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Limitation of Bacterial Growth by Dissolved Organic Matter and Iron in the Southern Ocean

Matthew J. Church

College of William and Mary - Virginia Institute of Marine Science

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Limitation of Bacterial Growth by Dissolved Organic Matter and Iron in the Southern Ocean

A Thesis
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
Master of Science


by
Matthew J. Church
1999

Approval Sheet

This thesis is submitted in partial fulfillment of

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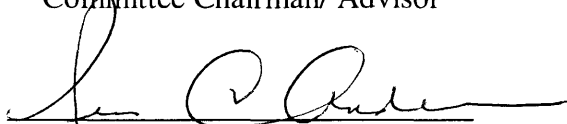
Master of Science


Matthew J. Church

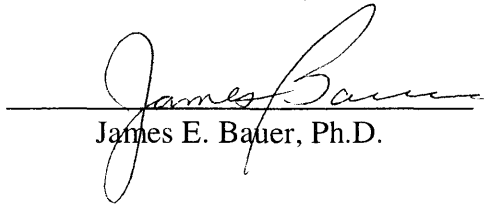
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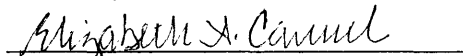
Hugh W. Ducklow, Ph.D.
Committee Chairman/ Advisor

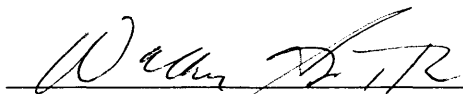


Iris C. Anderson, Ph.D.



James E. Bauer, Ph.D.


Elizabeth A. Canuel, Ph.D.


Walker O. Smith, Jr., Ph.D.

*To my parents, Nancy and Larry Church, who taught me that the answers can
often be found between the questions.*

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Abstract

The importance of resource limitation to controlling bacterial growth in high nutrient-low chlorophyll (HNLC) regions of the Southern Ocean was experimentally determined. Organic and inorganic nutrient enrichment experiments were performed along 141°E between 42°S and 55°S. Bacterial abundance, mean cell volume, and ³H-thymidine and leucine incorporation rates were measured throughout the course of 4-5 day incubations. Bacterial biomass, production and growth rates were calculated based on changes in cell abundance, cell volume and rates of incorporation. Differences between treatments were statistically differentiated and used to make general conclusions about possible limitation to bacterial growth in HNLC oceans.

Bacterial biomass, production and rates of growth all responded to organic enrichments in three of the four experiments. These results indicate that bacterial growth was primarily constrained by the availability of dissolved organic matter. Bacterial growth in the Subtropical Convergence, Subantarctic Zone and Subantarctic Front responded most favorably to additions of dissolved free amino acids or glucose + ammonium. Bacterial growth in these regions appears limited by input of both organic matter and reduced nitrogen. Bacterial growth was relatively unchanged by additions of iron alone; however, additions of glucose + iron resulted in substantial increases in rates of bacterial growth and biomass accumulation relative to a glucose-alone treatments. These results imply that bacterial growth efficiency may be partly constrained by iron availability in the HNLC Southern Ocean. A temperature manipulation experiment in the Antarctic Convergence revealed an interaction between dissolved organic matter and temperature in this region of the Southern Ocean.

**Limitation of Bacterial Growth by Dissolved Organic Matter and
Iron in the Southern Ocean**

Introduction

Bacterial Growth Limitation

Determining the factors that regulate growth of heterotrophic bacteria in marine ecosystems is ecologically and biogeochemically important. Bacterioplankton are often the most numerous members of the planktonic food web and their role in nutrient and energy cycling is crucial to the organization of marine ecosystems (Azam and Hodson 1977, Hobbie et al. 1977, Fuhrman and Azam 1980, 1982, Fuhrman et al. 1989, Cho and Azam 1990). Defining the factors that regulate rates of bacterial biomass production and control biomass accumulation provides insights into the dynamics of microbial food webs. Furthermore, identification of the factors that limit the amount of bacterial biomass produced in an ecosystem aids understanding on how energy and material flow within the system.

Efforts to determine the constraints on rates and stocks of bacterioplankton in different ecosystems suggest that growth limitation is not consistent across marine environments. Factors that potentially limit bacterial growth include quality of dissolved organic matter (DOM) (Kirchman 1990, Carlson and Ducklow 1996, Cherrier et al. 1996), inorganic nutrients (Rivkin and Anderson, 1997, Thingstad et al. 1997), temperature (Pomeroy and Deibel 1986, Shiah and Ducklow 1994), viral infection (Proctor and Fuhrman 1990) and grazing (Wright and Coffin 1984, Ducklow and Hill 1985). The importance of each of these factors must be assessed with respect to temporal and spatial scales upon which these potential limitations act (Ducklow 1992).

Bacterial growth and production of biomass can be tightly controlled by resource limitation in some ecosystems. Trophic levels that are controlled principally by resources (food supply) are termed “bottom-up” limited, in contrast to “top-down” limitation where predation controls growth (McQueen et al. 1986, Weisse 1991). In systems where resources limit bacterial biomass production and growth rates, positive relationships between the rate of substrate supply and the rate of bacterial production and growth may be observed (Cole et al. 1988, Billen et al. 1990). Alternatively, bacterial growth can be more strictly determined by the *quality* of the available resources, rather than by the *rate* at which organic matter is added to the system (Carlson and Ducklow 1996, Vallino et al. 1996).

The seasonal accumulation of dissolved organic material (DOM) in the euphotic zone of various marine environments indicates bacterial utilization of DOM is limited by some factor other than the rate that substrate is added to these systems (Carlson et al. 1994, Copin-Montegut and Avril 1993, Williams 1995). Dissolved organic matter constitutes a potentially large, exportable pool of reduced carbon and quantification of DOM fluxes are essential to balancing global carbon budgets (Carlson et al. 1994). Bacteria are the principal consumers of DOM, and the persistence of DOM in marine ecosystems suggests that DOM production and bacterial consumption processes are uncoupled in space and time. In such systems, the rate of organic matter input may not strictly define the rates or stocks of bacterial biomass production.

Experimental manipulations may be used to test the specific processes that control bacterial growth. Such manipulations are often a useful means of

understanding how bacteria respond to the presence or absence of growth limiting factors. Enrichment experiments are one way to examine the nature of DOM limitation on bacterial growth in marine ecosystems. In these experiments model DOM substrates are added to natural seawater batch cultures and bacterial growth properties (cell abundance, cell volume, and production) are measured over time. Williams (submitted) synthesized the results of a number of “dosing” experiments conducted by a variety of investigators in different marine environments and concluded that bacterial growth in the open ocean is frequently limited by input of labile DOM.

Bacterial growth requires carbon, nitrogen and phosphorus for synthesis of macromolecules such as proteins and nucleic acids. Studies from both open ocean and coastal environments suggest that most marine bacterial nitrogen requirements can be met by dissolved free amino acids (DFAA) or ammonium (NH_4^+) (Kirchman 1994, Keil and Kirchman 1991, Keil and Kirchman 1993). The specific carbon sources that fulfill oceanic bacterial carbon demands are not as well defined, but small monosaccharides like glucose appear to support a large fraction of bacterial carbon requirements (Rich et al. 1997). Measurements of primary amines and monosaccharides in the open ocean suggest that these organic substrates play an important biochemical role in DOM cycling in the oceans (Rich et al. 1997, Benner et al. 1992, Amon and Benner 1996).

Substrate Quality

Several investigations have evaluated how substrate quality affects bacterial growth efficiency and bacterial growth rates in marine systems. Bacterial growth efficiency (BGE) is a measure of the amount of bacterial biomass produced relative to

the amount of substrate utilized for total cellular metabolism. BGE depends in part on the quality of substrates that sustain bacterial growth (Goldman et al. 1987, Goldman and Dennett 1991, Kirchman 1990, Moran and Hodson 1990, del Giorgio and Cole 1998). Organic matter quality is generally defined by the elemental ratio (C: N: P) of the food or energy source. It may also be operationally defined on a temporal scale, where “high quality” (labile) organic material is processed by heterotrophic bacteria on time scales of minutes to days, while moderate or poor quality substrates (semi-refractory or refractory) may take months to years to degrade (Carlson and Duklow 1996, Cherrier et al. 1996).

Goldman et al. (1987) and Goldman and Dennett (1991) estimated bacterial growth rates and growth efficiencies in laboratory bacterial cultures on various sources of carbon and nitrogen. They found that bacterial growth rates and efficiencies were more strongly related to the stoichiometric C : N ratio of the substrate than to specific organic substrates. Bacteria grew just as well on glucose + NH_4^+ as on DFAA when the C: N ratios of the substrates were similar. Bacterial nitrogen and phosphorus requirements are large because they have high cellular nucleic acid and protein contents that require them to sustain low intracellular C: N: P ratios (Goldman et al. 1987, Goldman and Dennett 1991). Kirchman (1990) found bacterial growth rates in the subarctic Pacific were considerably larger in DFAA treatments relative to glucose + NH_4^+ treatments when both treatments were added at similar C: N molar ratios. He argued that bacterial preference for DFAA in the subarctic Pacific indicated that direct assimilation of DFAA is energetically more advantageous than construction of amino acids from simple carbon, nitrogen and phosphorus building blocks (Kirchman 1990).

Bacterial growth on organic nitrogen may be more energetically favorable than growth on simple sugars such as glucose. Although glucose catabolism provides a larger energy yield relative to nitrogen rich substrates like DFAA (as estimated by free energy changes), utilization of DFAA provides the bacterial cell with carbon, nitrogen and energy. Nitrogen rich substrates such as DFAA can be directly assimilated or catabolized depending on the net energy yield from each process. Direct assimilation of amino acids provides an energy saving step towards protein synthesis (Payne and Wiebe 1978, Goldman and Dennett 1991). Amino acid assimilation requires that the total energy expenditure from transporting the amino acid across the cell membrane is less than the energy required synthesizing the amino acid from individual precursor elements (Payne and Wiebe 1978).

Glucose may provide bacteria with an energy-rich substrate to fuel growth processes. Glucose catabolism generates energy by dissimilatory oxidation reactions which yield ATP and reaction by-products. The energy gained from glucose catabolism must be transferred to sites within the cell where cellular synthesis occurs. Additionally, bacterial growth on glucose requires acquisition of mineral nutrients before construction of nucleic acids and proteins may occur. Bacterial growth on glucose may be relatively inefficient because acquisition and transport of mineral nutrients can consume a large fraction of the cell's energy (Gottschalk 1979, Fenchel and Blackburn 1979).

Kirchman (1990) and Cherrier et al. (1996) hypothesized the bacterial preference for amino acids relative to glucose + NH_4^+ in the subarctic and northeast Pacific resulted because the bacteria were constrained by *energy* limitation. They

argued that the specific types of substrates utilized for bacterial growth dictated growth rates and growth efficiencies (Kirchman 1990, Cherrier et al. 1996). Bacterial preference for DFAA over glucose + NH_4^+ in the subarctic Pacific indicated that DFAA substrates provided the necessary constituents the cells required for growth. Bacterial growth on DFAA may sustain higher rates of bacterial growth because DFAA catabolism or assimilation releases cells from multiple growth limiting factors including carbon, nitrogen and energy (del Giorgio and Cole 1998).

Carlson and Ducklow (1996) performed a series of organic amendment experiments in the Sargasso Sea and assessed the bacterial responses to different types of organic and inorganic treatments. They observed significant increases in bacterial growth rates and growth efficiencies by additions of glucose, glucose + NH_4^+ and DFAA. Additions of glucose and glucose + NH_4^+ resulted in greater increases in growth rates and biomass production than DFAA treatments, indicating that bacterial growth in the Sargasso Sea was controlled by input of labile organic carbon. In contrast, Rivkin and Anderson (1997) found that glucose additions in the Sargasso Sea resulted in only slight increases in bacterial growth rates. They found that additions of PO_4^{3-} or glucose + PO_4^{3-} + NH_4^+ resulted in large increases in bacterial growth rates, leading them to hypothesize that PO_4^{3-} availability limited bacterial utilization of DOC in some ecosystems (Rivkin and Anderson 1997).

Nutrient Limitation of Bacterial Growth

Although bacterial growth in the open ocean may often be controlled by DOM quality, inorganic nutrient limitation could lead to the inability of bacterioplankton to fully utilize DOM in some ecosystems. Several studies indicate that bacterial growth rates are stimulated by additions of inorganic nutrients (Zweifel et al. 1993, Pomeroy

et al. 1995, Rivkin and Anderson 1997). Bacteria have been shown to be effective competitors with phytoplankton for ammonium and phosphate (Kirchman 1994, Currie and Klaff 1984) and in some systems bacterial uptake of mineral nutrients dominates nutrient fluxes (Wheeler and Kirchman 1986, Cotner and Wetzel 1992). Inorganic nutrient control over bacterial growth could have profound influence on fluxes of organic materials through ocean systems, particularly carbon fluxes into and out of the microbial loop (Thingstad et al. 1997). Nutrient limitation may block complete utilization of labile or semi-labile DOM, resulting in accumulation and eventual export of DOM from the surface oceans (Thingstad and Rassoulzadegan 1995, Thingstad et al. 1997).

Trace nutrients may also control bacterial growth rates. Large areas of the world's oceans are characterized as having subnanomolar concentrations of dissolved iron. Such low concentrations of dissolved iron can limit phytoplankton growth. Bacteria are widely regarded as more efficient competitors for limiting nutrients than phytoplankton because of their high abundance and high surface to volume ratios. However, the few investigations into bacterial growth in iron deplete oceans indicate that iron may restrict bacterial growth in HNLC oceans (Pakulski et al. 1996, Tortell et al. 1996). Pakulski et al. (1996) found that additions of iron to bacterial batch cultures in the Southern Ocean enhanced biomass production rates. Tortell et al. (1996) measured bacterial cellular Fe concentrations and found them to be relatively high compared to phytoplankton. Iron is an essential component of cytochrome c in the respiratory system of heterotrophic bacteria. Iron deficient cells may have considerably lower growth efficiencies if their respiratory system operates at sub-

maximal capacity (Tortell et al. 1996). Hutchins et al. (1998) observed increases in bacterial growth rates in response to an iron enriched phytoplankton bloom. They suggest that the bacterial response may have been due to input of DOM from the iron induced phytoplankton bloom rather than to iron directly. Their observations point to the importance of considering the effect of a resource limitation across entire food webs. A cascade of limitations may result from iron deficiencies at the primary producer level, including low fluxes of organic material for sustaining bacterial growth (Hutchins et al. 1998).

Temperature Constraints on Bacterial Growth

An additional consideration when evaluating possible limitation to bacterial growth is the role of temperature. In some ecosystems temperature can be an important limitation to bacterial growth rates (Kirchman et al. 1993, Wiebe et al. 1993, Shiah and Ducklow 1994, Kirchman and Rich 1997). Bacterial cells exhibit a temperature range where optimal growth occurs. Outside this temperature range, growth proceeds sub-optimally or not at all. Some of the cellular processes exhibiting temperature dependence include the active transport system, cytoplasm structural conformation, and protein synthesis pathways (Farrell and Rose 1967). Temperature induced changes in any of these processes could influence bacterial growth rates.

Pomeroy et al. (1991) and Wiebe et al. (1993) demonstrated that bacterial growth at cold temperatures required higher substrate concentrations. They observed that lower temperatures required longer bacterial response times to DOM input. Kirchman and Rich (1997) supported these observations finding that temperature and DOM interacted to control bacterial growth rates in the equatorial Pacific. They found bacteria responded more slowly to input of labile DOM at lower temperatures.

Additionally, incubations performed at *in situ* temperatures in the equatorial Pacific resulted in lower biomass yields than incubations at warmer temperatures (Kirchman and Rich 1997). They hypothesized that lower temperatures depressed bacterial affinity for DOM requiring higher labile substrate concentrations to sustain bacterial production in cold waters.

Bacterial Growth in HNLC Oceans

The motivation behind my study was to decipher the limitations to bacterial growth in the pelagic Southern Ocean. The pelagic regions of the Southern Ocean have been characterized as persistently HNLC waters. The equatorial Pacific, the subarctic Pacific and the Southern Ocean are the major HNLC regions of the world oceans. While many world's oceans undergo seasonal or continuous depletion of major nutrients, the defining characteristic of an HNLC oceans is the annual persistence of high concentrations of NO_3^- , PO_4^{3-} and often SiO_4 in surface waters (Banse 1996). Many hypotheses have been proposed to explain the existence of HNLC regions, but all of them have one common principle: some factor or combination of factors (iron, light, temperature, and/or grazing) limit the accumulation of phytoplankton biomass and restrict carbon export and new production (Walsh 1976, Martin and Fitzwater 1988, and see Limnology and Oceanography volume 36, 1991 especially reviews by Cullen, Miller et al., and Frost).

In an effort to synthesize various studies of food web dynamics in HNLC oceans, Landry et al. (1997) developed a conceptual model directed at evaluating possible constraints on phytoplankton growth in the equatorial Pacific. They concluded that both iron and grazing are complementary mechanisms that control rates of growth and biomass in HNLC systems. Studies from both the equatorial Pacific

and the subarctic Pacific indicate that phytoplankton cell division rates are relatively high while phytoplankton biomass is noticeably low. Landry et al. (1997) concluded that the importance of grazing to these systems may be more than just control of standing phytoplankton biomass. Grazing contributes to regenerated production by recycling nutrients (including iron) that support phytoplankton growth. The view that food webs in HNLC oceans might be constrained by either top-down or bottom-up pressures is probably too simplistic. An array of both types of control likely limits primary production, trickling up and down the food web to control the flux of energy through the microbial loop.

An examination of the factors that limit phytoplankton biomass and therefore rates of primary production in HNLC oceans will likely yield information on what limits other components of the food web. Primary producers are the principal source of fresh, labile DOM to marine systems. Intense grazing pressure observed in these systems could result in larger fluxes of DOM to the microbial loop through release of dissolved organic matter by sloppy feeding (Ducklow et al. 1995). Studies in the equatorial and subarctic Pacific indicate that the processes of production and consumption of DOM may be tightly coupled (Kirchman et al. 1993, Carlson and Ducklow 1995, Ducklow et al. 1995, Kirchman et al. 1995). Surface waters in the equatorial Pacific and the subarctic Pacific were hypothesized to have an active and efficient microbial loop resulting in a retentive euphotic zone that exported little DOM (Carlson and Ducklow 1995, Ducklow et al. 1995, Kirchman et al. 1995).

In support of these observations, several investigations found that DOM quality controlled bacterial growth and biomass production rates in the equatorial and

subarctic Pacific (Kirchman 1990, Kirchman and Rich 1997). Despite increases in growth rates, these DOM “dosing” experiments resulted in no significant increases in bacterial cell abundance. Bacterial growth rates were a function of DOM additions but grazing pressure apparently restricted bacterial biomass accumulation (Kirchman 1990, Kirchman et al. 1993, Kirchman and Rich 1997).

Despite tight couplings in DOM-bacterial interactions in HNLC oceans, the magnitude of fluxes of organic matter through bacteria in these systems may be more restricted than other ocean systems. Low phytoplankton biomass in HNLC oceans might translate to restricted input of DOM to the microbial community. Studies in both the equatorial and the subarctic Pacific indicated that the ratio of bacterial production: primary production may be considerably lower than other oceanic systems (<0.2) (Ducklow and Carlson 1992, Ducklow et al. 1995, Kirchman et al. 1995, Kirchman et al. 1993). All of these factors make HNLC oceans particularly interesting systems to examine how DOM limitation affects patterns of utilization by bacterioplankton.

The purpose of the present study was to determine whether resources (bottom-up controls) limited bacterial growth in the pelagic Southern Ocean. Specifically, I sought to test whether additions of labile organic material (glucose and amino acids) or inorganic nutrients (ammonium, phosphate, and dissolved iron) would stimulate rates of bacterial growth. By assessing how rates of bacterial production change relative to changes in standing biomass, I address the relative importance of dissolved organic matter and iron in controlling rates of bacterial growth in the Southern Ocean.

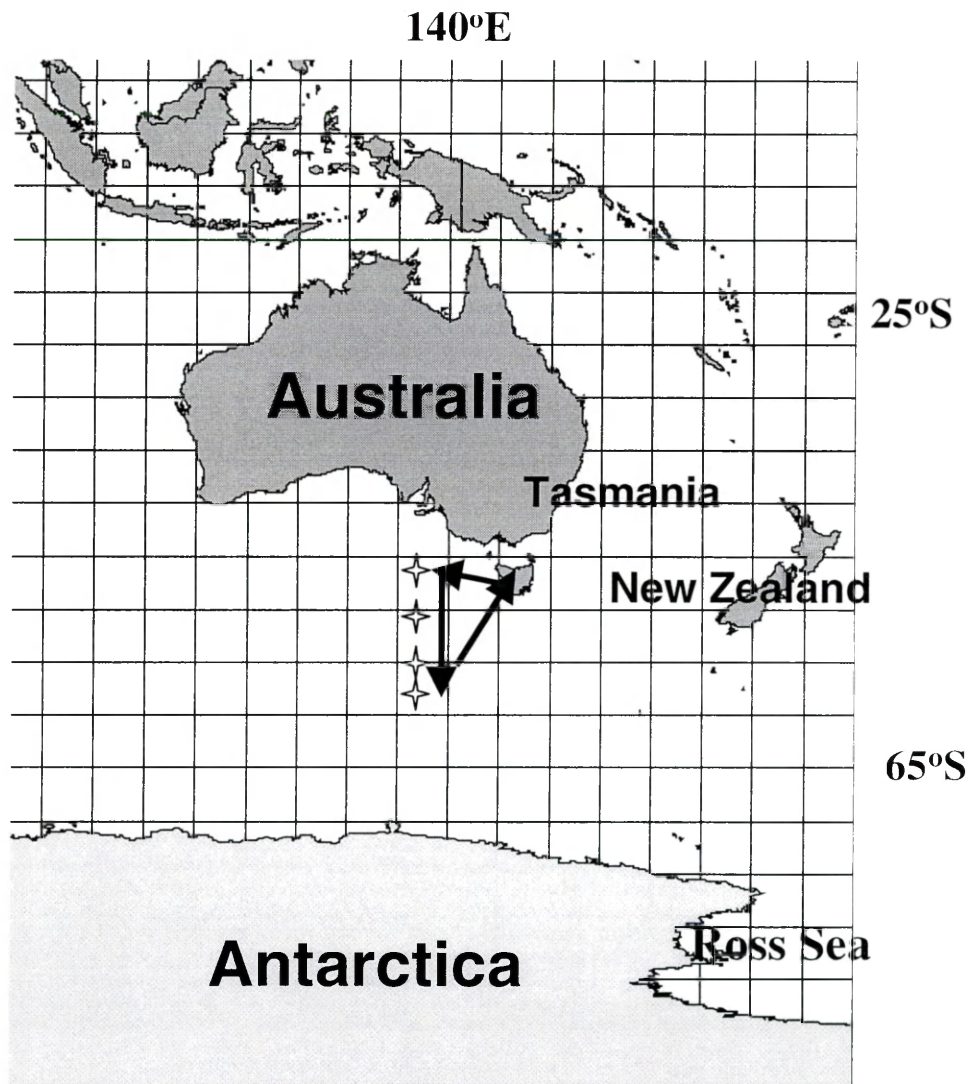
Materials and Methods

Study Site

Sampling for these experiments took place aboard the Australian vessel *R/V Aurora Australis* between February 28-April 3, 1998. The cruise followed a southerly transect along 141°E between 42°S and 55°S (**Fig. 1**). The cruise track intersected several water-mass frontal systems including the Subtropical Convergence (STC) (42°S), the Subantarctic Polar Front (SPF) (51°S) and the Antarctic Polar Front (APF) (54°S). The Southern Ocean is a spatially heterogeneous environment partly as a result of these frontal systems. Experiments were performed in each of these fronts and at one location inside the Subantarctic Zone (SAZ) at 47°S. These convergent zones export large volumes of the oceans surface water, making them particularly important to the biogeochemistry of the region. Although variable in their location, each frontal system has a characteristic chemical and physical hydrographic signature (Belkin and Gordon 1996, Rintoul et al. 1997).

