EFFECT OF EXOPOLYMERS ON THE CONSUMPTION OF *SYNECHOCOCCUS* BY *OXYRRHIS MARINA*

An Undergraduate Research Scholars Thesis

by

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Submitted to Honors and Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Daniel CO Thornton

May 2014

Major: Zoology

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ABSTRACT

Effect of Exopolymers on the Consumption of *Synechococcus* by *Oxyrrhis marina*. (May 2014)

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Microscopic organisms play an important role in carbon cycling in the ocean. Because of their large role, understanding how their activity is affected by other organisms and by particles in the water is important to understand the oceanic carbon cycle and flow of energy through food webs. This experiment was designed to determine what effect, if any, transparent exopolymer particles (TEP) have on the consumption of *Synechococcus* by *Oxyrrhis marina*. *O. marina* is a heterotrophic marine dinoflagellate that has the potential to be a model organism for heterotrophic dinoflagellates and protists. *Synechococcus* is a cyanobacterium that is responsible for a quarter of yearly marine net primary production. Because TEP are adhesive, they form aggregates of small particles such as *Synechococcus*. In this experiment, cultures containing *O. marina* and *Synechococcus* with TEP added saw a significant increase in the average number of *Synechococcus* consumed by *O. marina*. This is likely because the size of the aggregates of *Synechococcus* formed by TEP are more appealing to *O. marina* than individual *Synechococcus* cells. The results of this experiment suggest that the presence of TEP will increase the amount of carbon flow to upper trophic levels by causing higher consumption at lower levels due to prey aggregation.

ACKNOWLEDGMENTS

I would like to thank Chen Jie and Audra Hinson for showing me how to perform filtering, culturing, and microscopy in the lab. I would like to thank the Undergraduate Research Scholars Program and Texas Sea Grant for providing funds for this project. I would also like to thank Dr. Thornton for allowing me to work in his lab, teaching me various laboratory techniques, and advising me throughout this project.

CHAPTER I

INTRODUCTION

Role of heterotrophic flagellates in marine food webs

Heterotrophic flagellates are microorganisms that play an important role in the ocean's microbial loop. The microbial loop is the process by which particulate organic matter (POM) and dissolved organic matter (DOM) are consumed by bacteria and other microbes which are then eaten by larger microbes such as heterotrophic flagellates. This leads to nutrients and energy in POM and DOM becoming available to organisms at higher trophic levels. Without this process, nutrients and energy lost to detritus may never be recycled (Azam et al. 1983).

Heterotrophic flagellates also play a role in regulating populations of phytoplankton. They can influence the outcome of competition for nutrients between different species of bacteria and phytoplankton by reducing populations of certain species which leaves more nutrients available for use by others (Azam et al. 1983). They also serve as food for larger heterotrophic zooplankton. This process helps make energy available to larger organisms which are not able to feed on small phytoplankton directly (Azam et al. 1983).

Oxyrrhis marina

Oxyrrhis marina (Figures 1 & 2) is a heterotrophic marine dinoflagellate. It is somewhat pear-shaped, has pink pigmentation (Montagnes et al. 2011), and two flagella which it uses to swim in a tumbling motion (Lowe et al. 2011). *O. marina* is abundant in coastal waters worldwide, has a wide range of temperature and salinity tolerance (Lowe et al. 2010), and can survive on a variety

of food sources. Because of its wide range of tolerances and food sources, *O. marina* has been suggested as a model organism for heterotrophic dinoflagellates (Montagnes et al. 2011).

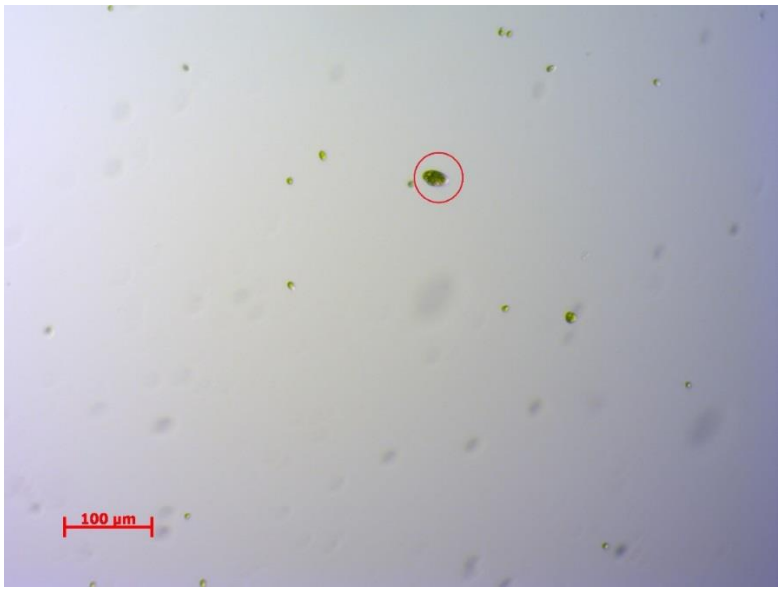

Figure 1: *Oxyrrhis marina* (circled) and *Dunaliella tertiolecta* (used as food for *O. marina* during culturing)

