: Ambient nutrient, temperature, and chlorophyll data compared to growth and grazing rate.

LATITUDE	GROWTH RATE (DAY ⁻¹)	TEMP (°C)	NITRATE (μM)	SILICATE (μM)	PHOSPHATE (μM)	GRAZING RATE (DAY ⁻¹)	R-SQUARED	CHLOROPHYLL (NG/L)
-5	0.07	30.24	1.55	0.42	0.36	0.52	0.97	5.15
-2	0.72	30.46	1.45	1.36	0.35	-9.62E-04	0.97	4.76
-1	1.96	30.03	2.12	1.26	0.39	2.37	1.00	9.15
-1	1.87	30.03	2.12	1.26	0.39	2.29	1.00	9.15
0	2.92	29.64	2.25	1.65	0.46	2.59	0.27	9.84
0	3.41	29.64	2.25	1.65	0.46	3.10	0.74	9.84
4	4.49	29.64	1.03	2.18	0.34	2.83	0.98	2.67
4	4.38	29.64	1.03	2.18	0.34	2.64	0.82	2.67
4	4.59	29.64	1.03	2.18	0.34	2.82	0.76	2.67
5	4.52	29.93	0.17	1.02	0.25	2.97	0.94	2.68
5	4.73	29.93	0.17	1.02	0.25	3.15	0.93	2.68

