



Examining the possibility of phagotrophy in the polar alga, *Micromonas* sp.

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ABSTRACT

Micromonas CCMP2099 is the most common picophytoplankton in the Arctic waters and one of the main contributors to CO₂ fixation in the ocean, which is very important for the carbon cycle. There are some scientists who suggest that *Micromonas* CCMP2099 can do phototrophy but also phagotrophy when there are bad conditions for them to obtain energy through photosynthesis. Mixotrophy may change the way that the carbon cycle works as well as the climate change. To further understand if *Micromonas* CCMP2099 is mixotroph and how that would affect to the carbon cycle, we are studying the ingestion of particles called “beads” in stressful conditions by *Micromonas* with flow cytometry.

INTRODUCTION

Phytoplankton are algae that obtain energy through photosynthesis.

Phytoplankton are the primary contributors to CO₂ fixation in the ocean and important globally fixing 50% of total CO₂ on Earth (Field et al, 1998). It has been observed that some phytoplankton are not only photosynthetic but are also mixotrophic, the ability to combine phototrophy and heterotrophy within the same individual (Flynn et al, 2012). This

means that they do photosynthesis but they can also ingest other cells. Recent studies have revealed how important mixotrophic phytoplankton are in low nutrient oligotrophic oceanic waters, as well as shown the additional importance of mixotrophy in the survival of the smallest protists in those waters (Hartmann et al, 2012). Limiting nutrients concentrations and darkness are believed to be conditions that favor mixotrophy because without light or nutrients they can't do photosynthesis so they would need to ingest other organisms to get those nutrients and the carbon that they need. For example, in the mixotrophic *Prymnesium parvum*, the contribution of nutrients from their prey increases when growing under P or N deficiency (Carvalho and Granéli, 2010). Another experiment with *Ochromonas sp.* revealed that in this strain, the phagotrophy increases under conditions of limiting light and nutrients. This also occurs with many of the freshwater algae in the genera *Dinobryon* and *Poterioochromonas* for example (Keller et al, 1994).

Mixotrophy can affect the way that the carbon cycle works in the ocean and helps to explain the carbon budgets at the community level (Hartmann et al, 2012). Not considering mixotrophy will make predictions of the future climate change less accurate (Mitra et al, 2014).

Micromonas sp CCMP2099 is a polar strain of *Micromonas*. They are considered to be picophytoplankton because of their size between 0.2-2 μm . For the rest of this paper I will be referring to this strain simply as *Micromonas*.

Micromonas is the most common picoeukaryote in Arctic waters (Lovejoy et al. 2007) where there's 24 hours of darkness in winter months. This makes the Arctic waters potentially a place where we would find mixotrophs because, as we introduced before, prolonged darkness may favor mixotrophy (Sanders and Gast, 2012). In the changing Arctic Ocean, the smallest phytoplankton would have more possibilities to thrive because of a variety of reasons, including reduced the upwelling of nutrients. In this case, the impact of these picoeukaryotes may be even more important if global climate change the Arctic Ocean (Li et al, 2009).

There are some scientists who suggest that *Micromonas* is a mixotroph based on feeding experiments examining how light and nutrients affect phagotrophic activity in this strain by

identifying ingested particles using microscopy (McKie-Krisberg and Sanders, 2014).

Our experiment will extend this idea with an experiment where we study the ingestion of beads by *Micromonas* with flow cytometry. The hypothesis is that the cultures of *Micromonas* grown in deplete nutrient media and darkness would be more likely to ingest particles than those cultures grown under replete nutrients and light.

MATERIAL AND METHODS

CULTURE ORIGIN

Our cultures of *Micromonas* were maintained at 6°C in L1ASW low salinity media made with artificial sea water (ASW). The cultures were kept at an irradiance level of $\sim 68 \mu\text{Em}^{-2}\text{s}^{-1}$. These lights were on at 9:00 and off at 18:00 and the unit didn't follow daylight saving time.

