Bacterial growth rates and bacterivory around the Galápagos Islands

[Running title: Bacterial dynamics around the Galápagos]

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2 March, 2006

Non-technical summary

Phytoplankton, which rely on sunlight and nutrients such as nitrogen, phosphate, and iron, serve as the basis of the oceanic food chain. While the phytoplankton in some areas of the world's oceans are limited by a lack of sunlight, nitrogen and/or phosphorus-containing nutrients, there are areas that are high in these nutrients but still exhibit limited growth due to a lack of iron. When iron is added to one of these high-nitrate, low chlorophyll (HNLC) regions, the phytoplankton begin to grow and reproduce more quickly, or 'bloom.' This not only increases the food supply for organisms feeding on phytoplankton, but particles released by phytoplankton, in addition to particles left behind by 'sloppy' feeding habits grazers, serve as food for bacteria. The equatorial Pacific is an HNLC region, with the exception of the area around the Galápagos Islands, where there is commonly a relatively large standing stock of phytoplankton. In this experiment, the growth rates of bacteria and the grazing rates of the organisms that feed on bacteria were measured at two stations around the Galápagos, one thought to have higher iron concentrations than the other. It was predicted that when iron was added to water samples from east of Isabela island, a large increase in growth rate would be seen, because it usually has less phytoplankton, presumably due to having less iron. The results did not support that prediction. In addition, the addition of iron did not significantly increase bacterial growth rates at either station.

Acknowledgements

I must first acknowledge my family and closest friends: my grandmothers, Montana and Eva, Dad, Mom, Darcy, Jon, Kaitlyn, Kelly, Tish. You have always been there for me – if a paper about bacteria doesn't make you proud, abandon hope now. I love you all so much!

My sincerest thanks to all of my classmates and the Leg 2 participants in particular. I have thoroughly enjoyed the unending torture that is 443/444 and am grateful for your support and friendship.

I would like to thank the faculty, Gabrielle, Seelye, Roy, Mark, and yes you, Llyd, without whom the unending torture would not have existed. I have been inspired, contemplative, excited, confused, triumphant, seasick, determined, and exhausted in the course of this psychological experiment you call “443/444” – clearly you are doing your jobs well.

I would like to express my gratitude to the other Iron Siren, Wendy NoMI Guo, without whose visits to the soft-serve stand in Puerto Ayora I might still be able to fit into my ‘skinny jeans.’ Would you rather be a noun-verb, or ice cream?

A special thanks to Pamela Maynard, my partner in bacteria for her support and understanding – do you ever look up at the stars…and absent-mindedly start counting them?

My thanks, as well, to the crew of the Thompson, the ARCS ladies, the sea lions, the boobies, the rubies, the grubies, and anyone who takes the time to read this paper.

I pledge my undying gratitude, as promised, to my mentor and source of courage, the immortal Dr. Richard Keil.

Abstract

Growth rates of and grazing rates on bacteria collected from two sites around the Galapagos Islands were determined using 24-hour dilution experiments modeled after Landry et al. (2000). Stations were chosen because of the expected higher concentration of iron at one of the stations, BIO-2. Bacterial abundance was measured using epifluorescence microscopy. Growth rates were expected to increase with the addition of iron. Statistically significant growth and grazing rates were not determined in either experiment, with or without added iron. Net growth rates from all dilutions indicated that bacteria were growing very slowly, although qualitatively iron addition appeared to increase the net growth rate at station BIO-2. Overall, these results suggest that bacteria at these two stations were not limited by iron.

Introduction

The equatorial Pacific, the subarctic Pacific, and the Southern Ocean are characterized by high nitrate and low chlorophyll concentrations (HNLC). The paradox of HNLC regions has been explained, albeit with the complexity and ensuing questions of any scientific 'answer,' by the 'iron hypothesis' first posited by John Martin. Martin's theory that HNLC regions' productivity was limited by lack of available iron was tested in the first mesoscale iron-enrichment experiment, IronEx I, in 1993 (Martin et al. 1994). Since then, a number of open-ocean iron-seeding experiments have taken place, each with results supporting the iron hypothesis (de Baar, et al. 2005).

In cases where phytoplankton grow so quickly that grazers cannot easily control their population increase, a portion of their production sinks down through the water column, where some of it is eventually buried in the sediment. If this biological carbon export results in the partial pressure of carbon dioxide on the water side of the air-sea interface becoming lower than that of the atmosphere, then carbon dioxide will flux from the atmosphere into the ocean (Coale, et al. 2004). The role of iron in the export of carbon in the ocean has become increasingly important as attention is brought to the effects of anthropogenic carbon dioxide on climate change (Tortell et al. 1996).