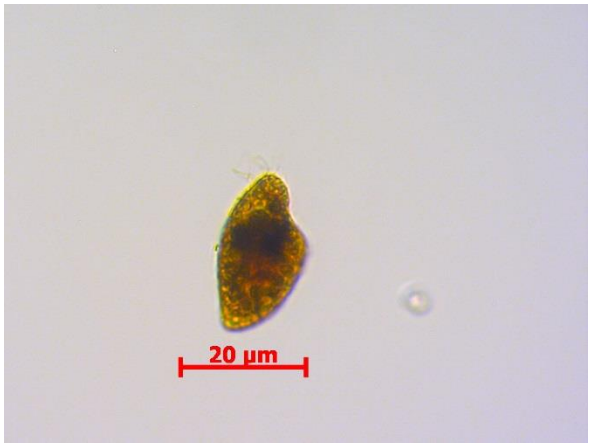

Figure 2: *Oxyrrhis marina* stained with Lugol's Iodine

O. marina was chosen for this experiment for two main reasons. The first is that it is easy to culture. It can survive in a wide range of salinities and temperatures, consumes a variety of prey items, and can be ordered from the National Center for Marine Algae and Microbiota (NCMA).

The second is that because it is a model organism, the results of this experiment may be extrapolated to estimate how other heterotrophic dinoflagellates and similar protists are likely to be affected by the presence of exopolymers.

Synechococcus

Synechococcus is a genus of marine cyanobacteria. The members of this genus are the most abundant members of the picoplankton (0.2-2µm) size group (Raven 1998; Trujillo & Thurman 2011). *Synechococcus* can occur at concentrations of up to 10^5 cells ml^{-1} in the open ocean and 10^6 cells ml^{-1} during blooms. It generates approximately one-fourth of global ocean primary production (Wang et al. 2011). Because of this, *Synechococcus* plays an important role in the oceanic carbon cycle. Information on factors that affect the flow of carbon fixed by *Synechococcus* will help aid understanding of a large portion of the marine carbon cycle.

Transparent exopolymer particles (TEP)

Transparent exopolymer particles (TEP) are acidic polysaccharides produced by marine microorganisms. Most TEP are formed from precursor compounds released by phytoplankton, but TEP may also form from mucus sloughing off cell surfaces or by the dissolution of adhesive compounds that hold colonial organisms together (Passow 2002). Because TEP are adhesive particles, they can hold small particles together forming aggregates. These aggregates may make it easier for heterotrophic microbes to consume small prey items such as *Synechococcus*. *Synechococcus* that are too small to be ingested efficiently or in large quantities alone may be more easily ingested when held together in aggregates by TEP (Wurl et al. 2011). As aggregates increase in mass, they sink more easily so aggregation by TEP may also increase the amount of

carbon in the surface ocean that sinks to the deep sea (Passow 2002). Carbon that is sequestered in the deep sea is effectively removed from the oceanic carbon cycle, potentially for hundreds of years (Rowe et al. 1991).

Information on the effect of TEP on consumption of prey by heterotrophic protists is important in order to increase understanding of the oceanic carbon cycle. If aggregation by TEP increases the amount of *Synechococcus* consumed by protists then this would lead to more carbon becoming available to higher trophic levels and lead to higher numbers of organisms in those levels. Decreased consumption would lead to more of the aggregates sinking from the surface to the deep sea. This is especially interesting due to the concern over climate change. Carbon that sinks to the deep sea may be sequestered there. Organic carbon that enters ocean floor sediments has an estimated residence time of 11 years in continental shelves and 756 years in deep sea plains (Rowe et al. 1991). Carbon sequestered in those areas is effectively removed from the carbon cycle for that period (Trujillo & Thurman 2011). If TEP aggregation deters protists such as *O. marina* from consuming *Synechococcus* then it could serve as a mechanism for decreasing the amount of carbon available to enter the atmosphere. The effect of TEP aggregation on grazing habits of other organisms would play a major role in determining what actually happens but only *O. marina* will be examined here.