The STC marks the zone of convergence where the Southern Hemisphere's anti-cyclonic subtropical gyres intersect with the Antarctic Circumpolar Current (ACC). The convergent zone identifies the northern extent of the Southern Ocean's Pacific basin and it is operationally defined by the latitudinal position of the 11° C isotherm at a depth of 150 m (Rintoul et al. 1997). Although the boundary shifts seasonally and displays spatial variability, it is generally located between 44° S and 47° S (**Fig. 2**; Belkin and Gordon 1996, Rintoul et al. 1997). Immediately north of this front is the Subtropical Zone (STZ) and to the south lies the Subantarctic Zone (SAZ). Typically, water masses to the north and south of the STC are vertically structured through the summer and deeply mixed throughout the winter. Mean annual

Figure 1. Study site and cruise track of R/V *Aurora Australis* Voyage 6, February 28- April 3, 1998. Stars represent experiment stations (42°S, 47°S, 51°S, 54°S).



sea surface temperatures just north of the STC are typically near 15° C, while surface water temperatures just south of the STC (in the ACC) drop to near 11°C. Nutrients also vary across the front. Nitrate concentrations are generally <0.5 μM to the north of the STC, increasing to 8-10 μM in the subantarctic water south of the front (Longhurst 1998). Dissolved iron decreases across the STC, remaining <1.0 nM throughout the SAZ and APF (Sedwick et al. 1997).

South of the STC (within the SAZ) lies the northern edge of the ACC. The ACC has the largest volume flux of any major world ocean current (180 Sv) (Pond and Pickard 1978). Driven primarily by circumpolar westerly winds, the current circulates unrestricted by landmasses from west to east. The southern boundary of the SAZ is marked by the SAF. The SAF is technically defined by the region of temperature transition from 3-8° C at 300 m depth (Rintoul et al. 1997). Convergence at this frontal interface forms Antarctic Mode Waters. These relatively light water masses form through winter convective cooling of subtropical waters. Mode waters flow north from the SAF, below subtropical surface waters and above Antarctic intermediate waters. Waters within the SAF undergo strong seasonality with vertical stratification in the summer months due to solar warming followed by deep convective mixing in early autumn. Despite the seasonal variability of the water column, chemically and biologically the water column remains remarkably consistent.. Surface nitrate concentrations range between 10-15 μM, while chlorophyll remains low, averaging 0.25 mg chl m⁻³ (Longhurst 1995).

The APF separates the warmer SAZ water from colder polar water. The front is defined as the northernmost extent of the 2° C isotherm (Rintoul et al. 1997, Park

and Gamberoni 1997). Sea surface temperature south of this isotherm rapidly drops to temperatures characteristic of the cold Antarctic waters, while north of the front temperatures rise towards those of the subtropical gyres. The polar front is a site of convergence where southward flowing subtropical waters force subduction of cold Antarctic surface water. This is the process that forms the Antarctic Intermediate Waters (**Fig. 2**). The northern edge of the polar front meanders between 50° and 52° S, with surface temperatures ranging between 2° and 4° C. Nitrate increases from ~15 to near 30 μM poleward of the APF. Also noteworthy is the rise in surface water silicate concentrations south of the APF (Longhurst 1995).

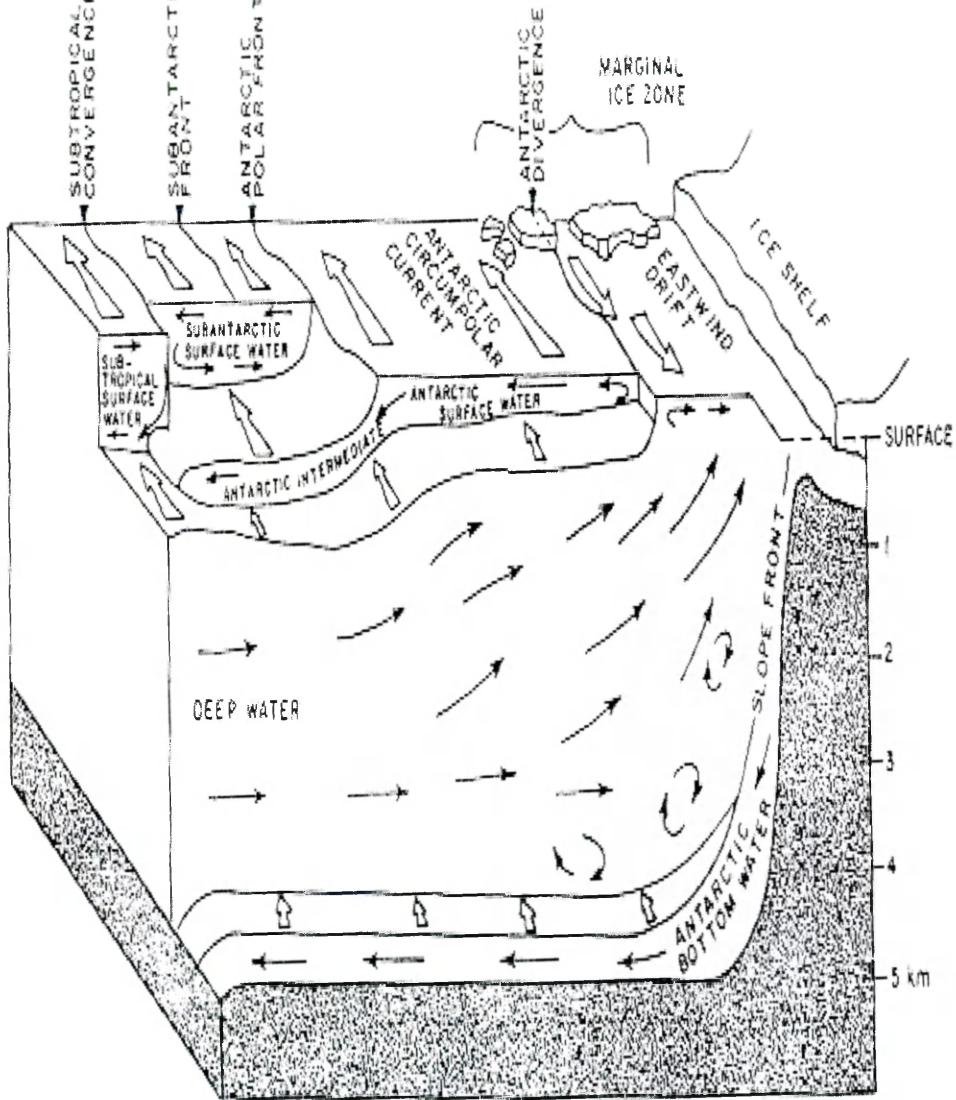
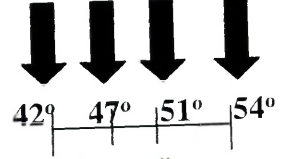
Experimental Design and Sample Collection

The overall design of these experiments was to add various amendments (both organic and inorganic) to whole seawater, incubate the water at in situ temperatures and monitor changes in bacterial growth, abundance, and biomass over a 4-5 day incubation period. All samples were incubated in the dark to separate heterotrophic and autotrophic processes. The decision to use unfiltered rather than size fractionated seawater (ie. grazer reduced treatments) was made to minimize risks of potential contamination of samples with metals or dissolved organic material as an artifact of filtration (Carlson and Ducklow 1996).

Water for the experiments was collected before sunrise and supplemented with amendments then immediately placed in darkened incubators for the duration of the experiment. Water for all experiments was collected from between 15 and 20 m depth with an all-Teflon trace-metal clean pump system (Hutchins et al., 1998). Whole seawater was pumped directly into a trace-metal clean incubation van, where

Figure 2. Characterization of large-scale circulation and frontal systems in the Southern Ocean, dark arrows indicate location of stations sampled for experiments (modified from Lutjeharms et al. 1985).

Station Positions



water was dispensed directly from the pump tubing into 2-liter polycarbonate bottles (**Fig. 3**). All incubation bottles were soaked for 48 hours in 10% HCl, then rinsed three times with sample water. The two-liter polycarbonate bottles were filled, capped and carried to a positive pressure hood, where water was dispensed into 175 ml polyethylene bottles for each experiment treatment. Prior to the addition of sample water, various amendments (**Table 1**) had been added to the 175 ml polyethylene bottles. Duplicate treatments were prepared and sampled for all experiments. Sample handling and set up was designed to reduce possible metal contamination, although no possible contamination was not directly measured. Such steps included pipette tips were rinsed with HCl and MilliQ- water and the time necessary to transfer water and amendments to open sample bottles was minimized.

Substrates were prepared from commercially available reagents. Glucose, ammonium and phosphate additions were made from dry stocks dissolved in MilliQ-water. Initial iron stocks were made in 0.01% N HCl. Stocks were made in 1 liter polycarbonate bottles and frozen until use. Small volumes of the stock were sterilized by filtering through 0.2 μm Acrodiscs (HT Tuffryn® membrane) prior to dispensing into 175ml polyethylene bottles. To minimize possible organic contamination Acrodiscs were flushed several times with MilliQ- water prior to sample filtration

The control treatment consisted of untreated, whole seawater. Combined nutrient and substrate additions contained the same concentrations of glucose, ammonium, phosphate, and iron as treatments where these amendments were added individually (**Table 1**). Concentrations of glucose amendments differed in each experiment, while all other treatment concentrations were constant among

Figure 3. Diagram of design and setup of enrichment experiments.

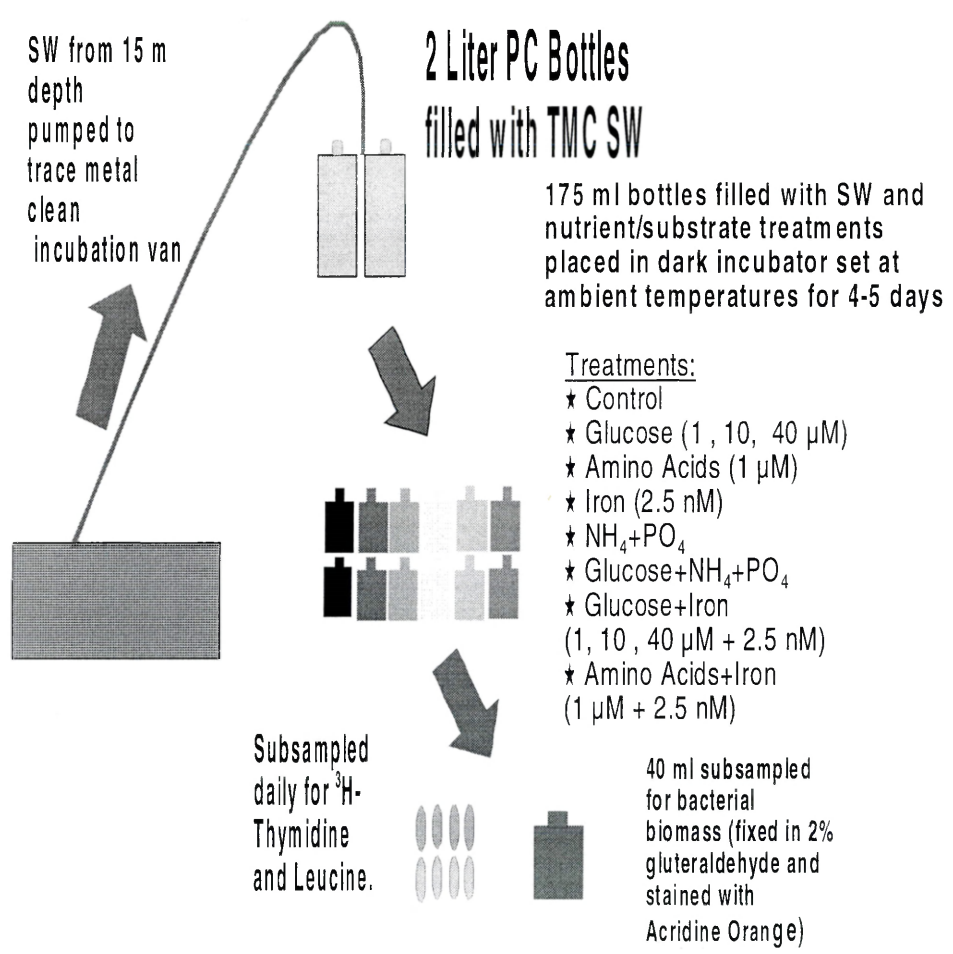


Table 1. Dates, Locations and Concentrations of amendments used for addition experiments

<u>Date</u>	<u>Location</u>	<u>Treatment</u>	<u>Final Concentration of Addition</u>	<u>Experiment Duration (days)</u>
March 2, 1998	42°S 141°E Subtropical Convergence	Glucose	6.7 μM Glucose	4
		Fe	2.5 nM FeCl ₃	4
		Glucose+Fe	6.7 μM+2.5 nM	4
		NH ₄ +PO ₄	10 μM+1.6 μM	4
		Glucose + NH ₄ +PO ₄	6.7μM+10μM+1.6μM	4
		Glucose+NH ₄ +PO ₄ +Fe	6.7μM+10μM+1.6μM+2.5nM	4
March 9, 1998	47°S 141°E Subantarctic Zone	Glucose	1 μM Glucose	5
		Fe	2.5 nM FeCl ₃	5
		Glucose+Fe	1 μM+2.5 nM	5
		DFAA	1 μM DFAA	5
		DFAA+Fe	1 μM+2.5 nM	5
March 26, 1998	51°S 142°E Subantarctic Polar Front	Glucose	10 μM Glucose	4
		Fe	2.5 nM FeCl ₃	4
		Glucose+Fe	10 μM+2.5 nM	4
		DFAA	1 μM DFAA	4
		DFAA+Fe	1 μM +2.5 nM	4
		Glucose Fe	5 μM Glucose 2.5 nM FeCl ₃	5
March 18, 1998	54°S 141°E Antarctic Convergence (Polar Front)	Glucose+Fe	5 μM+2.5 nM	5
		DFAA	1 μM DFAA	5
		DFAA+Fe	1 μM +2.5 nM	5
		Glucose	5 μM Glucose	5
		Fe	2.5 nM FeCl ₃	5

experiments. Glucose and nutrient treatments were added at roughly the molar ratio of bacterial biomass (C : N : P of 45 : 9 : 1; Goldman et al. 1987). Iron was added at 16000 : 1 C : Fe ratios to insure that iron concentrations in those treatments would be high enough to overcome potential growth limitation (C: Fe of iron deficient bacteria may be as high as 130000:1; Tortell et al. 1996). Amino acid additions were from a commercially available mixture of 20 amino acids (Pierce Chemical). Amino acid solution was obtained from Prof. David Hutchins (University of Delaware) and had been treated on a Chelex resin column to remove metals.

Samples for initial time points were collected in separate bottles and measurements were made by the techniques described below. Upon addition of amendments to various treatments, 175 ml incubation bottles were immediately transferred to a temperature controlled, darkened incubator. In all experiments except at the STC, bottles were sampled daily for bacterial abundance, biovolume and rates of incorporation of ^3H -thymidine (TdR) and ^3H -leucine (Leu). For the experiment in the STC, bottles were sampled only at days 0, 2 and 4. All sampling was done in a positive pressure, trace metal clean incubation van. Daily samples of approximately 30 ml were poured from polyethylene incubation bottles into acid cleaned, MilliQ-water rinsed 50ml polycarbonate Oak Ridge tubes. Tubes were then transferred to a separate radiation van for preparation of incubations measuring ^3H -thymidine and leucine incorporation rates.

A temperature manipulation experiment was conducted on water collected in the AC. Four treatments were incubated for the experiment, two at in situ temperature (4.4°C) and two at the surface water temperatures of the SAF (7.5°C).

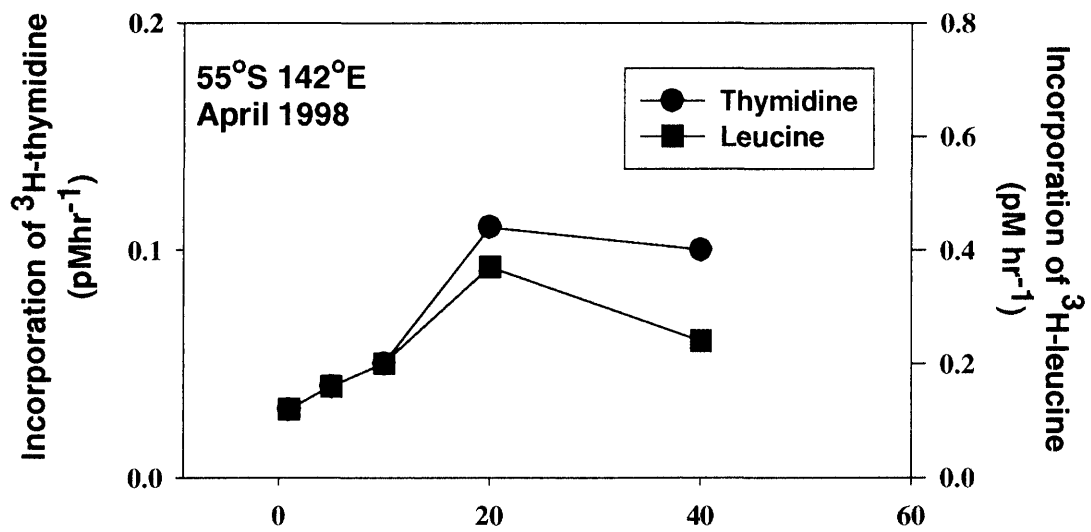
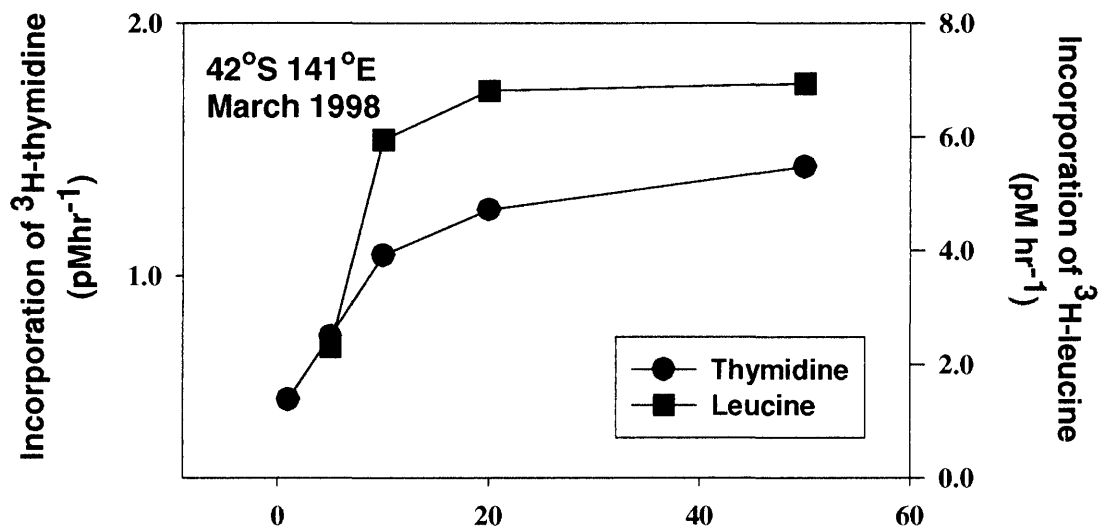
Treatment 1 consisted of unamended seawater incubated at in situ temperature (4.4°C). Treatment 2 received a 10 µM addition of glucose incubated at 4.4°C, treatment 3 was unamended seawater incubated at 7.5°C, and treatment 4 was seawater that received a 10 µM addition of glucose and was incubated at 7.5 °C. Bacterial abundance, cell volume and rates of ³H-leucine incorporation were measured daily for four days.

Measurements

TdR and Leu Incorporation

Incubations for measurements of ³H-thymidine and leucine were carried out in on-deck, flow through incubators or in shipboard refrigerated incubators. On-deck incubators were maintained at surface water temperatures by use of the ship's pump system which circulated surface seawater through the incubators. Incorporation of TdR and Leu was measured following the microcentrifugation procedure described by Smith and Azam (1992). Incubations were performed in 2.0 ml microcentrifuge tubes. High specific activity tritiated thymidine and leucine (79 Ci mmol⁻¹ TdR and 179 Ci mmol⁻¹ Leu, New England Nuclear) were added to each tube, followed by the addition of 1.5 ml of sample water to start the incubations. Final concentrations of both TdR and Leu were 20 nM. These concentrations were determined to achieve rate saturation for two locations in the sampling site (**Fig. 4**). Triplicate samples of both TdR and Leu were incubated for each treatment bottle and each time point. To correct for abiotic incorporation, time zero blanks for both TdR and Leu were determined for each sample. The blank consisted of 20 nM of TdR or Leu in 1.5 ml sample killed with 5% final concentration of trichloroacetic acid (TCA). Microcentrifuge tubes for TdR and Leu incorporation were placed in floating racks covered with dark tape in shaded incubators. Samples for uptake assays were

Figure 4. Curves indicating rate-saturating concentrations of ^3H -thymidine and ^3H -leucine. Concentration of both isotopes used in all experiments and station sampling was 20 nM.



Concentration of ³H-thymidine and leucine

incubated for 4-16 hours depending on the expected activity of the samples and the temperature of the water.

Incubations were terminated by the addition of 100 μ l of 100% TCA (final concentration 5 %). Samples were immediately frozen for subsequent radioassay following the cruise (Hollibaugh 1988). Upon return to VIMS, sample tubes were placed in a refrigerated microcentrifuge at 2°C and spun at 14,000 rpm for 7 minutes, removed from the centrifuge and placed on ice. The supernatant liquid was then aspirated from each sample, leaving behind the centrifuged pellet containing DNA, RNA, and proteins precipitated by the TCA. After an additional 5% TCA rinse, spin, and removal of the supernate, a final rinse with 80% ethanol was used to remove lipids left in the centrifuged pellet. After removal of the ethanol, the DNA/RNA/protein pellets were dissolved in Packard Ultima Gold scintillation cocktail, and the radioactivity of each sample was counted with a scintillation counter. Rates of isotope incorporation for each sample were calculated as the average of three replicates minus the value of the blank.

Cellular Abundance and Volumes

Samples for bacterial abundance and cell volume were collected in 50 ml polyethylene tubes. Samples were preserved in 1% 0.2 μ m filtered gluteraldehyde and filtered immediately. Samples were filtered onto blackened 0.2 μ m polycarbonate membrane filters (Poretics Corp.). The volume filtered varied depended on cell density, with the objective of evenly distributing 100-300 cells per microscope field. A 0.005% solution of acridine orange was added to the last 2 ml of water on the filter (Hobbie et al. 1977). Filters were removed from filter towers and

immediately placed on microscope slides and affixed with a small drop of Resolve® immersion oil and mounted with a cover slide. Filters were frozen until return to VIMS for image analysis.

