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# Collaborative Research: The Effect of Iron-Complexing Ligands on Iron Availability to Phytoplankton in HNLC Waters of the Subarctic Pacific Ocean

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Collaborative Research: The Effect of Iron-Complexing Ligands on Iron Availability to Phytoplankton in HNLC Waters of the Subarctic Pacific Ocean

**Project Participants****Senior Personnel****Name:** Wells, Mark**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Perry, Mary**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Trick, Charles**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Graduate Student****Undergraduate Student****Technician, Programmer****Other Participant****Research Experience for Undergraduates****Organizational Partners****Other Collaborators or Contacts**

Dr. William Cochlan has the collaborative proposal joining this proposal. As a group we are interactive on the research planning, implementation, data reduction and interpretation, and writing stages of the project

**Activities and Findings****Research and Education Activities:**

The research activities during this project have centered on five research cruises; one to

the Western subarctic Pacific, two to the Eastern Subarctic Pacific and three to the coastal upwelling waters off Washington State and British Columbia, Canada. In each case specific hypotheses about the role of iron complexing ligands, and high affinity iron uptake systems were tested using both standard batch cultures to measure the magnitude of the population response to perturbations, and continuous cultures operated at dilution rates of 0.5 per day to identify the selective pressures of our manipulation experiments on phytoplankton community structure. These major field efforts were supported by laboratory experiments growth experiments. In both cases the research focus was on the impact that different iron complexing ligands have on iron availability to large and small phytoplankton, and the degree that copper-based high affinity iron uptake systems are involved.

Experimental outcomes of our manipulations were assessed using increases in size-fractionated chlorophyll a concentrations, macronutrient utilization, iron uptake kinetics, and bacterial abundance and productivity. We assessed the physiological condition of the phytoplankton community using three criteria: community growth responses (using the FACSCalibur flow cytometer in association with the measured extracted chlorophyll measurements), the carbon fixation efficiency (photosynthetic rates against the amount of light available, P vs. E) and the quantum yield efficiency of the photosystems (Fv/Fm).

The first of the field programs supported the second mesoscale Fe enrichment experiment conducted in the western subarctic Pacific by our Japanese colleagues (SEEDS II). The central findings of our work showed that Fe remained limiting in the fertilized patch despite Fe concentrations being well above the diffusion-limited threshold for rapid diatom growth. Growth in the patch was an order of magnitude lower than observed in SEEDS I, performed 2 years earlier in the same general location, and our ligand manipulation experiments suggest that strong Fe(III) complexation by natural organic ligands in patch waters was responsible for this outcome. We also showed that heterotrophic bacteria activity was not suppressed in the patch, and that light was not the primary limiting factor. Detailed Fe(II) measurements demonstrated that a major fraction of dissolved Fe existed as reduced (Fe(II)) species in the photic zone during daylight hours, both in the fertilized patch as well as in the surrounding ambient surface waters. The finding of continued Fe limitation in the patch therefore is perplexing, given that current models for Fe uptake by phytoplankton suggest Fe(II) is an essential substrate. However, we showed that Fe(II) oxidation rates were substantially slower than derived from empirical oxidation rate calculations at higher Fe(II) concentrations; the implication being that Fe(II) oxidation was slowed by weak Fe(II) complexation in these surface waters. We showed that increasing Cu availability partially alleviated Fe limitation in the presence of siderophores in our manipulation experiments, confirming our hypotheses that Cu is involved in the induction of high affinity Fe uptake systems in diatoms. There were several other findings with respect to cyanobacteria and nanoplankton responses to different Fe(III) complexing ligands in these Fe-enriched patch waters.

