Inter-island comparison of bacterial growth rates and bacterivory in unamended and iron-enriched incubations in the Galapagos

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Project Summary

Growth rate and grazing rate of heterotrophic bacteria will be determined from samples collected at four locations around the Galapagos Islands. The samples will be collected from the *R/V Thomas G. Thompson*, diluted with varying amounts of filtered seawater, with and without added iron, and incubated for 24 hours. After being stained, the bacteria present will be counted using epifluorescence microscopy. It is expected that the addition of iron will increase the abundance (at least temporarily) of phytoplankton, which provide a source of dissolved organic carbon (DOC) for the bacteria, thus increasing the growth rate of the bacteria. Bacterial abundance, however, is expected to remain fairly consistent as grazing rates on the bacteria are also expected to increase, resulting in little or no net increase in the number of bacteria present.

Introduction

The importance of iron in the world's oceans gained much attention when John Martin proposed that not only were high-nitrate, low chlorophyll (HNLC) regions limited by iron, but that large-scale iron fertilization induces phytoplankton blooms that could cause sufficient carbon drawdown to affect global climate change. When the first *in situ* iron fertilization investigation, IronEx, was carried out (about 500 km south of the Galapagos Islands, in fact), an almost three-fold increase in chlorophyll was observed (Martin et al. 1994). Since then, a number of such *in situ* iron-seeding experiments in HNLC regions have taken place (IronEx II, SOIREE, EISENEX, SOFeX), all of which indicate that that iron-amended waters increase phytoplankton production. However, as the iron hypothesis and the fertilization experiments come under increasing scrutiny from other researchers, the question of iron seeding has become increasingly complex.

Perhaps beginning with Karl Banse, who noted that IronEx investigators had not carefully accounted for grazing (Banse 1995 a), scientists began to more carefully consider parameters involved in carbon and iron cycling in the oceans in light of the *in situ* fertilization experiments. The image of phytoplankton as the end-all be-all control in the oceanic cycling of carbon is being replaced by an increasingly complex schematic involving layer upon layer of causes, effects, and control by multiple sources. One such layer found to be increasingly significant is bacteria. For example, it has been suggested that the majority of oceanic biomass and metabolism are now attributed to bacteria (Azam 2004). As a whole, bacteria play an integral role in nutrient cycling by nitrogen-fixing, feeding on DOC, and serving as a source of recycled nutrients (including iron) to trophic levels above them (Azam 2004, Azam et al. 1983).

Increased bacterial abundance and production has been shown to occur shortly after phytoplankton blooms induced by iron addition (Cochlan 2001, Hutchins 2001). Some studies suggest that this is caused directly by the addition of iron, indicating that the bacteria were limited by low iron concentrations (Pakulski 1996). However, most researchers seem to agree that the higher number of bacteria is more likely due to increased availability of dissolved organic carbon (DOC) from phytoplankton (Banse 1995, Cochlan 2001, Hutchins 2001, Landry and Kirchman 2002). The apparent complexity caused by heterotrophic bacteria directly competing for iron with phytoplankton, their primary source of DOC, is commonly reconciled by the assumption that bacteria, due to their higher surface area-to-volume ratio and the presence of siderophores, have such a distinct advantage over phytoplankton in taking up iron that bacterial iron-limitation is not considered (Tortell et al. 1999).

During “normal” (that is, non-El Nino) years, the water around the Galapagos have an area of comparatively high chlorophyll on the west side of the islands, due to increased nutrients

from upwelled and Aeolian sources (Feldman 1984). (The cruise will be occurring during a time when "normal" conditions are expected.) The existing natural juxtaposition of a higher-chlorophyll area (west side) and a low-chlorophyll area (east side) offers a unique opportunity to compare bacterial growth rates in waters presumably having different iron concentrations. (See Figure 1.)

For this project, dilution experiments (similar to those used for phytoplankton in Landry, et al. (2000)) will be used to find the growth and grazing rates of heterotrophic bacteria collected from four sites. Bacterial abundance will be measured using epifluorescence microscopy. Rates will be measured using incubations with and without the addition of iron.

