The Microbial Fate of Carbon in High-Latitude Seas:
Impact of the Microbial Loop on
Oceanic Uptake of CO_2

by

Patricia L. Yager

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Chairperson of Supervisory Committee

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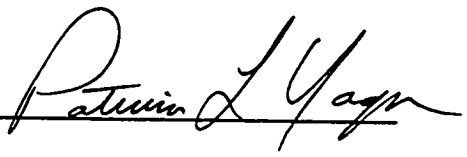
Signature 

Date 2/29/96

UNIVERSITY OF WASHINGTON

Abstract

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by Patricia L. Yager

Chairperson of the Supervisory Committee: Professor Jody W. Deming
School of Oceanography

This dissertation examines pelagic microbial processes in high-latitude seas, how they affect regional and global carbon cycling, and how they might respond to hypothesized changes in climate. Critical to these interests is the effect of cold temperature on bacterial activity. Also important is the extent to which marine biological processes in general impact the inorganic carbon cycle. The study area is the Northeast Water (NEW) Polynya, a seasonally-recurrent opening in the permanent ice situated over the northeastern Greenland continental shelf. This work was part of an international, multi-disciplinary research project studying carbon cycling in the coastal Arctic.

The first chapter describes a simple model which links a complex marine food web to a simplified ocean and atmosphere. This model is designed to test the sensitivity of the air-sea flux of carbon to microbial food web structure and behavior, particularly those processes which might be sensitive to warming. Preliminary results suggest that organisms can impact short term air-sea carbon flux.

The second chapter investigates the inorganic carbon inventory of the summertime NEW Polynya surface waters to establish the effect of biological processes on the air-sea pCO_2 gradient. A unique one-way sink for atmospheric carbon is hypothesized for the NEW Polynya and other seasonally-ice-covered seas. If this type of one-way carbon sink occurs on a global scale, it may provide a significant feedback to greenhouse warming.

The third and fourth chapters use a kinetic approach to examine microbial activities in the NEW Polynya as a function of temperature and dissolved organic substrate concentration, testing the so-called "Pomeroy hypothesis" that microbial activity is disproportionately reduced at low environmental temperatures owing to increased organic substrate requirements. With field experiments, responsive microbial communities of mostly psychrophilic (cold-loving) organisms were often found to exhibit high affinities to and high incorporation efficiencies on nitrogen-rich organic matter; their response to temperature was heterogeneous, however, indicating that controls on microbial behavior may not be as simple as previously believed. Together, the suite of data collected on microbial activities, cell size, and grazing pressure suggest how unique survival strategies adopted by an active population of high-latitude bacteria may contribute to, rather than detract from, an efficient biological carbon pump.

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PREFACE

". . . the professional duty of a scientist confronted with a new and exciting theory is to try to prove it wrong. That is the way science works. That is the way science stays honest. Every new theory has to fight for its existence against intense and often bitter criticism. Most new theories turn out to be wrong, and the criticism is absolutely necessary to clear them away and make room for better theories. The rare theory which survives criticism is strengthened and improved by it, and then becomes gradually incorporated into the growing body of scientific knowledge."

–F. Dyson (1988) from *Infinite in All Directions*

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Words do not well express the sincere appreciation and admiration that I have for my advisor, Dr. Jody Deming. Her faith and encouragement provided a world where I could safely develop the strength to trust myself and my ideas; her scientific vision and leadership provided a framework on which those ideas could grow; her constructive editing skills helped me learn how to express them. Thanks to Jody, I understand the meaning of the word Mentor.

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The National Science Foundation also provided financial support to my advisor, Jody Deming, as part of the ARCSS multidisciplinary investigation of the Northeast Water Polynya. This grant enabled me to go to sea and collect the data presented in Chapters 2, 3, and 4 of this dissertation, as well as to attend several valuable planning and data workshops with the other NEWP investigators. I was very fortunate to be a part of the scientific team for the NEW Polynya project. This inspirational group of scientists treated me as a colleague, rather than an underling, and contributed greatly to my scientific development. I am particularly grateful to Doug Wallace for his synergistic collaboration on Ch. 2. Walker Smith and Peter Minnett provided supporting data and were also co-authors on the published version of Ch. 2.

I am greatly indebted Dr. Katharina Kurz Six ("Tinka") who showed open-mindedness to my wild ideas and patience in teaching me how to use models as tools. With great optimism and with the good-natured push-me-pull-you compromises required for a carbon modeler and a microbiologist to work together, Tinka and I initiated what I hope are long careers working together to find answers to all those questions we asked during the Summer of 1991.

Any talent for analytical chemistry that may appear on these pages is due to the patient tutelage of Doug Wallace, Ken Johnson (who designed the SOMMA instrument I used to measure total inorganic carbon in Ch. 2), Rick Wilke (who showed me how to take apart and then rebuild a functioning SOMMA), Dr. Catherine Goyet (who provided generous guidance as well as access to her alkalinity titration system), Maren Tracey and Bob Adams (who provided technical support with the titrator).

I am grateful to the captains, officers, and crew of both the US Coast Guard Cutter *Polar Sea* and the German research vessel *Polarstern* for getting us both into and out of the polynya safely and successfully.

My early-season experience in the polynya, so pivotal in the development of the Rectification Hypothesis, would not have been possible without the generous invitation by Dr. Kim Juniper and the collaboration of Dr. Telesphore Sime-Ngando. On board *Polarstern*, assistance and invaluable support came from Dr. Michel Gosselin, Stéphane Pesant, Paul Renaud, Annelie Skoog, and Kendra Daly. The birthday polar bear was a nice touch.

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Dedication

This dissertation is dedicated to all the women who walked before and beside me, leaving enough footprints to reveal that not all paths nor ideas are linear.

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INTRODUCTION

Air-sea carbon flux and the role of marine ecosystems

Missing sink. The striking feature of nearly all global carbon-cycle models is their inability to match the observed atmospheric increase in pCO_2 with the larger anthropogenic source. One aspect of my research focuses on the extent that marine ecosystems, particularly at high latitudes, could be part of the as yet unaccounted for (missing) carbon sink. Although the marine biosphere is considered a relatively small reservoir of carbon in the global scheme (3 Gt C; Siegenthaler and Sarmiento, 1993), the relative flux of carbon through this reservoir (>300% yr^{-1}) is by far the greatest of all pools in the system, providing the greatest potential for feedback.

Modeling assumptions. One reason for underestimating the air-to-sea carbon flux and the role of marine biota may be the coarse resolution of existing models, not only in space but also in time. Biological processes are often patchy as well as cyclic or episodic. As a result, annual or even seasonal time-stepping by models can underestimate the biological effect. Existing global carbon models are also incomplete in their parameterization of recycling (e.g. Armstrong, 1994; Najjar *et al.*, 1992; Sarmiento *et al.*, 1989). These models assume steady state and emphasize new production that results from the utilization of "preformed" or "new" nutrients (from upwelling, atmospheric deposition, or in situ N-fixation; Redfield *et al.*, 1963). In this scenario, new production equals export production (Eppley and Peterson, 1979) according to a constant stoichiometry (Redfield *et al.*, 1963), and only new production plays a significant role in the sequestration of carbon.

My approach has been to query the assumption that the processes of carbon export function at steady state, coupled spatially and temporally to nitrogen cycling, with constant Redfield ratios. I use a type of sensitivity analysis which purposefully incorporates episodicity and biological variation to test for biological influence on short- and long-term air-sea carbon exchange. Chapter 1 of this dissertation discusses these ideas further and introduces the first results from an ongoing modeling exercise designed to determine the extent to which complex microbial processes can influence carbon flux in the ocean.

Perturbation. Also of interest is whether marine biota can impact anthropogenic carbon dioxide (Broecker, 1991; Smith and Mackenzie, 1991) or affect global climate change on longer time scales. The critical issues of global change research, particularly in biological oceanography, are concerned with how and at what rate a system which may or may not be in steady state responds to perturbation. In order for a physical, chemical, or biological system to be a sink for anthropogenic CO_2, the flux of carbon related to that system must have increased relative to its pre-perturbation value (Smith and Mackenzie, 1991). Though it would be much simpler to assume that biological processes have been and always will be the same as observed today, there is no reason to believe that biological systems will not respond to significant changes in their physical or chemical environment, particularly when temperature is involved. Changes in ultraviolet (UV) radiation exposure is also likely to have an influence on biological processes.