Located in the equatorial Pacific, the Galápagos Islands create an anomalous patch in the surrounding waters, which often exhibit a plume of phytoplankton near the islands, generally to west of Isabela island, attributed to iron supplied by upwelling of the Equatorial Undercurrent (Feldman 1984, Gordon, et al. 1998). The existing natural juxtaposition of a higher-chlorophyll area (west side) and a lower-chlorophyll area (east side) offered a unique opportunity to compare growth rates in waters presumably having different iron concentrations (Figure 1, Feldman 1984, http://oceancolor.gsfc.nasa.gov/cgi/tiles.pl?sub=region_timeseries_table&rgn=GalapagosIs).

In addition to increased phytoplankton biomass and growth rates, increased bacterial abundance and production have been shown to occur shortly after iron-induced blooms (Cochlan 2001, Hutchins 2001). 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 DOC from phytoplankton (Banse 1995, Kirchman 2000, 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 insignificant (Tortell et al. 1999).

Collection sites were chosen based on satellite data commonly showing a higher standing stock of phytoplankton at BIO-2 (Figures 1 and 2). This was presumed to indicate that BIO-2, on the west side of Isabela, contained a higher concentration of iron than BIO-6, located on the east side of Isabela. Different results were expected from samples taken in high versus low chlorophyll areas. The stations we chose (Table 1, Figure 2) include areas in which we expected to find variability in chlorophyll concentration. For those areas of higher chlorophyll, little increase in bacterial growth rate associated with iron was expected, based on the assumption that the higher chlorophyll was due to higher iron concentrations. That is, the change in magnitude of growth rates in seawater containing lower concentrations of iron would be larger than that of the growth rates in seawater containing higher concentrations of iron.

Methods

This experiment utilized dilution experiments modeled after Landry (2000). Dilution experiments operate 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 experiments were carried out in collaboration with Wendy Guo, who measured the growth rate of and grazing rates on phytoplankton with iron enrichment. Incubations were carried out in the same bottles. Samples were collected at two using standard CTD rosette sampling techniques (Table 1, Figure 2) at a depth of around 20 m.

Our method of preparation for the incubation experiments was based on that found in Landry et al. (2000), including the addition of 0.5 μM ammonium, 0.03 μM phosphate, and 0.1 nM manganese (final concentrations) (Figure 3). These nutrients were added to the samples to ensure that the phytoplankton were not limited by nutrients other than iron and to encourage constant growth of phytoplankton. For each station, we used 20 transparent 2-L bottles. Ten bottles received the following treatments in duplicate: seawater without added nutrients and nutrient-amended bottles with seawater diluted 1:0, 1:1, 1:4, and 1:9 with 0.2μm filtered seawater. Another 10 bottles received the same treatments but also added iron (2.0 nM $FeCl_3$, final concentration). The bottles were placed in incubators on the ship deck, through which seawater was circulated to maintain consistent temperature. The incubator was then covered with netting to simulate 50% photosynthetically active radiation. Incubations lasted roughly 24 hours, to include an entire diel cycle.

10 ml samples from each bottle were fixed with 0.5 ml 37% formaldehyde (final concentration ~1.8%), then filtered using a Hoeffer box onto 0.2-µm polycarbonate filters. The filters were then placed on slides and the bacteria on the filter stained with 4',6-diamidino-2-phenylindole (DAPI), a DNA-specific fluorochrome, in the mounting medium DAPI-Vectashield. Bacterial enumeration was carried out using an epifluorescence microscope at 1000x magnification. The bacteria in 20 fields per slide were counted, giving an estimate of the number of bacteria per liter of seawater. Samples were taken at the beginning and end of the 24-hour cycle and then used to determine the difference between the two time points to yield an apparent growth rate per dilution, with growth rate calculated assuming exponential growth.

