Iron and carbon limitation of prokaryotic growth in the ocean

Jacques L. Oliver
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IRON AND CARBON LIMITATION OF PROKARYOTIC GROWTH IN THE OCEAN

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Jacques L. Oliver
2005
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

The requirements for the degree of

Doctor of Philosophy

Approved, June 2005

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University of California – Santa Barbara
Santa Barbara, California
To Jane B. Lowry and William J. and Martha G. Payne...

for the gift of family.
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ABSTRACT

Prokaryotes are a significant component of the ocean biosphere. They are the most abundant living organisms in the ocean and are ecologically significant due to their ubiquity and numerical dominance. Prokaryotes act as mediators of organic carbon, one of the largest exchangeable pools of carbon on earth, and nutrient fluxes. The objective of this dissertation is to provide insights into how iron and carbon directly govern prokaryotic dynamics in the ocean and, in turn, the subsequent biogeochemical transformations which they mediate.

Studies were undertaken to examine the roles of iron and carbon in modulating prokaryotic growth in the ocean. The context of the first study was an open-open iron fertilization experiment in the high nutrient, low chlorophyll (HNLC) regime in the Southern Ocean. This study focused on the direct and indirect effects of iron enrichment on prokaryotic growth, carbon production, and community structure. The context of the second study was the oligotrophic, iron-replete, and organic carbon-limited northwest Sargasso Sea. The aim of this study was to examine the effects of an iron-binding compound, the siderophore desferrioxamine B (DFOB) on organic carbon utilization by heterotrophic prokaryotes.

In the first study prokaryotic abundance, carbon production, and growth rate increased in response to iron in two experimental locations, one north and one south of the Antarctic Polar Front Zone (North Patch and South Patch, respectively). However, prokaryotes responded indirectly to iron-induced phytoplankton production. Prokaryotic production was highly correlated to particulate primary production ($r^2 = 0.80$), but it was only a small fraction of particulate primary production (1%). Prokaryotes comprised a larger percentage of particulate organic carbon (POC) in the North versus the South Patch relative to non-fertilized waters.

Analysis of prokaryotic community structure was also examined as part of the first study. Results showed that unique prokaryotic communities existed in the North and South Patch for both iron-fertilized and non-fertilized waters. Additionally, the community composition shifted over time in the South Patch and overall was distinct from non-fertilized waters. Measures of community diversity indicated an increase in taxonomic richness and diversity in iron-fertilized waters over time. Specific taxonomic groups monitored over time in the South Patch exhibited a differential response to the iron-induced phytoplankton bloom. At the domain level, the biomass response was greater for Eubacteria compared to Archaea. At the clade level, Cytophaga-Flavobacteria net biomass yields outpaced SAR11, although both exhibited significant increases ($p<0.05$) in net growth rate over time in the South Patch.

In the second study DFOB did not limit utilization of organic carbon (glucose), an indication that iron limitation using the siderophore was not achieved. Conversely, DFOB stimulated prokaryotic growth in a dose-dependent manner. The trend of the response to DFOB was similar to glucose; however, the magnitude of the response (i.e. growth rate and biomass yield) at higher equivalent carbon doses was greater than that of glucose. Additionally, DFOB and glucose elicited a differential taxonomic response.
IRON AND CARBON LIMITATION OF PROKARYOTIC GROWTH IN THE OCEAN
INTRODUCTION

The ocean carbon cycle - The intermediate and deep ocean represent the largest reservoir of carbon that is exchangeable with the atmosphere, with approximately 50 times the carbon dioxide (CO$_2$) found in the atmospheric pool (Falkowski et al. 2000). Considering the strong influence of atmospheric CO$_2$ in the earth’s climate over geological time (Knox and McElroy 1984, Sarmiento and Toggweiler 1984, Siegenthaler and Wenk 1984), even a small flux of CO$_2$ from the oceans could have important climatic consequences. CO$_2$ is exchanged between the oceans and the atmosphere over time scales ranging from days to thousands of years. In general, the oceans are believed to act as a net long-term sink for carbon because the carbon that enters the ocean from the atmosphere or land is not equally exchanged back on short time scales (Falkowski et al. 1998, Falkowski et al. 2000). The fate of anthropogenic CO$_2$ produced since the start of the industrial age is another indication of the absorptive capabilities of the oceans with respect to carbon (Sabine et al. 2004). There are three mechanisms that facilitate the storage of carbon in the ocean: the biological pump, the solubility pump, and continental runoff and accumulation of organic and inorganic carbon.

The first mechanism, the biological pump (Volk and Hoeffert 1985, Longhurst and Harrison 1989), is driven by primary production in the euphotic zone. Photosynthesis mediates the chemical transformation of dissolved inorganic carbon (DIC) to organic carbon as biomass (particulate organic carbon, POC) or dissolved material (dissolved organic carbon, DOC). Photosynthetic production of POC is primarily attributed to eukaryotic marine phytoplankton and cyanobacteria, although there is emerging evidence that marine prokaryotes also possess the potential to capture carbon through light-mediated reactions.
(Kolber et al. 2000, Beja et al. 2000). Initially, photosynthesis maintains a thermodynamically-favorable state for net uptake of inorganic carbon by reducing the partial pressure of CO$_2$ (pCO$_2$) in seawater. Newly produced POC either sinks to the deep ocean or is cycled through the ocean’s food web where it can be transformed into more rapidly sinking POC. Additionally, active transport of vertically migrating zooplankton facilitate delivery of organic carbon, ingested in the surface ocean, to the ocean interior (i.e. the meso- and bathypelagic) (Steinberg et al. 2000). In either case some fraction of POC ultimately finds its way to the ocean interior for long-term storage or oxidation. Phytoplankton and zooplankton also produce DOC during photosynthesis and heterotrophic processes, respectively. The fate of plankton-derived DOC is heterotrophic utilization by marine prokaryotes (Pomeroy 1974) or convective transport to the ocean interior (Carlson et al. 1994). Under the former process, also known as remineralization, DOC is respired to CO$_2$, converted into biomass (POC), and cycled through the microbial food web with potential for transfer to higher trophic levels (Azam et al. 1983). Alternatively, convective transport can deliver unused and/or refractory DOC to the ocean interior prior to complete remineralization of the DOC pool providing another mechanism of carbon sequestration in the deep ocean (Carlson et al. 1994, Carlson and Ducklow 1995, Ducklow et al. 1995a, Hansell and Carlson 1998). The overall balance between autotrophic and heterotrophic processes in the surface ocean and the time scales on which they operate control the efficiency of the biological pump and the extent to which organic carbon is exported to the ocean interior for long-term sequestration.

The second mechanism, the solubility pump, is chemically and physically mediated through the dissolution of atmospheric CO$_2$ in the surface ocean where it forms bicarbonate, HCO$_3$$. This is a process which occurs at all latitudes, but prevails at high latitudes where
there are more frequent wind-driven mixing events; colder ocean temperatures also enhance CO$_2$ solubility (Takahashi et al. 2002). Similar to convective DOC export, deep convective mixing at high latitudes enables CO$_2$ to be transported to the ocean interior.

The third mechanism is continental runoff of organic matter and inorganic carbon as crustal material (Field et al. 1998). Organic and inorganic carbon enters coastal waters through surface and groundwater flow. Terrestrially-derived organic carbon is either remineralized to CO$_2$, transformed to refractory carbon, and/or accumulates in sediments. The remainder that escapes these processes in coastal waters enters the open ocean for further cycling.

Taken together, these three mechanisms drive the ocean carbon cycle. They function as delivery mechanisms of carbon to the ocean interior where it undergoes further cycling, remains suspended, settles on the ocean benthos, or is transported across ocean basins via deep ocean circulation. The latter process occurs over thousands of years and results in eventual ventilation of remineralized carbon to the atmosphere, thereby completing the cycle (Wallace 2001).

Iron limitation of the biological pump - The biological pump is a major driving force of the ocean carbon cycle. In a more reduced form, this mechanism is a mosaic of biological processes superimposed on one another. Photosynthesis is carried out by a variety of phytoplankton species all working in concert with one another to fix carbon. Respiration, on the other hand, is the destruction of that fixed carbon by mixed assemblages of marine prokaryotes and zooplankton species. Fundamentally, the rates of photosynthesis and respiration are dependent upon the underlying nutrients which support them. Thus, carbon
export and the efficiency of the biological pump are directly linked to the availability of nutrients.

Traditionally, nutrient limitation of phytoplankton growth was either by nitrogen or phosphorus (Smith 1984, Bjoerkman et al. 2000, Wu et al. 2000, Sanudo-Wilhelmy et al. 2001). Both elements are building blocks of biological macromolecules essential for growth (nucleic acids, lipids, and proteins) and are considered macronutrients. The proposal that nitrogen and phosphorus limit growth stems from the relative scarcity of inorganic forms in surface waters, the minimal sources of each to the open ocean, and the dependence of phytoplankton growth on regenerated forms of nitrogen and phosphorus. Thus, there is less new carbon available for export and the biological pump operates at reduced efficiency.

However, there are regions of the ocean where nitrate and phosphate abound. They include the subarctic Pacific Ocean, the equatorial Pacific Ocean, and the Southern Ocean. But in contrast to the expectation of unfettered phytoplankton growth and biomass accumulation with coincident macronutrient depletion, low standing stocks of biomass persist. Together these features describe a paradox known as the high nutrient, low chlorophyll (HNLC) condition. The HNLC condition is also symptomatic of a less efficient biological pump and implies that one or more factors apart from nitrogen and phosphorus pre-empt phytoplankton growth.

Just as nitrogen and phosphorus were identified as likely rate-limiting factors for phytoplankton growth due to their low concentrations, it is instructive to examine other elements in HNLC regions that are in short supply which are also critical to phytoplankton growth. In this sense, iron stands out in HNLC regions due to its extremely low dissolved concentrations in seawater relative to macronutrients. Furthermore, there is a high biological
demand for iron due to its role in key photochemical and respiratory processes. Unlike nitrate and phosphate, dissolved iron concentrations are three orders of magnitude lower, ranging from <0.5 nmol kg\(^{-1}\) (0-1000 meters) to 1.0 nmol kg\(^{-1}\) (>1000 meters) (Johnson et al. 1997). A major input of iron to the surface ocean is through aeolian deposition and subsequent dissolution of mineral aerosols (Zhuang et al. 1990, Duce and Tindale 1991, Jickells 1999, Fung et al. 2000). Therefore, the frequency and magnitude of airborne iron deposition, as well as the rates of dissolution, are the ultimate constraints on upper ocean iron content. Upwelling of remineralized iron is another source to the surface ocean, although it represents only a small fraction (<1%) of new iron (Fung et al. 2000). Fluxes of iron from the surface ocean through the sinking of iron-associated particles or convective transport are other important physical constraints on upper ocean iron content (de Baar and de Jong 2001).

The abiotic and biotic transformations of iron that occur following inputs determine the speciation, distribution, concentrations, and bioavailability of iron in seawater. Iron undergoes complex inorganic and organic speciation upon dissolution to both of its monomeric forms, \(\text{Fe}^{2+}\) and \(\text{Fe}^{3+}\). Dissolved free iron occurs most frequently in its oxidized state, \(\text{Fe}^{3+}\), where it readily undergoes speciation to form complexes with hydroxides or other dissolved inorganic species (Moffett 2001). \(\text{Fe}^{3+}\) can also be associated with particulate matter, both inorganic and organic, and thus can be lost from surface waters through irreversible complexation or sinking. In sunlit waters photoreduction of \(\text{Fe}^{3+}\) can produce the more soluble \(\text{Fe}^{2+}\), but \(\text{Fe}^{2+}\) will not endure in this form due to its vulnerability to rapid oxidation and preference by phytoplankton (Sunda 2001). Another significant interaction of iron is with dissolved and colloidal organic matter (Wells 2002). Both forms of organic
matter are major sinks for iron; however, their composition, origin, and chemical kinetics with iron are not fully known.

The importance of iron is underscored by the universal role it plays in phytoplankton physiology. Iron is a co-factor for numerous enzymes and a key component of redox reactions, two fundamental intracellular processes which form the basis of cell division and the construction of biomass. For phytoplankton, iron is essential to the process of light harvesting and electron transport (Greene et al. 1992), the synthesis of chlorophylls (Porra et al. 1997), and assimilative reduction of nitrate to form amino acids intracellularly (Rueter and Ades 1987, Timmermans et al. 1994). The critical role of iron in plankton nutrition is manifested as a strong biological demand for iron. This demand, coupled with physical fluxes and chemical transformations of iron, limit the available iron pool in the ocean. An extension of this collective constraint is the limitation placed on biologically-mediated fluxes of carbon, specifically the biological pump.

From Liebig to Martin: Testing the iron hypothesis - The earliest contemplation of resource limitation on growth came from Justus von Liebig (von Liebig 1842) in which he posited that agricultural plant yields could increase if the nutrient in least availability, relative to biological demand, was provided. This concept, known as Liebig’s Law of the Minimum, was later mis-applied to ocean primary productivity in which nitrate was viewed as the limiting factor for algal growth (de Baar 1994). Gran (1931) and Hart (1934, 1942) challenged the nitrate-limitation paradigm after discovering the HNLC condition in the Antarctic. In place of nitrate, they hypothesized that iron might alleviate phytoplankton growth limitation in Antarctic waters, and in turn explain the paradox of the HNLC.
condition. However, analytical limitations in measuring iron precluded successful testing of the hypothesis. A revival of the hypothesis, spurred by analytical improvements and trace metal-clean techniques, was led by the late John Martin. In a series of proof-of-principle studies using shipboard bottle experiments in the subarctic Pacific Ocean (Martin and Fitzwater 1988, Martin and Gordon 1988, Martin et al. 1989), the Southern Ocean (1990a, 1990b), and the equatorial Pacific Ocean (Martin et al. 1991), Martin and colleagues demonstrated the *in vitro* stimulatory effect of iron on primary production and the accumulation of autotrophic biomass. Scaled to the natural environment, it was thought that *in situ* additions of iron could lead to full macronutrient drawdown and a departure from the HNLC condition. This became known as the HNLC-iron hypothesis (Cullen 1991, 1995).

Martin (1990) extended the revised iron hypothesis by suggesting a corollary link between iron and global climate. Martin postulated that iron flux to the ocean over geological time could be the key driver of glacial-interglacial atmospheric CO$_2$ oscillations, a cycle that was previously linked with ocean biogeochemistry (Knox and McElroy 1984, Sarmiento and Toggweiler 1984, Siegenthaler and Wenk 1984). In other words, by employing Liebig’s Law of the Minimum at the phytoplankton community scale, Martin argued that long-term supply of iron to the oceans, particularly in the Southern Ocean, would continuously stimulate the biological pump by the drawdown of macronutrients, the fixation of dissolved CO$_2$ into biomass, and support net export of carbon resulting in the eventual drawdown of atmospheric CO$_2$. Martin’s focus on iron in this way drew skepticism that widespread iron fertilizations would not be sufficient to ameliorate global atmospheric CO$_2$ (Broecker 1990, Sarmiento and Orr 1991). Other views, crystallized in a paper by Banse (1990), were skeptical of the HNLC-iron hypothesis for ecological considerations. These
arguments did not discount the stimulatory effects of iron \textit{in situ}, but rather contended that iron did not have a singular role in controlling phytoplankton productivity and biomass in HNLC waters. Other proximal controls such as light limitation (Mitchell et al. 1991) and zooplankton grazing (Frost 1990, Miller et al. 1991), factors not adequately tested in Martin et al.'s shipboard bioassays, could also account for the lack of complete macronutrient utilization in HNLC regions. Morel et al. (1991) postulated a more holistic version of the iron hypothesis which took into account these considerations, calling it the 'ecumenical iron hypothesis'. Price et al. (1994) supported this interpretation after observing preferential grazing on non-iron-limited, autotrophic picoplankton versus reduced grazing on large, iron-limited phytoplankton (>3 µm) in the HNLC equatorial Pacific Ocean.

In response to challenges of iron's role in phytoplankton growth and broader ocean biogeochemistry, an unprecedented field study was carried out in an open-ocean iron fertilization experiment in the eastern equatorial Pacific Ocean (IronEx I, Martin et al. 1994). A single iron addition to surface waters resulted in unequivocal stimulation of phytoplankton growth on the scale of 64 km$^2$ in area, an \textit{in situ} confirmation of the original iron hypothesis. This was a significant finding because unlike shipboard bioassays, the response occurred in a phytoplankton community exposed to natural losses, i.e. sinking, viral lysis, and grazing. Despite the phytoplankton response, the experiment failed to show drawdown of CO$_2$ and macronutrients or export of carbon, an apparent rejection of the HNLC-iron hypothesis. However, Martin and colleagues argued that iron loss from the system, co-limitation by another trace element, and grazing losses could explain the failure of phytoplankton to fully utilize macronutrients. They emphasized, though, that retention of iron in the system for
fueling growth was the critical factor. Collectively, these factors left the HNLC-hypothesis unresolved (Cullen 1995).

Coale et al. (1996) re-tested the HNLC-iron hypothesis, focusing on the aforementioned potential limiting factors, in a series of similar iron fertilization experiments in the eastern equatorial Pacific Ocean (IronEx II). Following multiple iron additions over the course of 14 days, a massive phytoplankton bloom (120 km$^3$) ensued in which community dominance shifted from small autotrophic picoplankton to large pennate diatoms (Cavender-Bares et al. 1999). Overall increases in phytoplankton photochemical efficiency and cell division rates re-confirmed iron limitation of growth (Behrenfeld et al. 1996). In contrast to IronEx I, there was also a measurable drawdown of dissolved CO$_2$ (Cooper et al. 1996), modest drawdown of macronutrients with a concomitant increase in organic carbon production (75% POC, 25% DOC), and partial export of new carbon from surface waters (Coale et al. 1996). While IronEx II confirmed the physiological and geochemical basis for the HNLC-iron hypothesis (i.e. stimulation of the biological pump) iron fertilization did not lead to sustained productivity, full macronutrient exhaustion, and sustained carbon export. Losses due to sedimentation of large celled-phytoplankton (Bidigare et al. 1999) and microzooplankton grazing (Landry et al. 2000a) were invoked to explain the under-utilization of macronutrients. Mesozooplankton grazing did occur, however, it was not significant enough to prevent the diatom bloom (Landry et al. 2000b). Nonetheless, the presence of zooplankton grazing confirmed the suspicions previously articulated by Banse and colleagues and pushed the interpretation of the HNLC condition towards one that embodies the balance between dual constraints, iron limitation and grazing. In retrospect, Morel et al. (1991) and Price et al. (1994) were prescient in their interpretation of the HNLC condition in which
phytoplankton reside in an iron-limited system but whose populations are controlled by grazers.

The initial constraint of iron limitation, harkening back to the interpretation of IronEx I (Martin et al. 1994), may have recurred despite the repeated iron additions. Surface concentrations of dissolved iron returned to their original, pre-fertilization state by the end of the experiment, implying loss of dissolved iron from the system. Iron loss associated with sinking POC may have occurred; however, the fate of the iron was not fully determined. Additionally, circumstantial evidence suggested that iron was "lost" from the system through chelation to natural organic ligands (Rue and Bruland 1997). The ligands were produced rapidly following individual iron additions and the transformed iron was not available for phytoplankton uptake. The origin of the ligands was not known, although their chemical characteristics were similar to siderophores, iron-binding ligands produced by autotrophic and heterotrophic bacteria.

Despite the mixed results, IronEx II was a successful test of the iron hypothesis and to an extent a confirmation that macronutrient and inorganic carbon drawdown was possible. Coale et al. (1996) suggested that a foundation had been laid for testing Martin's glacial-interglacial iron hypothesis in the Southern Ocean. Boyd et al. (2000) assumed this challenge and initiated an ambitious iron fertilization experiment in the polar waters south of New Zealand (SOIREE). The size, scale, and responses were remarkably similar to those of IronEx II (Boyd 2002). A 200 km² phytoplankton bloom resulted over the course of thirteen days as a result of four iron additions. There were increases in photosynthesis and photochemical efficiency, a floristic shift from small cells to larger diatoms, and modest macronutrient and inorganic carbon drawdown (Boyd et al. 2000). Also, zooplankton
grazing, particularly by microzooplankton, played a part in shaping the composition of the phytoplankton community. Like IronEx II, this appeared to fit the Morel et al.'s model of the ecumenical iron hypothesis (1991). However, much of the carbon produced during the bloom remained in the surface waters and carbon export was not detected during the experiment. The most surprising departure from IronEx II was the duration of the iron-induced bloom. Whereas the bloom in IronEx II began to decline after 2 weeks, satellite remote sensing of the SOIREE bloom indicated a lifespan of 50 days and an increase in size to 1100 km². A combination of favorable ocean conditions and horizontal mixing processes may have allowed the bloom to persist (Boyd 2002).

Implicit in an extended bloom such as the one observed in SOIREE, as well as blooms necessary for the hypothetical departure from the HNLC condition, is continued iron availability for phytoplankton growth. Boyd et al. (2000) noted the production of organic iron-binding ligands during SOIREE, a feature that was also present during IronEx II, which maintained dissolved iron concentrations above those limiting for phytoplankton growth. However, the timing of ligand production and the form of iron that was chelated differed from IronEx II. Production occurred after 12 days in SOIREE and the ligands chelated Fe^{+2} rather than Fe^{+3} (Croot et al. 2001). The origin of the ligands and whether they persisted beyond the 13 day experiment was not known. Boyd (2002) speculated that the ligands may have been produced by heterotrophic bacteria in response to inadvertent iron limitation brought on by alleviation of DOM limitation.

*Marine prokaryotes and the ocean carbon cycle: The traditional view* - Marine prokaryotes are a major biological component of the oceanic carbon cycle (Cho and Azam 1988). They
are the numerically dominant living organisms in the ocean and compose a significant proportion of oceanic biomass (Ducklow 1999, 2000). Prokaryotes are comprised of members of both the Eubacterial and Archaeal lineages (Giovannoni and Rappé 2000) and are ubiquitous throughout the oceans (Whitman et al. 1998).

One way in which prokaryotes function as part of the ocean carbon cycle is through assimilating and remineralizing primary production in the euphotic zone. Prokaryotic production, as a proportion of particulate primary production, ranges from 4-26% (Ducklow 2000). By incorporating primary production into biomass prokaryotes reduce the efficiency of the biological pump and the ratio of carbon produced to carbon exported. Prokaryotic remineralization and production occurs through the utilization of phytoplankton-derived DOC (Lancelot 1979, Goldman et al. 1992), zooplankton-derived DOC (Lampert 1978, Nagata and Kirchman 1992), virally-derived DOC (Proctor and Fuhrman 1991, Fuhrman 1992, Fuhrman 1999), and by hydrolyzing non-living POC (Smith et al. 1992, Urban-Rich 1999, Bidle and Azam 1999). Marine prokaryotes utilize DOC across the size spectrum from low (<1 kDa) to high molecular weight (> 1 kDa) (Amon and Benner 1996) and can transform DOC into refractory material that is resistant to further microbial assimilation and transformation (Ogawa et al. 2001). Marine prokaryotes are also links in the oceanic food web (Pomeroy 1974, Azam et al. 1983). This occurs through the assimilation and transformation of non-living POC and DOC into prokaryotic biomass where grazing processes transfer carbon through the food web. In turn, the transformation of carbon at each trophic level collectively serves as an additional pathway for the release of biologically important dissolved compounds such as ammonium, urea, and amino acids (Bronk 2002), dissolved organic phosphorus (Karl and Bjorkman 2002), as well as trace metals (Hutchins et
food web and higher trophic levels can also dramatically reduce the carbon export ratio
(Michaels and Silver 1988).

In addition to the release of inorganic nutrients through trophic transfer, prokaryotes
can simultaneously regenerate inorganic nutrients, such as ammonium and phosphate, during
their assimilatory metabolic processing of nutrient-rich organic matter (Kirchman 2000).
They are also instrumental in collectively regenerating various forms of nitrogen through
nitrate reduction and denitrification (Capone 2000), and nitrification (Ward 2000),
dissimilative metabolic processes which are coupled to prokaryotic remineralization (i.e.
oxidation) of organic matter and prokaryotic respiration, respectively. Nitrogen-fixing
prokaryotes are also significant contributors to the ocean carbon cycle by introducing
biologically-available nitrogen to the ocean and by fixing carbon (Capone et al. 1997, Karl et
al. 1997). Thus, the array of prokaryotic metabolisms in the ocean serve as important
feedback mechanisms which aid in fueling oceanic production, both new and regenerative
(Dugdale and Goering 1967), and in turn, export of carbon (Eppley and Peterson 1979).