Microbiological processes may be especially important in perturbation scenarios for several reasons. They occur over a large range of temporal and

spatial scales, and only occasionally is it safe to assume steady state. Bacteria play an active if not primary role in every ecosystem on the planet through the global cycling of carbon, nitrogen, and other biogeochemically important elements. Of all the biological systems, bacteria are the best equipped physiologically and genetically to respond quickly to change.

This dissertation illustrates in several ways how ecosystems potentially impact the oceanic uptake of anthropogenic carbon: (1) via episodicity (non-steady state processes); (2) via ice cover and its effect as a wintertime lid to outgassing, and (3) via temperature effects on microbes (rates or efficiencies sensitive to warming).

High-latitude coastal ecosystems

Biological pump. One of the regions where global climate changes are expected to have the greatest impact is the high latitudes (Hansen *et al.*, 1984). High-latitude oceans are also unique and critical to the global carbon cycle for several reasons, including strongly seasonal, short-term but high rates of biological productivity and the formation of deep water (Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984). The geochemical model of Knox and McElroy (1984) shows how the "efficiency of the biological pump" (which depends inversely on the extent of remineralization) in high-latitude seas significantly determines the degree to which carbon is sequestered in the global deep sea. Coastal arctic ecosystems are among the most productive in the world, with spatial and temporal heterogeneity as common characteristics. Seasonally ice-covered regions such as marginal ice zones and some polynyas are sites where the strong influences of light, nutrients, and temperature merge to create short-term but dramatic

blooms which can occur rapidly (Smith *et al.*, 1991; Sullivan *et al.*, 1988). In an environment characterized by strong localized pulses of primary production, the response of microorganisms within the euphotic zone may determine the rate of carbon, nitrogen, and phosphate remineralization and also the fraction of total production that is exported from the euphotic zone (Wiebe and Pomeroy, 1991).

The Northeast Water (NEW) Polynya. The NEW Polynya was the study area for an intensive, multi-disciplinary research project investigating biogeochemical cycling in high-latitude oceans in 1992 and 1993 (NEWater Investigators, 1993; Overland *et al.*, 1995; Hirche and Deming, in press). The polynya is a seasonally-recurrent opening in the permanent ice situated over the continental shelf of northeastern Greenland (77-81°N, 6-17°W; Fig. A-1). Typically the ice begins to open in May and close in October. Ice algal blooms dominate primary production early in the season, while phytoplankton blooms (mostly diatoms) develop later. Because of the heterogeneity in the summertime ice coverage, however, all stages of this succession may be present in the polynya at any one time. I participated in research cruises to this region in July-August 1992, May-June 1993, and July-August 1993.

Rectification Hypothesis. Seasonally ice-covered seas at high latitudes may be potential one-way (rectified) sinks for atmospheric carbon, driven by a unique linkage between strongly seasonal biological productivity and sea-ice formation (Ch. 2; Yager *et al.*, 1995). This scenario is sensitive to climate change because of the predicted increase in the areal extent of these regions due to global warming (Ingram *et al.*, 1989) and provides a negative feedback to increasing anthropogenic CO_2. The rectification scenario, however, also

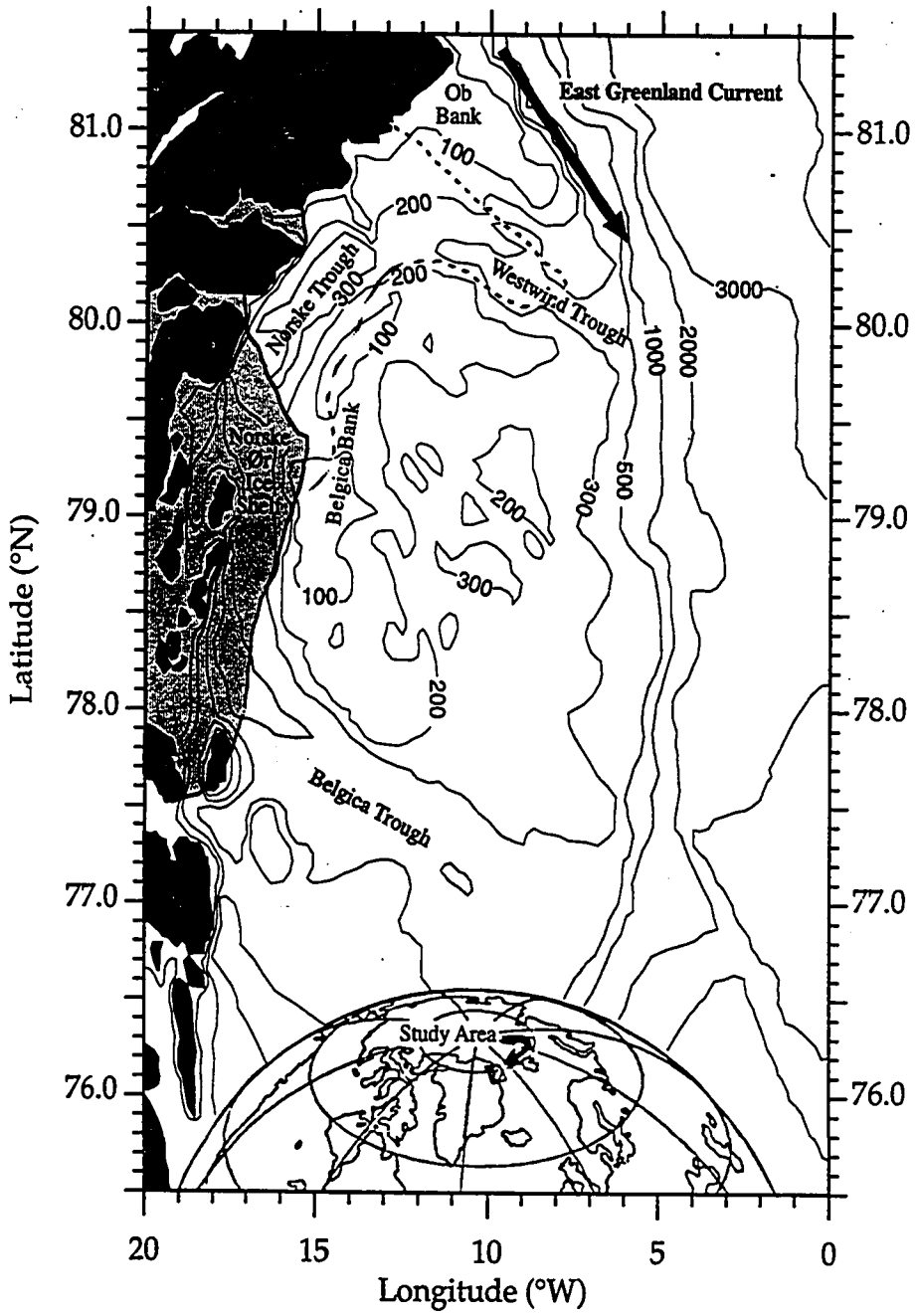


Figure A-1. Map of the Northeast Water (NEW) Polynya showing bathymetry (depth contours in meters), geographic features, and maximum extent of open water during August 1992 (dashed line; polynya opened north of Norske Ør Ice Shelf, over Norske Trough region).

depends on low pelagic respiration rates during summer which allow primary production to undersaturate inorganic carbon in the surface waters. It further assumes that biological activity will remain the same if high-latitude oceans warm. This may not be a reasonable expectation since many of the marine biota in these regions have adapted to a narrow range in temperature and may show especially strong sensitivity to warming (Baross and Morita, 1978). I investigate this idea in Chapters 3 and 4.