Results

The initial concentrations of bacteria in the unamended (no added nutrients, no added iron), unincubated samples were 2.7×10^9 and 1.2×10^9 cells per liter of seawater at stations BIO-6 and BIO-2, respectively. Apparent growth rates were plotted against the dilution factors (the fraction of unfiltered seawater in each bottle) for both the iron-unamended (top graphs) and iron-amended (bottom graphs) bottles (Figure 4, Figure 5). The slightly negative slope in both treatments at both stations is consistent with possible grazing, though is not statistically different from zero. The extrapolated y-intercept for each graph represents the maximum growth rate of the bacteria, which was about 0.02 day^{-1} for both treatments at BIO-6 and 0.02 day^{-1} and 0.04 day^{-1} for without and with iron, respectively. For both stations, the net growth rate of the bottles with added nutrients was higher for those without iron, but lower for those with added iron (Figure 6).

Discussion

The initial bacteria concentrations of 2.7 x 10^9 and 1.2 x 10^9 cells per liter of seawater fall within the range of previously observed marine bacteria abundances of 10^6 to 10^{11} cells per liter (Chase and Price 1997, Cochlan 2001, Skoog 2002, Maynard 2006). The initial bacterial concentration from BIO-6 was more than twice that of BIO-2. This was unpredicted, as bacterial abundance was expected to be higher at BIO-2, the station exhibiting a higher phytoplankton standing stock at the time of sampling (Guo 2006). However, primary production rates were likely greater at BIO-6 than at BIO-2 (BIO-6 had limited production data) (Gilmore 2006), and assuming that the majority, if not all, net production is lost to grazing, this could plausibly provide increased DOC to bacteria, resulting in a higher bacterial concentration. (It should also be noted that the slides for the first station were counted on the ship, while the slides from the second station were counted back at the University of Washington and with a different microscope.) On the other hand, the concentrations were of the same order of magnitude and were relatively small for such large numbers and so may reflect the error inherent in the counting process.

Although most of the growth rates were positive, a few were negative. Though it was assumed that the concentrations of organisms were relatively consistent, perhaps certain bottles randomly ended up with more grazers (or viruses) than others in the sampling process. Bottles were filled from the same containers, but it may have been possible for the water in a specific

Niskin bottle containing a higher concentration of grazers or viruses to have been transferred into a single carboy and used entirely in certain bottles.

The growth rates ranged from 0.004 day^{-1} to 0.044 day^{-1}. These rates are one to two orders of magnitude smaller (slower) than both expected growth rates and previously recorded growth rates of marine bacteria (Pakulski 1996, Cochlan 2001). The negligible growth rates of and grazing rates on bacteria at both stations is consistent with the initial hypothesis, as little to no growth of and grazing on phytoplankton were observed (Guo 2006).

The addition of iron had no demonstrable effect on the rates observed for either phytoplankton or bacteria at BIO-6, the station that was thought to have less iron (Guo 2006). There appeared to be a small increase in bacterial growth rate at BIO-2, at which larger phytoplankton (>20µm) showed a slight increase in growth with the addition of iron (Guo 2006). These differences were not, however, statistically significant. The hypothesis that growth rates would increase with the addition of iron was not supported. These results accord with the results of a study carried on another leg of the same cruise, in which bacterial growth exhibited no change in growth with the addition or removal of iron (Maynard 2006). Together these findings suggest that the waters neither west of nor east of Isabela are iron-limited.

The comparison of net growth rates between those treatments receiving nutrients and those receiving no nutrients was worth noting (Figure 6). For those not receiving iron, the nutrient-amended samples yielded higher growth rates. However for those receiving iron, the nutrient-amended samples yielded lower growth rates. The highest growth rates (for whole seawater) were for those bottles receiving iron, but receiving no nutrients. Perhaps the addition of iron better served grazers or viruses present in the samples than the bacteria. It is also interesting to note that this nutrient/growth trend was not consistently found in similar data with phytoplankton (Guo 2006). This could be interpreted to indicate that the nutrients encouraged growth only when iron was not added or that the nutrients themselves were detrimental to growth when iron was added. These results were unexpected and do not have a clear cause.

One possible explanation is that the adding of nutrients induced unforeseen behavioral changes which led to changes in growth rates of any or all of the organisms in the bottles or altered the community structure of the bacterial or grazer populations. For example, it has been suggested that diminishing nutrient competition can cause growth rate changes comparable to reduction of predation in such experiments (Dolan 2005). Clearly, unaccounted-for growth was not an issue in this project. However, such observations suggest that the complexity of trophic dynamics with varying nutrient availability allows for the admission that processes, which were unknown or not understood, were at work. If this was the case, then it is possible that the results of the experiment were not representative of the in situ phytoplankton/grazer/bacteria dynamics.

