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# *Prochlorococcus* Death by a Ubiquitous Zooplankton Filter Feeder

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*Prochlorococcus* Death by a Ubiquitous Zooplankton Filter Feeder

by

Avery Harman

An undergraduate honors thesis submitted in partial fulfillment of the

requirements for the degree of

Bachelor of Science

in

University Honors

and

Biology

Thesis Advisor

Anne W. Thompson, PhD

Portland State University

2024

## Abstract

*Prochlorococcus* is a globally abundant cyanobacterium that can help reveal the ecology of microorganisms in the ocean. Specifically, mortality by zooplankton such as appendicularians is globally important and *Prochlorococcus* can help reveal some of the choices made around feeding for this ubiquitous filter feeder. Two ecotypes of *Prochlorococcus* were introduced to appendicularians to determine if appendicularian feed selectively between these ecotypes. Further, a single ecotype of *Prochlorococcus* was fed to appendicularians over a variety of life stages to determine if retention rate changed with appendicularian life stage. My results reveal that there is no significant selection between the two *Prochlorococcus* ecotypes (as supported by a *p-value* of 0.35), and retention of *Prochlorococcus* cells by appendicularians increased with grazer developmental stage. Lack of discrimination between the two ecotypes reveals that the two similar *Prochlorococcus* ecotypes fulfill a similar feeding trend for the appendicularians. Further work should be performed to determine if increased retention rate of *Prochlorococcus* cells coincides with appendicularian physiological changes during development. This research helps in understanding the differences and similarities in the microorganisms that appendicularians choose to feast upon and why these microorganisms are chosen over others.

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## Introduction

Climate change has resulted in a global increase in temperature by 1.1°C since the latter 19<sup>th</sup> century (*Climate Change Widespread, Rapid, and Intensifying – IPCC — IPCC, 2021*). Due to the oceans' ability to absorb heat from the atmosphere, the global rise in temperature is not as substantial as it could have been (Levitus et al., 2012). Another climate-related role for the oceans is in the carbon cycle, which affects the atmospheric concentrations of greenhouse gases, such as carbon dioxide. Much of the carbon cycling that occurs in oceans is managed by marine microorganisms. Coupled with oceans covering 71% of the Earth's surface, marine microbial activity is key in understanding climate change on global scales. Thus, marine microorganisms must be taken into account when considering the implications of climate change.

Marine microorganisms play a vital role in carbon and nitrogen cycling and oxygen production. Like the role of blood to the human body, these microbial cells deliver oxygen and transform gases that contribute to the composition of the atmosphere and sustain life on Earth (Flombaum et al., 2013; Johnson et al., 2006; Moisaner et al., 2010). The demands and function of these cells is paramount amid the changing climate. It has been long known that increased atmospheric levels of CO<sub>2</sub> are directly correlated to rising temperatures (Foote, 1856; Manabe & Wetherald, 1975). CO<sub>2</sub> levels that have been generated by humans since the industrial revolution are currently hovering around 400 parts per million; a staggering 50% more than before this time (*Carbon Dioxide Now More than 50% Higher than Pre-Industrial Levels | National Oceanic and Atmospheric Administration, 2022*). We must ask ourselves how do marine microorganisms participate in this process?

Picocyanobacteria are one lineage of marine bacteria that are diverse and globally abundant. Picocyanobacteria exist in all sunlit water, from oceans to freshwater, and among a

variety of oceanographic conditions, including temperature and nutrient gradients.

Picocyanobacteria perform photosynthesis to meet their energy and carbon requirements, thus play a role in global carbon cycles. Since the discovery of marine picocyanobacteria 40-50 years ago, research has revealed many of the chemical and physical factors (i.e. abiotic factors) of the oceans that support the growth of these cells (Chisholm et al., 1988; Waterbury et al., 1979).

*Prochlorococcus* is the most abundant picocyanobacterium on Earth (Biller et al., 2015; Johnson et al., 2006). The diversity of *Prochlorococcus* has been divided into two ecological categories (Moore et al., 1998). A high-light adapted ecotype (HL) is associated with oceanic surface waters. A low-light adapted ecotype (LL) is associated with deeper waters. Within these major clades, many subclades exist. The two most abundant subclades are HLI (MED4 is a representative strain) and HLII (MIT9312 is a representative strain) (Biller et al., 2015). The success of *Prochlorococcus* is attributed in part to this diversity as well as highly efficient photosynthesis when compared with other photosynthetic cells, due to their unique pigmentation and high surface-area-to-volume ratio (Biller et al., 2015). These characteristics makes *Prochlorococcus* a formidable presence that can live up to 200 m deep and across vast latitudinal gradients.

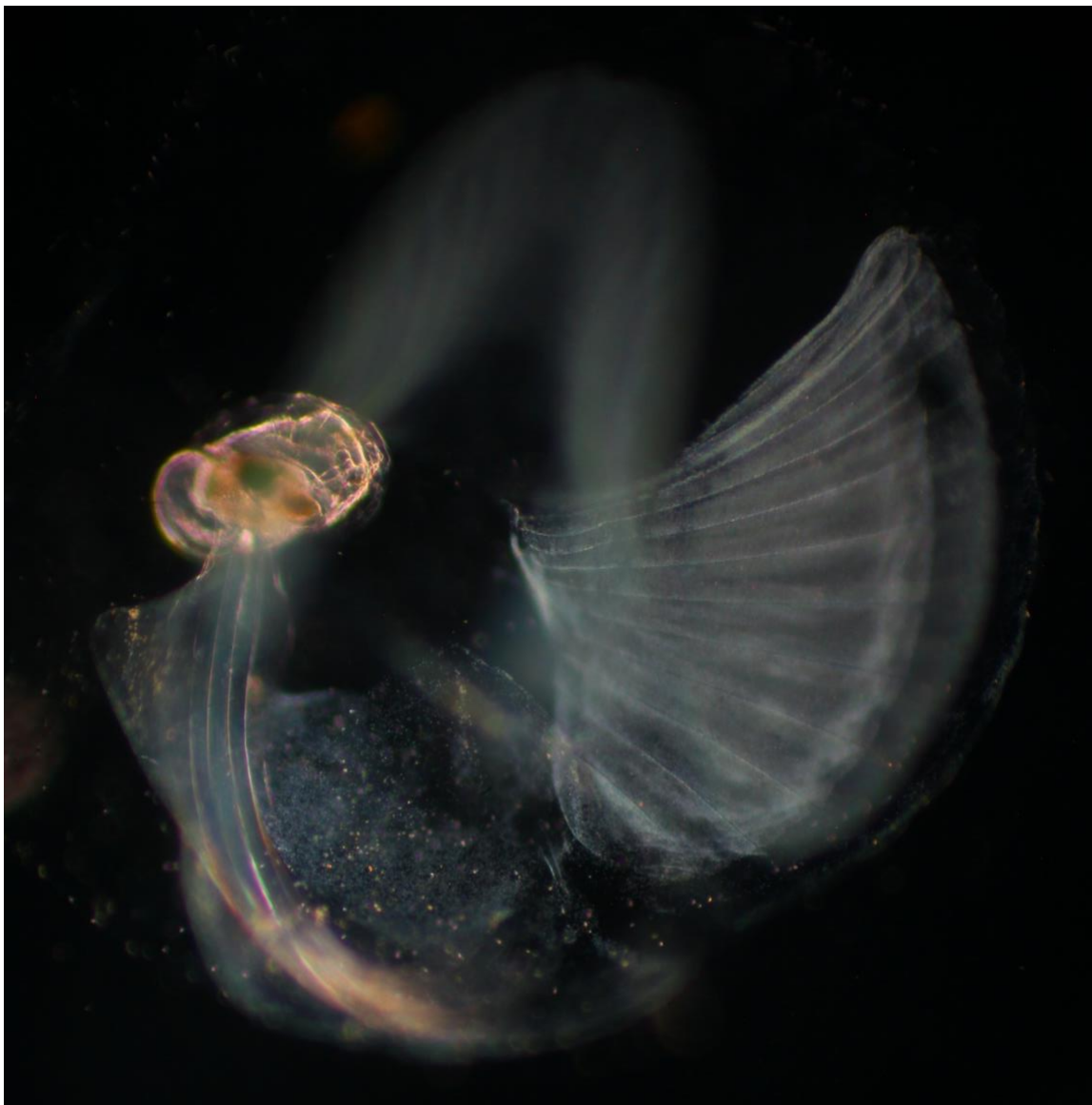
The life and growth of *Prochlorococcus* has been well-documented by researchers, however, the processes that remove these cells from the ocean are severely understudied, despite being critical to fully understanding the ocean. We do know that *Prochlorococcus* suffers mortality from oxidative damage via hydrogen peroxide and plastic leachates, cyanobacterium-specific viruses, and protist grazing (Morris et al., 2011; Quevedo & Anadón, 2001; Sullivan et al., 2003; Tetu et al., 2019). However, this information may be only a portion of *Prochlorococcus* mortality. Thus, there is a gap in knowledge on the relationship of deceased

*Prochlorococcus* cells and carbon cycling and ecosystem structure. As the carbon cycle is an important topic amidst escalating carbon pollution, the existence of *Prochlorococcus* is important for the fixation of carbon, estimated to be roughly 4 gigatons per year (Flombaum et al. 2013). Less *Prochlorococcus* would result in a reduction of carbon fixation, potentially catalyzing other climate events. Even though *Prochlorococcus* is necessary for our planet, we still do not have a complete comprehension as to all the factors that remove these microorganisms from the ocean and how that process drives carbon cycles.