Temperature effects on microbial activity: optimal foraging and survival strategies

Cold adaptations. Unlike in the temperate or tropical surface ocean, pulse-responsive bacteria in polar oceans must also be cold-adapted (i.e. able to respond quickly to a food source while living at sub-zero temperatures). Prevailing wisdom suggests that most organisms operate very slowly when they are cold. Yet, slow organisms, designed for a steady food supply, seem ill-suited for the dynamic polar environment. This apparent contradiction has been one of the underlying themes of my research, directing my focus toward those organisms that seem to do best, by some measure, at low temperature. Psychrophilic (cold-loving) bacteria have been studied in the laboratory for decades and, while I do not review that literature here (see for example recent reviews by Russell, 1990, Gounot, 1991, and Karl, 1993, and references therein, especially earlier reviews of Baross and Morita, 1978, and Morita, 1966, 1975), there are indications that strategies for survival and definitions of optimality may be fundamentally different from those we consider standard in the temperate environment. The same case can be made for organisms living in especially low-nutrient environments, or oligotrophs (see reviews by Morgan

and Dow, 1986, or Fry, 1990). During my dissertation research, I developed a sense that these two types of "extreme" environments, and the constraints on organisms associated with them, probably come together in the coastal Arctic, much like they do in the deep sea (Deming, 1986, who suggested a link between oligotrophy and barophily). While there are times in the polar spring and summer when phytoplankton bloom and dissolved organics may become readily available (either directly or via grazing), the most common condition in these permanently cold environments is probably low organic nutrients. As a consequence, the most competitive organism might have a rapid response to high nutrient pulses, but overall a greater tendency for storage (Amy *et al.*, 1983) and low endogenous metabolism (e.g., more active and/or efficient respiratory chain, lower energy of maintenance, and lower minimal growth rate; Morgan and Dow, 1986). Transport systems that capture the ephemeral food supply must be constitutive, i.e., always ready (Koch, 1979), but catabolic enzymes that utilize specific substrates should be sensitive to available substrate concentrations (Novitsky and Morita, 1977; Harder and Dijkhuizen, 1983). There also appear to be advantages in being a generalist (Button, 1994).

"Pomeroy Hypothesis." The link between temperature and substrate concentration lies at the center of the polar microbial activity debate. At issue is whether bacteria in permanently cold (<5°) waters respond differently to pulses of substrates than do bacteria in permanently or seasonally warm water. The initial results of Pomeroy and co-workers (Pomeroy and Deibel, 1986; Pomeroy *et al.*, 1990, 1991; Wiebe *et al.*, 1992, 1993) suggest that the microbial community in northern high-latitude seas require relatively high

concentrations of organic matter at low temperatures. These greater food requirements might result in an under-utilization of primary production and thus provide a greater chance for carbon export (Pomeroy and Wiebe, 1988). Recent laboratory work with cultured psychrophilic bacteria (Pomeroy *et al.*, 1995), however, show a heterogeneous response to organic nutrient concentration and suggest that the situation may be more complicated than originally thought. This project examined temperature and substrate effects on bacterial utilization and incorporation efficiency, directly testing the Pomeroy hypothesis for its application in the NEW Polynya.

Uptake kinetics. Bacteria in low-nutrient or pulsed-nutrient environments can increase their potential uptake of substrate in two ways: by increasing the number of binding proteins on the cell surface (thereby increasing their maximum specific uptake rate, V_{max}), or by synthesizing a binding protein with a greater affinity for substrate (thereby lowering the half-saturation constant, K_m; Matin and Veldkamp, 1978). ANT-300, a psychrophilic marine *Vibrio* from Antarctic waters, has at least two transport systems with different affinities for arginine (Geesey and Morita, 1979). Some bacteria from low nutrient environments have more affine amino acid transport systems than their copiotrophic counterparts (Ishida *et al.*, 1982).

Button (1985; 1991; 1994) argues that V_{max} and K_m are not independent and that the specific affinity for a substrate (a^0_A; initial slope of the hyperbolic response curve) better reflects nutrient collection ability. All three of these parameters can be estimated using radiolabeled substrate additions to natural samples or cultured populations. Chapter 3 discusses how I measured these three parameters as a function of temperature using short term incubations of

natural communities from the NEW Polynya. Although there are limitations to using the kinetics approach in the marine environment (see for example the discussion in Karl, 1986), it is still the most direct means of addressing how bacteria direct their food capture and utilization mechanisms to survive and perhaps even thrive in cold, nutrient-limited environments.

Incorporation efficiency. In an environment were the food supply is often scarce, it makes sense for organisms to use the energy wisely. Deming and Yager (1992) suggested that polar deep-sea sediment bacteria may show uniquely high incorporation efficiencies on certain organic substrates. My dissertation research has further investigated and confirmed this phenomenon for the NEW Polynya surface waters (Ch. 4). The metabolic pathway of dissolved organic carbon taken up by heterotrophic bacteria can vary according to the quantity and type of compounds available (Goldman *et al.*, 1987). Griffiths *et al.* (1984; 1978) report respiration efficiencies for the Beaufort Sea which depend on substrate type, temperature, and also on the season. Growth efficiency in protozoa (e.g., bacterivores) can depend on the physiological state of the cell (Fenchel and Finlay, 1983) and temperature (Choi and Peters, 1992). I also found that efficiency sometimes depends on temperature (decreasing with warming) and organic substrate concentration (decreasing with increasing food). These results have implications for both global biogeochemistry and microbial ecophysiology which are discussed at the end of chapter 4 and in the final conclusions of the dissertation.

Extreme environments (from a temperate-living human point of view) provide unique opportunities for examining adaptation or optimal foraging behaviors in microorganisms. In any discussion of adaptation, one

must be certain to frame the question properly with respect to the organism (Stephens and Krebs, 1986). Fitness depends on the time-scales over which it is measured and the currency used in its evaluation. An optimal strategy for cold-adapted bacteria may incorporate other constraints such as food supply and grazing pressure. In some cases, stability rather than maximization may be optimal (Maynard Smith, 1982; Stephens and Krebs, 1986).

Research goals

My research project set forth the following five goals: (1) determine with modeling whether biology, through non-steady state processes, could potentially impact the air-sea flux of carbon; (2) establish the extent of biological impact on the seasonal and annual inorganic carbon cycle in the present-day NEW Polynya, using it as a model system for seasonally ice-covered high-latitude oceans; (3) determine the role of pelagic bacteria in the carbon cycle of the NEW Polynya region; (4) determine the sensitivity of this role to temperature; and (5) predict the sensitivity and feedback response of the high-latitude oceanic carbon cycle to expected warming.

I. Modeling the Impact of Marine Ecosystems on Air-Sea Carbon Exchange.

Background

During the summer of 1991, while participating in a course on *Climate and the Marine Biosphere* at Friday Harbor Marine Laboratories, Katharina Kurz Six (Max Plank Institut für Meteorology, Hamburg, Germany) and I built a model designed to test the then-prevailing idea that marine biological processes did not have any influence over the oceanic uptake of anthropogenic CO_2 (Broecker, 1991). We began by noting that existing global carbon cycle models were unable to match the observed atmospheric increase in pCO_2 with the well-known anthropogenic source function (recently discussed by Siegenthaler and Sarmiento, 1993). Tans *et al.* (1990) proposed that the missing sink had to be in the northern hemisphere and that it was probably the extensive northern forests. Having recently read the three classic box models that revealed the importance of high latitude oceans in controlling atmospheric pCO_2 levels over glacial-interglacial time scales (Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984), my first thought was that some of the missing sink could be in the Arctic ocean, assisted by the unique marine biota of the high-latitude continental shelves, particularly if the high-latitude community was responsive to temperature changes and thus to the anthropogenic perturbation. This idea was not entirely inconsistent with the Tans *et al.* (1990) analysis, since they grouped all oceanic data north of 50°N latitude; the

average ΔpCO2 used (-37 ppm) to estimate air-sea flux for northern oceans was certainly an underestimate for some regions of the Arctic (see Chapter 2).

Biological processes were essentially ignored in most of the oceanic geochemical models built during the late 1980's and early 1990's (e.g., Bacastow and Maier-Reimer, 1990; Maier-Reimer and Hasselmann, 1987; Toggweiler *et al.*, 1989). Even when biology was acknowledged as the primary mechanism controlling atmospheric pCO_2, it was modeled as a strictly geochemical or stoichiometric process (e.g. Sarmiento *et al.*, 1988).

