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The impact of nutrient depletion on the effect of HHQ on *Emiliana huxleyi*

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The impact of nutrient depletion on the effect of HHQ on *Emiliana huxleyi*

Introduction

Phytoplankton are both drivers of marine biogeochemical cycles (Simó, 2001) and the basis of the marine food web (Legendre & Rassoulzadegan, 1995). Phytoplankton population dynamics are mediated by interactions with bacteria and bacterial compounds (Azam & Malfatti, 2007), and these interactions are often species-specific (Harvey et al., 2016). An example of a species-specific interaction is that between bacteria in the *Pseudoalteromonas* genus and the marine phytoplankton *Emiliana huxleyi*.

Emiliana huxleyi is a major bloom forming coccolithophore that impacts carbon cycling (Balch et al., 1992). *Pseudoalteromonas* spp. have been found to produce algicidal effects in a variety of types of phytoplankton (Mitsutani et al., 2001; Skerratt et al., 2002), due to the production of 4-heptyl-2-quinolone (HHQ) (Harvey et al., 2016), a quorum sensing precursor molecule (Whalen et al., 2019).

HHQ arrests cell division in *E. huxleyi*, slowing growth while simultaneously protecting it from viral mortality (Pollara et al., 2021), affecting bloom dynamics and nutrient cycling (Harvey et al., 2016).

Previous investigations into the impact of HHQ on *Pseudoalteromonas* have all been conducted with *E. huxleyi* being grown under ideal conditions, in order to isolate effects by reducing all other forms of stress. However, this is not indicative of the marine environment. Environmental stressors, such as low nutrient conditions, could have implications for relationships between bacteria and phytoplankton. This study aimed to explore the impact that nutrient depletions have on the effect of HHQ on *E. huxleyi*.

Methods

The strain of *E. huxleyi* used in all experiments (CCMP 2090) was cultured in 0.2 μm filtered artificial seawater (ASW) amended with F/2 nutrients minus silica. Cultures were reared in an incubator set to 18°C, 80-100 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ light intensity, and a 12:12 light:dark cycle. *E. huxleyi* cultures were not axenic, but care was used to not introduce additional bacteria to the cultures. All *E. huxleyi* cultures were transferred to fresh media weekly.

ASW F/2	Modification
<i>Unmodified</i>	replete nutrients
<i>Deplete Fe</i>	5 nM Fe
<i>Deplete Zn</i>	4 pM Zn
<i>Deplete Co</i>	1 pM Co
<i>Deplete P</i>	5 μM P
<i>Deplete N</i>	25 μM N

Table 1: Versions of ASW used in experiment with relevant modification

Media was created under six experimental conditions: first, an unmodified control media was created (“replete”), adding nutrients in the same ratio as found in F/2. Second, five experimental medias were made. In each, an essential nutrient (iron, cobalt, zinc, nitrogen, or phosphorus) was added at a concentration that previous research had shown to reduce growth rates by 50%, and other nutrients remained at original levels.

One low nutrient media was tested at a time, for a total of six experiments. Experiments were completed in 19 mL glass tubes. At the start of the experiment, concentrations of the control and experimental cultures were measured once they had reached exponential growth stage, and cultures were appropriately diluted with their respective medias to a starting concentration of $\sim 6 \times 10^4$ cells mL⁻¹, in a total of 10 mL. Replete and deplete cultures were then exposed to either a control (dimethyl sulfoxide [DMSO]) or 1 of 7 concentrations of HHQ (final concentration in the experiment: 1, 10, 50, 100, 250, 500 ng mL⁻¹ and 1 μ g mL⁻¹) in triplicate, for a total of 48 tubes. Immediately after dosing (t = 0 hours), 200 μ L samples of each treatment were loaded onto a 96-well plate and run on a Guava EasyCyte HT flow cytometer. Prior experiments were used to find the correct settings to enumerate the concentration, mean forward scatter (size proxy), and mean side scatter (calcification proxy) for *E. huxleyi* populations. The experiment was sampled daily for four days (t = 24 – 96 days). This experimental design was repeated for each deplete nutrient media.

Data analysis

Percent survival for each HHQ treatment at t = 96 hours was calculated in Excel by dividing, for each replicate, the concentration of the HHQ dosed sample by the concentration of the control sample, and multiplying by 100. Using this method, the percent survival of each control replicate was 100%.

Percent survival data and raw mean forward/side scatter data was loading in to GraphPad Prism 9. Replicates were averaged in this program, and 15 graphs were produced, comparing the replete and deplete trends of these criteria for each nutrient. Using Prism, t-tests at each HHQ concentration were used to determine significance between nutrient replete and nutrient deplete percent survival, forward scatter, and side scatter. The p-value for all tests was set at 0.05.