Pelagic tunicates, known predators of marine microorganisms, deserve a closer look on the question of *Prochlorococcus* mortality. The pelagic tunicates are complex gelatinous organisms, some of which can form colonial structures, and are found globally (Damian-Serrano et al., 2023; Luo et al., 2022). As gelatinous zooplankton, these animals have been long-underappreciated due to their destruction in net-based sampling surveys. A defining characteristic of pelagic tunicates is a complex mesh web to filter and digest marine microorganisms of various sizes (Fernández et al., 2004; Katija et al., 2020; Sutherland & Thompson, 2022). This mesh supports the pelagic tunicate's ability to feed upon marine cyanobacteria, which may be an integral component of the carbon cycle and oceanic food webs (Sutherland & Thompson, 2022). Once thought to graze non-discriminately, we now know that their feeding can be highly selective as well (Deibel & Turner, 1985).

This thesis will focus on the interaction between the pelagic tunicates, appendicularians and *Prochlorococcus*. Appendicularia use an intricate, self-made mesh, mucous structure (“mucous house”) to filter and concentrate marine microorganisms (Alldredge, 1977; Conley, Gemmell, et al., 2018; Fernández et al., 2004) (Figure 1). Sinusoidal beating of the appendicularian tail brings new marine microorganisms into the house and propels them along

the thin mucous filaments of the food-concentrating filter (Conley, Gemmell, et al., 2018). From the food-concentrating filter, the marine microorganisms are moved into the appendicularian's buccal tube and mouth. The mucous house that contains this complex series of filters is discarded and remade between 2 and 40 times per day (Sato et al., 2003).



**Figure 1.** An appendicularian, *Oikopleura dioica*, observed by Carey Sweeney at the Michael Sars Centre in Bergen, Norway on October 16, 2023. The food-concentrating filter is clear, with *Prochlorococcus* cells attached.



Appendicularian - *Prochlorococcus* interactions are also of interest in understanding the global carbon cycle. The export of carbon via the discarded house and fecal pellets is estimated to contribute 83 to 100% of the carbon accumulated on the sea floor (Jaspers et al., 2023). When paired with *Prochlorococcus*' abundance, the appendicularian-aided transfer of *Prochlorococcus* carbon to other parts of the oceanic food web is a salient puzzle during a time of escalating carbon pollution.

Appendicularian abundance and fecundity has been found to increase with ocean acidification and rising ocean temperatures, indicating that they will be a main consumer of marine microorganisms in our changing climate (Taucher et al., 2024). During acidic conditions, appendicularians outperformed other zooplankton in sequestration of carbon – inhibiting the available prey microorganisms for other zooplankton species (Taucher et al., 2024). In addition, appendicularians occupy all the world's oceans and have been observed to consume microorganisms from 0.2 to 0.75  $\mu\text{m}$  (Flood, Deibel, and Morris, 1992; Lawrence et al., 2018). With looming threats of increased removal of phytoplankton, it is necessary to understand the current state of *Prochlorococcus* demise and appendicularians as a predator zooplankton.

The data presented in this thesis will act as a clarifying lens when considering how appendicularian blooms could lead to a decrease in *Prochlorococcus* populations. My thesis addresses the factors that control appendicularian feeding on *Prochlorococcus*. Specifically, I examined feeding selectivity on different *Prochlorococcus* and changes in retention rate of *Prochlorococcus* over the lifecycle of appendicularians. My approach includes a model appendicularian, *Oikopleura dioica*, and a model picocyanobacterium, *Prochlorococcus* (strains MED4 and MIT9312), in a controlled lab setting. The outcome of the work is understanding the preference and feeding rates of *Prochlorococcus* by *O. dioica*. These measurements will be

helpful in determining the impact that appendicularians have upon picocyanobacteria mortality within larger ecosystem models. Ultimately revealing the details of the interaction between appendicularians and picocyanobacteria amidst climate change. This information will be especially useful in assisting scientists predict the influence that climate change is having upon the small aquatic creatures that impact our planet's health.

## **Materials and Methods**

### *Appendicularian Rearing*

The cultivation design of appendicularians was followed from Bouquet et al., (2009) and performed at the Michael Sars Centre in Bergen, Norway by Carey Sweeney and Anne Thompson in the Bluewater Lab. Sweeney and Thompson designed all experiments and performed them at the Michael Sars Centre. Sweeney returned samples to Portland State University, where she trained the thesis author (Harman). Harman took ownership of a subset of the samples and applied the training provided, which resulted in a thesis of original data, synthesized in the context of all results, including Sweeney's M.S. thesis (Sweeney, 2024).

Appendicularians (*Oikopleura dioica*) were reared in 8 L tanks filled with 6 L of natural seawater from 4 to 8 meters depth, at a salinity ( $29 + 28/_{\infty}$ ), and pH ( $8.0 + 0.1$ ). The temperature of the water was brought to 12° C in the culture facility and strained through a series of filters (1  $\mu$ m), activated charcoal, and UV-light prior to use.

Appendicularians and algal media were maintained at a constant suspension using a rotating polyvinyl chloride paddle within the tanks. The diurnal light cycle was imitated by providing the appendicularians with 10 – 12 hours of light and 12 – 14 hours of dark each day. The appendicularian life cycle was set to 7 days using temperature control of their life cycle

progression. The appendicularians were transferred to new beakers with fresh seawater on days of life 3 through 7 (“D3-D7”).

The appendicularian’s standard algal food sources were provided to each 8 L tank, as described in Bouquet et al. (2009). Briefly, in the morning: 2000 cells mL<sup>-1</sup> of *Isochrysis* sp. & *Chaetoceros calcitrans*, 1000 cells mL<sup>-1</sup> *Rhinomonas reticulata*, and 80000 cells mL<sup>-1</sup> *Synechococcus* sp. were provided. In the evening: 1000 cells mL<sup>-1</sup> of *Isochrysis* sp. & *C. calcitrans*, 1000 cells mL<sup>-1</sup> *R. reticulata*, and 40000 cells mL<sup>-1</sup> *Synechococcus* sp. were provided. After day 3 of the lifecycle, the algal food sources are doubled.

#### *Prochlorococcus Cultivation*

The *Prochlorococcus* strains used in this research, MIT9312 and MED4, were reared in a plant growth chamber (Caron Products & Services Inc., Marietta, Ohio) set to 20 °C. Lighting conditions included 12-hour light intervals with a 25 μE meter<sup>-2</sup> second<sup>-1</sup> daytime light. Artificial seawater (AMP1) with PRO99 medium amendments, was utilized for *Prochlorococcus* incubation as described in a previous protocol (Moore et al., 2007). *Prochlorococcus* cultures were transported from Portland, Oregon to Bergen, Norway by Sweeney and Thompson in cell culture flasks (Thermo Fisher Scientific Inc., Waltham, Massachusetts), secured with Parafilm<sup>®</sup> M All-Purpose Laboratory Film (Amcor, Zürich, Switzerland), and plastic zipper bags. Cultures were stored at room temperature (about 70 °C) after arrival in Bergen, Norway.

#### *Feeding Study Design*

Feeding designs were formulated by Sweeney and Thompson of the Bluewater Lab in Portland, Oregon, and published in Sweeney’s M.S. thesis (Sweeney, 2024).

### *Survival on a Prochlorococcus Diet*

The survival of appendicularians on a diet of only *Prochlorococcus* was tested during the first experiment to make sure the appendicularians would survive and thrive in the experimental designs. Results of this experiment are reported on in Sweeney's M.S. thesis (Sweeney, 2024). This first design of feeding included three treatments in two replicate chambers per treatment, with ten appendicularians (D4 of lifecycle) per chamber. The three diets involved can be viewed in Supplemental Table 2. Briefly, the first diet consisted of  $10^5$  *Prochlorococcus* cells  $\text{mL}^{-1}$ ; the second diet consisted of the algal food source as described in *Appendicularian Rearing*; the third diet consisted of  $10^5$  *Prochlorococcus* cells  $\text{mL}^{-1}$  as well as the algal food source as described in *Appendicularian Rearing*.

### *Prochlorococcus Ecotype Preference*

In the second feeding setup, I investigated whether appendicularians had preference for two different strains of *Prochlorococcus*: MED4 and MIT9312. Thus, the second design of feeding introduced MED4 and MIT9312 *Prochlorococcus* cells to D7 appendicularians. There were two replicate chambers per treatment, with eight appendicularians per chamber. Times were measured for each individual appendicularian. The time of incubation was measured through notation of a start time for all appendicularians, and then the time from which each individual appendicularian was removed from incubation.