Marine prokaryotes continually operate in the aforementioned ways and have the
ability to respond rapidly to new inputs of organic carbon and nutrients. In this way
prokaryotes become essential and ubiquitous features in carbon and nutrient cycling during
phytoplankton blooms. Prokaryote blooms have been associated with seasonally-driven
phytoplankton blooms (Carlson et al. 1996, Ducklow et al. 1993, 2001b), as well as blooms
driven by episodic climatic events (Ducklow et al. 1995b, Ducklow et al. 2001a) and unique
physical oceanographic features (Ducklow and Hill 1985).
Marine prokaryotes ubiquity, numerical dominance, and modulation of carbon flow in the ocean demonstrate their ability to successfully meet their nutritional demands. The extent to which prokaryotic growth and biomass is constrained has been widely studied. Temperature can be influential in slowing growth (Pomeroy and Deibel 1986, Shiah and Ducklow 1994, Kirchman and Rich 1997) although it does not necessarily limit growth. Ducklow et al. (2001b) found significant accumulation of prokaryotic biomass in the Ross Sea over the spring and summer despite low temperatures. Losses due to bacterivory (Pomeroy 1974) and viral mortality (Proctor and Fuhrman 1990) can also constrain biomass.

Resource limitation has also been proposed as a proximal control on prokaryotic growth. In oligotrophic waters growth limitation has been linked to phosphorus (Pomeroy et al. 1995, Thingstad and Rassoulzadegan 1995, Cotner et al. 1997, Rivkin and Anderson 1997, Thingstad et al. 1998) and nitrogen (Caron et al. 2000), although there is evidence which shows carbon availability or quality can also limit growth (Carlson and Ducklow 1996, Carlson et al. 2002). In HNLC regions resource limitation by inorganic macronutrients is not likely to limit prokaryotic growth, although growth could be indirectly limited by lack of organic carbon production by iron-limited phytoplankton.

Like phytoplankton, iron has received attention as a direct limiting nutrient for prokaryotic growth in HNLC waters. Iron plays a prominent role in heterotrophic metabolism as a component of cytochromes and iron-sulfur proteins. To test the direct influence of iron on growth, iron was added to trace-metal clean seawater cultures in the HNLC Southern Ocean (Pakulski et al. 1996). The results showed that bacterial cell density and production increased upon enrichment with iron. In contrast, in nutrient-addition experiments across three frontal zones in the Southern Ocean (HNLC and non-HNLC)
(Church et al. 2000) enrichment with iron alone did not stimulate bacterial growth and production. Rather, a combination of temperature, carbon, nitrogen, and iron limitation constrained heterotrophic bacterial growth. Similar results were found in the California Current HNLC (Kirchman et al. 2000). Iron alone did not stimulate bacterial growth in iron-depleted waters. Rather the inclusion of glucose (i.e. DOC) with iron enhanced growth and production. The observations that prokaryotic growth can be co-constrained by DOM is consistent with previous studies which showed prokaryotes preferentially responded to additions of DOM as dissolved free amino acids (Kirchman 1990), neutral monosaccharides (Rich et al. 1996, Carlson et al. 2002), or both (Carlson and Ducklow 1996) over inorganic nutrients alone.

The effects of iron on prokaryotic growth and biomass were also studied in IronEx II (Cochlan 2001) and SOIREE (Hall and Safi 2001). Both studies showed bacterial production in surface waters increased in response to iron additions, with modest biomass accumulation during IronEx II. While neither study showed conclusive evidence of iron limitation, Hall and Safi (2001) argued that prokaryotic production increased as an indirect result of iron-stimulated production of organic carbon by phytoplankton. They reasoned that the lag in the response of prokaryotic production following the initial iron addition and the close association between prokaryotic and particulate primary production over the course of the bloom reflected a stronger dependence of prokaryotes on organic matter rather than iron. There was intense grazing of prokaryotes which prevented biomass accumulation during SOIREE (Hall and Safi 2001), a conceptual confirmation the ecumenical iron hypothesis, albeit on a separate member of the food web. Regardless of the reason for the prokaryotic
responses in IronEx II and SOIREE, they appeared to function predictably as sinks and links for carbon.

Marine prokaryotes, iron, and the ocean carbon cycle: A revised view - Coupled with physical and chemical interactions, biological processes also constrain iron availability in the ocean. Marine prokaryotes, by virtue of their high demand and affinity for iron, have the potential to greatly impact both iron and carbon dynamics in the ocean. For example, prokaryotic uptake of iron constituted 20-45% of the total uptake by the plankton community in the HNLC subarctic Pacific (Tortell et al. 1996). Isolates of heterotrophic bacteria possessed a higher intracellular Fe:C relative to phytoplankton when both groups were exposed to iron deficient conditions (Tortell et al. 1996). These experiments also showed that heterotrophic bacterial growth efficiencies increased once iron deficient conditions were lifted.

This illustration of prokaryotic iron demand, affinity, and uptake fits conceptually with the current model of iron acquisition in microbes. Siderophores are low molecular weight molecules (0.5-1.0 kDa) with varying affinities for Fe$^{3+}$ and are expressed in response to low iron availability (Butler 1998, Witter et al. 2000). They are widely studied in clinical microbiology because pathogenic fungi and bacteria employ them as virulence factors (Raymond et al. 1984). Siderophores are typically expressed in response to iron deficient conditions. They are released extracellularly into the surrounding medium and then reacquired with attached iron through membrane-bound receptors or by diffusive uptake (Raymond et al. 1984). The chemical characteristics (Reid and Butler 1991, Reid et al. 1993), expression (Trick 1989, Wilhelm and Trick 1994, 1995, Wilhelm et al. 1996, 1998),
and uptake kinetics (Granger and Price 1999, Maldonado and Price 1999, Hutchins et al. 1999a, Barbeau et al. 2001) of marine prokaryotic siderophores, including autotrophic prokaryotes, have been described in vitro. However, very few studies have detected and characterized siderophores occurring in situ (Macrellis et al. 2001).

Although the in situ dynamics of siderophore production and siderophore-mediated iron uptake remains largely unexplored, it may be instructive to look at the iron fertilization experiments for hints as to the properties or processes that govern their dynamics. A recurrent theme in the development and continuation of the three iron-induced phytoplankton blooms was the concentration, speciation, and bioavailability of iron. Low iron bioavailability was first suspected as a factor in the muted phytoplankton and geochemical response during IronEx I (Martin et al. 1994). This was addressed with multiple iron additions in IronEx II (Coale et al. 1996). However, repeated additions did not necessarily restore iron to non-limiting concentrations. The appearance of iron-binding organic ligands seemed to act as an iron-sequestration mechanism and confounded uptake by phytoplankton (Rue and Bruland 1997). In SOERE similar iron-binding ligands were also noted, but they appeared to play a role in enhancing phytoplankton activity rather than restricting it (Croot et al. 2001).

The occurrence of iron-binding ligands during IronEx II and SOERE raises a paradox. The production of iron-binding ligands directly implicated a source that is widely known to produce them: prokaryotes. Prokaryotic properties were not considered during IronEx I and were believed to be of only peripheral importance to carbon dynamics during IronEx II. Their importance in the proliferation of phytoplankton blooms may have been underestimated in light of later suspicions that prokaryotes could be the source of organic
ligands (Rue and Bruland 1997, Croot et al. 2001, Boyd 2002). However, the observations of organic ligand production following iron additions do not fit with the first principles observed in laboratory culture studies of siderophore expression. Laboratory studies showed that an iron-deficient environment was a pre-condition for siderophore expression. As previously mentioned, the chemistry, dynamics, sources, fates, and conditions for the in situ expression of siderophores have been poorly characterized. Thus the factors that govern in vitro siderophore dynamics may not be a full representation of what governs in situ dynamics.

Examination into the composition of the natural DOM pool in the ocean has revealed a subset of compounds whose chemical characteristics are in surprising agreement to those of siderophores. The compounds are low-molecular weight organic ligands with high affinities for Fe$^{3+}$. Studies in the North Pacific Ocean (Rue and Bruland 1995) and the northwest Atlantic Ocean (Wu and Luther 1995) showed that the overwhelming majority of dissolved inorganic iron (>99.9%) was complexed to these organic ligands. The ligands fell into two classes, L1 and L2, and their distribution and abundance in the water column varied with depth. Organic complexation of iron by similar ligands has been observed in the equatorial and South Atlantic Ocean (Powell and Donat 2001) and the western Mediterranean Sea (vanden Berg 1995). The origins of the naturally-occurring compounds and the extent to which they enhance bioavailability of iron are not known. Nonetheless, their discovery again forces a re-examination of our understanding of the assumptions that govern siderophore dynamics and further underscores the complexities of iron chemistry in the ocean.

Marine prokaryotes are clearly important as links in the ocean food web and as the major remineralizers of ocean carbon. However, the convergence of these four separate
areas of study - prokaryotic iron uptake and demand, laboratory characterization of siderophores, iron fertilization experiments, and oceanic DOM characterization – provides motivation to re-examine the traditional role of prokaryotes in the ocean. The past studies described above indicate that the manifestation of prokaryotic physiology as it pertains to iron (i.e. siderophores) may play a more prominent role, directly and indirectly, in modulating carbon flow in the ocean. From the view point of DOC dynamics, increased iron availability, facilitated by siderophores, may enhance DOC remineralization and reduce the carbon export ratio provided other nutrients do not become limiting. Indirectly, a byproduct of prokaryotic demand and affinity for iron is a hindrance placed on phytoplankton to meet their physiological iron demand. In this way, prokaryotes clearly have the potential to be antagonists of phytoplankton growth.

Indirectly, the latter effect has been demonstrated in iron limitation studies of natural phytoplankton populations in iron-replete waters. Rather than study the stimulatory effects of iron on phytoplankton growth in iron-depleted waters, the effects of iron removal on phytoplankton growth in iron-replete waters were examined (Wells et al. 1994, Wells 1999). This was achieved by using a terrestrially-derived fungal (Streptomyces pilosus) siderophore, desferal \textsuperscript{®} or desferrioxamine B (DFOB). The results showed that DFOB limited the growth of *Synechococcus* in the equatorial Pacific Ocean (Wells et al. 1994) and that DFOB could limit iron uptake in the 0.2-5.0 \textmu m size fraction of the plankton community (Wells 1999). A subsequent study demonstrated taxon-specific growth limitation of phytoplankton by DFOB (Hutchins et al. 1999b). Heterotrophic bacterial growth was also observed to be adversely affected by DFOB additions. However, the lack of growth might have been due to an indirect limitation by low dissolved organic carbon (DOC) production by phytoplankton.
rather than low iron availability (Hutchins et al. 1999b). The objective in each of these studies was to observe the effects of iron limitation on phytoplankton growth rather than focus on a scaled interpretation of siderophore-induced iron limitation in the ocean. Nonetheless, they may serve as a proof-of-concept provided that the mechanisms, frequency of siderophore expression and abundances in situ, can be confirmed.

Parenthetically, recent advancements in analytical and molecular-based methodologies have also led to the discovery of new prokaryotic processes in the ocean. Historically, photosynthesis by single cells in the ocean has been regarded as a process restricted to the algae, cyanobacteria, and prochlorophytes. However, Kolber et al. (2000, 2001) found that up to 20% of surface heterotrophic bacteria utilize a novel form of photoautotrophy to fix carbon. Simultaneously, Beja et al. (2000, 2001) found that rather than fixing carbon, some prokaryotes employ photoheterotrophy to generate ATP and biomass through light-mediated reactions. The inclusion of prokaryotes into the primary producer community, coupled with the known inherent iron acquisition capabilities of heterotrophic prokaryotes, will lead to refinements in the understanding of ocean ecology and carbon cycling.

*Marine prokaryote diversity, composition, and community structure in the ocean* - Knowing the distributions, abundances, and identities of prokaryotes is an important step in understanding how their ecology affects biogeochemical processes and vice versa. Prokaryotes are a dynamic mosaic of taxa with variable phylogenetic and metabolic diversity. In fact, phylogenetic investigations have shown that prokaryotes possess broad diversity with a high degree of community structure in the water column (Giovannoni et al.

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1990, 1996, Rappé et al. 1998, González et al. 2000, Morris et al. 2002, 2004). However, the advances in determining the phylogeny of prokaryotes have not necessarily translated into an understanding of their functional roles in the ecosystem. For example, extensive surveys of *Archaea* have shown that they occur in a variety of oceanic environments - temperate coastal waters (DeLong 1992, Fuhrman et al. 1992, Massana et al. 1997, Murray et al. 1999), Antarctic coastal waters (DeLong et al. 1994), the open-ocean (Fuhrman and Davis 1997, Massana et al. 2000), and the meso- and bathypelagic (Karner et al. 2001) – and that their populations display seasonality (Murray et al. 1998, Church et al. 2004). Archaeal relevance to these environments and the factors which alter their ecology are not well understood.

Conversely, it can be instructive to review the environmental contexts in which prokaryotic communities are dynamic as a means to connect diversity with function. Seasonal, episodic, and annual climatic perturbations are known to affect prokaryotic dynamics. However, the composition, diversity, and structure of prokaryotic communities has often been overlooked. For example, decadal climatic variability is responsible for ecosystem-wide changes in the ecology and biogeochemistry in the North Pacific Subtropical Gyre (NPSG), one of the largest oceanic domains on earth (Karl et al. 2001). The basis for this finding was a major shift in nutrient stoichiometry in the NPSG between 1988 and 1994, which favored phosphorus limitation over nitrogen limitation of phytoplankton growth (Karl and Tien 1997). In turn, changes in resource limitation provided a driving force for a shift in phytoplankton community structure (Karl et al. 1997). Changes in community structure also appeared to alter DOM biogeochemistry in the NPSG (Church et al. 2002). The significance of DOM source shifts with respect to prokaryotic diversity and community structure, however, are still not known.
On a smaller scale, the open-ocean iron fertilization experiments provided an opportunity to study the ecological and biogeochemical responses of a plankton community to an analogous natural perturbation (in the form of an episodic dust input of iron to the surface water, for example). The experiments were a clear demonstration of how a change in nutrient chemistry can bring about major changes to the phytoplankton community structure and subsequent biogeochemical pathways (Coale et al. 1996, Boyd et al. 2000). Iron additions also shifted zooplankton community structure to varying degrees (Landry et al. 2000a, b, Hall and Safi 2001). Iron did affect prokaryotic stocks and rates (Cochlan 2001, Hall and Safi 2001), but it was not clear how iron affected prokaryotic community structure. Potential alterations to prokaryotic community composition could have profound effects on the biogeochemical cycles that they mediate.

Studying prokaryotic diversity, independent of the environmental conditions in which they reside, and vice versa, limits the interpretation of how prokaryotic ecology is linked to their functional roles in the ocean. By combining information on diversity and identity with environmental conditions, changes in prokaryotic communities can be considered in context. This principle was demonstrated in bottle experiments in the Sargasso Sea (Carlson et al. 2002). Additions of organic and inorganic nutrients altered prokaryotic community composition and, in turn, affected the utilization of naturally-occurring DOC. More recent evidence from the northwest Atlantic Ocean points to the bottom-up forcing implied in NPSG DOM dynamics (Church et al. 2002). Convective export of spring bloom production (POC and DOC) was coincident with changes in the diversity of the prokaryotic assemblage below the mixed layer (Morris et al. 2005). The timing of convective transport of semi-labile organic matter, coincident with a prokaryotic community shift, suggested a cause and effect
association (Morris et al. 2005). Previously, Carlson et al. (2004), recreating the convective mixing events in controlled bottle experiments, showed that physical mixing of DOM and prokaryotes could result in community structure shifts similar to the ones observed by in the field by Morris et al. (2005).

Investigations into prokaryotic community composition and structure have been greatly facilitated by culture-independent methodologies (Amman et al. 1995). Culture-independent techniques such as polymerase chain reaction (PCR), gene cloning, gene sequencing (Olsen et al. 1986), whole-cell ribosomal RNA probing (DeLong et al. 1999), and terminal restriction fragment length polymorphism (tRFLP) analysis (Liu et al. 1997) have become widely used tools to detect, identify, and characterize the prokaryotic community in situ. Powerful ordination tools such as principle component analysis and non-metric multidimensional scaling couple prokaryotic community data with disparate environmental data with the objective of revealing coherent trends and patterns (McCune and Grace 2002).

**Motivation** - This dissertation contains studies which describe prokaryotic dynamics in response to simulated natural perturbations. The goal of this work is to provide insights into prokaryotic biogeochemical processes as a function of environmental forcings and community structure. Chapter 1 describes the bulk prokaryotic response to a mesoscale, open-ocean iron fertilization in the Southern Ocean. It addresses the question of whether iron or carbon directly limits prokaryotic growth in HNLC waters. It also describes the prokaryotic response as part of the broader biological and geochemical response to iron. Chapter 2 documents prokaryotic community composition dynamics during the iron fertilization experiment. Specifically, it addresses the fundamental question of whether iron
enrichment affects prokaryotic community structure. Chapter 3 describes the prokaryotic response to nutrients and siderophores in the Sargasso Sea. The aim of this chapter is to study prokaryotic dynamics in controlled nutrient-amended systems.
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CHAPTER 1

THE PROKARYOTIC RESPONSE TO IRON ENRICHMENT
IN THE SOUTHERN OCEAN
ABSTRACT

Prokaryote dynamics were studied as part of the Southern Ocean Iron Experiment (SOFeX), January – February 2002. Two phytoplankton blooms were monitored following infusions with iron sulfate (FeSO₄). The first bloom was initiated north of the Antarctic Polar Front Zone (APFZ) in silica-poor waters (North Patch) and was observed on days 11 and 38 following iron enrichment, whereas the second was south of the APFZ in silica-rich waters (South Patch) and was continuously observed for 30 days. In both experiments iron additions resulted in increased Chl \( a \), particulate organic matter (POC + PN), and a drawdown of inorganic nutrients. Marine prokaryotes responded by increasing their abundance by 110% and 60% in the North and South Patch, respectively, relative to non-enriched waters. Thymidine (TdR) and leucine (Leu) incorporation rates in the North Patch increased by 400% and 120%, respectively, with more modest increases in the South Patch (80% and 70%, respectively). In the South Patch prokaryotic production (BP) was significantly correlated with net particulate primary production (PP) and Chl \( a \). Prokaryotic abundance was also significantly correlated with Chl \( a \). The net prokaryotic accumulation rate in the South Patch was 0.02 d⁻¹ over 17 days, not considering physical dilution by mixing with water outside the South Patch. Despite the close association of BP to PP, BP remained a small fraction of PP (<10%). Evidence is considered suggesting that prokaryotic growth during SOFeX was limited by labile carbon rather than by iron.
INTRODUCTION

After more than a decade of testing the Iron Hypothesis (Martin et al. 1994), it is clear that iron plays a central role in regulating phytoplankton production in the ocean, particularly in high nutrient, low chlorophyll (HNLC) regions. Repeated mesoscale open-ocean iron experiments have also improved the overall understanding of ocean geochemistry (Bakker et al. 2001) and food webs (Landry et al. 2000). These experiments have provided insights into paleo-biogeochemistry (Bidigare et al. 1999) and even sparked controversy over the potential role of iron in ameliorating global climate warming (Chisholm et al. 2001).

Iron enrichment experiments also demonstrated the stimulation of one component of natural carbon storage in the ocean: the biological pump (Ducklow et al. 2001a). In HNLC regions like the Southern Ocean, dissolved iron is the primary rate-limiting factor of the first component of the biological pump, primary production. While iron enrichments have demonstrated priming of the biological pump, they have demonstrated only modest carbon export to the ocean interior (Bidigare et al. 1999). Marine prokaryotes can influence the efficiency of carbon export through the remineralization of organic material (either POM or DOM), thus retarding its export from the surface ocean. During the creation of prokaryotic biomass, prokaryotic respiration returns organic matter back to the inorganic constituents. This process counteracts the autotrophic carbon fixation process and serves as a balance to organic carbon production (POC and DOC).

Understanding growth constraints on prokaryotes can provide insights into the degree to which they can mediate organic carbon fluxes in the ocean. Bottle experiments have revealed direct iron limitation (Pakulski et al. 1996), DOM limitation (Kirchman et al. 2000),
and co-limitation by iron and DOM together (Church et al. 2000) in HNLC regions. In the context of open-ocean iron enrichment experiments, there is a growing body of evidence that has shown the positive effects of iron on prokaryotic growth. During IronEx II in the equatorial Pacific Ocean, Cochlan (2000) observed enhanced growth and production of prokaryotes to the extant that growth exceeded removal by grazing, advection, and/or natural mortality. During the Southern Ocean Iron Release Experiment (SOIREE), Hall and Safi (2001) observed a similar enhancement of bacterioplankton growth and production in response to inputs of iron, but growth and removal were equivocal leading to no net accumulation of prokaryotic biomass. Arrieta et al. (2004), working in the Atlantic sector of the Southern Ocean, observed periodic enhancement of production with modest accumulation of biomass throughout EISENEX. As a whole, previous iron enrichment experiments have successfully documented the prokaryotic response within the context of the microbial food web; however, the response has not been thoroughly evaluated within the broader geochemical context.

The objective of my study was two-fold. The first objective was to measure the response of prokaryotes to iron enrichment and to determine whether the response was directly or indirectly stimulated by iron. I hypothesized that prokaryotic production would increase as a direct consequence of increased DOC production from iron-stimulated phytoplankton, rather than as a direct response to iron. Furthermore, I predicted that while prokaryotic production would increase, prokaryotic biomass would not (e.g. Hall and Safi 2001). My second objective was to understand the prokaryotic response as part of a broader plankton and biogeochemical response to iron enrichment. Overall, this study was conducted to understand the controlling factor(s) of prokaryotic activity in the ocean and the degree and
time scales upon which this activity modifies biological pump efficiency during phytoplankton blooms in the Southern Ocean.

**MATERIALS AND METHODS**

*Study site* - Two iron enrichment experiments were performed in the HNLC Pacific Ocean sector of the Southern Ocean during the late austral summer 2002 (January through February) (Coale et al. 2004). The first experiment took place north of the Antarctic Polar Front Zone (APFZ) in low iron, low silica, high NO$_3$ waters (<0.1 nmol Fe L$^{-1}$, 3 µmol Si L$^{-1}$, 22 µmol N L$^{-1}$, respectively) near 56$^\circ$S, 172$^\circ$W and was designated the North Patch. The second experiment took place south of the APFZ in low iron, high silica, high NO$_3$ waters (0.1 nmol Fe L$^{-1}$, 60-64 µmol Si L$^{-1}$, 28 µmol N L$^{-1}$, respectively) near 66$^\circ$S, 171$^\circ$W and was designated the South Patch. Water temperatures in the North and South Patches were 7°C and -1°C, respectively. All prokaryotic, primary production, and POM measurements reported herein were made aboard the *R/V Melville*. Initial hydrographic measurements were made aboard from the *R/V Revelle* during surveys prior to the iron additions. Three iron fertilizations in the North Patch occurred on January 13, 17, and February 9; three iron fertilizations in the South Patch took place on January 25, 28, and February 1. Periodically, *R/V Melville* conducted transects of the South Patch to determine its dimensions and measure standing stocks and biological rates.

*Marine prokaryotes* - Water was collected with Niskin bottles from depths of 5, 10, 20, 30, 40, and 50 m for the North and South Patch or during transects across the South Patch from
depths of 10 and 50 or 60 m. Prokaryotic abundance was determined by flow cytometry (FCM) following the methods of Troussellier et al. (1999). Flow cytometry samples were fixed with 0.2 μm-filtered formalin (1% final concentration) and stored at -80°C until analysis. Triplicate 1 ml subsamples were stained with Syto13 (Molecular Probes) and counted on a Beckman-Coulter Epics Altra flow cytometer equipped with an Enterprise II laser at 488 nm. Count calibration was performed by adding 1.0 μm beads (Molecular Probes, Fluo Spheres) at a concentration of 5 x 10⁴ beads ml⁻¹ to each subsample. A minimum of 10,000 cells was counted from each subsample. Intra-sample variation was less than 1%. High DNA- (HDNA) and low DNA-containing (LDNA) cells were differentiated by fluorescence (Gasol and Moran 1999).

Prokaryotes were also enumerated via the acridine orange direct count method of Hobbie et al. (1977). Briefly, samples were preserved with 0.2 μm-filtered gluteraldehyde (1% final concentration) and stored at -80°C until analysis. Five to twenty milliliters were filtered through a blackened, 0.2 μm polycarbonate filter (Poretics) and stained with acridine orange. Stained filters were affixed to glass slides and stored at -20°C with until enumeration. Cells were visualized by epifluorescence microscopy on an Olympus Provis AX-70 microscope (1000X) using ultraviolet excitation. For statistical purposes a minimum of 300 cells were counted (Kirchman et al. 1982).

Prokaryotic production (BP) was estimated via tritiated thymidine (³H-TdR) and leucine (³H-Leu) incorporation using the microcentrifuge method described by Smith and Azam (1992). Briefly, separate incubations for ³H-TdR and ³H-Leu incorporation were carried out in triplicate 2 ml microcentrifuge tubes containing 20 nmol L⁻¹ ³H-TdR (specific activity: 88.7 Ci mmol⁻¹) or 20 nmol L⁻¹ ³H-Leu (specific activity: 172 Ci mmol⁻¹). Killed

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controls contained the same concentrations of ^3H-TdR and ^3H-Leu but with 100 μl 100% trichloroacetic acid (TCA). Incubation times ranged between 8 and 14 h depending on the ambient water temperature (Church et al. 2000). All incubations were carried out in the dark at the ambient temperature in a circulating water bath. Incubations were stopped by additions of 100% TCA, samples centrifuged to a cell pellet, and nucleic acids and proteins extracted. Ultima Gold scintillation cocktail (Packard) was added to the remnant cellular material in the microcentrifuge tubes and radiolabeled biochemicals were detected by counting the samples on a scintillation counter.