Results

Percent survival

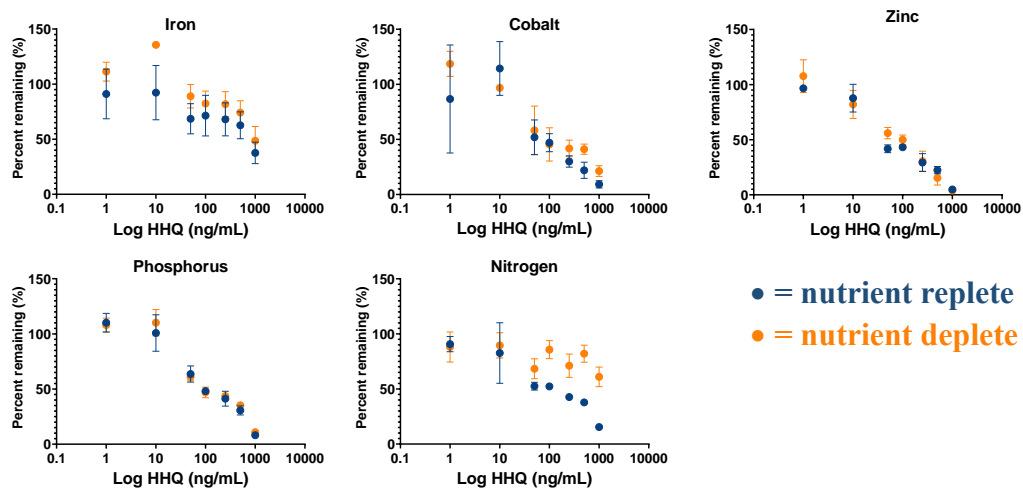


Figure 1 Graphs showing percent survival of cultures in replete and deplete nutrients for a) Fe, b) Co, c) Zn, d) P, and e) N.

In all treatments percent survival decrease with increased HHQ dose. There were limited, significant differences in percent survival between replete and deplete conditions for Fe, Co, Zn, and P. However, there was a significant increase in percent survival of cells under deplete N conditions compared to replete nitrogen ($p < 0.05$), starting only at HHQ concentrations higher than 10 ng mL⁻¹.

Cell size

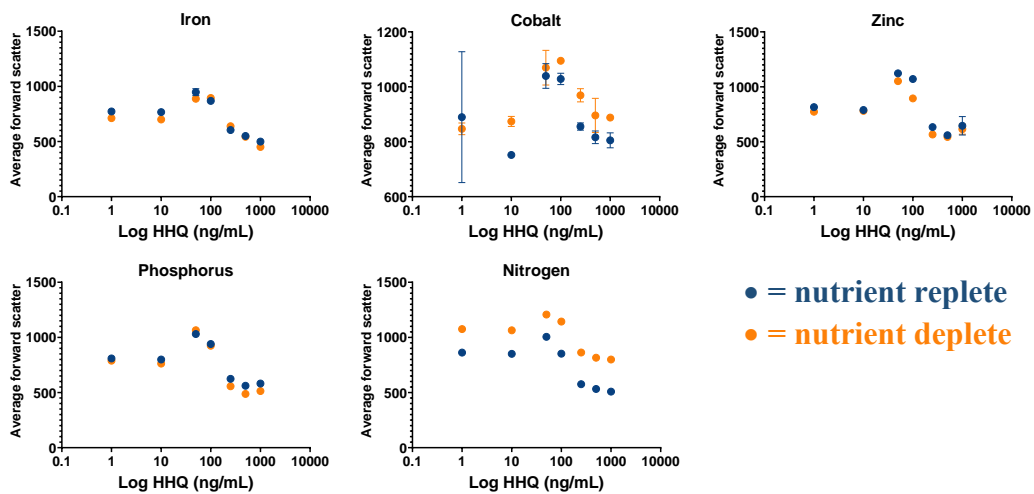


Figure 2 Graphs showing average forward scatter of cultures in replete and deplete nutrients for a) Fe, b) Co, c) Zn, d) P, and e) N.