One reason for underestimating the role of marine biota has been the scale, both temporal (annual) and spatial (km), used in most physical models. Biological processes are often patchy and cyclic or episodic, particularly in the Arctic. If export is sensitive to the scale over which it is averaged (extremely probable in the temporally and spatially heterogeneous Arctic), it can thus be incorrectly estimated by large scaling. Since we began our investigation, an entire NATO ASI workshop was devoted to developing an oceanic carbon cycle model with improved biogeochemistry. The problem of scale was identified as a key issue (Evans and Fasham, 1993) with the recommendation that "detailed high resolution models rich in vertical structure and biological detail" be developed (Murphy *et al.*, 1993).

A related problem in the existing geochemical models was incomplete parameterization of nutrient recycling. Historically, modelers had only considered new production important to determining carbon flux from the euphotic zone. By definition (Eppley and Peterson, 1979), this parameterization assumes a system at steady state. Having studied the deep-sea benthos, I was aware that export from the euphotic zone could be highly episodic (Theil *et al.*, 1988), and that assumptions of steady state were often

invalid or highly sensitive to the chosen time scale. Also, remineralization of CO_2 was traditionally linked to the nitrogen or phosphate cycle using Redfield ratios. No allowances were made for decoupling of these processes. Organismal carbon-to-nitrogen ratios are often out of balance with the Redfield ratio (see for example Chapter 2 of Parsons *et al.*, 1984), but only recently has it been recognized that entire oceanic regions may not always follow Redfield ratios either (Sambrotto *et al.*, 1993).

Awareness of these issues was not lacking for at least some of the modelers, who acknowledged that the potential impact of including biological processes in modeling the oceanic uptake of atmospheric pCO_2 was of the same order of magnitude as the anthropogenic source itself (Sarmiento *et al.*, 1989). The problem was building a biological model simple enough to incorporate into a computationally-large global general circulation model (GCM). Somehow simplicity had to be accomplished with the integrity of the biological system still intact. To somewhat ease this dilemma, the NATO workshop recommended building models designed to address specific questions (Murphy *et al.*, 1993). When asking questions about the linkage between marine biota and climate change, they recommended that future models focus on those biotic components likely to be sensitive to variations in temperature, for example.

At the time (and still to some extent today), modeling in biological oceanography was focused on understanding the controls on primary production rather than how biological processes influence biogeochemical cycling. In our examination of existing models of biological processes we typically found either no linkage to the inorganic carbon cycle (Evans and Parslow, 1985; Frost, 1987; Fasham *et al.*, 1990) or, if there was linkage, poor

representation of any reasonably complex marine ecosystem (e.g., Taylor *et al.*, 1991, and more recently, Antoine and Morel, 1995a, 1995b, although both models found biological influence on oceanic pCO_2 despite their simplified biological parameterizations). The Fasham *et al.* (1990) model was probably the best of the genre in that it captured most of the basic biological components, but structural sensitivity and the role of complexity remained untested, nor was it linked to the atmosphere. Ultimately, their model would be incorporated into the Princeton GCM (Fasham *et al.*, 1993; Sarmiento *et al.*, 1993) for sensitivity analyses and comparison to satellite (surface chlorophyll) observations (Slater *et al.*, 1993). The effects of complex marine food webs, particularly the role of the microbial loop, on air-sea flux of CO_2 remain unexamined, however, as do perturbation studies addressing anthropogenic CO_2.

We constructed from existing models a unique hybrid that linked a complex marine food web, such as those developed by Fasham et al. (1990), to an oceanic inorganic carbon cycle and a local atmosphere, letting the CO_2 flux vary as a function of the short term gradients developed between the surface ocean and the atmosphere. Our specific goal was to test the sensitivity of air-sea carbon flux and the uptake of the anthropogenic transient to biological processes using a more sophisticated biological model than used previously, but with a simple ocean and atmosphere. To this day, testing for biological sensitivity in oceanic uptake of the anthropogenic CO_2 transient remains a unique aspect of our modeling approach.

Our hypothesis was that the storage of carbon in organic biomass, even if only during certain times of the year, could have an effect on the annual uptake capacity of the ocean. In addition to the traditional expectation that

export of carbon from the surface to deep ocean occurs via the vertical flux of particulate and dissolved organic carbon, we hypothesized that export might also occur in the form of dissolved inorganic carbon if the ocean was not in steady state and the inorganic carbon and nitrogen remineralization cycles were decoupled. This process seemed possible due to the fact that most of the CO_2 in the ocean is not in the form of pCO_2 (e.g., the Revelle buffer factor; Broecker and Peng, 1982), yet the air-sea flux is driven by the pCO_2 gradient alone. For every ppm of pCO_2 that moves from the atmosphere to the pCO_2 of the ocean because of a gradient, ~10x that amount also gets shunted into the rest of the oceanic carbonate system before the gradient is reduced.

We emphasized at the time that the model we constructed was a preliminary attempt to test our hypothesis. The biological processes we included in our model were still poor representations of a complex biological system (resembling the Fasham *et al.*, 1990 model) and the simple ocean model was missing some key ingredients (e.g. advection or loss terms). We also never incorporated unique Arctic characteristics, (e.g., boreal light, low temperature, or ice cover) since we started with parameterizations published for Weather Station "Papa" in the sub-arctic Pacific (Frost, 1987). After describing the preliminary model and what we were able to determine from it, I will conclude this chapter with funded plans to refine the model as a postdoctoral fellow. Despite the fact that the project remains unfinished, and advances to the field have been made during the interim, the exercise of initiating it had great impact on my thinking. Ideas that originated during the building and testing of this model are woven throughout my graduate research efforts and the scientific approach I take with me into the future. As such, the preliminary model has earned a place in my dissertation.

Model Description

Physical dynamics

We developed a one-dimensional box diffusion model for the ocean with high vertical resolution (5 m) in the upper 100 m. This resolution gradually decreases towards the deepest box at 1000 m depth (Fig. I-1).

Ocean dynamics are defined by turbulent mixing with a diffusion coefficient of $K = 1 \times 10^{-6}$ m^2 s^{-1} for the deep ocean (in retrospect, this value is too low and likely resulted in a sluggish deep ocean). A seasonally sinusoidal mixed-layer depth (ranging from 25 m in the summer to 125 m in the winter) is included (Fig. I-2). Within the mixed layer, we increase the turbulent mixing by two orders of magnitude to a diffusion coefficient of $K = 1.0 \times 10^{-4}$ m^2 s^{-1}. Deepening of the mixed layer entrains water properties from beneath.

We solved the diffusion equation using an implicit finite-difference numerical method. For all grid points, the concentration at the new time step is calculated simultaneously. Since implicit methods are unconditionally stable, it allows us to specify a time step of one day.

To investigate the atmospheric response, we added an atmosphere (single box; Fig. I-1) which connects to the ocean surface by a simple flux equation. The only property transferred across the air-sea interface is carbon dioxide concentration. We determined the flux of CO_2 by a constant gas exchange coefficient, $\lambda = 0.05$ mol l^{-1} yr^{-1} ppm^{-1} (Broecker and Peng, 1974).