Cell volumes were determined using a video image analysis system. A Zeiss Axiophot epifluorescence microscope was used to visualize cells (at 1000 X magnification) and video images of the 24 x 24 μm microscope field were captured and stored by computer driven image analysis software (VIDAS VIDEOPLAN). Fluorescence was achieved using blue excitation (450-490 nm) and a 520 nm emission filter from a 200 watt mercury lamp. Sufficient video images were captured from each filter to yield between 300-1000 measurements of individual cells. Cell images were digitized and digital images were used for cell size measurements. Volumes were estimated by measuring the length, width, area and perimeter of each cell, and applying algorithms that derive cell volumes from estimates of perimeter and area (Ducklow et al. 1992). Cell abundance was determined by visual cell counts where at least 300 cells per filter were counted.

Conversion Factors

Conversion factors were used to translate incorporation and biovolume (abundance x mean cell volume) into carbon-based estimates of production and biomass. Use of ^3H -thymidine incorporation to estimate bacterial production requires the use of two conversion factors, one that derives the number of cells produced per mole of thymidine incorporated, and another to derive the amount of carbon per cell. For this study, I used 2×10^{18} cell mole⁻¹ TdR and $120 \mu\text{g C } \mu\text{m}^{-3}$, a commonly used factor for open ocean studies (Fuhrman and Azam 1982, Lee and Fuhrman 1987,

Ducklow and Carlson 1992). Leucine based estimates require the use of only one conversion factor: $3.1 \text{ kg C mole}^{-1} \text{ Leu incorporated}$ (Simon and Azam 1989). However, leucine incorporation could not be measured in the DFAA and DFAA + Fe additions because the amino acid mixture contained unlabeled leucine and extracellular isotope dilution prevented signal detection. Thus, estimates of bacterial production ($\mu\text{gC L}^{-1} \text{ d}^{-1}$) were derived exclusively from ^3H -thymidine incorporation rates. Leucine incorporation was employed as an extra index of growth limitation in all other treatments. All production estimates result from the combination of thymidine incorporation rates multiplied by mean cell volumes. These estimates were then converted to carbon using the two conversion factors described. Biomass was determined from cell biovolume (cell abundance \times mean cell volume) multiplied by $120 \text{ fgC } \mu\text{m}^{-3}$.

Data Analysis

Duplicate incubation samples were analyzed for each treatment. Data were analyzed statistically using two-way ANOVA with both treatment and incubation time as factors. Homogeneity of variance was tested using Levene's test and data were transformed if necessary to achieve heterogeneity. Statistically significant results were analyzed using *a posteriori* Student-Newman-Kuels (SNK) multiple comparison tests, with statistical significance determined at $p < 0.05$ (Underwood 1997). SNK multiple comparison tests are similar to sequential t-tests. Sample means are ranked, a pooled standard error of the means is calculated, and means are compared against each other by use of a test statistic (Q). For these experiments, SNK tests were used to distinguish differences between treatments and significant differences over time for

the variables cell abundance, cell volume, rates of leucine and thymidine incorporation, rates of incorporation of TdR and Leu per cell, and total biovolume.

The rates of change of various properties were estimated using regression coefficients from Model I least-squares fits on the natural logarithm of the individual data versus incubation time. Growth rates were estimated by several methods. Production rates divided by standing biomass (P/B) for given time points yielded estimates of the instantaneous specific growth rates in the presence of bacterivores and took into effect changes in abundance and biovolume. Net accumulation rates were determined from the rate of increase of cell abundance over time, providing an indication of population growth based on cell division. Growth rates computed for the total biovolume used the rate of increase in the natural logarithm of cell abundance \times mean cell volume, which accounts for increases in cell size and cell division. Regressions were performed over appropriate intervals following inspection of the experimental time course plots.

Results

Bacterial Production and Distribution

A strong zonal gradient in surface water temperatures was observed along the cruise transect. The northern most station had warm surface waters (14°C) characteristic of the subtropical gyres, while surface water temperatures at the southern stations fell to \sim 4°C (**Table 2**). The prominent biological and chemical properties for each of the experimental stations are listed in **Table 2**. Major nutrient concentrations increased from north to south. Surface nitrate and phosphate in the

Table 2. Prominent Chemical and Biological Surface Water Properties for Aurora Australis Voyage 6 March-April 1998 Southern Ocean

Location	Water Temp. (°C)	NO ₃ +NO ₂ (µM)	Chl (mg Chl m ⁻²)	Primary Production ¹ (mgC.m ⁻² .d ⁻¹)	Mixed Layer Depth (m)	Bacterial Abundance x 10 ⁸ (cells liter ⁻¹)	Bacterial Biomass (µg C liter ⁻¹)	Bacterial Production ² (µg C liter ⁻¹ d ⁻¹)
Station 1 42°S 141°E	13	6.68 0.60 0.70	1.36	43.17	10	12	9	0.33
Station 2 47°S 141°E	11	9.38 0.74 0.07	4.24	196.52	30	10	5	0.30
Station 3 51°S 142°E	8.5	22.0 1.62 <0.2	45.91	321.25	122	5	2	0.40
Station 4 54°S 141°E	4.5	25.5 1.22 0.97*	6.21	118.87	90	6	4	0.03

¹Primary Production integrated through depth of mixed layer.

²Bacterial Production determined by ³H-thymidine incorporation and conversion factors cited in text.

* Contamination of sample suspected

Subtropical convergence were $7\mu\text{M}$ and $0.6\mu\text{M}$ respectively, while concentrations in the Antarctic Polar Front were $26\mu\text{M}$ and $1.2\mu\text{M}$ respectively.

To estimate rates of bacterial production and distributions of bacterial biomass in this region of the Southern Ocean, samples for incorporation of ^3H -thymidine and leucine, bacterial abundance, and cell volume were collected at the four main stations located inside the frontal zones. Depth profiles for these properties are shown for the upper water column at the four main stations (**Figs. 5-8**). Contour plots of bacterial cell abundance, cell volumes and rates of thymidine and leucine incorporation in the upper 150 m of the water column are shown in **Fig. 9**. Bacterial abundance in the upper 150 m of the water column generally decreased with depth. Surface water bacterial cell density displayed a north-south gradient in surface waters with higher cell numbers at the northern end of the transect decreasing to the south. Zonal north-south gradients in surface production were observed with rates in the APF are nearly an order of magnitude lower than rates in the SAF or SAZ.

Responses to Potential Growth Limiting Substances

Organic and inorganic compounds were added to unfiltered, whole seawater to examine the effects of various treatments on bacterial growth. The specific goal of the project was to determine whether inorganic nitrogen, iron and or labile forms of dissolved organic carbon and nitrogen limit bacterial growth in the Southern Ocean. The results from each of the experimental locations varied, so evaluations of the prominent trends from each experiment are presented and discussed in turn.

The major result of the four addition experiments performed in this study was that labile DOM stimulated rates of bacterial growth and enhanced biomass production (**Table 3**). Bacterial growth responded favorably to both glucose and

Figure 5. Bacterial properties in the surface water of the Subtropical Convergence (42°S 141°E). a.) Cell abundance, b.) Mean cell volume, c.) Total bacterial biovolume, d.) ³H-thymidine and leucine incorporation.

Subtropical Convergence

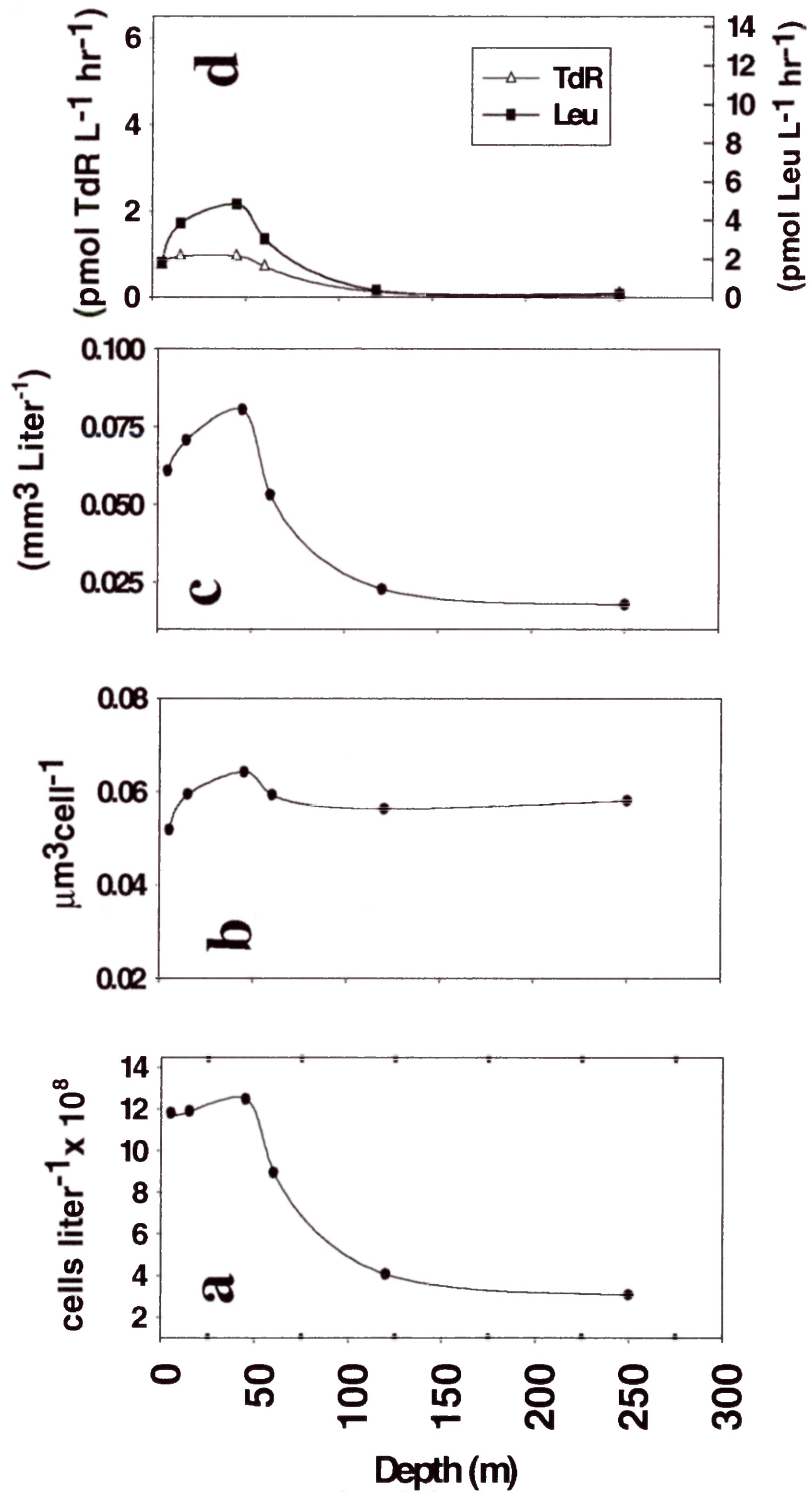


Figure 6. Bacterial properties in the surface water of the Subantarctic Zone (47°S 141°E). a.) Cell abundance, b.) Mean cell volume, c.) Total bacterial biovolume, d.) ³H-thymidine and leucine incorporation.

Subantarctic Zone

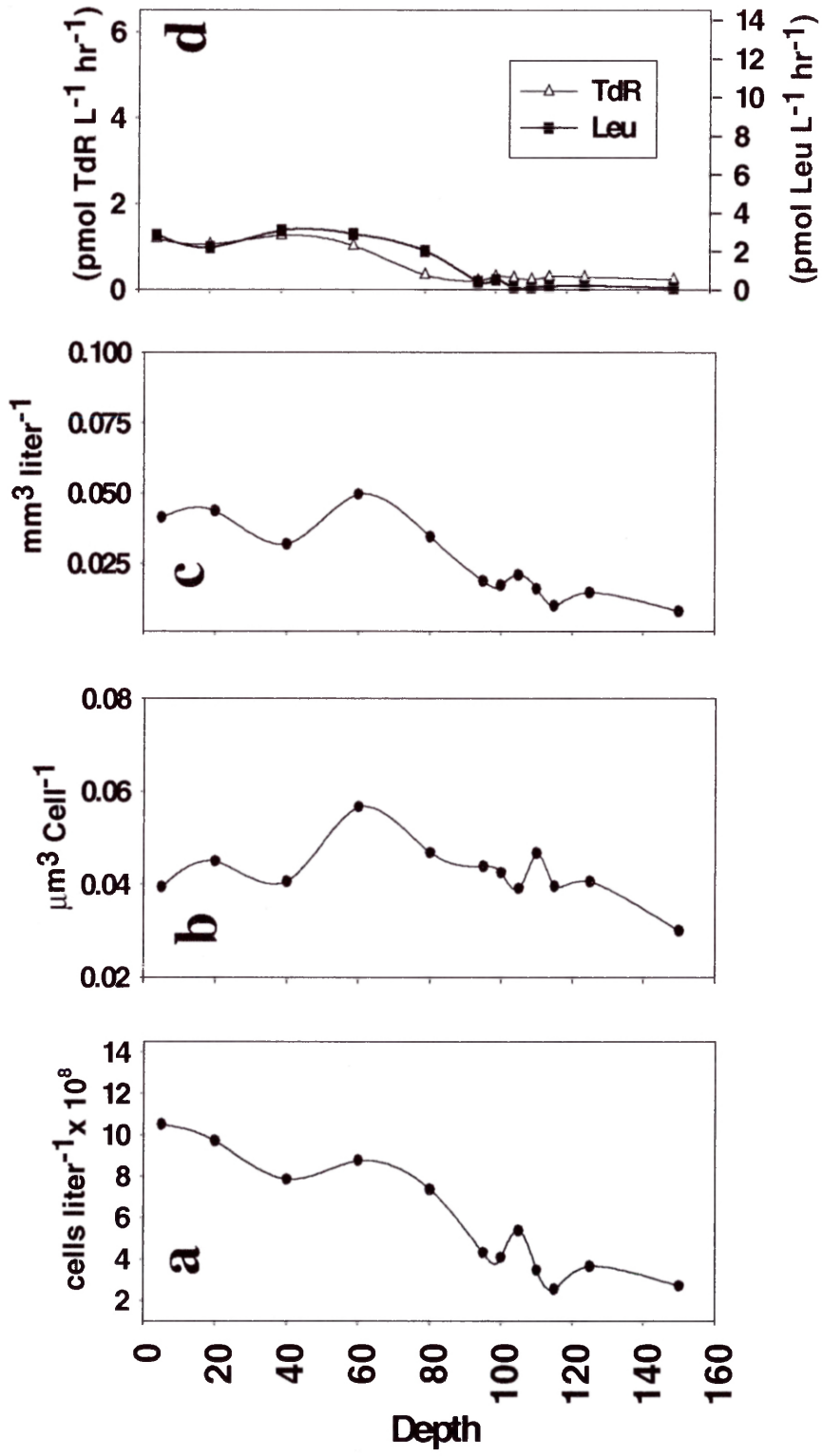


Figure 7. Bacterial properties in the surface water of the Subantarctic Front (47°S 141°E). a.) Cell abundance, b.) Mean cell volume, c.) Total bacterial biovolume, d.) ³H-thymidine and leucine incorporation.

Subantarctic Front

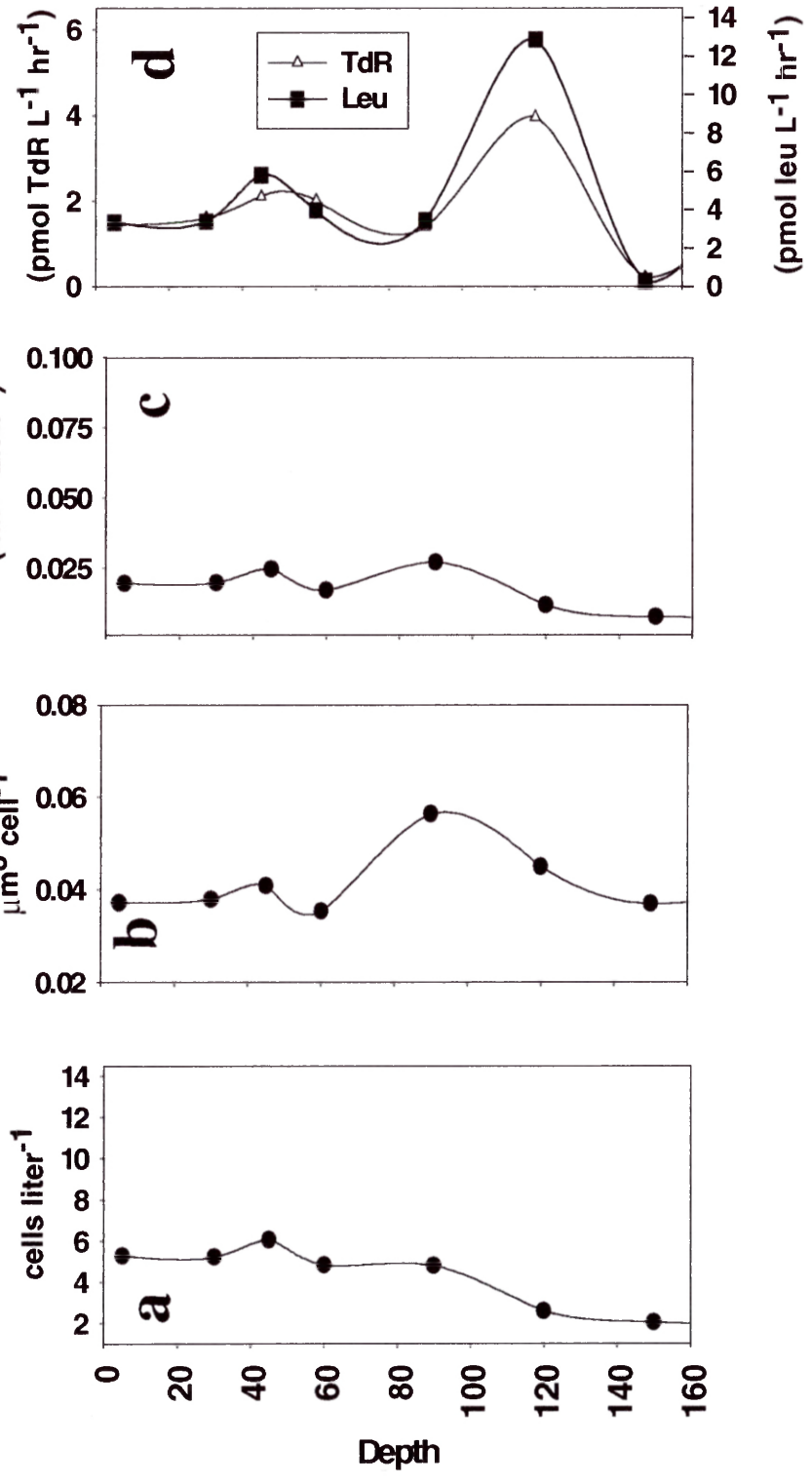


Figure 8. Bacterial properties in the surface water of the Antarctic Convergence (51°S 141°E). a.) Cell abundance, b.) Mean cell volume, c.) Total bacterial biovolume, d.) ³H- thymidine and leucine incorporation.

Antarctic Convergence

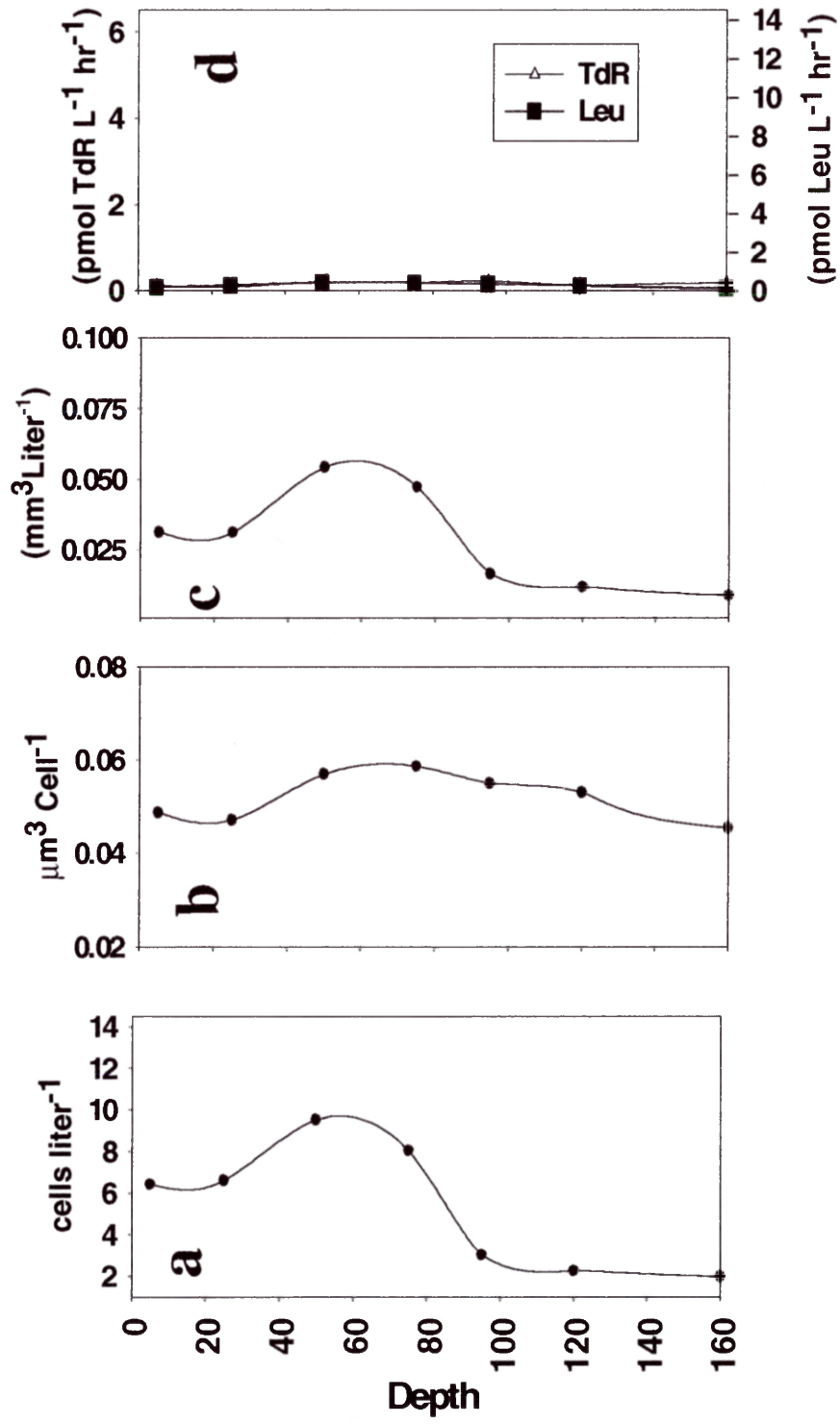
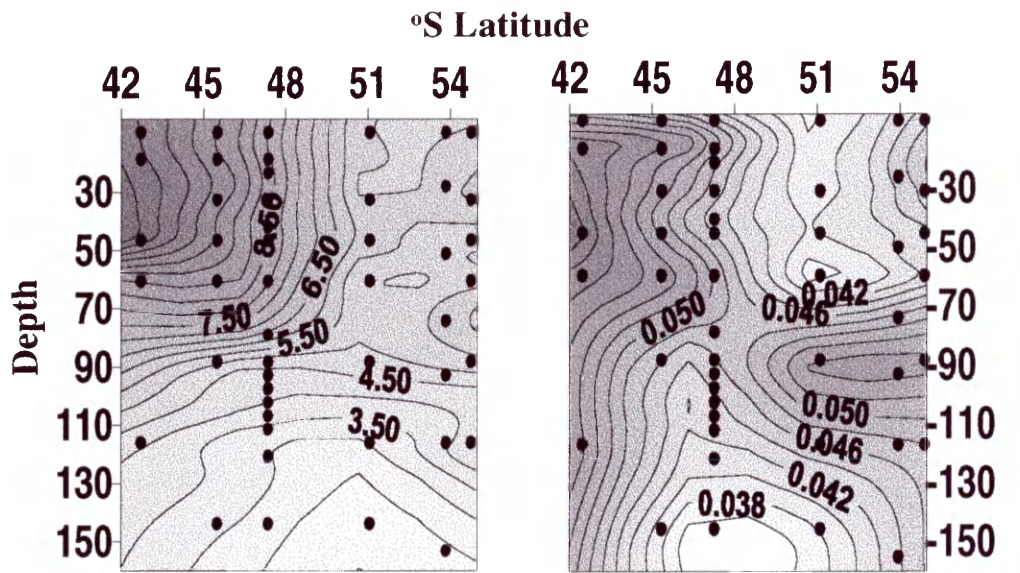
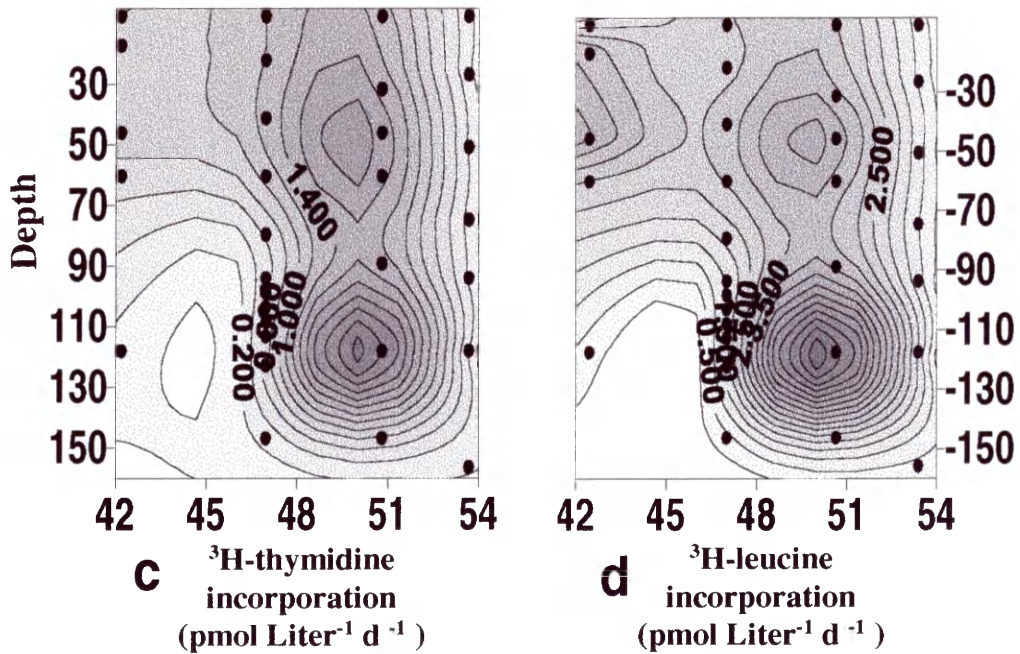