Prokaryote carbon was calculated using a conservative carbon conversion factor (CCF) of 12.4 fg carbon per cell (Fukuda et al. 1998). To calculate total POC, and in turn, prokaryotic carbon: total POC, POC was corrected assuming that ~50% of the prokaryotic cells passed through a pre-combusted Whatman GF/F filter (Total POC = POC + [0.5 x Prokaryote carbon]). Prokaryote nitrogen was calculated by dividing prokaryote carbon by a prokaryotic C:N of 4.5 (Goldman and Dennett 1991). Total PN was corrected following equation as above. Cell production rates based on thymidine incorporation were calculated using a thymidine conversion factor (TCF) of 2.4 x 10^{18} cells produced per mole thymidine incorporated (Fuhrman and Azam 1982). Specific growth rates (d^{-1}) were derived by dividing the cell production rate (cells L^{-1} d^{-1}) by the total abundance (cells L^{-1}) as determined by FCM. Prokaryotic carbon production rates derived from leucine incorporation utilized a leucine conversion factor (LCF) of 1.5 kg carbon produced per mole leucine incorporated (Simon and Azam 1989). Turnover rates of prokaryotic biomass were computed by dividing the carbon production rate, P (μg C L^{-1} d^{-1}), by the prokaryotic carbon biomass, B (μg C L^{-1}).
Grazer-free seawater cultures were initiated during occupation of the South Patch for the purpose of providing another estimate of prokaryotic specific growth rate. Water was collected from the mixed layer (< 20 m) from within iron-fertilized waters on days 6, 12, and 19 using a trace-metal clean rosette. Water for reference seawater cultures was collected from non-fertilized waters on days 7, 13, and 21. Water was filtered from the rosette through a pre-combusted Whatman GF/F into darkened, acid-washed, 1-L polycarbonate bottles. Bottles were capped and placed in darkened incubators with flowing ambient seawater. Bottles were sampled every other day for prokaryotic abundance. Specific growth rates (d\(^{-1}\)) were calculated as the slope of the natural log-transformed curve of prokaryotic abundance versus time excluding the lag and stationary phases of growth. Linear regressions of prokaryotic abundance versus time were also tested for significance (\(\alpha = 0.05\)).

Mixed layer depth - To determine mixed layer depth (MLD)-averaged rates, Leu-based BP was integrated through the depth of the mixed layer and divided by its depth. MLD was determined using a criterion of a change of 0.02 from surface \(\sigma_t\) for 1-m binned density measurements (U.S. JGOFS, http://usjgos.whoi.edu/jg/dir/jgos/, nbp97_8, W. Smith, Ross Sea Mixed Layer Depths). In instances where full water column prokaryotic data were lacking for integration, an average of abundances and rates measured within the mixed layer were used. The MLD, sample sizes, and sample sources are provided in Appendix 1.1.

Statistical analysis - Ordinary linear least squares regression (OLS) analyses were performed for total and HDNA prokaryotic abundance, TdR and Leu incorporation, and BP and PP versus time. Model II or geometric mean regression analyses were performed for BP versus
PP, size-fractionated PP, size-fractionated chlorophyll a (Chl a), and POC and PN. Simple linear correlations between prokaryotic and biological/geochemical variables were also performed. Pearson's product-moment correlation coefficients were calculated and significance of those correlations were accepted or rejected using an $\alpha = 0.05$. Statistical comparisons of mean prokaryotic properties and processes between IN and OUT stations were performed using two-sample $t$-tests (95% confidence interval, $\alpha = 0.05$). Means were computed two ways: by averaging all IN station discrete depth data and comparison to the mean of all OUT station discrete depth data, and by averaging each integrated property over all IN stations and comparison to the mean of all integrated OUT stations.

**Primary production, Chl a, and POM** - Primary production (PP) measurements were obtained from Dr. R. T. Barber. Primary production, Chl a, and POM data were MLD-averaged as previously described.

**RESULTS**

**North Patch** - Iron enrichment positively enhanced primary production (Table 1.1). Primary production was 140% and 70% higher on days 11 and 39, respectively, than on day 0. IN station PP on day 11 was 43% higher than OUT station PP on day 12. The magnitude of total PP decreased by day 39, but IN station PP remained 40% higher than OUT station PP. While there was an increase in PP between days 0 and 11 in the North Patch, there was no detectable shift in size-fractionated PP. Total PP was dominated by the 0.7-5.0 $\mu$m fraction for both IN and OUT stations. A notable shift in size-fractionated PP was observed during
the latter stage of our observations. On day 39 the IN station percentages of 5-20 and >20 μm fraction PP were 26% and 26%, respectively, and the 0.7-5.0 μm fraction PP decreased to 48% of total PP. At the OUT station, total PP remained dominated by the 0.7-5.0 μm fraction. Chl $a$ also increased over time within the North Patch. Initial Chl $a$ concentrations were 0.07 μg L$^{-1}$ and rose to 0.39 and 0.61 μg L$^{-1}$ by days 11 and 39, respectively. Chl $a$ concentrations within the iron-enriched waters were also higher than non-enriched waters.

Marine prokaryotes also responded positively to iron enrichment in the North Patch (Table 1.1). Opportunity to sample the North Patch was limited by ship logistics so only longer-term changes and comparison of enriched and non-enriched waters are presented. IN station prokaryotic abundance and biomass were 80% greater than OUT station abundance by day 11-12. By day 39 IN station abundance was 170% higher than day 12 OUT station abundance and 110% higher than day 38 OUT station abundance. The net increase in prokaryotic biomass between days 11 and 39 in the North Patch was 50%. This compares to an apparent net increase in non-enriched waters of 26% over the same time period. The absolute abundance of HDNA cells was higher for IN stations on days 11 and 39 relative to OUT stations (data not shown); however, the HDNA cells did not change over time in fertilized waters as a fraction of the total abundance.

Thymidine and leucine incorporation rates followed a similar trend to abundance. IN station TdR and Leu on day 11 were two-fold higher than OUT station rates. TdR was higher on days 38 and 39 for both IN and OUT stations; but, TdR in iron-enriched waters was still higher by a factor 1.5 over non-enriched waters. Incorporation of leucine exhibited little change in both IN and OUT stations late in the bloom; however, IN station Leu remained nearly two-fold higher than OUT station rates. The trends for BP were the same as
those for Leu since a single conversion factor was used to obtain carbon production.

Prokaryotic production as a percentage of total PP was relatively uniform in time and space, ranging from 4-9%.

South Patch - Iron addition also significantly enhanced phytoplankton and prokaryotic properties in the South Patch. The response to enrichment is summarized by comparison of days 5 and 22 following enrichment (Table 1.2). Values at the reference OUT stations are reported as an average of four MLD-averaged measurements made on days 7, 9, 13, and 20. Due to the net eastward movement of the iron-fertilized patch over time, the reference stations were determined where SF6 concentrations in the mixed layer reached analytical background. Thus, OUT stations were not temporally or spatially consistent and may reflect higher variability than if one non-enriched water mass had been followed over time.

Marine prokaryotes abundance and biomass - A significant (p<0.05) increase in prokaryotic abundance was observed in the fertilized patch between days 5 and 22 (Table 1.2 and Figure 1.1). Abundance and biomass increased by 60%. Variability in OUT station abundance is evident (Figure 1.1); however, the change in OUT station abundance over time was not significant. HDNA cell abundance increased steadily between days 5 and 22 (Figure 1.1; p<0.05). The percentage of HDNA cells within the South Patch increased from 24% to 45%, whereas the mean of OUT station %HDNA was 36% (Table 1.2). Least-squares regression of OUT total and HDNA abundance indicated that there were no significant trends over time. A statistical comparison of means at the IN and OUT stations, using discrete depth data, for abundance, HDNA, and biomass showed no statistical differences (t-test, p<0.05) (Table
1.3). However, when days 5 and 8 IN station data were removed from the calculation of the mean, there were significant differences \((p<0.05)\). When the same comparison was made using a computation of the mean with integrated data, no statistical differences were observed (Table 1.3). Prokaryotic mortality via microzooplankton grazing and/or viral lysis and/or dilution from surrounding non-enriched waters (0.08 \(d^{-1}\), M. A. Brzezinski, pers. comm.) may have accounted for the low observed net increase in prokaryotic abundance, thus underestimating the gross numerical response.

**Prokaryotic production** - Incorporation rates between days 5 and 22 followed the trend of abundance (Table 1.2 and Figure 1.2A). TdR increased three-fold while Leu increased 1.5-fold. Increases in TdR and Leu were significant over time \((p<0.05)\) and depth-averaged IN station TdR and Leu were higher than depth-averaged OUT station rates. Mean OUT station TdR and Leu were comparable to day 5 rates. However, IN station TdR and Leu were higher than OUT station rates by day 22 (Table 1.2). Significant differences between mean IN and OUT station TdR and Leu \((t\text{-test}, p<0.05)\) were apparent for two separate estimates of the mean (average of all discrete depth data and average of all integrated data) with the exception of IN versus OUT, days 5-22 integrated TdR (Table 1.3). Cell-specific incorporation of thymidine (SpTdR) and leucine (SpLeu) were highly variable for both IN and OUT stations, with neither SpTdR nor SpLeu showing any clear trends (Figure 1.2B). Leu IN was significantly different from OUT and peaked on days 12 and 21 within iron-enriched waters. Periodic maxima in specific incorporation rates may indicate episodes of enhanced growth in response to iron addition on days 8 and 11. Prokaryotic production doubled from 0.2 to 0.4 \(\mu g \text{ C L}^{-1} \text{ d}^{-1}\) (Figure 1.4A). OUT station BP averaged 0.2 \(\mu g \text{ C L}^{-1}\)
and was consistently less than IN station BP over the course of 17 days.

**South Patch Transects** - Transects across the South Patch revealed temporal and spatial variability in prokaryotic abundance and incorporation rates (Figure 1.3). Trends in prokaryotic abundance were not apparent within the South Patch transects (Figure 1.3A, B, C) and abundance did not show a strong correlation to SF$_6$ concentrations (data not shown). Nonetheless, abundance exhibited variability in both space and time. Incorporation rates displayed higher spatial and temporal variability than abundance (Figure 1.3D, E, F). When incorporation rates were linearly regressed against SF$_6$ concentrations, spatial and temporal trends could be seen (Figure 1.3G, H, I). During transect 1, 49% and 58% of the variability in TdR and Leu, respectively, could be explained by SF$_6$ concentrations. In transect 2, this changed to 45% and 32% for TdR and Leu, respectively. By transect 3, 86% and 85% of the variability could be explained by SF$_6$ concentrations for TdR and Leu, respectively. The slopes of the linear regression lines also increased over time. This is reflection of the non-conservative nature of SF$_6$ due to loss of the gas through ventilation to the atmosphere.

**Marine prokaryotes and primary production** - Primary production in the South Patch increased four-fold over 17 days before declining sharply on day 22 (Figure 1.4A). The increase over time was not statistically significant ($p>0.05$); however, when the final time point is excluded, the relationship becomes significant ($p<0.05$). A shift in the size distribution of PP was also observed within the South Patch as PP in the 5-20 μm fraction increased from 17% to 27% of total PP from days 0 to 21 (Table 1.2). The relative percentage of the total PP <5 μm decreased from 54% to 30%, whereas the %PP in the >20
μm fraction increased from 29% to 43% by day 21. 51% of the average of OUT station PP was in the > 20 μm fraction. Despite the larger relative increase in PP, BP was closely correlated with total PP (Figure 1.4B). Geometric mean regression revealed that 80% of the variation in BP was attributed variability in total PP demonstrating that BP and PP were thus closely coupled. However, BP was only 1% of PP (Figure 1.4B) and 3% of PP on days 5 and 22 (Table 1.2). Prokaryotic production was not significantly correlated with the 0.7-5.0 μm fraction of PP, but was significantly correlated with the 5.0-20 and > 20 μm fraction of PP (Figure 1.5, Table 1.4).

Stocks of Chl a, POC, and PN also increased as a result of iron enrichment (Table 1.2). Chl a increased from 0.1 to 3.6 μg L\(^{-1}\) between days 0 and 21. Particulate organic carbon and nitrogen both increased more than two-fold over this period. Prokaryotic production was strongly correlated with total Chl a (Figure 1.6A; \(r = 0.86\), Table 1.4), correlated most strongly with the > 20 μm fraction (Figure 1.6A; \(r = 0.94\), Table 1.4), and less strongly with the 5-20 μm fraction (Figure 1.6A; \(r = 0.73\), Table 1.4). BP was also significantly correlated with PN (Figure 1.6B; \(r = 0.77\), Table 1.4). Prokaryotic abundance correlated significantly with Chl a, but did not correlate significantly with POC, PN, or PP (Table 1.4). Prokaryotic biomass, as a fraction of total POC and PN, decreased from day 5 to 22 (Table 1.2). The decrease of prokaryotic carbon:total POC is most likely a dilution effect due to the larger increase in autotrophic biomass relative to prokaryotic biomass (600% vs. 50%).

Prokaryotic specific growth rates - Prokaryotic specific growth rates were estimated using cell-specific \(^3\)H-TdR incorporation and grazer-exclusion seawater cultures. IN station TdR-
based growth rates were equal to OUT station rates during the early and middle stages of the bloom (Table 1.5). Late in the experiment (day 19) TdR-based growth rate was higher than its reference OUT station. There was a temporal increase in the TdR-based growth rate within the South Patch. The specific growth rate increased from 0.14 d\(^{-1}\) (days 5-7) to 0.19 d\(^{-1}\) (days 12, 19). The same trend and magnitude in specific growth rates were observed in the grazer-free seawater cultures. The specific growth rates were equal early in the experiment (days 5-7), but they increased from 0.11 d\(^{-1}\) (days 5-7) to 0.14 and 0.15 d\(^{-1}\) (days 12 and 19, respectively). In one instance the specific growth rate outside the iron-fertilized patch was higher than inside the patch (days 12, 13) in the grazer-free seawater cultures. However, taken together all other estimates of specific growth rates in iron-fertilized waters were equal to or higher than specific growth rates outside iron-fertilized waters. Instances where there were comparable specific growth rates inside and outside iron-fertilized waters suggests that prokaryotes were not nutrient-limited in non-fertilized waters. In fact, nearly a third of the prokaryote population in OUT stations were HDNA cells, an indication of an actively growing subpopulation free from growth constraints (Table 1.2).

*Flow cytometry versus microscopic counts* - Prokaryotic abundance was measured using flow cytometry and acridine orange direct counts. The fidelity of cytometric counts, a more recent enumeration method, to the traditional microscopic counts is shown in Figure 1.7. Model II regression of cytometric versus microscopic counts showed general agreement between the two methods \((y = 0.85x + 0.28; r^2 = 0.68, n = 137)\) with a significant Pearson's correlation coefficient \((r)\) of 0.82 \((p<0.05)\).
DISCUSSION

*Prokaryotic dynamics during SOFeX* - Following iron fertilizations north and south of the APFZ, BP increased as hypothesized. The magnitude of the increase (two-fold) was less than that observed by Hall and Safi (2001) during SOIREE and Cochlan (2001) in the Equatorial Pacific Ocean during IronEx II, but the increase was comparable to BP observed by Arrieta et al. (2004) during EisenEx. In the latter study prokaryotes responded to iron enrichment with a three-fold increase in BP. Contrary to the hypothesis that prokaryotic abundance would not increase, a two-fold net accumulation of prokaryotic biomass was observed over the course of 40 days in the North Patch and 22 days in the South Patch. This is in contrast to the observations of Hall and Safi (2001) who saw no net accumulation in biomass over 13 days, but is consistent with those of Arrieta et al. (2004) who observed a two-fold increase in prokaryotic abundance by the end of EisenEx. It is also consistent with the observations of Cochlan (2001) who saw a similar net increase over 14 days in tropical waters during IronEx II in the equatorial Pacific. Landry et al. (2000), also working in IronEx II, calculated a net accumulation rate for prokaryotes of 0.08 d⁻¹, higher than the net accumulation rate observed herein (0.02 d⁻¹). Furthermore, a net increase in HDNA cells in the South Patch was observed. This suggests an actively growing subpopulation of prokaryotes (Lebaron et al. 2001) and is corroborated by increasing thymidine incorporation rates over time in the South Patch.

Enhancement of prokaryotic growth in iron-fertilized waters is evident from the two independent estimates of the specific growth rate. The specific growth rate, as measured by $^3$H-TdR incorporation, showed a modest increase over time. Cochlan (2001) observed an
increase in the specific growth rate using $^{3}$H-Leu from 0.10 to 0.45 d$^{-1}$ following iron enrichment. The microzooplankton exclusion cultures also showed an increase in the specific growth rate over time in the fertilized patch (Table 1.5). Both estimates of specific growth rate were also in general agreement with respect to the magnitude of the rates.

Comparison of prokaryotic dynamics between the North and South Patches is limited by the paucity of observations in the North Patch and the lack of time-zero measurements. However, a comparison can be made between the responses to iron enrichment assuming that OUT stations were similar to initial conditions. By day 11 in the North Patch, prokaryotic biomass had nearly doubled, whereas there was no change in biomass by day 12 in the South Patch. Incorporation rates in the North Patch had doubled by day 11 with the same change in TdR and a slightly lower increase in Leu in the South Patch. Thymidine-based growth rate did not change substantially in either the North or South Patch after 11 and 12 days, respectively.

The prokaryotic response during SOFeX was also consistent with previous mesoscale enrichments in terms of the relative proportion of biomass (BB:PB) and the correlation of BP to PP. There was a disproportionate increase in phytoplankton relative to prokaryotic biomass. Using Chl $a$ as a proxy for phytoplankton biomass, North Patch phytoplankton biomass increased eight-fold compared to a two-fold increase in prokaryotic biomass over 40 days. In the South Patch, Chl $a$ increased twenty-seven-fold compared to a two-fold increase in prokaryotic biomass. Cochlan (2001) observed the same pattern with a peak increase in phytoplankton biomass of fourteen-fold with only a two-fold increase in prokaryotic biomass. Hall and Safi (2001) observed a peak increase in integrated Chl $a$ of six-fold with no increase in prokaryotic biomass. A proximal cause for this disproportionate increase in
biomass is a high rate of prokaryotic mortality relative to phytoplankton mortality and a
closer coupling between prokaryotes and their predators. In fact, prokaryotic mortality
during SOFeX could have balanced growth or to allowed only minor accumulation of
biomass (M. R. Landry et al., pers. comm.). Increases in heterotrophic nanoflagellates and
the uncoupling of BB from BP during SOIREE were evidence of a high rate of prokaryotic
mortality (Hall and Safi 2001).

Prokaryotic growth efficiency (BGE) may have changed in response to the increase in
PP, but direct measurements of BGE were not made. The observation that prokaryotic
carbon production in the South Patch was significantly correlated to net particulate primary
production (PP) (i.e. that the ratio of BP to PP remained constant) serves as circumstantial
evidence for ruling out the effect of BGE on the dampened response in prokaryotic biomass.
Hall and Safi (2001) found a similar positive correlation between BP and PP. The
relationship we observed between the two parameters is striking ($r = 0.80$) and both BP and
PP trends were similar in time and space. This suggests that bacteria were responding
positively to PP. del Giorgio and Cole (2000) concluded that BGE generally increases as BP
increases and also reported that BGE is proportional to PP.

Substrate limitation: The case for carbon - During SOFeX primary production was
unequivocally stimulated by multiple iron additions (Figure 1.4). Drawdown of inorganic
nutrients (DIC, NO$_3$, and PO$_4$), a response observed in previous experiments, was also
observed. While the evidence for iron limitation of phytoplankton during SOFeX is
unequivocal, the argument for iron limitation of prokaryotes is not as clear.
First, prokaryotic incorporation rates increased over time in the low iron (<1 nmol L⁻¹), OUT stations in the North Patch (Table 1.1). Also, TdR-specific growth rate was higher in non-enriched waters by day 38 and higher than the growth rate measured in the North Patch on day 39. Leucine incorporation, an indicator of biomass synthesis, was higher by 20% on day 38 compared to day 12 in the reference stations outside the North Patch. If one assumes that iron directly limits prokaryotic growth, then the observed higher TdR and Leu in low iron OUT stations over time and relative to IN stations are paradoxical.

Second, BP in the South Patch showed a lag of approximately 8 days behind the initial enrichment with iron. A positive response was initiated after three enrichments with iron (Figure 1.4A). Primary production, on the other hand, increased after 5 days, with shifts in size-fractionated PP to larger cells (Table 1.2). It is assumed that BP at time zero in the South Patch was similar to that of OUT station BP and BP on day 5. In fact, using the Model II regression of BP and PP (Figure 1.4B) and calculating BP using a time zero PP of 7.4 µg C L⁻¹ d⁻¹, BP is computed to be 0.2 µg C L⁻¹ d⁻¹, nearly the same as day 5 in iron-fertilized waters. Considering prokaryotes have a high iron demand (Tortell et al. 1996) and are highly competitive in taking up iron compared to phytoplankton (Hutchins et al. 1999), it is doubtful that in an iron-limited system prokaryotic growth would lag behind phytoplankton growth following new inputs of iron if iron were the primary control on prokaryotic growth.

Third, growth rates estimated by ³H-TdR incorporation and from grazer-exclusion cultures from low iron reference stations outside the South Patch are comparable to those in iron-fertilized waters. If prokaryotes were iron-limited in the Southern Ocean like phytoplankton, there would be an expectation to see lower growth rates in the OUT stations.

It should be noted, however, that there are methodological differences between the
two specific growth rate estimates. The TdR-based specific growth rate is independent of cell biovolume, but assumes all prokaryotes take up and incorporate thymidine. Additionally, the grazer-exclusion cultures reflect the prokaryotic community that passed through the nominal 0.7 μm pore size of the filter. Larger cells would not pass through the filter and would not contribute to the estimate of specific growth rate. Despite these methodological differences and assumptions, the two specific growth rates for prokaryotes are in general agreement in magnitude, but also in trend for the iron-fertilized patch.

Given the paucity of iron, how are prokaryotes able to grow in non-iron fertilized waters? As mentioned above, prokaryotes are highly competitive with phytoplankton for iron due to their ability to acquire iron by expressing iron-binding ligands known as siderophores (Butler 1998). Prokaryotes are capable of acquiring iron using their own expressed siderophores and have been shown to acquire iron by utilizing exogenous siderophores as well (Hutchins et al. 1999). Thus, siderophore-mediated iron uptake is one possible way in which prokaryotes likely circumvented iron limitation during SOFeX. It would explain the comparable specific growth rates from seawater cultures and rates of substrate incorporation observed inside and outside the South Patch. Another possibility is the acquisition of iron bound to bulk DOM. The majority (>99%) of dissolved iron in the ocean has been found to be organically complexed (Rue and Bruland, 1997), a pool that is poorly characterized. It is possible that prokaryotes are capable of acquiring iron from bulk DOM using strategies that are currently unknown.

The exclusion of iron as a limiting factor leaves macronutrients and organic carbon as potential growth-limiting factors. Macronutrients (nitrate and phosphate) were replete throughout the experiment. Ammonium concentrations increased in iron fertilized waters to
0.1 – 0.2 μmol L$^{-1}$ (Cochlan, pers. comm.). Thus, dissolved organic carbon limitation likely constrained prokaryotic growth. There are numerous sources of DOM to prokaryotes within ocean food webs, and it is produced through a variety of mechanisms (Nagata 2000). DOM composition and lability exhibits a wide range (Carlson 2002), and these (rather than DOM concentration) directly affect prokaryotic growth. In the open ocean one source of DOM to prokaryotes is extracellular release by phytoplankton (ER; Nagata 2000, Carlson 2002). ER varies as a function of light, nutrient availability, and species composition (Nagata 2000). Despite its variation over ocean systems, ER tends to be linearly related to PP and averages 13% of PP (Baines and Pace 1991). It is possible to demonstrate that direct excretion by phytoplankton during SOFeX was sufficient to meet prokaryotic carbon demand (BCD) and alleviate carbon limitation. First, a conservative value for prokaryotic growth efficiency (BGE) of 20% is assumed (Carlson et al. 1999, Ducklow et al. 2000). Next, averaged BP from OUT stations and day 22 IN station (Table 1.2) to calculate BCD are employed. Finally, a constant ER of 13% over time using PP concurrent with BP is assumed (Table 1.2). In non-enriched waters, ER could meet only 60% of BCD. By difference this means that 40% of BCD was met by other carbon sources. In fertilized waters on day 22, ER could meet 95% of BCD, thus obviating prokaryotic need for other carbon sources. Because PP was elevated above 8 μg C L$^{-1}$ d$^{-1}$ in the South Patch after day 6 (Figure 1.4A), it is likely that ER provided a constant and sufficient source of carbon to prokaryotes during the bloom. The close association of BP to PP and to size-fractionated PP (5-20 and > 20 μm) (Figures 1.4 and 1.5) supports the argument that prokaryotes were indeed reliant upon freshly produced photosynthate. Results for other values of BGE are listed in Table 1.6.
With the exception of one study in the Antarctic (Pakulski et al. 1996), there are no field observations of direct iron limitation of prokaryotes. To date most evidence supports DOM limitation across a range of ocean systems: the sub-arctic and equatorial Pacific Ocean (Kirchman 1990), the northwest Atlantic Ocean (Carlson et al. 2002), and the Southern Ocean (Carlson et al. 1998, Hall and Safi 2001, Ducklow 2003). Nutrient amendment experiments carried out in the Southern Ocean (Church et al. 2000) and off the coast of California (Kirchman et al. 2000) suggested a co-limitation of bacterial growth by iron and DOM. Iron and carbon (as amino acids or glucose) in these studies stimulated bacterial growth above carbon additions alone. Tortell et al. (1996) illustrated the dynamic interplay between iron and DOM limitation. They measured growth efficiencies of bacterial isolates in culture under iron-replete and depleted conditions and found reduced growth efficiencies under iron limitation. The implication was that carbon flow through the microbial food web could be altered such that bacteria could serve as carbon sinks or carbon links to higher trophic levels as a function of iron availability, assuming that carbon is not limiting. It remains an open question whether this occurs in natural environments.