### *Retention Rates of MIT9312*

The third design of feeding measured retention rates of *Prochlorococcus* by the appendicularians across different life stages (D1, D2, D4, and D5). A concentration of  $8.8 \times 10^4$

MIT9312 *Prochlorococcus* cells  $\text{mL}^{-1}$  were fed to the D1 and D2 appendicularians and can be seen outlined in Table 3. A concentration of  $8.5 \times 10^3$  MIT9312 *Prochlorococcus* cells  $\text{mL}^{-1}$  were fed to the D4 and D5 appendicularians and can be seen outlined in Table 3. There were two replicate chambers per treatment, with eight appendicularians per chamber. Feeding times were measured for each individual appendicularian. The time of incubation was measured through notation of a start time for all appendicularians, and then the time from which each individual appendicularian was removed from incubation.

All designs sampled the prey field prior to starting the experiment ( $t_0$ ) and at the end of the experiment ( $t_F$ , about 15 minutes after the start) for each chamber in triplicate. All samples were stored in 100  $\mu\text{L}$  of DNA/RNA Shield™ (Zymo Research Corporation, Irvine, California) and kept at 4°C. The prey fields were sampled to give a measure of background *Prochlorococcus* concentration in the seawater, and to determine if there were any concentration differences that occurred over the duration of appendicularian grazing.

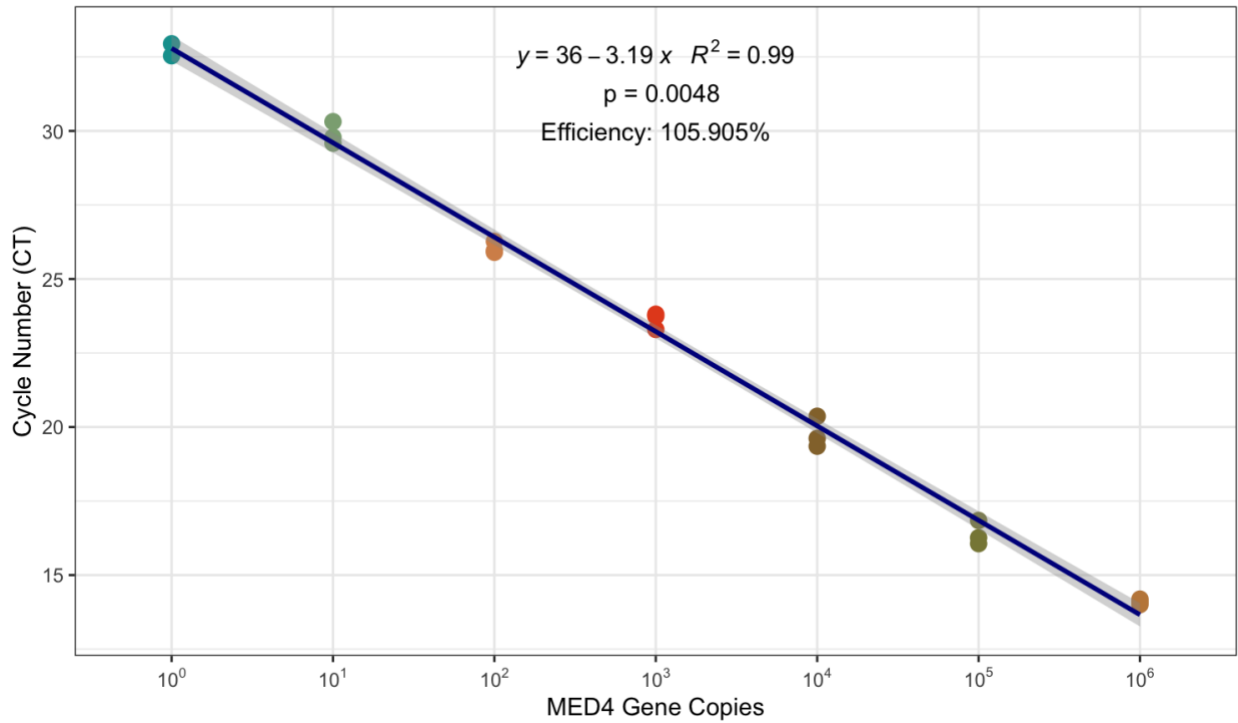
#### *DNA Extraction*

To quantify the number of *Prochlorococcus* cells retained by each appendicularian, qPCR was applied to the experimental appendicularians and the surrounding prey fields. The templates used for qPCR were DNA derived from whole grazing appendicularians and experimental seawater samples. DNA was extracted from all samples using the Quick-DNA™ Miniprep Kit (Zymo Research Corporation, Irvine, California) with modifications including using two 400  $\mu\text{L}$  aliquots of lysate during the extraction process and eluting in 105  $\mu\text{L}$  of DNase/RNase Free Water (Zymo Research Corporation, Irvine, California). The 105  $\mu\text{L}$  elution volume was chosen to provide excess sample for determining DNA concentrations. DNA

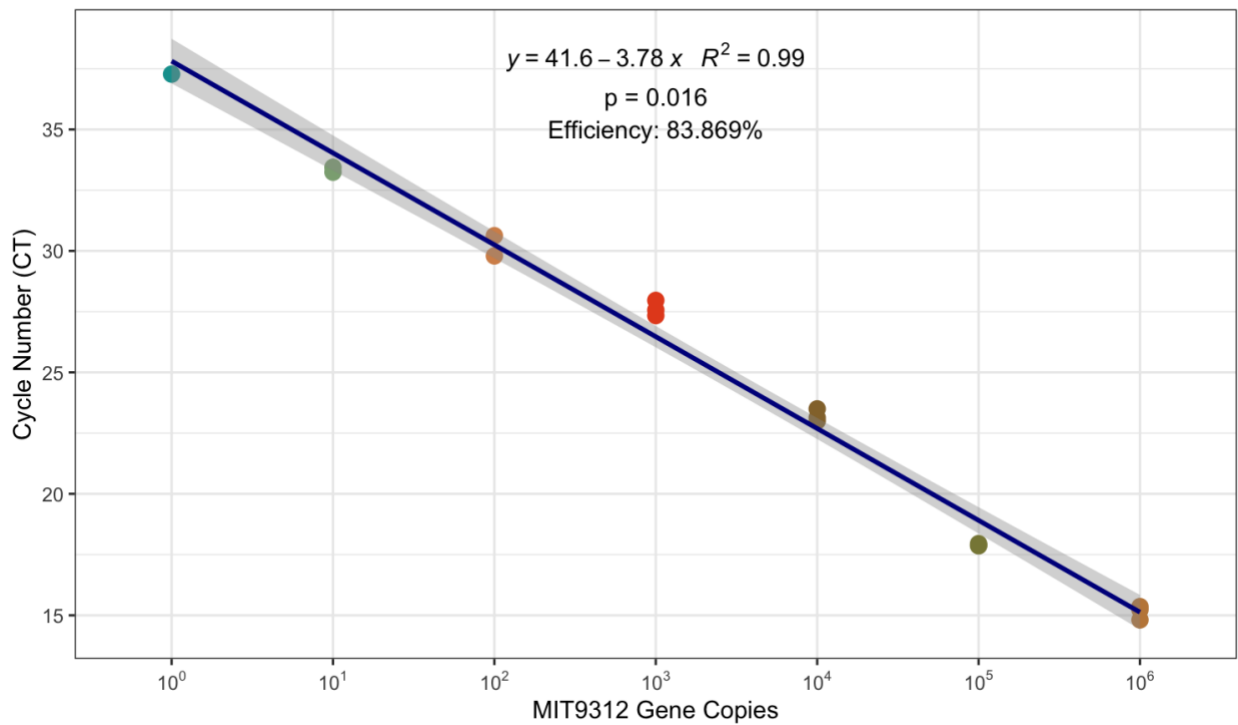
concentrations were quantified using the NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts).

