Hungry Hungry Phytoplankton: the effect of limited nutrients on HHQ-induced mortality of *Emiliania huxleyi*

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Background

- Phytoplankton are drivers of marine biogeochemical cycles (1), and phytoplankton population dynamics are mediated by bacteria and bacterial compounds (2).
- *Emiliania huxleyi* is a bloom forming coccolithophore that impacts carbon cycling (3). 2-heptyl-4-quinolone (HHQ) is a quorum sensing quinolone produced by *Pseudoalteromonas* spp. (4)

Figure 1: Satellite image of *E. huxleyi* bloom in the English Channel

- HHQ arrests cell division in *E. huxleyi*, slowing growth while simultaneously protecting it from viral mortality (5), affecting bloom dynamics and nutrient cycling (6).
- All previous research with HHQ and *E. huxleyi* has been conducted under ideal nutrient conditions, which is not representative of the environment.
- **Research question**: Does nutrient depletion change the effect of HHQ on *E. huxleyi*?

Experimental Methods

- Grew *E. huxleyi* in 6 different artificial seawater (ASW) F/2 media types (Table 1) at 18˚C for 12 hours (light): 12 hours (dark)

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

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

- Exposed *E. huxleyi* cultures (control and low nutrient) to 8 concentrations of HHQ (0M [DMSO], 1 µM, 10 µM, 50 µM, 100 µM, 250 µM, 500 µM, 1 mM) in triplicate.
- Measured cell abundance, average forward scatter (proxy for cell size), and average side scatter (proxy for cell calcification) using flow cytometry daily for four days.
- Repeated experiment for all ASW F/2 medias

Data Analysis

- Calculated percent survival at T96 in Excel.
- Graphed percent survival, average forward scatter, and average side scatter (at T96) in GraphPad Prism 9

Survival | **Cell Size** | **Calcification**

Iron ● = nutrient replete ● = nutrient deplete

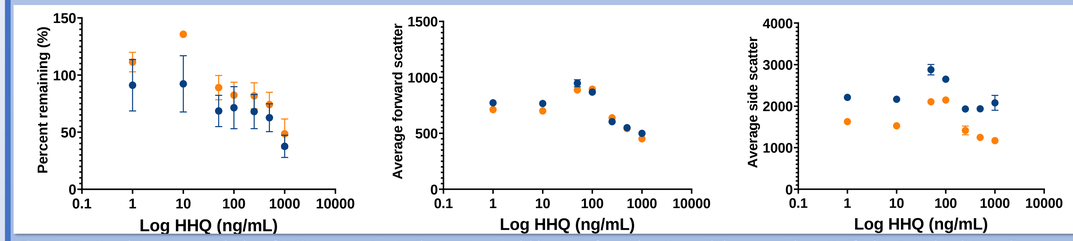

Figure 2: Scatter plots comparing the log of HHQ concentration to a) percent survival, b) average forward scatter, and c) average side scatter for low Fe and replete medias.

Zinc

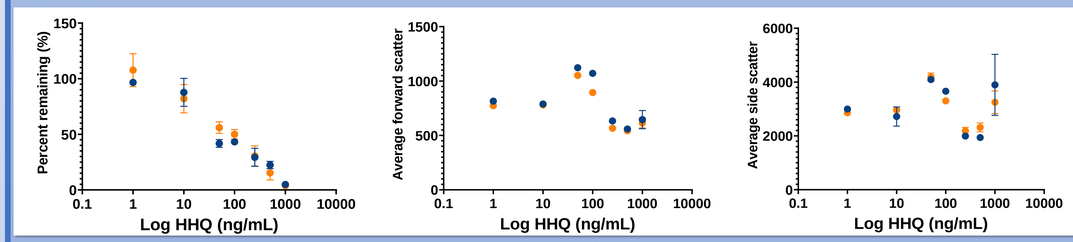

Figure 3: Scatter plots comparing the log of HHQ concentration to a) percent survival, b) average forward scatter, and c) average side scatter for low Zn and replete medias.