Conclusion

Unamended, unincubated samples yielded initial bacterial concentrations of 2.7×10^6 and 1.2×10^9 bacteria L^{-1} for stations BIO-6 and BIO-2, respectively. Growth rates were extremely low, but most were positive, indicating that the bacteria present are reproducing. Results supported the hypothesis that bacteria would respond to the addition of iron only with a response in phytoplankton. The predicted trend of an increase in growth rate with the addition of iron was supported only with the results from BIO-2, and then only qualitatively. The results of this project, combined with those of projects carried out on the same cruise, indicate that the waters immediately surrounding the Galápagos Islands were not iron-limited at the time of this study.

References

Banse, K. 1995. Zooplankton: pivotal role in the control of ocean production. J. Mar. Sci. **52**: 265-277.

Chase, Z., N. Price. 1997. Metabolic consequences of iron deficiency in heterotrophic marine protozoa. Limnol. Oceanogr. **42**: 1673-1684.

Coale, K., et al. Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science. **304**: 408-414.

Cochlan, W. 2001. The heterotrophic bacterial response during a mesoscale iron enrichment experiment (IronEx II) in the eastern equatorial Pacific Ocean. Limnol. Oceanogr. **46**: 428-435.

de Baar, H. 2005. Synthesis of iron fertilization experiments: from the iron age in the age of enlightenment. J. Geophys. Res. **110**: C09S16, doi:10.1029/2004JC002601.

Dolan, J. 2005. Interactive comment on “Reality checks on microbial food web interactions in dilution experiments: responses to the comments of Dolan and McKeon” by M.R. Landry and A. Calbet. European Geosciences Union: Ocean Science Discussions. **1**: S22-S25.

Feldman, G., D. Clark, D. Halpern. 1984. Satellite color observations of the phytoplankton distribution in the eastern equatorial Pacific during the 1982-1983 El Nino. Science. **226**: 1069-1071.

Gilmore, B. 2006. Primary production around the Galápagos Islands and the effects of cloud cover and differing light regimes. Unpublished Bachelor's Thesis, University of Washington.

Gordon, R., K. Johnson, K. Coale. The behaviour of iron and other trace elements during the IronEx-I and PlumEx experiments in the equatorial Pacific. Deep-Sea Research II. **45**: 995-1041.

Guo, Wendy. 2006. Inter-island comparison of phytoplankton growth rates and herbivory rates. Unpublished Bachelor's Thesis, University of Washington.

Hutchins, D., B. Campbell, M. Cottrell, S. Takeda, S. Cary. 2001. Response of marine bacterial community composition to iron additions in three iron-limited regimes. Limno. Oceanogr. **46**: 1535-1545.

Kirchman, D., B. Meon, M. Cottrell, D. Hutchins. 2000. Carbon versus iton limitation of bacterial growth in the California upwelling regime. Limnol. Oceanogr. **45**:1681-1688.

Landry, M., J. Constantinou, M. Latasa, S. Brown, R. Bidigare, M. Ondrusek. 2000. Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II).III.Dynamics of phytoplankton growth and microzooplankton grazing. Mar. Ecol. Prog. Ser. **201**: 57-72.

Landry, M., D. Kirchman. 2002. Microbial community structure and variability in the tropical Pacific. Deep sea research II. **49**: 2669-2693.

Maynard, P. 2006. Bacterial abundances around the Galápagos Islands. Unpublished Bachelor's Thesis, University of Washington.

Martin, J. et al. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature. **371**: 123-129.

Pakulski, J., R. Coffin, C. Kelley, S. Holder, R. Downer, P. Aas, M. Lyons, W. Jeffrey. 1996. Iron stimulation of Antarctic bacteria. Nature. **383**: 133-134.

Skoog, A., K. Whitehead, F. Sperling, K. Junge. 2002. Microbial glucose uptake and growth along a horizontal nutrient gradient in the north Pacific. Limnol. Oceanogr. **47**: 1676-1683.

Tortell, P., M. Maldonado, N. Price. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature. **383**: 330-332.

Tortell, P., M. Maldonado, J. Granger, N. Price. 1999. Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiology Ecology. **29**: 1-11.

Table 1. The latitude and longitude of each station.

Station	Date	Deg.	Min.	N/S	Deg.	Min.	W/E	Depth
1 (BIO-6)	21/01/06	0°	55.02’	S	89°	59.96’	W	23.4
2 (BIO-2)	23/01/06	0°	36.98’	S	91°	19.00’	W	21.7

Figure Captions

Figure 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.