There was a consistent peak in mean forward scatter at 100 μM HHQ for all treatments, suggesting larger cell size at this concentration. Interestingly, mean forward scatter decreased in all treatments, in response to increasing HHQ concentrations. There was no significant difference in cell size between deplete and replete conditions in the iron, zinc, and phosphorus treatments. Deplete cobalt cells were larger than replete cells, but was only significant ($p < 0.05$) at 250 ng mL^{-1} and 1 $\mu\text{g mL}^{-1}$ HHQ. However, cells in deplete nitrogen conditions had a significantly higher mean forward scatter ($p < 0.05$) suggesting that the cells in deplete nitrogen were larger than those in replete nitrogen for all HHQ concentrations tested.

Calcification

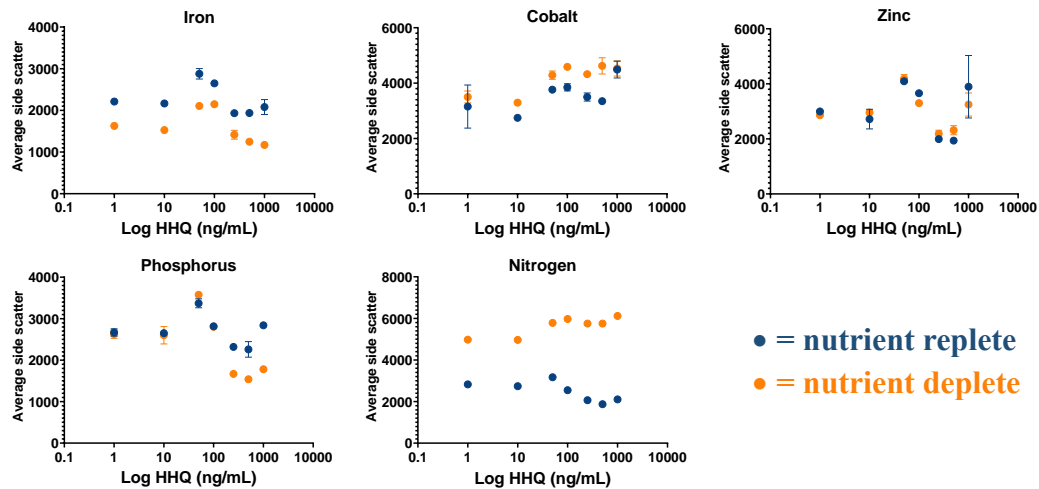


Figure 3 Graphs showing average side scatter of cultures in replete and deplete nutrients for a) Fe, b) Co, c) Zn, d) P, and e) N.

Changes in calcification data varied under different nutrient conditions. There was a general increase in calcification up to $\sim 100 \mu\text{g mL}^{-1}$ HHQ followed by a decrease at higher concentrations of HHQ, seen in Fe, Co, Zn, and P. There was not a significant difference in calcification in replete and deplete Zn. In the P treatment, cells under nutrient deplete conditions had a significantly ($p < 0.05$) lower average side scatter relative to P replete conditions, only at the highest three HHQ concentrations, suggesting these cells are less calcified. Similarly, cells in replete iron were had significantly ($p < 0.05$) lower mean side scatter under deplete conditions. This was observed across all HHQ concentrations tested. Conversely, cells in deplete N had significantly ($p < 0.05$) higher average side scatter under nutrient deplete conditions suggesting that these cells are more highly calcified relative to nutrient replete conditions. This difference was observed across all HHQ concentrations tested.

Discussion

Nitrogen protection

Of the five nutrients analyzed, nitrogen depletion had the greatest effect on percent survival, cell size, and calcification, with significant differences from a replete control in all three categories. Though cell concentration was lower in N deplete culture due to difficulty of rearing cells in poor conditions, there was a higher percent survival of N deplete cells than N replete cells (see Figure 1.c). It can thus be inferred that nitrogen protects *E. huxleyi* from high concentrations of HHQ. While the exact mechanism of this protection is unknown, a possible explanation is that deplete nitrogen shuts down the cell cycle, putting the cell in stasis before HHQ can affect the cell. Since HHQ also affects the cell cycle, if low N conditions shut it down before HHQ can, its effects would be lessened. The arresting of the cell cycle by lack of N would affect cell division, but not cell growth; cells would continue to get larger. This is seen in Figure 2-e, as replete cells were significantly larger than deplete cells.

Calcification

There was more variation in calcification trends than in percent survival and size. Overall, higher doses of HHQ tended to result in less calcification. High HHQ concentrations (>100 μM) showed a greater difference in calcification between replete and deplete Fe and P; depletion of those nutrients may result in less calcification of *E. huxleyi* when exposed to high concentrations of HHQ. The opposite was true for the N treatments, and depletion of N may result in greater calcification when exposed to high concentrations of HHQ. Overall, though, the effect that nutrient depletion had on this relationship for this strain of *E. huxleyi* was minimal to none.