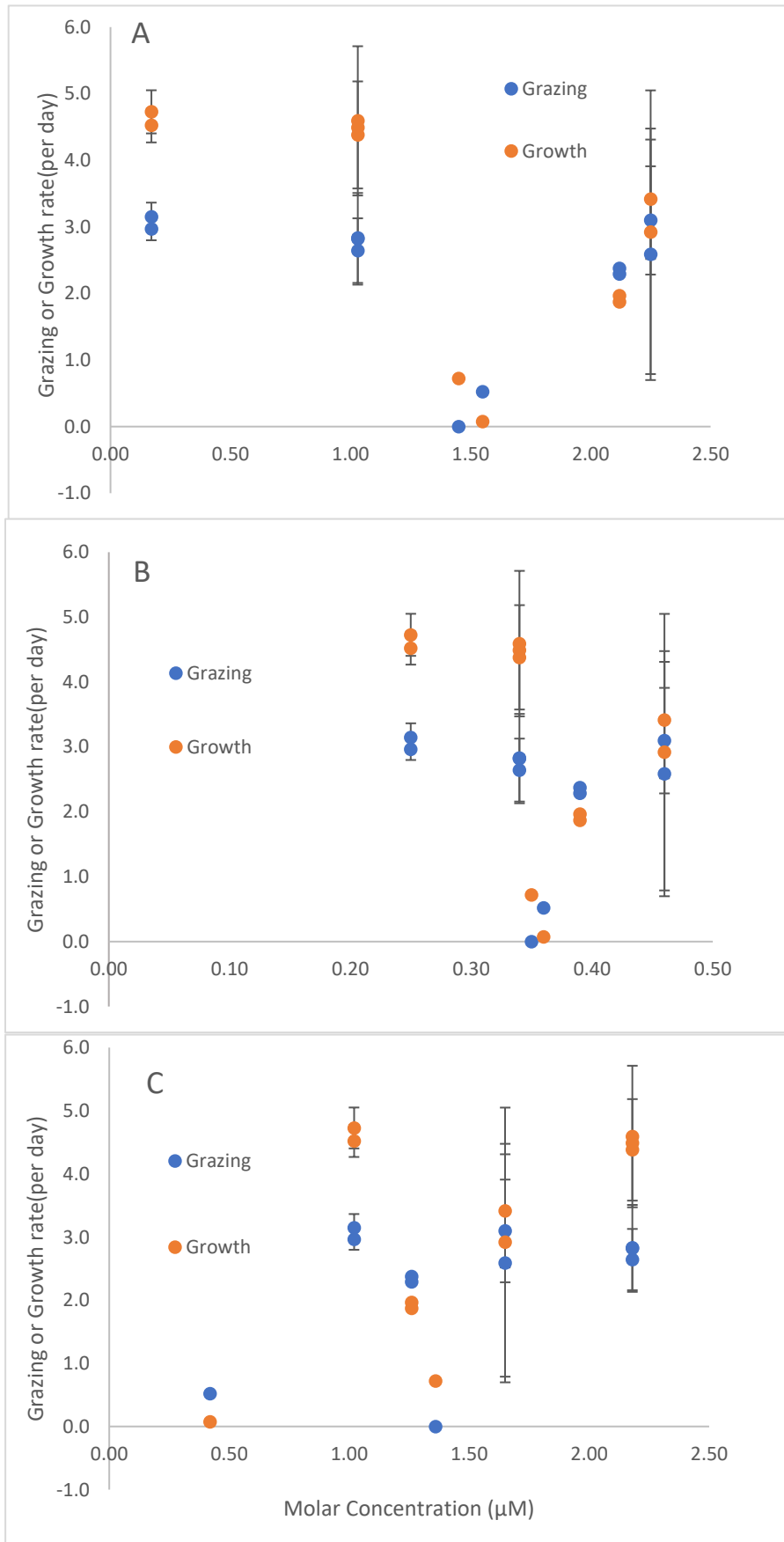


Figure 2: Grazing rate and growth rate by in situ (A) Nitrate, (B) Phosphate, and (C) Silicate concentrations. Error bars were fit using percent confidence from linear regression analysis of growth rate by dilution from the corresponding incubation.

Response to temperature and other environmental values:

Surface water temperatures ranged from 29.6 °C to 30.5 °C across the transect (Table 2). The grazing rate exhibited a negative relationship to temperature, p -value = 0.001 reaching a zero-grazing level at 30.4 °C (Fig 3.). Growth rates also followed a decreasing trend with temperature and reached zero growth at 30.4 °C, p -value = 0.005 (Fig. 4). Additionally, the highest variance in grazing rates was at lower temperatures. Dissolved Oxygen ranged from 131.34 mM Kg⁻¹ to 177.6 mM Kg⁻¹ and showed no significant relationship to grazing rate. The pH of the seawater ranged from 8.069 to 8.132, and grazing and growth rates showed no trend with pH (Table 2). There was no significant response in grazing rate due to in situ dissolved oxygen concentrations, pH, or salinity.

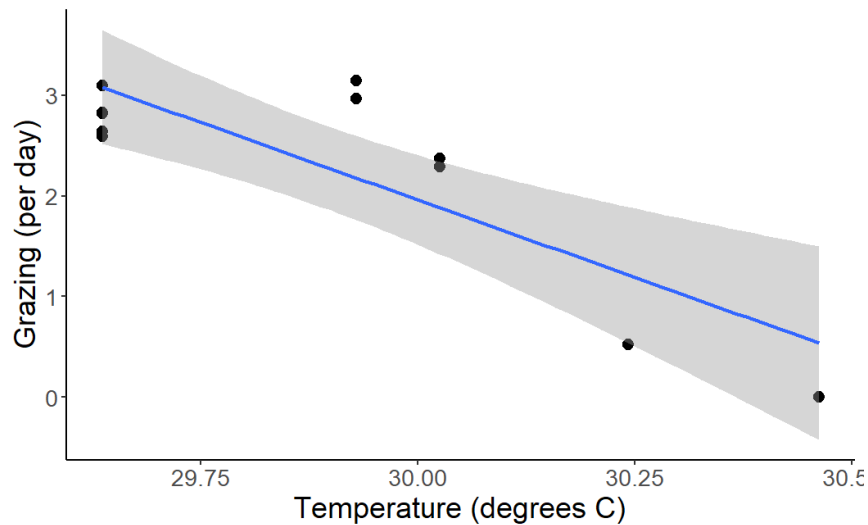


Figure 3: Grazing rate by grazers smaller than 10 microns per day by ambient temperature, with 95 percent confidence intervals (shaded regions).

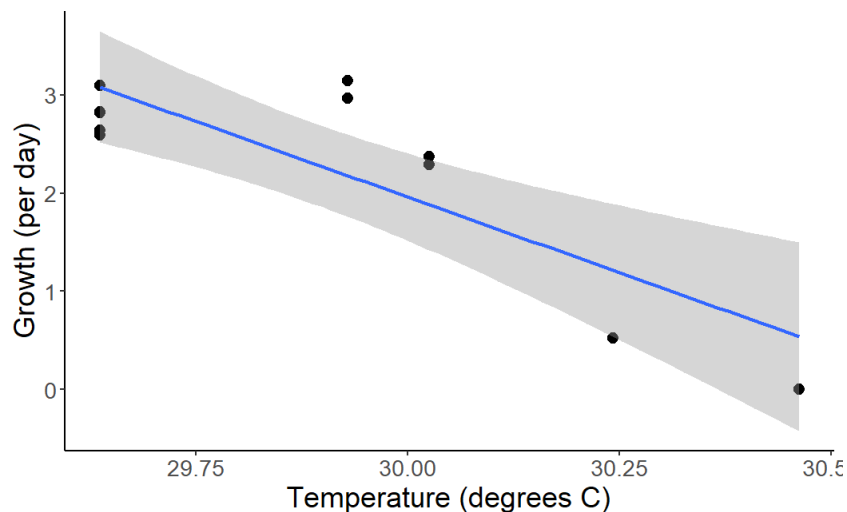


Figure 4: Bulk Phytoplankton growth rate at 100 percent dilution per day by temperature in degrees Celsius. 95 percent confidence region (shaded).