Experimental design

In this experiment, I investigated the effects of TEP on *O. marina*'s consumption of *Synechococcus*. There is little published on how TEP affects grazing by heterotrophic microorganisms, and no information available on how TEP affects the consumption of

Synechococcus by *O. marina*. There is information on how exopolymer secretions affect *O. marina*'s consumption of *Aureoumbra lagunensis*. *A. lagunensis* is an alga that is responsible for brown tide in Texas (Liu and Buskey 2000). When the amount of exopolymer was increased in sample cultures containing *O. marina* and *A. lagunensis*, the number of *A. lagunensis* consumed by *O. marina* decreased. The researchers in this experiment offer two possible explanations for this result. The first is that exopolymers are distasteful to *O. marina* so it will be less likely to consume any exopolymer coated *A. lagunensis* encountered. The second is that the exopolymers inhibits *O. marina*'s feeding mechanisms and it may try to consume *A. lagunensis* coated with exopolymers but will not be able to do so (Liu and Buskey 2000).

It is possible that the presence of TEP would decrease *O. marina*'s consumption of *Synechococcus* by being distasteful or inhibiting *O. marina*'s feeding mechanisms. It is also possible that TEP would increase the number of *Synechococcus* ingested by *O. marina* by forming aggregates which *O. marina* may prefer over individual *Synechococcus*. In a feeding experiment with artificial particles, Roberts *et al.* (2011) showed that *O. marina* ingested 4 μm beads four times faster than 1 μm beads (about the size of *Synechococcus*). This preference for larger prey size may be enough to overcome the reasons suggested for the decreased consumption of *A. lagunensis* in the presence of exopolymers. This effect may not be seen in *O. marina*'s consumption of *A. lagunensis* because *A. lagunensis* is larger than *Synechococcus*. If there are only *Synechococcus* available for feeding, *O. marina* may preferentially feed on *Synechococcus* aggregates despite TEP distastefulness or adverse effects on feeding mechanisms.

I hypothesized that aggregation of *Synechococcus* due to TEP would increase the number of *Synechococcus* ingested by *O. marina* and that the preference *O. marina* has for larger prey would be enough to overcome any negative impact TEP has on its ingestion of *Synechococcus*. I tested this by culturing *O. marina* and *Synechococcus* then combining them in experimental cultures with and without TEP added. After feeding, samples were taken from the experimental cultures and stained with Alcian Blue for TEP and DAPI for nucleic acids to identify *Synechococcus* and *O. marina*. I took pictures from each slide using a Zeiss microscope and then analyzed them using ImageJ software.

CHAPTER II

METHODS

Culture preparation

Artificial seawater and growth medium

The medium used for culturing organisms for this experiment was artificial seawater with added f/2 nutrients from the National Center for Marine Algae and Microbiota (NCMA). This organization is run by the Bigelow Laboratory for Ocean Sciences. The components of f/2 medium and their concentrations are listed in Table 1.

Table 1: f/2 medium components (NCMA)

Component	Final concentration (μM)
$NaNO_3$	882
$Na_2SiO_3 \cdot 9H_2O$	106
$NaH_2PO_4 \cdot H_2O$	36.2
$FeCl_3 \cdot 6H_2O$	11.7
$Na_2EDTA \cdot 2H_2O$	11.7
$MnCl_2 \cdot 4H_2O$	0.910
Thiamine HCl (vitamin B_{12})	0.296
$Zn_2SO_4 \cdot 7H_2O$	0.0765
$CoCl_2 \cdot 6H_2O$	0.0420
$CuSO_4 \cdot 5H_2O$	0.0393
$Na_2MoO_4 \cdot 2H_2O$	0.0260
Biotin (vitamin H)	2.05×10^{-3}
Cyanocobalamin (vitamin B_{12})	3.69×10^{-4}

The artificial seawater for culturing was prepared according to Berges et al. (2001). The components for this mixture are listed in Table 2.

Table 2: artificial seawater components and their concentrations (Berges et al. 2001)

Compound	g/L in solution	Molar concentration in solution
NaCl	21.19	363 mM
$MgCl_2 \cdot 6H_2O$	9.592	41.2 mM
Na_2SO_4	3.55	25.0 mM
$CaCl_2 \cdot 2H_2O$	1.344	9.14 mM
KCl	0.599	8.04 mM
$NaHCO_4$	0.174	2.07 mM
KBr	0.0863	725 μM
H_3BO_3	0.0230	372 μM
$SrCl_2 \cdot 6H_2O$	0.0218	82 μM
NaF	0.0028	65.7 μM