In the surface ocean, we calculated the full carbon chemistry (e.g., the formation of bicarbonate and carbonate dependent on alkalinity and temperature; Broecker and Peng, 1982). Solubility of carbon dioxide changes

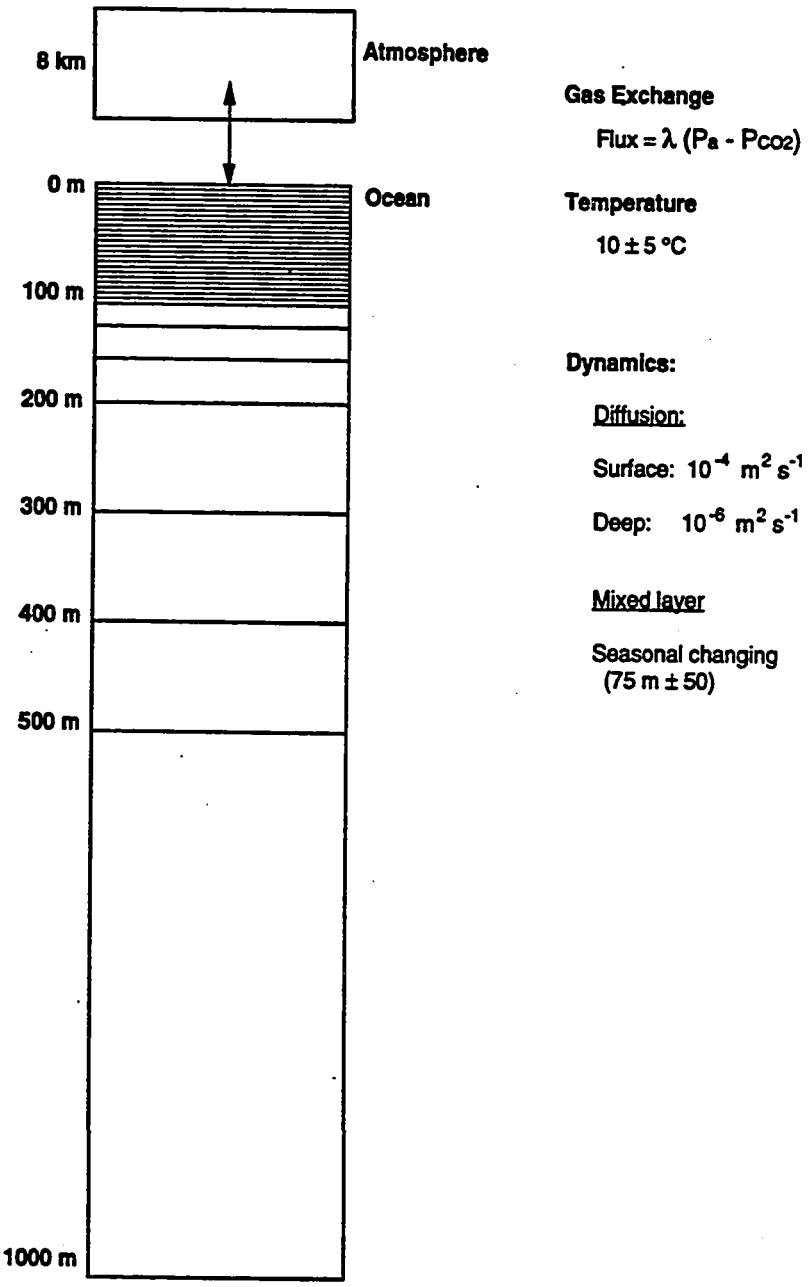


Figure I-1. The dynamical model. Twenty-eight oceanic boxes decreasing in resolution with depth. Box number starts with 1 at the surface and increases to 28 on the bottom.

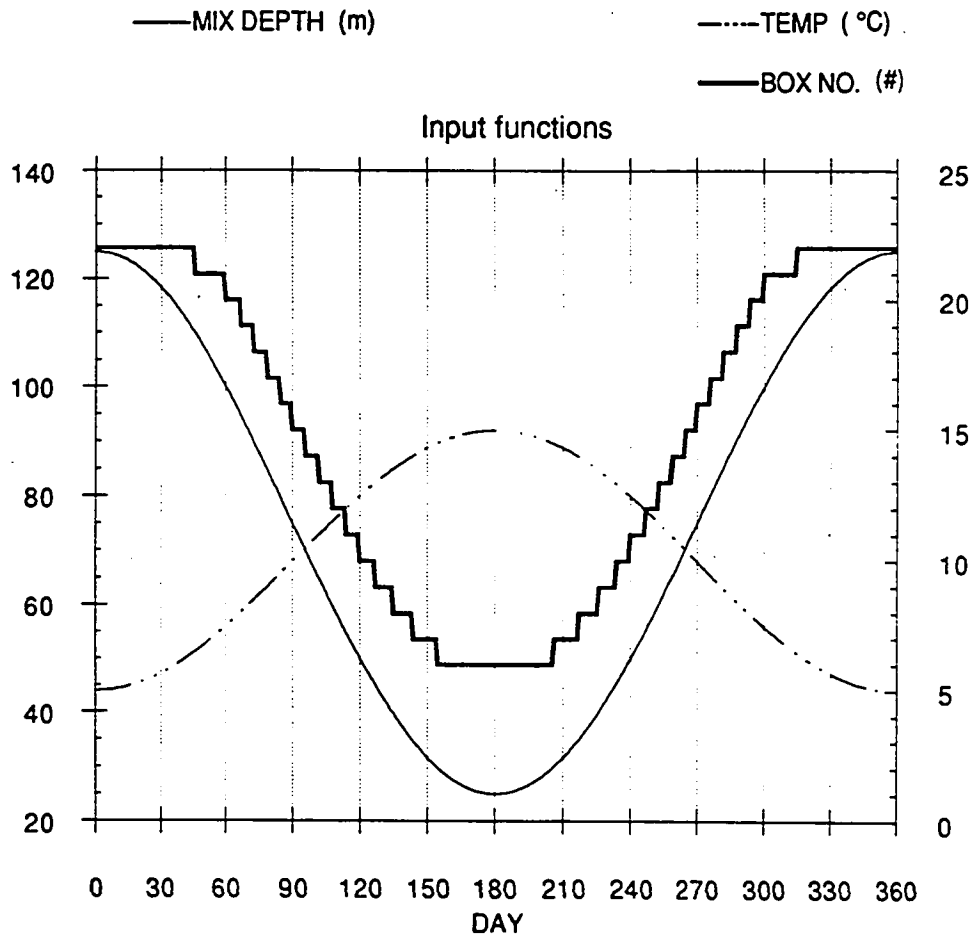


Figure I-2. Input functions of the dynamical model as a function of time (Day 1 = January 1; 1 y = 360 d). Mixed-layer depth (m; thin solid line) varies sinusoidally between a maximum of 125 m and a minimum of 25 m. Box number (thick solid line) refers to the box which contains the base of the mixed-layer. Temperature (°C; dashed line) is also forced by a sinusoidal function which ranges from 5 to 15 °C.

with temperature. Seasonal variations of temperature are prescribed as a sinusoidal function within the range from 5° to 15° C (Fig. I-2).

Biology

Our biological model is a simplified version of the pelagic microbial loop (*sensu* Deming and Baross, 1993; Jumars *et al.*, 1989; originally inspired by classic papers of Pomeroy, 1974, and Azam *et al.*, 1983). We chose three major groups of organisms (phytoplankton, zooplankton, and bacteria) as simplified representatives of three different trophic levels. A single "chimeric" zooplankton group consumed both phytoplankton and bacteria. To model the biological cycle, organisms as well as other organic and inorganic species were considered as concentrations of carbon or nitrogen per unit volume of seawater (Fig. I-3). Exchanges between them were described by fluxes of carbon or nitrogen per time step (Fig. I-3; arrows). Interactions between pools were either modeled as Michaelis-Menten kinetic processes (e.g. uptake of dissolved nutrients and grazing rates) or prescribed as fractions of present concentration (e.g., excretion, respiration, and death). Uptake rates and half-saturation constants were chosen or estimated from published values (Table I-1). The carbon to nitrogen ratio used for all pools of organic matter was 106:17 (Redfield *et al.*, 1963).