Grazing versus Growth and Chlorophyll:

Growth and grazing rates followed similar trends across the parameters of the study. Each rate showed similar trends across the different nutrient concentrations (Fig. 2) and temperatures (Fig. 3). On average, the grazing rate accounted for around 55 percent of the production (Fig. 5). The Grazing rate versus growth rate followed a linear trend which is roughly proportional (Fig. 5). This trend was highly significant, with a p-value of 0.0005. The grazing rate did not show any significant relationship with ambient chlorophyll concentrations, demonstrating a p-value of 0.94 (Fig. 6).

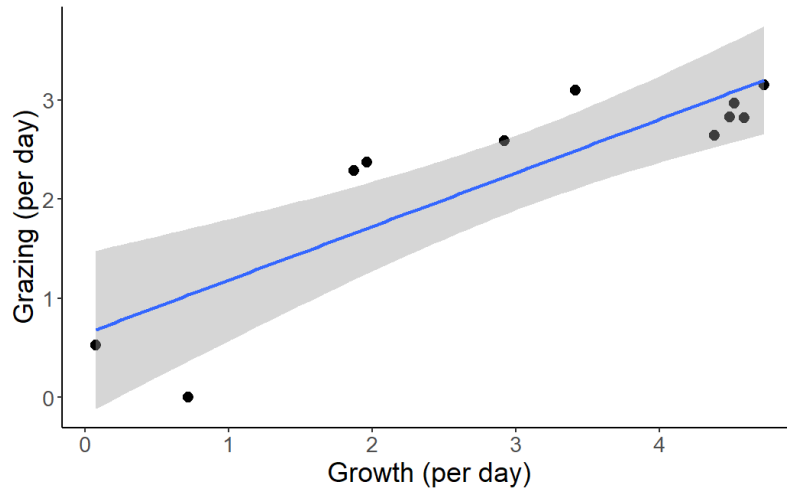


Figure 5: Grazing rate versus growth rate. The slope of this trend can be interpreted as the average fraction of grazed growth. 95 percent confidence region (shaded).

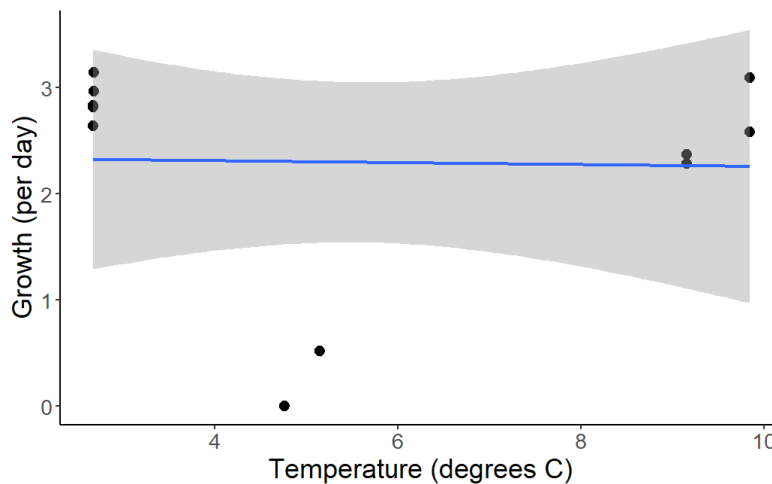


Fig 6: Grazing rate in nanograms per liter per day versus ambient chlorophyll concentration in nanograms per liter. 95 percent confidence region (shaded).

Discussion:

The results of this study show that the growth and grazing rates of plankton and protozoans smaller than 10 microns follow near identical trends due to temperature, nutrient concentrations, and other environmental factors. This is concurrent with prior knowledge that growth and grazing rate responses to temperature remain proportional (Pulsifer and Laws 2021). It is known that nutrient limitations have resulted in a decreasing thermal tolerance in some freshwater phytoplankton (Bestion et al. 2018). Anomalously high temperatures were measured in situ during sampling for this study. Argo float data from Simon CMAP shows an average surface temperature of 27.42 degrees Celsius with a standard deviation of 1.25 degrees from 2002 to 2021. All the temperatures seen during this experiment were above this temperature and upper range of the average standard deviation (Table 2). Figures 3 and 4 show that the grazing and growth plankton exhibited a strong negative trend with temperature. However, the current understanding is that both growth and grazing rates increase with ambient water temperatures, which contradicts the findings of this experiment (Pulsifer and Laws 2021; Vaque et al. 1994). Due to the strong relationship of growth and grazing rates versus temperature in this study, it is not likely that experimental error could have caused this discrepancy. However, phytoplankton populations in the study region may have reached a thermal tolerance, possibly due to nutrient limitations, and consequently, higher temperatures caused a decrease in production and in response, grazing.