Culture setup

Oxyrrhis marina (CCMP 1739) and *Dunaliella teriolecta* (CCMP 1320) were purchased from NCMA. *D. tertiolecta* is a green alga that was used to feed *O. marina* during initial culturing. Initial cultures were shipped in 15 mL plastic tubes. When they were received, cultures were prepared in the following way: 1) 40 mL of artificial seawater was filtered through a syringe-mounted 0.2 μm filter. Water volume was measured in a 100 mL graduated cylinder, poured into

125 mL Erlenmeyer flasks and then autoclaved; 2) f/2 medium nutrients were added to each flask; 3) into some flasks, 1 mL of *D. teriolecta* was added. Into others, 2 mL of *D. tertiolecta* and 2 mL *O. marina* were added; 4) flasks were placed in an incubator at 20 ºC. The incubator had a light-dark cycle of 14 hours of light then 8 hours dark. The light intensity was about 76.5 µmol photons m^{-2}.

After this initial culturing, cultures were prepared from previously made cultures. These cultures were prepared in the same way as the initial cultures except for the *O. marina* and *D. tertiolecta* cultures. In these sub-cultures, only 1 mL of each was added. *Synechococcus* (1379) cultures were already present in the lab and were subcultured in the same way. These cultures were stored in the same incubator as *O. marina* and *D. tertiolecta*. The cultures were placed behind a screen which lowered light intensity to about 6.9 µmol photons m^{-2}.

Cells were counted using a counting chamber slide and a method similar to that described by Schoen (1988). Each square on the slide had area 6.25×10^{-4} cm^2. The coverslip sat 0.02 cm above the counting surface so a volume of 1.25×10^{-5} mL was above each square. This meant 80,000 squares made up one milliliter. Distilled water was spread lightly on the raised edges of the slide, and a coverslip was placed on top so it covered both grids and was gently pressed down. After swirling culture flasks to suspend the cells, 1 mL was taken and combined in a small tube with one drop of Lugol's iodine. A Pasteur pipet was used to draw in stained cells. The tip of the pipet was placed at the junction between coverslip and slide and the stained cell suspension was slowly expelled from the pipet until the entire area under the coverslip was covered. For *Synechococcus*, cells were counted until 400 total had been counted. For *O. marina*, cell counts

were limited by time so less than 400 were counted. After counting, the number of squares which had been observed to count the cells was used to calculate cell concentration according to Equation 1.

$$cells\ ml^{-1} = \frac{cells\ counted}{number\ of\ squares} x\ 80{,}000\ squares\ mL^{-1} \qquad \text{(Equation 1)}$$

TEP production

For the preliminary experiment, xanthan gum was used as a substitute for TEP. Xanthan gum was chosen because it has been used in the past as a TEP analog for TEP assays (Passow 2002). A stock solution of 12 μg mL^{-1} xanthan gum was prepared in artificial seawater. This solution was stored in a refrigerator until the day of the experiment.

TEP for the main experiment was produced by the diatom *Thalassiosira weissflogii* (1051). A *T. weissflogii* culture in a 20 mL glass vial was filtered through a syringe-mounted 2.0 μm filter. The amount of collected TEP solution was measured using a graduated cylinder and divided equally among all cultures requiring TEP.

Experiments

Preliminary experiment

The procedure for both feeding experiments was adapted from Apple et al. (2001). Several days before the experiment, the cultures of *O. marina* and *D. tertiolecta* were placed in a dark room. This was done prevent *D. tertiolecta* from photosynthesizing and reproducing and remove it from the cultures. For this experiment, xanthan gum was used as a substitute for TEP. Four sets

of cultures were prepared: 1) *O. marina* + *Synechococcus*, 2) *O. marina* + *Synechococcus* + xanthan gum, 3) *Synechococcus* + xanthan gum, and 4) *Synechococcus* only.

On the day of the experiment, the concentrations of *O. marina* and *Synechococcus* were determined. The final concentrations in experimental cultures were 200 cells mL^{-1} *O. marina*, 10^5 cells mL^{-1} *Synechococcus*, and 1 μg mL^{-1} xanthan gum. This xanthan gum concentration was used because Passow (2002) lists it as the common maximum of TEP concentration during plankton blooms. The components were mixed together according to Table 3. The total volume of all components was 48.215 mL

Table 3: Volumes of components for experimental flasks

	1	2	3	4
O. marina	0.355 mL	0.355 mL	NA	NA
Synechococcus	3.93 mL	3.93 mL	3.93 mL	3.93 mL
Xanthan Gum	NA	3.93 mL	3.93 mL	NA
Water	43.93 mL	NA	40.355 mL	44.285 mL

After preparation, flasks were placed in an incubator at 20 ºC for 1 hour. The feeding period was chosen to allow time for *O. marina* to acclimate and still have enough time to feed. After this time, the entire contents of each flask was poured into a 50 mL centrifuge tube and mixed with formalin for preservation. The final concentration of formalin in each tube was 2%. This preserved the cells for staining with 4',6-diamidino-2-phenylindole (DAPI), a fluorescent nucleic acid dye (Porter & Feig 1980). Preserved cultures were stored in a refrigerator until filtering.

