Bacterial abundances and their relationship to iron around the Galápagos Islands, Ecuador

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March 11th, 2006

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Non-Technical Summary

Iron plays a large role in regulating how much primary production occurs in the ocean. Studies have shown that in High-Nitrate-Low-Chlorophyll (HNLC) regions where there are plenty of nutrients but low phytoplankton biomass, phytoplankton can be limited by a lack of iron. Phytoplankton produce carbon that bacteria use for growth and can that is recycled back into the food web when bacteria are eaten/lysed. Bacteria also need iron for their life processes and research has show that seawater iron concentrations may affect how many bacteria are present in a certain volume of seawater at any depth. This project hypothesized that the islands provided some form of iron source for the bacteria. In an attempt to characterize the influence of an iron source on bacteria around the Galapagos Islands, bacteria were sampled at the surface, the shallow subsurface (10-25m), and at 250m, 500m, and 1000m, from the R/V Thomas G. Thompson during 13-15 January 2006. The bacteria in these samples were then counted to get an idea of bacterial abundances at each depth. Total bacterial numbers decreased with depth, in concurrence with what others have found. Below 250m bacterial concentrations were approximately equal at all stations. However, the surface and shallow subsurface bacteria concentrations varied when across sampling sites. At two stations the total number of bacteria was greatest in the shallow subsurface, where there was a chlorophyll maximum, while at the rest the bacterial concentration was highest at the surface. No distinct cause was found for why this variability among stations occurred, but it may be explained by carbon availability for bacteria to feed upon. In addition, water samples incubated unamended, with added iron, or with a chemical intended to remove iron to examine possible effects of iron on bacterial growth over 72 hours. There was no significant differences among treatments, suggesting that bacteria around the islands are not strongly influenced by iron availability.

Acknowledgements

I would like to thank the generous Achievement Rewards for College Students (ARCS) donors who graciously funded a major portion of this project. The University of Washington's School of Oceanography also contributed both support and additional funding. Many thanks to Professor Roy Carpenter for leading this venture and Professors Gabrielle Rocap, Seelye Martin, Mark Holmes, for their comments, corrections and criticisms. Additionally, a big thanks to Llyd Wells for his encouragement, enthusiasm, and extra effort and time helping out another crazy bacteria fiend. Lastly I would like to thank my classmates for their encouragement and editing skills.

Abstract

The highly productive waters around the Galapagos Islands are an anomaly in the eastern equatorial Pacific, an otherwise High-Nitrate-Low-Chlorophyll (HNLC) region are characterized by relatively low phytoplankton biomass despite available nutrients. The islands in this area may contribute to this phenomenon by influencing seawater iron concentrations. Studies have shown that phytoplankton growth and productivity are limited when iron is deficient. Bacteria can also be iron limited and show an increase in number corresponding to an increase in phytoplankton growth when iron is added to HNLC waters. During 13-22 Jan 2006, bacterial concentrations at seven stations around the Galapagos Islands were determined aboard the R/V Thomas G. Thompson to investigate the relationship of bacterial concentration to proximity to potential iron sources. Samples were taken at the surface, the shallow subsurface (10-25m), and at 250m, 500m, and 1000m and counted via epifluorescence microscopy. Bacterial concentrations decreased by an order of magnitude from the surface to 1000m at all sites. At the surface and shallow subsurface (10-25m), they were considerably more variable, ranging between 0.64×10^6 - 3.13×10^6 bacteria ml^{-1} and 0.58×10^6 and 2.14×10^6 bacteria ml^{-1}, respectively. No recognizable pattern or relationship to water properties or distance to the islands was noted to explain these variations. In addition, an incubation experiment was conducted from water collected at the chlorophyll maximum from a relatively low chlorophyll standing stock site and incubated for 72 hours either unamended, with added $FeCl_3$, or with an added iron chelator (DFOM, intended to remove iron) amendments to determine bacterial response to iron addition and removal. No statistically significant difference between amendments was observed after 72 hours suggesting that iron had little effect on bacterial growth. Thus, bacterial numbers may not be directly influenced by iron in this region but rather by organic carbon, nutrient concentrations, or biological interactions.

Introduction

The highly productive Galapagos region exists as an anomaly in the Equatorial Pacific. Past research has demonstrated that the Equatorial Pacific is an area of high nutrients including notably high levels of nitrate, but unusually low productivity, commonly referred to as an high-nitrate-low-chlorophyll (HNLC) zone (Cullen 1991). In contrast, around the Galapagos Islands, satellite data show relatively high chlorophyll concentrations for the Equatorial Pacific, especially on the western side of Isabela Island (Feldman 1986). This area of anomalous chlorophyll sometimes extends past 98º W into the Equatorial Pacific (Feldman 1986). Higher primary production has also been measured west of the Galapagos Islands at 92° W as compared to low production within surrounding waters some distance from the islands (Chavez and Barber 1987).

The distinct physical oceanography of the region contributes to an explanation of this anomaly. The Equatorial Undercurrent (EUC) surfaces in the area of the Galapagos Islands when it encounters the steep topography of the Galapagos Platform on the western side of Isabela Island (Feldman 1986, plate 1b; Barber and Chavez 1991; Chavez and Brusca 1991; Binder et al. 1996). This upwelling is thought to supply surface waters with both nutrients from depth and iron derived from iron-rich platform sediments (Martin and Gordon 1988; Barber and Chavez 1991,). This additional supply of iron near the air-sea interface may stimulate and support the high levels of chlorophyll and productivity observed (Chavez and Barber 1987; Cullen 1991), as suggested by in-situ iron enrichment experiments in nearby HNLC regions of the Equatorial Pacific (Martin et al. 1994).

In addition to phytoplankton, bacteria also appear to benefit from increased iron concentrations. For example, the IronEx (II) experiment in the Eastern Equatorial Pacific documented that when iron was added, bacterial abundances increased as phytoplankton

numbers increased (Cochlan 2001). Even so, it's not clear whether this iron addition directly or indirectly stimulated bacterial growth (Cochlan 2001).

The increase in bacterial numbers as a direct result of iron stimulation requires further study. Bacterial population increases due to iron addition have also been observed in the Antarctic Ocean and in other studies of the Eastern Equatorial Pacific but not in all HNLC regions (Price et al. 1994; Pakulski et al. 1996; Church et al. 2000; Cochlan 2001). Where bacterial increase is noted, the increase in relative numbers may be directly due to iron addition or indirectly related by increased dissolved organic carbon (DOC) concentrations as a result of an iron-induced increase in phytoplankton concentrations or productivity, as demonstrated in previous bottle experiments (Kirchman et al. 2000; Cochlan 2001; Fig 1).

This study assumed that iron concentrations decreased with distance from the islands and that bacteria concentrations would reflect this by also decreasing with distance from the islands. Bacteria were enumerated along an east-west transect and at multiple water depths in a productive region and at two relatively low phytoplankton standing stock regions around the Galapagos Islands to provide data relevant to how iron concentration distributions in this region affected bacterial numbers. Additionally, an iron incubation experiment was conducted to determine how bacterial populations responded to iron addition or removal using a siderophore. These data were then compared with the survey of in-situ bacterial abundance to try to determine iron's influence upon bacteria in the region.

Methods

Water column sampling and bacterial enumeration

To assess in-situ bacterial numbers in relation to distance from the islands, sampling occurred at 7 stations located around the Galapagos Archipelago (Fig. 2 and Table 1). Stations PM1 and PM2 were located in areas of relative low phytoplankton standing stock, WT0 in an

area of high chlorophyll, and stations PM3-PM6 were sampled along a proposed iron gradient reflecting distance from land. Sampling of stations PM3-PM6 proceeded along 0º 37'S from 92ºW into Elizabeth Bay, located near Isabela Island. Stations PM1 and PM2 were sampled to the southwest of Isabela Island while station WT0 was sampled to the northwest of Fernandina Island.