### *Quantitative PCR*

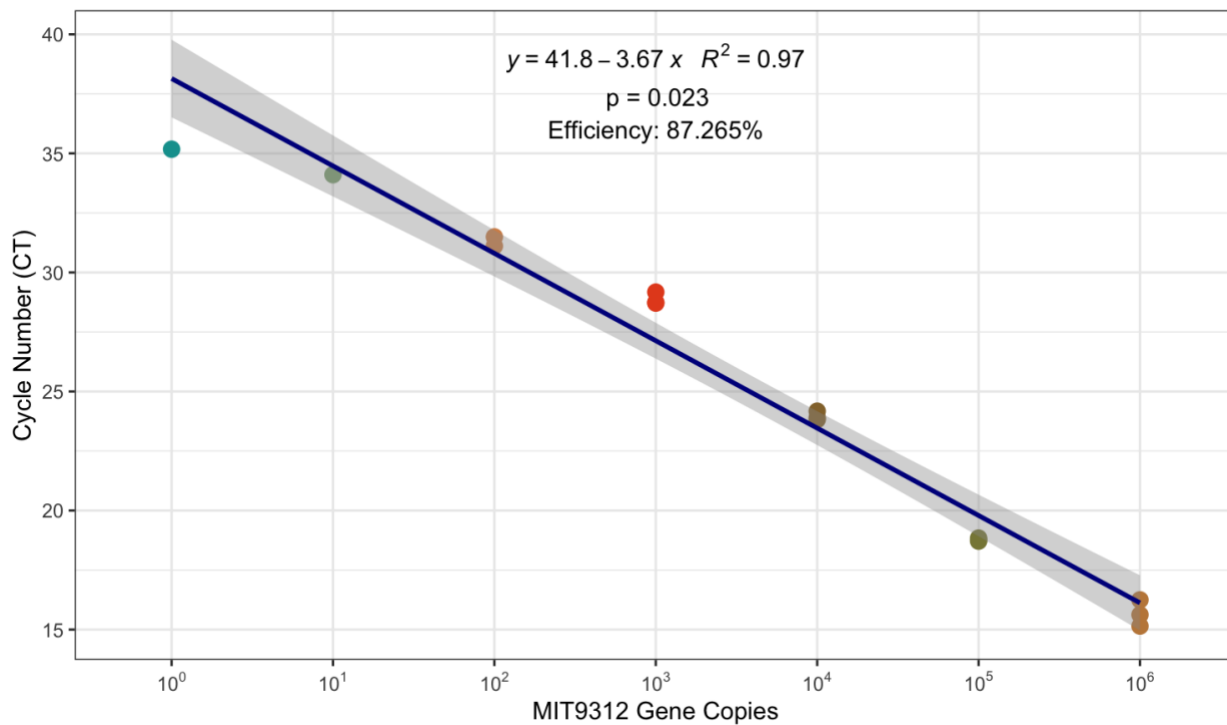
Quantitative PCR was performed using the Applied Biosystems® ViiA™ 7 Real-Time PCR System with the Power SYBR™ Green PCR Master Mix (Applied Biosystems, Waltham, MA). The *Prochlorococcus* eMED4 and eMIT9312 assay protocols used are included in Supplemental Table 1, as previously published by (Zinser et al., 2006). gBlocks™ Gene Fragments (Integrated DNA Technologies, Coralville, IA) serial dilutions were used for the standards and were diluted using DNase/RNase Free Water (Zymo Research Corporation, Irvine, California), with sequences shown in Supplemental Table 1. Standards from  $10^0$  to  $10^6$  gene copies per reaction were run in triplicate. No template controls with water were run in duplicate. qPCR conditions were adapted from (Zinser et al., 2006). qPCR conditions targeting eMED4 were 95°C for 15 min, followed by 40 cycles of 95°C for 45 seconds (s), 58°C for 45 s, and 72°C for 30 s. qPCR conditions targeting eMIT9312 were 95°C for 15 min, followed by 40 cycles of 95°C for 45 s, 56°C for 45 s, and 72°C for 10 s. qPCR thermocycling for both ecotypes ended with 72°C for 5 min. Primers for the *Prochlorococcus* ecotypes, eMED4 and eMIT9312, used during qPCR have been previously published in (Zinser et al., 2006). The standard curves used for the four separate qPCR experiments resulted in the following reaction efficiencies: 105.905% (Figure 2), 83.869% (Figure 3), 87.265% (Figure 4), and 95.454% (Figure 5). Melt curve analysis was performed for each qPCR run and confirmed the presence of a single amplicon.



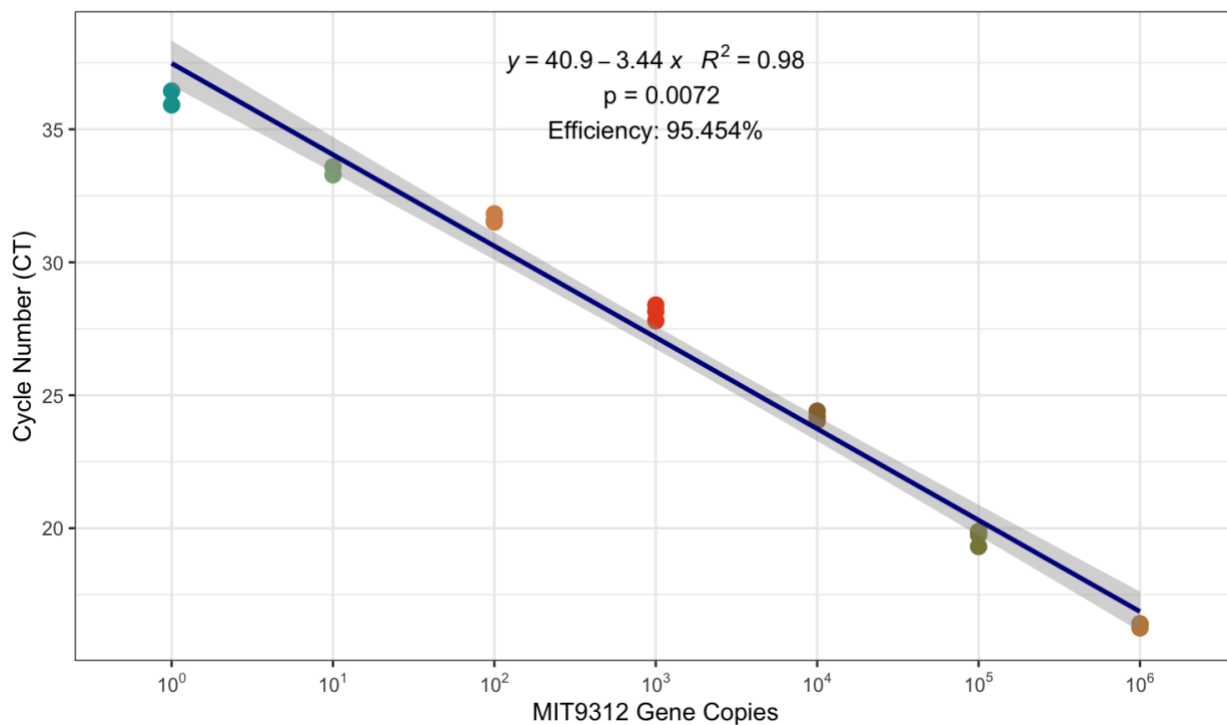
**Figure 2.** Standard curve for MED4 gene copies during qPCR with samples 148 through 169.



**Figure 3.** Standard curve for MIT9312 gene copies during qPCR with samples 148 through 169.



**Figure 4.** Standard curve for MIT9312 gene copies during qPCR with samples 246 through 283.



**Figure 5.** Standard curve for MIT9312 gene copies during qPCR with samples 284 through 321.



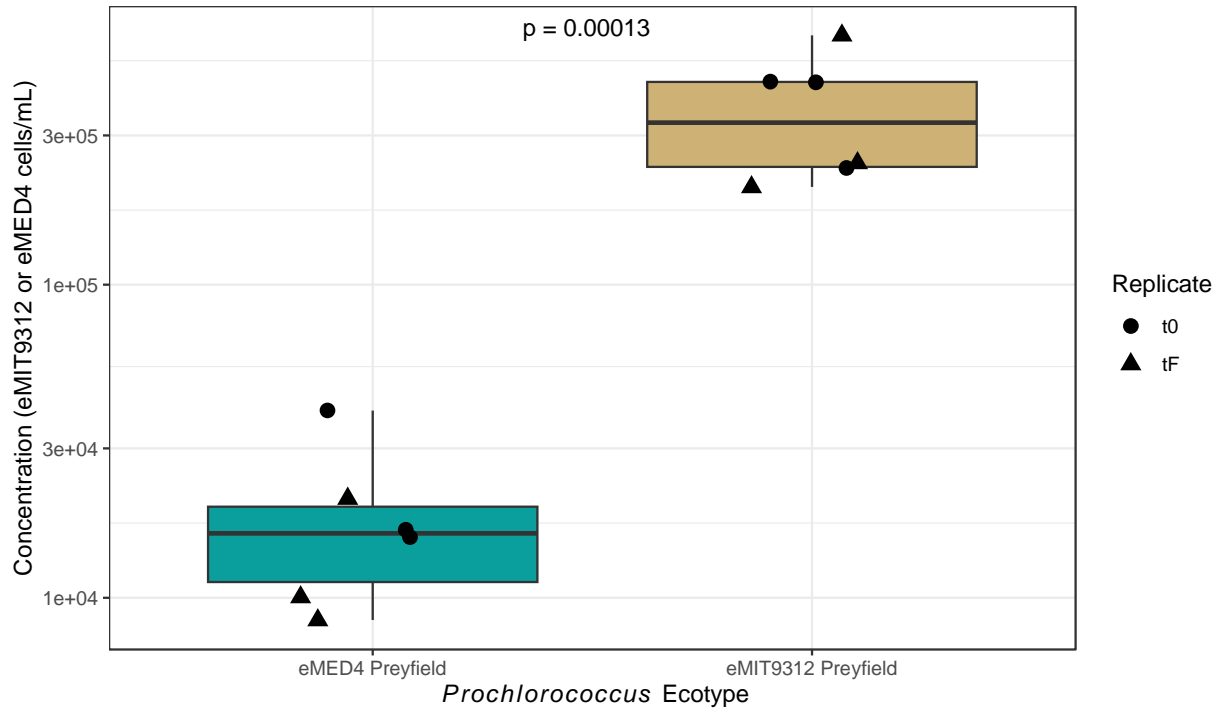
## *Data Analysis and Visualization*

All data analysis and visualization were performed with R version 4.3.2 in RStudio (Posit team, 2023; v4.2.3; R Core Team 2023). Plots were made using *ggplot2* (Wickham, 2016). The values for variation and error were calculated using the Wilcoxon t-test for the replicate samples. The Kruskal-Wallis test was utilized to compare retention rates (copy number/individual/time of incubation) across multiple groups. Normalization of samples to prey concentrations was performed by dividing each sample's cells/animal/minute (for prey field samples: cells/mL) by the averaged to prey field cells/mL.