Each sample was stained with DAPI and then filtered to prepare a slide. Images were captured using the microscope and analyzed using ImageJ. The procedures for slide preparation and analysis are described below.

Main experiment

The main experiment was performed with cultures in 6 mL wells in a 12-well plate. To determine the *O. marina* concentration and feeding time that would give the clearest results, a series of wells were prepared with 6 mL artificial seawater and f/2 nutrients. Cultures were setup with *O. marina* concentrations of 200, and 600 cells mL^{-1}. *Synechococcus* was added to each culture at 10^5 cells mL. At 30 minutes and 1 hour, 1 mL was removed from each culture and was added to a microcentrifuge tube containing 20 µL formalin. These samples were then stained with DAPI and filtered to make slides for analysis.

TEP for the main experiment was obtained from a culture tube of *T. weisflogii* filtered through a syringe. For this experiment, the *O. marina* concentration was 600 cells mL^{-1} and the *Synechococcus* concentration remained 10^5 cells ml^{-1}. The same four sets of cultures were prepared and two 1mL samples were taken from each well at 30 minutes and one hour. The thirty minute sample was added to this experiment because I thought that the *Synechococcus* in each *O. marina* would be easier to see as individuals. The one hour sample was kept the same as in the previous experiment to allow time for acclimation and feeding. The samples were added to 20 µL formalin in 1.5 mL microcentrifuge tubes. Filtering and staining was performed as described below.

DAPI staining, filtering, and analysis

After the preliminary experiment, a 1 mL sample was taken from each preserved culture and added to an autoclaved 1.5 mL microfuge tube. 40 µL DAPI was added to each tube. The tubes were inverted five times to ensure mixing and then placed in the fridge for one hour. The protocol for staining main experiment samples was the same except 1 mL samples had been preserved rather than the entire culture.

After DAPI staining, 0.5 mL of each sample was diluted with 1.5 mL artificial seawater and filtered through a 0.4 µm membrane. To avoid contamination with precipitates and bacteria, the artificial seawater was filtered through a 0.2 µm filter before being used in sample filtering. After filtering, the membrane was washed by filtering through 2 mL artificial seawater. Membranes were mounted on white background cytoclear slides using immersion oil. A drop of oil was placed on the slide and the membrane was placed on top of the drop. Another drop of oil was placed in the center of a glass coverslip and placed on the membrane so that the drop of oil was over the center of the membrane. Slides were stored in the freezer when not in use.

The filtering for the preliminary and main experiments was done in the same way except for double staining. In the preliminary experiment, slides were stained with DAPI and Alcian Blue. In the main experiment, there was no double staining.

Each slide was examined using a Zeiss microscope with attached cameras. Images were captured using AxioVision software. An image was taken of each *O. marina* found on the slide using a fluorescent DAPI filter. This filter causes DAPI to fluoresce bright blue. Each image was 445

μm tall by 334 μm wide. In the main experiment, additional images were taken using a Sytox 3.5 fluorescent filter. This caused *Synechococcus* to fluoresce red. This is likely due to the pigment phycoerythrin in *Synechococcus*. Phycoerythrin fluoresces red when exposed to fluorescent light (Uysal 2001). *Synechococcus* that had been consumed did not appear to fluoresce so this filter could be used to indicate the boundary of the *O. marina* being examined. Cells were counted using ImageJ software from the National Institutes of Health. *Synechococcus* cells were counted by clicking on each individual cell. The number of *Synechococcus* consumed by each *O. marina* was recorded in a Microsoft Excel workbook. A t-test was performed in Excel to determine whether or not the difference in *Synechococcus* consumed with and without added TEP was significant.

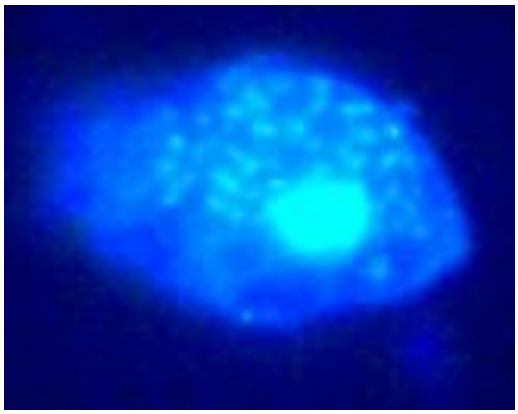

Figure 3: DAPI stained *Oxyrrhis marina* and *Synechococcus*. The large fluorescent area is the nucleus of *O. marina*. Each smaller fluorescent particle in an individual *Synechococcus* cell. The outline of the *O. marina* cell can be seen enclosing most of the fluorescent areas in the images.