Cobalt

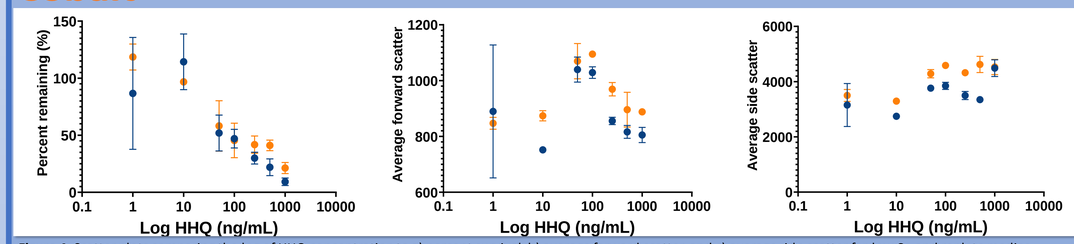

Figure 4: Scatter plots comparing the log of HHQ concentration to a) percent survival, b) average forward scatter, and c) average side scatter for low Co and replete medias.

Phosphorus

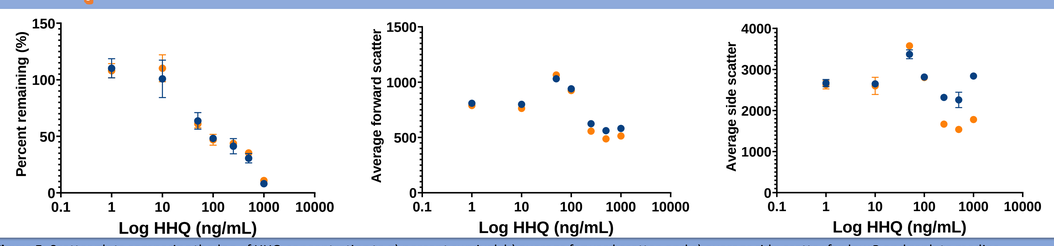

Figure 5: Scatter plots comparing the log of HHQ concentration to a) percent survival, b) average forward scatter, and c) average side scatter for low P and replete medias.

Nitrogen

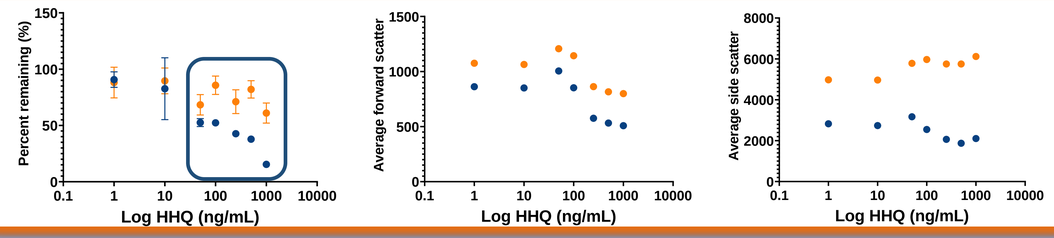

Figure 6: Scatter plots comparing the log of HHQ concentration to a) percent survival, b) average forward scatter, and c) average side scatter for low N and replete medias.

Discussion

- **Low Nitrogen protects *E. huxleyi* from high concentrations of HHQ**
  - Hypothesis: low N shuts down the cell cycle before HHQ can affect the cell
  - Connects the nitrogen cycle to biogeochemical bloom dynamics
- For all nutrients there is a peak in cell size at ~100 µM, consistent with literature
  - Low N cells have a larger size at all HHQ concentrations, supports theory that low N puts *E. huxleyi* in stasis, limiting HHQ effect
- Low metal concentrations do not significantly alter the impact that HHQ has on *E. huxleyi*
  - May not be low enough to mimic environmental conditions
- Limiting nutrients has varying effects on calcification (increase, decrease, no effect)
  - Addition of HHQ does not have an effect on calcification (similar responses in replete and deplete)

Future Research

- Limit nutrients more, specifically metals
- Explore low nitrogen HHQ protection
  - Grow *E. huxleyi* in different (limited) concentrations of N and expose to 100 µM HHQ to see what concentration of N offers protection

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