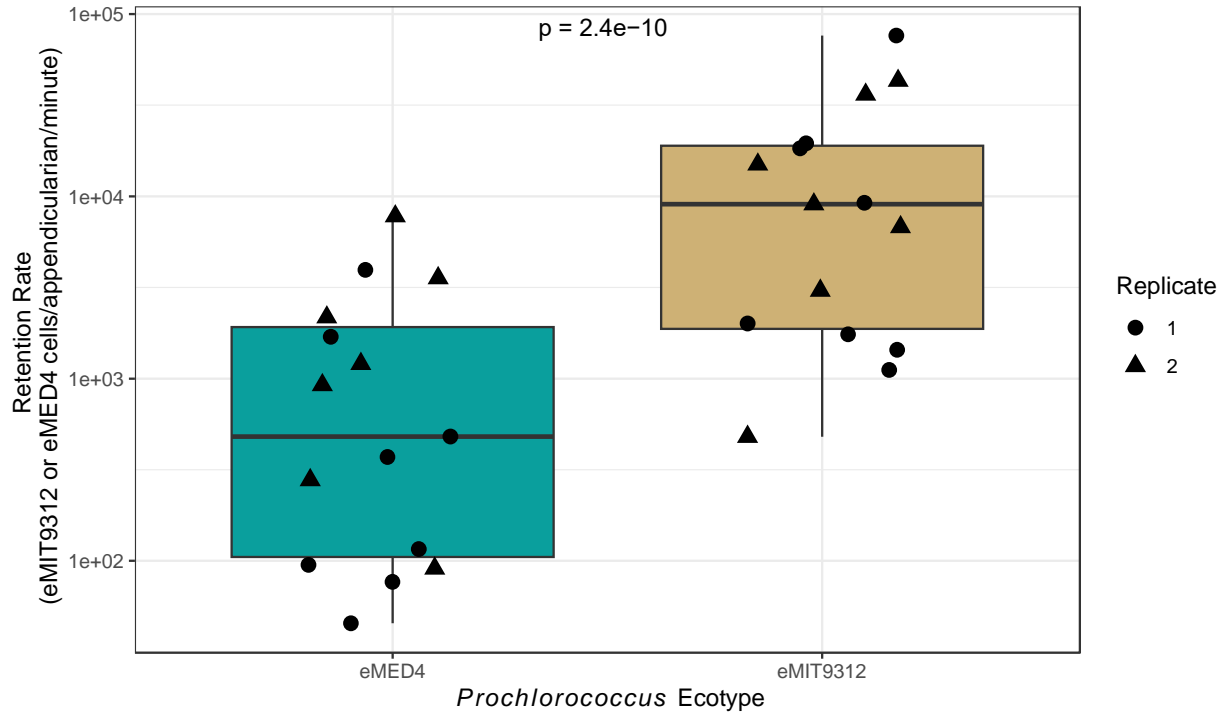
## **Results**

### *Equal Preference for Prochlorococcus Ecotypes*

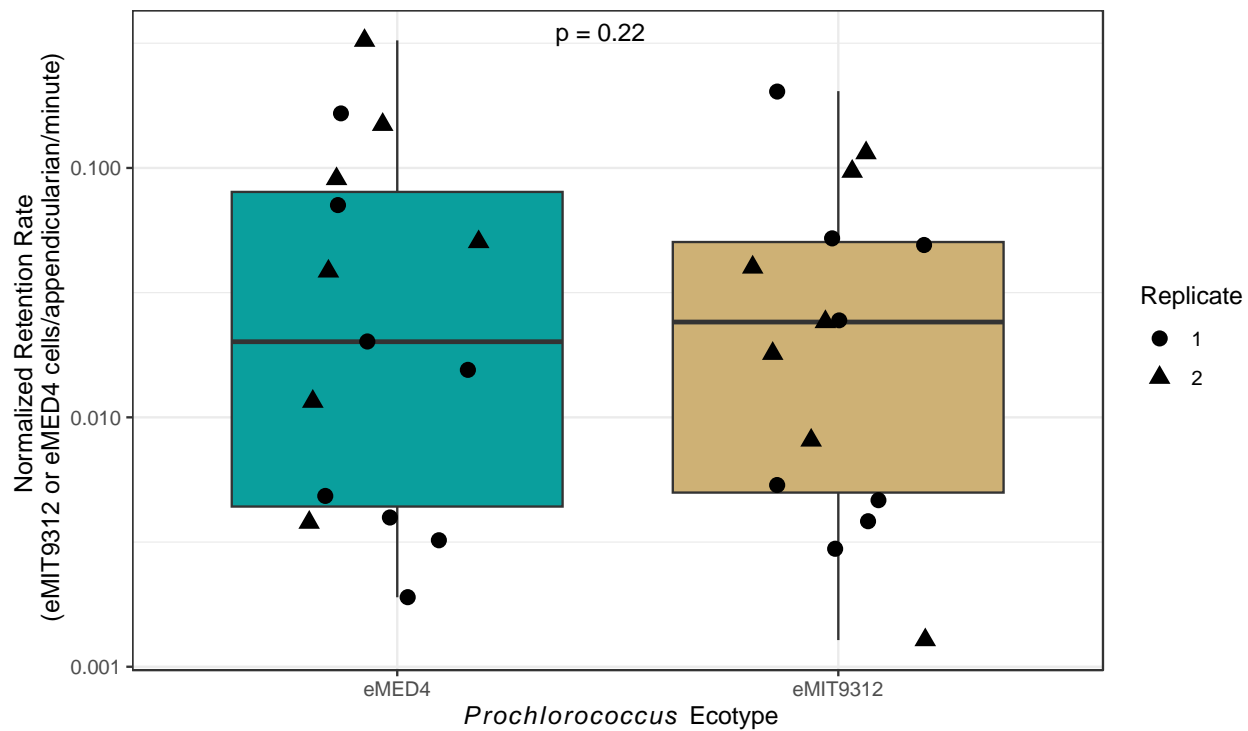
In order to examine whether closely related *Prochlorococcus* ecotypes have different susceptibility to grazing, D7 lifecycle appendicularians were presented with two ecotypes of *Prochlorococcus*: MED4 and MIT9312. The background prey concentration of MIT9312 was  $3.75 \times 10^5$  cells mL<sup>-1</sup> (Figure 6). The background prey concentration of MED4 was  $2.4 \times 10^4$  cells mL<sup>-1</sup> (Figure 6). Retention rates (Figure 7) were normalized to these prey field concentrations (Figure 8). The non-normalized prey field samples were not significantly different (Wilcoxon t-test,  $p$ -value=0.37). After normalization, the *Prochlorococcus* ecotype samples were compared (Figure 8). The normalized retention rates (*Prochlorococcus* cells/appendicularian/minute) were not significantly different (Wilcoxon t-test,  $p$ -value=0.22).



**Figure 6.** The prey field of MED4 and MIT9312, with shapes used to indicate  $t_0$  and  $t_F$ . The prey field concentrations are significantly dissimilar ( $p$  is “ $p$ -value” from the Wilcoxon t-test). Colors indicate the *Prochlorococcus* strain.



**Figure 7.** The retention rates observed prior to normalization. Within the second feeding design, an average prey field concentration of  $3.75 \times 10^5$  eMIT9312 cells and  $2.4 \times 10^4$  eMED4 cells were observed. The prey field concentration was adjusted to account for the greater amount of eMIT9312 cells.  $p$  is “ $p$ -value” from the Wilcoxon t-test. Shapes indicate the incubation replicate. Colors indicate the *Prochlorococcus* strain.