MICROSCOPING IMAGING OF *MICROMONAS*

For epifluorescent micrographs, samples of *Micromonas* were fixed with glutaraldehyde (final concentration of 1%) concentrated on 0.8-mm pore black Poretics PC filters and stained with 40,6-diamidino-2-phenylindole (DAPI). Filters were mounted on slides and examined under an epifluorescent microscope to ensure that there weren't any bacteria in our cultures.

EXPERIMENT

Our experiment had three treatments with three replicates each making a total of 9 flasks. The starting concentrations of *Micromonas* were between 1.6-2.2 million cells/mL for each flask, which were maintained in exponential growth prior to start of the experiment.

The treatments were:

- Control: cultures were grown in replete nutrient media with light/dark cycle.

- Deplete nutrients: cultures were grown in deplete nutrient media (with 20% of nutrients) with light/dark cycle.
- Continuous darkness: cultures were grown in replete nutrient media but the flasks were wrapped with aluminum foil to exclude the light.

At the beginning of the experiment (T₀), we added 2 mL of 0.5 μ m Fluoresbrite YG Carboxylate Microspheres (Polysciences) (beads) with a concentration of 1.7×10^8 beads/mL to each flask.

In a experiment totaling 5 days, samples were taken at 3 hour intervals for the first, then sampled once daily for the reminder of the experiment. We ran these samples on a BD Accuri C6 flow cytometer (BD Biosciences) to measure the abundance of *Micromonas* for each flask. The populations of *Micromonas* were determined based on clustering seen in cytograms with red fluorescence and forward scatter parameters (Figure 1). The cells of *Micromonas* that may have ingested the beads were determined in the same way but using cytograms with different parameters (red-APC fluorescence and green fluorescence) (Figure 2).

With these measurements abundance, we found daily growth rates (Equation 1):

$$\text{Growth rate} = \frac{\ln \left(\frac{p_2}{p_1} \right)}{t_2 - t_1}$$

P₁: concentration day 2

P₂: concentration day 1

T₁: day 1

T₂: day 2

RESULTS

CONCENTRATIONS

The concentrations of *Micromonas* cells from the control treatment ranged from 2.17 million cells/mL to 10.96 million cells/mL, which is very similar to the concentration of the

cultures from the deplete nutrient treatment, that ranged from 1.92 million cells/mL to 9.20 millions cells/mL over the entire time of the experiment. However, the concentration of the cultures grown in the continuous darkness treatment ranged from 1.85 millions cells/mL to 1.88 millions cells/mL which is approximately the same value throughout the experiment (Figure 3).

GROWTH RATES

We calculated the growth rates (Equation 1) for each treatment. For the cultures grown under control conditions, the average growth rate over the entire experiment is 0.33 (Figure 4). This value is very similar to the growth rate for the cultures grown under deplete nutrients conditions, which is 0.31 (Figure 4). However, the cultures grown under continuous darkness has a growth rate of 0.0015 (Figure 4).

MICROMONAS INGESTING THE BEADS

Micromonas ingested particles under all the experimental conditions with a different percent of total cells that may have ingested beads on each one.

The average percent of *Micromonas* “containing beads” for the cultures grown under control condition is 0.48% and for the cultures grown under deplete nutrients conditions is 0.40% (Figure 5). The highest phagotrophy activity was observed in the cultures grown under continuous darkness conditions with 0-64% of total cells that may have ingested beads (Figure 5).

DISCUSSION

Our cultures of *Micromonas* from the control and deplete nutrients conditions, were growing with a very similar ranged of cells/mL (Figure 3).

Under replete nutrients conditions and darkness, the cultures of *Micromonas* weren't growing at all but they weren't dying either, just maintained in a stationary phase. During their life cycle, *Micromonas* start with a lag phase; they grow in the exponential phase and then they get into the stationary phase before dying. When *Micromonas* doesn't have light to do photosynthesis they can't grow, but it doesn't necessary means that they will die immediately, which will explain why after 5 days of experiment, our cultures from the continuous darkness treatment, are still alive (Figure 3). If we continued with this experiment, the cells of *Micromonas* may die.