While the results lead to the conclusion that prokaryotes were carbon-limited during SOFeX, two caveats regarding nutrient limitation of prokaryotes should be emphasized. First, widespread carbon limitation across the Southern Ocean is not purported, nor can iron limitation elsewhere in the Southern Ocean at other times be excluded. The Southern Ocean encompasses a range of ecological provinces that possess dynamic food web structures and nutrient regimes (Treguer and Jacques 1992). Thus, caution should be taken in making broad generalizations regarding nutrient limitation of prokaryotes in the Southern Ocean. Second, it cannot be claimed with certainty that all members of the prokaryotic community
experienced nutrient/substrate limitation. The methods employed in this study restrict interpretations to the bulk community of prokaryotes and the members of that community that are capable of assimilating radiolabeled organic substrates. Therefore, it is possible that subpopulations of prokaryotes do in fact experience iron or carbon limitation or respond differently to iron fertilization (see Chapter 2).

**Carbon cycle implications** - SOFeX provided an opportunity to study the role of prokaryotes in the carbon cycle during a phytoplankton bloom. The blooms initiated during SOFeX are not unlike others that occur in the Southern Ocean and elsewhere throughout the year. Prokaryotic remineralization is an important component of the ocean carbon cycle because it prevents net community production from approaching true net primary production. This occurs through the remineralization of DOC, a pool that would otherwise accumulate or be exported by deep ocean convection. DOC production during phytoplankton blooms is a consistent phenomenon (Carlson et al. 1998) and its flow into the microbial food web has been well documented (Landry et al. 2000).

Several features of the prokaryotic response during SOFeX are consistent with blooms elsewhere in the Southern Ocean. Carlson et al. (1998) found that 90% of the organic carbon produced during the spring bloom in the Ross Sea accumulated as POC and the remaining 10% as DOC. They reasoned that little DOC accumulated as a result of bacterial utilization and that 72% of the supply of freshly produced DOC fueled bacterial growth. Ducklow (2003) and Carlson and Hansell (2003) recently reviewed bacterial dynamics and DOM dynamics, respectively, in the Ross Sea. Collectively, the authors found that semilabile DOM at near Redfield stoichiometry accumulated in the Ross Sea during the
austral spring with almost complete removal by the onset of winter due to bacterial remineralization.

The net accumulation of prokaryotic biomass is another feature observed in the Ross Sea. Ducklow et al. (2001b) observed an order of magnitude net increase in bacterial biomass in the upper water column over the course of the spring and summer at a net rate of 0.03 d\(^{-1}\). This is surprisingly close to the net increase we observed in our study, 0.02 d\(^{-1}\). Despite the large accumulation of bacterial biomass, the authors concluded that bacterial mortality remained a significant process over the same time scale such that two-thirds of bacterial carbon was processed by the microbial food web. The data suggest that similar resource-predator-prey dynamics occurred during SOFeX considering the comparable growth and mortality rates estimated by microzooplankton dilution experiments (M. Landry, pers. comm.). Ducklow et al. (2001b) also found BP to comprise a small fraction of PP (1-10%), with increases in the ratio from spring to summer. My finding that BP was only 1% of PP is in line with their observation; however, it is possible that the BP:PP ratio might have similarly been more dynamic during the declining stage of the bloom (Ducklow et al. 2002). A prolonged observation of an iron-induced bloom is needed in the future to more fully characterize the response of prokaryotes.

In addition to the effects on dissolved and particulate organic carbon flows, prokaryotes can affect silica and iron cycle dynamics. Silica is a key element in diatom frustules and is known to limit new production in HNLC waters. The concentration of bioavailable silicate (Si(OH)\(_4\)) in the upper ocean reflects the balance between vertical fluxes of the two pools, production of biogenic silica (bSiO\(_2\)), and its dissolution. Recently, evidence has emerged that prokaryotic activity could accelerate the rate of bSiO\(_2\) dissolution.
through hydrolytic enzymatic degradation of protective organic coatings around diatom frustules (Bidle et al. 2003). The implication from these studies is that the efficiency of silicate pump would decrease permitting a higher degree of production that was based on recycled silicate. This mechanism could have implications for DIC removal in low silicate HNLC regions like the North Patch in SOFeX. It is possible that prokaryotes played a role in maintaining silicate supply, and, in turn, production by diatoms through the mechanism described above. Conversely, prokaryotic remineralization of diatoms frustules could reduce carbon export and biological pump efficiency. The relative importance of this type of positive feedback mechanism (PP stimulating BP, which then enhances Si recycling, further enhancing PP and so forth) as a factor in the persistence of phytoplankton blooms and carbon export remains unclear.

Iron cycling within the plankton food web during SOFeX may have also played a role in both blooms’ dynamics. As is the case with carbon, iron can be cycled through the microbial food web (Tortell et al. 1999). During IronEx II microzooplankton biomass (heterotrophic nanoflagellates and dinoflagellates, ciliates) increased over the course of the iron-enriched bloom (Landry et al. 2000) with microzooplankton grazing occurred across phytoplankton taxa with particularly efficient removal of diatoms. Landry et al. suggested that an efficient recycling of iron and silicate allowed phytoplankton growth rates to remain high. The plankton food web can also be an important source to the dissolved and particulate iron pools (Hutchins et al. 1993). It is unclear whether prokaryotes would be important sources or sinks of iron. They may represent a significant source of recycled iron to phytoplankton based on their high intracellular Fe:C relative to phytoplankton (Tortell et al. 1996), but this may be dependent upon the activity and composition of the microbial food
web as well as the composition and rate of supply of iron to the dissolved pool. Clearly more work is needed to more completely elucidate the role of prokaryotes in modifying carbon, silica, and iron dynamics.

The prokaryotic response during SOFeX is both similar to and in contrast with previous open-ocean iron enrichment experiments. The differences between this study and previous ones likely reflect subtle differences in the initial structure and composition of the microbial food web and the nutrient resources available at the time. The increase in prokaryotic production to new inputs of organic carbon, accelerated growth rate, and high prokaryotic mortality appear to be consistent features of \textit{in situ} iron enrichment. These features are also consistent with previous studies describing prokaryotic dynamics during phytoplankton blooms in the ocean. The response of prokaryotic processes to iron enrichment was quantitatively less than that of phytoplankton. This is a function of the time scales of the observations, but may also be an indicator of the effects of cold temperatures in slowing the prokaryotic response. \textit{In situ} prokaryotic dynamics during the initial stages of iron-stimulated blooms are well-characterized; however, the dynamics and the relative importance to ocean carbon cycling during the latter stages of blooms remain a poorly studied.


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TABLE 1.1. North Patch mixed layer depth-averaged stocks and rates of prokaryotes and phytoplankton. Time denotes days after initial iron fertilization by R/V *Revelle*. Days 11 and 12 are averages of 2 stations inside and outside iron-fertilized waters, respectively. Days 38 and 39 are one station, each. Total and size-fractionated primary production (PP) are expressed as µg C L\(^{-1}\) d\(^{-1}\). Bold type entries indicate IN stations.

<table>
<thead>
<tr>
<th>Time</th>
<th>Prokaryote abundance (10^6 cells L(^{-1}))</th>
<th>Prokaryote biomass (µg C L(^{-1}))</th>
<th>Thymidine incorporation (pmol L(^{-1}) h(^{-1}))</th>
<th>Specific growth rate (µ, d(^{-1}))</th>
<th>Leucine incorporation (pmol L(^{-1}) h(^{-1}))</th>
<th>Prokaryote production (BP) (µg C L(^{-1}) d(^{-1}))</th>
<th>%HDNA(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 11 IN</td>
<td>7.26</td>
<td>9.01</td>
<td>0.93</td>
<td>0.07</td>
<td>10.1</td>
<td>0.36</td>
<td>35%</td>
</tr>
<tr>
<td>Day 12 OUT</td>
<td>4.01</td>
<td>4.97</td>
<td>0.44</td>
<td>0.06</td>
<td>4.91</td>
<td>0.18</td>
<td>31%</td>
</tr>
<tr>
<td>Day 39 IN</td>
<td>10.9</td>
<td>13.5</td>
<td>2.37</td>
<td>0.13</td>
<td>11.0</td>
<td>0.40</td>
<td>35%</td>
</tr>
<tr>
<td>Day 38 OUT</td>
<td>5.07</td>
<td>6.28</td>
<td>1.54</td>
<td>0.17</td>
<td>5.88</td>
<td>0.21</td>
<td>40%</td>
</tr>
<tr>
<td>Total PP</td>
<td>PP: 0.7-5.0 µm(^2)</td>
<td>PP: 5-20 µm(^2)</td>
<td>PP: &gt;20 µm(^2)</td>
<td>POC (µg C L(^{-1}))</td>
<td>PN (µg N L(^{-1}))</td>
<td>Chl a (µg L(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 0 IN</td>
<td>3.14</td>
<td>2.24 (72%)</td>
<td>0.54 (17%)</td>
<td>0.35 (11%)</td>
<td>43.6</td>
<td>7.75</td>
<td>0.073</td>
</tr>
<tr>
<td>Day 11 IN</td>
<td>7.50</td>
<td>5.26 (71%)</td>
<td>1.29 (17%)</td>
<td>0.95 (12%)</td>
<td>39.1</td>
<td>8.04</td>
<td>0.387</td>
</tr>
<tr>
<td>Day 12 OUT</td>
<td>4.27</td>
<td>3.76 (88%)</td>
<td>0.38 (9%)</td>
<td>0.13 (3%)</td>
<td>35.9</td>
<td>5.07</td>
<td>0.154</td>
</tr>
<tr>
<td>Day 39 IN</td>
<td>5.28</td>
<td>2.56 (48%)</td>
<td>1.35 (26%)</td>
<td>1.37 (26%)</td>
<td>46.0</td>
<td>10.0</td>
<td>0.614</td>
</tr>
<tr>
<td>Day 38 OUT</td>
<td>3.20</td>
<td>2.34 (73%)</td>
<td>0.51 (16%)</td>
<td>0.35 (11%)</td>
<td>38.2</td>
<td>7.66</td>
<td>0.334</td>
</tr>
</tbody>
</table>

BP: Total PP | BP: 0.7-5.0 µm \(^2\) | BP: 5-20 µm \(^2\) | BP: >20 µm \(^2\) | Prokaryote C: Total POC\(^3\) | Prokaryote N: Total PN\(^3\) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 11 IN</td>
<td>0.057±0.035</td>
<td>0.07</td>
<td>0.28</td>
<td>0.38</td>
<td>0.21</td>
</tr>
<tr>
<td>Day 12 OUT</td>
<td>0.039±0.021</td>
<td>0.05</td>
<td>0.47</td>
<td>1.41</td>
<td>0.13</td>
</tr>
<tr>
<td>Day 39 IN</td>
<td>0.079±0.056</td>
<td>0.15</td>
<td>0.29</td>
<td>0.29</td>
<td>0.26</td>
</tr>
<tr>
<td>Day 38 OUT</td>
<td>0.088±0.037</td>
<td>0.09</td>
<td>0.41</td>
<td>0.60</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^1\)%HDNA = (HDNA cell count/Prokaryotic abundance) \times 100.  \(^2\)Size-fractionated primary production as a percentage of total primary production is given in parentheses.  \(^3\)Ratios were corrected for prokaryote carbon and nitrogen retained on filter (see Methods).
TABLE 1.2. South Patch mixed layer depth-averaged stocks and rates of prokaryotes and phytoplankton. Time denotes days after initial iron fertilization by R/V Revelle. Total and size-fractionated primary production (PP) are expressed as μg C L⁻¹ d⁻¹. Bold type entries indicate IN stations.

<table>
<thead>
<tr>
<th>Time</th>
<th>Prokaryote abundance (10⁸ cells L⁻¹)</th>
<th>Prokaryote biomass (μg C L⁻¹)</th>
<th>Thymidine incorporation (pmol L⁻¹ h⁻¹)</th>
<th>Specific growth rate (μ, d⁻¹)</th>
<th>Leucine incorporation (pmol L⁻¹ h⁻¹)</th>
<th>Prokaryote production (BP) (μg C L⁻¹ d⁻¹)</th>
<th>% HDNA¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5 IN</td>
<td>3.28</td>
<td>4.07</td>
<td>0.56</td>
<td>0.10</td>
<td>5.37</td>
<td>0.19</td>
<td>24%</td>
</tr>
<tr>
<td>Day 22 IN</td>
<td>5.25</td>
<td>6.51</td>
<td>1.57</td>
<td>0.17</td>
<td>8.35</td>
<td>0.30</td>
<td>45%</td>
</tr>
<tr>
<td>Avg. OUT (n=4)</td>
<td>3.43±0.67</td>
<td>4.25±0.83</td>
<td>0.87±0.20</td>
<td>0.15±0.05</td>
<td>4.93±0.83</td>
<td>0.18±0.03</td>
<td>36%±8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Total PP</th>
<th>PP 0.7-5.0 μm²</th>
<th>PP 5-20 μm²</th>
<th>PP &gt;20 μm²</th>
<th>POC (μg C L⁻¹)</th>
<th>PN (μg N L⁻¹)</th>
<th>Chl a (μg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 IN</td>
<td>7.41</td>
<td>3.99 (54%)</td>
<td>1.26 (17%)</td>
<td>2.15 (29%)</td>
<td>75.1</td>
<td>14.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Day 5 IN</td>
<td>7.17</td>
<td>3.34 (47%)</td>
<td>0.90 (13%)</td>
<td>2.93 (40%)</td>
<td>90.7</td>
<td>19.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Day 22 IN</td>
<td>10.5</td>
<td>3.08 (30%)</td>
<td>2.75 (27%)</td>
<td>4.49 (43%)</td>
<td>168</td>
<td>38.5</td>
<td>3.61</td>
</tr>
<tr>
<td>Avg. OUT</td>
<td>4.25±1.07</td>
<td>1.48 (35%)</td>
<td>0.60 (14%)</td>
<td>2.17 (51%)</td>
<td>74.8±6.02</td>
<td>15.5±1.58</td>
<td>0.44±0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>BP:Total PP</th>
<th>BP:0.7-5.0 μm PP</th>
<th>BP:5-20 μm PP</th>
<th>BP:&gt;20 μm PP</th>
<th>Prokaryote C: Total POC³</th>
<th>Prokaryote N: Total PN³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5 IN</td>
<td>0.022±0.009</td>
<td>0.06</td>
<td>0.21</td>
<td>0.07</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Day 22 IN</td>
<td>0.010±0.004</td>
<td>0.10</td>
<td>0.11</td>
<td>0.07</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Avg. OUT</td>
<td>0.049±0.019</td>
<td>0.12</td>
<td>0.30</td>
<td>0.08</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

¹ %HDNA = (HDNA cell count/Prokaryotic abundance) x 100. ² Size-fractionated primary production as a percentage of total primary production is given in parentheses. ³ Ratios were corrected for prokaryote carbon and nitrogen retained on filter (see Methods).
TABLE 1.3. Comparison of IN versus OUT station prokaryote properties in the South Patch. Statistical significance was based on T-tests (α=0.05). ns denotes comparisons that were not statistically significant. Sample sizes are denoted for both IN and OUT stations in parenthesis.

<table>
<thead>
<tr>
<th>Prokaryote property or process</th>
<th>Mean - all IN stations&lt;sup&gt;1&lt;/sup&gt; days 5-22 (n&lt;sub&gt;IN&lt;/sub&gt;=32, n&lt;sub&gt;OUT&lt;/sub&gt;=20)</th>
<th>Mean - all IN stations&lt;sup&gt;1&lt;/sup&gt; days 8-22 (n&lt;sub&gt;IN&lt;/sub&gt;=27, n&lt;sub&gt;OUT&lt;/sub&gt;=20)</th>
<th>Mean - all IN stations&lt;sup&gt;1&lt;/sup&gt; days 12-22 (n&lt;sub&gt;IN&lt;/sub&gt;=22, n&lt;sub&gt;OUT&lt;/sub&gt;=20)</th>
<th>Mean - all integrated IN stations&lt;sup&gt;2&lt;/sup&gt; days 5-22 (n&lt;sub&gt;IN&lt;/sub&gt;=7, n&lt;sub&gt;OUT&lt;/sub&gt;=4)</th>
<th>Mean - all integrated IN stations&lt;sup&gt;2&lt;/sup&gt; days 8-22 (n&lt;sub&gt;IN&lt;/sub&gt;=6, n&lt;sub&gt;OUT&lt;/sub&gt;=4)</th>
<th>Mean - all integrated IN stations&lt;sup&gt;2&lt;/sup&gt; days 12-22 (n&lt;sub&gt;IN&lt;/sub&gt;=5, n&lt;sub&gt;OUT&lt;/sub&gt;=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance</td>
<td>ns</td>
<td>p=0.047</td>
<td>p=0.0037</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Biomass</td>
<td>ns</td>
<td>p=0.047</td>
<td>p=0.0037</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HDNA abundance</td>
<td>ns</td>
<td>p=0.017</td>
<td>p=0.0018</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>TdR</td>
<td>p=0.0025</td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>ns</td>
<td>p=0.015</td>
<td>p=0.023</td>
</tr>
<tr>
<td>Leu</td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>p=0.028</td>
<td>p=0.015</td>
<td>p=0.0051</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means were calculated by averaging all IN station discrete depth data for the specified time period and comparing the mean of all OUT station data. <sup>2</sup>Means were calculated by averaging all integrated IN station data for the specified time period and comparing the mean of all integrated OUT stations.
TABLE 1.4. Simple linear correlations of prokaryote production and abundance versus size-fractionated chlorophyll $a$ (Chl $a$), POC, PN, and size-fractionated primary production in the South Patch. $+$ denotes statistically significant correlations at $p<0.05$. ns denotes non-significant correlations.

<table>
<thead>
<tr>
<th>Prokaryote production versus</th>
<th>$p &lt; 0.05$</th>
<th>Correlation coefficient ($r$)</th>
<th>Prokaryote abundance versus</th>
<th>$p &lt; 0.05$</th>
<th>Correlation coefficient ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF/F Chl $a$</td>
<td>$+$</td>
<td>0.79</td>
<td>GF/F Chl $a$</td>
<td>$+$</td>
<td>0.94</td>
</tr>
<tr>
<td>0.7 - 5.0 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.65</td>
<td>0.7 - 5.0 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.91</td>
</tr>
<tr>
<td>5.0 - 20 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.72</td>
<td>5.0 - 20 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.96</td>
</tr>
<tr>
<td>&gt;20 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.92</td>
<td>&gt;20 $\mu$m Chl $a$</td>
<td>$+$</td>
<td>0.80</td>
</tr>
<tr>
<td>POC</td>
<td>ns</td>
<td>0.63</td>
<td>POC</td>
<td>ns</td>
<td>0.71</td>
</tr>
<tr>
<td>PN</td>
<td>ns</td>
<td>0.70</td>
<td>PN</td>
<td>$+$</td>
<td>0.79</td>
</tr>
<tr>
<td>Primary production</td>
<td>$+$</td>
<td>0.84</td>
<td>Primary production</td>
<td>ns</td>
<td>0.51</td>
</tr>
<tr>
<td>0.7 - 5.0 $\mu$m PP</td>
<td>ns</td>
<td>-0.01</td>
<td>0.7 - 5.0 $\mu$m PP</td>
<td>ns</td>
<td>-0.32</td>
</tr>
<tr>
<td>5.0 - 20 $\mu$m PP</td>
<td>$+$</td>
<td>0.82</td>
<td>5.0 - 20 $\mu$m PP</td>
<td>ns</td>
<td>0.19</td>
</tr>
<tr>
<td>&gt;20 $\mu$m PP</td>
<td>$+$</td>
<td>0.80</td>
<td>&gt;20 $\mu$m PP</td>
<td>ns</td>
<td>0.44</td>
</tr>
</tbody>
</table>
TABLE 1.5. Specific growth rates of heterotrophic prokaryotes in the South Patch.

<table>
<thead>
<tr>
<th>Location in South Patch</th>
<th>Day</th>
<th>Grazer-exclusion $^1$ (μ, d$^{-1}$)</th>
<th>TdR $^2$ (μ, d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (early)$^3$</td>
<td>5-7</td>
<td>0.11$^4$</td>
<td>0.14</td>
</tr>
<tr>
<td>IN (middle)$^3$</td>
<td>12</td>
<td>0.14$^4$</td>
<td>0.19</td>
</tr>
<tr>
<td>IN (late)$^3$</td>
<td>19</td>
<td>0.15$^4$</td>
<td>0.19</td>
</tr>
<tr>
<td>OUT</td>
<td>7</td>
<td>0.11$^4$</td>
<td>0.14</td>
</tr>
<tr>
<td>OUT</td>
<td>13</td>
<td>0.17$^4$</td>
<td>0.19</td>
</tr>
<tr>
<td>OUT</td>
<td>21</td>
<td>0.10$^4$</td>
<td>0.09</td>
</tr>
</tbody>
</table>

$^1$Specific growth rates were measured using grazer-excluded GF/F filtrate. $^2$Mixed layer depth-averaged TdR was used to calculate growth rates. Bold type indicates IN stations. $^3$Stage of the iron-induced phytoplankton bloom. $^4$Denotes specific growth rates that were significantly greater than zero ($p<0.05$).
TABLE 1.6. Estimation of the fraction of prokaryotic carbon demand potentially supported by phytoplankton extracellular release (ER). BGE, prokaryote growth efficiency; BP, prokaryote production (µg C L\(^{-1}\) d\(^{-1}\)); BCD, prokaryotic carbon demand (µg C L\(^{-1}\) d\(^{-1}\)); PP, particulate primary production (µg C L\(^{-1}\) d\(^{-1}\)); ER, extracellular release, expressed as a fraction of the estimated particulate primary production (µg C L\(^{-1}\) d\(^{-1}\)). Assumed values of BGE are listed. BP and PP are from averaged OUT stations and day 22 IN station (Table 1.3). BCD is calculated as BP BGE\(^{-1}\). ER is fixed at 13% of PP (Baines and Pace 1991). The fraction of BCD supported by PER is calculated as PER BCD\(^{-1}\). Bold type indicates IN stations.