TEP filtering, staining, and analysis

TEP was stained on the same slides as DAPI staining for the preliminary experiment and separately for the main experiment. Half a milliliter was taken from the preserved culture

samples and combined with 1.5 mL of artificial seawater then filtered through a 0.4 μm membrane. The TEP collected on the membrane was stained with 1 mL of 0.02% Alcian Blue. On slides that were stained with DAPI and Alcian Blue, 2 mL of artificial seawater was used to wash the membranes after filtering. On slides with only Alcian Blue staining, 2 mL of UHP water were used to wash the membranes. Slides were mounted on cytoclear slides in the same manner as DAPI.

Ten images of TEP (Figure 4) were taken from each slide at 400 x magnification. The images were 445 μm tall by 334 μm wide. The field of view for each image was selected randomly. Images were analyzed using ImageJ software from the National Institutes of Health. To determine the TEP concentration, an image was opened and the ImageJ pencil tool was used to eliminate any non-blue particles. The image's color channels were split, the green and blue channels closed, and the red channel kept open. This channel was converted to black and white by setting a threshold based on blue intensity. At this point, the area above the threshold was black and everything else white. Black areas above 10 pixels (4.12 μm^2) were defined as TEP particles and counted. This minimum size was used to decrease the effect of background noise (Thornton 2012). The area of TEP for each image was recorded in Excel. A T-test was used to determine if the amount of TEP at thirty minutes and one hour was significantly different.

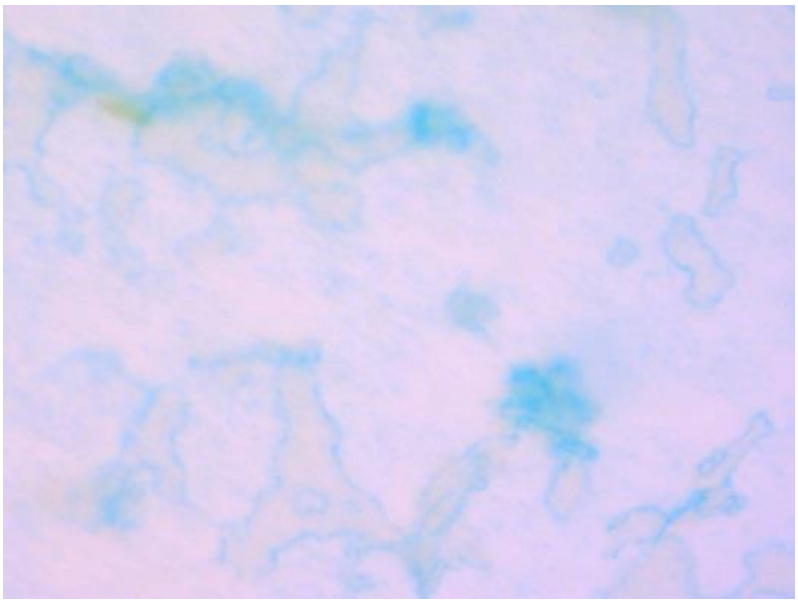

Figure 4: Alcian Blue stained TEP. This image is of an area 445 µm tall by 334 µm wide.

CHAPTER III

RESULTS

The results from the preliminary experiment are summarized in Table 1. Only slides from culture sets 1 (*Oxyrrhis Marina* + *Synechococcus*) and 2 (*O. marina* + *Synechococcus* + xanthan gum) were analyzed. Slides from the *Synechococcus* alone and *Synechococcus* and xanthan gum cultures were not analyzed because they did not appear to have any useful information. During preservation *Synechococcus* released TEP so these cultures would not have been able to provide information on how TEP affected *Synechococcus* aggregation. The difference in the average number of *Synechococcus* consumed by *O. marina* in each culture set was 9.77. The p-value for a T-test of the results was 0.00265 which indicated a significant difference between cultures.