Response of Micromonas under different treatments

The cultures grown with deplete nutrients media and light have a growth rate very similar to the one that the cultures grown with replete nutrients media and light have, between 0.3-0.33 (Figure 4). The deplete nutrient media still have 20% of nutrients, which means that maybe this percent of nutrients is enough for the culture to grow in the same way as the cultures growing with replete nutrients and light does. It could also be that the 5 days of the experiment is not enough time for deplete nutrient *Micromonas* to get out of the exponential phase or to stop growing.

However, when *Micromonas* is grown under continuous darkness, the value of the growth rate decrease to 0.0015 because, as we said before, without any light they can't do photosynthesis so they can't grow at all (Figure 4).

Phagotrophy activity

The percent of cells that may have ingested the beads is very low for all the treatments, nearly 1% of total cells of *Micromonas* (Figure 5).

The cultures from the continuous darkness conditions have a higher percent of cells with "ingested beads" than the rest of the treatments. When *Micromonas* is in the dark, they need to ingest particles or organisms to get the nutrients and carbon that they can't make without light, which will explain why the percent is higher. There's also some percent of

cells from the deplete nutrient condition that may have ingested beads. This would confirm our hypothesis from the beginning.

We found that the culture from the control condition also has some percent of *Micromonas* that may have ingested beads (Figure 5). If the control condition has replete nutrients media and light, why are they ingesting beads? It could be that *Micromonas* it's not actually ingesting the beads and they perhaps release some exudate that make the beads stick to the surface of the cells and change the fluorescence (appearing the same on the flow cytometer as cells that would have actually ingested beads). This release of exudates could also potentially increase when the cultures are in the dark (i.e. stressful conditions) and this could explain why the percent of cells "containing beads" is higher for the continuous darkness treatment.

CONCLUSION

After this experiment, we realized that we can't either confirm or deny that *Micromonas* is mixotroph because we don't have enough information for that. Right now, we even have more questions than at the beginning of the experiment, but no answers of why they can survive in a place where there are prolonged periods of darkness.

For the future, we should extend the length of experiment, use more dilute nutrient deplete media (less percent of nutrients for the media), use bacteria instead of beads so we will have a more realistic prey for our cultures and expand methods to examine the cultures (e.g microscopy and flow cytometry sorting).

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Figures:

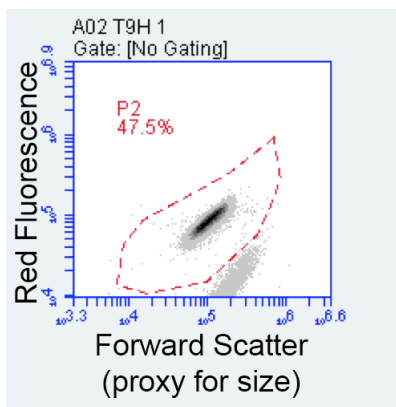


Figure 1. Representation of a culture of *Micromonas* using the flow cytometry.

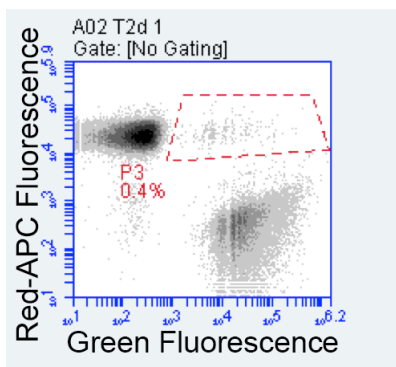


Figure 2. Representation of a culture of *Micromonas* with the red fluorescence, the beads with green fluorescence and the cells of *Micromonas* that may have ingested the beads inside the gated with the same fluorescence and size as *Micromonas* and the same fluorescence as the beads.