A Seabird SBE 911 CTD package mounted upon a rosette generated a depth profile of salinity, oxygen concentration, chlorophyll concentrations, and ambient light levels at all stations. Water samples were collected at the surface, shallow subsurface (10-25m), and at most stations 250m, 500m, and 1000m using PVC Niskin sampling bottles mounted upon the CTD rosette (Table 2). Shallow subsurface samples were intended to be taken at the chlorophyll maximum, but only stations PM2, PM4 actually sampled the chlorophyll maximum. (Chlorophyll maxima depths ranged from 5-30m.) One depth was sampled in triplicate at each station (except WT0, where no replication was attempted) such that all the depths were replicated at least once over the course of sampling (Table 3).

Once brought to the surface, water from the CTD rosette was transferred to 500 ml containers and brought into the laboratory for analysis. Aliquots of 10 ml seawater were withdrawn and fixed with 0.2-µm filtered 1.5% formaldehyde (final concentration) and filtered onto an Osmotics white poretics 0.2-µm pore size, 25-mm diameter polycarbonate membrane using a Hoeffer box filtration system. These filters were then stained upon a glass slide with one drop of the DNA-specific stain 4',6-diamidino-2-phenylindole (DAPI) mixed with Vectashield mounting medium and set with gentle pressure on the slide cover to force the DAPI to the edges of the filter. Fixed and stained slides were frozen until analysis to prevent decay of the dye.

Some samples were counted shipboard, while others were counted upon return to the University of Washington. Onboard enumeration involved thawing and then exciting samples

with ultraviolet (UV) light under an epifluorescence microscope at 1000x magnification. Twenty fields per slide were counted for good statistical accuracy. Slides not counted shipboard were kept as cold as possible in a cooler packed with ice packs during transit to the University of Washington and were placed in freezers or refrigerators when possible. Once there, further counts were conducted using the protocol outlined above for ship-based analysis, but at a higher magnification of 1562.5x on a Zeiss epifluoresence microscope. To confirm that samples which are immediately fixed, filtered and stained, and thereafter returned cold to the laboratory, have insignificant cell losses (as reported by Turley and Hughes 1992), one slide counted shipboard was recounted at the University of Washington and showed negligible decay.

Incubation Experiment

In concert with Trina Lichendorf, one iron enrichment bottle experiment was conducted at station PM1, an area of relatively low pigment concentration when compared to the surrounding region (Fig. 2), to investigate whether iron stimulates an increase in bacterial abundance. This study examined the effect of both adding and removing iron from the system, compared to an un-amended control. Seventy liters of water from the chlorophyll maximum was filtered through a 200µm Nitex filter to remove larger zooplankton, collected, and transferred into five 10 L and one 20 L acid washed carboys and brought into the lab. One ml of 2nmol L^{-1} $FeCl_3$ solution mixed in a 1:1 ratio with the chelator ethylendiamintetraacetic acid (EDTA), was added to 3 of the 10 L carboys to a final concentration of 2 nmol L^{-1}. This concentration is almost twice the highest concentration (1.3 nmol L^{-1}) reported by Martin et al. (1994) just west of Isabela Island. The remaining carboys were left un-amended and used for both survey and experimental work.

The incubation was conducted in a total of 9 acid-washed polycarbonate 500 ml bottles that were wrapped in foil in an effort to limit photosynthetic production. Three control bottles

were filled from the un-amended 20 L carboy and had no further alterations made to them. Three 500 ml acid-washed, foil-wrapped bottles were filled with iron-amended seawater while Trina used the remainder of the amended seawater for her incubations. A further three 500 ml bottles were filled with seawater amended with 100 nmol L^{-1} Deferoxamine Mesylate Salt (DFOM), a siderophore here intended to remove bioavailable iron from the system (Hutchins et al. 1999; Wells 1999). These nine bottles were incubated in a screen-covered, on-deck incubator at sea surface temperature for 72 hours. In addition to enumerating initial (t_0) concentrations, sampling took place at 29, 49, and 72 hours to estimate increases in bacterial abundances. Samples were filtered, stored, transferred, and counted as described above.

Results

Survey of bacterial abundance

Overall, surface bacterial counts were non-uniform in distribution over the entire sampling area, showing no distinct correlation with distance to the Galápagos (a likely iron source). Over the full 1000m depth-profile, overall bacterial concentrations consistently declined with depth at all stations (Fig 3). All bacterial counts were on the order of 10^6 in the surface waters and about an order of magnitude lower at 1000m. At station PM1, located in an area of relatively low phytoplankton standing stock, surface bacteria numbers were 1.5 x 10^6 bacteria ml^{-1}, half highest bacterial concentration found in surface waters (at PM6, an area of noted higher chlorophyll; Fig. 4A). Station PM2 surface bacteria counts (located in an area of low chlorophyll stock) were low but similar to stations to those PM3 and PM4, which were located in areas of higher chlorophyll concentrations (Fig. 2, 4A). Station PM6, located directly within Elizabeth Bay, recorded the highest bacterial counts at the surface of surface samples, on the order of 3.1 x 10^6 bacteria ml^{-1} and was similar to station PM5 surface bacteria numbers. WT0

was sampled in the region of highest chlorophyll, had fewer bacteria than in Elizabeth Bay, but more than stations PM1-PM4 (Fig. 4A).

Additionally, at stations PM2 and PM4, bacteria maxima corresponded to the local fluorescence maxima as measured by the CTD (Figs. 5A, 5B). These bacterial maxima did not correspond consistently with any other water property measured by the CTD (Figs. 5A, 5B) or with SeaWiFS sea surface chlorophyll data (Fig. 2). Furthermore, at all stations, surface and shallow subsurface bacteria concentrations showed more spatial variation than at depths of 250 to 1000m (Fig. 3)

Surface bacteria concentrations taken along the proposed iron gradient, which formed an eastward transect from 92ºW toward Elizabeth Bay, also showed no distinct correlation to distance from Isabel Island. Bacterial numbers from surface samples taken at stations PM 3 – PM6, though all on the order of 10^6 bacteria ml^{-1}, were a factor of about two or three higher at near-shore stations (PM5 & PM6) than the other two stations (PM2 & PM3) (Fig 4A and Table 4). Since the standard error of bacteria concentrations at stations PM5 and PM6 do not overlap with those at PM3 and PM4, these differences are likely significant.

Bacteria concentrations in the shallow subsurface were slightly lower than at the surface and had a different spatial distribution (Fig. 4A, 4B). Bacterial concentrations were highest at PM5 (2.14×10^6 bacteria ml^{-1}) with abundances at the additional stations statistically similar to one another within standard error (Fig 4B).

Hydrographic Conditions

High bacterial abundances did not correlate with any individual hydrographic property or with any set of conditions at a given station. Though there is often a correlation between phytoplankton production and bacteria, high fluorescence values did not correspond with high bacterial numbers. Fluorescence was highest at WT0 (7.6 CTD fluorescence units) (Fig. 5A) but

bacterial values were highest at PM6 ($3.13x10^6$ bacteria ml^{-1}) which had a low fluorescence of ~1.4 CTD fluorescence units (Fig. 5B). Conversely, PM2 had the lowest bacterial abundances ($0.64x10^6$ bacteria/ml) and the second lowest fluorescence (2.99 CTD fluorescence units) (Fig . 6A). Chlorophyll maxima were located at 13m at PM1, 21m at PM2, 8m at PM3, 6m at PM4, 11m at PM5, 33m at PM6, and 6m at WT0 showing no relationship between maxima depth and abundance. Abundances also showed no relationship to oxygen, but oxygen did correlate to fluorescence. WT0 the highest concentrations of phytoplankton and thus the highest surface oxygen concentrations (4.5 ml L^{-1}), due to phytoplankton oxygen production (Fig. 5A), but had only the third-highest bacterial surface concentrations (Fig .4A). Surface oxygen concentrations at PM2 were neither the highest nor the lowest though it had the least number of surface bacteria. Salinity appeared to have no correlation to bacteria concentrations. PM6 had second–highest salinity, second only to PM4 where bacterial numbers were also low ($0.83x10^6$ bacteria ml^{-1}) (Figs. 5B, 6B). PM2, having the lowest number of bacteria, also had a low salinity, negating the idea that salinity corresponds to bacteria numbers in this region. Temperature effects, though they are known to influence growth rates, showed little correspondence in this region either. Stations PM1 and PM2 were warmer than PM3-WT0 but do not show a clear pattern relating temperature (Fig. 7). Stations of the highest and lowest abundance are different temperature, with PM2 warmer than PM6. However, PM1 must be included with the other stations with high abundance (>10^6 bacteria ml^{-1}) (Fig. 4A) and since PM1 was warmer than PM6, cooler temperatures do not necessarily indicate higher bacterial concentrations. Additionally, no station displayed characteristics that made it stand out against the other stations and be correlated to high or low abundances. Therefore, no direct connection between water properties and abundances was noted.