Figure 2. Map showing the three stations at which samples were taken. [Image from 23 January, 2006, taken by satellite from NASA’s SeaWiFS (Sea-viewing Wide Field-of-view Sensor) Project, available at: <http://oceancolor.gsfc.nasa.gov/cgi/tiles.pl?sub=region_timeseries_table&rgn=GalapagosIs>]

Figure 3. Schematic of the dilution experiment, showing added nutrients and iron.

Figure 4. Results of the dilution experiment from Station 1. Top graph shows growth rates without iron; bottom graph shows growth rates with iron (all with added nutrients).

Figure 5 Results of the dilution experiment from Station 2. . Top graph shows growth rates without iron; bottom graph shows growth rates with iron (all with added nutrients).

Figure 6 Growth rates of whole seawater samples with and without nutrient and iron additions, from both Stations 1 and 2.

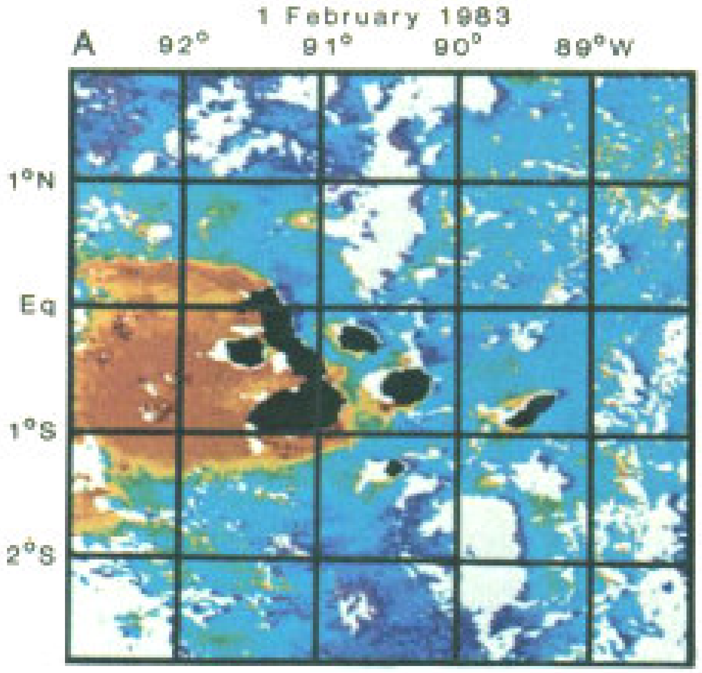

Figure 1

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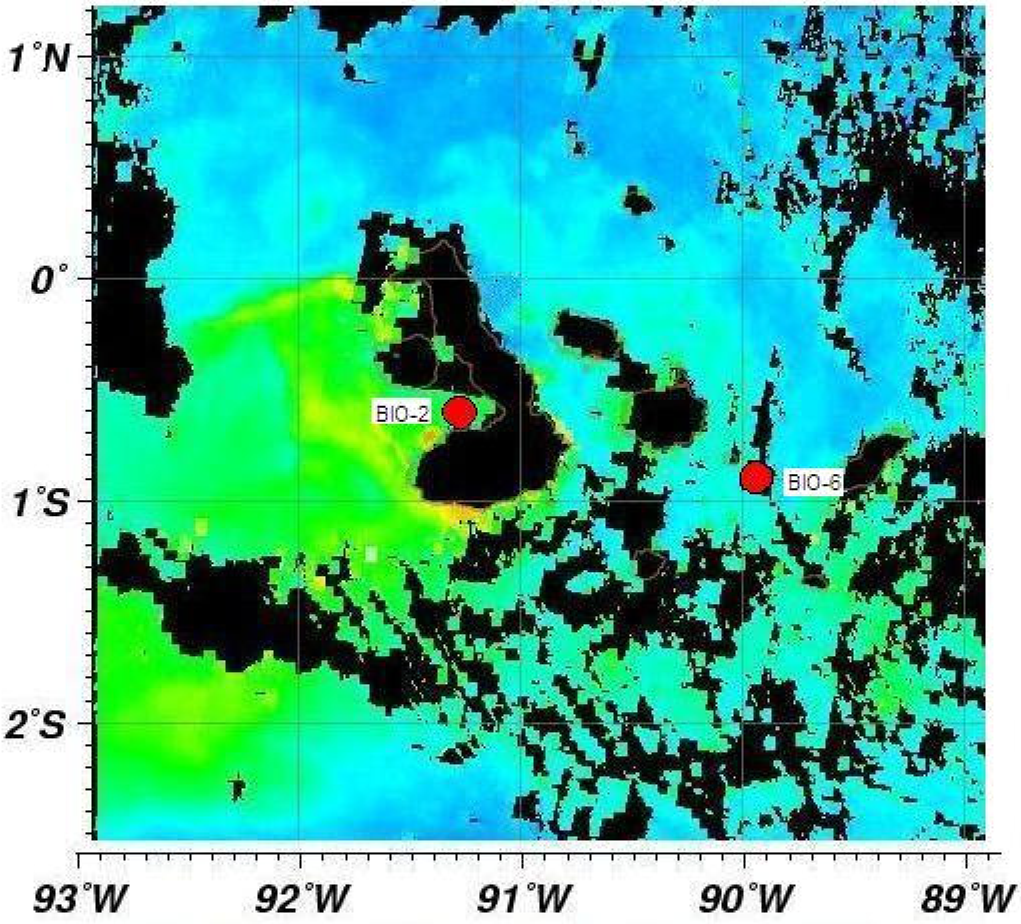