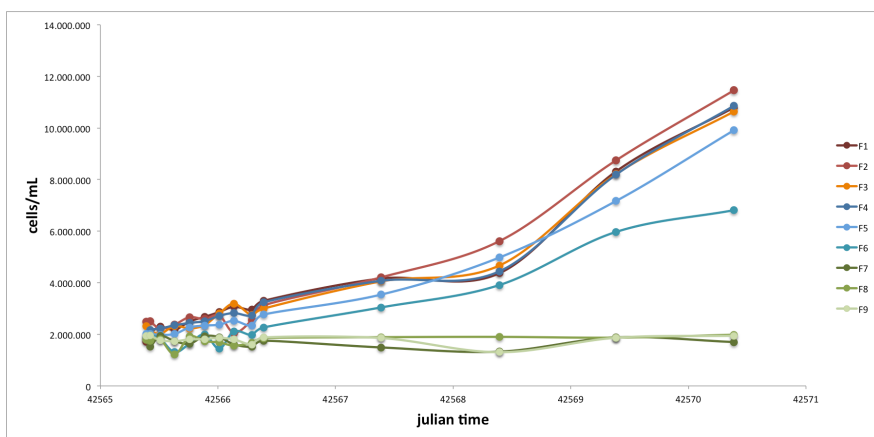


Figure 3. Concentrations of cells over Julian time from the 9 different flaks.

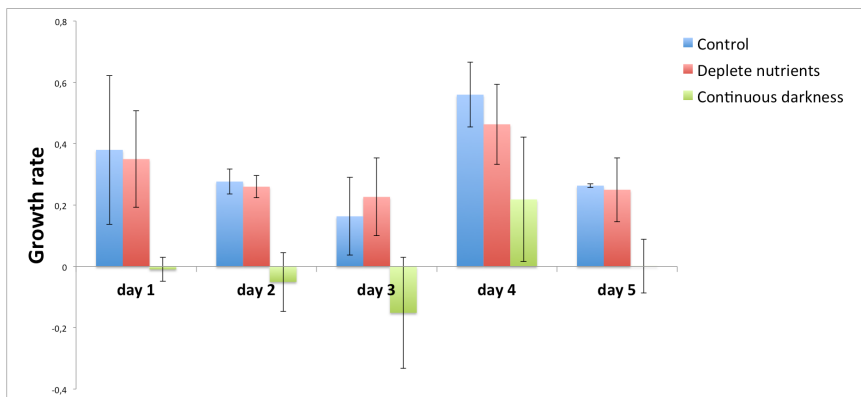


Figure 4. Growth rate over days of experiment from the 3 different treatments where our cultures of *Micromonas* were growing.

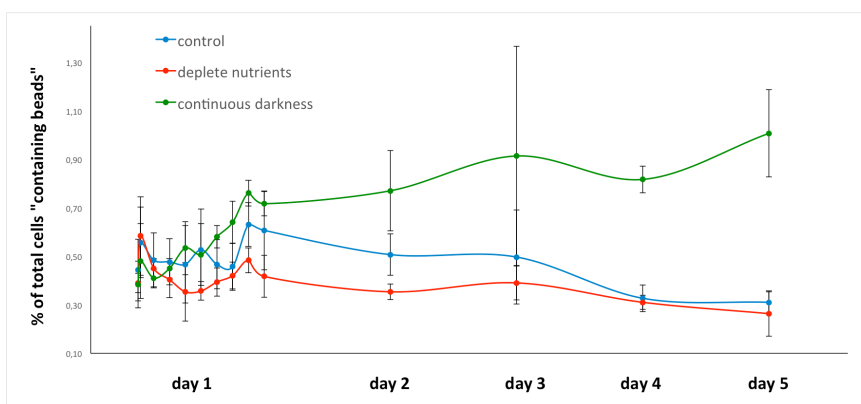


Figure 5. Percent of total cells that may have ingested beads over day of experiment from the 3 different treatments.