The subsequent cruises focused on studying the role of Fe(III) complexing ligands in regulating community composition and growth responses to low level Fe enrichment. Our findings show that communities in nearshore Fe-replete, nearshore Fe-deplete and offshore HNLC regions were shaped by elevated growth rates of community members (based on flow cytometric measurements) rather than the removal of cells through grazing. Thus, the in situ cells obtained an ability to obtain iron from an environment with growth limiting levels of free inorganic iron. In our experimental manipulations the addition of different artificial ligands (e.g. Desferal) improved the growth kinetics of selected community members but access to the iron bound by these artificial ligands was accelerated if cells were supplemented with a low level of copper (less than 2 nM). These conditions only accelerated the growth of the larger phytoplankton with the smaller prokaryotic phytoplankton (cyanobacteria) showing minimal-to-negative responses to the added ligand/copper combination. Thus the availability of an organic source of iron seemed beneficial but the mechanism was not taxon specific (i.e. not a one-to-one

association with cellular iron transporters) but semi-specific with the iron presumably reduced and made available at the cell surface of the benefiting large phytoplankton cells. Thus the value and extent of reduced iron was assessed in the later stages of the experiment. These findings provided a fundamental shift in our understanding of the mechanism of iron procurement in the large phytoplankton and confirmed that the iron dynamics is extremely important in shaping the large phytoplankton community.

We confirmed that none of the treatments improved the ecological position of the cyanobacteria. While the community remained ecologically robust in numbers the numbers were maintained at a constant rate by grazing and by a relatively low growth rate. In contrast the heterotrophic bacterial community had constant numbers in situ, but the dilution experiments indicate that the intrinsic growth rates were exceptionally high with robust remineralization rates. Not all bacteria responded equally to our ligand treatments. One subgroup (referred to as the high DNA/cell group) elevated growth rates to greater than 3 per day when ligand iron mixture were added. In contrast the low DNA/cell group maintained a growth rate of ~ 1.1 divisions per day under all treatments. This confirms the form of iron in seawater shapes the activities and community structure for three levels of marine microbes: heterotrophic bacteria, cyanobacteria and large diatoms.

Our nearshore cruises in upwelling coastal waters off Washington State studied the effects of Fe ligand additions on community trajectories. The findings were consistent with those from offshore environments, whereby strong ligand analogs (siderophores) strongly curtailed growth of eukaryotic phytoplankton but not that of cyanobacteria, while weaker ligands (porphyrins) enhanced the growth of eukaryotic phytoplankton with no effect on cyanobacteria. We also showed the toxin domoic acid produced by toxigenic *Pseudo-nitzschia* spp., which is a weak Fe(III) and Cu(II) complexing ligand, selectively enhances the growth of *Pseudo-nitzschia* spp. over other diatoms. To our knowledge, this is the first demonstration of a metal ligand release by eukaryotic phytoplankton that provides a competitive advantage to the producer.

The addition of data collected for the variable cell fluorescence measurements (using our PAM fluorometer) should confirm the iron status of the larger phytoplankton cells and the time course of the shift between iron-limitation and iron-satiated growth. The measurement of the fluorescent efficiency of cells from the waters of the 2007 cruise path indicate that we have observed waters that were iron and nutrient replete ( $F_v/F_m$  of greater than 0.750 to iron deplete, nutrient rich ( $F_v/F_m$  if below 0.3)).

For the subarctic Pacific cruise we also studied phytoplankton variable fluorescence ( $F_v/F_m$ ) in water samples collected from 8-12 meters with a trace-metal free pumping system. Hourly sampling, conducted over 24 hours, examined diel changes in  $F_v/F_m$ . Night-time values were low (0.35), consistent with results in other Fe-limited regions. When cloud cover was significant and day-time light levels were low, there was little to no change in variable fluorescence during the daytime. However, when light levels were high, variable fluorescence decreased ( $O(0.20)$ ) during the day, consistent with solar quenching of fluorescence and suggestive of damage to photosynthetic reactions centers.

Additional water samples were collected from the CTD rosette system every morning between surface and 80 meters. The general pattern was low variable fluorescence at the surface and higher variable fluorescence at depth. Over the course of the cruise, chlorophyll concentration in the deep chlorophyll maximum at 60 meters increased and variable fluorescence decreased (0.2).

We co-supported a two high school teachers to join the research cruises and participate in the research activities. They each maintained a daily web-log not only for their classrooms but also across their districts. They fielded e-mail questions daily from students, and created incident or science topic-specific web pages to inform students and

parents alike on the research goals and activities. On return to their home institutions, both teachers participated in outreach lectures.