Figure 2

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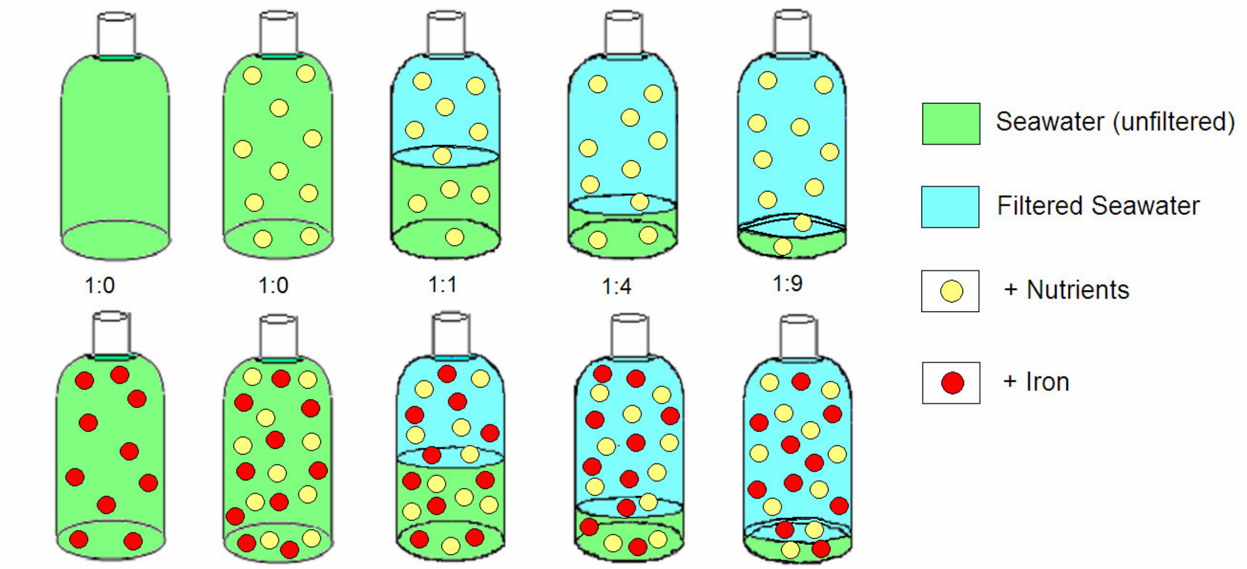