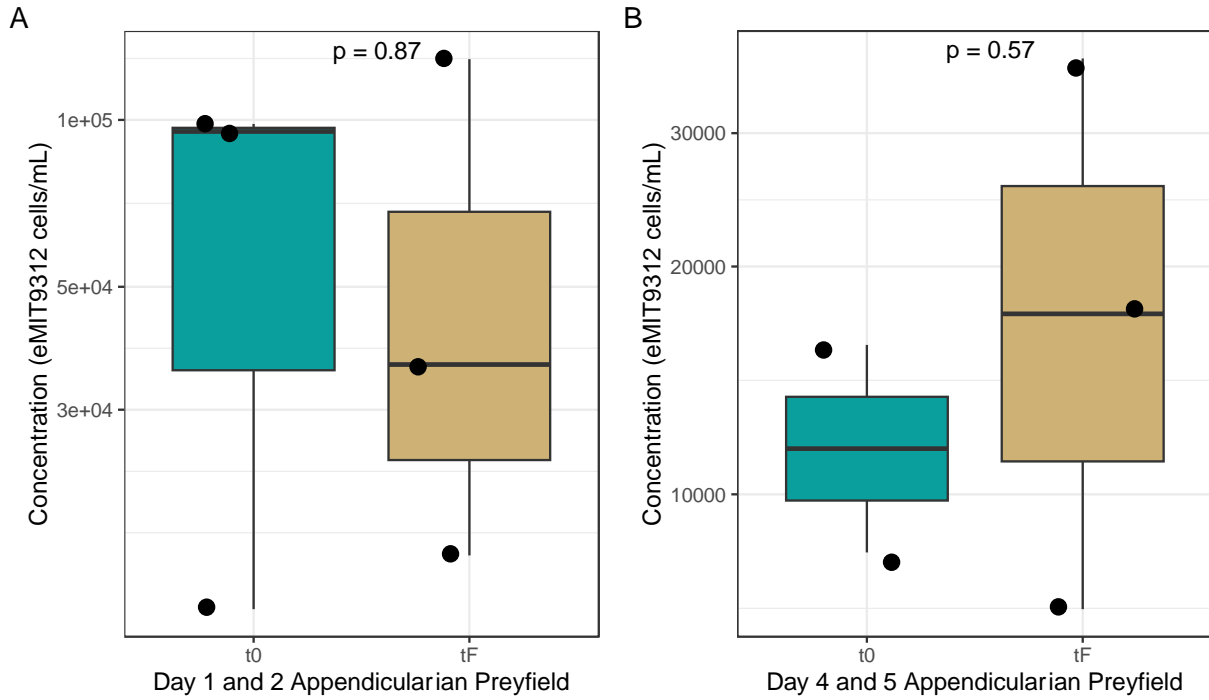


**Figure 8.** Normalized retention rates, which indicate equal retention of MED4 and MIT9312 ( $p$  is “ $p$ -value” from the Wilcoxon t-test). Shapes indicate the incubation replicate. Colors indicate the *Prochlorococcus* strain.

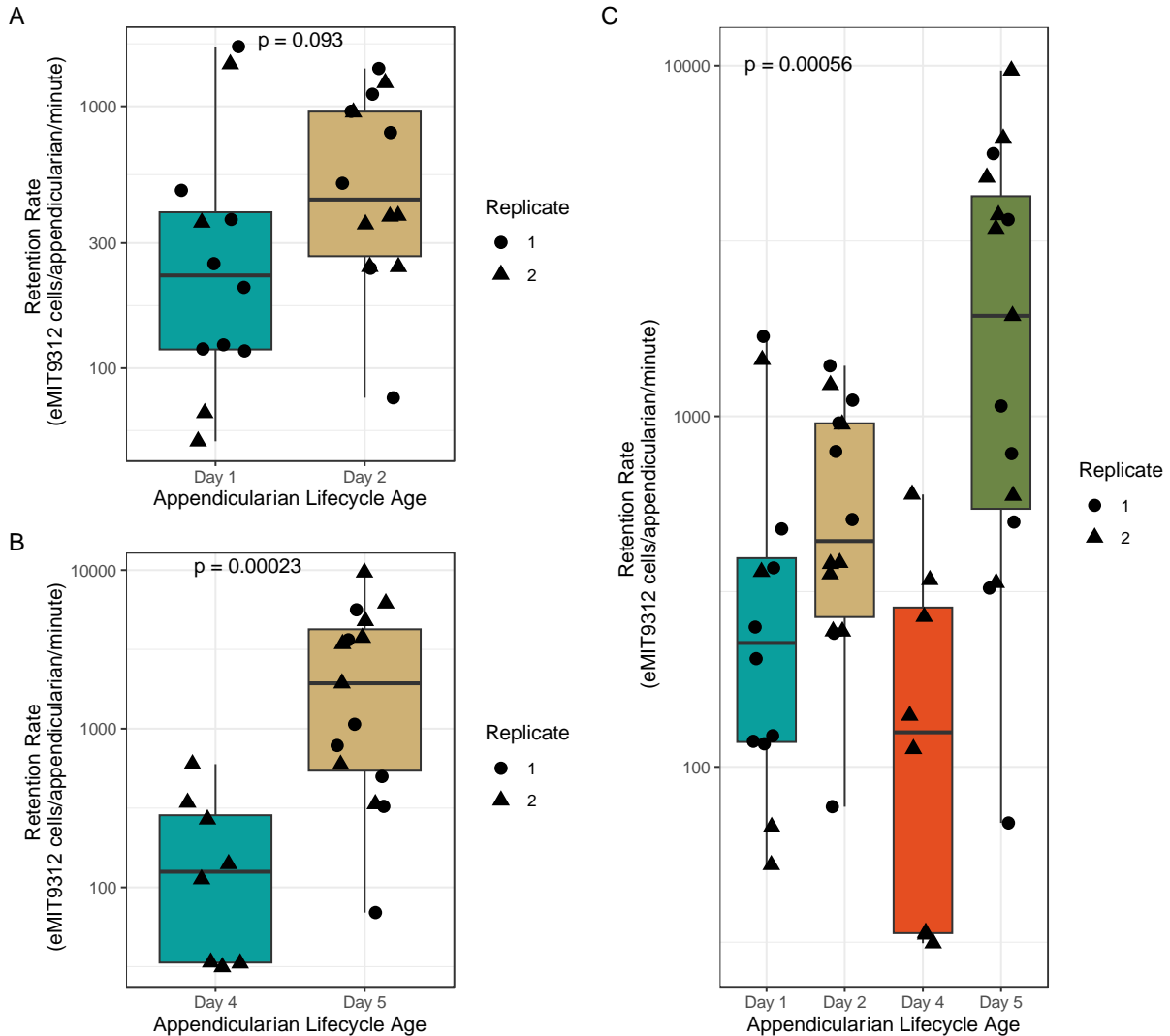
### *Feeding Rate Changes with Appendicularian Life Stage*

In order to compare the retention rates of *Prochlorococcus* by appendicularians as they reach advanced life stages, MIT9312 cells were presented to appendicularians progressively along in lifecycle age (D1, D2, D4, and D5). Pairwise incubations of D1 vs D2, and D4 vs D5 were performed (on separate days). The background prey concentration (cells/mL) of MIT9312 cells were greater for the lifecycle D1 & D2 appendicularians than that of the lifecycle D4 & D5 appendicularians (Figure 9). The starting concentrations of MIT9312 cells,  $t_0$ , during the D1 & D2 appendicularians are similar to the  $t_F$  values (Wilcoxon t-test  $p$ -value=0.87, Figure 9 left). Similar results were observed for comparison of the  $t_0$  and  $t_F$  for D4 & D5 appendicularians (Wilcoxon t-test  $p$ -value=0.57, Figure 9 right). Retention rates for each treatment, before normalization, are in Figure 10.