<table>
<thead>
<tr>
<th>Location</th>
<th>BGE</th>
<th>BP</th>
<th>BCD</th>
<th>PP</th>
<th>ER</th>
<th>Fraction of BCD supported by ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUT</td>
<td>0.10</td>
<td>0.18</td>
<td>1.80</td>
<td>4</td>
<td>0.52</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.18</td>
<td>1.20</td>
<td>4</td>
<td>0.52</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.18</td>
<td>0.90</td>
<td>4</td>
<td>0.52</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.18</td>
<td>0.72</td>
<td>4</td>
<td>0.52</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.18</td>
<td>0.60</td>
<td>4</td>
<td>0.52</td>
<td>87%</td>
</tr>
<tr>
<td>IN</td>
<td>0.10</td>
<td>0.30</td>
<td>3.00</td>
<td>11</td>
<td>1.43</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.30</td>
<td>2.00</td>
<td>11</td>
<td>1.43</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.30</td>
<td>1.50</td>
<td>11</td>
<td>1.43</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.30</td>
<td>1.20</td>
<td>11</td>
<td>1.43</td>
<td>119%</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>1.00</td>
<td>11</td>
<td>1.43</td>
<td>143%</td>
</tr>
</tbody>
</table>
FIGURE 1.1. South Patch total prokaryote abundance. IN stations are denoted by filled circles and high DNA-containing cell abundance (HDNA) are denoted by filled triangles. OUT stations are denoted by open circles (total prokaryotic abundance, $r^2=0.72$, $p<0.05$) and open triangles (HDNA, $r^2=0.81$, $p<0.05$). Asterisks denote transect stations where an average of 10-meter abundance was calculated. Hashed areas denote iron enrichment by R/V Revelle. Dashed lines denote linear least squares regressions of IN station abundances versus time.
FIGURE 1.2. South Patch prokaryote incorporation rates. (A) Volumetric incorporation rates of thymidine, $^3$H-thymidine (filled circles, $r^2=0.53$, $p=0.01$), and $^3$H-leucine, Leu (filled squares, $r^2=0.63$, $p<0.05$). Open circles and open squares denote OUT station TdR and Leu incorporation rates, respectively. Dashed lines denote linear least squares regressions. (B) South Patch cell-specific TdR (not significant) and Leu incorporation (not significant) rates. Rates are normalized to total prokaryotic abundance. Asterisks denote transect stations where an average of 10-meter rates was calculated. Hashed areas denote iron enrichment by R/V Revelle.
Thymidine incorporation (10^{-12} mol TdR cell^{-1} h^{-1})

Leucine incorporation (10^{-12} mol Leu cell^{-1} h^{-1})

Time (days after initial Fe fertilization)

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FIGURE 1.3. Transects of South Patch. (Top panels – A, B, C) 10 m prokaryote abundance (cells L⁻¹) (filled circles). Error bars represent instrument variation within a sample. Open diamonds denote SF₆ concentrations (fM). Station numbers are given along the x-axis. (Middle panels – D, E, F) 10 m Volumetric uptake of ³H-thymidine (open triangles) and ³H-leucine (filled squares) (pmol L⁻¹ hr⁻¹). Error bars represent variability at 10 m. Station numbers are given along the x-axis. (Bottom panels – G, H, I) 10 m incorporation rates linearly regressed against SF₆ concentrations. Symbols are the same as in the middle panels. Linear regression lines are denoted by the solid lines.
FIGURE 1.4. South Patch prokaryote and particulate primary production. (A) Prokaryotes (filled circles, \( r^2 = 0.63, p < 0.05 \)) and particulate primary production (filled squares, \( r^2 = 0.23 \), not significant). Open circles and open squares denote OUT station prokaryote production and primary production, respectively. Dashed regression lines represent ordinary linear least-squares regressions of BP versus time. The solid horizontal line represents the level of PP at time zero. The dotted horizontal line represents the level of BP at time zero following the model II (geometric mean) regression in panel B. Hashed areas denote iron enrichment by \textit{R/V Revelle}. Asterisks denote transect stations where an average of 10-meter rates was calculated. (B) Model II regression of prokaryote vs. primary production from all IN stations (filled circles) in the South Patch. OUT stations are denoted by open circles.
Bacterioplankton production

\[ BP = 0.14 + 0.01 \text{ PP} \]

\[ r = 0.84, p < 0.05 \]
FIGURE 1.5. Regressions of prokaryote versus particulate primary production in the South Patch. (A) Model II regression of prokaryote production vs. 0.7 – 5.0 μm size-fractionated primary production (not significant) from all IN stations in the South Patch. OUT stations are denoted by open symbols. (B) Model II regression of prokaryote production vs. 5.0 – 20 μm (filled squares, $r=0.82, p<0.05$) and >20 μm (filled circles, $r=0.820 p<0.05$) size-fractionated primary production from IN stations in the South Patch. Respective OUT stations for each size fraction of primary production are denoted by open symbols.
FIGURE 1.6. Regressions of prokaryote production versus chlorophyll, POC, and PN in the South Patch. (A) Model II regression of prokaryote production vs. total fluorometric chlorophyll \( a \) (filled circles, \( r=0.79, p<0.05 \)), 5–20 \( \mu \text{m} \) fluorometric chlorophyll \( a \) (filled squares, \( r=0.72, p<0.05 \)), and >20 \( \mu \text{m} \) fluorometric chlorophyll \( a \) (filled triangles, \( r=0.92, p<0.05 \)) from all IN stations in the South Patch. Respective OUT stations for each size fraction of fluorometric chlorophyll \( a \) are denoted by open symbols. (B) Model II regression of prokaryote production vs. particulate organic carbon, POC (filled circles, \( r=0.63 \), not significant), and particulate organic nitrogen, PN (filled squares, \( r=0.70 \), not significant), from all IN stations in the South Patch. Respective OUT stations for POC and PN are denoted by open symbols.
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FIGURE 1.7. Regression of cytometric versus microscopic prokaryote counts. Model II regression of prokaryotic abundance measured by microscopic counts (acridine orange) and flow cytometric counts in the upper 50 m for both the North and South Patch. Samples from non-fertilized waters are also included. The model II regression line is:

\[ y = 0.85x + 0.28; \quad r^2 = 0.68, \quad n = 137. \]
CHAPTER 2

PROKARYOTE COMMUNITY STRUCTURE DYNAMICS

IN RESPONSE TO IRON ENRICHMENT
ABSTRACT

Prokaryotic community structure and diversity was studied in response to iron enrichment. This study was part of the Southern Ocean Iron Experiment (SOFeX) which comprised two open-ocean iron fertilizations conducted in Antarctic waters north (North Patch) and south (South Patch) of the Antarctic Polar Front Zone. 16S rRNA probes for fluorescence in situ hybridization (FISH) were used to enumerate Eubacteria and Archaea at the domain-level. Eubacteria varied between 46% and 73% of total prokaryotes over space and time in the mixed layer in the North Patch. Archaea exhibited little variation in space and time and on average comprised 20% of total prokaryotes in the mixed layer. In the South Patch Eubacteria and Archaea were 72-88% and 11-30%, respectively, of the total prokaryotic abundance in the mixed layer. Prokaryotes were also enumerated at the Cytophaga-Flavobacteria (CF) and SAR11 subdivision level. CF and SAR11 comprised a smaller proportion of the total prokaryotic community in the North Patch compared to the South Patch. The relative proportion of CF and SAR11 in the North Patch was 7-14% and 36-44%, respectively. In the South Patch these two groups represented 14-29% and 31-45%, respectively. Absolute abundances of CF and SAR11 increased over time in the South Patch; however, the net growth rate of CF ($\mu = 0.09 \, d^{-1}$) was significantly higher than SAR11 ($\mu = 0.04 \, d^{-1}$). Non-metric multidimensional scaling (NMS) of 16S rDNA ribotypes identified by terminal restriction fragment length polymorphism (tRFLP) showed that community structure in the North and South Patch were distinct from one another in both iron-fertilized and non-fertilized waters. Further NMS analyses showed that the community structure in the South Patch shifted over time and was distinct from non-fertilized waters. Taxonomic (ribotype) richness and diversity were higher in iron-fertilized waters compared
to non-fertilized waters in the North Patch. In the South Patch taxonomic richness and
diversity increased over time. The results of this study show systematic variability of
prokaryotic groups in response to iron enrichment.
INTRODUCTION


The linkage of prokaryotes with organic matter cycling makes them ubiquitous features associated with enhanced phytoplankton production to the extent that 50% or greater of primary production flows through heterotrophic prokaryotic production (Ducklow and Hill 1985, Ducklow et al. 1995, 2001a, Carlson et al. 1996). In many instances prokaryotic blooms develop in response to new inputs of labile dissolved organic carbon (DOC) and particulate organic carbon (POC) derived from stimulated phytoplankton production and subsequent dissolved organic matter (DOM) production processes (Ducklow et al. 1997, 2001b). The prokaryotic response to iron-induced phytoplankton blooms and phytoplankton-derived organic carbon has also been demonstrated in previous open-ocean iron fertilization experiments (Cochlan 2001, Hall and Safi 2001, Arrieta et al. 2004, Oliver et al. 2004). Heterotrophic activity of prokaryotes is important in the context of phytoplankton blooms because it can affect the export efficiency (carbon exported:carbon produced) and the long-term sequestration of carbon in the ocean interior. Prokaryotic modulation of carbon export was aptly illustrated by Boyd et al. (2004) during an iron fertilization experiment in the
subarctic North Pacific Ocean. They concluded that more than 50% of the POC lost from the mixed-layer during an iron-stimulated phytoplankton bloom was solubilized by prokaryotes, thereby reducing the export efficiency to 8%.

The dynamics of prokaryotes and their ecological function in response to phytoplankton blooms have been well-documented (Ducklow et al. 2001a, b, Ducklow et al. 2002). However, prokaryotic community composition, structure, and dynamics during periods of enhanced phytoplankton production remain largely uncharacterized. While the phytoplankton community structure is known to shift in response to increased resource availability, it is not known whether such changes also occur within the prokaryotic community and which prokaryotic taxa are ecologically relevant.

Characterization of prokaryotic community structure historically has been constrained by methodology. Advances in understanding the composition and relatedness within prokaryotic communities have arisen from culture-independent molecular techniques (Amann et al. 1995, Suzuki and Giovannoni 1996, Suzuki et al. 1998). Specific techniques such as PCR amplification and sequencing of 16S rRNA genes (Olsen et al. 1986), whole-cell fluorescence in situ hybridization (FISH) (DeLong et al. 1989), and terminal restriction fragment length polymorphism (tRFLP) (Liu et al. 1997, Blackwood et al. 2003) have been instrumental in helping to observe the ecology of specific prokaryotic taxa with varying degrees of resolution (species to domain). Ultimately, molecular tools such as these help to understand the linkage between prokaryotic community structure and function.

The purpose of this study was to characterize the prokaryotic composition during an iron-induced phytoplankton bloom in the Southern Ocean and document potential shifts in prokaryotic community structure. Culture-independent molecular techniques were employed.
to detect the relative abundance of prokaryotes and to track specific groups thought to be ecologically relevant during the phytoplankton bloom. Whole-cell FISH was used to track two domains of prokaryotes, Eubacteria and Archaea, as well as two major subdivisions within the Eubacteria, SAR11 and Cytophaga-Flavobacteria (CF), thought to have ecological significance to ocean biogeochemistry (Morris et al. 2002, Kirchman 2002). In addition, Eubacteria community fingerprint data derived from tRFLP was analyzed by non-metric multidimensional scaling (NMS) (Kruskal 1964, Mather 1976) to observe patterns in prokaryotic community structure and to assess its potential shifts over the course of the phytoplankton bloom.

MATERIALS AND METHODS

Study Site and Sample Collection - SOFeX was comprised of two open-ocean iron enrichment experiments in the high nutrient, low chlorophyll (HNLC) Pacific Ocean sector of the Southern Ocean. The first experiment, or North Patch, took place north of the Antarctic Polar Frontal Zone (APFZ) in low silica, high NO\textsubscript{3} waters (3 μM, 22 μM, respectively) near 56°S, 172°W. The second experiment, or South Patch, took place south of the APFZ in high silica, high NO\textsubscript{3} waters (60-64 μM, 28 μM, respectively) near 66°S, 171°W. Both experiments took place during the late austral summer 2002 (January through February). A more detailed description of the study sites and iron enrichments can be found in Coale et al. (2004).

Prokaryotes were collected with Niskin bottles from depths of 5, 10, 20, 30, 40, and 50 meters within (IN stations) and outside (OUT stations) the iron-fertilized waters.
Sampling days are listed in Appendix 1.1. Water was dispensed into clean, acid-washed 2-L polycarbonate bottles. For whole-cell FISH water was transferred to four sterile 5 ml cryovials and fixed with 0.2 μm-filtered, neutral-buffered formalin to a final concentration of 2% at 4°C for 2 hours. Four replicates were made for each depth. Fixed samples were stored at -80°C. For prokaryotic community characterization via tRFLP analysis, 500-1000 ml of water was filtered onto 0.2 μm pore-size polysupone filters (Supor-200, Gelman Sciences Inc.) under gentle vacuum (80-100 mm Hg). Filters were transferred to clean plastic pouches containing 2 ml of cell lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl pH 9.0) (Suzuki et al. 1998). Pouches were heat sealed and stored at -80°C until DNA extraction.

**Mixed layer depth (MLD)** - The MLD was determined using a criterion of a change of 0.02 from surface σ, for 1-m binned density measurements (http://usigofs.whoi.edu/jg/dir/jgosf/, nbp97_8, W. Smith, Ross Sea Mixed Layer Depths). The MLD is provided in Appendix 1.1.

**Whole-cell FISH** - Multiple 5', Cy3-labeled rRNA probes (Qiagen, Inc., Valencia, CA) were synthesized for the domains *Eubacteria* and *Archaea*, and for two subdivisions within the *Eubacteria*, SAR11 and *Cytophaga-Flavobacteria* (CF). The probes for each taxonomic group were combined into respective probe suites (Table 2.1). Probe suites for each taxonomic group were prepared in the appropriate hybridization solution (see below, Table 2.1).

Cells were prepared for FISH analysis following the methods of Morris et al. (2002). Three different hybridization solutions were prepared as a function of formamide
concentration to control for the stringency of the hybridization. Hybridization solutions were composed of 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), and 0.01% (wt/vol) sodium dodecyl-sulfate (SDS) with varying formamide concentrations (15%, 20%, and 35%). Multiple probes targeting each taxonomic group were combined together in the appropriate hybridization solutions to form a probe suite. The final concentration of each probe in the probe suite was 2 ng probe μl⁻¹. Frozen cryovials containing fixed prokaryotes were quickly thawed in a water bath. Samples obtained from the mixed layer (5, 10, 20, and 30 m) were pooled to obtain a sufficient number of cells for enumeration. Between 15 and 20 ml of pooled samples were filtered onto white, 0.2 μm pore-size polycarbonate filters (GE Osmonics, Minnetonka, MN) under gentle vacuum (80-100 mm Hg). Filters were air-dried and cut into six equal portions and each filter fragment affixed to a clean glass slide. Filter fragments were covered with 40 μl of hybridization solution containing the appropriate rRNA probe suite and cover slips. Slides were incubated in air-tight bags in the dark for 6-12 hours at 37°C or 46°C depending on the optimal annealing temperatures for each probe suite (Table 2.1). At the end of the incubation, cover slips were removed and the slides were placed in Coplan jars containing a pre-warmed hybridization wash (37°C or 46°C). Two different hybridization washes were prepared as a function of NaCl concentration to control for the stringency of the wash. Hybridization washes consisted of 20 mM Tris-HCl (pH 7.4), 0.01% (wt/vol) SDS, and 5 mM EDTA with varying NaCl concentrations (0.07 M or 0.15 M NaCl). Slides with affixed filter fragments were washed in two consecutive 10 minutes washes. This was followed by a 10 minute wash in cold (4°C) DAPI (4',6-diamidino-2-phenylindole). Following the successive washes, filters were air-dried and transferred to clean glass slides. Filters were covered with Citifluor (Citifluor Ltd., Leicester, England) and

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cover slips and then sealed with nail polish to protect the filter from moisture. Slides were stored at -20°C. The appropriate incubation temperatures, hybridization solutions and washes, and wash temperatures for each probe suite is provided in Table 2.1.

Probe-positive and DAPI-positive cells were enumerated using epifluorescence microscopy under CY3 and UV excitation and emission, respectively. Probe-positive and DAPI-positive cells were identified individually using imaging software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD) and an imaging/masking protocol (R. Parsons, pers. comm.). Briefly, cells in six replicate fields were enumerated for both probe-positive and DAPI-positive cells. Images of each field were taken using a digital camera. Images were processed individually to account for variation in signal intensity. The intensity range for each image was adjusted so as to retain probe- and DAPI-positive cells while simultaneously reducing non-specific background noise (i.e. non-cellular objects). To attain true probe-positive cells, i.e. cells which were both probe-positive and DAPI-positive, a separate image mask of probe-positive cells was created. A similar masked image of DAPI-positive cells was created. The probe-positive masked image was overlaid on top of the DAPI-positive masked image to yield cells which were coincident to both images. These coincident cells were quantified as probe-positive cells. Probe-positive cell counts were summed and corrected by subtracting out probe-positive cells which were incubated and hybridized with negative control probes (i.e. non-specific binding of probes or false positives). Negative control hybridizations were carried out using the least stringent conditions of all the hybridization conditions (lowest % formamide, highest NaCl concentration, lowest temperature). Summed net probe-positive cells were expressed as a fraction of summed DAPI-positive cells.
Prokaryotic and taxon-specific net growth rates were determined as the slope of the least-squares regression of the linear portion of natural log-transformed prokaryotic abundance versus time. Net growth rates were tested for significance from zero ($p<0.05$) and subsequently compared using a two-tailed t-test to determine significant differences between net growth rates ($p<0.05$).

**PCR and tRFLP** - Total genomic DNA (gDNA) was extracted from mixed layer frozen filter samples containing lysed prokaryotic cells following the methods of Giovannoni et al. (1990a). Briefly, individual samples were thawed at room temperature and the cell lysis mixture was treated with 200 μl proteinase K (10 mg ml$^{-1}$) and 20 μl 10% SDS for 1 hour at 37°C on an orbital shaker. Genomic DNA was isolated using a series of phenol-chloroform-isoamyl alcohol extractions and salt-isopropanol precipitations. The purified gDNA was resuspended in a sterile Tris-EDTA (TE) buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.0). Isolated gDNA was quantified on a GeneQuant spectrophotometer (Biochrom, Ltd., Cambridge, England) at 260 and 280 nm. Working stocks of gDNA were prepared in sterile TE buffer at a concentration of 10 ng gDNA μl$^{-1}$. All gDNA was stored at -20°C.

Working stocks of gDNA served as a template for PCR (Suzuki et al. 1998). A portion of the 16S rRNA gene was amplified by PCR using Taq polymerase (Invitrogen, San Diego, CA), *Eubacteria* primers, Eub27F (5'-AGA GTT TGA TCC TGG CTC AG-3', Hicks et al. 1992) and Eub519R (5'-GYC GMC GGC GCC ATT AWG-3', Lane et al. 1985), and 10 mM dNTPs. The forward primer, Eub27F, was 5'-labeled with an IRdye™ for infrared visualization at 700 nm (IR-700 Eub27F, LI-COR, Inc., Lincoln, NE). PCR was performed in a DNA Engine Thermal Cycler (MJ Research, Cambridge, MA) using the following
conditions: denaturing for 1 minute at 94°C, annealing for 1 minute at 55°C, extension for 2 minutes at 72°C. Thermal cycles were kept to 25 to minimize PCR bias (Suzuki et al. 1998).

To test reproducibility of the PCR and dependability of the primers, gDNA from three bacterial isolates was procured and a portion of their 16S rRNA genes amplified by PCR using the same primers and PCR conditions employed for the environmental samples. Reproducibility in the PCR product was tested within and between different PCR machines. Results showed consistent PCR amplification of the 16S rRNA gene within and between PCR machines (data not shown). Secondary PCR and restriction digests of the same environmental sample consistently yielded the same band pattern and band intensities (data not shown).

For tRFLP analysis, PCR products were subjected to a restriction enzyme digest using 10 units of HaeIII and 2 ml 10X M Buffer (100 mM Tric-HCl, pH 7.5, 100 mM MgCl₂, 10 mM Dithiothreitol, 500 mM NaCl) (Takara Bio, Inc., Otsu, Japan) for 6 hours at 37°C. A 1:10 dilution of the unrestricted PCR products and the restriction digests were loaded onto a 25 cm x 0.25 mm polyacrylamide gel (Kbpplus, LI-COR, Inc.). IR700-labeled molecular weight standards (50-700 bp) were also loaded. The gel was run on a Global IR2 4200 dual-dye DNA analyzer (LI-COR, Inc.) for 3 hours under the following conditions: 1500 Volts, 20 mA, 25 W, and 45°C. Whole and terminal restricted PCR fragments were visualized using e-Seq software (LI-COR, Inc.). PCR products and terminal fragments were resolved using Gene ImageIR 4.03 (Scanalytics, Inc., Fairfax, VA) and compared to molecular weight standards for size calculations.
Analysis of prokaryotic community structure - Non-metric multidimensional scaling (NMS) was employed to observe patterns and changes in the *Eubacteria* community structure for both the North and South Patch using the PC-ORD software package (MJM Software Design, Gleneden Beach, OR) (McCune and Grace 2002). The following software options were selected for the NMS analysis: autopilot mode, slow and thorough, and Sorensen distance. Autopilot mode was selected for ease in maintaining ordination parameters over multiple ordinations. The slow and thorough setting under autopilot mode was selected so as to find the most stable solution using the strictest parameters (400 iterations, an instability criterion of 0.00001, 6 axes, 40 real runs, and 50 randomized runs). Sorensen distance is a computed measure of the dissimilarity between two samples in species or taxonomic space (McCune and Grace 2002). A total of 52 samples (14 North Patch, 38 South Patch, including reference OUT stations) were screened by tRFLP and restriction fragments (ribotypes) were identified in all 52 samples. The following modifications were made to the tRFLP data prior to NMS analysis: (1) fragments were transformed into relative area units by dividing the integrated fragment area by the total area of all the fragments for a given sample, (2) fragment sizes were binned by rounding up to the nearest whole number, (3) fragments occurring in less than 10% of samples were excluded, (4) an outlier analysis using the PC-ORD outlier function was performed in which samples lying three standard deviations from the mean distance were excluded. NMS analysis was performed on the North and South Patch as a function of IN (29 samples, 29 ribotypes) and OUT (23 samples, 52 ribotypes) stations. To identify potential shifts in *Eubacteria* community structure within the South Patch, NMS analysis was performed on South Patch IN stations, relative to South Patch OUT stations, as a function of time (38 samples, 29 ribotypes).
Diversity indices were also calculated using PC-ORD. Taxonomic richness, $S$ ($S =$ number of ribotypes), the Shannon index, $H$ ($H = - \sum p_i \log p_i$, where $p_i$ is the proportion of individuals belonging to ribotype $i$), and Evenness, $E$ ($E = H \ln[S]^{-1}$, where $H$ is the Shannon index) were calculated for all samples.

RESULTS

FISH - Prokaryotic community dynamics were investigated using rRNA probes for domain-level and clade-level characterization of prokaryotic community dynamics. Domain and clade-level abundances varied in the North Patch IN and OUT stations (Table 2.2).

*Eubacteria* abundance decreased from day 11 to day 39 for the IN stations while *Archaea* abundance increased over that same time period. The same trend was seen for *CF* and SAR11: a decrease in the former group and an increase in the latter group over the same time period. The relative proportion of *Archaea* to the total prokaryotic abundance measured by DAPI did not change over time. The relative proportions of *Eubacteria* and *CF* decreased over time while SAR11 relative proportion increased. Both domain and clade-level abundances in the North Patch OUT stations increased over time; however, their relative proportion to the total prokaryotic abundance did not vary.

*Eubacteria* abundance increased from $4.0 \times 10^8$ cells L$^{-1}$ on day 5 to $7.0 \times 10^8$ cells L$^{-1}$ on day 22 (Figure 2.1A). OUT station abundances on days 7, 9, and 13 showed little variability for both domains; OUT station abundance for both domains on day 22, however, was higher than on previous days. Both *CF* and SAR11 increased from $0.8$ and $1.8 \times 10^8$ cells L$^{-1}$, respectively, on day 5 to $2.0$ and $3.4 \times 10^8$ cells L$^{-1}$, respectively, on day 22 (Figure
2.1B). SAR11 abundance in OUT stations was invariable over time; CF abundance also did not vary on days 7, 9, and 13, but was higher on day 22 than on previous days.

The relative proportion of the prokaryotic domains and groups varied over time in the South Patch IN stations (Table 2.3). Eubacteria increased from 74% of the total prokaryotic abundance to 88% from day 5 to day 22. Archaea did not show a discernable trend and the relative proportion ranged from 18% to 30%. The sum of the proportions of both domains ranged from 100 to 111%. The relative proportion of CF increased over time from 14% on day 5 to a maximum of 29% on days 15 and 19. The relative proportion of SAR11 was higher than that of CF. SAR11 relative proportion showed periodic increases over time with an increase between days 5 and 12 (31 to 45%), followed by a decrease to 33% on day 15, then an increase to 41% on day 22. The relative proportion of CF and SAR11 to the total prokaryotic abundance increased from 46% on day 5 to a maximum of 66% on day 19. OUT station relative proportions of Eubacteria ranged from 72% to 79% (Table 2.3). The relative proportion of Archaea, however, increased from 11% to 28% (Table 2.3). The sum of the relative domain proportions ranged from 83 to 103%. CF and SAR11 relative proportions did not exhibit any trend with time. CF and SAR11 ranged from 8 to 21% and 34 to 44%, respectively. The sum of the proportions of the two groups ranged from 51 to 59% of the total prokaryotic abundance. As with the IN stations, the relative proportion of SAR11 was higher than that of CF.

Domain and clade-level prokaryotic net growth rates were calculated (Table 2.4). The net growth rates for Eubacteria and Archaea were 0.04 and 0.03 d⁻¹, respectively. The net growth rate for Archaea was not significantly different from zero. These net growth rates
were similar to those calculated for the total prokaryotic community measured by both DAPI and flow cytometry. The net growth rate for CF, 0.09 d\(^{-1}\), was twice that for SAR11, 0.04 d\(^{-1}\), and was significantly higher (\(p<0.05\)).