Figure 9. Contour plots of bacterial cell properties in surface water along 141°E *Aurora Australis* Voyage 6 transect. X-axis are °S latitude, Y-axis are depth in meters. Dark dots indicate discrete sampling depths and locations. a.) cell abundance (cells liter⁻¹ x 10⁸), b.) Mean cell volume (μm³ cell⁻¹), c.) ³H-thymidine incorporation (pmol liter⁻¹ d⁻¹), d.) ³H-leucine incorporation (pmol liter⁻¹ d⁻¹).



a Cell Abundance x 10⁸
(cells/Liter)

b Cell Volume
(μm³)



c ³H-thymidine
incorporation
(pmol Liter⁻¹ d⁻¹)

d ³H-leucine
incorporation
(pmol Liter⁻¹ d⁻¹)

Table 3. Net Accumulation Rates, Specific Growth Rates and Biomass Yields

Experiment	Treatment	Net Accumulation (Abundance) (d ⁻¹)	Net Accumulation (Biovolume) (d ⁻¹)	Maximal Specific Growth Rates (P/B) (d ⁻¹)	Biomass Yield (μgC Liter ⁻¹)
Station 1 42°S 141°E	Control	0.13	0.02 ^{ns}	0.27	0.38
	Glucose	0.28	0.50 ^{ns}	0.51	24.50
	Fe	0.21	0.22	0.23	4.14
	Glucose+Fe	0.31	0.56 ^{ns}	0.44	31.56
	NH ₄ +PO ₄	0.22	0.10 ^{ns}	0.25	1.90
	Glucose + NH ₄ +PO ₄	0.44	0.73 ^{ns}	0.91	67.66
	Glucose+NH ₄ +PO ₄ +Fe	0.42	0.62 ^{ns}	0.78	38.87
Station 2 47°S 141°E	Control	0.04	0.10 ^{ns}	0.19	0.57
	Glucose	0.13	0.17	0.15	1.69
	Fe	0.05 ^{ns}	0.10 ^{ns}	0.18	3.86
	Glucose+Fe	0.13	0.16	0.33	4.40
	DFAA	0.36	0.71	2.02	51.28
	DFAA+Fe	0.33	0.79	1.97	46.61
				0.06	
Station 3 51°S 142°E	Control	0.04 ^{ns}	0.07 ^{ns}		1.08
	Glucose	0.14 ^{ns}	0.35	0.52	10.28
	Fe	0.09 ^{ns}	0.11 ^{ns}	0.06	2.39
	Glucose+Fe	0.16	0.53	0.70	21.36
	DFAA	0.32	0.64 ^{ns}	0.79	40.96
	DFAA+Fe	0.33	0.69	1.13	38.41
Station 4 54°S 141°E	Control	0.11	0.23	0.05	2.98
	Glucose	0.06 ^{ns}	0.06 ^{ns}	0.05	0.94
	Fe	0.10 ^{ns}	0.14 ^{ns}	0.05	2.34
	Glucose+Fe	0.12	0.15	0.03	3.29
	DFAA	0.11	0.15 ^{ns}	0.03	2.82
	DFAA+Fe	0.07	0.07 ^{ns}	0.04	4.32

Notes: Net accumulation rates derived from linear regression of natural log of cell abundance or biovolume through day 4 in all treatments. Conversion factors for P/B ratio cited in text. Biomass yield calculated from (biomass at final time point -biomass at t=0). ns=regression coefficient (slope) not significantly different from zero (p>0.05)

amino acids, indicating that bacterial growth may be constrained by both organic carbon and nitrogen. Additions of dissolved iron or ammonium and phosphate alone had no significant impact on rates of bacterial growth or biomass production. The two experiments in the SAZ and SAF indicated that combined additions of glucose +Fe resulted in higher growth rates and biomass yields. With the exception of the experiment in the AC, the experiments showed dependence on the type of DOM substrate (glucose versus DFAA) used to support bacterial growth (**Tables 4-7**). General conclusions for each group of experiments will be provided in the Discussion.

Experiment in the Subtropical Convergence (42°S)

Cellular Abundance, volume and biomass

The addition of glucose and combinations of glucose and ammonium, phosphate and iron all significantly stimulated bacterial growth rates and the production of bacterial biomass in seawater collected from the STC (**Fig. 10**). Bacterial abundance increased in all treatments, including the control. No significant differences in bacterial cell abundance between any of the treatments were observed until day 4 (**Table 4**). At day 4, all glucose treatments displayed elevated cell abundance relative to the control treatments. Treatments receiving combinations of glucose and $\text{NH}_4^+ + \text{PO}_4^{3-}$ resulted in significantly greater cell abundance than all other treatments. Cell abundance in the glucose + $\text{NH}_4^+ + \text{PO}_4^{3-}$ and glucose + $\text{NH}_4^+ + \text{PO}_4^{3-} + \text{Fe}$ additions increased by roughly three times the control and yielded cell abundance five times greater than measured at day 0 (**Fig. 10a, Table 4**). Significant differences in cell abundance between the

Figure 10. Responses of various bacterial properties to enrichment treatments in the Subtropical Convergence (42°S 141°E). a.) Cell abundance, b.) Mean cell volume, c.) ³H-thymidine incorporation, d.) ³H-leucine incorporation, e.) ³H-thymidine incorporation per cell, f.) ³H-leucine incorporation per cell. Error bars are standard error of duplicate samples.

Subtropical Convergence (42°S)

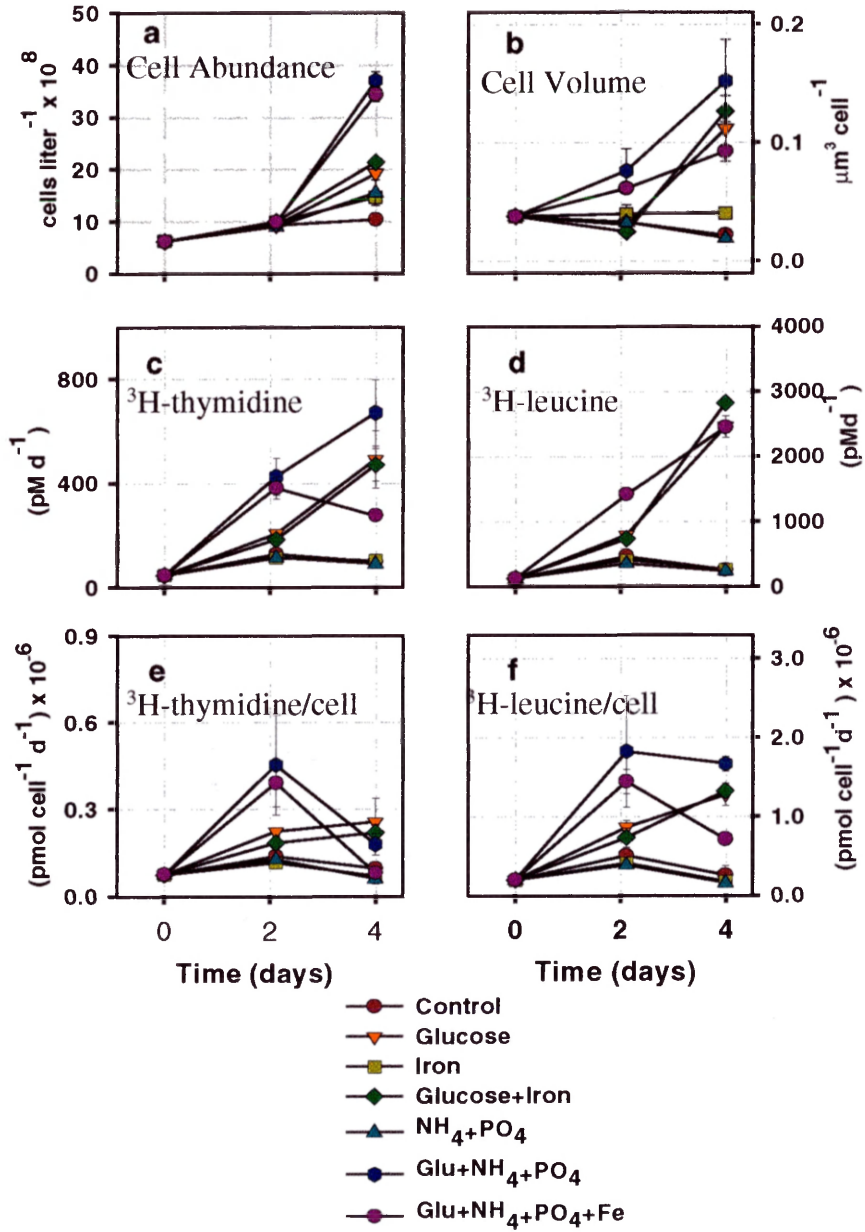


Table 4. Bacterial Cell Properties from Experiment at 42°S 141°E

Time (days)	Treatment	Bacterial Abundance (cells Liter ⁻¹) × 10 ⁸	±SE	Thymidine Incorporated (pmol TdR Liter ⁻¹ d ⁻¹)	±SE	Cell Volume (μm ³ cell ⁻¹)	±SE	TdR cell ⁻¹ (pmol TdR cell ⁻¹ d ⁻¹) × 10 ⁸	±SE	Bacterial Biovolume (μm ³ Liter ⁻¹) × 10 ⁸	±SE
0	T=0	6.21		46.73		0.048		0.08		0.030	
2	Control	9.17	0.06	A 126.12	3.48	A 0.043	0.00	A 0.14	0.00	A 0.39	0.03
	Glucose	9.19	0.15	A 205.32	1.08	B 0.041	0.00	A 0.22	0.00	A 0.38	0.02
	Fe	9.81	0.35	A 113.28	3.60	A 0.050	0.01	A 0.12	0.01	A 0.49	0.13
	Glucose+Fe	10.00	0.32	A 183.00	10.20	B 0.035	0.00	A 0.18	0.02	A 0.35	0.02
	NH ₄ +PO ₄	9.05	0.06	A 114.60	3.96	A 0.043	0.00	A 0.13	0.01	A 0.39	0.05
	Glucose + NH ₄ +PO ₄	9.64	1.03	A 425.64	70.68	C 0.086	0.03	A 0.45	0.12	B 0.81	0.16
	Glucose+NH ₄ +PO ₄ +Fe	9.82	0.33	A 382.20	43.08	C 0.034	0.00	A 0.39	0.06	B 0.70	0.03
	Control	3.94	0.44	A 97.68	0.48	A 0.03	0.00	A 0.19	0.02	A 0.33	0.04
	Glucose	28.05	8.94	B 491.88	109.80	B 0.26	0.06	B 0.51	0.12	B 2.34	0.75
	Fe	8.85	0.22	C 102.96	2.88	A 0.07	0.00	A 0.14	0.00	A 0.74	0.02
4	Glucose+Fe	35.01	0.02	B 470.64	61.68	B 0.22	0.02	B 0.44	0.04	B 2.92	0.00
	NH ₄ +PO ₄	5.45	1.06	C 89.64	3.72	A 0.06	0.01	A 0.12	0.02	A 0.45	0.09
	Glucose + NH ₄ +PO ₄	71.22	18.58	D 671.04	126.96	B 0.18	0.03	B 0.36	0.05	B 5.94	1.55
	Glucose+NH ₄ +PO ₄ +Fe	42.43	1.50	D 275.04	3.84	C 0.08	0.00	B 0.16	0.01	A 3.54	0.13
	Control	3.94	0.44	A 97.68	0.48	A 0.03	0.00	A 0.19	0.02	A 0.33	0.04

SE=Standard errors of duplicate samples. Letters indicate statistical evaluation of growth properties. Treatments with same letters within a time point are statistically indistinguishable at p>0.05.

Figure 11. Bacterial biomass response to treatments in the Subtropical Convergence (42°S 141°E). Shown are days 2 and 4 of the incubation. Colors indicate statistically significant ($p < 0.05$) similarities and differences among responses to treatments, colors do not apply to statistical differences between days. Error bars are standard error of duplicate samples.

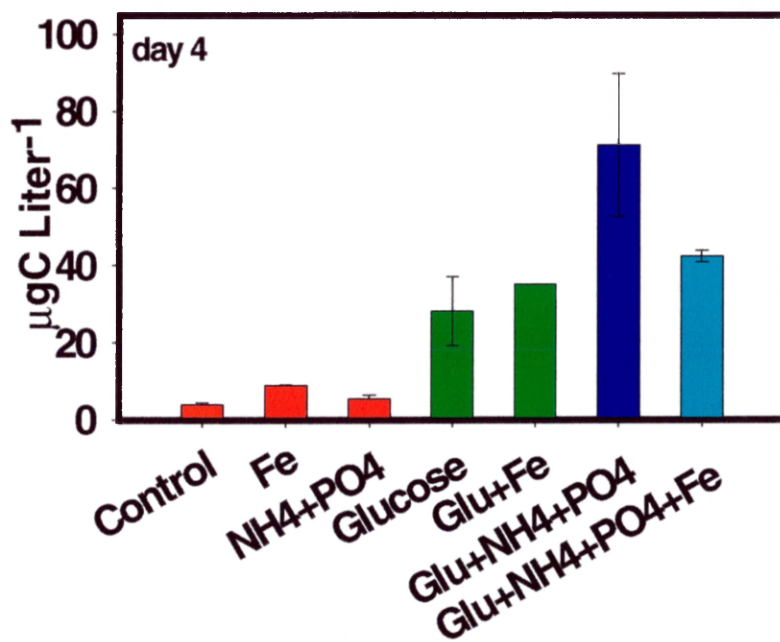
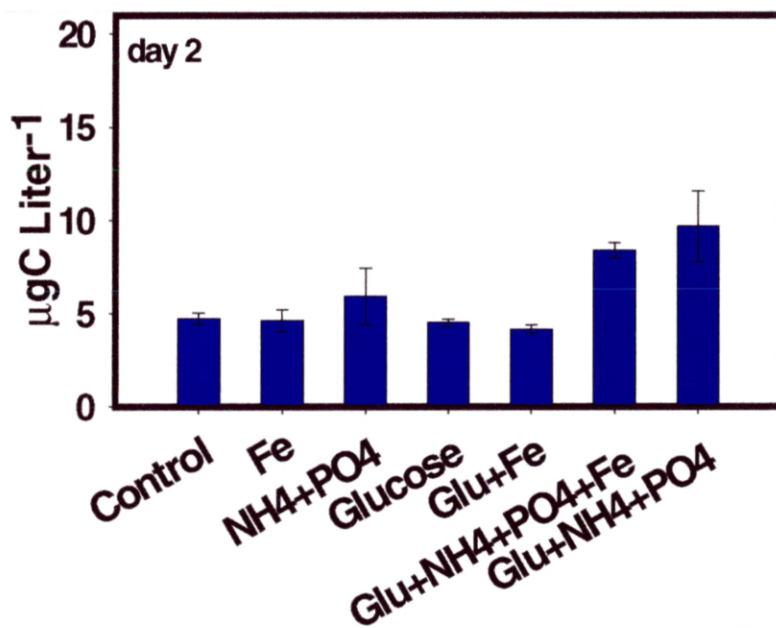


Figure 12. Bacterial production ($^3\text{H-TdR}$) response to treatments in the Subtropical Convergence (42°S 141°E). Shown are days 2 and 4 of the incubation. Colors are the same as for Figure 11. Error bars are standard error of duplicate samples.

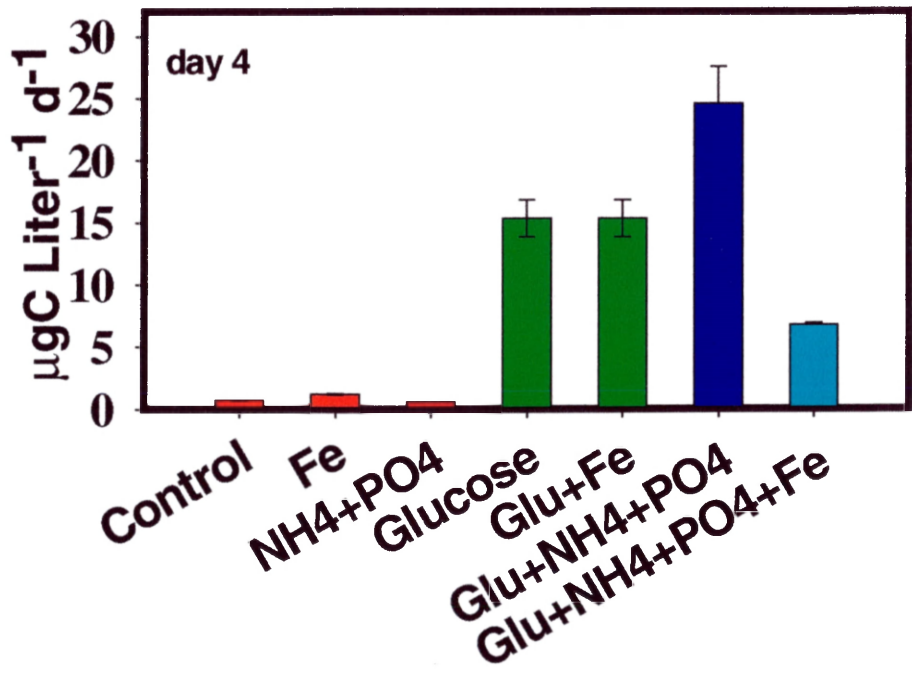
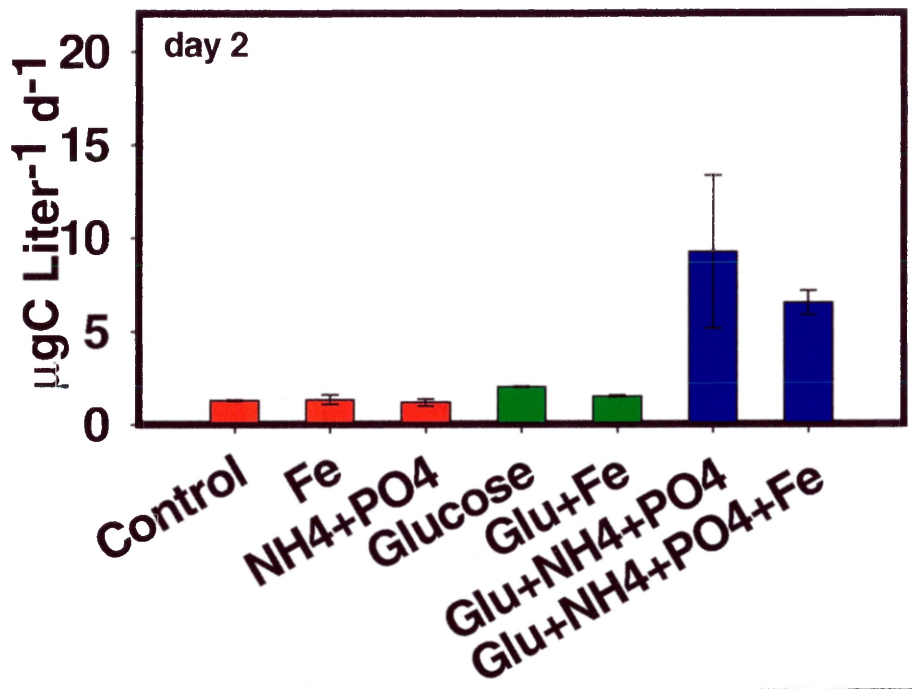
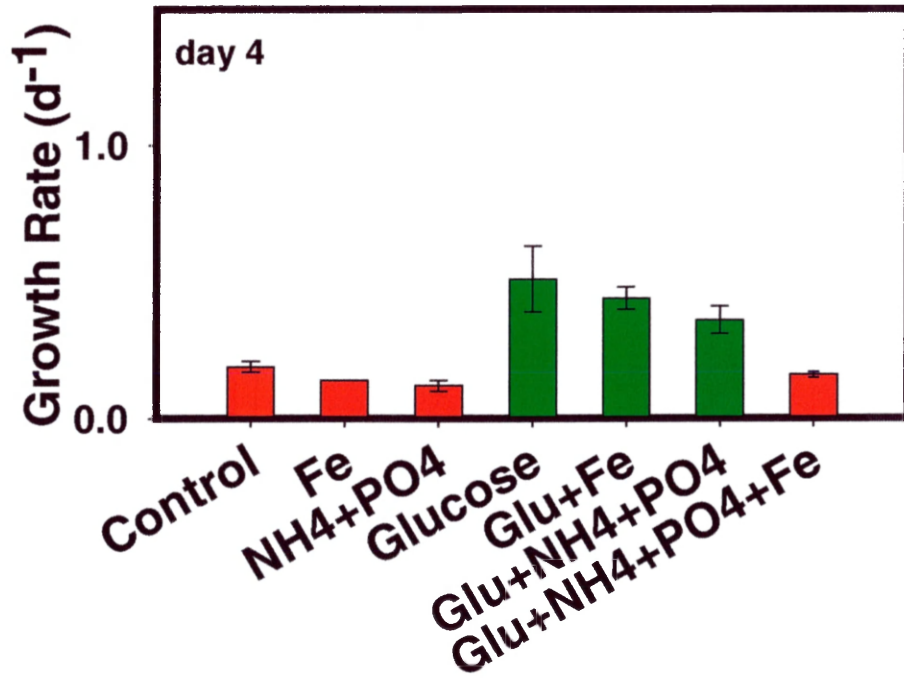
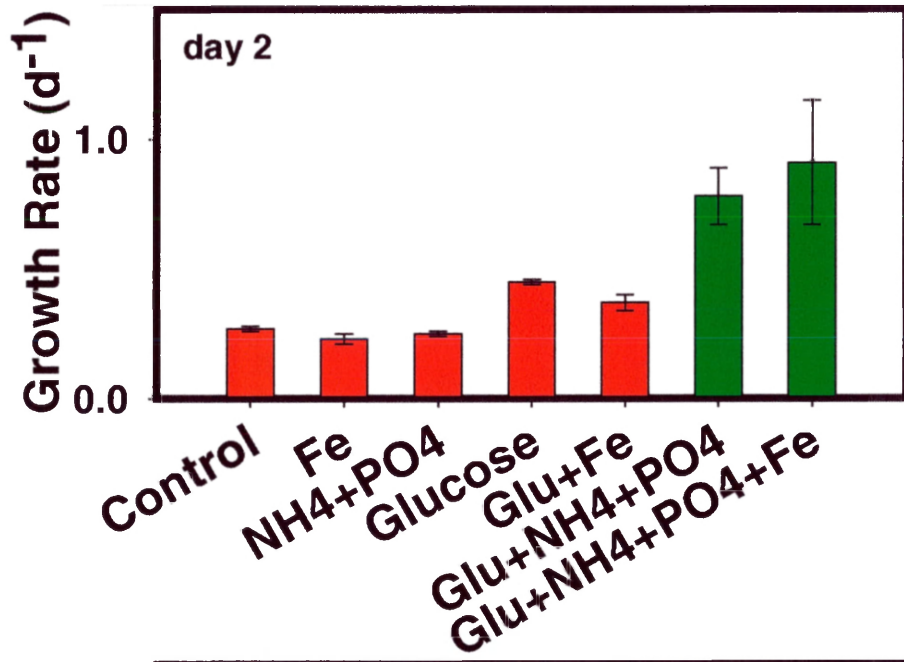