Table 1: Results of the preliminary experiment for average consumption of *Synechococcus* by *O. marina* in cultures with and without xanthan gum added as a TEP substitute

Culture	Number of *O. marina* analyzed	Average number of *Synechococcus* consumed per *O. marina*
O. marina + *Synechococcus*	57	39.25
O. marina + *Synechococcus* + xanthan gum	81	49.02

The results for the main experiment are summarized in Table 2. The difference in the average number of *Synechococcus* consumed by *O. marina* in each culture set was 7.87. A T-test on the collected data gave a P-value of 0.00383 which indicates that the difference between the two sets was significant. The combined results of both experiments are shown in Figure 5.

Table 2: Results of the main experiment for average consumption of *Synechococcus* by *O. marina* in cultures with and without TEP added

Culture	Number of *O. marina* analyzed	*Synechococcus* consumed per *O. marina*
O. marina + *Synechococcus*	94	49.15
O. marina + *Synechococcus* + TEP	48	57.02

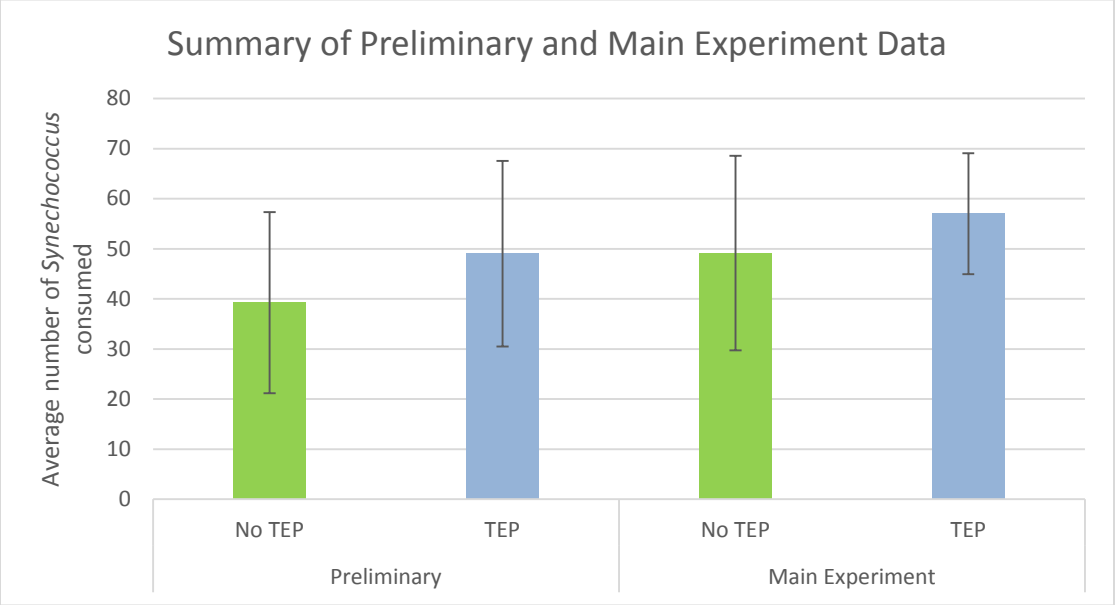

Figure 5: Graph of combined data on *Synechococcus* consumption by *O. marina* from the preliminary and main experiment with and without TEP added. Error bars represent ± one standard deviation

TEP area was determined for the main experiment only. The average area of TEP at 30 minutes was 12,391 μm^2. At 1 hour it was 10,877 μm^2. This difference was not significant. Because of TEP leakage in both experiments, the difference in *Synechococcus* aggregation between cultures with and without TEP added could not be determined. The data was interpreted as presence versus absence of TEP instead.

In the main experiment, some data concerning the rate of *Synechococcus* consumption by *O. marina* was gathered. The average number of *Synechococcus* consumed by each *O. marina* at 30 minutes was 86.58 cells h^{-1} in TEP added cultures and 82.28 cells h^{-1} in cultures with no added TEP. After one hour, the averages were 57.02 cells h^{-1} and 49.15 cells h^{-1} respectively. These results are summarized in Table 3.

Table 3: The average rate of *Synechococcus* consumption by *O. marina* at 30 minutes and one hour in cultures with and without TEP added

Culture	Rate of *Synechococcus* consumption by *O. marina* at 30 minutes (cells h^{-1})	Rate of *Synechococcus* consumption by *O. marina* at 1 hour (cells h^{-1})
O. marina + *Synechococcus*	82.28	49.15
O. marina + *Synechococcus* + TEP	85.58	57.02

CHAPTER IV

DISCUSSION

Sources of error in analysis

Synechococcus aggregation differences were not analyzed in this experiment. Before each experiment, clumps of *Synechococcus* were broken up by swirling cultures and passing cells through a syringe. During preservation, TEP was released by *Synechococcus* so *Synechococcus* aggregates formed in all cultures. This meant that there was no apparent difference in aggregation between cultures with and without TEP added. This is why I do not have data on *Synechococcus* aggregation differences and why a reason for the increase in *O. marina*'s *Synechococcus* consumption cannot be determined.