_Eubacteria community structure and diversity - _Eubacteria_ community structure dynamics were assessed using NMS analysis of tRFLP banding patterns. Non-metric multidimensional scaling was used to observe patterns in the total sample set (\(n = 52\)), i.e. North and South Patch IN and OUT samples. Analysis of the total sample set failed to show any discernable patterns or trends (data not shown). The total sample set was subdivided further to observe patterns and investigate potential differences in community composition at smaller spatial and temporal scales. This was done by comparing the following samples: (1) North and South Patch IN samples, (2) North and South Patch OUT samples, (3) South Patch IN and OUT samples, and (4) South Patch IN samples on each day of sampling. The final stress for the each of the four NMS analyses was 11.0, 7.9, 10.6, and 8.1, respectively. Stable solutions for all analyses were identified with instability near zero. The probability of a similar stress obtained by chance was determined for 40 real and 50 randomized Monte Carlo runs (0.0196).

These analyses revealed patterns and differences which were not discernable when the whole sample set was analyzed. Analysis of the North and South Patch as a function of IN and OUT samples revealed the strongest differences in community structure. The ordination axes were not significantly correlated with time and depth. Results showed South Patch IN samples exhibited community composition that was different from North Patch IN samples (Figure 2.2A). There was variability in the community composition of the North
and South Patch samples; however, clustering of the respective samples suggested the response of the *Eubacteria* communities in the two locations to iron enrichment was different. NMS ordination of the North and South Patch OUT samples showed that the *Eubacteria* community structure in non-iron fertilized waters north and south of the APFZ were distinct from one another (Figure 2.2B). Differences in the *Eubacteria* community structure were also apparent within the South Patch (Figure 2.3). Despite variability in both IN and OUT samples, both samples exhibited clustering associated with Axis 2 and 1, respectively. South Patch IN *Eubacteria* community structure patterns were also examined (Figure 2.4). The ordination showed that there was a shift in community structure from days 5 to 12. The shift in samples is associated along Axis 2 as day 5 samples clustered at the top of Axis 2 with day 8 and 12 samples shifting down Axis 2. There was variability in the distribution of IN samples as a function of depth. For example, while day 5 samples clustered together with Axis 2, day 22 samples showed a bimodal clustering along the same axis. The community structure from two depths on day 22 was distinct from day 5 samples; however, the community structure from the other two depths from day 22 appeared to be similar to that of day 5.

Diversity indices were also calculated for each of the 52 samples (Table 2.5). Mean values were calculated as a function of day for the North and South Patch. Mean ribotype richness in the North Patch was higher within iron-fertilized waters (day 11) versus outside (day 12) (S = 21 and S = 15.3, respectively). Later in the North Patch the mean IN sample ribotype richness (day 39) remained higher than the mean OUT sample ribotype richness (S = 16.0 and S = 14.8, respectively). The same trend in the Shannon index of diversity was observed for the same samples. The mean Shannon index for the IN samples on day 11 was
2.62 compared to 2.16 for the OUT samples on day 12. On day 39, the mean Shannon index decreased to 2.3 for the IN samples, but remained higher than that for the OUT samples on day 38 (H = 2.22).

Mean ribotype richness and Shannon index in the South Patch IN samples increased over time (Table 2.5). On day 5 mean ribotype richness and Shannon index were 2.67 and 0.87, respectively. By day 22 they increased to 12.8 and 1.58, respectively. Ribotype richness peaked on day 15 at 14.5 while the Shannon index peaked on day 19 at 1.96. Compared to the South Patch OUT samples, though, the two indices of diversity were lower. Ribotype richness ranged from 12.0 to 16.5 and Shannon index ranged from 2.10 to 2.46 with no trend over time.

Evenness (E), a measure of the equitability among ribotypes in a given sample, was higher in the North Patch IN sample on day 11 compared to the OUT sample on day 12 (0.86 versus 0.80) (Table 2.5). There was no difference in evenness between OUT and IN on days 38 and 39, respectively. Evenness in the South Patch IN samples decreased over time from 0.95 to 0.70 between days 5 and 22. The decrease in evenness in the South Patch suggests a shift towards more dominant ribotypes in response to the iron-induced bloom. Evenness in the South Patch OUT samples ranged from 0.80 to 0.90 with no trend over time.

**DISCUSSION**

Community structure dynamics in response to two iron fertilization experiments (North and South Patch) were observed in this study using culture-independent methods. Ribosomal probing by FISH detected *Archaea* in both locations at abundances comparable to
those found elsewhere in the Antarctic (Church et al. 2003), although their populations exhibited less variability relative to that observed in specific *Eubacteria* groups. Other FISH results showed that specific groups of *Eubacteria* were present and their populations were dynamic over time in both locations. Specifically, *CF* and SAR11 abundances increased over time in the South Patch following iron enrichment. Furthermore, the net growth rate of *CF* was twice that of SAR11 indicating either preferential growth of *CF* over SAR11, differential mortality (viral lysis, grazing) of SAR11 over *CF*, or a combination of both. Also, the increase in the relative proportions of *CF* and SAR11 as a fraction of total prokaryotic abundance, individually and in combination, was suggestive of their relative importance during the iron-stimulated phytoplankton bloom. *Cytophaga-Flavobacteria* increased to a quarter of the total prokaryotic community by the end of the experiment, although this did not coincide with the end of the phytoplankton bloom. In combination with SAR11 the two groups comprised two-thirds of prokaryotic abundance.

The use of multivariate statistical analyses (ordination) with culture-independent methods increases the analytical power in which community structure trends and patterns can be discerned. In this study non-metric multidimensional scaling, was used to analyze the distribution of ribotypes from 52 different samples identified by tRFLP. Results showed that the *Eubacteria* community structure response to iron enrichment in the North Patch and South Patch was different (Figure 2.2A). NMS also revealed spatial differences in the *Eubacteria* community structure in non-iron fertilized waters (Figure 2.2B). It is possible that environmental variation (temperature and silica) as well as variation in phytoplankton and zooplankton community structure (Coale et al. 2004) may have influenced *Eubacteria* community structure. Differences in community structure and diversity within iron-fertilized
waters over time and between iron-fertilized and non-fertilized waters were also revealed (Figure 2.3). The sum of the data demonstrates that the *Eubacteria* community in the Southern Ocean was responsive and dynamic to environmental perturbations. The data also show that *Eubacteria* community structure was heterogeneous in the Southern Ocean over space and time (Figure 2.3, 2.4). Nonetheless, the data herein that supports shifts in community structure and diversity following iron enrichment contrast with the findings found in bottle experiments in three different oceanic regimes (Hutchins et al. 2001) and EISENEX, another Southern Ocean iron fertilization experiment (Arrieta et al. 2004). In the latter, Arrieta et al. observed that prokaryotic community structure and diversity was invariable over time in response to iron enrichment. The three studies taken together do not support a specific model of prokaryotic ecology in response to iron fertilization and more studies are needed to achieve a consensus on prokaryotic community structure behavior.

The numerical dominance of the two groups of *Eubacteria*, *Cytophaga-Flavobacteria* and SAR11, in this study supports the existing hypotheses that both groups are ecologically significant in marine environments. SAR11 is a diverse subdivision of $\alpha$-proteobacteria known to have a global distribution, including the Antarctic, and is a numerically dominant prokaryote in surface waters (Field et al. 1997, Morris et al. 2002). Ecological studies of SAR11 in the northwest Atlantic Ocean has shown this group to be associated with seasonal convective overturn events in the upper mesopelagic zone as well as with the surface environment in the Sargasso Sea during the summer (Morris et al. 2005, in press). The implication is that SAR11 may play a significant role in carbon cycle dynamics because its ecology fits contemporaneously with the seasonal cycle of DOC. The physiology of this group is largely unknown although recent success in cultivating it may lead to new insights.
into its functional role in ocean biogeochemistry (Rappe et al. 2002). *Cytophaga-Flavobacteria* is also a cosmopolitan group of prokaryotes found in abundance throughout the ocean (Kirchman 2002). The CF are chemoorganotrophs capable of degrading high-molecular weight dissolved organic matter (DOM) (Reichenbach and Dworkin 1991). The occurrence of CF in marine environments, specifically natural (Fandino et al. 2001) and mesocosm phytoplankton blooms (Riemann et al. 2000), is consistent with its physiology. The CF are known to be attached to suspended particles (DeLong et al. 1993, Rath et al. 1998) and can consume low- and high-molecular weight DOM (Cottrell and Kirchman 2000). In this study direct enumeration of specific prokaryotic groups suggests their biogeochemical role during the phytoplankton bloom. Further statistical analysis by NMS ordination implicated a variety of taxa, perhaps included within CF and SAR11, which may also be important biogeochemically.

Ordination analysis provides an opportunity to observe communities in association with environmental variables, thus leading to the development of testable hypotheses. A more detailed analysis (cloning and sequencing) of the dominant ribotypes identified in this study might be instructive in designing additional ribosomal probes for prokaryotic enumeration in future iron enrichment experiments. These probes may prove useful in additional culture-independent methodologies capable of simultaneously detecting the organism(s) and its metabolic activity.

Marine prokaryotes can have an enormous impact in the fate of phytoplankton blooms. Boyd et al. (2004) observed that over 50% of lost POC from an iron-induced phytoplankton bloom was solubilized by prokaryotes prior to export from the mixed layer. Clearly this has profound implications for carbon cycling; however, the specific organisms
responsible for the heterotrophic activity remain unknown. Investigations into prokaryotic community structure and diversity, as well as their dynamics and the factors that modulate them, need to become routine components of ocean carbon cycling studies considering the biogeochemical significance of this group of plankton.
LITERATURE CITED


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49:799-808.


polymorphism data analysis for quantitative comparison of microbial communities.

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P. J. Harrison, R. Strzepek, J. Gower, R. M. McKay, E. Abraham, M. Arychuk, J.
Barwell-Clarke, W. Crawford, D. Crawford, M. Hale, K. Harada, K. Johnson, H.
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Psychometrika. 29:115-129.


TABLE 2.1. Probes and hybridization conditions used for whole-cell FISH. CF denotes Cytophaga-Flavobacteria and % Form. denotes the percent formamide used in the hybridization solution.

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th>Probe Suite</th>
<th>Probe Sequence (5' to 3')</th>
<th>Hybridization Solution</th>
<th>Hybridization Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Form.</td>
<td>Temp. (°C)</td>
</tr>
<tr>
<td>Eubacteria</td>
<td>EUB-27R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-CTG AGC CAK GAT CRA ACT CT-</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>EUB-338Rpl&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-GCG GCC WCC CGT AGG WGT-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUB-700R&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-CTA HGC ATT TCA CYG CTA CAC-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUB-700Ral&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-CTA CGA ATT TCA CCT CTA CAC-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eub-1522R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-AAG GAG GTG ATC CAN CCV CA-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea</td>
<td>Arch344&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-TCG CGC CTG CTG CRC CCC GT-</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Arch915&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-GTG CTC CCC CGC CAA TTC CT-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAR11</td>
<td>SAR11-152R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-ATT AGC ACA AGT TTC CYC GTG T-</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>SAR11-441R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-TAC AGT CAT TTT TTT CCC CGA C-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SAR11-542R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-TCC GAA CTA CGC TAG GTC-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SAR11-732R&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-GTC AGT AAT GAT GAT CCA GAA AGY TG-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>CF319a&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-TGG TCC GTG TCT CAG TAC-</td>
<td>35</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>CF319b&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-TGG TCC GTA TCT CAG TAC-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>338F&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-TGA GGA TGC CCT CCG TCG-</td>
<td>15</td>
<td>37</td>
</tr>
</tbody>
</table>

<sup>1</sup>Morris et al. 2002.<br><sup>2</sup>Stahl and Amann. 1991.<br><sup>3</sup>Manz et al. 1996.<br><sup>4</sup>Glockner et al. 1996.
TABLE 2.2. North Patch prokaryotic taxonomic abundance. Abundance is expressed as cells L$^{-1}$. The relative proportion of the group is expressed as a percentage of DAPI counts and is given in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>DAPI</th>
<th><strong>Eubacteria</strong></th>
<th><strong>Archaea</strong></th>
<th>CF</th>
<th>SAR11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 11</td>
<td>1.2 x 10$^9$</td>
<td>8.9 x 10$^8$ (73%)</td>
<td>2.6 x 10$^8$ (21%)</td>
<td>1.7 x 10$^8$ (14%)</td>
<td>4.6 x 10$^8$ (38%)</td>
</tr>
<tr>
<td>Day 39</td>
<td>1.4 x 10$^9$</td>
<td>7.6 x 10$^8$ (53%)</td>
<td>3.2 x 10$^8$ (22%)</td>
<td>1.0 x 10$^8$ (7%)</td>
<td>6.3 x 10$^8$ (44%)</td>
</tr>
<tr>
<td><strong>OUT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>1.1 x 10$^9$</td>
<td>5.3 x 10$^8$ (49%)</td>
<td>2.2 x 10$^8$ (20%)</td>
<td>8.1 x 10$^7$ (7%)</td>
<td>3.9 x 10$^8$ (36%)</td>
</tr>
<tr>
<td>Day 38</td>
<td>1.5 x 10$^9$</td>
<td>7.1 x 10$^8$ (46%)</td>
<td>2.9 x 10$^8$ (19%)</td>
<td>1.6 x 10$^8$ (7%)</td>
<td>5.8 x 10$^8$ (38%)</td>
</tr>
</tbody>
</table>
TABLE 2.3. South Patch prokaryotic community dynamics. Group relative proportion is expressed as a percentage of DAPI counts. E+A Sum denotes the sum of %Eubacteria and %Archaea. CF+SAR11 Sum denotes the sum of %CF and %SAR11.

<table>
<thead>
<tr>
<th>IN</th>
<th>% Eubacteria</th>
<th>% Archaea</th>
<th>E+A Sum %</th>
<th>% CF</th>
<th>% SAR11</th>
<th>CF+SAR11 Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5</td>
<td>74%</td>
<td>25%</td>
<td>100%</td>
<td>14%</td>
<td>31%</td>
<td>46%</td>
</tr>
<tr>
<td>Day 8</td>
<td>81%</td>
<td>27%</td>
<td>108%</td>
<td>16%</td>
<td>41%</td>
<td>56%</td>
</tr>
<tr>
<td>Day 12</td>
<td>81%</td>
<td>23%</td>
<td>104%</td>
<td>17%</td>
<td>45%</td>
<td>62%</td>
</tr>
<tr>
<td>Day 15</td>
<td>82%</td>
<td>18%</td>
<td>100%</td>
<td>29%</td>
<td>33%</td>
<td>62%</td>
</tr>
<tr>
<td>Day 16</td>
<td>85%</td>
<td>19%</td>
<td>104%</td>
<td>22%</td>
<td>32%</td>
<td>54%</td>
</tr>
<tr>
<td>Day 19</td>
<td>81%</td>
<td>30%</td>
<td>111%</td>
<td>29%</td>
<td>37%</td>
<td>66%</td>
</tr>
<tr>
<td>Day 22</td>
<td>88%</td>
<td>21%</td>
<td>109%</td>
<td>24%</td>
<td>41%</td>
<td>65%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OUT</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>72%</td>
<td>11%</td>
<td>83%</td>
<td>16%</td>
<td>43%</td>
<td>59%</td>
</tr>
<tr>
<td>Day 9</td>
<td>72%</td>
<td>14%</td>
<td>86%</td>
<td>8%</td>
<td>43%</td>
<td>51%</td>
</tr>
<tr>
<td>Day 13</td>
<td>79%</td>
<td>25%</td>
<td>103%</td>
<td>15%</td>
<td>44%</td>
<td>59%</td>
</tr>
<tr>
<td>Day 20</td>
<td>73%</td>
<td>28%</td>
<td>101%</td>
<td>21%</td>
<td>34%</td>
<td>56%</td>
</tr>
</tbody>
</table>
TABLE 2.4. South Patch domain and clade-level net growth rates. Net growth rates are expressed as d$^{-1}$. The statistical significance of each net growth rate is denoted by the p value for a 95% confidence interval. The time period over which the net growth rate was calculated is given. A denotes net growth rates which were significantly different from one another ($p<0.05$)

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th>$\mu$ (d$^{-1}$)</th>
<th>$p$ value</th>
<th>$\mu$, Time Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eubacteria</td>
<td>0.04</td>
<td>0.001</td>
<td>5 – 22</td>
</tr>
<tr>
<td>Archaea</td>
<td>0.03</td>
<td>0.172</td>
<td>5 – 19</td>
</tr>
<tr>
<td>CF</td>
<td>0.09$^A$</td>
<td>0.009</td>
<td>5 – 19</td>
</tr>
<tr>
<td>SAR11</td>
<td>0.04$^A$</td>
<td>0.001</td>
<td>5 – 22</td>
</tr>
<tr>
<td>Prokaryotes (DAPI)</td>
<td>0.03</td>
<td>0.002</td>
<td>5 – 22</td>
</tr>
<tr>
<td>Prokaryotes (FCM)$^1$</td>
<td>0.02</td>
<td>0.003</td>
<td>5 – 22</td>
</tr>
</tbody>
</table>

$^1$Oliver et al. (2004).
TABLE 2.5. Diversity measures in the North and South Patch. S denotes species richness, E denotes evenness, and H denotes the Shannon index of species diversity.

<table>
<thead>
<tr>
<th>North Patch Depth (m)</th>
<th>Day</th>
<th>S</th>
<th>E</th>
<th>H</th>
<th>South Patch IN Depth (m)</th>
<th>Day</th>
<th>S</th>
<th>E</th>
<th>H</th>
<th>South Patch OUT Depth (m)</th>
<th>Day</th>
<th>S</th>
<th>E</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>21</td>
<td>0.86</td>
<td>2.60</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>1.00</td>
<td>0.69</td>
<td>20</td>
<td>7</td>
<td>10</td>
<td>0.83</td>
<td>1.91</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>21</td>
<td>0.87</td>
<td>2.64</td>
<td>30</td>
<td>5</td>
<td>2</td>
<td>0.93</td>
<td>0.64</td>
<td>30</td>
<td>7</td>
<td>17</td>
<td>0.85</td>
<td>2.40</td>
</tr>
<tr>
<td>Mean 21.0</td>
<td></td>
<td></td>
<td>0.86</td>
<td>2.62</td>
<td>Mean 2.67</td>
<td></td>
<td></td>
<td>0.95</td>
<td>0.87</td>
<td>Mean 12.0</td>
<td></td>
<td></td>
<td>0.86</td>
<td>2.10</td>
</tr>
<tr>
<td>OUT</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>0.83</td>
<td>2.12</td>
<td>10</td>
<td>8</td>
<td>0.78</td>
<td>1.51</td>
<td>10</td>
<td>9</td>
<td>17</td>
<td>0.81</td>
<td>2.31</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>14</td>
<td>0.82</td>
<td>2.17</td>
<td>20</td>
<td>8</td>
<td>7</td>
<td>0.77</td>
<td>1.73</td>
<td>20</td>
<td>9</td>
<td>14</td>
<td>0.88</td>
<td>2.32</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
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FIGURE 2.1. Prokaryotic taxonomic abundances in the South Patch. (A) Total prokaryotic abundance (cells L$^{-1}$) measured by DAPI (IN - filled circles, OUT – open circles), Eubacteria (IN - filled triangles, OUT – open triangles), and Archaea (IN - filled squares, OUT – open squares). (B) Group-specific Eubacteria, Cytophaga-Flavobacteria (IN - filled circles, OUT – open circles) and SAR11 (IN - filled triangles, OUT – open triangles).
Bacterioplankton Abundance (cells liter⁻¹)

Days Post-Initial Fe Fertilization

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FIGURE 2.2. North versus South Patch non-metric multidimensional scaling (NMS) of *Eubacteria* 16S rRNA terminal restriction fragments. (A) North (open squares) and South Patch (filled squares) IN samples. (B) North (open squares) and South Patch (filled squares) OUT samples. Each symbol represents a sample from a specific depth on a specific day within the mixed layer (10, 20, 30, and 40 meters).
FIGURE 2.3. South Patch non-metric multidimensional scaling (NMS) of *Eubacteria* 16S rRNA terminal restriction fragments from fertilized and non-fertilized waters. IN samples are denoted by filled circles and OUT samples are denoted by open circles. Each symbol represents a sample from a specific depth on a specific day within the mixed layer (10, 20, 30, and 40 meters). Symbol labels designate IN or OUT, depth, and day (e.g. IN20d5 refers to IN, 20 m, day 5).
FIGURE 2.4. South Patch non-metric multidimensional scaling (NMS) of *Eubacteria* 16S rRNA terminal restriction fragments over time. Symbols are as follows: day 5 (open circles), day 8 (filled circles), day 12 (open diamond), day 15 (filled diamond), day 19 (open square), and day 22 (filled square). Each symbol represents a sample from a specific depth on a specific day within the mixed layer (10, 20, 30, and 40 meters). Symbol labels designate IN or OUT, depth, and day (e.g. IN40d5 refers to IN, 40 m, day 5).
CHAPTER 3

STIMULATION OF MARINE PROKARYOTIC GROWTH
BY THE SIDEROPHORE, DESFERRIOXAMINE B (DFOB)
ABSTRACT

Marine prokaryotes acquire iron through the expression, release, and reacquisition of iron-binding compounds known as siderophores. Marine prokaryotes are also capable of acquiring iron through the uptake of exogenous siderophores. The use of siderophores as a carbon source, however, has not been previously reported for marine prokaryotes. This study demonstrates the growth enhancement of marine prokaryotes in seawater cultures from the oligotrophic Sargasso Sea using a commercially available siderophore, desferrioxamine B (DFOB). Initial experiments suggested carbon limitation of growth relative to nitrogen and phosphorus limitation. Upon enrichment with DFOB, prokaryotic specific growth rates and yields were higher than cultures receiving either glucose and/or inorganic nutrients. When cultures were amended with varying concentrations of DFOB, prokaryotic growth responded in a dose-dependent fashion. Furthermore, differential shifts in prokaryotic community composition in seawater cultures receiving either glucose and DFOB were observed. 

*Alteromonas macleodii* dominated a glucose-amended culture comprising up to 60% of DAPI-stained cells. In cultures amended with DFOB, SAR11 cells dominated the community comprising up to 45% of DAPI-stained cells. From these results it appears that DFOB, and possibly siderophores in general, represent an organic DOM source that supports prokaryotic production in the ocean.
INTRODUCTION

Marine heterotrophic prokaryotes represent a major entry point of dissolved organic carbon (DOC) into the marine plankton food web by serving as a conduit for DOC-derived carbon transfer to higher trophic levels (Azam et al. 1983). Prokaryotes also modify ocean biogeochemistry through the remineralization of dissolved organic matter (DOM). In the latter process, heterotrophic prokaryotes affect the lability (Ogawa et al. 2001) and composition (Church et al. 2002) of DOM, as well as the rate of supply of regenerated nitrogen (Kirchman 2000) and silica (Bidle and Azam 1999) to the phytoplankton community. In addition, the time scale of heterotrophic prokaryotic remineralization of DOC relative to the frequency of convective mixing events influences the amount of DOC available for export to the ocean interior (Carlson et al. 1994). Thus, it is important to understand the factors that enhance and constrain DOC remineralization by heterotrophic prokaryotes.

Nutrient limitation is one potential factor that limits heterotrophic prokaryotic growth and, in turn, remineralization of organic carbon and inorganic nutrients. Growth limitation has been observed for different substrates in a variety of locales: inorganic macronutrients in the Gulf of Mexico (Pomeroy et al. 1995) and DOC in the subarctic (Kirchman 1990) and equatorial Pacific (Kirchman and Rich 1997). Considerable attention has been focused on the northwest Sargasso Sea with respect to nutrient limitation due to the seasonal accumulation and subsequent export of DOC to the ocean interior that occurs there (Carlson et al. 1994). Macronutrient limitation (Rivkin and Anderson 1997, Cotner et al., 1997, Caron
et al. 2000) and quality of accumulated carbon (Carlson and Ducklow 1996, Carlson et al.
2002) have been suggested as mechanisms that allow accumulation to occur.

Iron limitation, while known to constrain phytoplankton growth in high nutrient, low
chlorophyll (HNLC) regions (Martin et al. 1994), also affects heterotrophic prokaryotic
growth. Studies in the Antarctic reported that prokaryotic production was directly stimulated
by iron (Pakulski et al. 1996) and iron plus labile organic carbon in seawater cultures
(Church et al. 2000). Furthermore, studies in the sub-Arctic Pacific Ocean demonstrated that
iron enhanced prokaryotic growth efficiencies (BGE) in monocultures (Tortell et al. 1996).
These results have profound implications for HNLC regions because they couple DOC
remineralization to iron bioavailability. Such a coupling may take on less importance in
regions like the northwest Sargasso Sea where aeolian inputs of iron are high (Jickells 1999)
and surface water (<20 m) concentrations of total iron are typically above those which would
be considered limiting to phytoplankton (<0.1 nM) (Wu and Luther 1996). However, large
fluxes and standing stocks of iron do not necessarily equate to increased bioavailability.
Powell and Donat (2001) demonstrated that despite high atmospheric iron deposition in the
equatorial and South Atlantic Ocean, greater than 99% of total dissolved iron was complexed
to organic ligands. Other studies support the phenomenon of ubiquitous organically-bound
iron (Rue and Bruland 1995, Wu and Luther 1995), thus bringing into question the forms of
iron that are available to both phytoplankton and prokaryotes.