Figure 3

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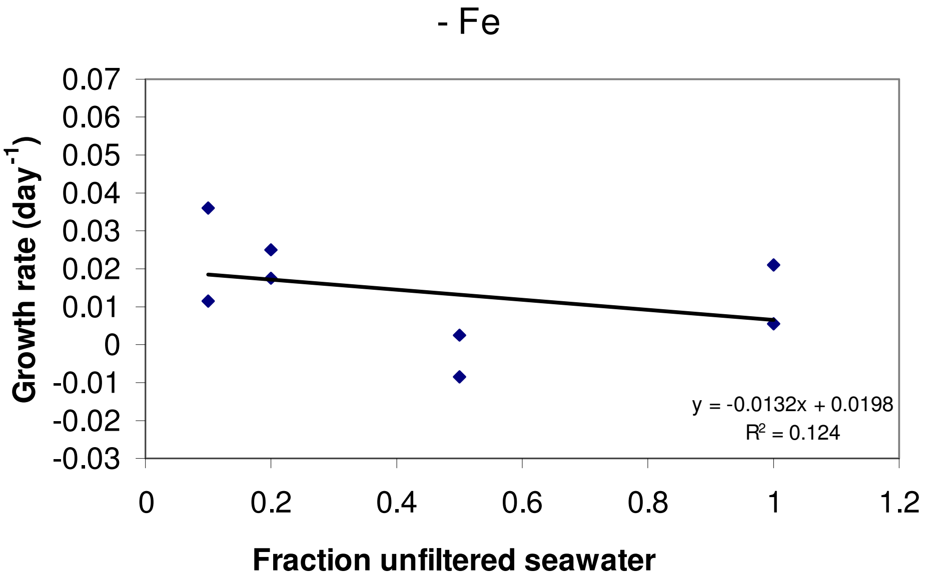

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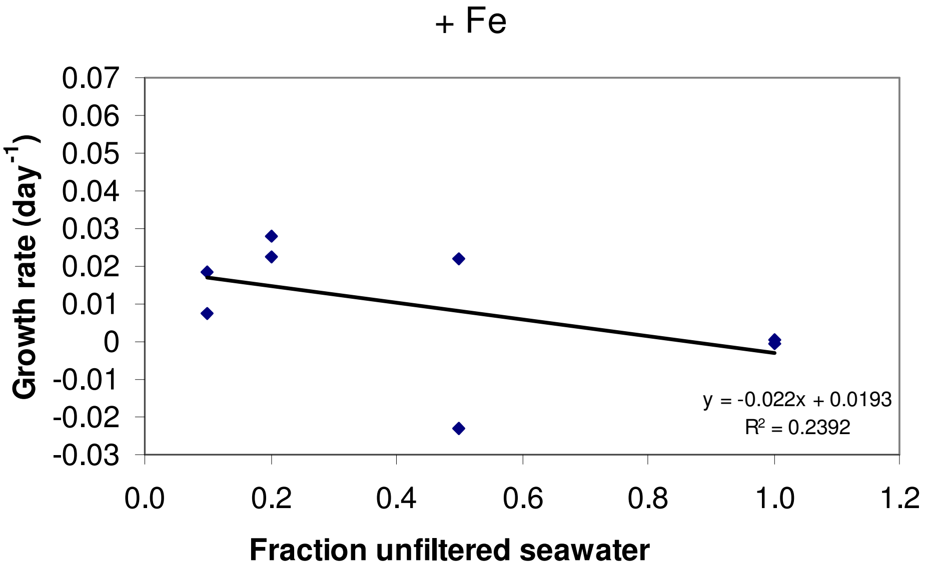

Figure 4

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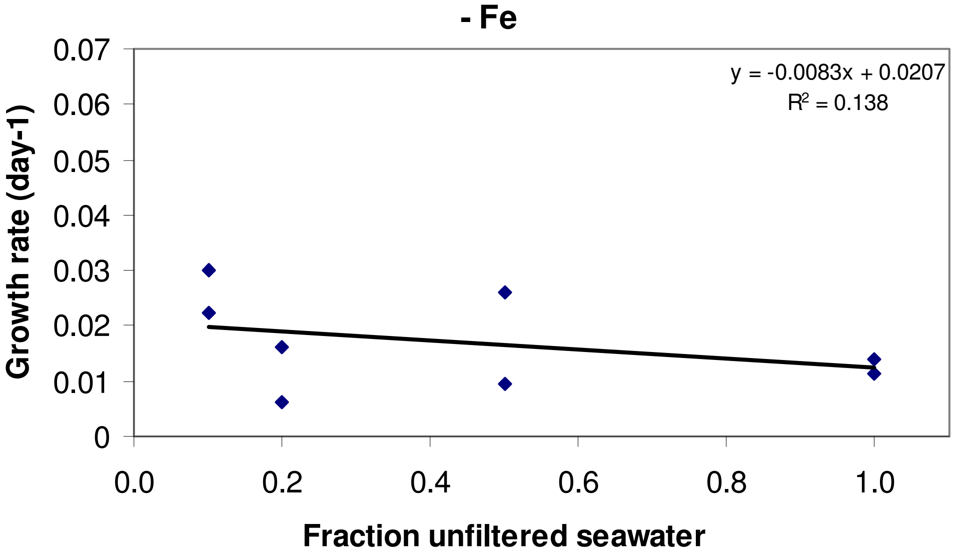

