Bacterial and viral abundances along a proposed iron gradient at the Galapagos Islands

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Pamela Maynard
2648 W. Plymouth St. Seattle WA 98199
(206)-979-7857
plm@u.washington.edu

Project Summary:

Upwelling of the Equatorial Undercurrent (EUC) provides the bulk of the excess nutrients in the Equatorial Pacific, including iron that may stimulate and support the high levels of chlorophyll and productivity in an otherwise iron-poor (High-Nitrate-Low-Chlorophyll) region (Cullen 1991 Chavez and Barber 1987, Chavez 1989). Experimentation has documented that in addition to phytoplankton, bacteria in this area can also be iron-limited. Iron enrichment experiments show increases in bacterial abundance corresponding to phytoplankton abundance increase (Cochlan 2001). Thus, this research attempts to enumerate bacteria at along an east-west transect at multiple depths to determine the differences in bacterial concentration as influenced by proximity to the Islands and thus an iron source through aeolian transport and EUC upwelling. Samples will be taken at various depths including the chlorophyll maximum and enumerated using SYBR Green-1 epifluorescence microscopy as detailed in Noble and Fuhrman (1998). To further determine iron's influence upon bacteria within the region, two incubation experiments will be set up from samples at the beginning and terminating ends of the transect. For each experiment, 3 samples from 15 meters depth will be amended with iron, incubated over 72 hours, and sub sampled for enumeration according to the technique described above. Three additional samples from 15 m depth will have the current iron concentrations removed using a siderophore and also be incubated in the dark for 72 hours and sub sampled for each experiment. One control per experiment will be un-amended and incubated as the others. All samples will be incubated in the dark to minimize the effect of phytoplankton production upon bacterial abundances. Bacterial abundances will then be determined and analyzed for relationships between iron addition and any increases.

Introduction:

Past research around the Galapagos has demonstrated that the Equatorial Pacific is a high-nitrate, low chlorophyll (HNLC) zone, an area of high nutrients but notable low productivity (Cullen 1991). However, satellite data analyzed by Feldman (1986) indicate a pattern of increased chlorophyll concentrations on the western side of Isabella Island, as compared to areas a far distance from the Galapagos. This area of anomalous chlorophyll extends west into the Equatorial Pacific past 98 degrees west (see Feldman 1986, plate 3). Studies by **Chavez and Barber (1987)** have also measured higher primary production downstream of the Galapagos Islands at 92° W as compared to the low productivity within surrounding waters.

In conjunction with this area of higher chlorophyll levels and increased productivity, the Equatorial Undercurrent (EUC) surfaces in the area of the Galapagos Islands when it encounters the steep topography of the start of the Galapagos Platform on the western side of Isabella Island, bringing nutrients with it from at depth (Feldman 1986; plate 1b, Chavez and Brusca 1991, Barber and Chavez 1991, Binder et al. 1996).

According to Cullen (1991) upwelling provides the bulk of the excess nutrients near the air-sea interface, including providing iron that may stimulate and support the high levels of chlorophyll and productivity (Chavez and Barber 1987, Chavez 1989). When the EUC hits the Galapagos platform it deflects and/or upwells, causing iron-rich sediments to mix up into the water column (Barber and Chavez 1991, **Martin and Gordon 1988**). This mixing re-mineralizes the iron contained within the sediments, making it available for bio-utilization. Indeed, iron enrichment experiments have demonstrated iron to be a limiting nutrient in the Equatorial Pacific (Martin and others 1994, Price et al. 1994, Coale et al. 1996, Cochlan 2001) and thus it can be inferred that the iron supplied by the Equatorial Undercurrent may be one stimulus of this plume of high chlorophyll concentrations.

Studies by Martin et al (1994) have determined that iron limitation in HNLC regions such as the Equatorial Pacific drives many of the population dynamics amongst phytoplankton within the system. Further investigation by the Iron Ex (II) (2001) experiment documented an increase in bacterial abundance in the Equatorial Pacific as phytoplankton abundances increased in the presence of added iron, leading researchers to hypothesize that iron deficiencies may also inhibit bacterial growth (Cochlan 2001). While this research has demonstrated that iron addition does indeed stimulate small increases in bacterial biomass, it has not definitively confirmed whether this increase is due to iron addition directly or to increased DOC concentrations as a result of an induced phytoplankton bloom (Cochlan 2001). Bacterial population increase due to iron addition has been documented in the Arctic Ocean, but not shown in all HNLC regions (Price et al. 1994, Pakulski et al. 1996, Church et al. 2000). Even so, Ducklow et al. (1995) have determined that HNLC regions support high levels of bacterial biomass, suggesting that in iron-limited areas, already high bacterial abundances may increase in the presence of added iron. Furthermore, a chlorophyll plume noted by Feldman (1986) may serve as a proxy for identifying an external iron supply from up-welled EUC waters and give insight into gradients of bacterial abundances. The location of the plume is correspondent with the location of The Southern Equatorial Current (SEC) which is driven by the trade winds blowing to the West. As the water moves, it carries with it any chlorophyll and nutrients brought to the surface by upwelling from around the Galapagos. Therefore, sampling from roughly West to East to the Galapagos Islands along a transect through waters increasing in iron concentration will provide additional information on how iron concentrations affect bacterial numbers, while iron incubations will enable determination of relative iron-stress between the bacterial populations.