### Findings:

The major findings from the research efforts are listed below:

1. Eukaryotic phytoplankton have difficulty accessing iron bound to strong iron-complexing ligands, but grow readily on weaker classes of iron complexing ligands
2. Low level iron additions to HNLC waters yields very different outcomes depending on whether natural UV light is present or blocked. UV light stimulates growth of diatoms in HNLC waters, while blocking of UV light prevents their growth. The findings show the importance of photochemical cycling in providing a source of ambient iron to phytoplankton in HNLC waters.
3. UV light photoreduces Fe(III) to the extent that a major fraction (up to 50%) of total dissolved Fe in sunlight surface waters can exist in Fe(II) species. The findings strongly suggest that the chemical speciation of Fe in surface waters cannot be described adequately by equilibrium theory, as is presently done.
4. Fe(II) oxidation rates in the western subarctic Pacific surface waters are significantly slower than predicted by theory, and measured in UV oxidized (i.e., organic free) seawater. The implication is that Fe(II) complexation by organic chelators serve to buffer Fe(II) in surface waters
5. Fe(II) oxidation rates in the eastern subarctic Pacific surface waters are significantly faster than in UV oxidized seawater, or in deep waters. Experiments show that acceleration of Fe(II) oxidation can be associated with strong Fe(III) complexing ligands. Faster rates of Fe(II) oxidation correlate with concentrations of Chl a, indicating that phytoplankton are the main source of these Fe(III) complexing ligands.
6. Copper amendments increase the ability of large phytoplankton to utilize Fe complexed by strong Fe(III) complexing ligands, consistent with a these eukaryotic phytoplankton possessing copper-based high affinity Fe transport systems used to acquire Fe from bacterially produced siderophores. Adding further support are observations that decreasing copper availability by adding Cu(I) and Cu(II) complexing ligands decreases phytoplankton growth.
7. Changes in Fv/Fm measured at Station PAPA in early spring suggest that phytoplankton growth in the deep chlorophyll maximum reduced available Fe, leading to an intensification of Fe-limitation.
8. The two iron additions during the SEEDS II mesoscale Fe enrichments were insufficient to meet the growth requirements of diatoms. Although Fe concentrations in the patch were well above ambient, they remained below the concentration of strong Fe(III) complexing ligands. As a consequence, further Fe amendments were needed. The results suggest a new paradigm for describing the ecosystem effects of iron additions to HNLC waters, and raise significant question about whether natural dust deposition events seen today can routinely cause diatom blooms in HNLC waters.
9. UV oxidation of HNLC waters to destroy metal complexing ligands results in increased growth of all phytoplankton size classes. This finding demonstrates that metal complexing ligands shape and restrict phytoplankton community trajectories.
10. Release of the metal chelating toxin domoic acid by the pennate *Pseudo-nitzschia* to

the dissolved phase alters metal chemistry to provide a competitive advantage over other diatoms.

11. Offshore Pseudo-nitzschia spp. previously thought to be non-toxic were found to produce domoic acid in oceanic subarctic Pacific waters. The effect that dissolved domoic acid exerts on metal chemistry in HNLC waters remains to be determined.

The project has resulted in over 25 published abstracts at international meetings, with multiple oral and poster presentations by all the PIÆs and graduate students.

#### **Training and Development:**

There are 5 graduate students and 6 undergraduate students who have participated in the research of this project. Each of these students have significantly improved their research skills and teaching skills. The project was the central thesis topic for one PhD student, who successfully defended her dissertation research shortly after completion of the grant. There were two high school teachers who participated in the research as full team members. Each took back to their classrooms a heightened awareness of research planning, hypothesis derivation, and new methods for communicating these skills to their students.

#### **Outreach Activities:**

There were two high school teachers who participated in the research cruises as full team members. They each maintained a daily web-log not only for their classrooms but also across their districts. They fielded e-mail questions daily from students while on board, and created incident or science topic-specific web pages to inform students and parents alike on the research goals and activities. On return to their home institutions, both teachers participated in outreach lectures. Each took back to their classrooms a heightened awareness of research planning, hypothesis derivation, and new methods for communicating these skills to their students.