Bacterial dynamics during incubation

The unamended, $FeCl_3$- and DFOM-enriched incubations differed little from one another in terms of bacterial concentration change over time (Table 7). Each bottle had approximately 24 hours of delay before a sharp increase in bacterial concentration occurred over a 24hr period (Fig. 8). Bacterial numbers reached their peak around 48 hours and a slight decline in numbers thereafter. A net increase in number compared to initial populations over the 72-hour period was observed in all treatments.

Discussion

Although surface concentrations of bacteria were variable, they showed no distinct correlations with distance from the Galápagos Islands (and thus an iron source) (Figs 3, 4A). Additionally, no decrease in surface bacterial abundance was noted along the W-E transect from 92ºW, contrary to expectations of an iron gradient and associated bacteria gradient. Supporting these observations the incubation experiment showed no effect of iron addition or presumed removal on bacterial growth or total concentration. The lack of difference between treatments may be due to a number of factors. The sample collection was not done in a trace-metal-free fashion and so contamination may have been introduced at any step of the process. Even if free of contamination, the area may not be iron-depleted due to the strong influence of the EUC. Thus adding iron would not have influenced the bacterial concentrations positively. Research has shown that bacteria use siderophores for iron-uptake (Mioni et al. 2005); therefore, DFOM may have acted to make the iron more bio-available contrary to the goal of its addition. Alternately, the bacteria may have used the DFOM as a source of carbon. Finally, iron simply may not be important to bacterial dynamics in this region at least during this sampling period. The upwelling of the EUC and Aeolian transport of iron may supply in full the iron needs of the bacteria in this region.

Although there was no difference between treatments, each group of bottles in the incubation experiment demonstrated no increase in bacterial number for 24 hours, then saw a large increase between 24 and 48 hours, and then finally a decline in numbers after 48 hours. This pattern of growth is consistent with findings in other grow out experiments in coastal waters where no change in numbers was noted during exponential growth of the phytoplankton: in one experiment bacterial numbers increased noticeably after nitrate was depleted and phytoplankton began to die and release DOM, and numbers continued to increase for several days before falling, presumably after substrates became limiting (Wetz and Wheeler 2004). In the case of the incubations reported here, bacterial increases in all bottles may have resulted from DOM release due to phytoplankton stressed by sunlight deprivation. Additionally, this incubation was conducted in 500ml bottles, which might have biased overall numerical values or changed overall population dynamics. The 24-hour lead-time for bacterial growth may be the time it took the population to shift to species that favored the bottle conditions. Small bottles have the potential to facilitate bacterial growth on bottle walls or skew bacteria/grazer relationships (Wetz and Wheeler 2004). Samples were whole water filtered through a 200μm mesh, which was intended to only filter out the larger zooplankton grazers and thus may also have shifted ecosystem dynamics within the bottles by removing the largest predators.

Since iron did not appear to be regionally important to the explanation of surface bacterial distribution, other factors were considered. No distinct water properties measured by the CTD correlated with surface bacterial abundances, however nutrient or dissolved organic carbon (DOC) concentration was not determined during this cruise but may be the largest influencing factor upon bacterial surface concentrations leading to the variability since these are food and energy sources. In fact, research has shown that in eutrophic regions, bacterial numbers are regulated by access to nutrients whereas in oligotrophic regions bacteria concentrations are

first limited by grazing/lysis and then by nutrient concentrations (Gasol et al. 2002). In prior work in the Equatorial Pacific, Kirchman and Rich (1997) and Kirchman et al (2000) found that DOC availability was a more important factor regulating bacterial abundance than iron. The high, spatially variable productivity suggested by the abundance of chlorophyll in the region may serve as a source for DOC (Fig. 1) as phytoplankton blooms die off or are grazed upon (Wetz and Wheeler 2004). Variations in bacterial abundance may also reflect larger ecosystem dynamics including bactivory and viral lysis.

Maximum surface bacteria numbers from stations PM5 and PM6 are up to 9 times the surface concentrations of bacteria at 131º30'W (Hyun and Yang 2005). This can possibly be accounted for by the eutrophic conditions around the Galápagos Islands. Many of this study's stations can possibly considered coastal stations while samples at 131º31'W are from open-ocean, oligotropic conditions. JGOFS averages for equatorial heterotrophic bacteria in the top 80m at the equator between 130ºW and 160ºW in the Pacific range from 0.5-0.9 x 10^6 bacteria ml^{-1}, which is roughly equivalent to bacterial numbers seen at stations PM2-PM4 (Landry and Kirchman 2002). This then suggests that when stations PM2-PM4 are considered against PM5 and PM6, PM2-PM4 may be more open-ocean-like, with different ecological controls than the stations nearer Isabela Island.

In the subsurface there is little evidence for a correspondence between bacterial abundances and proximity to the islands, or between water properties (Fig. 4B). There is some relationship between chlorophyll concentration and bacterial concentrations, as noted in the bacterial maximum at stations PM2 and PM4 (Fig 5) but this is not consistent across the data set. This may be due to failure to sample precisely at the chlorophyll maximum. On several occasions the true maximum was missed by a number of meters.

The consistent decline of bacterial numbers over the 1000m depth-profiles (Fig. 3) parallels similar observations throughout equatorial region (Karner et al. 2001; Landry and Kirchman 2002; Brown et al 2003; Hyun and Yang 2005). This decrease in bacterial numbers may be attributed to multiple factors. Studies have shown that C:N:P ratios vary between surface and deep waters and that DOC concentrations are much less in deep waters than at the surface due to remineralization processes (Martin and Fitzwater 1992; Hopkinson and Vallino 2005). Thus, since the major source of DOC is surface primary production, bacterial access to DOC for life processes decreases with depth and so should limit growth. Temperature decreases with depth also slow bacterial reproduction rates, which could impact bacterial numbers and contribute to the decline in numbers with depth (Shia and Ducklow 1994). Lastly, the community makeup of bacteria shifts with depth and so may be reflected in bacterial numbers (Karner et al. 2001; DeLong et al. 2006).

Conclusion

Bacterial abundances among the islands have many complex factors that may influence them. Iron does not appear to be a major influencing factor on bacterial distribution however, as indicated by the absence of significant differences between iron-amended or iron-removed treatments within the incubation experiment. Instead, other factors must play a role. No discernable correlation between water properties and bacterial abundances were noted, however. Nutrients and DOC concentrations were not sampled during the course of this research so their influence could not be confirmed. Even so, the possibility exists that nutrient or DOC availability plays a strong role in influencing total concentrations.

References

Barber, R. T. and Chavez, F. P. 1991. Regulation of primary productivity rate in the Equatorial Pacific. Limnol. Oceanogr. **36**:1803-1815

Binder, B. J., S. W. Chisholm, R. J. Olson, S. L Frankel, A. Z. Worden. 1996. Dynamics of picophytoplankton, ultraphytoplankton and bacteria in the central equatorial Pacific. Deep-Sea Res. Pt. II. **43**:907-931

Brown, S.L., M.R. Landry, J. Neveux, C. Dupouy. 2003. Microbial community abundance and biomass along 180º transect in the equatorial Pacific during an El Niño-Southern Oscillation cold phase. Jour. Geophys. Resear. 108:C12,8139

Chavez, F.P. and R.T. Barber. 1987. An estimate of new production in the Equatorial Pacific. Deep-Sea Res. **34**:1129-1243

---------- and R.C. Brusca. 1991. The Galapagos Islands and their relation to Oceanographic processes in the Tropical Pacific, p 9-33. *In* M. J. James [ed.], Galapagos Marine Invertebrates – Taxonomy, Biogeography, and Evolution in Darwin's Islands. Academic.