Proposed Research:

Field Methods

To assess bacterial numbers and their response to iron addition, sampling will occur along 35’ south, moving from west of 92°W toward Elizabeth Bay, located within Isla Isabella. An additional station within Elizabeth Bay at a depth of 15 m will be sampled to compare bacterial abundances in the euphotic zone in a highly productive area to abundances in areas of lower productivity found at a distance from the Islands.

A CTD rosette will generate a profile of salinity, oxygen concentration, chlorophyll concentrations, and ambient light conditions at 4 stations along the transect. The rosette will collect water samples to a depth of 1000m, at intervals of 15m, 250 m, 500m, 750 m, and 1000m using Niskan sampling bottles able to close independently at discrete depths. The HNLC chlorophyll max, as described by Chavez and Brusca (1991) will also be sampled. Replicates will be sampled at 5 depths along the transect to estimate degree of error in enumerating the bacteria and viruses.

Laboratory Analysis

Once brought to the surface, water from the CTD rosette will be collected into carboys and brought into the laboratory for analysis. Samples of 10 mL volume will be fixed with .02 um filtered 2% formalin and then filtered onto a .02 um pore size, 25 mm diameter aluminum oxide Anodic filter using a vacuum pump and filter system according to the methods outlined in Noble and Fuhrman (1998). Under low light conditions, 5 uL of SYBR Green I epifluorescence DNA marker will be mixed with 92 uL of deionized water in one drop per well in a Hoeffer Box. The freshly filtered sample will be laid sample side up in the SYBR Green 1 drop and left for 15 minutes in the dark to allow the stain to penetrate the sample. After being dried, the filter containing the sample will be mounted upon a glass slide with 30 ul glycerol anti-fade mounting solution and frozen until analysis.

Analysis will begin while aboard ship and continue upon return to Seattle. During analysis, samples will be removed from cold-storage, excited with blue light under an epifluorescence microscope and counted. At least 200 bacteria will be counted per field for a total of 10 fields per slide. Final counts would be compared along the east-west continuum at all depths to determine if bacterial abundances increase with proximity to the Galapagos Islands.

Additionally, an iron enrichment bottle experiment modeled after Church et al. (2000) will be conducted to investigate whether iron addition will stimulate bacterial abundance increases in the areas sampled. While Church chose to amend his bottles with several different substances, this study will examine the effect both of adding and removing iron from the system.

To begin, 500 ml sub-samples taken from water at the chlorophyll maximum will be taken from the carboys and placed into 500 mL, HCl washed plastic incubation bottles. These bottles will be covered with aluminum foil to block out all light and control for dissolved organic carbon (DOC) stimulation of bacterial numbers by phytoplankton production. Little to no research has yet established the relative importance of iron limitation and DOC concentrations to bacterial growth in HNLC regions (Cochlan, 2001). Some research has demonstrated that iron addition does stimulate a small increase in bacterial biomass, but it is questionable as to whether it is due to the iron directly or to increased DOC concentrations as a result of an induced phytoplankton bloom (Cochlan 2001). Thus, incubating in the dark will allow for a higher degree of certainty regarding iron's influence upon the bacterial populations. Phytoplankton production of DOC is limited or non-existent without sunlight. Thus, no new addition of carbon will be introduced to influence bacterial numbers.

Iron will be added to 3 samples bottles in the form of ______________ to a final concentration of ________, _______ times the average iron concentration within the Galapagos HNLC region. In 3 other bottles, ambient iron will be removed using a siderophore to determine what effect iron limitation has upon bacterial numbers. To control for possible DOC production by phytoplankton within the sample, which may affect bacterial numbers, all bottles will be incubated for 72 hours in the dark and sampled every 24 hours to determine any increase in bacterial numbers. If samples taken from more westerly stations demonstrate higher levels of iron stimulation, and experimental conditions are stringent enough to eliminate other stimulating factors, this could be used as an indirect indication of higher iron limitation to the west.