#### **Journal Publications**

Roy, E. and Wells, M.L., "The Persistence of Fe(II) in Surface Waters of the Western Subarctic Pacific", *Limnology and Oceanography*, p. 89, vol. 53, (2008). Published,

Roy, E., Jiang, C., Wells, M.L., and Tripp, C., "Determining subnanomolar iron concentrations in oceanic seawater using siderophore-modified film analyzed by infrared spectroscopy", *Analytical Chemistry*, p. 4689, vol. 80, (2008). Published,

Pickell, L., Wells, M.L., Trick, C.G., and Cochlan, W. P., "A sea-going continuous culture system for investigating phytoplankton response to macro- and micronutrient (trace metal) manipulations", *Limnology and Oceanography*, p. , vol. , (2009). Accepted,

Wells, M.L., Trick, C.G. Cochlan, W. P., and Beall, B., "Iron Inputs and the Persistence of Iron Limitation in the Western Subarctic Pacific SEEDS II Mesoscale Fertilization Experiment", *Deep Sea Research Part II*, p. , vol. , (2009). Accepted,

Roy, E. and Wells, M.L., "Regulation of Fe(II) oxidation rates by organic complexing ligands in the Eastern Subarctic Pacific", *Environmental and Science Technology*, p. , vol. , (2008). Submitted,

Pickell, L., Wells, M.L., Trick, C.G., and Cochlan, W. P., "Dissolved domoic acid: a competitive advantage for pseudo-nitzschia in coastal waters", *Limnology and Oceanography*, p. , vol. , (2008). Submitted,

Pickell, L., Wells, M.L., Trick, C.G., and Trainer, V.L., "The effects of continuous iron, copper and domoic acid supply on shaping phytoplankton assemblages from contrasting subarctic Pacific regions", *Limnology and Oceanography*, p. , vol. , (2008). Submitted,

Pickell, L., Wells, M.L., Trick, C.G., and Cochlan, W. P., "Using continuous cultures to determine the effects of strong iron(III) complexing ligands on a natural coastal phytoplankton community", *Limnology and Oceanography*, p. , vol. , (2009). Submitted,

### **Books or Other One-time Publications**

#### **Web/Internet Site**

##### **URL(s):**

<http://www.marine.maine.edu/~wellsm/Homepage.htm>

##### **Description:**

This site explains to the public and prospective graduate students the central goals and reasoning behind the research of this project

### **Other Specific Products**

#### **Contributions**

##### **Contributions within Discipline:**

The project supported the development, testing, and application of a novel, sea-going continuous culture system for use in deckboard incubation experiments with natural seawater. The system has a number of improvements over the initial system developed by Hutchins and colleagues, and should provide a robust platform design for other investigators wishing to move beyond batch culture experiments to determine the trajectory of planktonic ecosystems responding to chemical or physical perturbations.

##### **Contributions to Other Disciplines:**

A central finding of the project was that small additions of Fe, such as normally would occur during aerosol deposition events, has a restricted effect on diatom growth due to excess concentrations of strong Fe(III) complexing ligands. These findings are in contrast to those of most mesoscale enrichment experiments, which initially swamp the free ligand concentrations and precipitate colloidal and particulate iron oxyhydroxides that can continue to supply iron to eukaryotic phytoplankton. The work thus brings into greater question whether natural perturbations in iron supply to HNLC waters have the ability to influence climate change.

##### **Contributions to Human Resource Development:**

There are 5 graduate students and 6 undergraduate students who have participated in the research of this project. Each of these students have significantly improved their research skills and teaching skills. The project was the central thesis topic for one PhD student, who successfully defended her dissertation research shortly after completion of the grant.

##### **Contributions to Resources for Research and Education:**

There were two high school teachers who participated in the research as full team members. Each took back to their classrooms a heightened awareness of research planning, hypothesis derivation, and new methods for communicating these skills to their students.

##### **Contributions Beyond Science and Engineering:**

As part of our efforts we showed that iron enrichment of subarctic Pacific HNLC waters resulted in increased production of pennate diatoms of the genus *Pseudo-nitzschia*, and that these organisms produced the potent neurotoxin domoic acid as found in nearshore waters. These findings strongly suggest that discussions and any planning for large scale iron enrichments to mitigate climate change should take into account this previously unknown threat.

**Categories for which nothing is reported:**

Organizational Partners

Any Book

Any Product