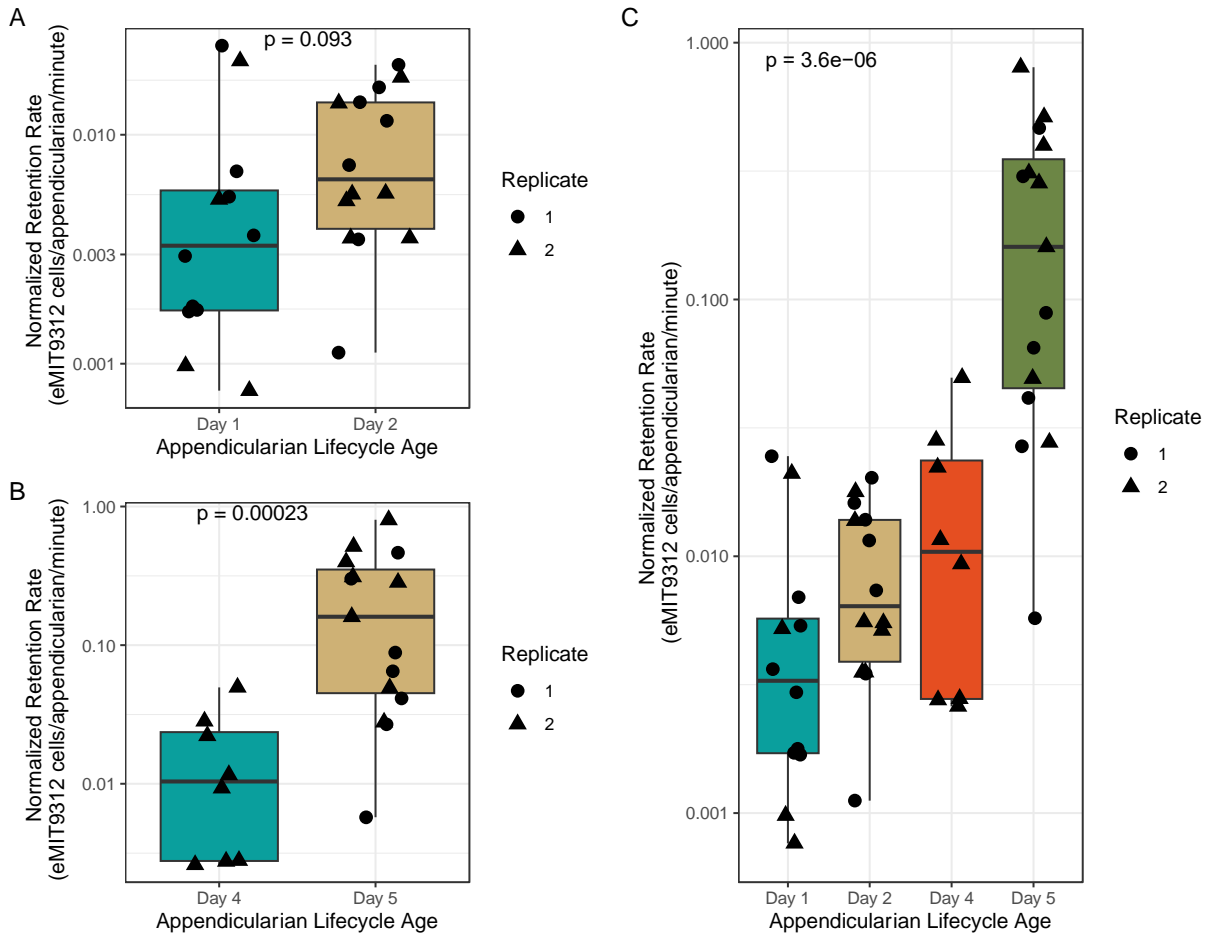
After normalization, the appendicularians of lifecycle D1, D2, D4, and D5 were compared via boxplot (Figure 11). The comparison of appendicularians of D1 and D2 showed significantly different retention rates (eMIT9312 cells/appendicularian/minute), where lifecycle D2 appendicularians had increased retention (Wilcoxon t-test  $p$ -value=0.093, Figure 11 top left). In parallel, the comparison of appendicularians of lifecycle D4 and D5 showed significantly different retention rates, where lifecycle D5 appendicularians had increased retention (Wilcoxon t-test  $p$ -value=0.00023, Figure 11 bottom left). When comparing all days together, it is observed that appendicularians of lifecycle D2 and D4 are similar, but there is a general tendency towards an increase in retention rate for appendicularians with increased age (Wilcoxon t-test  $p$ -value=3.6e-6, Figure 11 right).



**Figure 9.** The initial ( $t_0$ ) and final ( $t_F$ ) prey fields of eMIT9312, in reference to the appendicularian lifecycle during days A) D1 and D2, and B) D4 and D5.  $p$  is  $p$ -value from the Wilcoxon t-test). Colors indicate the *Prochlorococcus* initial ( $t_0$ ) and final ( $t_F$ ) prey fields of eMIT9312.



**Figure 10.** Comparison of retention rate of appendicularians when considering lifecycle age before normalization. A) Days 1 and 2 retention rates comparison indicates an increased in consuming eMIT9312 by day 2 appendicularians (Wilcoxon t-test  $p$ -value=0.093). B) A comparison of the retention rates of days 4 and 5 indicate an increased consumption by appendicularians (Wilcoxon t-test  $p$ -value=0.00023). C) An increase in retention rate is observed by increasing age of appendicularians through all life stage days, save for day 4 (Wilcoxon t-test  $p$ -value=0.00056). Shapes indicate the incubation replicate. Panel C colors indicate the day of lifecycle age of the appendicularians.



**Figure 11.** Normalized retention rates of appendicularians when considering lifecycle age. A) Days 1 and 2 retention rates comparison indicates an increased in consuming eMIT9312 by day 2 appendicularians (Wilcoxon t-test  $p$ -value=0.093). B) A comparison of the retention rates of days 4 and 5 indicate an increased consumption by appendicularians (Wilcoxon t-test  $p$ -value=0.00023). C) An increase in retention rate is observed by increasing age of appendicularians through all life stage days (Wilcoxon t-test  $p$ -value= $3.6 \times 10^{-6}$ ). Shapes indicate the incubation replicate. Panel C colors indicate the day of lifecycle age of the appendicularians.



## Discussion

This thesis addressed the feeding interaction between two abundant organisms in the sunlit surface oceans. I combined *Prochlorococcus*, a picocyanobacterium that is responsible for an estimated carbon export of 4 gigatons year<sup>-1</sup> with an appendicularian, a pelagic tunicate whose fecal pellets are estimated to contribute up to 100% of the carbon accumulated on the sea floor (Jaspers et al., 2023). My results have implications to understanding the flow of carbon in the oceans and the structure of food webs.

### *Appendicularians & Prochlorococcus are a Useful Model to Measure Predation Rates*

I found that appendicularians can retain MIT9312, a representative of the most globally abundant *Prochlorococcus* ecotype (HLII). While retention of *Prochlorococcus* has been shown in earlier studies this is the first insight into retention of a specific ecotype (Dadon-Pilosof et al., 2023; Gorsky & Fenaux, 1998; Scheinberg et al., 2005). This knowledge indicates that MIT9312 does not poison appendicularians and that appendicularians can collect MIT9312 cells. Further, this result indicates that appendicularians do not immediately reject all MIT9312 cells, and appear to accumulate them, as will be discussed in *Aging Appendicularians and Retention Rate*.

Because MIT9312 is from the most globally abundant *Prochlorococcus* clade (about 100,000 cells mL<sup>-1</sup> in surface ocean waters (Zinser et al., 2006)) its retention by appendicularians has several implications to global marine microbial ecology. First, the retention rates I measured are similar to those measured for wild populations of cells (Dadon-Pilosof et al., 2023), indicating that experiments with cultivated appendicularians may reproduce ocean-relevant feeding interactions. The ability of this experimental system to mimic field retention rates make it attractive for future experiments. Second, appendicularians contribute to global carbon cycling

in several distinct ways, thus feeding on *Prochlorococcus* creates previously unrecognized pathways of *Prochlorococcus*-derived carbon into the carbon cycle (Jaspers et al., 2023; Taucher et al., 2024). I expect some *Prochlorococcus* would be present in discarded appendicularian houses. Once discarded, the houses transport mineral dense packets as marine snow and sink slowly to other trophic layers of the ocean (Lombard et al., 2013). The interaction between *Prochlorococcus* and appendicularians could be a mechanism by which *Prochlorococcus* becomes a component of marine snow. Third, discarded appendicularian houses are an important food source for *Microsetella norvegica*, *Oncaea mediterranea*, and other invertebrate larvae diets in the lower euphotic zone, thus this is a mechanism by which *Prochlorococcus* production could fuel deep ecosystems (Alldredge, 1972; Koski et al., 2007).

*Prochlorococcus* could also be integrated into nutrient-rich appendicularian fecal pellets, which would drive the carbon cycle in additional ways. Regarding the sinking rates, the average discarded appendicularian house travels  $121 \text{ m d}^{-1}$  and fecal pellets sink at a considerably slower rate between 10 to  $100 \text{ m d}^{-1}$  (Gorsky et al., 2005; Hansen et al., 1996; Legendre, L. & Michaud, J., 1998). This indicates that fecal pellets would linger for a longer period within the euphotic zone before travelling to greater depths. The sinking appendicularian house means that the discarded house could act as a mode of carbon transport to the ocean floor. The amount of carbon present within fecal pellets is less as well, being  $0.025 \mu\text{g C pellet}^{-1}$ , where the discarded house contains  $3.0 \pm 1.2 \mu\text{g C house}^{-1}$  (Taguchi, 1982; Taucher et al., 2024). Half of discarded houses also contain fecal pellets, which would contribute even more to the carbon present in the house (Sato et al., 2003). The size and concentration of carbon of the house would also inform which organisms feed upon the house: a variety of adult and larval fish, medusae, ctenophores, and carnivorous zooplankton consume appendicularian houses (Gorsky & Fenaux, 1998; Koski

et al., 2007). The primary consumers of appendicularian fecal pellets are dominant within the euphotic zone and include zooplankton, protozooplankton, and bacteria (Dagg et al., 2014). These differences lead one to think that appendicularian fecal pellets are important for carbon recycling within the euphotic zone, where appendicularian discarded houses are necessary for transferring carbon to much deeper areas of the ocean.

Appendicularians are bountiful in all the world's oceans euphotic layer, indicating that there would be contact between appendicularians and MIT9312. Past studies have found that appendicularian fecal pellets contain diatoms, flagellates, and coccoliths – but these were dominant within the ocean waters where sampling occurred (Hopcroft et al., 1998; Landry et al., 1994). It would be beneficial for further *in situ* studies to be performed on how *Prochlorococcus* are integrated into appendicularian houses and fecal pellets.

#### *Appendicularians Do Not Select Between Prochlorococcus Ecotypes*

While viruses are known to be host-specific (Sullivan et al., 2003), thus select between *Prochlorococcus* ecotypes, this concept has not been tested for protist or zooplankton predators of *Prochlorococcus*. It is unknown to what extent non-viral *Prochlorococcus* predators select between the different *Prochlorococcus* ecotypes. Determining whether non-viral predators choose between ecotypes will be important to understand if predator interactions contribute to global biogeography patterns of the different ecotypes (Johnson et al. 2006).