The scarcity of iron in the ocean has led to the evolution of efficient iron acquisition
strategies by prokaryotes which employ strong iron chelators known as siderophores
(Raymond et al. 1984, Butler 1998). Prokaryotes genetically express and release
siderophores extracellularly, then reacquire siderophore-iron complexes for uptake (Reid et
There are also cases where prokaryotes can acquire exogenous siderophores from other taxa (Granger and Price 1999, Hutchins et al. 1999a, Maldonado and Price 1999), to obtain iron. Recent evidence suggests that a portion of the organic ligands responsible for widespread iron complexation share chemical characteristics with those of siderophores (Macrellis et al. 2001), a hypothesis proposed earlier (Rue and Bruland 1997). Therefore, exogenous siderophore-mediated iron uptake may be an important pathway for iron acquisition by prokaryotes.

I conducted a series of experiments in the northwest Sargasso Sea near Bermuda to examine the effects of a commercially available siderophore, desferrioxamine B (Desferal®, DFOB), on prokaryotic growth. Specifically, my study examined the effects of iron limitation on prokaryotic growth with respect to DOC cycling. The oligotrophic northwest Sargasso Sea provides a convenient context for conducting such experiments because during the summer and early fall a predictable stratified surface mixed layer (< 20 m) persists, preventing surface DOC from being diluted via mixing. This results in elevated and relatively constant DOC concentrations. Stocks of inorganic nitrogen and phosphorus remain low and heterotrophic prokaryotes are reliant upon freshly produced labile DOM and regenerated nutrients for growth (Carlson and Ducklow 1996). Thus, the oceanographic conditions during this time permit the observation of the growth dynamics of the resident prokaryotic community under nutrient amendments.

DFOB, along with other trihydroxamate siderophores, is produced by a genus of Gram-positive soil bacteria, Streptomyces spp., which belong to the order Actinomycetales (Keller-Schierlein et al. 1964, Fiedler et al. 2001). Previous studies using DFOB in seawater cultures have shown its effectiveness in chelating iron and, in turn, restricting phytoplankton
growth (Hutchins et al. 1999b) and rendering iron unavailable to prokaryotes (Wells 1999). My hypothesis was that DFOB would limit heterotrophic prokaryotic growth in a similar manner. Cultures were amended with a combination of labile DOC (glucose), inorganic nutrients, and DFOB to examine the response of prokaryotes to the addition of labile DOC under Fe limiting conditions. Initial results showed the opposite effect: DFOB did not suppress prokaryotic growth but appeared to stimulate growth more than glucose. The stimulatory effects of DFOB were unexpected and led to the new hypothesis that the siderophore might stimulate prokaryotic growth directly as a carbon source, a phenomenon not previously observed.

The goals of the present study were to: (1) determine whether DFOB could stimulate prokaryotic growth in dose-dependent manner, (2) compare the growth dynamics of prokaryotes in response to glucose and DFOB, (3) determine whether DFOB differentially stimulates specific prokaryotic taxa.

MATERIALS AND METHODS

Seawater cultures - Water for seawater cultures was collected from the surface mixed layer (<20 m) aboard the R/V Weatherbird II at Hydrostation S (32°10.00’N, 64°0.00’W) and various nearby stations within the Bermuda Atlantic Time-Series (BATS) region. Water was collected in 12-L Niskin bottles attached to a CTD (conductivity, temperature, depth probe) rosette and emptied into acid-washed, polycarbonate 20-L carboys. Water was kept in the dark at 20° to 22°C until the start of the experiments (< 6 hours from the time of collection). The first experiment testing the potential suppressive effects of DFOB was carried out in late...
spring (April 2001). Experiments which tested DFOB as a growth stimulant were carried out in the summer (July 2003a, July 2003b). Seawater collected for the July 2003b experiment coincided with the passage of a cyclonic mesoscale feature in the BATS region.

Seawater cultures (70% dilutions of whole seawater with 0.2 μm seawater filtrate) were initiated following the methods of Carlson et al. (2002). The purpose of the cultures was two-fold. First, microzooplankton grazing pressure was reduced by dilution. This facilitated prokaryotic growth and biomass accumulation thus permitting an estimate of community specific growth rate (Ammerman et al. 1984). Second, by incubating cultures in the dark, dissolved photosynthate production was halted and prokaryotic growth was constrained by ambient DOC and nutrients. In this way prokaryotic growth dynamics could be observed and compared after amendments with external supplies of DOC and nutrients.

Briefly, whole water was gravity filtered through 0.2 μm, 142 mm nitrocellulose filters (Millipore) into a clean, acid-washed carboy. The nitrocellulose filters were pre-rinsed with 2-L of deionized, distilled water to remove any potential organic and inorganic contaminants. After generation of 0.2 μm filtrate, unfiltered seawater was added to the filtrate to achieve a 70% dilution of the plankton. Water was dispensed into acid-washed, 2-L polycarbonate darkened bottles and subsequent nutrient amendments were made.

Cultures were amended with ammonium (NH₄Cl) and phosphate (K₂HPO₄), to final concentrations of 1.0 and 0.1 μM, respectively. Glucose was chosen as a labile DOC enrichment because it is the most abundant neutral sugar in oceanic systems and some reports indicate that it is preferentially utilized relative to other monosaccharides (Rich and Kirchman 1994). Iron (FeCl₃) was added in preliminary experiments, but had no effect on
prokaryotic growth and was not included in seawater culture experiments described below (data not shown).

In the initial experiment DFOB was added at three different concentrations (0.1, 1.0 and 10 μM corresponding to 2.5, 25, and 250 μM carbon) while keeping glucose (10 μM glucose or 60 μM carbon) and inorganic nutrient concentrations constant. A fourth seawater culture was amended with the same glucose and inorganic nutrient concentrations as the others, but excluded DFOB. A fifth seawater culture was included without any amendments.

Following the first experiment, new seawater cultures were initiated to examine the potential stimulatory effects of DFOB on prokaryotic growth. Adjustments were made to the April 2001 experimental design to ensure that amendments with glucose or DFOB were made in terms of organic carbon equivalents. Inorganic nutrient amendments were made as previously described. Two controls were included: a seawater culture amended with only NH₄Cl and K₂HPO₄ and a seawater culture with no amendment. In the July 2003a experiment, both glucose and DFOB were added to seawater cultures at organic carbon equivalents of 0.1, 1.0, 5, and 10 μM final concentrations. Also in July 2003a, a side experiment was carried out to test the potential stimulatory effects of a salt in the commercial DFOB formulation. This salt, known as mesylate (CH₄O₃S), enhances the solubility of DFOB. In order to distinguish the effects of DFOB-mesylate from mesylate alone, methane sulfonic acid, a solubilized form of mesylate, was added in equal organic carbon concentrations to those occurring in the DFOB additions (0.004, 0.039, 0.195, and 0.395 μM final concentrations). In the final experiment, July 2003b, glucose and DFOB were added to seawater cultures in equal organic carbon concentrations (10 μM final concentrations as carbon) along with inorganic nutrients.
Prokaryotic abundance and growth - Prokaryotic cell abundance in the April 2001 experiment was measured by epifluorescence microscopy of DAPI-stained cells (Porter and Feig 1980). Prokaryotic cell abundance in the July 2003a and July 2003b experiments was measured by flow cytometry following the methods of Troussellier et al. (1999). Flow cytometry samples (4.5 ml) were drawn from seawater cultures, fixed with 0.2 µm-filtered formalin (1% final concentration), and stored frozen at -80°C until analysis. Triplicate 1 ml subsamples were stained with Syto13 (Molecular Probes) and counted on a Beckman-Coulter Epics Altra flow cytometer equipped with an Enterprise II laser at 488 nm. Count calibration was performed by adding 1.0 µm beads (Molecular Probes, Fluo Spheres) at a concentration of 5 x 10^4 beads ml^{-1} to each subsample. A minimum of 10,000 cells was counted from each subsample. Intra-sample or instrumental variation was less than 1%. High DNA (HDNA) and low DNA-containing (LDNA) cells were differentiated by fluorescence and side scatter following the methods of Gasol and Moran (1999). Percent HDNA (%HDNA) was expressed as the fraction of total abundance as HDNA multiplied by 100.

Taxon-specific prokaryotic cell abundance was determined using fluorescence in situ hybridization (FISH). Two ribosomal probes were employed: SAR11, due to its ubiquity in the upper surface ocean (<50 m) (Morris et al. 2002), and Alteromonas macleodii, due to its ability to grow rapidly and dominate community cell counts in response to glucose, nitrogen, and phosphorus amendments (C. Carlson, unpubl. data). Samples (50 ml) were drawn from seawater cultures, fixed with 0.2 µm-filtered formalin (1% final concentration), and stored at 4°C for 6-10 hours. Fixed subsamples (5-20 ml) were filtered onto white, polycarbonate 0.2 µm filters under vacuum. Filters were cut into smaller fragments and affixed to glass slides using adhesive stickers. Hybridization took place with each fragment incubated
with a suite of oligonucleotide ribosomal probes for the SAR1 clade (SAR11-152R, SAR11-441R, SAR11-542R, SAR11-732R; Morris et al. 2002) or *A. macleodii* (AC-137R, 5'-TGT TAT CCC CCT CGC AAA-3'). SAR11 and *A. macleodii*-positive cells and DAPI-positive cells were enumerated using epifluorescence microscopy under CY3 and UV excitation and emission, respectively. Probe-positive and DAPI-positive cells were identified individually using imaging software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD) and an imaging/masking protocol (R. Parsons, pers. comm.). Briefly, cells in six replicate fields were enumerated for both probe-positive and DAPI-positive cells. Images of each field were taken using a digital camera. Images were processed individually to account for variation in signal intensity. The intensity range for each image was adjusted so as to retain probe- and DAPI-positive cells while simultaneously reducing non-specific background noise (i.e. non-cellular objects). To attain true probe-positive cells, i.e. cells which were both probe-positive and DAPI-positive, a separate image mask of probe-positive cells was created. A similar masked image of DAPI-positive cells was created. The probe-positive masked image was overlaid on top of the DAPI-positive masked image to yield cells which were coincident to both images. These coincident cells were quantified as probe-positive cells. Probe-positive cell counts were summed and corrected by subtracting out probe-positive cells which were incubated and hybridized with negative control probes (i.e. non-specific binding of probes or false positives). Negative control hybridizations were carried out using the least stringent conditions of all the hybridization conditions (lowest % formamide, highest NaCl concentration, lowest temperature). Summed net probe-positive cells were expressed as a fraction of summed DAPI-positive cells.
Prokaryotic and taxon-specific specific growth rates were determined as the slope of the least-squares regression of the linear portion of natural log-transformed prokaryotic abundance versus time. Least-squares regressions excluded lag and stationary phases of growth. Specific growth rates were tested for significance \((p<0.05)\) and subsequently compared using a two-tailed t-test to determine significant differences between specific growth rates \((p<0.05)\).

RESULTS

Initial experiments were designed to examine the potential suppressive effects of DFOB on prokaryotic growth. In the April 2001 experiment concentrations of glucose (G), ammonium (N), and phosphate (P) were kept constant and the concentration of DFOB was varied. Prokaryotic growth increased with higher concentrations of added DFOB (Figure 3.1). In comparison, when prokaryotes were provided only G, N, and P, growth was not significantly different from that observed in unamended control.

Dose-response to DFOB - In subsequent experiments (July 2003a and July 2003b), the potential of DFOB to stimulate prokaryotic growth as the sole carbon source was tested. One additional dose of DFOB \((5 \mu M)\) and comparable cultures receiving equal doses of organic carbon as DFOB (Figure 3.2A) and glucose (Figure 3.2B) were included. Additionally, mesylate \((\text{CH}_4\text{O}_3\text{S})\), a solubility-enhancing salt that accompanies DFOB, was tested as a potential growth stimulant (Figure 3.2C). The carbon ratio of DFOB to mesylate is 25:1. This means that a 10 \(\mu M\) carbon addition as DFOB is actually 9.61 \(\mu M\) carbon as DFOB and
0.39 μM carbon as mesylate. To test the effects of mesylate, methanesulfonic acid (CH₄O₃S) was added at equivalent carbon concentrations to those of the DFOB amendments. For example, 0.39 μM carbon as methanesulfonic acid was added to simulate the concentration that was added in a 10 μM carbon addition as DFOB-mesylate. In this way, the prokaryotic growth response to DFOB-mesylate could be distinguished from that of mesylate alone.

Results from carbon additions as DFOB demonstrated a dose-response (Figure 3.2A, 3.2B, Table 3.2). The highest dose of DFOB (10 μM carbon) resulted in the largest yield in cell abundance (maximum abundance = 7.0 x 10⁸ cells L⁻¹) and a significant specific growth rate (1.2 d⁻¹, Figure 3.2A and Table 3.2) relative to the unamended or NP control. The next highest dose of DFOB (5 μM carbon) also enhanced cell abundance (maximum abundance = 5.0 x 10⁸ cells L⁻¹) and specific growth rate (0.75 d⁻¹). Additions of DFOB in the two lowest doses, 0.1 and 1.0 μM carbon, did not significantly stimulate growth above that of the NP control, nor the unamended controls.

Doses of glucose also appeared to stimulate prokaryotic growth significantly (Figure 3.2B, 3.2D, Table 3.2) but only the specific growth rate in the 10 μM dose was significantly different from the NP or unamended controls. While large doses of glucose with replete N and P demonstrated similar trends to that of DFOB additions, the magnitude of the response was significantly greater with DFOB as the carbon source versus glucose (Figures 3.1, 3.2, Table 3.2). Some significant specific growth rates in treatments receiving carbon as DFOB or glucose that also lack statistical differences with the controls reflects variability (standard deviation in the regression) in growth. Amendments with methanesulfonic acid did not significantly alter the growth kinetics of prokaryotes and are indistinguishable from the unamended or NP controls (Figure 3.2C).
One advantage of flow cytometric analysis is the ability to distinguish HDNA from LDNA cells. The presence of HDNA cells has been suggested as an indication of an actively growing subpopulation of prokaryotes (Lebaron et al. 2001). The percentage of cells that were HDNA cells represented only 30% of the total count at the initiation of the July 2003a experiment and varied over time depending on the treatment. The timing of the appearance of HDNA cells in both the DFOB and glucose additions (Figure 3.3A and B, respectively) coincided with the timing of increases in cell abundance (Figure 3.2A and B, respectively).

Percent HDNA over time in the July 2003a experiment for both the DFOB and glucose-amended cultures is given in Table 3.2. In line with the prediction that actively growing cells increase their DNA content, cultures amended with varying doses of glucose exhibited increases in %HDNA. Maximum %HDNA occurred for all doses of glucose on day 1.5 (Figure 3.3B). Furthermore, the magnitude of the increases appeared to be proportional to the dose of glucose. Similarly, cultures amended with varying doses of DFOB exhibited the same proportional response in %HDNA as those amended with glucose. However, there were two subtle differences: the maximum %HDNA and the length of time in which the maximum %HDNA was sustained. Maximum %HDNA in the 5 μM DFOB (day 3) and 10 μM DFOB (day 1) cultures exceeded their counterpart cultures receiving glucose (Table 3.3). Also, in DFOB-amended cultures, particularly in the 5 and 10 μM doses, near maximum %HDNA was sustained through day 4 whereas their glucose-amended counterparts exhibited decreases in %HDNA through day 4 following their peaks (Figure 3.3).
Community composition shifts - Organic carbon amendments also produced differential taxonomic shifts in the prokaryotic community depending upon the type of organic carbon provided (Figure 3.4). SAR11 comprised one-fifth of the prokaryotic community at the start of the experiment (Table 3.3). A. macleodii, however, comprised only 2% of the community (Table 3.3). Despite comprising a marginal fraction of the total community, A. macleodii responded more rapidly to glucose and numerically dominated over SAR11 (Figure 3.4A). Within one day A. macleodii accounted for 53% of DAPI-stained cells compared to 16% for SAR11 (Figure 3.4A, Table 3.3). Maximum A. macleodii abundance and relative percent composition occurred on day 2 comprising 62% of DAPI-stained cells. The specific growth rate for A. macleodii, 2.7 d\(^{-1}\), was nearly four times higher than that of SAR11, 0.70 d\(^{-1}\) (Table 3.4). Furthermore, SAR11 responded only modestly to glucose with a two-fold increase over time (Figure 3.4A).

In contrast to the glucose-amended culture, SAR11 dominated the culture receiving DFOB. SAR11 relative percent composition doubled from 22% to 47% (Table 3.3) and abundance increased eight-fold by day 1 (Figure 3.4B). Although A. macleodii relative percent composition increased six-fold from 2% to 13% by day 1, it never exceeded 24% of the community. Despite higher SAR11 abundance throughout the experiment, A. macleodii displayed higher growth kinetics in response to DFOB. The specific growth rate for A. macleodii was twice that for SAR11, 1.9 d\(^{-1}\) compared to 0.80 d\(^{-1}\) (Table 3.4).

Total prokaryotic specific growth rates in response to 10 \(\mu\)M carbon as glucose or DFOB varied in comparison to the July 2003a experiment conducted a week earlier. While the general trend was similar, that is growth stimulation in response to organic carbon, the magnitude of the response was different. In July 2003b the specific growth rates were 1.2
and 1.4 d\(^{-1}\) for glucose and DFOB, respectively (Table 3.4). The time period over which specific growth rates were calculated for DFOB and glucose were 0.5 to 1.5 days and 0 to 1.5 days, respectively. In contrast, specific growth rates in July 2003a were 1.2 and 0.87 d\(^{-1}\) for DFOB and glucose, respectively (Table 3.2), over comparable time scales. In addition to the variation in response to organic carbon, the specific growth rate for N+P was higher in the July 2003b experiment compared to the July 2003a experiment (0.58 d\(^{-1}\) versus 0.28 d\(^{-1}\)). The specific growth rate in the unamended control did not vary from July 2003a. The July 2003b experiment coincided with the passage of a mesoscale eddy (D. McGillicudy, pers. comm.). The integrated prokaryotic production (0 – 200 m), via the \(^3\)H-thymidine method (Smith and Azam 1992), measured during the coincident BATS cruise was approximately ten-fold greater than the previous cruises in May and June of 2003 (BATS data set).

**DISCUSSION**

DFOB has been employed as an alternative way to explore iron limitation of the plankton community in the absence of trace metal clean sampling systems (Hutchins et al. 1999b, Wells 1990). In practice, the siderophore is added to natural seawater cultures wherein it chelates free Fe\(^{3+}\), rendering it unavailable for uptake by the plankton community. Hutchins et al. (1999b) demonstrated that DFOB suppressed taxon-specific algal growth in upwelled waters near California, reduced iron uptake by different size fractions of the plankton community (including heterotrophic prokaryotes, but perhaps as an indirect effect), and reduced nitrate utilization. Wells (1999) also demonstrated DFOB’s inhibitory effect on
iron uptake by the plankton community. Seawater cultures from the California and Oregon coasts were incubated with DFOB and $^{59}$Fe and size fractionated to determine the fate of the radiolabeled iron. DFOB was effective in inhibiting iron uptake within the 0.2-5.0 μM size fraction over short time scales (hours). Despite the effectiveness of DFOB in restricting iron uptake, other studies demonstrated DFOB enhanced iron uptake. These include the natural plankton community in the subarctic Pacific (Maldonado and Price 1999), natural and cultured cyanobacteria from the Sargasso Sea (Hutchins et al. 1999a), and cultured bacterial isolates (Granger and Price 1999). Despite the evidence for and against the inhibitory effects of DFOB on iron uptake, none of the previous studies addressed the effects of DFOB in the context of prokaryotes growth and metabolism of DOC. My study hypothesized that DFOB would inhibit prokaryotes growth by restricting the bioavailability of iron and, in turn, restricting the metabolism of labile DOC (as glucose). Initial experiments carried out in the Sargasso Sea with DFOB showed that the siderophore did not suppress prokaryotes growth. In fact, it appeared that DFOB, in the presence of elevated inorganic macronutrients, N and P, enhanced growth of prokaryotes over and above prokaryotes which received glucose, N, and P. This led me to reformulate my hypothesis and test whether DFOB could directly stimulate growth.

Three pathways in which DFOB could stimulate prokaryotic growth can be postulated. First, the siderophore could facilitate iron uptake by prokaryotes as demonstrated in studies previously described. This could, in turn, increase bacterial growth efficiency on carbon sources \textit{in situ}, an effect demonstrated by Tortell et al. (1996). Second, the siderophore might serve as the actual carbon source, a phenomenon not previously observed in marine environments. Third, a combination of the two mechanisms might be operating
simultaneously either for a specific prokaryote, whereby both iron and carbon are obtained from the siderophore, or for different members of the community where some are obtaining iron and others are obtaining carbon.

The results herein consistently demonstrated that DFOB was effective in stimulating prokaryotic growth relative to unamended and inorganic amended treatments. In the first experiment, April 2001, DFOB plus glucose and inorganic nutrients, N and P, stimulated prokaryotic growth in a dose-dependent manner. The cell yields were above that of the culture receiving only glucose, N, and P. Corresponding specific growth rates were also higher in cultures receiving the two highest doses of DFOB suggesting that DFOB was the determining factor in the responses. In the second experiment, July 2003a, seawater cultures were amended with glucose and DFOB at equal carbon concentrations to make the experiments comparable. DFOB stimulated growth in a manner analogous to that of glucose, a labile carbon source, in the presence of N and P. The prokaryotic response was an order of magnitude higher in the April 2001 compared to July 2003a due to the significantly higher doses of DFOB in the former experiment. The trend, however, is the same: a proportional response in cell yield and specific growth rate to DFOB. It should be noted that equivalent carbon doses as DFOB and glucose resulted in different biomass yields. For example, by stationary phase prokaryotic biomass reached $7 \times 10^8$ cells L$^{-1}$ in the treatment receiving 10 $\mu$M carbon as DFOB. In contrast, prokaryotic biomass in the equivalent carbon dose as glucose reached only $4 \times 10^8$ cells L$^{-1}$. Differences in the biomass yield may have been a result of differences in the growth efficiency on the different substrates. Lower growth efficiency on glucose compared to DFOB could have resulted in the lower biomass yield that was observed. Rates of respiration were not measured and, in turn, growth efficiencies could
not be determined. Another possibility is the inadvertent addition of reduced nitrogen as a component of DFOB. Each molecule of DFOB possesses 6 atoms of nitrogen as NH$_2$. Thus, in the July 2003a experiment the DFOB doses as 10, 5, 1, and 0.1 μM carbon also equate to 2.28, 1.14, 0.23, and 0.2 μM additions of nitrogen, respectively. This nitrogen is in addition to the 1 μM ammonium added to each treatment. The glucose has no equivalent nitrogen source to DFOB. Thus, this difference in nitrogen dose might have also accounted for the differential biomass yields provided that nitrogen became limiting during the exponential growth phase.

In the last experiment, July 2003b, taxonomic shifts in the prokaryotic community concomitant with the increase in prokaryotic abundance in response to DFOB were observed. SAR11, a group with cosmopolitan distribution and comprising a large percentage (20 - 40%) of the surface prokaryotic community in the northwest Sargasso Sea (Morris et al. 2002), responded rapidly to inputs of DFOB and quickly dominated the seawater culture community. Furthermore, the SAR11 specific growth rate in the DFOB-amended culture was twice that of the glucose-amended culture. *Alteromonas macleodii*, a gamma proteobacteria species that comprises only a small percentage (less than 5%) of the surface prokaryotic community in the northwest Sargasso Sea (C. Carlson, unpubl. data), grew at comparable rates in both cultures; however, it accounted for up to 25% of the community receiving DFOB compared to as much as 62% of the community receiving glucose. These experiments demonstrate that specific taxa of prokaryotes are capable of responding to both DFOB and glucose and that the quality of the organic matter may play a role in selecting the responding community structure.
The observed shift in the magnitude of the community response to these carbon sources may have been a function of when the experiments were conducted. July 2003b specific growth rates in both treatments receiving DFOB and glucose varied in comparison to their counterparts in the July 2003a experiment conducted a week earlier. The July 2003b experiment coincided with a mesoscale cyclonic eddy that passed through the BATS study area. Mesoscale cyclonic eddies have been shown to enhance biological production due to entrainment of nutrients into the euphotic zone (Ducklow 1986, Falkowski et al. 1991, McGillicuddy et al. 1998). During the July 2003b experiment, there was a significant increase in integrated prokaryotic production indicating that bottom up or top down controls were altered in the eddy, relative to the ambient background. While the general experimental trend of increased specific growth rate with DFOB amendment was maintained, the magnitude of the response was higher compared to previous experiments. In addition, the specific growth rate in the inorganic N+P amendment was higher than the previous experiment and was higher than the unamended control. The variability in response to the same treatments between experiments suggests that bottom up controls on prokaryotic growth vary in space and time in the Sargasso Sea (Caron et al. 2000; Carlson et al. 2002) with superimposed mesoscale perturbations, such as cyclonic eddies, possibly contributing to this variability.