Another problem that I encountered was getting an accurate count of *Synechococcus* in each *O. marina*. In many of the pictures I took, the outlines of *Synechococcus* inside *O. marina* were fuzzy. I think that I was able to determine where each individual *Synechococcus* ended but I am not convinced that I got an accurate count. I think that this problem was mostly caused by the formalin added to each sample. Partial digestion of *Synechococcus* may also have caused this.

Consumption of TEP by *Oxyrrhis marina*

There was no significant difference between average TEP area in the images taken at 30 minutes and 1 hour. This result suggests that *Oxyrrhis marina* does not consume TEP by itself. There was a difference in TEP concentration at 30 minutes and 1 hour but it was not a significant difference. The results support previous work by Liu & Buskey (2000) who suggested that

exopolymers, like TEP, were not a food source for *O. marina* and prevented the dinoflagellate from feeding on *Aureoumbra lagunensis*. Liu & Buskey (2000) hypothesized that exopolymers are either distasteful or inhibit the feeding mechanisms or *O. marina*.

TEP and the consumption of *Synechococcus* by *Oxyrrhis marina*

The average number of *Synechococcus* consumed by individual *O. marina* in cultures with TEP added was significantly higher than in cultures without TEP. This result was found in both experiments. A positive connection between *Synechococcus* consumption and TEP presence is present but the cause of this connection is uncertain but the cause is uncertain. I do not have any data on *Synechococcus* aggregation differences between cultures without TEP and cultures with TEP added so it cannot be concluded that *Synechococcus* aggregation by TEP was the cause of this. If increased *Synechococcus* aggregation is the cause of the increase, it is likely due to *Synechococcus*' size. *Synechococcus* is grouped by size with picophytoplankton which is a group made up of organisms that are smaller than 2 μm in diameter (Raven, 1998). Burkill et al. (1993) examined *Synechococcus* from the Indian Ocean and found that cell diameter was 0.7-1.2 μm. *O. marina* preferentially feeds on particles larger than this. Roberts *et al.* (2011) showed that *O. marina* ingested 4 μm beads four times faster than 1 μm beads. When fed live prey, *O. marina* feeds best on flagellates greater than 4 μm in diameter (Roberts et al., 2011). Individual *Synechococcus* cells are too small to be preferential prey items for *O. marina* but their aggregation due to TEP would create particles in this size range. Because of this, aggregation should make *Synechococcus* more attractive to *O. marina* by increasing food particle size. The results of this experiment support this and suggest that the positive effect of aggregation is enough to counteract any negative effect of TEP unpleasantness. This aggregation would help

extend the range of prey items for *O. marina* by joining individual cells that are too small to be preferentially consumed alone into aggregates that are of preferred size.

This experiment also provided some data on the rate at which *O. marina* consumes *Synechococcus*. In the main experiment, the rate of *Synechococcus* consumption by *O. marina* was higher at 30 minutes than it was at 1 hour. Assuming that each *O. marina* started with no ingested *Synechococcus* then this shows that ingestion slows down after the first 30 minutes. The fact that TEP sub-cultures had less difference between 30 minutes and 1 hour may also be due to aggregation keeping more cells in an area and making them easier to find.

Because *O. marina* is a potential model organism for heterotrophic dinoflagellates and other protists (Roberts et al. 2011), the results from this experiment can be used to infer how other organisms will respond to *Synechococcus* aggregation of TEP. This experiment suggests that other similar sized protists will also increase *Synechococcus* consumption when TEP is present. This would increase the amount of energy available to higher order consumers that cannot consume *Synechococcus* directly. This would also help with nutrient recycling. Small organisms are able to use nutrients that are free in the water. Larger organisms cannot do this so, without the small organisms, these nutrients would be lost from the food webs. Increased consumption of organisms like bacteria that can recycle nutrients would increase the amount of nutrients that get added back into the food web (Azam 1993).

Microscopic organisms play an important role in carbon cycling in the ocean. This experiment investigated the effect that TEP, an adhesive exopolymer that forms aggregates of small

particles, has on the consumption of *Synechococcus* by the dinoflagellate *Oxyrrhis marina*. The results showed that the presence of TEP increases the rate of *Synechococcus* consumption by *O. marina*. This shows that TEP may increase the flow of organic matter to higher trophic levels and increase the rate of nutrient recycling into these higher levels.

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