Figure 13. Instantaneous bacterial specific growth rate calculated from thymidine incorporation rates and bacterial biomass (P/B) in the Subtropical Convergence (42°S 141°E). Shown are days 2 and 4 of the incubation. Colors are the same as for Figure 11. Error bars are standard error of duplicate samples.



glucose + NH_4^+ + PO_4^{3-} and glucose + NH_4^+ + PO_4^{3-} +Fe treatments were not observed, nor were differences between the glucose and glucose + Fe treatments (Table 4). Additions of NH_4^+ + PO_4^{3-} and Fe alone resulted in insignificant differences in cell abundance by day 4 relative to control treatments.

All treatments receiving glucose showed significant increases in bacterial cell volume. Glucose amended treatments showed increases in cell size by ~ 300% relative to the control (**Fig. 10b**). Biovolume increases were greatest in the two treatments that contained glucose + NH_4^+ + PO_4^{3-} . Mean biovolume increases in the glucose additions were by day 4 more than seven times larger than the control, NH_4^+ + PO_4^{3-} and iron treatments (**Table 4**). There were no significant differences in total biovolume between the glucose and the glucose +Fe treatments. After four days, increases in bacterial biomass were greatest in the glucose + NH_4^+ + PO_4^{3-} treatments (**Fig. 11**).

Bacterial Production

Rates of bacterial production in glucose treatments were significantly greater than other treatments (**Fig. 12**). The addition of glucose + NH_4^+ + PO_4^{3-} resulted in the largest overall increase in ^3H -thymidine incorporation (**Fig. 10e**). The temporal response of production rates to the various glucose treatments is striking. Both the glucose + NH_4^+ + PO_4^{3-} and glucose + NH_4^+ + PO_4^{3-} + Fe increased significantly by day 2 relative to the other treatments. By day 4, there were no significant differences in rates of thymidine incorporation in the glucose + NH_4^+ + PO_4^{3-} , glucose + Fe and glucose treatments (**Fig. 12, Table 4**). Rates of ^3H -leucine incorporation in the glucose + NH_4^+ + PO_4^{3-} treatment increased more than 25 times the control by day 4

(**Fig. 10d**), while the other glucose treatments increased at roughly 10 times the control. There were no significant differences in rates of ^3H -leucine incorporation between the glucose, glucose + Fe, and glucose + NH_4^+ + PO_4^{3-} + Fe additions by day 4.

Growth rates

Additions of glucose + NH_4^+ + PO_4^{3-} and glucose + NH_4^+ + PO_4^{3-} + Fe dramatically increased rates of ^3H -TdR incorporation per cell after two days relative to the control (**Fig. 10e, Table 4**). Two days later, bacterial cell abundance increased in both treatments resulting in ~ 75% decrease in thymidine per cell. Overall, glucose + NH_4^+ + PO_4^{3-} resulted in greater enhancement of cell growth rates than the glucose and glucose + Fe treatments (**Fig. 13**). Calculated specific growth rates (P/B) are consistent with trends in cell specific thymidine incorporation. Control, Fe and NH_4^+ + PO_4^{3-} growth rates were statistically indistinguishable after four days and ranged from 0.07-0.19 d^{-1} . At day 2, cell growth rates (P/B) in glucose and glucose + Fe treatments were 0.45 and 0.36 d^{-1} , while glucose + NH_4^+ + PO_4^{3-} and glucose + NH_4^+ + PO_4^{3-} + Fe were 0.95 and 0.77 d^{-1} . Glucose, glucose + Fe, and glucose + NH_4^+ + PO_4^{3-} treatments showed no significant differences at day 4, ranging between 0.35-0.55 d^{-1} (**Fig. 13**). Overall, additions of glucose + NH_4^+ + PO_4^{3-} and glucose + NH_4^+ + PO_4^{3-} + Fe resulted in the largest increases in growth rates and biomass production than any of the other treatments.

Combined additions of glucose and NH_4^+ + PO_4^{3-} resulted in more rapid (i.e. by day 2) increases in bacterial growth rates and production than any other treatments. Rates of thymidine incorporation in additions containing both glucose and NH_4^+ + PO_4^{3-} increased about four-fold by day 2, and roughly twice as large as treatments

receiving glucose without a nitrogen or phosphorous source. Thymidine incorporation per cell followed similar patterns, significantly increasing by day 2, while glucose and glucose + Fe treatments remain statistically indistinguishable from the control. This temporal response to glucose + NH_4^+ + PO_4^{3-} treatments was not observed in cell abundance, cell volume, total biovolume or biomass (**Fig. 11, Table 4**).

Experiment in the Subantarctic Zone (47°S 141°E)

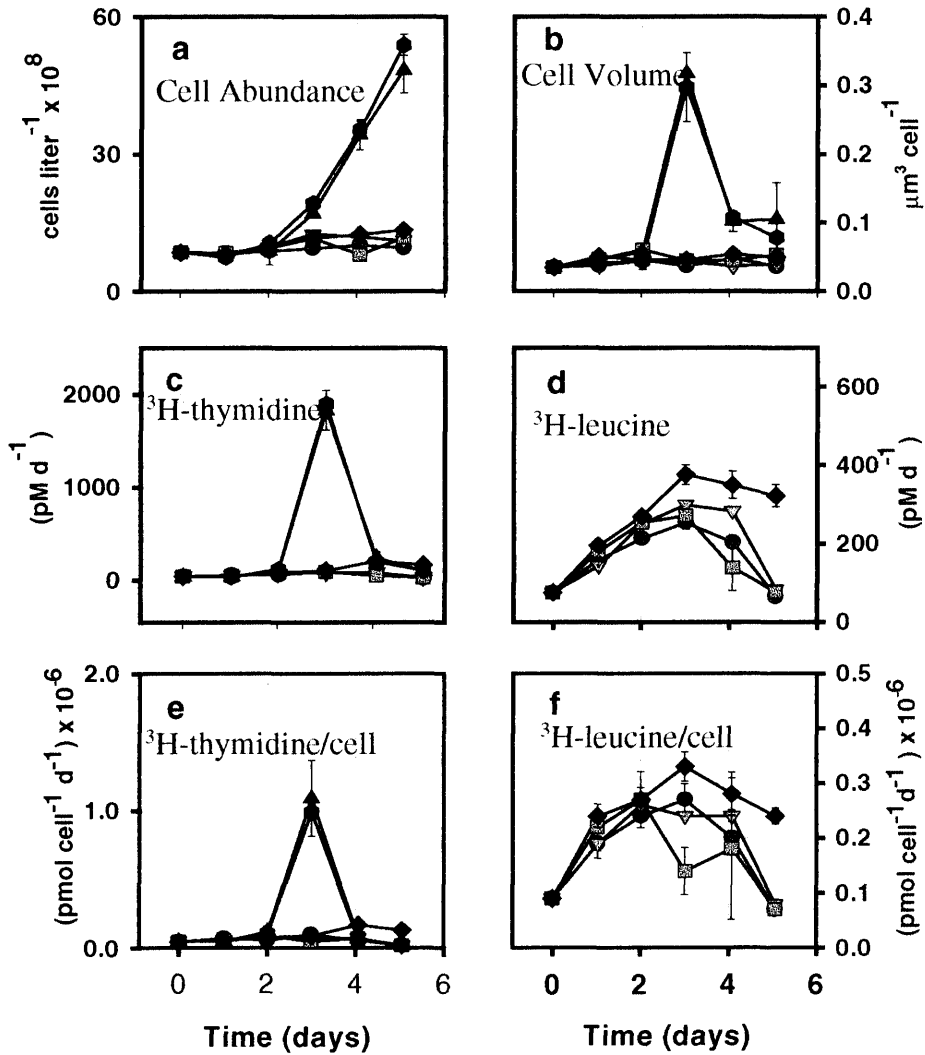
Cellular Abundance, volume and biomass

Dissolved free amino acid (DFAA) additions significantly stimulated rates of bacterial growth and enhanced bacterial biomass in amendment experiments performed in the Subantarctic zone. No significant changes in cell abundance were observed in the glucose, Fe and control treatments (**Table 5**). In contrast, the addition of DFAA and DFAA + Fe resulted in exponential increases in cell abundance over the course of the incubation (**Fig. 14a**). By day 5, DFAA and DFAA + Fe treatments produced roughly five times more cells than all other treatments. There were no statistical differences in cell abundance between the DFAA and DFAA + Fe treatments.

No significant differences in mean cell volumes were observed for glucose, iron or control treatments over the 5 days of incubation. The addition of DFAA and DFAA + Fe resulted in significantly larger cell volumes than with any other treatment by day 3, at which time cell volumes in the DFAA treatments had grown to

Figure 14. Responses of various bacterial properties to enrichment treatments in the Subantarctic Zone (47°S 141°E)
a.) Cell abundance, b.) Mean cell volume, c.) ³H-thymidine incorporation, d.) ³H-leucine incorporation, e.) ³H-thymidine incorporation per cell, f.) ³H-leucine incorporation per cell. Error bars are standard error of duplicate samples.

Subantarctic Zone (47°S)



- Control
- ▽ Glucose
- Fe
- ◆ Glucose+Fe
- ▲ DFAA
- DFAA+Fe

Table 5. Bacterial Cell Properties from Experiment at 47°S 141°E

Time (days)	Treatment	Bacterial Abundance (cells Liter ⁻¹) x 10 ⁸	±SE	Thymidine Incorporated (pmol TDR Liter ⁻¹ d ⁻¹)	±SE	Cell Volume (μm ³ cell ⁻¹)	±SE	TDR cell ⁻¹ (pmol TDR cell ⁻¹ d ⁻¹) x 10 ⁻⁴	±SE	Bacterial Biovolume (μm ³ Liter ⁻¹)	±SE					
0	T=0	8.44		40.56		0.04		0.05		0.03						
3	Control	9.41	0.10	A	89.28	13.20	A	0.04	0.00	A	0.10	0.02	A	0.03	0.00	A
	Glucose	12.50	0.01	A	82.44	4.68	A	0.04	0.01	A	0.07	0.01	A	0.05	0.01	A
	Fe	16.98	0.18	A	87.84	1.20	A	0.04	0.00	A	0.05	0.02	A	0.08	0.00	A
	Glucose+Fe	11.47	0.06	A	104.52	1.56	A	0.05	0.01	A	0.09	0.00	A	0.05	0.01	A
	DFAA	11.60	0.77	A	1827.20	214.40	B	0.32	0.01	B	1.01	0.30	B	0.62	0.09	B
	DFAA+Fe	19.23	0.70	A	1893.10	26.88	B	0.30	0.01	B	0.99	0.05	B	0.58	0.12	B
4	Control	10.05	0.54	A	63.12	4.56	A	0.05	0.00	A	0.06	0.00	A	0.05	0.01	A
	Glucose	11.92	0.41	A	82.20	7.80	A	0.04	0.00	A	0.07	0.00	A	0.04	0.00	A
	Fe	7.89	0.52	A	50.52	21.24	A	0.04	0.00	A	0.07	0.05	A	0.04	0.00	A
	Glucose+Fe	12.36	0.01	A	206.28	6.12	B	0.05	0.01	A	0.17	0.01	A	0.05	0.00	A
	DFAA	34.17	3.23	B	239.52	57.60	B	0.10	0.02	B	0.07	0.01	A	0.34	0.02	B
	DFAA+Fe	35.44	1.79	B	195.24	13.08	B	0.11	0.00	B	0.06	0.01	A	0.38	0.03	B
5	Control	9.45	0.19	A	19.20	5.28	A	0.04	0.00	A	0.02	0.01	A	0.03	0.00	A
	Glucose	11.02	0.31	A	26.88	1.44	B	0.04	0.00	A	0.02	0.00	A	0.04	0.00	A
	Fe	11.67	0.49	A	28.08	6.72	B	0.05	0.01	A	0.02	0.01	A	0.06	0.00	A
	Glucose+Fe	13.29	0.43	A	166.68	3.72	C	0.05	0.01	A	0.13	0.00	A	0.07	0.01	A
	DFAA	48.34	4.92	B	95.16	3.72	C	0.11	0.01	A	0.02	0.00	A	0.46	0.20	B
	DFAA+Fe	53.75	2.29	B	105.84	5.76	C	0.08	0.00	A	0.02	0.00	A	0.42	0.01	B

SE=Standard errors of duplicate samples. Letters indicate statistical evaluation of growth properties with letter designations same as Table 4.

Figure 15. Bacterial biomass response to treatments in the Subantarctic Zone (47°S 141°E). Shown are days 3, 4 and 5 of the incubation, there were no statistical differences within treatments after days 1 and 2. Colors indicate statistically significant ($p < 0.05$) similarities and differences among responses to treatments, colors do not apply to statistical differences between days. Error bars are standard error of duplicate samples.

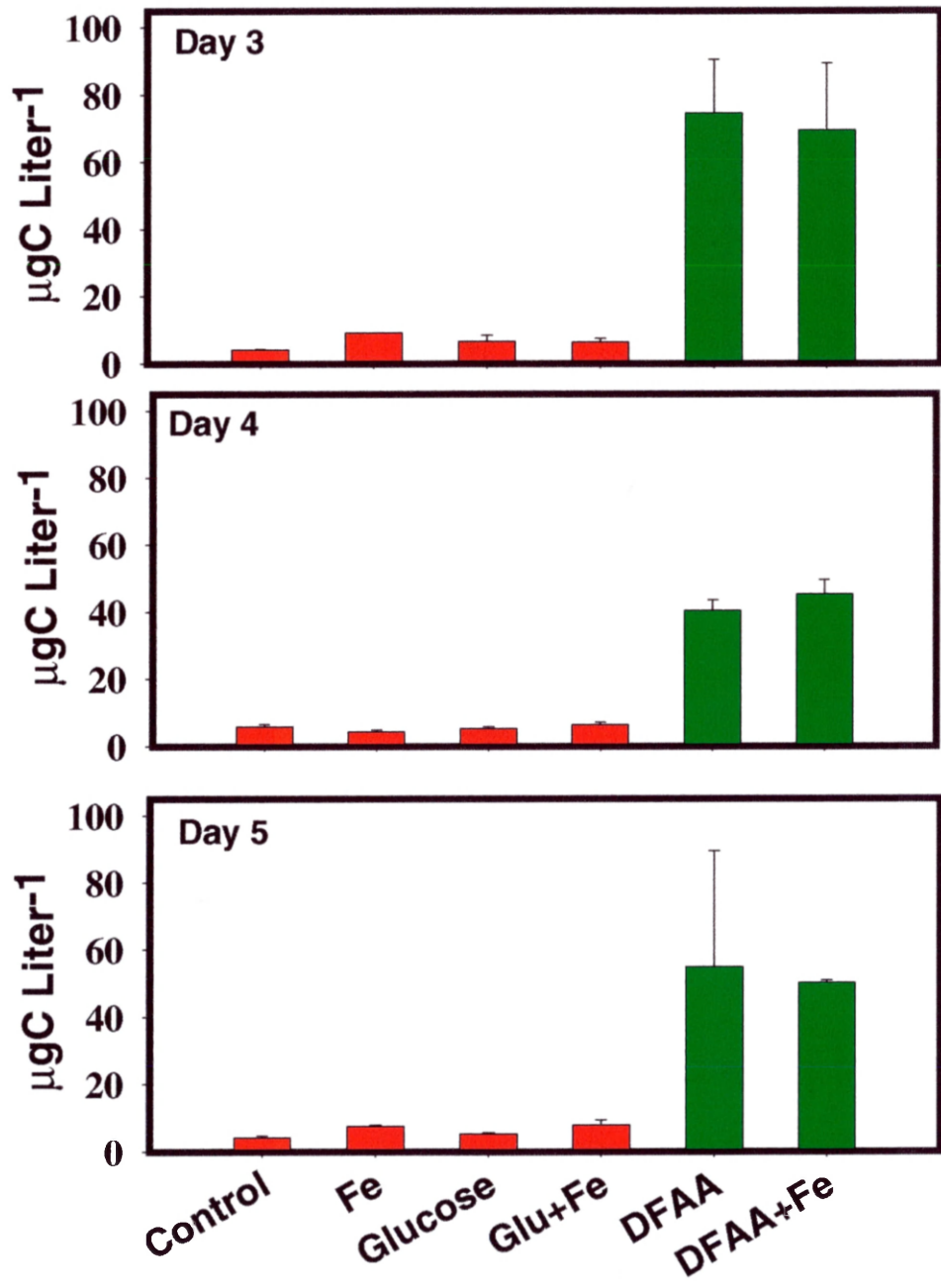


Figure 16. Bacterial production ($^3\text{H-TdR}$) response to treatments in the Subantarctic Zone (47°S 141°E). Shown are days 3, 4 and 5 of the incubation, there were no statistical differences within treatments after days 1 and 2. Colors are the same as for Figure 15. Error bars are standard error of duplicate samples.