Church, M.J., D.A. Hutchins, H.W. Ducklow. 2000. Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean. Appl. Environ. Microb. **66**(2):455-466

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

Cullen, J.J. 1991. Hypotheses to explain high-nutrient conditions in the open sea. Limnol. Oceanogr. **36**:1578-1599

Delong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, S.W. Chisholm, D.M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science **311**:496-503

Feldman, G.C. 1986. Patterns of phytoplankton production around the Galapagos Islands, p 77-106. *In* M. Bowman, R. Barber, C. Mooers [eds.], Coastal and Estuarine Studies: Tidal Mixing and Plankton Dynamics. Academic.

Gasol, J.M., C. Pedros-Alió, D. Vaqué. 2002. Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. Antoine van Leeuwenhoek **81**:435-452

Hutchins, D.A., V.M. Franck, M.A. Brzezinski, K.W. Bruland, 1999. Inducing phytoplankton iron limitation in iron replete coastal waters with a strong chelating liquid. Limnol. Oceanogr. **44**:1009-1018

Hopkinson, C.S. and J.J. Vallino. 2005. Efficient export of carbon to the deep ocean through dissolved organic matter. Nature **433**:142-145

Hyun, J. and E.J. Yang. 2005. Meso-scale spatial variation in bacterial abundance and production associated with surface convergence and divergence in the NE equatorial Pacific. Aquat. Microb. Ecol. **41**:1-13

Karner, B.K., E. F. Delong, D. M. Karl. 2001. Archeal dominance in the mesopelagic ocean. Nature **409**: 507-510

Kirchman, D. L. and J. H. Rich. 1997. Regulation of bacterial growth by dissolved organic carbon and temperature in the Equatorial Pacific Ocean. Micro. Ecol. **33**:11-20

---------- B. Meon, M.T. Cottrell, D. A. Hutchins, D. Weeks, K. W. Bruland. 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnol. Oceanogr. **45**:1681-1688

Landry, M.R. and D.L. Kirchman. 2002. Microbial community structure and variability in the tropical Pacific. Deep-Sea Res. Pt. II **49**:2669-2693

Martin D.L., B. Meon, M.T. Cottrell, D. A. Hutchins, D. Weeks, K. W. Bruland. 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnol. Oceanogr. **45**:1681-1688

Martin, J.H. and R.M Gordon. 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. Deep-Sea Res. **35**:177-196

---------- S.E. Fitzwater. 1992. Dissolved organic carbon in the Atlantic, Southern and Pacific oceans. Nature **356**:699-700

---------- K.H. Coale, K.S. Johnson, S.E. Fitzwater, R. M. Gordon, S.J. Tanner, C. N. Hunter, V.A. Elrod, J.L. Nowicki, T.L. Coley, R.T. Barber, S. Lindley, A.J. Watson, K. Van Scoy, C.S. Law, M.I. Liddicoat, R. Ling, T. Stanton, J. Stockel, C. Collins, A. Anderson, R. Bidigare, M.Ondrusek, M. Latasa, F.J. Millero, K. Lee, W. Yao, J.Z. Zhang, G. Friederich, C. Sakamoto, F. Chavez, K. Buck, Z. Kolber, R. Greene, P. Falkowski, S.W. Chisolm, F. Hoge, R. Swift, J. Yungel, S. Turner, P. Nightengale, A. Hatton, P. Liss, N.W. Tindale. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature **371**: 123-129

Mioni, C.E., L. Poorvin, S.W. Wilhelm. 2005. Virus and siderophore-mediated transfer of available Fe between heterotrophic bacteria: characterization using an Fe-specific bioreporter. Aquatic Micro. **41**:233-245

Pakulski, J.D., R.B. Coffin, C.A. Kelley, S.L. Holder, R. Downer, P. Aas, M.M. Lyons, W.H. Jeffrey. 1996. Iron Stimulations of Antarctic Bacteria. Nature. **383**:133-134

Price, N.M., B.A. Ahner, F. M. M. Morel. 1994. The equatorial Pacific Ocean: grazer-controlled phytoplankton populations in an iron-limited ecosystem. Limol. Oceanogr. **39**:520-534

Shia, F.K. and H.W. Ducklow. 1994. Temperature regulation of heterotrophic bacterioplankton abundance, production and specific growth in Chesapeake Bay. Limnol. Oceanogr. **39**:1243-1258

Turley, C.M. and D.J. Hughes (1992) Effects of storage on direct estimates of bacterial numbers of preserved seawater samples. Deep-Sea Res. A **39**: 375-394.

Wells, M. 1999. Manipulating iron availability in nearshore waters. Limnol. Oceanogr. **44**:1002-1008

Wetz, M.S. and P.A. Wheeler. 2004. Response of bacteria to simulated upwelling phytoplankton blooms. Mar. Ecol. Prog. Ser. **272**:49-57

Table 1. Station locations where bacteria samples were obtained around the Galapagos Islands

Station	Latitude	Longitude
PM1	1° 09.03’ S	90° 59.96’ W
PM2	1° 29.99’ S	91° 59.99’ W
PM3	0° 36.99’ S	91° 59.99’ W
PM4	0° 37.01’ S	91° 42.03’ W
PM5	0° 37.01’ S	91° 24.95’ W
PM6	0° 36.98’ S	91° 19.02’ W
WT0	0° 04.51’ S	91° 55.99’ W

Table 2 Actual depths sampled

	PM1	PM2	PM3	PM4	PM5	PM6	WT0
Surface	3m	2.6m	2.2m	2.4m	3.3m	2.4m	4m
Shallow	24m	14.05m	13.6m	21.89m	19.9m	17.8m	68m
Subsurface							
250m	254.2m	253.4m	253.3m	253.95m	254.2m		
500m	502.8m	503.1m	502.3m	502.8m	503.6m		
1000m	1002.6m	1002.2m	1001.8m	1002.3m	1002.7m		

Table 3. Replicate locations and number of bottles sampled per depth and location Note: Starred depths indicate replicates were taken from the same bottle; all other replicates were sampled from separate bottles. # Indicates that the sample was taken at 62m.

	PM1	**PM2**	**PM3**	**PM4**	**PM5**	**PM6**	**WT0**
Surface	3	1	1	1	1	3*	1
Shallow subsurface	1	3	1	1	1	1	1#
250m	1	1	3	1	1		
500m	1	1	1	3	3*		
1000m	1	1	1	3	1		

Table 4 Bacterial concentrations found at each depth for each station location (10^6 Bacteria ml^{-1}). Error is listed as standard error (10^6 Bacteria ml^{-1}) Note: NS means no sample was taken at that depth.

	PM1	**PM2**	**PM3**	**PM4**	**PM5**	**PM6**	**WT0**
Surface	1.54±0.28	0.64±0.26	1.02±0.26	0.83±0.21	2.46±0.33	3.13±0.56	1.86±0.43
Shallow Subsurface	0.86±0.24	1.03±0.20	0.58±0.20	1.10±0.25	2.14±0.32	1.3±10.34	0.63±0.16
250m	0.29±0.10	0.25±0.04	0.32±0.04	0.29±0.06	0.17±0.03	NS	NS
500m	0.13±0.06	0.12±0.03	0.16±0.03	0.14±0.03	0.15±0.03	NS	NS
1000m	0.09±0.04	0.08±0.04	0.07±0.07	0.06±0.05	0.07±0.06	NS	NS

Table 5. Incubation 1 Bacteria Concentrations (10^6 ml^{-1}) at t = 0, 29, 49, and 72 hours. Error is listed as standard error (10^6 Bacteria ml^{-1})

	t_0	t_{29}	t_{49}	t_{72}
Control	0.86±0.50	0.94±0.24	1.85±0.71	1.83±0.28
$FeCl_3$	0.86±0.50	1.14±0.24	1.92±0.39	1.72±0.25
DFOM	0.86±0.50	1.21±0.27	1.85±0.38	1.65±0.23

Figure Captions

Figure 1. Bacterial abundance in incubation experiments using enriched HNLC waters left untreated or amended with glucose, iron, or both glucose and iron (From Kirchman et al. 2000). This demonstrates the important coupling between DOM and iron for controlling bacteria growth and abundance, suggesting that DOM may be the limiting factor in this experiment rather than iron.