The response of growth and grazing rate due to nutrients was weak yet showed a parabolic pattern (Fig. 2). A weak correlation between the parameters makes the interpretation of this data difficult. However, the close patterns between the growth and grazing rate versus nutrient concentrations are consistent with prior knowledge that grazing is often proportional to the growth rate (Pulsifer and Laws 2021) (Fig. 5). It is possible that the addition of nutrients to the incubation samples caused any trends in growth due to seawater nutrient concentrations to be skewed. If this is the case, it would suggest that the nano-plankton in the samples responded to an increase in nutrient concentration within the timespan of a day. Despite the proportionality of grazing to growth rate, Figure 6 shows a lack of correlation between grazing rate and ambient chlorophyll concentrations. This suggests that grazers smaller than 10 microns are not limited by the presence of phytoplankton but are rather limited by different factors. The lack of correlation between grazing rate and nutrients, chlorophyll, and other factors suggests that the consumption of phytoplankton by grazing organisms is not controlled by these factors. Therefore, it is possible that any trends in grazing rate could instead be explained by changes in the growth rate of their prey, or by changes in temperature.

Due to the strong proportionality of grazing and growth rate across the parameters of this study, growth rate likely had a much greater effect on the grazing rate of protozoans than other environmental conditions. It is likely that any condition that may affect the growth rate indirectly will change the protozoan grazing rate. This is consistent with the findings of the study by Pulsifer and Laws in 2021 which found that freshwater phytoplankton grazing by microzooplankton was directly proportional to the growth rates. This consistency suggests that since protozoan grazing will remain proportional to phytoplankton growth, protozoan grazers are

an important top-down control on phytoplankton in this study region. Grazing by protozoans in this study region accounted for more than half of the nanoplankton growth. Additionally, nanoplankton could be grazed by protozoans above the size range of 10 μ m along the study transect such as ciliates larger than nanoflagellates (Tsai and Mukhanov, 2021). These protozoans could account for another portion of the growth in these size ranges. A decrease in grazing by protozoans and nano zooplankton means weaker transfer of energy to higher trophic levels (Park et al. 2003). However, this study suggests that even in thermally limiting conditions, grazing by protozoans remains proportional to the growth of their prey, and will remain an important top-down control.

The findings of this study suggest that nanoplankton populations experience dynamic changes in thermal tolerance during El Nino events in the equatorial West Pacific. This has caused a decrease in the growth of producers and the grazing rate of nanoplankton consumers. High-temperature circumstances at the time of this study give a window into the future and how the predator-prey relationship at microbe levels may change due to global warming. Any decrease in grazing or primary production in the future may make an oligotrophic environment such as the equatorial West Pacific less able to support important macrofauna. Lower productivity and transmission of energy to higher trophic levels through grazing would severely impact the number of valuable fishery organisms in the equatorial Pacific. It is evident from this data that future monitoring of primary production and grazing in various ambient conditions is important for predicting how the changing climate may affect ecosystems at large.

Conclusions:

New developments in marine science suggest that herbivorous protozoans are more important in ecosystem dynamics than previously believed. Their rapid adaptation to changing conditions makes them an important top-down control over primary producers, and an important link between primary production and higher trophic levels. Protozoan grazers may serve an important role in cycling carbon in environments dominated by small phytoplankton, such as the equatorial Pacific. This study suggests that growth rate constitutes a more important control of grazing rate in protozoan grazers. It was also found that the phytoplankton in the study region had likely reached their thermal tolerance which caused production to decrease with temperature. Additionally, grazing rate showed no correlation to phytoplankton abundance, which suggests other factors such as mixotrophy may be disrupting the predator-prey relationship. Understanding how these microbes respond to different environmental conditions will help scientists understand further changes in planktonic communities due to anthropogenic factors.