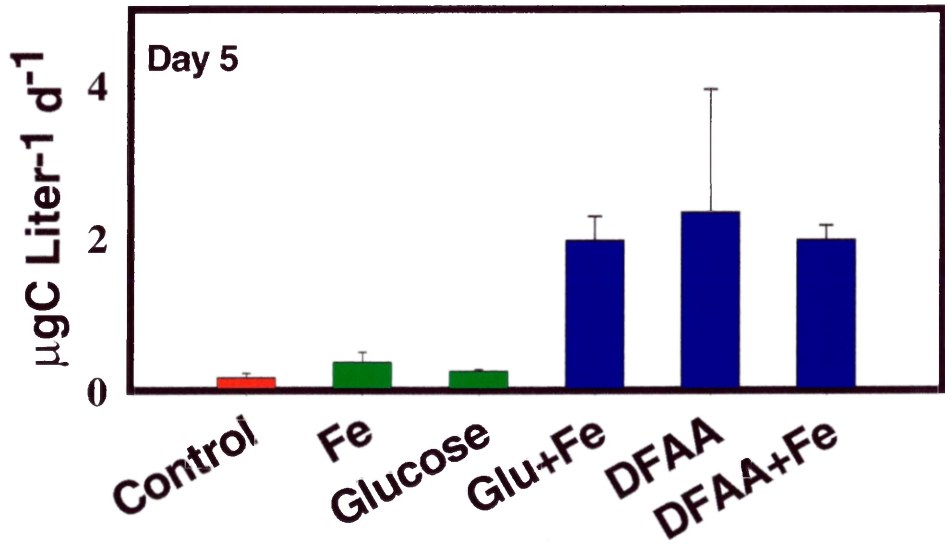
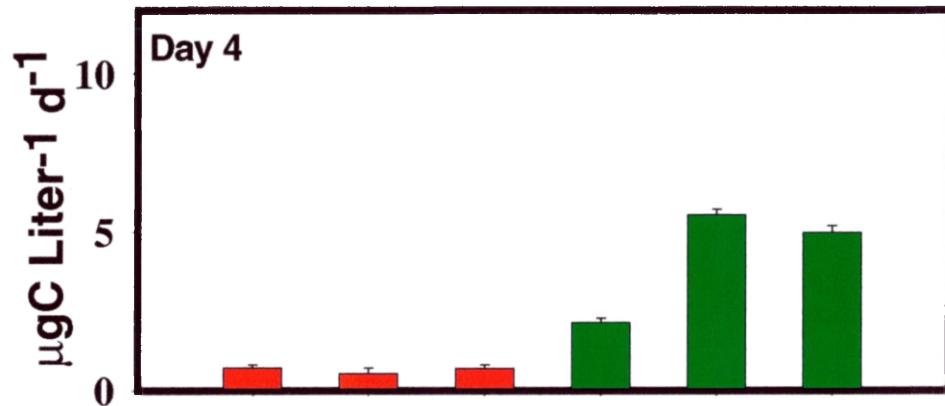
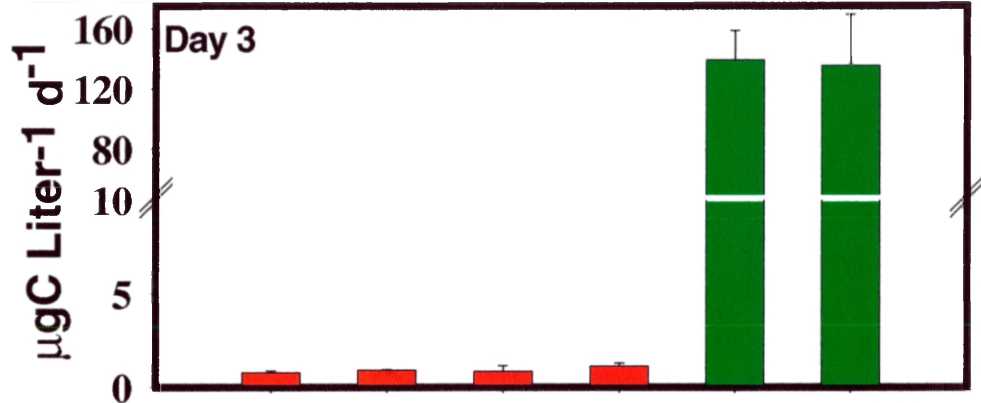
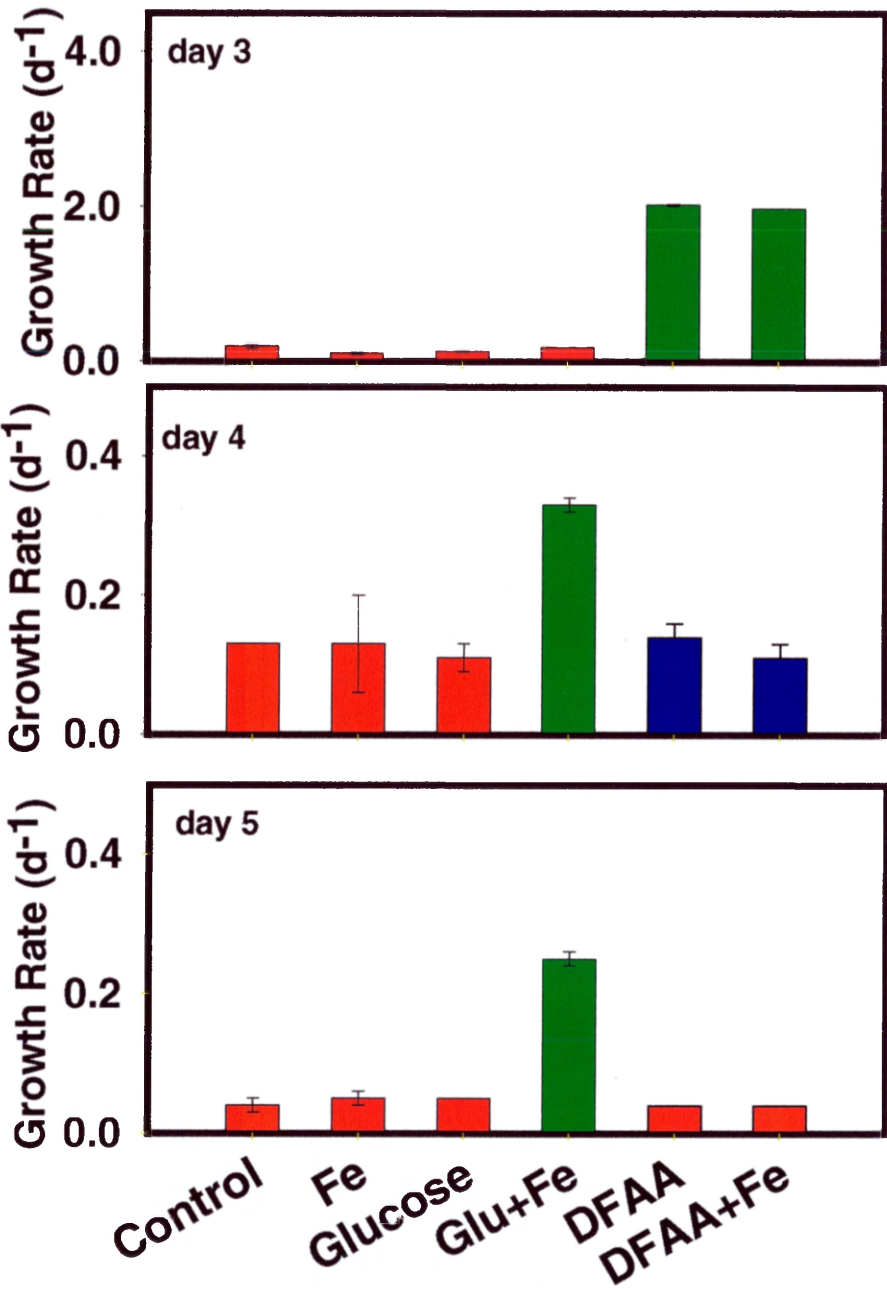


Figure 17. Instantaneous bacterial specific growth rate calculated from thymidine incorporation rates and bacterial biomass (P/B) in the Subantarctic Zone (47°S 141°E). Shown are days 3, 4 and 5 of the incubation, there were no statistical differences within treatments after days 1 and 2. Colors are the same as for Figure 15. Error bars are standard error of duplicate samples.



about eight times the size as all other treatments (Table 5). The large increases in cell volume in the DFAA treatments diminished by day 5, when no significant differences between any treatments were observed. Changes in total biovolume closely resembled changes in mean cell volume. Largest increases in total biovolume were seen in DFAA treatments after day 3, dropping by ~50% by day 4 (Table 5). There were no significant differences between DFAA and DFAA + Fe treatments throughout the experiment. Bacterial biomass in both DFAA treatments increased more than an order of magnitude through the experiment and was significantly larger than all other treatments for the remainder of the experiment (**Fig. 15**). These changes in biomass were driven primarily by the dramatic increases in cell volume observed throughout the incubation.

Bacterial Production

The additions of DFAA and DFAA + Fe resulted in large increases in rates of ³H-thymidine incorporation by day 3 of the experiment (**Fig. 14c, Table 5**). Peak rates were ~ 45 times higher than rates at day 0 and were more than twenty times greater than rates measured in the control treatment. Following the sharp rise in production rates at day 3, rates of incorporation dropped considerably by day 4 (**Fig. 14c, Table 5**). Rates of isotope incorporation ($\text{pmol}^{-1} \text{L}^{-1} \text{d}^{-1}$) in the glucose and glucose + Fe additions did increase significantly by day 4 and 5, but never reached the magnitude of the DFAA additions. By day 4, bacterial production in the glucose + Fe treatment was not significantly different from the DFAA treatments (**Fig. 16**). The addition of glucose alone never resulted in substantial increases in rates of production relative to the control.

Bacterial Growth Rates

DFAA treatments resulted in substantial increases in bacterial growth rates. Thymidine incorporation per cell increased by a factor of 10 between day 2 and day 3 in both DFAA and DFAA + Fe treatments (**Fig. 14e**). By day 4 incorporation per cell declined substantially, dropping to rates similar to those measured in the control treatments. No statistical differences in rates of isotope incorporation per cell were observed between DFAA and DFAA + Fe treatments. The addition of glucose alone had no significant effect on thymidine incorporation per cell over the 5 days of the experiment. By day 4 and 5 the addition of glucose + Fe resulted in more than a doubling in rates of thymidine incorporation per cell relative to the control and additions of glucose alone. Similar trends are seen in leucine incorporation per cell, where glucose + Fe treatments nearly doubled all other treatments by day 4 (**Fig. 14f**).

Organic matter-iron interactions

No significant differences in the DFAA and DFAA + Fe treatments were observed for any of the measured or calculated growth properties (**Table 5**). Treatments receiving glucose + Fe showed significantly greater rates of ³H-leucine incorporation and leucine incorporation per cell than glucose-alone treatments (**Fig. 14e,f**). No significant differences in cell volume, cell abundance or total biovolume were observed between glucose and glucose + Fe treatments (**Table 5**). Overall, DFAA treatments resulted in considerably larger growth rates than glucose treatments (**Table 3**). However, the combined addition of Fe and glucose did result in larger increases in growth rates than additions of glucose alone (**Fig. 17, Table 3**).

The addition of Fe to DFAA treatments had no measurable effect on growth rates relative to additions of DFAA alone (**Fig. 17**).

Experiment in the Subantarctic Front (51°S 142°E)

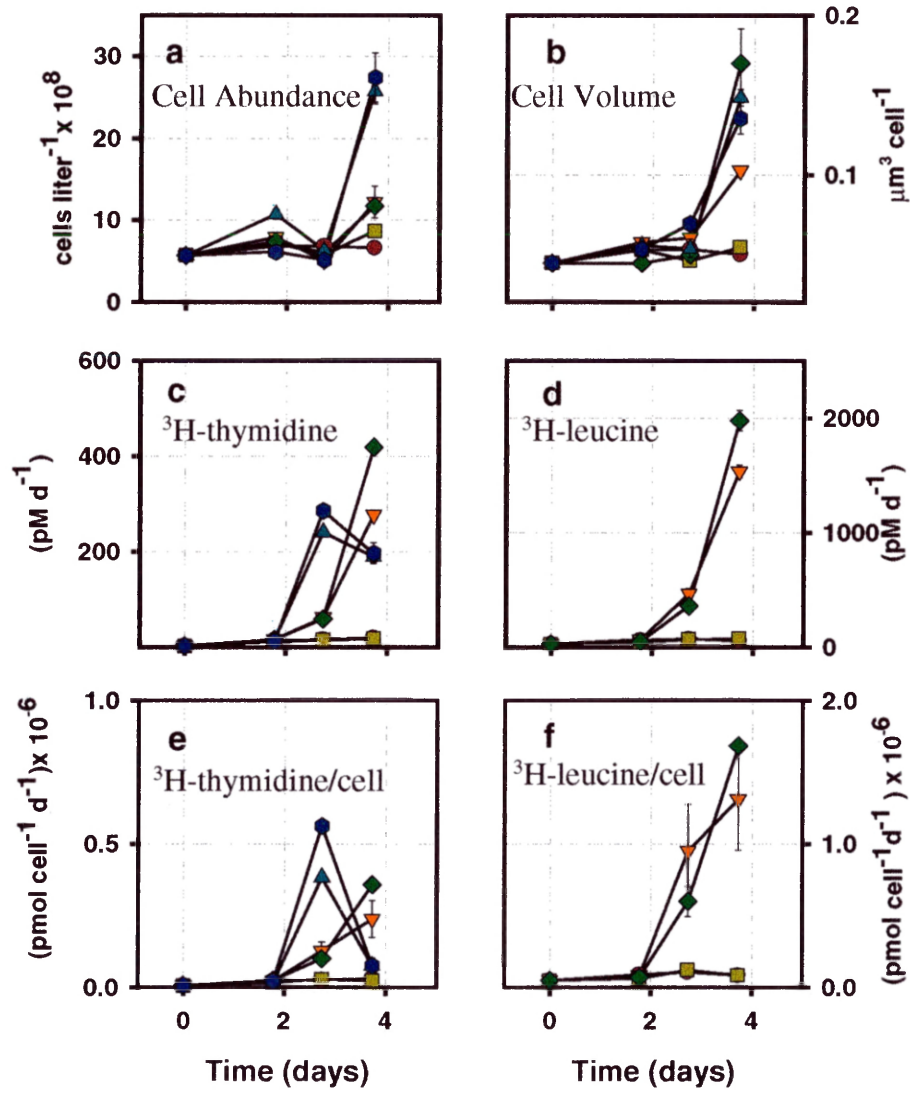
Cellular Abundance, volume and biomass

Bacterial growth in the Subantarctic Front increased in response to amendments of amino acids and glucose (**Figs. 18-21**). Bacterial cell abundance increased in both glucose treatments and both amino acid treatments, but cell abundance in amino acid treatments was roughly twice those of the glucose treatments (**Fig. 18a, Table 6**). No significant differences in cell abundance between Fe and control treatments were observed. Similarly, no significant differences in cell abundance were observed between DFAA and DFAA + Fe or glucose and glucose + Fe treatments.

Mean cell volume increased significantly in the all treatments receiving DOM by day 4. DFAA, DFAA + Fe and glucose + Fe treatments resulted in nearly three times greater cell volumes by day four relative to the control and roughly a five fold increase in cell volume from day 0. Total biovolumes in the DFAA and DFAA + Fe treatments were significantly greater than all other treatments by day 4 (**Table 6**). Total biovolumes in the glucose and glucose + Fe treatments were significantly different from one another. The glucose + Fe treatment resulted in about twice as large an increase in biovolume than the glucose treatment. DFAA additions resulted in significantly greater biomass than all other treatments by day 4. DFAA and DFAA + Fe treatments produced three times more biomass than the glucose treatment and about twice the biomass of the glucose + Fe treatment (**Fig. 19**).

**Figure 18. Responses of various bacterial properties to enrichment treatments in the Subantarctic Front (51°S 141°E).
a.) Cell abundance, b.) Mean cell volume, c.) ³H-thymidine incorporation, d.) ³H-leucine incorporation, e.) ³H-thymidine incorporation per cell, f.) ³H-leucine incorporation per cell. Error bars are standard error of duplicate samples.**

Subantarctic Front (51°S)



- Control
- ▼ Glucose
- Fe
- ◆ Glucose+Fe
- ▲ DFAA
- DFAA+Fe

Table 6. Bacterial Cell Properties from Experiment at 51°S 142°E

Time (days)	Treatment	Bacterial Abund. (cells L ⁻¹) x 10 ⁸	±SE	TdR Incorp. (pmol TdR L ⁻¹ d ⁻¹)	±SE	Cell Volume (µm ³ cell ⁻¹)	±SE	TdR cell ⁻¹ (pmol TdR cell ⁻¹ d ⁻¹) x 10 ⁻⁶	±SE	Bacterial Biovolume (µm ³ L ⁻¹) x 10 ⁸	±SE
0	T=0	5.67		3.18		0.04		0.01			
3	Control	6.82	0.03	A	17.04	0.72	A	0.05	0.00	A	0.03
	Glucose	5.06	0.52	A	62.64	1.44	B	0.06	0.00	A	0.13
	Fe	5.71	0.49	A	15.96	0.84	A	0.05	0.00	A	0.03
	Glucose+Fe	5.99	0.03	A	59.52	8.40	B	0.05	0.00	A	0.10
	DFAA	6.30	0.31	A	240.96	8.88	C	0.05	0.01	A	0.38
	DFAA+Fe	5.09	0.07	A	286.20	4.92	D	0.07	0.00	A	0.56
4	Control	6.58	0.70	A	20.04	1.56	A	0.05	0.00	A	0.03
	Glucose	12.20	1.96	B	277.20	10.08	B	0.10	0.00	B	0.24
	Fe	8.66	0.60	A	18.36	2.28	A	0.05	0.00	A	0.02
	Glucose+Fe	11.70	0.26	B	419.40	0.12	C	0.17	0.02	C	0.36
	DFAA	25.70	1.54	C	190.20	12.12	D	0.15	0.01	C	0.08
	DFAA+Fe	27.40	2.98	C	197.04	21.84	D	0.14	0.01	C	0.08

SE=Standard errors of duplicate samples. Letters indicate statistical evaluation of growth property with letter designations same as Table 4.

Figure 19. Bacterial biomass response to treatments in the Subantarctic Front (51°S 141°E). Shown are days 3 and 4 of the incubation, there were no statistical differences within treatments after days 2. Colors indicate statistically significant ($p < 0.05$) similarities and differences among responses to treatments, colors do not apply to statistical differences between days. Error bars are standard error of duplicate samples.

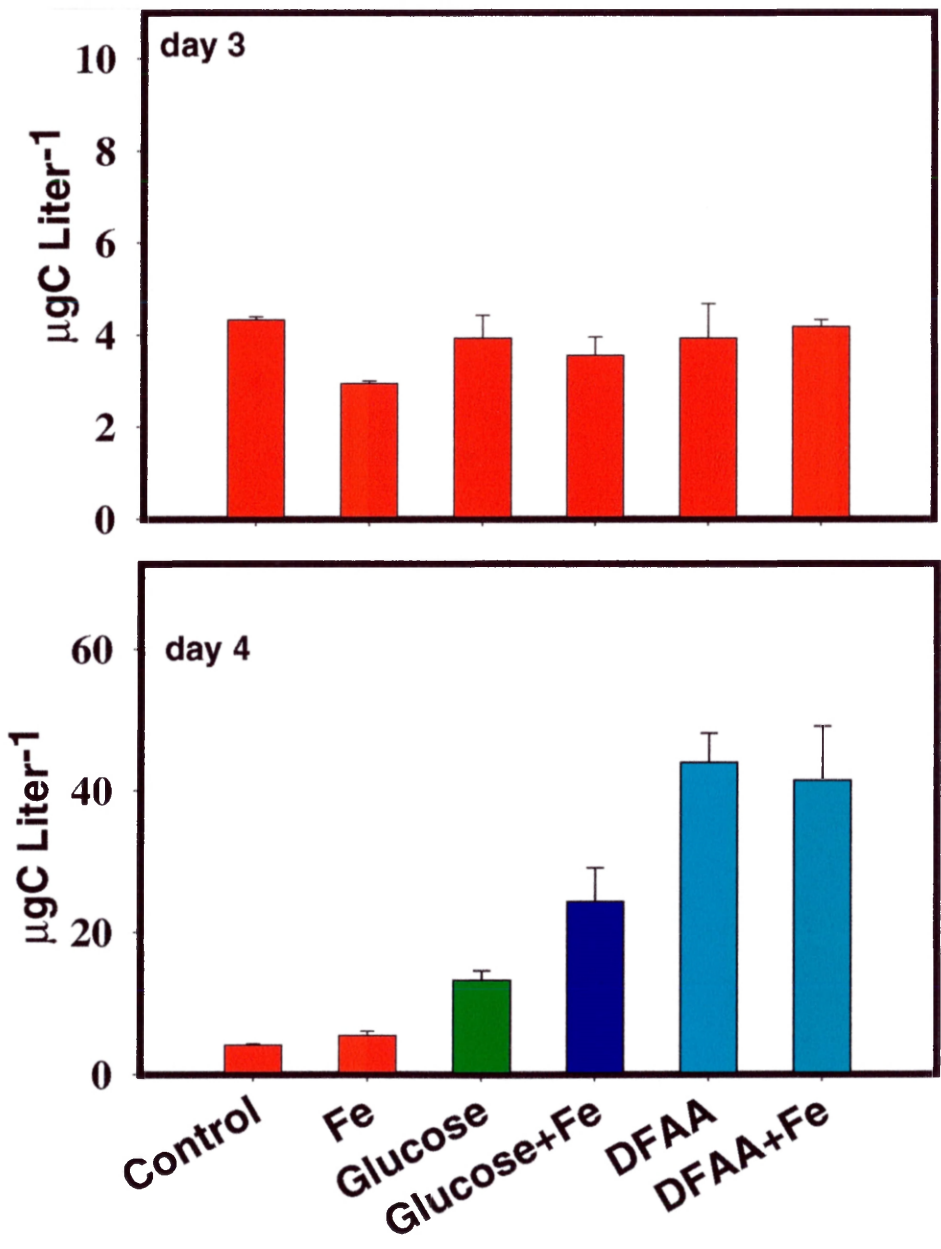


Figure 20. Bacterial production ($^3\text{H-TdR}$) response to treatments in the Subantarctic Front (51°S 141°E). Shown are days 3 and 4. Colors are the same as for Figure 19. Error bars are standard error of duplicate samples.

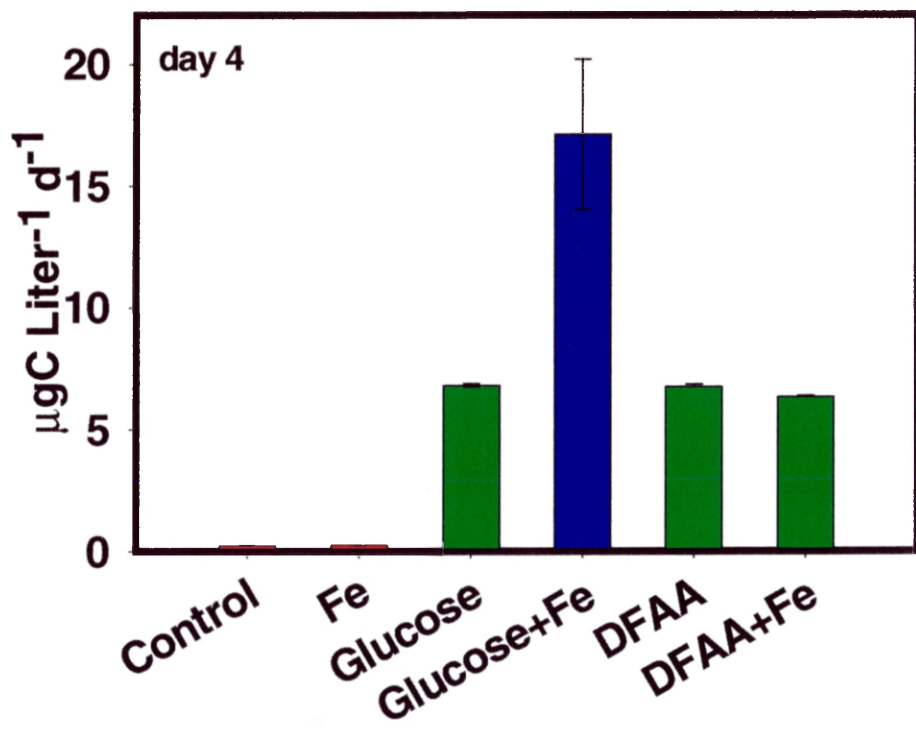
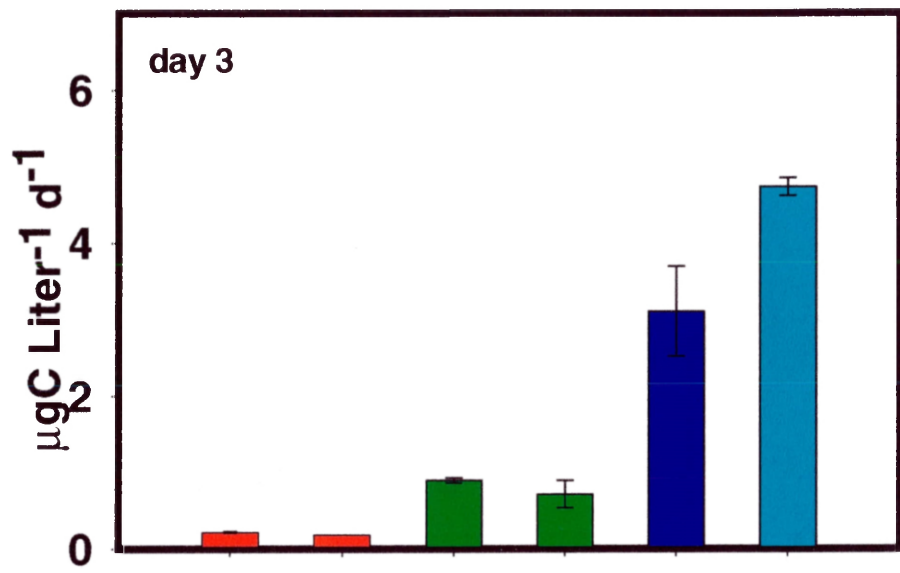
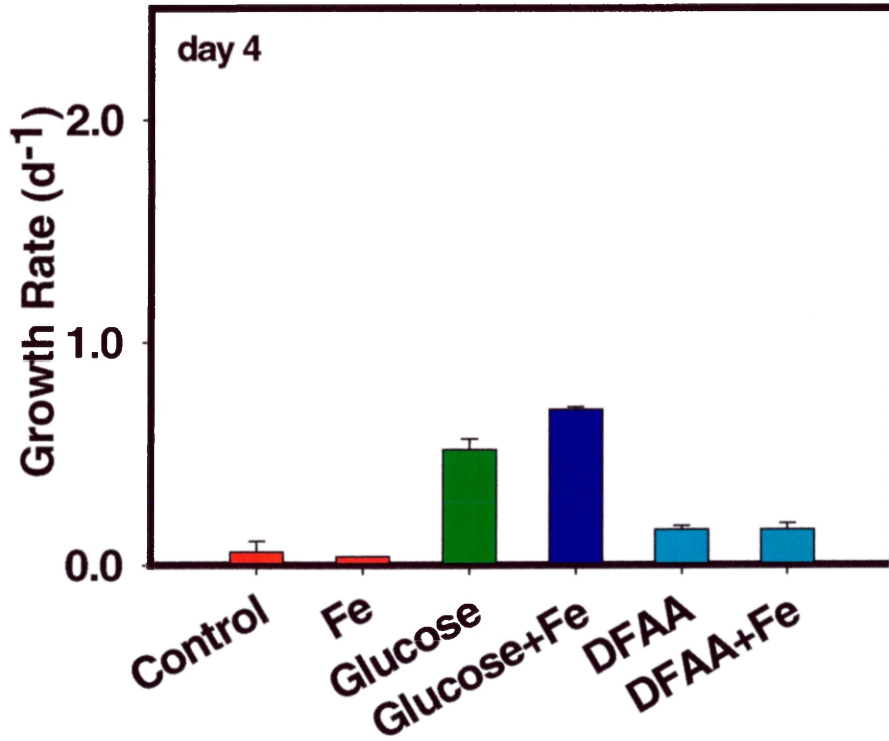
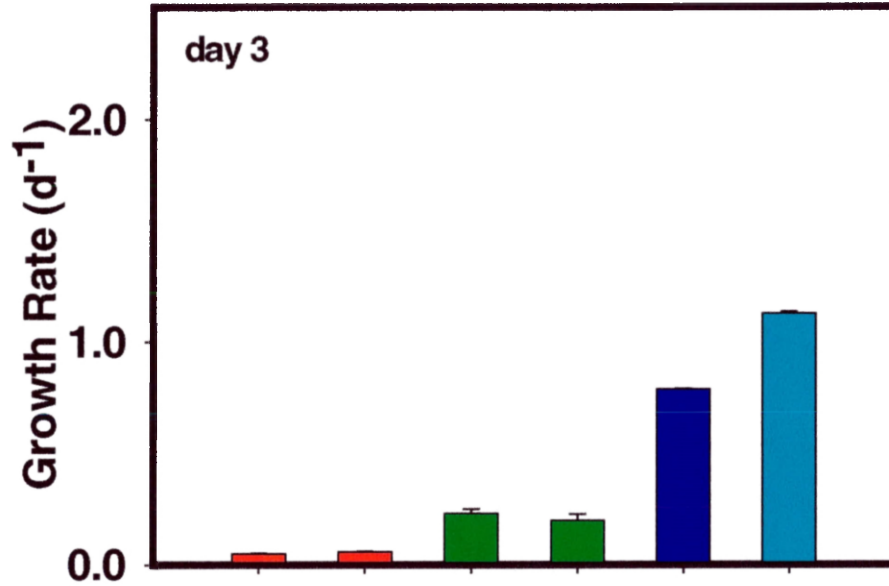