However, nutrient depletion did have a significant effect on overall calcification. Fe and N had the greatest differences. Cells in replete Fe were significantly more calcified, while cells in replete N were significantly more calcified. While implications of these findings are unknown, this may impact ecology of *E. huxleyi*, as well as its role in biogeochemical cycling. It is also unclear whether or not this is a species-specific occurrence, or even strain-specific. *E. huxleyi* 2090 is an uncalcified strain, so the differences in cells in replete and deplete cultures could be less pronounced in a calcified strain. Since coccoliths are a major source of calcite in the marine environment, and are thus connected to ocean acidification, the iron and nitrogen cycles may also be connected to the impacts of ocean acidification on coccolithophores.

Future research

This study did not find that low Fe, Zn, Co, or P significantly alter the impact that HHQ has on *E. huxleyi*. There may be several explanations for this. First, even the deplete nutrient treatments were still more concentrated than common nutrient concentrations observed in the open ocean. Therefore, cells may not have been properly limited by the respective nutrient. This experiment could be repeated, further limiting Fe, Zn, Co and P concentrations to see if they have an impact.

Another course of action would be further explore the HHQ protection offered by low nitrogen. *E. huxleyi* cultures would be grown in varying concentrations of N depletion and then exposed to 100 μ M HHQ to identify the concentration of N necessary. Further research could also determine whether the protection is due to N depletion induced stasis or by another mechanism. Research could also address the ecological impact of protection in low nitrogen environments, since HHQ could be less of a factor in bloom termination in these conditions, and blooms, though less abundant, could persist for longer periods of time.

Sources

Azam, F., & Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nature Reviews. Microbiology*, 5(10), 782–791. <https://doi.org/10.1038/nrmicro1747>

Balch, W. M., Holligan, P. M., & Kilpatrick, K. A. (1992). Calcification, photosynthesis and growth of the bloom-forming coccolithophore, *Emiliana huxleyi*. *Continental Shelf Research*, 12(12), 1353–1374. [https://doi.org/10.1016/0278-4343\(92\)90059-S](https://doi.org/10.1016/0278-4343(92)90059-S)

Harvey, E. L., Deering, R. W., Rowley, D. C., El Gamal, A., Schorn, M., Moore, B. S., Johnson, M. D., Mincer, T. J., & Whalen, K. E. (2016). A Bacterial Quorum-Sensing Precursor Induces Mortality in the Marine Coccolithophore, *Emiliana huxleyi*. *Frontiers in Microbiology*, 7. <https://www.frontiersin.org/articles/10.3389/fmicb.2016.00059>

Legendre, L., & Rassoulzadegan, F. (1995). Plankton and nutrient dynamics in marine waters. *Ophelia*, 41(1), 153–172. <https://doi.org/10.1080/00785236.1995.10422042>

Mitsutani, A., Yamasaki, I., Kitaguchi, H., Kato, J., Ueno, S., & Ishida, Y. (2001). Analysis of algicidal proteins of a diatom-lytic marine bacterium *Pseudoalteromonas* sp. Strain A25 by two-dimensional electrophoresis. *Phycologia*, 40(3), 286–291. <https://doi.org/10.2216/i0031-8884-40-3-286.1>

Pollara, S. B., Becker, J. W., Nunn, B. L., Boiteau, R., Repeta, D., Mudge, M. C., Downing, G., Chase, D., Harvey, E. L., & Whalen, K. E. (2021). Bacterial Quorum-Sensing Signal Arrests Phytoplankton Cell Division and Impacts Virus-Induced Mortality. *MSphere*, 6(3), e00009-21. <https://doi.org/10.1128/mSphere.00009-21>

Simó, R. (2001). Production of atmospheric sulfur by oceanic plankton: Biogeochemical, ecological and evolutionary links. *Trends in Ecology & Evolution*, 16(6), 287–294. [https://doi.org/10.1016/S0169-5347\(01\)02152-8](https://doi.org/10.1016/S0169-5347(01)02152-8)

Skerratt, J., Bowman, J., Hallegraeff, G., James, S., & Nichols, P. (2002). Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary. *Marine Ecology Progress Series*, 244, 1–15. <https://doi.org/10.3354/meps244001>

Whalen, K. E., Becker, J. W., Schrecengost, A. M., Gao, Y., Giannetti, N., & Harvey, E. L. (2019). Bacterial alkylquinolone signaling contributes to structuring microbial communities in the ocean. *Microbiome*, 7, 93. <https://doi.org/10.1186/s40168-019-0711-9>