Alternative carbon sources: Catabolism of siderophores - While there is ample evidence demonstrating facilitated iron uptake by exogenous siderophores, including DFOB, there are no known reports of catabolism of siderophores by marine prokaryotes, either in culture or in their natural environment. There are, however, reports of catabolism of siderophores by soil microbes. The earliest is by Warren and Neilands (1964) who reported the degradation of
ferrichromes by an unknown Pseudomonad. They later proposed a metabolic pathway for
found that a soil isolate (DFBC 5) was capable of catabolizing the non-ferrated form of
DFOB. DeAngelis et al. (1993) determined that the same isolate employed ‘nutritional
selectivity’ when obtaining carbon and iron when presented with two siderophores. While
DFBC 5 could obtain carbon from DFOB, it could not obtain iron from the ferrated form of
the siderophore. Conversely, DFBC 5 could not catabolize ferrirhodotorulic acid (ferriRA)
nor its non-ferrated form (RA), but it could acquire iron from ferriRA. Harwani et al. (1997)
proposed a metabolic pathway for degradation of DFOB whereby DFOB undergoes an initial
hydrolysis by a proposed enzyme, DFB hydrolase, to two different intermediate catabolites.
Each intermediate undergoes further hydrolysis to yield succinic acid, acetic acid, and
cadaverine. Harwani et al. (1997) further demonstrated that the two latter compounds (acetic
acid as acetate) could stimulate growth of DFBC 5. Based on its catalytic properties, DFB
hydrolase was characterized as a serine protease sensitive to sulfhydryl and metal inhibitors
(Zaya et al., 1998).

The paucity of reports of microbial catabolism of siderophores in the literature may
not truly reflect the frequency with which this phenomenon occurs in nature. Most of the
previous marine studies have reported on the utilization of siderophores as they pertain to
iron acquisition, not carbon catabolism. The results also contrast with previous studies in
which DFOB was successful in rendering iron unavailable for uptake by marine
phytoplankton in non-axenic seawater cultures (Wells 1999, Kirchman et al. 2000). An
important distinction between this study and previous ones is that previous studies were
carried out in coastal waters off of California. Differences in community composition in
coastal Pacific and Sargasso Sea waters could account for differences in the fate of DFOB and, in turn, the effectiveness of DFOB to immobilize iron. Nonetheless, the results herein represent a proof-of-principle in which a model siderophore, DFOB, stimulated prokaryotic growth.

This study may have important implications given the ongoing convergence of iron chemistry and DOM research. An overwhelming majority of dissolved iron is bound to organic ligands (Rue and Bruland 1995, Wu and Luther, III 1995), a suite of compounds whose composition, reactivity, and origin are still unknown. Recent evidence suggests that these ligands share chemical characteristics (binding affinities, molecular weight) that are common to purified siderophores (Macrellis et al. 2001). Perhaps acquisition and catabolism of this poorly characterized form of iron is an important metabolic pathway for prokaryotes to meet their iron and carbon demands, two properties that are intimately linked (Tortell et al. 1996).

The implications not only extend to prokaryotic nutrition, but also suggest complex food web interactions between microorganisms in the ocean that are consistent with previous hypotheses. Intense competition for iron exists between eukaryotic autotrophs and prokaryotes given their respective intracellular iron content (Tortell et al. 1996). Competitiveness for iron was further supported by studies which showed that different marine phytoplankton, including autotrophic prokaryotes, utilized different forms of organically bound iron (Hutchins et al. 1999a). This study supports the hypothesis of specialization and niche separation with respect to plankton iron acquisition. The evidence that SAR11 and A. macleodii responded favorably to DFOB suggests complex ecological interactions exist within plankton communities. Clearly not all plankton are subjected to iron
limitation induced by DFOB. Theoretically, metabolically flexible prokaryotes that can acquire and catabolize siderophores would have a physiological and ecological advantage over organisms that do not possess such pathways.

Despite evidence of DFOB-stimulated growth, the definitive carbon source utilized by prokaryotes in these experiments, including the fate of the iron complexed to the siderophore, needs to be determined. In addition, a variety of other siderophores with different chemical characteristics, particularly those of marine origin, should be tested as potential carbon sources. Finally, future studies should also focus on determining which members of the prokaryotic community are catabolizing and/or metabolizing via this pathway. Ultimately, such studies should seek to determine the relative importance of this generalized pathway of nutrition, if it imparts a competitive advantage to prokaryotes along with its geochemical consequences, and whether it explains the ubiquity and dominance of specific prokaryotic taxa in the oceans.


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TABLE 3.1. April 2001 prokaryotic response to nutrient amendments. Prokaryotic specific growth rates in response to inorganic nutrients (NH$_4$ and PO$_4$), DFOB, and glucose. Equivalent carbon concentrations are presented in parenthesis. Specific growth rates are accompanied by the standard error and are expressed as d$^{-1}$. Asterisks denote specific growth rates that are significantly different from zero ($p<0.05$). Specific growth rates that share the same superscript denote growth rates which were significantly different from one another (t-test, $p<0.05$). The time period over which specific growth rates were calculated is also given.

<table>
<thead>
<tr>
<th>NH$_4$, PO$_4$</th>
<th>DFOB</th>
<th>Glucose</th>
<th>April 2001 Specific growth rate, $\mu$ (d$^{-1}$)</th>
<th>$\mu$, Time Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0, 0.1 µM</td>
<td>10 µM (250 µM)</td>
<td>10 µM (60 µM)</td>
<td>*0.45$^{A,B}$ ± 0.2</td>
<td>0 - 6</td>
</tr>
<tr>
<td>1.0, 0.1 µM</td>
<td>1.0 µM (25 µM)</td>
<td>10 µM (60 µM)</td>
<td>*0.41 ± 0.06</td>
<td>0 - 6</td>
</tr>
<tr>
<td>1.0, 0.1 µM</td>
<td>0.1 µM (2.5 µM)</td>
<td>10 µM (60 µM)</td>
<td>0.13$^B$ ± 0.07</td>
<td>0 - 6</td>
</tr>
<tr>
<td>1.0, 0.1 µM</td>
<td>-</td>
<td>10 µM (60 µM)</td>
<td>0.22 ± 0.53</td>
<td>0 - 4</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.18$^A$ ± 0.01</td>
<td>0 - 4</td>
</tr>
</tbody>
</table>
TABLE 3.2. July 2003a prokaryotic response to nutrient amendments including prokaryotic specific growth rates and % HDNA in response to DFOB, glucose, and inorganic nutrients. Doses of DFOB and glucose are presented in equivalent carbon concentrations, C. N and P denote inorganic nutrients, 1.0 μM NH$_4$ and 0.1 μM PO$_4$, respectively. Specific growth rates are accompanied by the standard error and are expressed as d$^{-1}$. All specific growth rates were significantly different from zero (p<0.05). Specific growth rates that share the same superscript denote growth rates which were significantly different from one another (t-test, p<0.05). The time period over which specific growth rates were calculated is also given.

<table>
<thead>
<tr>
<th>Organic carbon source</th>
<th>Treatment</th>
<th>July 2003a Specific growth rate, μ (d$^{-1}$)</th>
<th>Time Period (days)</th>
<th>Day 0 % HNDA</th>
<th>Day 1 % HNDA</th>
<th>Day 3 % HNDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFOB</td>
<td>10 μM C + NP</td>
<td>*1.20$^A$X ± 0.27</td>
<td>0.5 - 2</td>
<td>30%</td>
<td>43%</td>
<td>82%</td>
</tr>
<tr>
<td></td>
<td>5.0 μM C + NP</td>
<td>*0.75$^BY$ ± 0.14</td>
<td>0.5 - 2</td>
<td>30%</td>
<td>63%</td>
<td>88%</td>
</tr>
<tr>
<td></td>
<td>1.0 μM C + NP</td>
<td>*0.23 ± 0.02</td>
<td>0.5 - 2</td>
<td>30%</td>
<td>42%</td>
<td>61%</td>
</tr>
<tr>
<td></td>
<td>0.1 μM C + NP</td>
<td>*0.35$^C$ ± 0.02</td>
<td>0.5 - 2</td>
<td>30%</td>
<td>44%</td>
<td>55%</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 μM C + NP</td>
<td>*0.87 ± 0.24</td>
<td>0 - 1.5</td>
<td>30%</td>
<td>73%</td>
<td>68%</td>
</tr>
<tr>
<td></td>
<td>5.0 μM C + NP</td>
<td>*0.80$^BZ$ ± 0.13</td>
<td>0 - 1.5</td>
<td>30%</td>
<td>63%</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>1.0 μM C + NP</td>
<td>*0.65 ± 0.27</td>
<td>0 - 1.5</td>
<td>30%</td>
<td>40%</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>0.1 μM C + NP</td>
<td>*0.36 ± 0.15</td>
<td>0 - 1.5</td>
<td>30%</td>
<td>35%</td>
<td>57%</td>
</tr>
<tr>
<td>Ambient</td>
<td>No Amendment</td>
<td>*0.27$^ABCD$ ± 0.03</td>
<td>0 - 4</td>
<td>30%</td>
<td>37%</td>
<td>45%</td>
</tr>
<tr>
<td>Ambient</td>
<td>NH$_4$ + PO$_4$ (NP)</td>
<td>*0.28$^XYZ$ ± 0.02</td>
<td>0 - 4</td>
<td>30%</td>
<td>37%</td>
<td>50%</td>
</tr>
</tbody>
</table>
**TABLE 3.3.** July 2003b prokaryotic taxonomic response to nutrient amendments. Relative percent composition of *Alteromonas macleodii* and SAR11 in response to 10 μM carbon as DFOB, 10 μM carbon as glucose, plus inorganic nutrients, 1.0 μM NH₄(N) and 0.1 μM PO₄(P). Percent composition is expressed as a fraction of total DAPI-stained cells.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>DFOB + NP <em>A. macleodii</em></th>
<th>DFOB + NP SAR11</th>
<th>Glucose + NP <em>A. macleodii</em></th>
<th>Glucose + NP SAR11</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2%</td>
<td>22%</td>
<td>2%</td>
<td>22%</td>
</tr>
<tr>
<td>0.9</td>
<td>13%</td>
<td>47%</td>
<td>53%</td>
<td>16%</td>
</tr>
<tr>
<td>1.9</td>
<td>24%</td>
<td>28%</td>
<td>62%</td>
<td>14%</td>
</tr>
<tr>
<td>2.9</td>
<td>16%</td>
<td>41%</td>
<td>47%</td>
<td>13%</td>
</tr>
</tbody>
</table>

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TABLE 3.4. July 2003b prokaryotic growth rate response to nutrient amendments. Specific growth rates, $\mu \, (\text{d}^{-1})$, for total prokaryotes, *Alteromonas macleodii*, and SAR11 in response to 10 μM carbon as glucose, 10 μM carbon as DFOB, plus inorganic nutrients, 1.0 μM NH$_4$ (N) and 0.1 μM PO$_4$ (P). ND denotes that the specific growth rate was not determined.

<table>
<thead>
<tr>
<th>Amendment</th>
<th>Glucose +NP</th>
<th>DFOB + NP</th>
<th>NP</th>
<th>No Amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Prokaryotes</td>
<td>1.2</td>
<td>1.4</td>
<td>0.58</td>
<td>0.30</td>
</tr>
<tr>
<td>A. macleodii</td>
<td>2.7</td>
<td>1.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SAR11</td>
<td>0.7</td>
<td>0.8</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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FIGURE 3.1. April 2001 prokaryotic biomass response to nutrient amendments. Dose-response of prokaryotes (cell abundance, cells L⁻¹) to DFOB plus 1.0 μM NH₄ (N), 0.1 μM PO₄ (P), and 10 μM glucose. Doses of DFOB were: 10 μM (filled triangle), 1.0 μM (open diamond), 0.1 μM (filled square). Equivalent organic carbon concentrations as DFOB are given in parenthesis. Controls were 1.0 μM N, 0.1 μM P, and 10 μM glucose (open triangle) and no amendment (filled circle).
FIGURE 3.2. July 2003a prokaryotic biomass response to nutrient amendments. 
(A) Dose-response of prokaryotes (cell abundance, cells L$^{-1}$) to DFOB plus 1.0 μM NH$_4$ (N) and 0.1 μM PO$_4$ (P). Controls were 1.0 μM N and 0.1 μM P (open triangle) and no amendment (filled circle). (B) Dose-response of prokaryotes (cell abundance, cells L$^{-1}$) to N, P, and glucose. Controls were the same as in (A). (C) Dose-response of prokaryotes (cell abundance, cells L$^{-1}$) to methanesulfonic acid (CH$_4$O$_3$S) plus 1.0 μM N and 0.1 μM P. Controls were the same as in (A). (D) Prokaryotic growth rate as a function of carbon dosage. The filled and open diamonds denote no amendment and N+P, respectively.
The graphical data shows the cell abundance over time for different treatments.

**A** shows the cell abundance for treatments with varying DFOB concentrations.

- **No Amendment**
- **NH₄⁺ - PO₄⁻ (NP)**
- **0.038 µM DFOB (0.1 µM C) + NP**
- **0.08 µM DFOB (1.0 µM C) + NP**
- **0.199 µM DFOB (5 µM C) + NP**
- **0.380 µM DFOB (10 µM C) + NP**

**B** shows the cell abundance for treatments with varying glucose concentrations.

- **No Amendment**
- **NH₄⁺ - PO₄⁻ (NP)**
- **0.0167 µM Glucose (0.1 µM C) + NP**
- **0.067 µM Glucose (1.0 µM C) + NP**
- **0.835 µM Glucose (5 µM C) + NP**
- **1.67 µM Glucose (10 µM C) + NP**

**C** shows the cell abundance for treatments with varying CH₃O₃S concentrations.

- **No Amendment**
- **NH₄⁺ - PO₄⁻ (NP)**
- **0.004 µM CH₃O₃S (0.004 µM C) + NP**
- **0.039 µM CH₃O₃S (0.039 µM C) + NP**
- **0.195 µM CH₃O₃S (0.195 µM C) + NP**
- **0.39 µM CH₃O₃S (0.39 µM C) + NP**

**D** shows the growth rate of cells over time.

- **DFOB**
- **Glucose**
- **No Amendment**
- **N+P**

Time (days) is plotted on the x-axis, and cell abundance (cells L⁻¹) is plotted on the y-axis.
FIGURE 3.3. Percent high DNA-containing (%HDNA) cells in July 2003a. (A) Dose-response of prokaryotes (cell abundance, cells L⁻¹) to DFOB plus 1.0 μM NH₄ (N) and 0.1 μM PO₄ (P). (B) Dose-response of prokaryotes (cell abundance, cells L⁻¹) to glucose, N, and P. Controls were the same as in (A).
FIGURE 3.4. July 2003b prokaryotic biomass and taxonomic response to nutrient amendments. (A) Nutrient amendments were 1.0 μM NH₄ (N), 0.1 μM PO₄ (P), and 10 μM carbon as glucose. Prokaryotic abundance is denoted as total cell abundance (filled circle, solid line), *A. macleodii* abundance (open circle, dotted line), and SAR11 abundance (filled circle, dashed line). (B) Nutrient amendments were 1.0 μM N, 0.1 μM P, and 10 μM carbon as DFOB. Prokaryotic abundance is denoted as total cell abundance (filled square, solid line), *A. macleodii* abundance (open square, dotted line), and SAR11 abundance (filled square, dashed line).
Cell Abundance (cells L\(^{-1}\))

A. 10 μM Glucose

- Total Cell Abundance
- *A. macleodii*
- SAR 11

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SUMMARY AND CONCLUSIONS

The objective of this dissertation was to study how iron and carbon govern prokaryotic growth in the ocean. The three studies presented herein describe the prokaryotic response to iron and organic carbon at varying temporal, spatial, and taxonomic scales. In addition to examining the proximal responses to iron and carbon, the three studies attempt to describe the relative importance of the prokaryotic response in the broader context of ocean biogeochemistry.

The first study encompassed the bulk prokaryotic response to iron enrichment in an *in situ* open-ocean iron fertilization experiment in the Southern Ocean (SOFeX, Coale et al. 2004). The overall goal of SOFeX was to observe the phytoplankton and subsequent geochemical response to iron fertilization. The experiment took place in two distinct nutrient regimes in the Southern Ocean: (1) a low-chlorophyll, high nitrate, low silica regime (North Patch), and (2) a low-chlorophyll, high nitrate, high silica regime (South Patch). Phytoplankton blooms ensued in both study areas with substantial organic carbon production and inorganic nutrient removal. Prokaryotes, as a whole community, also responded positively to iron enrichment in both study areas; however, it was an indirect response to organic carbon production by phytoplankton. Prokaryotic abundance, carbon production, and growth rate increased as a result of iron enrichment relative to non-fertilized waters. While the general response was the same in both study areas, there were differences in the absolute magnitude of each response relative to geochemical references. In the North Patch, prokaryotic carbon comprised 21-26% of particulate organic carbon (POC) and 25-30% of particulate nitrogen (PN) compared to 13-15% of POC and 18-22% in non-fertilized waters. In contrast, prokaryotes comprised a smaller proportion of POC and PN in the South Patch, 4-7% and 4-5%, respectively, relative to non-fertilized waters.
Also, in the South Patch prokaryotic production was found to be closely associated with particulate primary production. Over the course of the study in the South Patch (22 days), however, prokaryotic production comprised only a small fraction of particulate primary production (1%). Overall, this study supported the previous findings in similar iron enrichment experiments. There were subtle differences in the dynamics of the prokaryotic response compared to other iron enrichment experiments (Cochlan 2001, Hall and Safi 2001, Arrieta et al. 2004), but the overall response was the same: prokaryotes grew in response to inputs of iron (indirectly) and new organic material. The dynamics of prokaryotes in the declining stages of both iron-induced phytoplankton blooms was not thoroughly characterized and should become a focus of future iron enrichment studies.

The second study was also part of SOFeX, but attempted to resolve the prokaryotic response at higher taxonomic resolution. The dynamics of specific groups of prokaryotes thought to be significant in ocean biogeochemistry (Cytophaga-Flavobacteria and SAR11) were examined during both iron-induced phytoplankton blooms. In addition, prokaryotic community structure and bacterial diversity were resolved at the domain and clade levels using culture-independent molecular methodologies and ordination analyses. Results showed that two taxonomic groups of bacterioplankton thought to be biogeochemically significant in the oceans, Cytophaga-Flavobacteria and SAR11, were indeed detected in both study areas. At times the two groups comprised the majority (>50%) of the prokaryotic population in both study areas as well as in non-fertilized waters. Their populations also increased over time indicating a positive response to iron enrichment, with Cytophaga-Flavobacteria exhibiting a higher net growth rate compared to SAR11. Analysis of prokaryotic community structure showed that the communities in both study areas were uniquely different for iron-fertilized and non-fertilized waters,
respectively. Additionally, the community structure shifted and diversity increased over time in the South Patch. The communities within the South Patch were also distinct from the communities in non-fertilized waters. Taken together, the data demonstrate that the bulk prokaryotic response characterized in the first study is comprised of more complex responses by specific taxa of prokaryotes. It further implies that a more diverse prokaryotic community is involved in the biogeochemical cycling of phytoplankton blooms in the Southern Ocean. The complete composition of the prokaryotic community, including Archaea, has not been fully characterized in the context of iron fertilization experiments and warrant further study. Ultimately, future studies should elucidate the underlying composition and physiologies of various prokaryotic taxa in the biogeochemical cycling of natural and iron-stimulated phytoplankton blooms.

The third study was designed to characterize the response of prokaryotes to inputs of organic carbon in the presence of iron and under iron limitation. Rather than stimulate prokaryotic growth by enriching with iron as in the first and second studies, iron was removed using a chelating agent, the siderophore desferrioxamine B (DFOB). The experimental design of the study employed seawater cultures taken from the summer surface environment in the Sargasso Sea, an inorganic nutrient-limited, low labile organic environment. This provided an ideal context to test the effects of individual and combinations of nutrients, both inorganic and organic, on prokaryotic growth in a controlled setting. Results showed that DFOB failed to limit prokaryotic growth in the presence of inorganic nutrients and a labile carbon source (glucose). Rather than constraining growth as originally hypothesized, prokaryotic abundance increased over time in response to the siderophore. This result was similar to the prokaryotic response to glucose and inorganic nutrients. Redesigned experiments tested the hypothesis that the
siderophore served as a carbon source to prokaryotes. DFOB and glucose were added at varying carbon concentrations in separate seawater cultures. Results showed that prokaryotes responded to both DFOB and glucose in a dose-dependent manner. Furthermore, the taxonomic response differed as a function of the carbon source. SAR11, previously shown in the second study to grow in response to iron enrichment in situ, grew over time in response to DFOB. Alternatively, *Alteromonas macleodii*, a species found in low abundances in situ in the Sargasso Sea, dominated the prokaryotic community in response to glucose. The results from this study demonstrate that siderophores are probable carbon sources for prokaryotes, particularly in an organic carbon-limited environment, and that the type of organic carbon available elicits a different taxonomic response by prokaryotes. The broader implication of this study is that ocean carbon cycling and the ecology of prokaryotes in the ocean are intimately linked to modulation of iron chemistry by siderophores. Future studies should focus on the molecular characterization of siderophore metabolism, which taxa possess this capability as well as their ecology, and the significance of this physiology to iron and carbon cycling.

Prior to SOFeX, four open-ocean iron enrichment experiments were performed. Since the completion of SOFeX two more open-ocean iron fertilization experiments have been conducted (Table 4.1). Each experiment provided new insights into the role of iron in controlling phytoplankton growth in high nutrient, low chlorophyll oceanic regimes. Specifically, the experiments were instrumental in refining the iron hypothesis first speculated by Gran and Hart, then pursued by the late John Martin. Today the iron hypothesis embodies a balance between iron supply, light fields, silica supply, grazing pressure, and the physical environment. The experiments also provided an opportunity to study fundamental aspects of ocean food web dynamics, at varying taxonomic and trophic scales, in response to iron
enrichment. Included in these appended studies were prokaryotes and their role in modulating carbon cycle dynamics. The first study represents the fifth investigation into prokaryotic dynamics in an open-ocean iron fertilization experiment and third for the Southern Ocean (Table 4.1). However, the second study is only the second in which prokaryotic community structure and diversity were investigated (Arrieta et al. 2004). In the context of the iron hypothesis the sum of the evidence from prokaryotic studies show an active role for prokaryotes in the cycling of carbon and inorganic nutrients (including iron). While it remains speculative, prokaryotic remineralization might be significant to the extent that phytoplankton blooms are partially sustained by a combination of regenerated nutrients. The study (Boyd et al. 2004) was the clearest demonstration to date of the biogeochemical significance of prokaryotes in phytoplankton blooms in which substantial amounts of newly produced POC were quickly remineralized. In addition, the magnified presence of prokaryotes during iron-induced phytoplankton blooms inevitably stimulates carbon and nutrient flow through the microbial food web; however, the extent to which prokaryotic biomass supports higher trophic level production is not well understood.

Perhaps the most provocative role prokaryotes may play in iron-induced phytoplankton blooms is as a competitor for iron, thus constraining phytoplankton blooms from reaching their full potential. Such a hypothesis runs counter to the hypothesis that prokaryotes sustain phytoplankton blooms through remineralization of nutrients. The time scales upon which phytoplankton and prokaryotes compete for iron is not clear. However, the circumstances under which prokaryotes are more effective in acquiring iron, i.e. low iron concentrations, implies that competition between the two groups of plankton increases in the latter stages of iron-induced phytoplankton blooms. Siderophore expression is a common response by prokaryotes to low
iron availability. Theoretically, this physiological response by prokaryotes could negatively affect phytoplankton growth assuming phytoplankton do not possess similar iron acquisition mechanisms. The suggestion that prokaryotes might directly modulate the fate of a phytoplankton bloom suggests a complex trophic interaction that would be superimposed on an already complex iron cycle in the oceans (Figure 4.1). Iron availability is tightly controlled by a combination of chemical, physical, and biological interactions. The conceptual pathways in the ocean iron cycle are well-defined, however, the relative importance of those pathways in fueling and limiting phytoplankton production are not well understood. Future studies in this vein should examine the relative capacity for prokaryotes to act as a biological mechanism underlying the decline and fate of phytoplankton blooms in the ocean.
LITERATURE CITED


TABLE 4.1. Mesoscale iron enrichment experiments conducted in the open sea. Table adapted from Ducklow et al. 2004.

<table>
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<tr>
<th>Study</th>
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<td>SOFeX</td>
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1Martin et al. (1994).
2Coale et al. (1996).
3Boyd et al. (2000).
4Smetacek et al. (2001).
5Tsuda et al. (2003).
6Coale et al. (2004).
7Boyd et al. (2004).
8http://www.niwa.co.nz/rc/atmos/sage

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APPENDIX 1.1. South Patch mixed-layer depths (MLD), sample sizes, and sources for prokaryotic (abundance, TdR, Leu) and primary production (total and size fractionated). MLD was determined using a criterion of a change of 0.02 from surface $\sigma_t$ for 1-m binned density measurements (U.S. JGOFS, http://usjgofs.whoi.edu/jg/dir/jgofs/, nbp97_8, W. Smith, Ross Sea Mixed Layer Depths). The number of discrete data points used in MLD-averaged calculations and the depth from which samples were obtained are given. Asterisks denote instances where full water column casts samples were not available and samples were averaged in lieu of MLD-averaging.

<table>
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<th>Day</th>
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