Figure 2. A map of the Galapagos Island region, and relative surface chlorophyll concentrations on 16 January 2006 (SeaWiFS; http://www.oceancolor.gsfc.nasa.gov) including sample sites.

Figure 3. Bacterial concentration with depth at all stations. Concentrations are listed in 10^6 bacteria ml^{-1}. Closed points include all transect samples along proposed iron gradients. Open points represent stations not located along the transect. Data labels indicate depth sampled at. Inset: increased resolution of bacterial profile of all stations to 260 m.

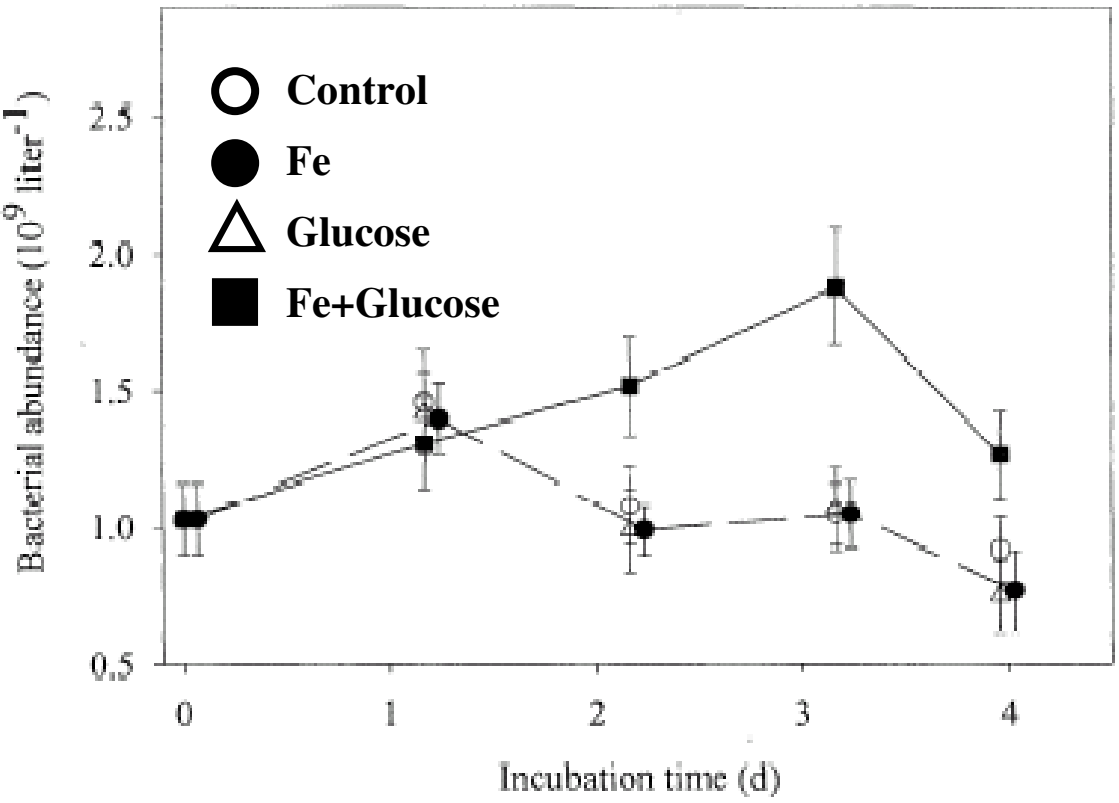
Figure 4. Bacteria concentrations from stations PM1-WT0 around the Galapagos Islands. Stations PM1 and PM2 were sampled southwest of Isabela Island. Stations PM 3-PM6 formed a West to East transect moving into Elizabeth Bay along 37'S. Error bars represent percent error. Data labels indicate depth sampled at. A. Surface total bacterial concentrations. B. Shallow subsurface total bacterial concentrations

Figure 5. Representative water profiles including bacterial concentrations. All values were scaled to fit on one graph. Numbers beside bacterial concentrations indicate sample depth. A.) Station WT0 B.) Station PM6

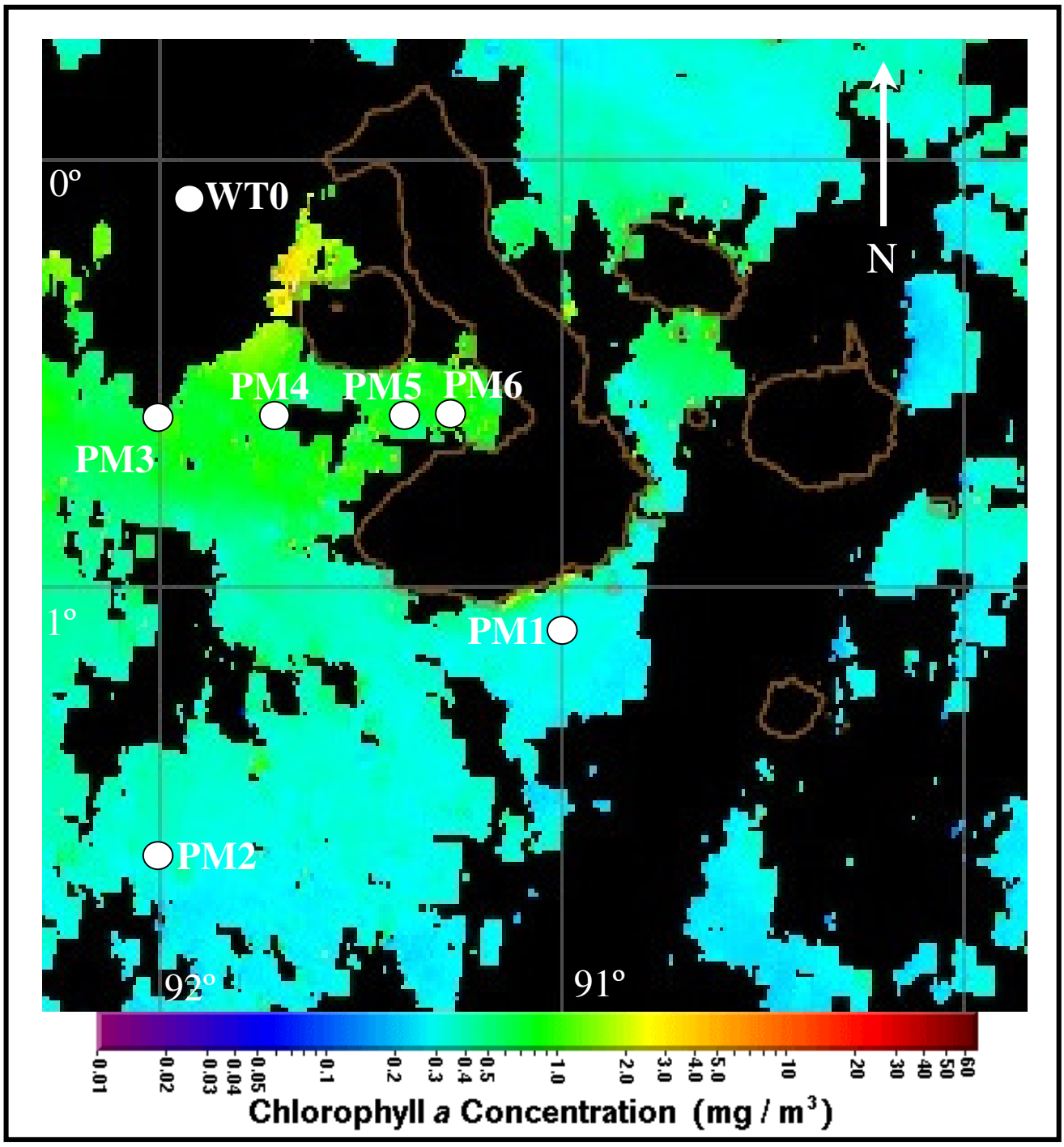
Figure 6. Representative water profiles including bacterial concentrations. All values were scaled to fit on one graph. Numbers beside bacterial concentrations indicate sample depth. A.) Station PM2; note the bacterial maximum located at the chlorophyll maximum. B.) Station PM4; note again the bacterial maximum located at the chlorophyll maximum.