Due to the lack of a large data set, and the removal of extraneous factors such as environmental variables, more data collection on the growth and grazing of nanoplankton populations in this region could shed light on the factors that control their population dynamics. Understanding the mixotrophic behavior of equatorial Pacific zooplankton populations could help determine the predator-prey relationships in microbial communities. Additionally, learning what drives changes in thermal tolerance of phytoplankton could help explain discrepancies in growth versus temperature compared to previous studies. Future research into predator-prey relationships based on marine food webs should consider the data presented in this study. The

range of seawater temperatures in this study was narrow and unusually high. More research into how nano-plankton and protozoans respond at thermally optimum temperatures could reveal more about how climate change may influence primary production in the future. Additionally, this study was unable to isolate extraneous variables such as sunlight exposure during incubation due to the nature of the experiment. By running carefully controlled experiments that isolate factors, it is possible that the effects of nutrient concentrations or other conditions on growth and grazing could be determined.

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References

- Beers, J., & Stewart, G. L. (1971). Micro-Zooplankters in Plankton Communities of Upper Waters of Eastern Tropical Pacific. *Deep-Sea Research*, 861-. doi:10.1016/0011-7471(71)90061-1
- BingZhing Chen. (2015). Patterns of thermal limits of phytoplankton. *Journal of Plankton research*, 285-292. Retrieved from <https://doi-org.offcampus.lib.washington.edu/10.1093/plankt/fbv009>
- Burford, M. A., Clementson, L., & Revill, S. J. (2012, December). Effect of sewage nutrients on algal production, biomass and pigments in tropical tidal creeks. *Marine Pollution Bulletin*, 2671-2680. Retrieved from <https://doi.org/10.1016/j.marpolbul.2012.10.008>
- Caron, D. A., & Hutchins, D. A. (2012, December). The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *Journal of Plankton Research*, 235-252. Retrieved from <https://doi.org/10.1093/plankt/fbs091>
- Hassett, M. L. (1982). Estimating the Grazing Impact of Marine Micro-zooplankton. *Marine Biology*, 283-288.
- Park, S., Brett, M. T., Muller-Navarra, D. C., Shin, S.-C., Liston, A. M., & Goldman, C. R. (2003, October 14). Heterotrophic nanoflagellates and increased essential fatty acids during *Microcystis* decay. *Aquatic Microbial Ecology*, 201-205. doi:10.3354/ame033201
- Pujari, L., Narale, D., Kan, J., Wu, C., Zhang, G., Ding, C., . . . Sun, J. (2021, March). Distribution of Chromophytic Phytoplankton in the Eddy-Induced Upwelling Region of the West Pacific Ocean Revealed Using *rbcL* Genes. *Sec. Aquatic Microbiology*. Retrieved from <https://doi.org/10.3389/fmicb.2021.596015>
- Pulsifer, J., & Laws, E. (2021). Temperature Dependence of Freshwater Phytoplankton Growth Rates and Zooplankton Grazing Rates. *Algae: Indices of Water and Ecological Quality*. doi:<https://doi-org.offcampus.lib.washington.edu/10.3390/w13111591>
- Regaudie-de-Gioux, A., S., S., & A., L.-U. (2015). Poor correlation between phytoplankton community growth rates. *Biogeosciences*, 1915-1923. doi:doi:10.5194/bg-12-1915-2015
- Schluter, L. (1998). The influence of nutrient addition on growth rates of phytoplankton groups, and microzooplankton grazing rates in a mesocosm experiment. *Experimental Marine Biology and Ecology*, 53-71. doi:[https://doi.org/10.1016/S0022-0981\(98\)00004-5](https://doi.org/10.1016/S0022-0981(98)00004-5)
- Simons CMAP. (2023). Retrieved from <https://simonscmap.com/>
- Taniguchi, D. A., Franks, P. J., & Landry, M. R. (2012). Estimating size-dependent growth and grazing rates and their associated errors using the dilution method. *Limnology and Oceanography: Methods*, 868-881. doi:10.4319/lom.2012.10.868
- Tillman, U. (2005, July 11). Interactions between Planktonic Microalgae and Protozoan Grazers. *Journal of Eukaryotic Microbiology*, 156-168. Retrieved from <https://doi-org.offcampus.lib.washington.edu/10.1111/j.1550-7408.2004.tb00540.x>
- Tillmann, U., & K., H. (1998). On the quantitative importance of heterotrophic microplankton in the northern German Wadden Sea. *Estuaries*, 585-596. doi:10.2307/1353297

Tsai, A.-Y., & Mukhanov, V. (2021). Response of Growth and Grazing Rate of Nanoflagellates on *Synechococcus* spp. to Experimental Nutrient Enrichment. *Biodiversity and Functionality of Aquatic Ecosystems*. doi:<https://doi.org/10.3390/w13192686>

Vaque, D., Gasol, J. M., & Marrase, C. (1994). Grazing rates on bacteria: the significance of. *Marine Ecology Progress Series*, 263-274.