Figure 21. Instantaneous bacterial specific growth rate calculated from thymidine incorporation rates and bacterial biomass (P/B) in the Subantarctic Front (51°S 141°E). Shown are days 3 and 4 of the incubation. Colors are the same as for Figure 19. Error bars are standard error of duplicate samples.



glucose additions increased biomass relative to the control, but biomass increases were still about half the as large as the DFAA treatments at day 4.

Bacterial Production

Rates of thymidine and leucine incorporation ($\text{pmol}^{-1} \text{L}^{-1} \text{d}^{-1}$) varied in time, but overall, the additions of glucose and amino acids stimulated rates of bacterial production (**Fig. 18 c,d, Fig. 20**). DFAA and DFAA + Fe treatments resulted in a seventeen-fold increase in thymidine incorporation relative to the Control and Fe-only additions by day 3. By day 4, however, glucose and glucose + Fe treatments enhanced thymidine incorporation to a greater extent than either DFAA treatment. The glucose + Fe increased rates of thymidine incorporation more than 150% above the glucose treatment, and ~ 215% higher than both DFAA treatments. Leucine incorporation in both glucose treatments increased relative to Control and Fe treatments after day 3 and day 4 (**Fig. 18d**). Rates of leucine incorporation increased most dramatically in the glucose + Fe addition.

Bacterial Growth rates

Thymidine incorporation per cell was substantially higher in the DFAA + Fe treatment after day 3 than in any other treatment. DFAA + Fe resulted in 1700% increases in thymidine per cell relative to control and Fe treatments. DFAA alone stimulated rates of thymidine incorporation per cell more than 1100% times greater than control and Fe-only treatments. Glucose additions did stimulate isotope incorporation per cell, but these changes were most pronounced at day 4. Isotope incorporation per cell in both glucose treatments roughly doubled between day 3 and day 4. Maximal thymidine incorporation rates in DFAA and glucose treatments were

comparable over the duration of the experiment, but increases in these rates were temporally out of phase.

The temporal response of bacterial growth was variable in both the treatment type and the measured cell property. For example, DFAA additions provoked exponential increases in rates of thymidine incorporation through the first three days of the incubation. Glucose treatments resulted in exponential increases in thymidine incorporation through the entire incubation. In contrast, cell abundance and cell sizes in both glucose and DFAA treatments did not increase substantially until day 4. Cell specific incorporation rates in both DFAA and DFAA + Fe treatments jumped ~2000% between day two and day three. By day four rates of incorporation per cell dropped by a factor of five, a result of nearly a 500% increase in cell abundance between the two days (**Fig. 18e, a**).

Organic matter-iron interactions

The experiment conducted in the Subantarctic Front provided additional indications that dissolved iron may play some role in constraining bacterial production in glucose amended experiments. Rates of thymidine and leucine incorporation in glucose + Fe treatments were significantly greater than rates measured in the glucose-alone addition. No statistical differences in cell abundance were observed for either glucose treatment at any of the time points; however, cell volume in the glucose + Fe addition was significantly greater than the glucose addition after day 4. The net result of increased cell size in the glucose + Fe addition was the accumulation of nearly twice as much as biomass as the glucose-alone addition by the end of the experiment (**Fig. 19**).

Experiment in the Antarctic Polar Front (54°S 141°E)

The most striking feature of the experiment conducted in the Antarctic Polar Front (54°S) is the lack of bacterial response to any of the treatments. Bacterial abundance, cell volume and rates of isotope incorporation all showed slight increases in every treatment with time, but none of the measured cell properties changed significantly relative to one another (**Fig. 22, Table 7**). Initial biomass at day 0 was lower than at any other station by a factor of three, while rates of thymidine and leucine incorporation at time zero were nearly the same as observed at the SAF (**Figs. 5-8**). Thymidine incorporation per cell ranged between 0.01 and 0.03 x 10⁶ pmol cell⁻¹ d⁻¹ for all treatments, over all time points.

Role of Temperature in Growth Limitation

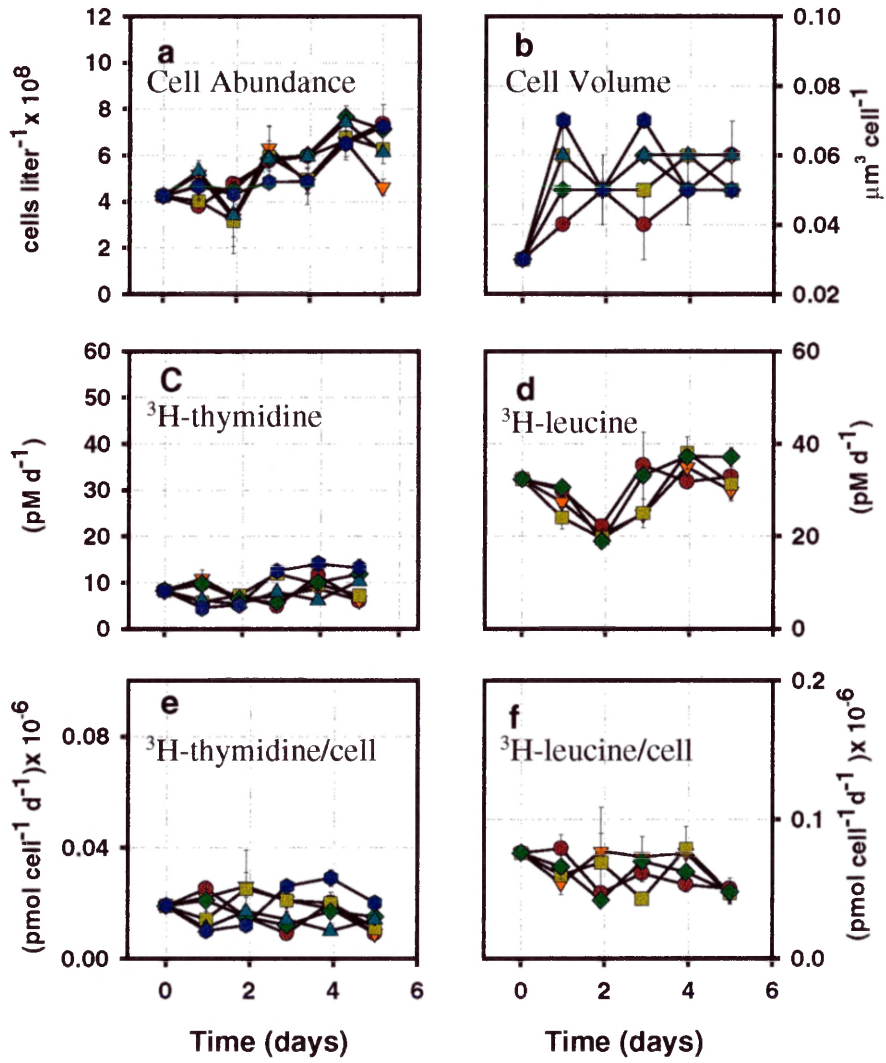
Bacterial growth in the Antarctic Polar Front did not appear limited by amino acids, glucose, iron or combinations of these treatments over the time scale of my observations. In an effort to determine the factors contributing to the relatively low rates of production and standing biomass measured at the Antarctic Polar Front, I performed an additional manipulation experiment to test the hypothesis that bacterial growth might be constrained by temperature or a combination of temperature and organic substrate (**Fig. 23**).

As in the previous experiment, cell abundance changed very little over the entire experiment. Changes in cell volume were not apparent until day four when cells in the glucose (7.5°C) treatment were significantly larger than the other

Figure 22. Responses of various bacterial properties to enrichment treatments in the Antarctic Convergence (54°S 141°E).

a.) Cell abundance, b.) Mean cell volume, c.) ³H-thymidine incorporation, d.) ³H-leucine incorporation, e.) ³H-thymidine incorporation per cell, f.) ³H-leucine incorporation per cell. Error bars are standard error of duplicate samples.

Antarctic Convergence (54°S)



- Control
- ▼— Glucose
- Fe
- ◆— Glucose+Fe
- ▲— DFAA
- DFAA+Fe

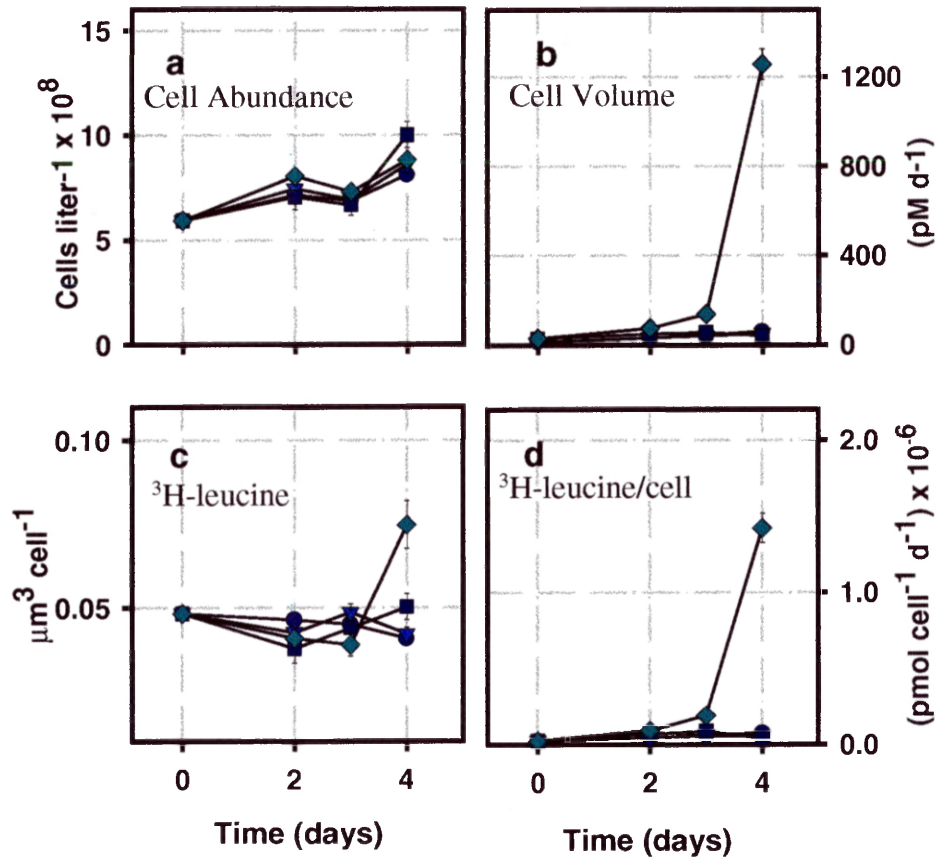
Table 7. Bacterial Cell Properties from Experiment at 54°S 141°E

Time (days)	Treatment	Bacterial Abundance (cells Liter ⁻¹) x 10 ⁸	±SE	Thymidine Incorporated (pmol TdR Liter ⁻¹ d ⁻¹)	±SE	Cell Volume (μm ³ cell ⁻¹)	±SE	TdR cell ⁻¹ (pmol TdR cell ⁻¹ d ⁻¹) x 10 ⁶	±SE	Bacterial Biovolume (μm ³ Liter ⁻¹) x 10 ⁸	±SE
0	T=0	4.25		8.20		0.03		0.02	0.00	0.14	
3	Control	5.76	0.62	4.92	0.12	A	0.04	0.01	0.00	A	0.08
	Glucose	3.53	0.73	6.96	0.00	B	0.05	0.02	0.00	A	0.02
	Fe	5.95	1.28	12.00	0.72	C	0.05	0.02	0.01	A	0.07
	Glucose+Fe	4.78	0.29	5.64	0.36	A	0.06	0.01	0.00	A	0.02
4	DFAA	5.85	0.77	7.92	0.48	B	0.06	0.01	0.00	A	0.03
	DFAA+Fe	4.84	0.18	12.48	0.96	C	0.07	0.03	0.00	B	0.02
4	Control	5.96	0.29	11.52	1.68	A	0.05	0.02	0.00	A	0.01
	Glucose	4.79	0.91	8.64	1.44	B	0.06	0.02	0.00	A	0.06
	Fe	4.94	0.55	9.60	0.00	B	0.06	0.02	0.00	A	0.04
	Glucose+Fe	6.01	0.22	9.96	0.36	B	0.05	0.01	0.00	A	0.04
5	DFAA	5.95	0.25	6.12	0.36	C	0.06	0.01	0.00	A	0.01
	DFAA+Fe	4.87	0.54	14.04	0.36	A	0.05	0.03	0.00	B	0.06
5	Control	6.64	0.01	6.00	0.00	A	0.06	0.01	0.00	A	0.04
	Glucose	6.60	0.81	6.12	0.36	A	0.06	0.01	0.00	A	0.07
	Fe	6.77	0.50	7.20	0.48	B	0.05	0.01	0.00	A	0.01
	Glucose+Fe	7.66	0.14	11.76	0.48	C	0.05	0.02	0.00	A	0.00
5	DFAA	7.44	0.71	10.32	0.00	B	0.06	0.01	0.00	A	0.01
	DFAA+Fe	6.51	0.56	13.20	1.92	C	0.05	0.02	0.00	A	0.05

SE=Standard errors of duplicate samples. Letters indicate statistical evaluation of growth properties with letter designations same as Table 4.

**Figure 23. Responses of various bacterial properties to temperature and substrate manipulation in the Antarctic Convergence (55°S 142°E).
a.) Cell abundance, b.) ³H-leucine incorporation, c.) Mean cell volume, d.) ³H-leucine incorporation per cell. Error bars are standard error of duplicate samples.**

Antarctic Convergence (55°S)



treatments (**Fig. 23c**). Leu incorporation increased dramatically in the glucose (7.5°C) treatment for the first three days of the experiment, while other treatments remained statistically indistinguishable. After four days of incubation, rates of ^3H -leucine incorporation in the glucose (7.5°C) treatment were more than thirty times greater than the control at the same temperature and more than twenty times greater than the glucose treatment at 4.4°C (**Fig. 23b**). ^3H -leucine incorporation per cell increased more than fifty times the initial rate and was ~3500% higher than the control at the same temperature (7.5°C). Calculated growth rates (P/B) increased from 0.01 d⁻¹ at day 0 to 0.5 d⁻¹ in the glucose (7.5°C) treatment, while the glucose (4.4°C) and control (7.5°C) treatments remained statistically unchanged from day 0 over the course of the experiment. The effects of the glucose (7.5°C) treatment at the Antarctic Convergence were similar to the effects of glucose additions in SAF where water temperatures were similar. Cell abundance, cell volume, rates of leucine incorporation, and leucine incorporation per cell in the glucose (7.5°C) treatment at day 4 were nearly equivalent to those measured in the glucose treatments in the SAF at day 4. Growth responses in this experiment appeared to be severely limited by temperature below 7.5°C in the Antarctic Polar Front.

Discussion

Predominance of DOM Stimulation of Bacterial Growth

Based on these experiments, bacterial production, biomass, and growth rates in the Southern Ocean appeared to be controlled primarily by glucose or DFAA availability. Interactive factors including dissolved iron and temperature may have been important in constraining complete utilization of labile dissolved organic matter,

but the primary limitation to bacterial growth was availability of labile organic substrates. These experiments indicate bacterial growth rates were constrained by inputs of both glucose and DFAA, while bacterial biomass production was more tightly coupled to the input of DFAA or glucose + NH_4^+ + PO_4^{3-} .

The most pronounced trend in three of the four studies was that the addition of dissolved organic matter, either as glucose or amino acids, enhanced rates of bacterial growth and resulted in net increases in bacterial abundance, production and biomass. Additions of glucose and amino acids consistently resulted in increased production and accumulation of larger cells than control treatments (**Table 3, Tables 4-6**). Ten out of the sixteen treatments receiving DOM additions showed significant increases in bacterial abundance and biomass yields. Eleven out of the sixteen treatments receiving either glucose or DFAA had significant increases in net accumulation rates (abundance) relative to the control and Fe treatments (**Table 3**). Mean rates of thymidine incorporation per cell in glucose and DFAA treatments were ~300% higher than control treatments in three of the four experiments. Twelve of the sixteen treatments receiving glucose or DFAA showed enhanced thymidine incorporation relative to the control treatments (**Tables 4-6**). Finally, large increases in specific growth rates (P/B) were seen in nearly all the DOM additions.

Patterns of DOM Utilization

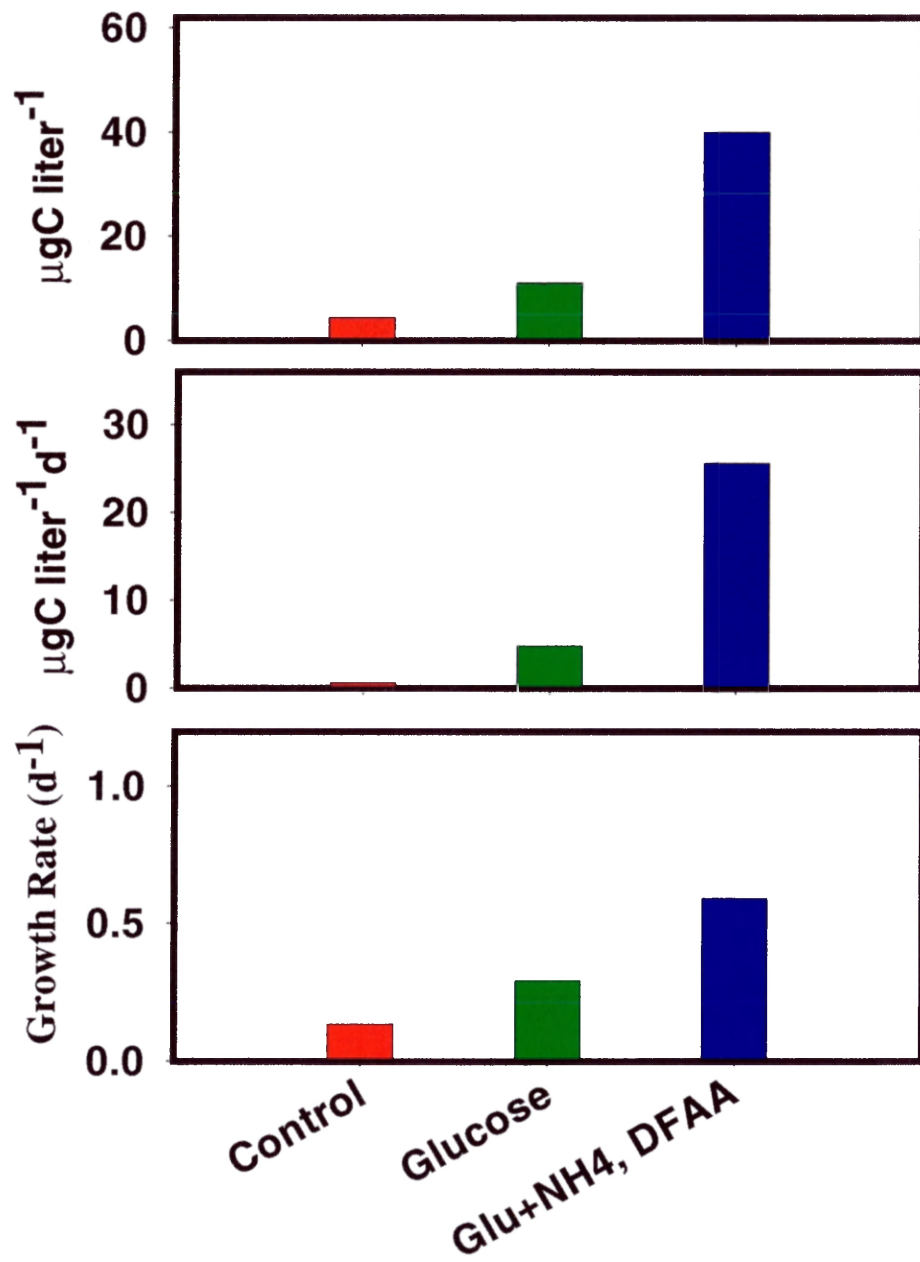
Patterns of bacterial growth changed in response to the types of organic substrates provided in these treatments. Enrichments of dissolved organic nitrogen (DFAA) or glucose + NH_4^+ + PO_4^{3-} stimulated rates of bacterial growth and production of biomass to a greater extent than additions of glucose alone. Six of the eight treatments receiving either DFAA or glucose + NH_4^+ + PO_4^{3-} resulted in larger

maximal specific growth rates (P/B) and produced between 2 and 25 times more biomass than glucose and glucose + Fe treatments (**Table 3, Figs. 11, 13, 15, 17, 19, 21**). Rates of bacterial production in DFAA treatments were larger than treatments receiving glucose alone (**Fig. 24**). Mean bacterial biomass, production and growth rates were larger in those treatments receiving DFAA or glucose + NH_4^+ + PO_4^{3-} than those treatments receiving glucose alone (**Fig. 24**). In two of the four experiments, maximal rates of thymidine incorporation occurred on either DFAA or glucose + NH_4^+ + PO_4^{3-} . DFAA treatments in the SAF yielded rates of production that were ~400% higher than all other treatments in any of the experiments.