Figure 7. Surface potential temperature profiles of each station

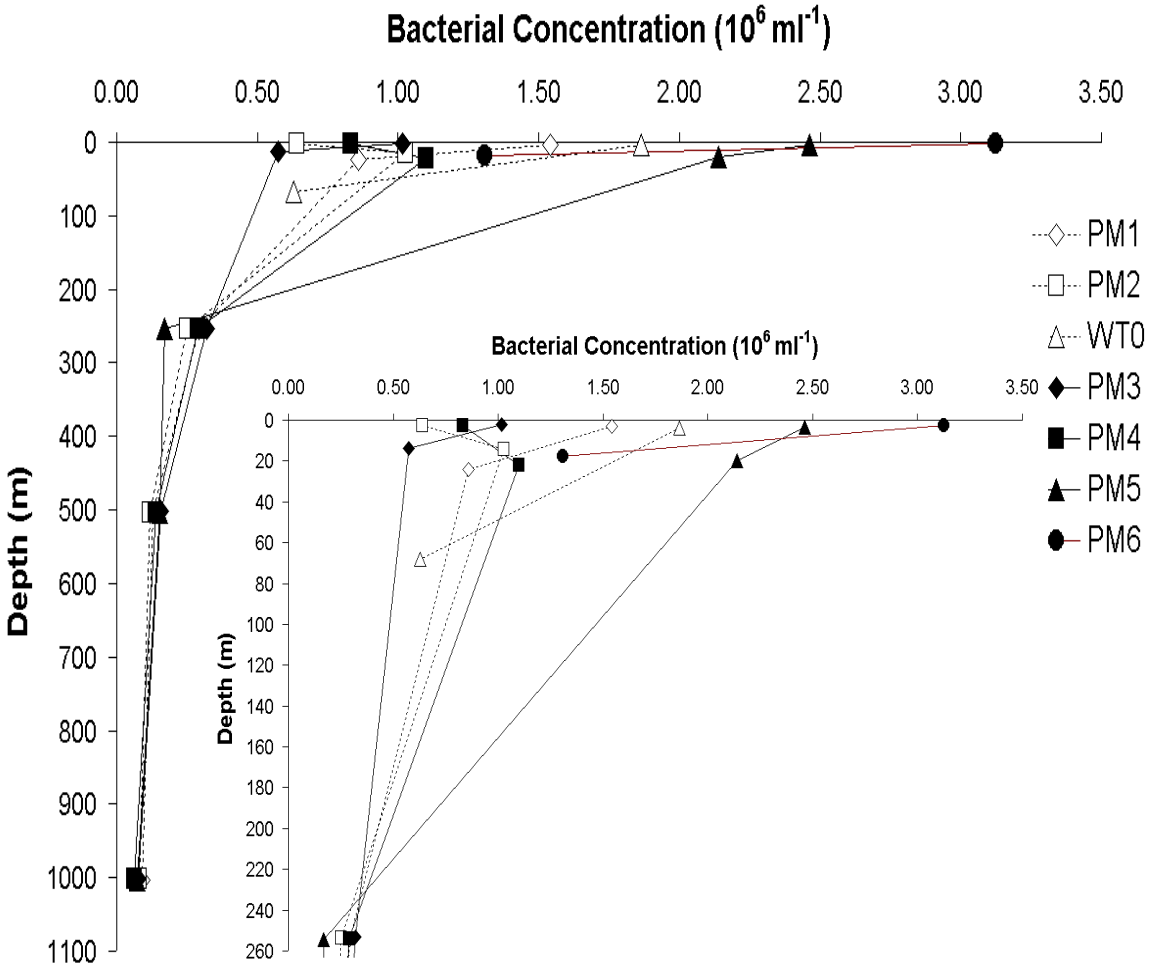
Figure 8. Incubation growth curves showing net bacterial abundances and growth over time from water collected at the PM1 chlorophyll maximum. Bottles were left untreated or amended with iron ($FeCl_3$) or DFOM and left to incubate for 72 hours with sub-sampling at t_0, t_{29}, t_{49}, and t_{72}. Error bars represent percent error.