I propose that, with the addition of iron, IF phytoplankton and phytoplankton-grazer growth rates increase, then bacterial growth rate will increase. The increased growth rate of bacteria would occur due to the increased amount of DOC, which would become available from "sloppy" feeding of grazers, excretion, dissolution of feces, and exudation of phytoplankton (Banse 1995 b). If phytoplankton do not increase, then there would be no expected incrase in bacteria; however, if phytoplankton increase and bacteria do not, then it would support the notion that the bacteria were iron-limited, rather than carbon-limited, or suggest that bacterial growth was controlled by viral lysis not accounted for in this study.

The system is expected to balance out; that is, an increase in phytoplankton leads to an increase in grazers, both of which lead to an increase in bacteria, but the bacteriovores will "catch up" (grazer abundance will increase to sufficiently control the increasing abundance of the bacteria) after some short time, as shown with phytoplankton and their grazers by Banse (1995 a).

Theoretically, there will be a time lag between an increase in phytoplankton, in grazers, and in bacteria, as each would be a direct effect of the previous. Such a time lag will not be observable for one of two reasons. First, the "catch up" of the grazers to the bacteria may occur within the 24-hour period, while only the initial and final bacterial abundances will be measured. In this case, no net increase in bacterial abundance will be observed (although growth rate increase should be observable). Second, the 24-hour incubations may not be long enough to capture the entire phytoplankton-grazer-bacteria-grazer increase/leveling dynamic. In this second scenario, bacterial count may be higher or similar to initial measurements, depending on where in the increase/leveling cycle the incubation ends.

In addition to these observations, different results are expected from samples taken in high vs. low chlorophyll areas. The stations we have chosen include areas at which we expect to find variability in chlorophyll concentration. For those areas of higher chlorophyll, little (or no) increase in bacterial growth rate is expected, based on the assumption that the higher chlorophyll is due to higher iron concentrations. That is, the change in magnitude of the growth rates in seawater containing lower concentrations of iron will be larger than that of the growth rates in seawater containing higher concentrations of iron.

Proposed Research

I will be working closely with Wendy Guo, who will be measuring the growth and grazing rates of phytoplankton with iron enrichment. We will be carrying out our incubations in the same bottles, so we can combine our analyses of phytoplankton -grazer and bacteria-grazer experiments to form a more complete explanation of the interactions present. In addition, while bacterial mortality due to viruses has not been accounted for in this experiment, I would like to

try to integrate the results from Pamela Maynard's project, which will be focusing on bacterial mortality due to viruses across an iron gradient.

Our cruise plan, shown in Figure 2, includes four stations at which Wendy and I will collect samples at about 15m depth using a CTD rosette with Niskin bottles. Latitude and longitude for each station are given in Table 1. For each station, we will use 22 2-L bottles: doubles of dilution factors of 0.2, 0.4, 0.6, 0.8, and 1 (undiluted seawater) for iron-enriched and unamended samples, and a filtered seawater control, to help us account for organisms that may pass through the filter. The bottles will be placed in incubators on the ship deck, through which seawater will be circulated to maintain consistent temperature. They will be covered with netting to simulate incident PAR at the depth from which the samples were taken. (Because our samples will be taken early in the cruise, we will use 15m PAR levels taken by the members of cruise leg 1 to estimate the required net covering.) Samples will be collected and incubations set up before sunrise so that our experiments, to include an entire diel cycle, will begin at sunrise and end 24 hours later.

Bacterial enumeration will be carried out by staining the bacteria filtered from a small amount of seawater (at each dilution factor) and viewing them under an epifluorescence microscope. The will be physically counted, giving an estimate of the number of bacteria per litre of seawater. These measurements will be taken at the beginning and end of the 24-hour cycle, the difference between which will yield an estimated net growth rate for one day. Plotting the net growth rate day^{-1} with the dilution factor will allow observation of differences in growth and grazing rates of amended and unamended samples.

The dilution experiments work on the principal that the more dilute the sample, the less physical interaction bacteria have with grazers. Grazing is thereby limited by increased space

between predator and prey, allowing for increased bacterial reproduction. Plotted growth rates in whole seawater and dilutions then allow for the extrapolation of data to a dilution factor of "zero," an imaginary dilution at which no grazing would occur, giving an estimated maximum growth rate.