This work will benefit from multiple investigations conducted concurrently with this project. Tamra Dickson will be conducting nutrient profiling in and around the Galapagos Islands which will provide me with nutrient data that will be comparable to and set the standard for my CTD measurements. These data will be used to examine possible alternate influences upon my iron incubation experiments. Trina Litchendorf will be investigating pH affects on iron solubility and phytoplankton, from which I may be able to extrapolate information regarding iron concentrations in my sampling area. Additionally, my data provides the opportunity to begin examining larger-scale carbon cycling and ecosystem responses. Natalie Tsui and Jennifer Nomura are enumerating and identifying zooplankton while Tasha Snow will be examining phytoplankton assemblages within the Islands. Our data, when examined together, may allow us to identify potential links and feedbacks between the various groups.

Project Budget:

Item	Cost/unit	# Units	# Days	Total Cost	My Cost
R/V Thompson	$18,000.00	1.00	9	$162,000.00	$0.00
Deck Incubator	$3.00	1.00	9	$27.00	$0.00
Filter Rack and vacuum pump	$6.00	1.00	9	$54.00	$0.00
CTD Seabird SBE-911+	$135.00	1.00	9	$1215	$0.00
Conductivity probe	$6.00	1.00	9	$54.00	$0.00
Oxygen meter	$15.00	1.00	9	$135.00	$0.00
Salinity meter	$15.00	1.00	9	$135.00	$0.00
Niskan bottles	?	1.00	9	?	$0.00
500 ml/1L Incubation bottles	?	14.00	9	?	$0.00
Lab fluorometer	$15.00	1.00	9	$135.00	$0.00
Secondary standard	$3.00	1.00	9	$27.00	$0.00
U of W bottles	$3.00	1.00	9	$27.00	$0.00
Salinity bottles (standard)	$3.00	1.00	9	$27.00	$0.00
Oxygen bottles	$6.00	1.00	9	$54.00	$0.00
Epifluorescence microscope	?	1.00	1	?	$0.00
480 nm epifluoresence filter	?	1.00	0	?	$0.00
Formalin	$61.84	1.00	0	$61.84	$61.84
.02 um Anodisk filters	$109.32	3.00	0	$327.96	$327.96
.8 um milipore filters	?	?	0	?	?
Kimwipes	$3.63	3.00	0	$10.89	$10.89
Iron for enrichment experiment	?	1.00	0	?	?
Iron siderophore	?	1.00	0	?	?
SYBR Green 1 stain	$176.00	1.00	0	$176.00	$176.00
Hydrochloric acid	$37.58	1.00	0	$37.58	$37.58
pipetter set	$857.44	1.00	0	$857.44	$0.00
10 ul tips	$47.03	1.00	0	$47.03	$0.00
50 ul tips	$54.00	1.00	0	$54.00	$0.00
200 ul tips	$79.94	1.00	0	$79.94	$0.00
1000 ul tips	$94.73	1.00	0	$94.73	$0.00
Glycerol	$69.83	1.00	0	$69.83	$69.83
PBS solution (10x)	$23.51	1.00	0	$23.51	$23.51
P-phenylenediamine	$37.67	1.00	0	$37.67	$37.67
20L water carboy	$178.39	1.00	0	$178.39	$178.39
Forceps	$22.65	2.00	0	$45.30	$45.30
Weigh boats (small)	$22.28	1.00	0	$22.28	$22.28
Aluminum foil	?	1.00	0	?	?
Chl a analysis standard	$3.00	1.00	0	$3.00	$3.00
Oxygen standard	$6.00	1.00	0	$6.00	$6.00
TOTAL PROJECT COST				**$166,023.39**	**$1000.25**

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Binder et al. (1996) notes in his paper a discrepancy between his and Ducklow (1995) et al.'s bacterial enumerations from samples both taken during the 1992 JGOFS Time Series 2 Cruise. Binder's flow cytometric enumerations were 25% lower than Ducklow's epifluorescence counts. Since epifluorescence enumeration and flow cytometry generally agree, Binder suggests that the discrepancy may be due to differences in sampling times. Binder sampled during dawn or dusk while Ducklow sampled during midday (Binder 1996, Ducklow 1995). Thus, to attempt to enumerate the most bacteria present in the water column, it would be nice to sample will ideally be collected at midday.