These results can be interpreted several different ways. First, bacterial growth rates in the Southern Ocean may simply be limited by the availability of reduced organic nitrogen. Alternatively, DFAA or glucose + NH_4^+ + PO_4^{3-} substrates may increase the efficiency of bacterial growth to a greater extent than growth on glucose alone. Finally, bacterial growth in the Southern Ocean may be constrained by *energy* rather than by one particular element. The energy limitation hypothesis assumes that amino acids are directly assimilated as ready-made cellular building blocks, resulting in cellular energy conservation by circumventing the cell's need to expend energy in construction of amino acids from simple carbon, nitrogen and phosphorus substrates (Cherrier et al. 1996, Kirchman 1990, Kirchman et al. 1990).

Clearly it would be an oversimplification to state that bacterial growth in the Southern Ocean is limited simply by carbon or by nitrogen. Similar to Cherrier et al. (1996) in the Eastern North Pacific and Kirchman (1990) in the Subarctic Pacific, we observed that combined additions of organic carbon and reduced nitrogen stimulated

Figure 24. Bacterial response to various enrichment treatments. Calculated cell properties averaged by treatment type and over time. Mean values of a.) biomass, b.) bacterial production (^3H -TdR), and c.) growth rates taken from all time points where significant responses between treatments were observed. Mean values for all control treatments, all treatments receiving glucose only, and those treatments receiving either glucose+ NH_4^+ + PO_4^{3-} or DFAA.



bacterial growth rates. However, the results of this study are more complicated than Kirchman's (1990) and Cherrier et al. (1996). Additions of DFAA or glucose + NH_4^+ + PO_4^{3-} in this study generally resulted in the largest increases in bacterial growth rates and production. However, additions of glucose and glucose + Fe also frequently resulted in increases in bacterial growth rates and increased biomass production. Labile organic matter primarily controlled bacterial growth in the Southern Ocean, however, specific limitation by the combination of reduced nitrogen and DOM was important in three of the four experiments.

Reduced nitrogen treatments may have increased bacterial growth efficiency, providing a possible explanation for the increased responses of bacterial growth to DFAA and glucose + NH_4^+ + PO_4^{3-} treatments. The efficiency at which bacteria convert DOM into biomass may be dependent on the nutritional quality of the substrate. Goldman et al. (1987) and Goldman and Dennett (1991) found no differences in BGE using laboratory enrichment cultures grown on DFAA or glucose + NH_4^+ as substrates provided the substrates were added in equimolar C : N ratios. They estimated growth efficiency on various organic carbon and nitrogen substrates and found a range between ~ 40-95%, with lower conversion efficiency on higher C : N substrates. Carlson and Ducklow (1996) estimated BGE in their amendment experiments in the Sargasso Sea and found a range of 4-30% for both DFAA and glucose treatments. Cherrier et al. (1996) found that DFAA treatments yielded considerably higher assimilation efficiencies (~9%) than other substrates (0.5-4.2%). Kirchman (1990) estimated BGE of ~34% for bacteria grown on DFAA in the Subarctic Pacific.

Bacterial utilization of reduced nitrogen substrates may increase BGE and result in larger growth rates and subsequently increase bacterial production relative to growth on simple carbon substrates. In my study four of the six treatments enriched in reduced nitrogen resulted in higher rates of bacterial production than glucose additions (disregarding the results of the experiment in the Antarctic Polar Front). Mean biomass yields and growth rates in the DFAA and glucose + NH_4^+ additions were consistently larger than glucose additions alone (**Fig. 24**). These results hint that bacteria grew less efficiently on glucose than on organic amendments containing reduced nitrogen, resulting in lower production of biomass. When available substrates offer poor nutritional value (such as glucose) bacterial catabolism may proceed at near maximal rates, but cellular synthesis rates may be low. Cells continue to catabolize substrates to provide basic maintenance energy for active transport systems and cell membrane upkeep (del Giorgio and Cole 1998), however, inefficient growth results in consumption and respiration of DOM (glucose), but no measurable increases in cell production or growth. Additions of reduced nitrogen (either as DFAA or NH_4^+) may have alleviated reduced nitrogen limitation and increased bacterial growth efficiency.

One reason bacterial growth in the Southern Ocean may be limited by reduced nitrogen is that the available DOM pool may be of poor nutritional quality (e.g. high C:N). Phytoplankton production is the predominant source of DOM to the surface waters in the open ocean. Phytoplankton growth in the Southern Ocean is at least partially constrained by low iron concentrations (Martin et al. 1990, Martin et al. 1991). One of the consequences of iron limitation to phytoplankton growth is a decrease in the nitrate uptake capacity of phytoplankton (Martin and Fitzwater 1988).

Martin et al. 1990, Dugdale and Wilkerson 1991). Phytoplankton stressed by their inability to assimilate nitrate might produce particulate and dissolved organic material rich in carbon and poor in nitrogen (Goldman et al. 1992, Smith et al. 1998). Thus, the bacterial response to glucose + NH_4^+ or DFAA in the Southern Ocean may be indirectly linked to iron limitation of the phytoplankton community, which results in production of high C : N DOM.

The major difference between my study in the HNLC region of the Southern Ocean and similar studies in the HNLC subarctic Pacific (Kirchman 1990, Kirchman et al. 1993) and equatorial Pacific (Kirchman and Rich 1997) was the large increase in cell abundance and biomass in response to DFAA and glucose + NH_4^+ additions. Kirchman (1990) found that additions of DFAA and glucose + NH_4^+ resulted in large increases in bacterial production but only small increases in bacterial abundance (Kirchman et al. 1990, **Figs. 1 and 2 c**). Amendment experiments in the equatorial Pacific indicated DFAA and glucose + NH_4^+ treatments limited rates of production, but had no substantial effect on bacterial abundance (Kirchman and Rich 1997). Removal processes (predation, viral lysis) apparently had sufficient capability to respond to increases in bacterial production.

The addition of model DOM substrates to bacterial incubations in the Southern Ocean frequently resulted in significant increases in bacterial abundance and biomass. In particular, three of the four experiments had large increases in bacterial abundance and biomass in response to DFAA and glucose + NH_4^+ + PO_4^{3-} treatments. In two of the four experiments, glucose and glucose + Fe treatments significantly increased cell abundance over the control treatment but the response was lower than treatments

receiving reduced nitrogen. Furthermore, DFAA and glucose + NH_4^+ + PO_4^{3-} treatments frequently led to production of larger cells. These large changes in cell abundance and mean cell volume produced significant increases in bacterial biomass. Bacterial production, rates of growth, and biomass all responded to input of labile DOM.

DFAA and glucose + NH_4^+ + PO_4^{3-} treatments also resulted in significantly larger maximal growth rates (P/B) than all other treatments despite the large increases in cell abundance and biomass (**Fig. 24**). Increases in the rates of bacterial production in DFAA and glucose + NH_4^+ + PO_4^{3-} treatments were coupled with increases in bacterial biomass. This indicates biomass production and growth rates were a function of the type of substrate that supported bacterial growth. Studies in other HNLC regions have found bacterial growth rates and rates of production are a function of DOM while biomass is tightly constrained by removal processes. In my experiments, additions of DFAA or glucose + NH_4^+ + PO_4^{3-} increased bacterial growth rates enough to escape control by grazers and viruses.

The function of the microbial loop in HNLC oceans

Differences in the apparent coupling between DOM and bacterial biomass in the Southern Ocean versus other HNLC oceans may be a reflection of fundamental differences in the structure of the microbial food webs among the different HNLC systems. **Table 8** lists bacterial biomass, production and growth rates from various ocean and estuarine systems around the world. Clear differences exist in all of these properties between these different systems. This indicates the mechanisms that control bacterial stocks and rates differ between the different HNLC regions. Results

of this study indicate bacterial biomass and production are not unlike those measured in the Ross Sea, Antarctica. Surprisingly, bacterial biomass in this study was considerably lower than biomass measured in the other two HNLC oceans. Rates of production and growth in this study appear roughly similar to those measured in the equatorial Pacific, where water temperatures are considerably higher.

The microbial loop may function differently in the Southern Ocean than it does in the other HNLC oceans. Kirchman (1990) and Kirchman and Rich (1997) observed a close coupling between bacterial growth and removal in the equatorial Pacific and subarctic Pacific despite additions of labile DOM. In the Southern Ocean my experiments indicated that increased fluxes of DOM could result in increased production of bacterial biomass, indicative of a temporal uncoupling between growth and removal. Unlike the equatorial and subarctic Pacific, bacterial consumption of DOM in the Southern Ocean appears to act as a net sink for organic carbon. On the time scale of these observations, bacterial growth and grazer removal rates in the DFAA additions were temporally uncoupled, potentially resulting in inefficient recycling of bacterial biomass through the microbial food web.

The temporal response of bacterial growth properties provides further indications that DFAA and glucose + NH_4^+ treatments increased growth rates enough to exceed grazer removal. Cell growth rates and thymidine incorporation per cell increased one or two days prior to significant increases in cell abundance. The temporary “shift-up” in growth rates in response to DFAA treatments was large enough to overcome removal rates. Treatments containing glucose only resulted in

Table 8. Bacterial biomass, production and growth rates in various oceanic and estuarine locations

<u>Region</u>	<u>Biomass</u> ($\mu\text{gC liter}^{-1}$)	<u>Production</u> ($\mu\text{gC liter}^{-1} \text{d}^{-1}$)	<u>Growth</u> <u>Rate</u> (d^{-1})	<u>Study</u>
<i>Ross Sea, Antarctica</i>	4.8	1.2	0.25	Carlson et al. 1998
<i>HNLC Equatorial Pacific</i>	11.3	1.8	0.15	Ducklow et al. 1992
<i>HNLC Subarctic Pacific</i>	14.3	0.7	0.05	Kirchman et al. 1993
<i>HNLC Southern Ocean</i>	6.0	1.1	0.19	This Study
<i>York River, VA.</i>	28	48	1.6	Schultz and Ducklow, in prep.

substantially lower maximal growth rates, often too low to overcome removal rates, resulting in considerably lower bacterial abundance and biomass yields.

Figures 25 and **26** show a schematic representation of the dependence of the microbial loop on the type of DOM available to bacterioplankton. In **Figure 25**, bacteria respond to additions of high C : N ratio substrates (such as glucose) with moderate increases in cell growth rates, but small increases in total biomass. Presumably, bacterial growth on the poor quality DOM (high C : N ratio) proceeds with low efficiency, and biomass accumulation is restricted as a result of the close coupling between growth and removal processes. **Figure 26** depicts a scenario where low C : N ratio substrate (DFAA) becomes available to bacteria and bacterial growth efficiency is high. The result is a large increase in bacterial growth rates and significant increases in cell size and abundance. Over the time scales of these observations (4-5 days) large increases in bacterial biomass occurred as a function of the substrate type and the relative efficiency that bacteria converted DOM into biomass.

Co-limitations: DOM, Iron and Temperature

Additions of iron alone did not significantly increase bacterial growth rates above the control in any experiment (**Table III**). However, combined additions of glucose and iron frequently resulted in significantly larger maximal growth rates, rates of production and biomass (see **Figs. 15, 16, 18, 19, 20**). In three of the four experiments, glucose alleviated primary growth limitations, while in two of the four

Figure 25. Schematic of the Southern Ocean microbial loop where DOM inputs are restricted to high C : N ratio material (glucose enrichments). Bacterial growth and removal terms are roughly balanced. Bacterial cell volumes often increased; however, cell abundance and biomass remained relatively unchanged over the course of the incubation.

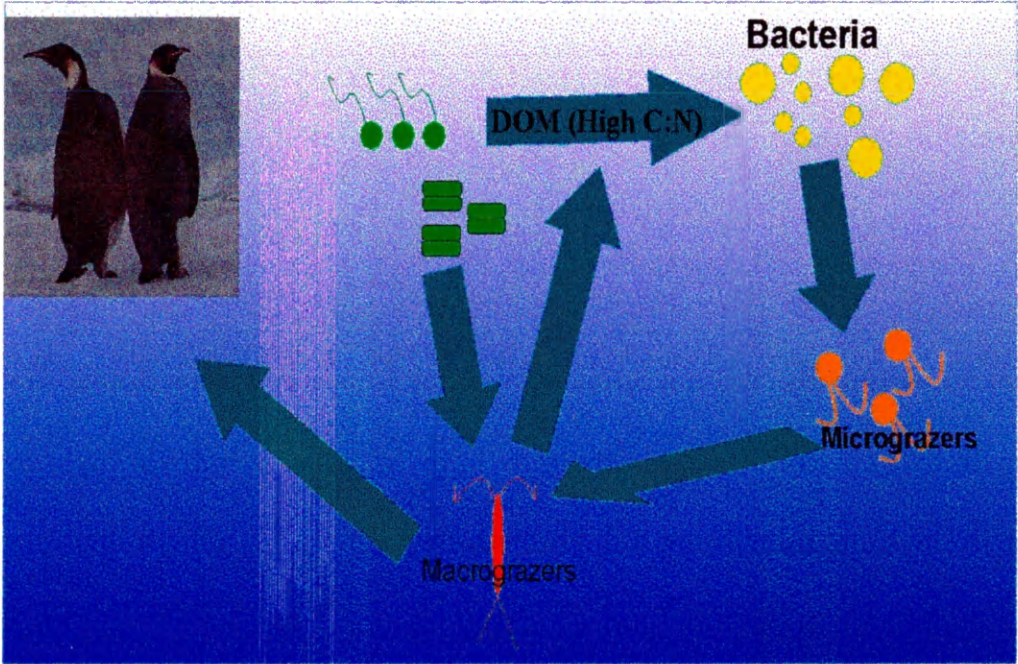
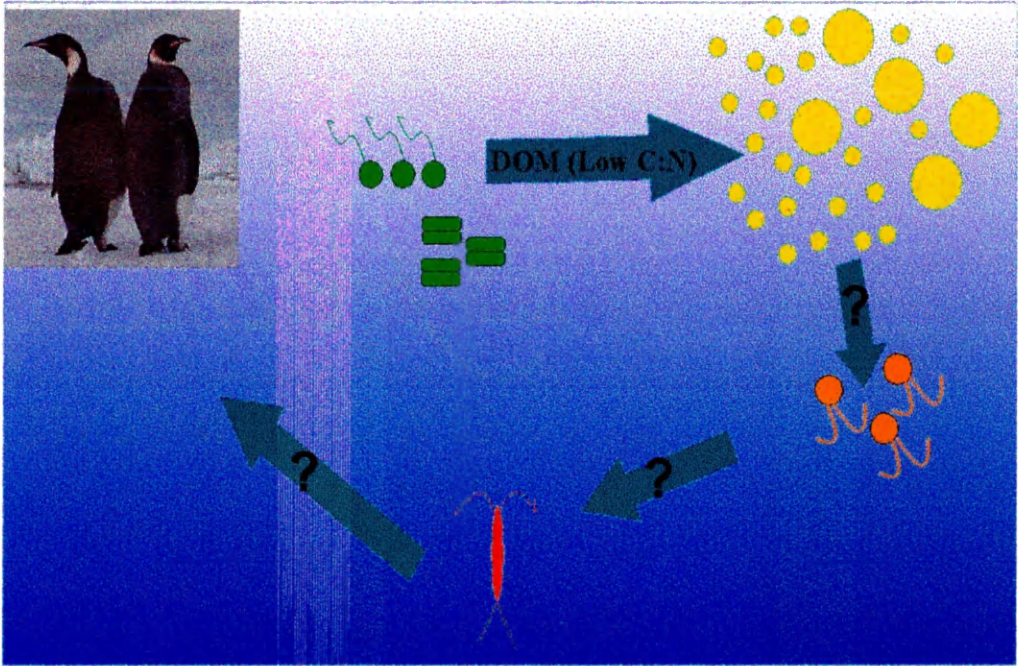


Figure 26. Schematic of the Southern Ocean microbial loop where DOM inputs are in the form of low C : N ratio material (DFAA or glucose + NH_4^+ enrichments). Bacterial growth rates increase and growth exceeds removal. Cell volumes and cell abundance often increased resulting in substantial increases in bacterial biomass. Over the time scales of these experiments, additions of low C : N DOM resulted in an uncoupling of bacterial growth and removal.



experiments the combined additions of dissolved iron and glucose resulted in a greater proportion of the glucose addition appearing as bacterial biomass. No increases in growth rates, rates of production or total biomass were seen in any of the DFAA + Fe versus DFAA treatments.

Although I did not measure BGE directly, based on growth yields and estimates of bacterial growth rates, it appears that combined additions of iron and glucose increased BGE relative to treatments receiving glucose alone. Iron appeared to have no significant effect on bacterial growth in DFAA treatments. Bacterial growth responses to DFAA + Fe and glucose + Fe treatments suggests that BGE in the DFAA treatments were already near maximal levels, while bacterial growth efficiencies in the treatments where glucose-alone was added may have been substantially lower. Iron additions did not alleviate primary growth limitations caused by poor quality DOM.

These results are consistent with Tortell et al. (1996), who found that bacterial growth efficiency could be limited by the availability of iron. Reduced iron is a vital component of the electron transport chain in the respiratory system of heterotrophic bacteria. The bacterial electron transport chain transfers electrons generated by oxidation of organic substrates (Gottschalk 1979). In iron limited systems such as the HNLC Southern Ocean, bacterial cells may lack iron required by the electron transport chain, thereby reducing the potential energy yield produced by electron chain phosphorylation. Iron limitation may decrease cell efficiency and result in ineffective utilization of energy produced through metabolism. Additions of iron to glucose

treatments may have increased bacterial conversion efficiency of glucose derived energy, allowing the cells opportunity to increase biomass production rates.

Temperature and DOM interacted to limit bacterial growth in the APF. Bacterial growth in the APF showed no enhancement due to organic enrichments, implying that some factor other than DOM exerted specific control over rates of bacterial biomass production. Over the course of a four-day incubation no significant change in bacterial growth in the glucose-enriched treatment occurred at *in situ* temperatures. Growth rates increased substantially in response to glucose at the elevated temperature. Furthermore, cell sizes increased in the glucose treatment at elevated temperatures.

There are several possible scenarios to account for the dependence of bacterial growth on both temperature and DOM. First, bacterial growth on substrates at lower temperatures may be less efficient than growth on the same substrates at higher temperatures. Cells at lower temperatures continue to metabolize available substrates, but the ATP derived from metabolism may be applied to basic cell maintenance such as active transport systems rather than growth (Farrell and Rose 1967). Alternatively, cellular metabolism may simply work at slower rates in cold waters. This implies that the bacterial community is not adequately adapted to maximize growth in the colder waters. Finally, lower temperatures may affect rates of substrate acquisition and uptake. Permease proteins and the cytoplasmic membrane undergo conformational changes at sub-optimal temperatures (Farrell and Rose 1967), resulting in strong temperature dependence of the substrate transport systems.

The Antarctic Polar Front marks the confluence of warm Subantarctic water and cold polar water. Bacterioplankton sampled in the Front may have adapted to the higher water temperatures characteristic of the subantarctic waters. Bacteria adapted to warmer water would show optimal rates of growth at temperatures greater than *in situ* temperatures found in the APF. Further South, bacteria may be adapted to the perennially cold waters and bacterial response to glucose amendments might be expected to occur more rapidly. Whatever the case, bacterial growth in the APF was limited by some combined interaction between DOM and temperature.

Conclusions

The results of these experiments suggest that bacterial growth in the Southern Ocean may be limited by organic matter quality (i.e. C : N). Moreover, these results indicate that both glucose and DFAA may contribute to fluxes of organic material through bacterioplankton in the Southern Ocean. Bacterial growth responded to enrichments of glucose and DFAA at the STC and the SAF. Experiments in the SAZ and SAF indicated that combined additions of dissolved iron and glucose resulted in larger growth rates and biomass production than treatments receiving glucose alone.

I hypothesize that no single factor limited rates of bacterial growth in the Southern Ocean. Rather a myriad of factors that include carbon, nitrogen, temperature and iron combined to restrict the growth of heterotrophic bacteria. Growth limiting factors may stem from an array of resource limitation across several trophic levels whose combined effect is to reduce *in situ* bacterial growth rates. Bacterial growth in the HNLC Southern Ocean appeared limited by inputs of dissolved organic nitrogen and carbon. However, in the APF the combination of DOM and temperature may interact to limit *in situ* growth. In the SAF and SAZ,

additions of carbon, nitrogen and iron may all interact to control bacterial stocks and rates of bacterial growth. Additions of ammonium and DFAA typically enhanced bacterial growth rates and biomass yields, implying that bacterial growth is at least in part controlled by the availability of reduced nitrogen.

Perhaps the most distinguishing feature of my study relative to other studies on bacterial growth in HNLC oceans was my observation that bacterial biomass accumulated in response to additions of DFAA and glucose + NH_4^+ + PO_4^{3-} . Additions of reduced nitrogen stimulated bacterial growth rates more so than additions of glucose. Nitrogen limitation of bacterial growth may result from the input of carbon-rich organic material by iron limited phytoplankton. The release of bacterioplankton from reduced nitrogen limitation in my experiments resulted in large increases in bacterial growth rates and concurrent uncoupling of growth and removal processes. Bacterial growth in the Southern Ocean appears to be constrained predominantly by organic matter and the availability of reduced nitrogen. These results indicate top-down pressure on bacterial growth in the Southern Ocean may be fundamentally different than other HNLC oceans. Future investigations need to explore the linkages between growth and grazing and how these processes respond to nutrient and organic material fluxes. Additionally, direct quantification of bacterial growth efficiencies are required to evaluate how bacterial cells in the Southern Ocean respond to carbon and nitrogen rich substrates. Finally, an evaluation of the linkages between bacterial growth efficiencies, dissolved iron and temperature would help constrain microbial food web processes in the Southern Ocean.

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Vita

Born in Buffalo, New York, December 27, 1970. Attended The Evergreen State College in Olympia, Washington from 1989-1994. Worked from 1994-1996 as a research technician at the Bermuda Biological Station for Research. Entered M.S. program of the School of Marine Science, Virginia Institute of Marine Science, College of William and Mary in August of 1996. Successfully defended M.S. thesis on April 15, 1999. Plans to pursue a Ph.D. in marine microbial ecology in the fall of 1999.