The expected result is that iron-enriched samples will exhibit both higher growth and grazing rates. This would be observable in the plotted results by higher maximum growth rates and steeper regression lines (indicating increased grazing). (See Figure 3 for a graphical example of predicted data.) In addition, a much smaller change is expected for those areas exhibiting relatively high chlorophyll concentrations (for example, in the bay on the west side of Isabela).

Project Budget

Item	Unit Cost	Number	Total	Provided (no actual charge to project budget)
Use and operation of the *R/V Thompson*	$18,000/ day	5 days	$90,000	provided
Deck incubator	$3.00/day	4 days	$12.00	provided
Filter rack/vacuum pump	$6.00/day	5 days	$30.00	
CTD	$135/day	4 days	$540.00	provided
Bottles	$3.00/day	5 days	$15.00	
Filters	?	1 box		
Slides	$8.07/box	1	$8.07	
Bateria-staining dye	?	1		
Epifluorscence microscope	?	1		
Time, patience, guidance of professors and TA			priceless	provided
Total	$18,155.07/day	4-5 days	$90,605.07	

The total cost of the project will be $90, 605.07

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Table 1. The latitude and longitude for each of our four proposed stations.

Deg.	Min.	N/S	Deg.	Min.	W/E
0˚	55.00’	S	90	0.00	W
0˚	55.00’	S	90	40.00	W
0˚	32.00’	S	91	19.50	W
0˚	01.00’	N	91	08.00	W

Fig. 1. Satellite image of chlorophyll concentrations around the Galapagos Islands taken from Feldman (1984).

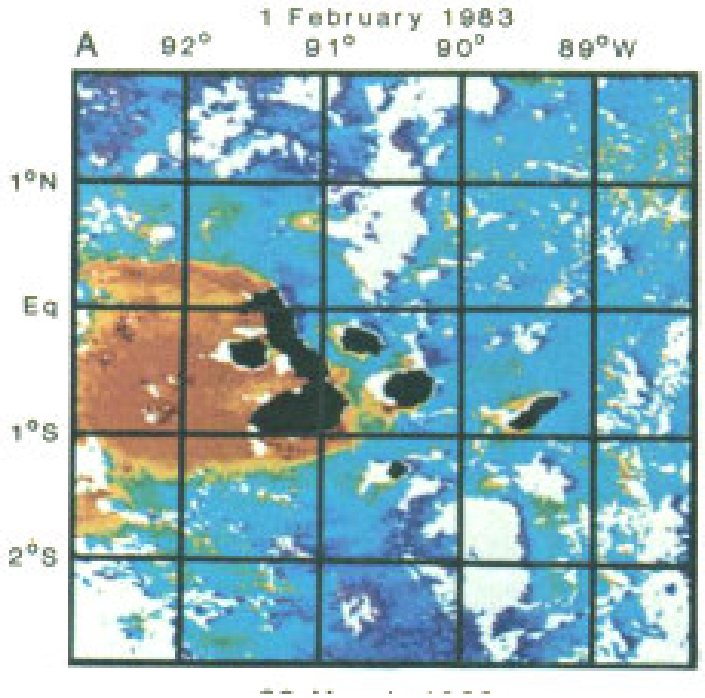

Fig.2. Map of the Galapagos Islands showing proposed cruise path and four stations to be used in this project.