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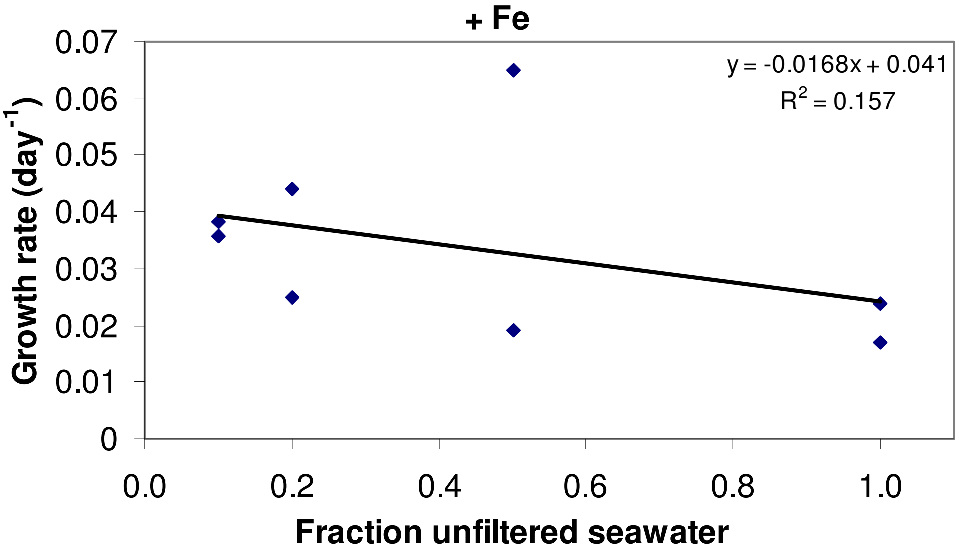

Figure 5

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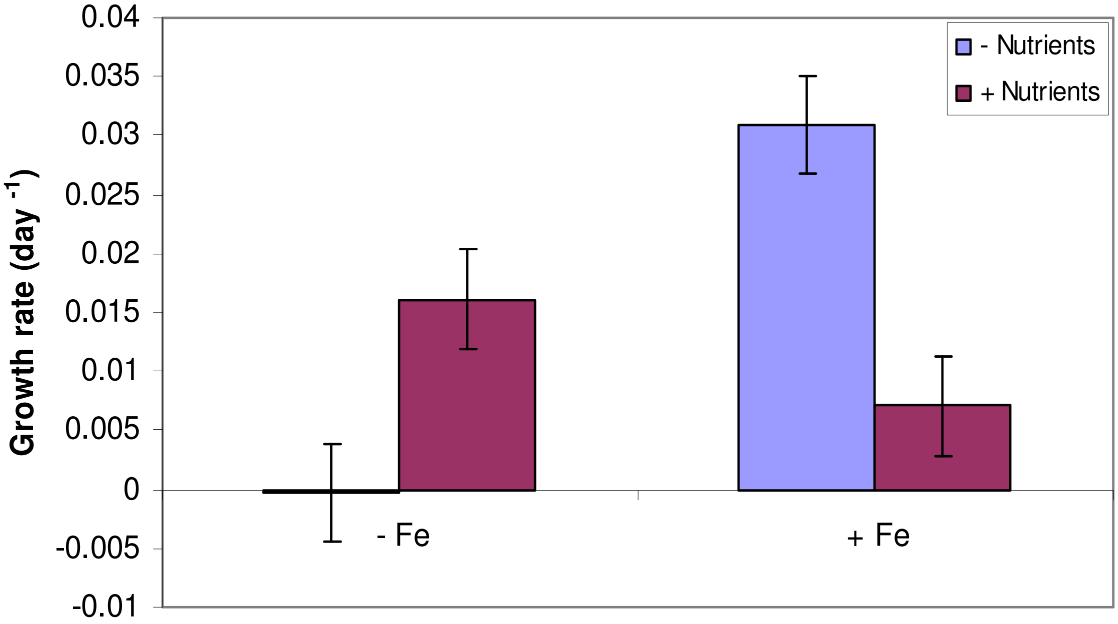

Figure 6

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