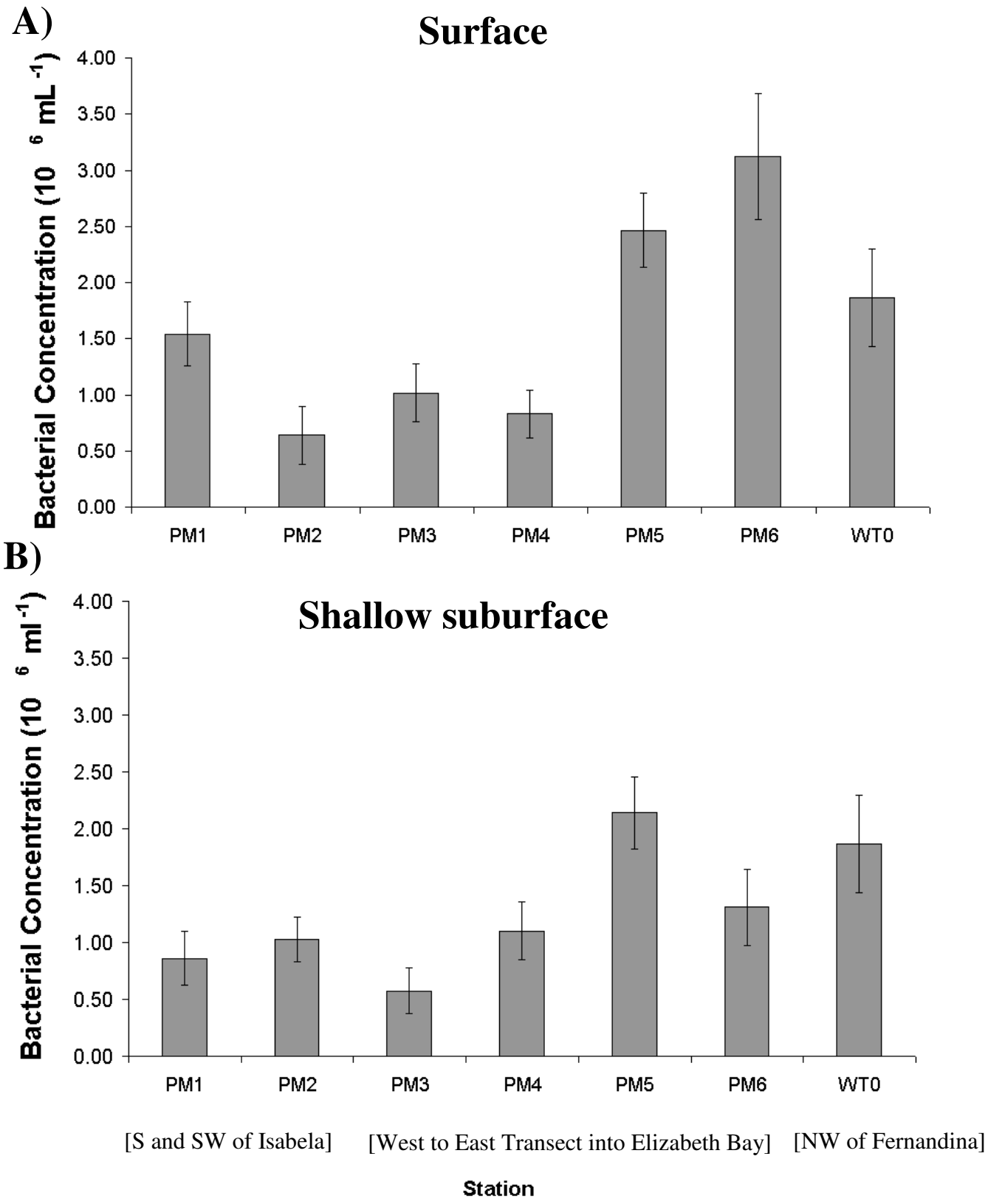
Pamela Maynard Figure 1



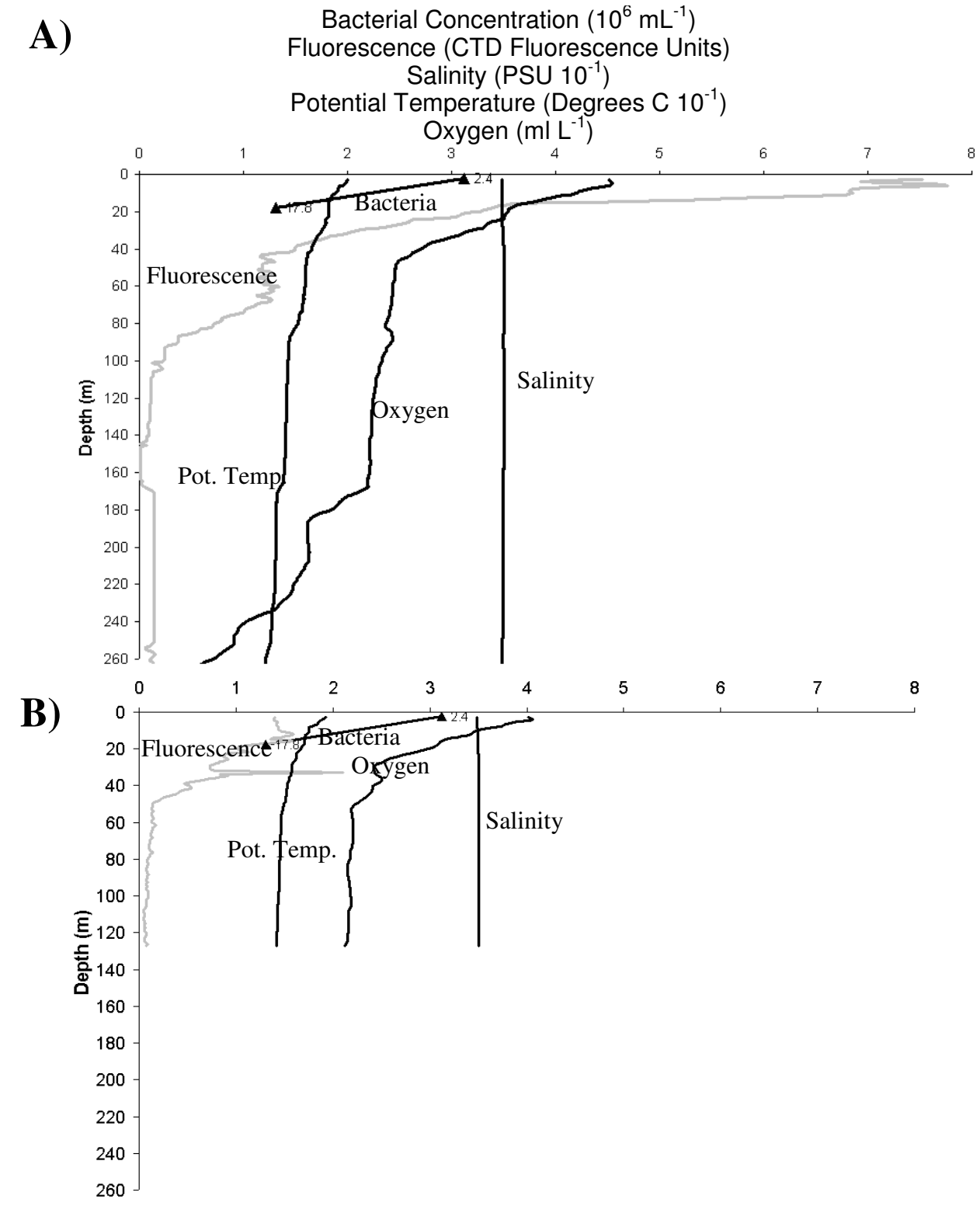
Pamela Maynard Figure 2



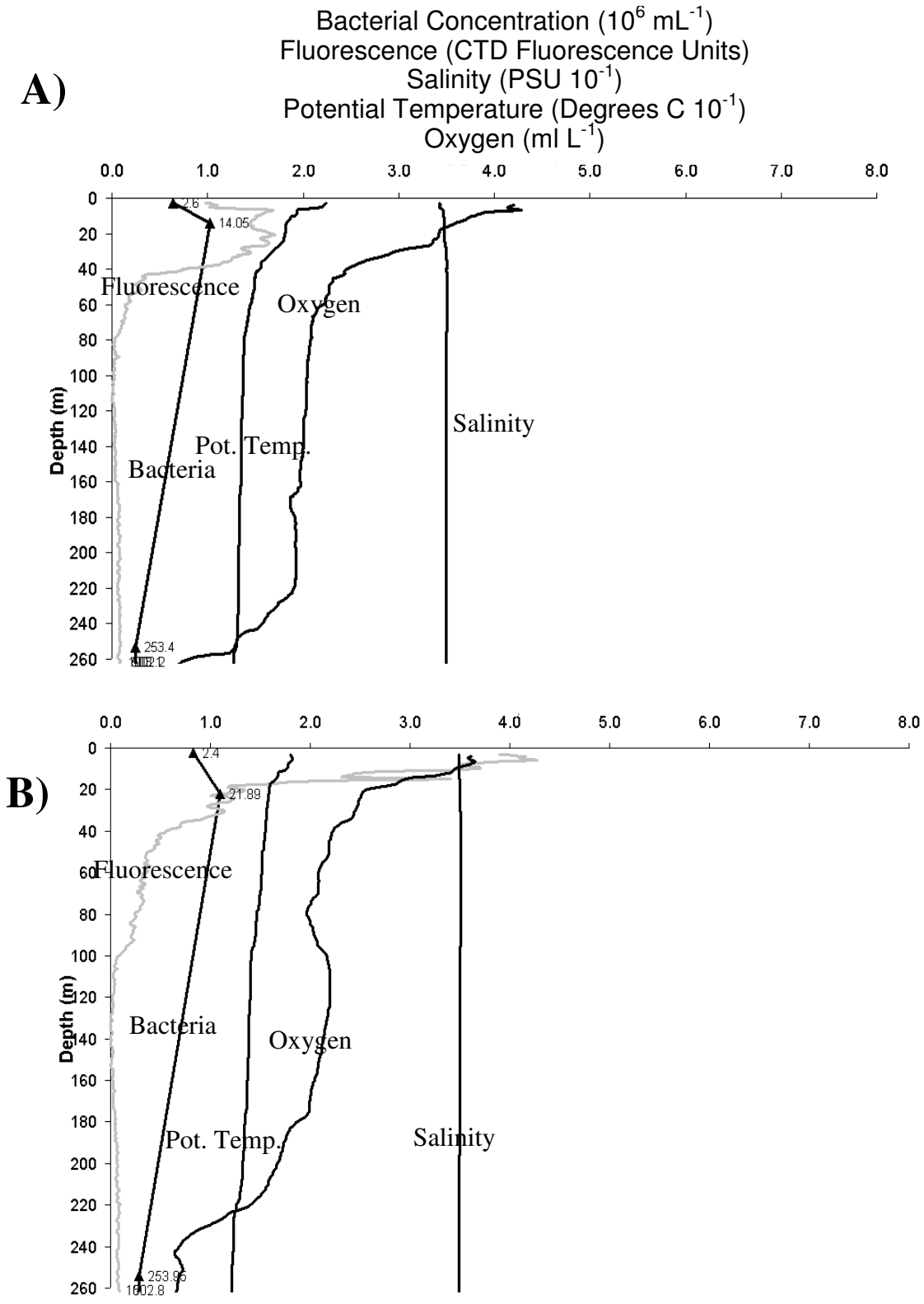
Pamela Maynard Figure 3