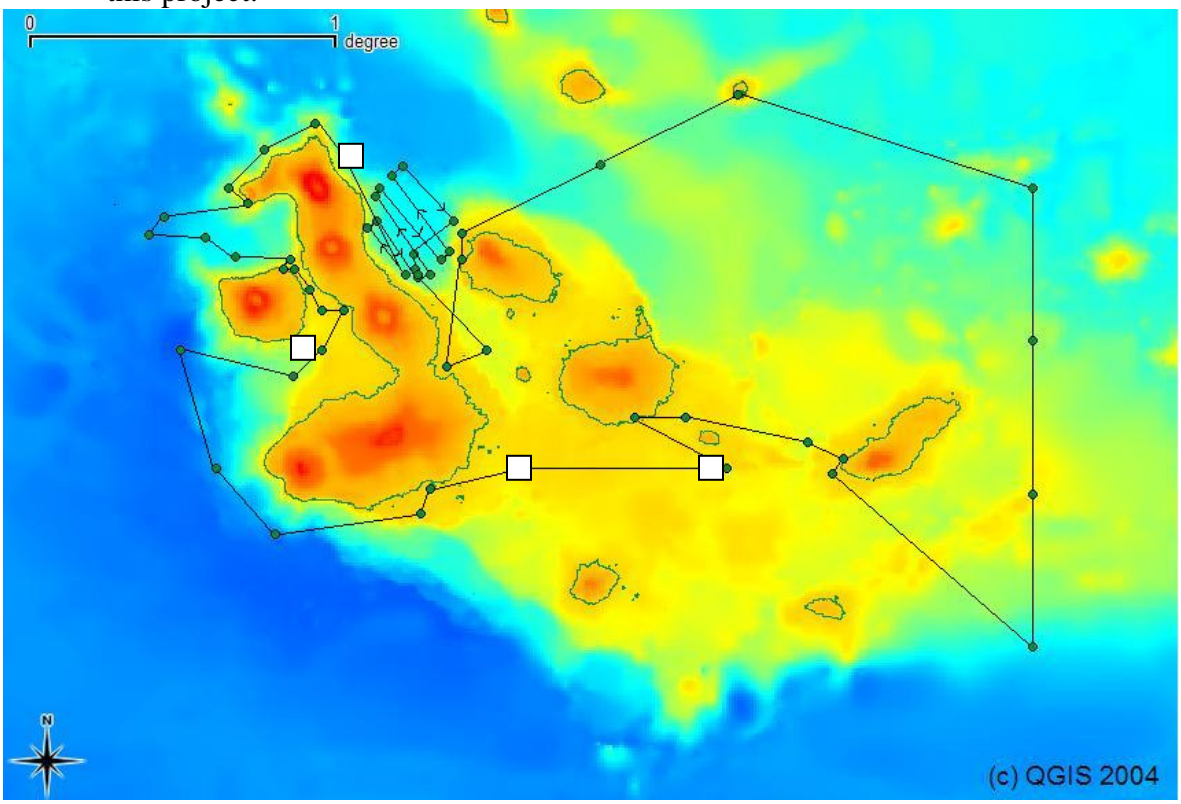

Fig. 3. Example of graphical analysis of a dilution experiment. Taken from Landry (2000).

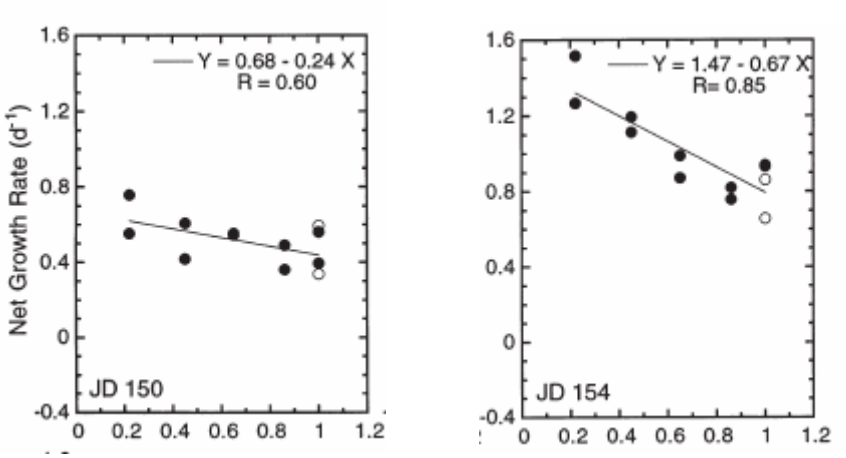

Figure Captions

Table 1. The latitude and longitude for each of our four proposed stations.

Fig.1. Satellite image from Feldman (1984) depicting chlorophyll concentration around the Galapagos Islands during "normal" (non-El Nino) conditions. The red and orange areas are those with higher chlorophyll concentrations; blue and dark blue represent lower chlorophyll concentrations.

Fig.2. Map showing the proposed cruise lines and stations. The four stations at which Wendy and I will be taking samples are shown by white squares.

Fig.3. Taken from Landry (2000), this figure is an example of graphical analysis of a dilution experiment. The left-hand graph represents data expected from an unamended sample, while the right-hand graph represents data from an iron-enriched sample. Note the difference in maximum growth rate (extrapolate to "zero" dilution factor) and slope of the line (indicating increased grazing).