This thesis compared MED4 and MIT9312, strains belonging to the two most globally abundant types of *Prochlorococcus*. I found no selection between the two cells (Figure 3) as appendicularian retention rates were the same between the two. This result suggests that the subtle differences between these two cell types may not be important for their predation by the appendicularians.

The *Prochlorococcus* ecotype, MED4, is present in the water column from 50 m to ~100 m (Zinser et al., 2006). eMED4 is prevalent within latitudes that are  $>40^{\circ}\text{N}$  and  $>40^{\circ}\text{S}$ , indicating that eMED4 occupies adjacent areas of the ocean to eMIT9312 (Zinser et al., 2007). This location within the water column, and among certain portions of the ocean, may also contribute to the nutrient content of each eMED4 cell. The nitrogen content of these cells have been previously measured to be 9.4 femtograms N cell<sup>-1</sup>, and the phosphorous has been measured to be 0.98 femtograms P cell<sup>-1</sup> (Bertilsson et al., 2003).

There are many similarities between MED4 and MIT9312, which may contribute to appendicularian's lack of selection between these two ecotypes. At the genomic level, MED4 and MIT9312 share 1574 genes, and their rRNA is only 0.8% different from one another (Coleman et al., 2006). While strain-specific genes are present in both ecotypes (Coleman et al., 2006), my work suggests that these genes do not encode for any predation defenses relevant to interaction with appendicularians. MED4 and MIT9312 also both have genes to assist during times of limited phosphorous and phosphorous is a limiting nutrient in the ocean (Coleman et al., 2006). Thus, feeding on *Prochlorococcus* may provide a beneficial source of phosphorous to appendicularians in an otherwise very P-limited environment, and both strains would provide this benefit.

MED4 and MIT9312 are also similar morphologically. Cells from these two strains are of roughly the same cell size (spherical diameter), at  $\sim 0.8 \mu\text{m}$  (Ribalet et al., 2019). The carbon (C) content for each is also similar at  $\sim 50$  femtograms C per cell (Ribalet et al., 2019). Due to the uniformity between the two ecotypes explored here, appendicularians may not discriminate between the two upon retention in their houses.

One important major difference between these two ecotypes is the locations within the ocean that they reside. From a depth perspective, MED4 occupies the upper and mid-levels of the euphotic zone water column and MIT9312 reigns over the top 50 m (Zinser et al., 2006). Within the global oceans, MED4 is dominant at higher latitudes than MIT9312, which is most abundant in the tropics (30°N to 30°S) (Johnson et al. 2006). Appendicularians are located over the continental shelf in tropic and temperate waters and share these areas with both ecotypes (Gorsky & Fenaux, 1998). Yet, *Prochlorococcus* is much more dominant in the band of ocean where eMIT9312 exists, signaling that appendicularian access to *Prochlorococcus* cells would be greatest in this range (Flombaum et al., 2013).

#### *Influence on Prochlorococcus Changes Over Life Stage*

I found that increasing appendicularian lifecycle age was positively correlated with increased retention rate of MIT9312. This result is consistent with other studies on eukaryotic phytoplankton prey (Troedsson et al., 2007). This result has important implications to predicting how *Prochlorococcus* retention might change during an appendicularian bloom. With older appendicularians reaching sexual maturity, then this may be an indicator of a greater need for nutrients during reproduction (Nishida, 2008). The cumulative increase in retention rates observed in older appendicularians in this study would support this idea.

As the ocean becomes more acidic, assuming the current trend of climate change, then it would be expected that blooms of appendicularians would be much more numerous and have greater impact upon *Prochlorococcus* populations (Taucher et al., 2024). Reduced availability of *Prochlorococcus* could have detrimental effects upon the oxygen necessary for respiration, with *Prochlorococcus* being responsible for producing up to 20% of the oxygen in Earth's atmosphere (Johnson et al., 2006).

### *Future Directions*

This study is an important piece in understanding the implications of appendicularian feeding upon *Prochlorococcus*. We can use this information to better understand our future relationship with our planet's climate. With increasingly acidic oceans, where appendicularians thrive, it is expected that appendicularian feeding upon prey microorganisms will only escalate (*Prochlorococcus* being included). My hope is that this information will assist future research into the lives of *Prochlorococcus*, the dynamic interactions of *Prochlorococcus* in our oceans, and how we can best ensure a healthy future for them and ourselves.

While the increasing retention rate of aging appendicularians can be showcased, it is more difficult to prove that the retention rate is correlated with ingestion. In Sweeney's M.S. thesis, it was noted that appendicularians were able to survive for 48 hours until day 7 in their life stage on a diet of only *Prochlorococcus* (deviating from the standard mixed algal diet fed by the Michael Sars Centre). The age of mortality was in keeping with the expected appendicularian lifecycle, suggesting that *Prochlorococcus* had no adverse effect on the appendicularians who consumed them (Bouquet et al., 2009). Past studies have also found that appendicularian houses accumulate phytoplankton cells that have not been ingested (Alldredge, 1976; Bedo et al., 1993; Davoll & Silver, 1986; Hansen et al., 1996). From this, we can determine that a diet of *Prochlorococcus* does not kill appendicularians, but it is unclear if ingestion is occurring, or only collection.

When considering the ability for appendicularians to create 40 houses per day, it is apparent that nutrients are necessary for this process to be successful (Conley, Lombard, et al., 2018). For another species of appendicularian, *Oikopleura longicauda*, it was found that 70% of discarded houses contained fecal pellets (Sato et al., 2003). This knowledge implies not all DNA

observed using qPCR would merely be due to cell adhesion to the appendicularian house, but my technique could have also counted *Prochlorococcus* in the fecal pellets. In conjunction with the awareness that appendicularians can only survive for 15 to 48 hours without food, it is evident that appendicularians must be consuming the *Prochlorococcus* cells that are being collected within their filters, Figure 1 (Lombard et al., 2005). From the data presented in *Feeding Rate Changes with Appendicularian Life Stage*, support is given for aging appendicularians successfully acquiring and ingesting greater *Prochlorococcus* cells. In the future, it would be meaningful to investigate if *Prochlorococcus* retention rate coincides with potentially expanding house size and increasing age during lifecycle. This would assist in understanding of how appendicularian populations impact phytoplankton blooms.

## Conclusion

This study presents novel findings on the feeding of *Prochlorococcus* by appendicularians in consideration to appendicularian lifecycle age and *Prochlorococcus* ecotype. During times of heightened appendicularian presence due to ocean acidification events, this study offers support for the expectation that *Prochlorococcus* consumption would also increase. This increase in *Prochlorococcus* ingestion could change the amount of carbon export, as well as limit nutrient capturing phytoplankton cells in the ocean (Jaspers et al., 2023; Taucher et al., 2024). Iterative research is advised to include a low-light (LL) *Prochlorococcus* ecotype to help understand any feeding preference when contrasting light ecotypes are involved (Biller et al., 2015), as LL *Prochlorococcus* may present more distinct morphological differences that could matter to feeding. Further studies should be performed to investigate eMIT9312 nutrient/mineral content, and any relationship present between appendicularian house size and age.

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## **Dedication**

I dedicate this thesis to all the queer scientists in the world.

“All that you touch, you Change. All that you Change, Changes you. The only lasting truth is Change.” Octavia Butler, Parable of the Sower



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## Supplementary Materials

Table 1. gBlock sequences for the three <i>Prochlorococcus</i> ecotypes being targeted during qPCR.		
<i>Prochlorococcus</i> ecotype target	Synthetic oligonucleotide standard sequences (gBlocks)	Assay reference
eMED4 operational taxonomic unit 1	TACATATATATAAAGAGGGAAATTGCTTTGAGTCGTGTCCTAATTT	(Zinser et al., 2006)
eMED4 operational taxonomic unit 2	TACATATAGTTGAAGTGGGAAATTGTTTTGAGTCGTGTCCTAATTT	(Zinser et al., 2006)
eMIT9312 operational taxonomic unit 1	CTTTGATCCGGGAATTTCCGAATGGGGCAACCCC	(Zinser et al., 2006)

Table 2. Feeding study design to determine appendicularian survival on a diet of only <i>Prochlorococcus</i> .	
Diet	Details
Only pro.	$10^4$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> .
Only algae	The algal food source as described in <i>Cultivation and collection of Appendicularians</i> .
Pro. and algae	$10^5$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> as well as the algal food source as described in <i>Cultivation and collection of Appendicularians</i> .

Table 3. Feeding study design to determine appendicularian consumption of <i>Prochlorococcus</i> , MIT9312, across life stages.	
Diet	Details
1	Day 1 appendicularians with $8.8 \times 10^4$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> .
2	Day 2 appendicularians with $8.8 \times 10^4$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> .
3	Day 4 appendicularians with $8.5 \times 10^3$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> .
4	Day 5 appendicularians with $8.5 \times 10^3$ <i>Prochlorococcus</i> cells mL <sup>-1</sup> .