Phytoplankton production rates were based on the temperature and light forcing scheme of Frost (1987; mimicking temperature and light levels at 50°N; Fig I-4). Phytoplankton could take up either ammonium (NH_4) or nitrate (NO_3). To model their overall preference for NH_4 (Goldman and Glibert, 1983), we used an exponential damping function such that high NH_4 concentrations inhibited NO_3 utilization. Zooplankton grazed on both

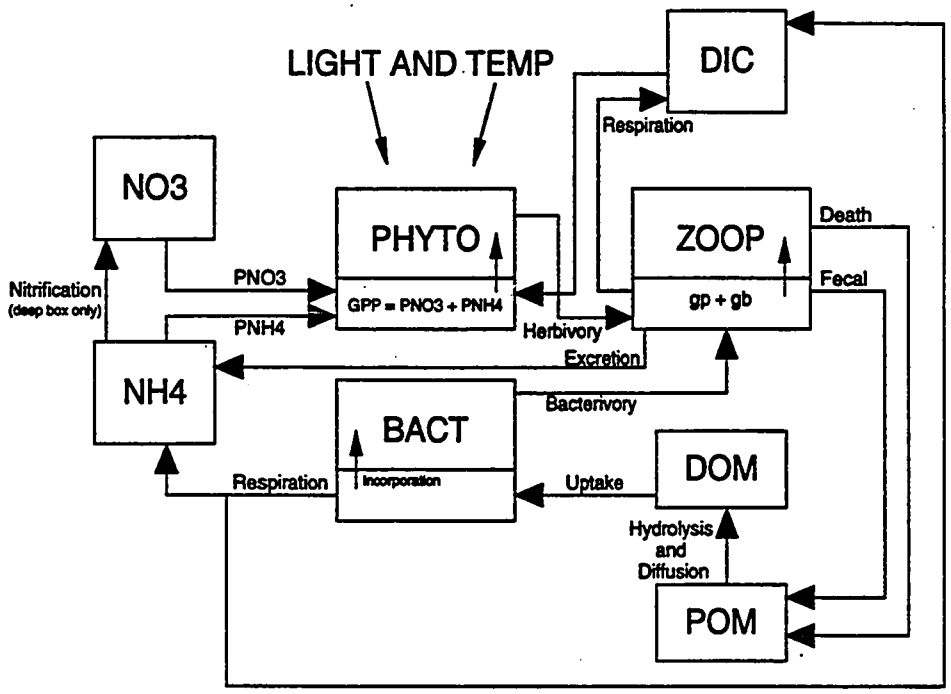


Figure I-3. The biological model where boxes indicate biomass or concentration in terms of C and/or N per unit volume seawater and arrows indicate fluxes of C and/or N per unit volume per unit time. PHYTO indicates phytoplankton; ZOOP, zooplankton; BACT, bacteria; DIC, dissolved inorganic carbon; DOM, dissolved organic matter; POM, particulate organic matter; NH4, ammonium; NO3, nitrate; PNH4, primary production using ammonium; PNO3, primary production using nitrate; GPP, gross primary production; gp, grazing of phytoplankton; gb, grazing of bacteria.

phytoplankton and bacteria only to prescribed refuge concentrations (Eccleston-Parry and Leadbeater, 1994; Heinbokel, 1978). Zooplankton contributed to the dissolved organic matter (DOM) pool via incomplete digestion that depended on fecal pellet production (Jumars *et al.*, 1989). Bacteria took up DOM only, but there was a hydrolysis function that allowed bacteria to turn particulate organic matter (POM) into DOM directly via extracellular enzymatic activity (Hoppe *et al.*, 1988; Hoppe *et al.*, 1993). Heterotrophic activities by both zooplankton and bacteria remineralized organic biomass to dissolved inorganic carbon (DIC) and ammonium.

Nitrate was recycled in the deepest box. All ammonium which was mixed to this layer was transformed into nitrate. Nitrate returned to the surface layers by mixing. All concentrations of organic and inorganic species were transported by the oceanic dynamics described above. Only POM was advected vertically, determined by its sinking rate.

Four sensitivity experiments tested the model response to changes in maximum grazing rates, bacterial incorporation efficiency (fraction of consumed carbon going to biomass), phytoplankton aggregation and sinking, and zooplankton excretion and respiration fraction (Table I-1).

RESULTS AND DISCUSSION

Standard model time-series

Our standard model ran to equilibrium for 300 y. We examined equilibrium results by integrating biological concentrations over the top 50 m (our euphotic zone). Observations of these values for the first 10 y after the 300-year run indicated that the model had reached steady state (data not

Table I-1: Parameters used in model and their assigned values.

Parameter:	Symbol	Standard Value	Units	Expt. #	New value
Light:[a]					
Light as function of time	I_o	225± 150	lyd^{-1}		
Light at surface	alight	30 to 140	-		
Attenuation	attu	0.1	m^{-1}		
Phytoplankton:					
Half-saturation for nutrient uptake[b]	hkn	1×10^{-6}	$molN\ l^{-1}$		
NH4 damp of NO3 uptake[b]	amdamp	1×10^{6}	$(molN\ l^{-1})^{-1}$		
Chlorophyll-specific maximum growth rate[a]	PMAX	20 to 80	$mgCmgChl\,a^{-1}\ d^{-1}$		
Carbon: chlorophyll ratio[a]	c	30	$mgCmgChl\,a^{-1}$		
Minimum concentration	Pmin	0.1×10^{-6}	$molCl^{-1}$		
Threshold concentration for aggregation (Expt. 3 only)	pocmax	-	$molCl^{-1}$	3	5×10^{-6}
Grazers:					
Half-saturation for herbivory	hkgp	0.1×10^{-6}	$molCl^{-1}$		
Mass-specific maximum herbivory	grazp	1	d^{-1}	1	10
Threshold concentration for herbivory	Pmin	0.1×10^{-6}	$molCl^{-1}$		

Table I-1 (continued)

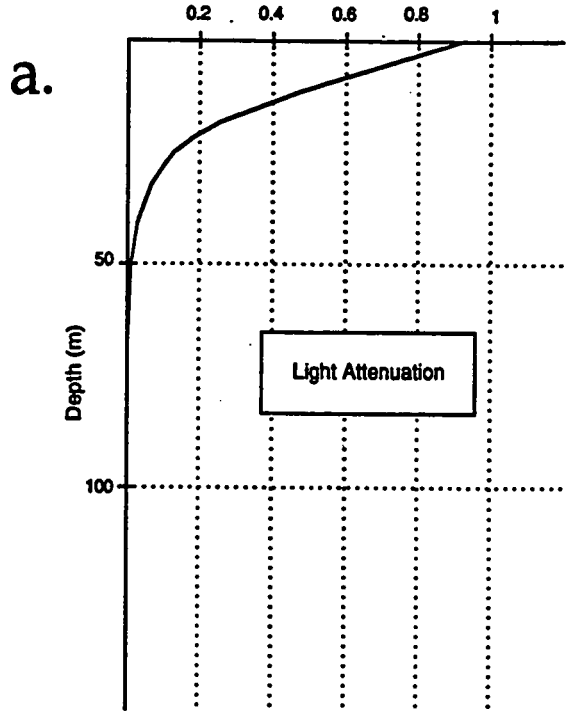
Half-saturation for bacterivory[b]	hkgb	0.1×10^{-6}	mol Cl^{-1}		
Mass-specific maximum bacterivory[b]	grazb	1	d^{-1}	1	10
Threshold concentration for bacterivory[b]	Bmin	0.1×10^{-6}	mol Cl^{-1}		
Excretion and respiration (fraction of intake)	excret	0.40	-	4	0.20
Fecal pellet fraction (fraction of intake)	fecal	0.30	-	4	0.15
Death of grazers (fraction of total biomass)	death	0.30	-		
Bacteria:					
Half-saturation for DOM uptake by bacteria[b]	hkb	5×10^{-6}	mol Cl^{-1}		
Maximum bacterial uptake rate[b]	Vb	2	d^{-1}		
Bacterial respiration efficiency	bacres	0.80	-	2	0.20
POM and DOM:					
Hydrolysis/diffusion half-saturation coefficient	hkhy	0.5×10^{-6}	mol Cl^{-1}		
Sinking distance per day	sinkrate	50	m		
Carbon: Nitrogen ratio[c]	rnc	106:17	mol C mol N^{-1}		

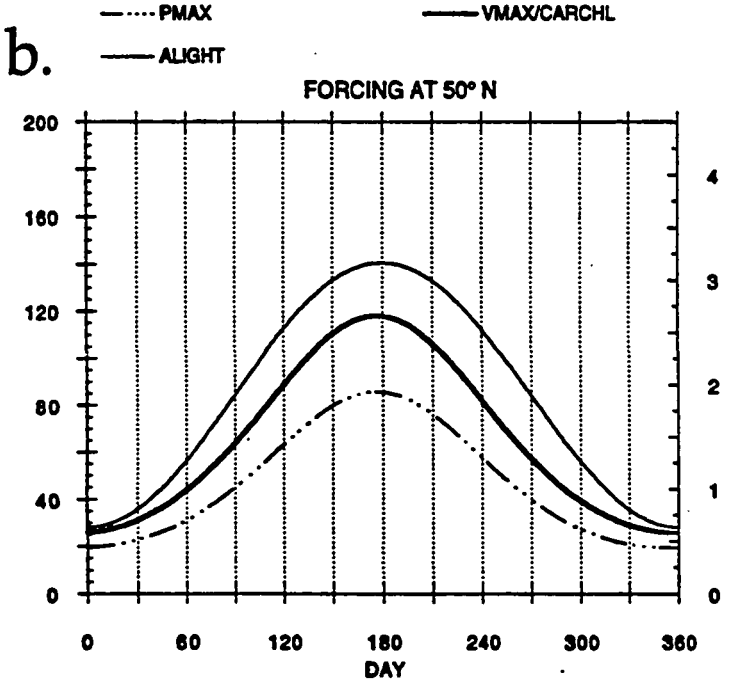
[a] from Frost (1987)