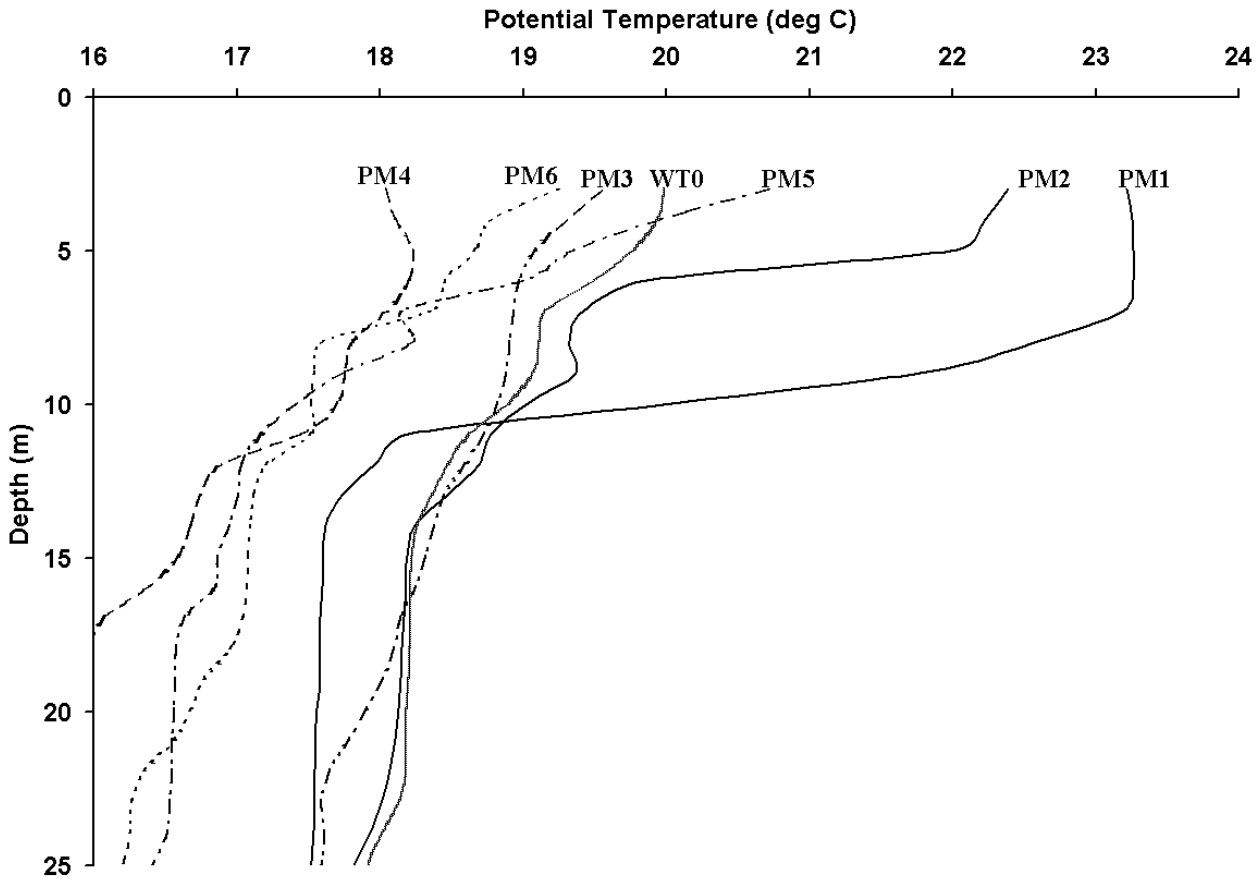
Pamela Maynard Figure 4



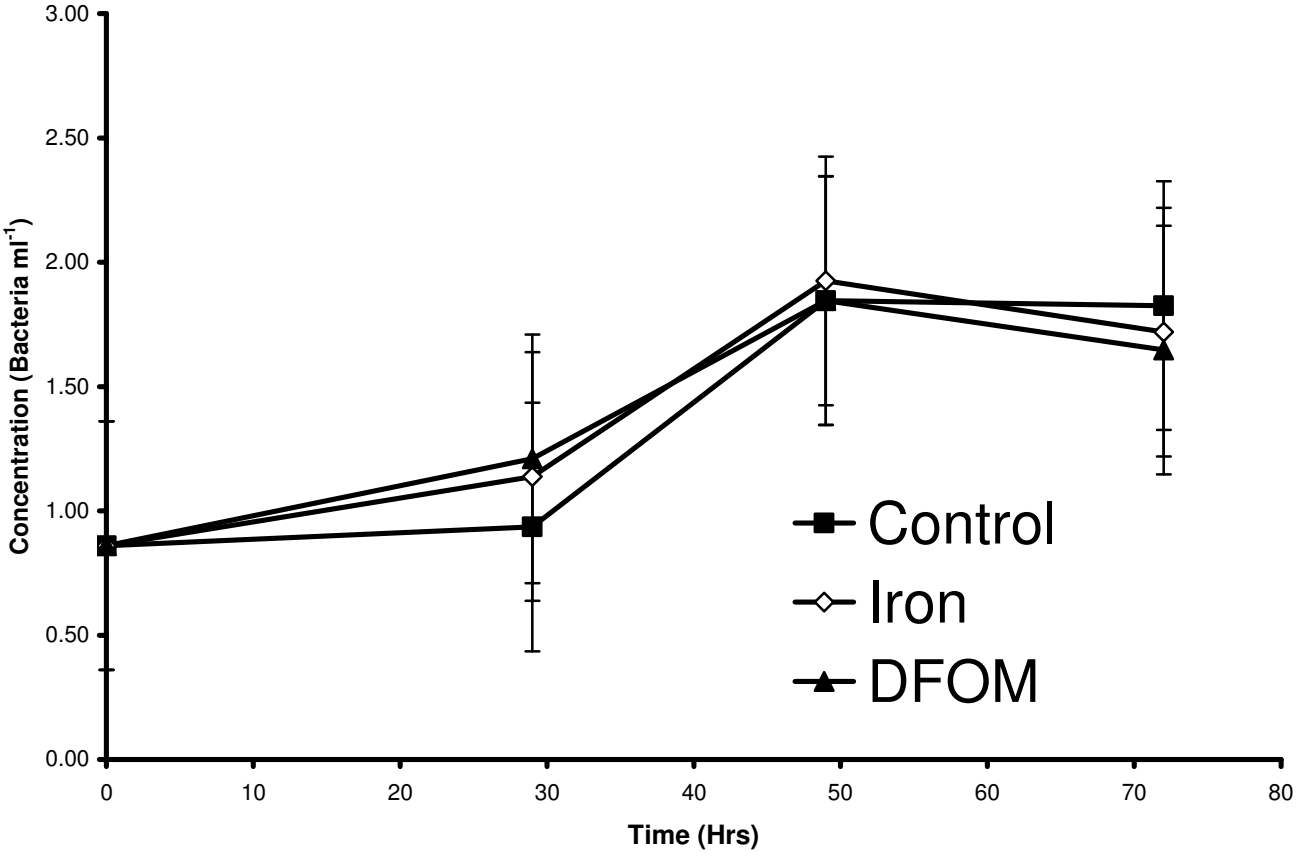
Pamela Maynard Figure 5



Pamela Maynard Figure 6



Pamela Maynard Figure 7



Pamela Maynard Figure 8