[b] from Sarmiento *et. al.*, (1989)

[c] from Redfield *et al.* , (1963)

Figure I-4. Light forcing functions: a) fraction of surface light decreasing with depth (m) creates a euphotic zone of about 50 m; and b) light and temperature effects on phytoplankton production (from Frost, 1987), where PMAX* (dashed line) indicates maximum uptake of phytoplankton (mg C per mg chlorophyll *a* per day) as a function of carbon to chlorophyll ratio, temperature, and daylength as defined by Frost (1987); ALIGHT (thin solid line), $\alpha PARz/e^{-(\text{attenuation x depth})}$ where αPARz is defined by Frost (1987) as the photosynthetically available radiation used by phytoplankton; and VMAX/CARCHL (thick solid line), (PMAX*)(tanh(αPARz/PMAX*)/c, where c is the carbon to chlorophyll ratio.


a.
0.2
0.4
0.6
0.8
1
50
100
Depth (m)
Light Attenuation


b.
PMAX
VMAX/CARCHL
ALIGHT
FORCING AT 50° N
200
160
120
80
40
0
4
3
2
1
0
0
60
120
180
240
300
360
DAY

shown). Annual cycling of primary production was strongly determined by mixed-layer depth and nutrients. However, since light could penetrate belowthe shallow summer mixed-layer, some production occurred there as well. A seasonal cycle was set up by light and temperature forcing on the phytoplankton (Fig. I-5a). As light increased in the early spring (~ Day 60), when bacterial biomass and nutrients were relatively high, phytoplankton production increased. Production with ammonium was much higher than that with nitrate. Springtime f-ratios (nitrate production to total production) reached the typical open-ocean level of ~0.06 (Eppley and Peterson, 1979). Phytoplankton biomass increased significantly enough by late Spring (Fig I-5b; ~ Day 160) to result in greater grazing rates and thus higher zooplankton biomass (Day 200). With greater zooplankton uptake, NH_4 excretion and CO_2 respiration increased, along with fecal pellet production and zooplankton death. This contributed to the DOM pool via POM such that bacterial uptake increased and bacterial biomass accumulated in the Fall (Fig. I-5c; ~ Day 270). This secondary increase of bacteria also stimulated a small secondary increase in zooplankton biomass. Increasing bacterial remineralization, coinciding with the deepening of the mixed-layer, contributed to the winter build-up of nutrients and the resulting spring bloom.

Standard model depth profiles

During springtime, nutrients were low in the surface layers (0-150 m), while production was limited to the upper 50 m (Fig. I-6a). High production rates caused low levels of nutrients. Nutrients increased with depth due to sinking POM, high bacterial biomass (Fig. I-6b), and resulting remineralization.

Sensitivity experiments

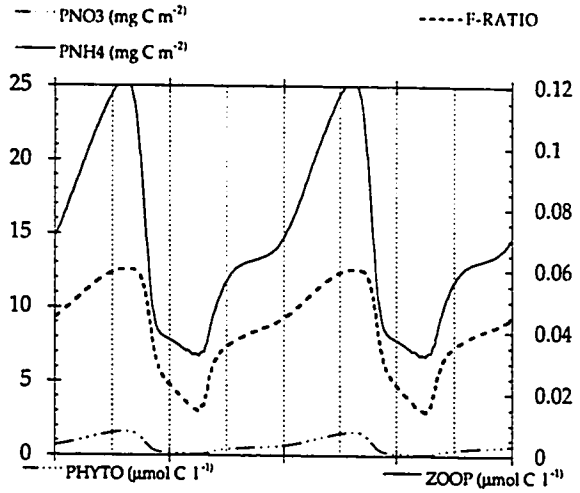
Our first sensitivity experiment tested the effect of increasing the maximum grazing rate by a factor of ten (Table I-1; grazb and grazp = 10). The resultant increase in zooplankton grazing and excretion dramatically increased ammonium concentration at the surface. Production with ammonium was low, however, because phytoplankton biomass was constantly grazed to its refuge level (Fig. I-7a). High ammonium concentration inhibited nitrate utilization, resulting in a relatively high surface concentration of nitrate, but an extremely low f-ratio (Fig. I-7a). Zooplankton biomass doubled (Fig. I-7b) and cycled in phase with primary production; a lag no longer existed between them. Bacterial biomass was also kept to the refuge level by grazers.

The second sensitivity experiment decreased the respired fraction of bacterial uptake by a factor of four, with more of the utilized carbon and nitrogen incorporated into bacterial biomass. This resulted in greater bacterial biomass in most of the water column and lower ammonium concentrations in the surface waters. Consequently, primary production with ammonium was lower such that the f-ratio increased (Fig. I-8).

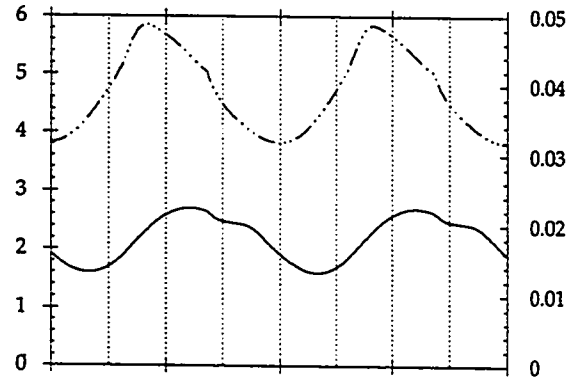
For the third sensitivity experiment, we set a threshold level of phytoplankton biomass ("pocmax"), above which cells aggregated and sank out of the surface as POM and the surface concentration of phytoplankton dropped to its refuge concentration (P_{min}). We anticipated that this process would result in a loss of carbon and nutrients from the surface, but because of the efficiency of the microbial loop and the degree of vertical mixing, the model achieved *faster* and more complete recycling through bypassing the

Figure I-5. Standard model results as a function of time, where Day 1 = January 1, 300 y after initial conditions, and 1 y = 360 d; a) phytoplankton production (mg C m^{-2}) integrated over the euphotic zone (upper 50 m) using nitrate (PNO3, thin dashed line) and ammonium (PNO4, solid line), where the F-ratio (heavy dashed line) is PNO3/(PNO3 + PNH4); b) phytoplankton (dashed line) and zooplankton (solid line) biomass averaged over upper 50 m (μmol C l^{-1}); c) bacterial biomass (dashed line) averaged over upper 50 m (μmol C l^{-1}).

a.


PNO3 (mg C m-2)
F-RATIO
PNH4 (mg C m-2)
25
20
15
10
5
0
0.12
0.1
0.08
0.06
0.04
0.02
0
PHYTO (µmol C l-1)
ZOOP (µmol C l-1)

b.


6
5
4
3
2
1
0
0.05
0.04
0.03
0.02
0.01
0

c.

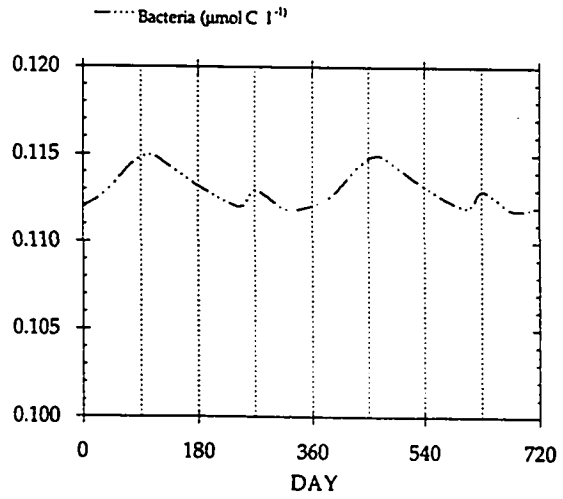
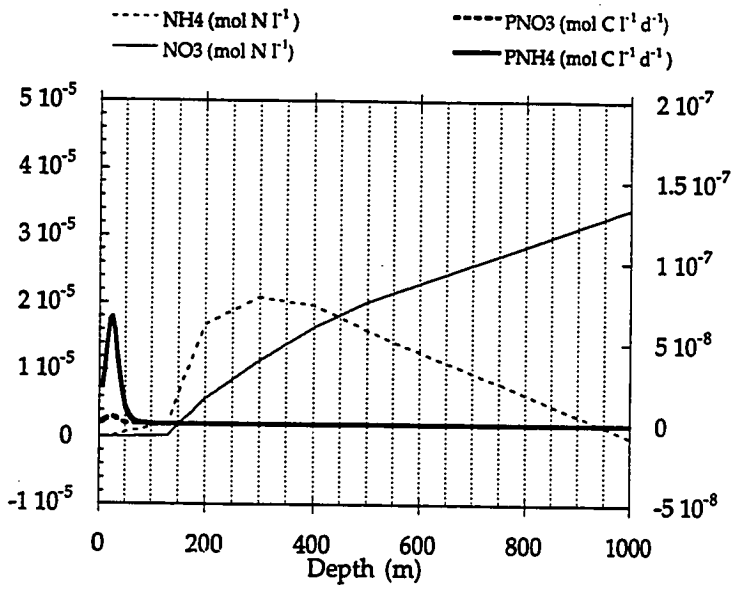

Bacteria (µmol C l-1)
0.120
0.115
0.110
0.105
0.100
0
180
360
540
720
DAY

Figure I-6. Standard model results as a function of depth (m) for Spring (Day 90) of year 300 for: a) nutrients and production, where NO3 (thin solid line) and NH4 (thin dashed line) are expressed in mol N l^{-1}, and phytoplankton production using nitrate (PNO3, thick dashed line) and ammonium (PNH4, thick solid line) are expressed in mol C l^{-1}; and b) phytoplankton (thick dashed line), zooplankton (solid line), and bacterial biomass (thin dashed line) expressed in mol C l^{-1}.

a.


NH4 (mol N l-1)
NO3 (mol N l-1)
PNO3 (mol C l-1 d-1)
PNH4 (mol C l-1 d-1)
5 10-5
4 10-5
3 10-5
2 10-5
1 10-5
0
-1 10-5
2 10-7
1.5 10-7
1 10-7
5 10-8
0
-5 10-8
0
200
400
600
800
1000
Depth (m)

b.

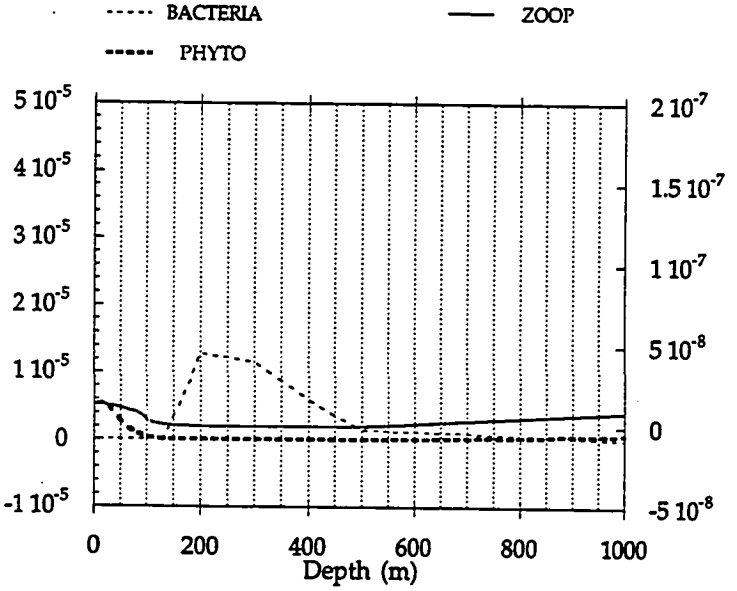
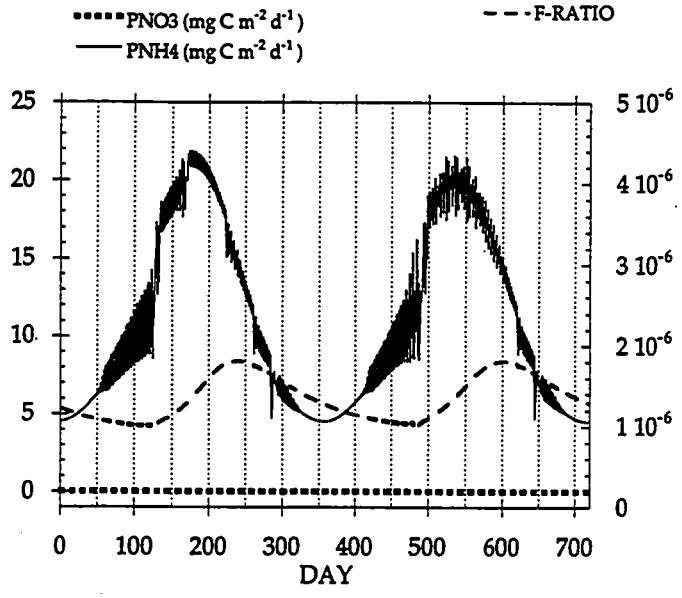

BACTERIA
PHYTO
ZOOP
5 10-5
4 10-5
3 10-5
2 10-5
1 10-5
0
-1 10-5
2 10-7
1.5 10-7
1 10-7
5 10-8
0
-5 10-8
0
200
400
600
800
1000
Depth (m)

Figure I-7. Model results from increased grazing rate (Experiment #1): a) phytoplankton production integrated over the euphotic zone (upper 50 m) using nitrate (PNO3; dotted line) and ammonium (PNH4; solid line, uppermost curve) in mg C m^{-2}, where F-ratio (dashed line, lowermost curve) is PNO3/(PNO3 + PNH4); b) phytoplankton (dotted line), zooplankton (solid line; uppermost curve) and bacterial biomass (dashed line, overlaps somewhat with PHYTO) averaged over upper 50 m (μmol C l^{-1}).

a.


PNO3 (mg C m-2 d-1)
PNH4 (mg C m-2 d-1)
F-RATIO
25
20
15
10
5
0
5 10-6
4 10-6
3 10-6
2 10-6
1 10-6
0
0 100 200 300 400 500 600 700
DAY

b.

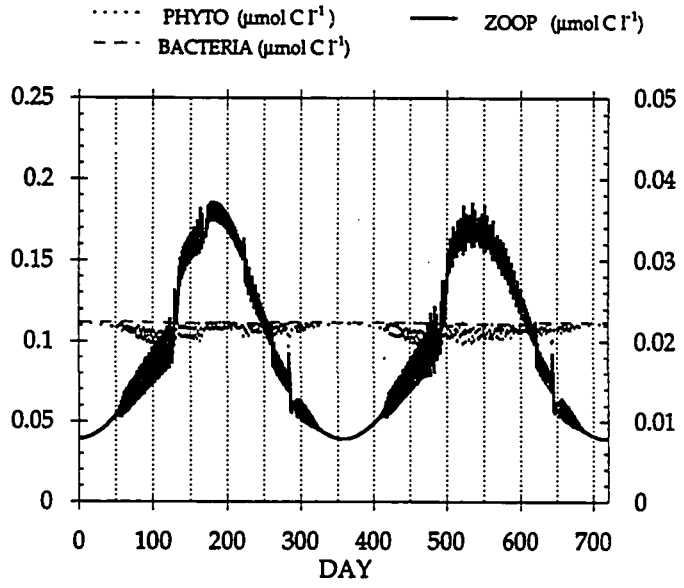
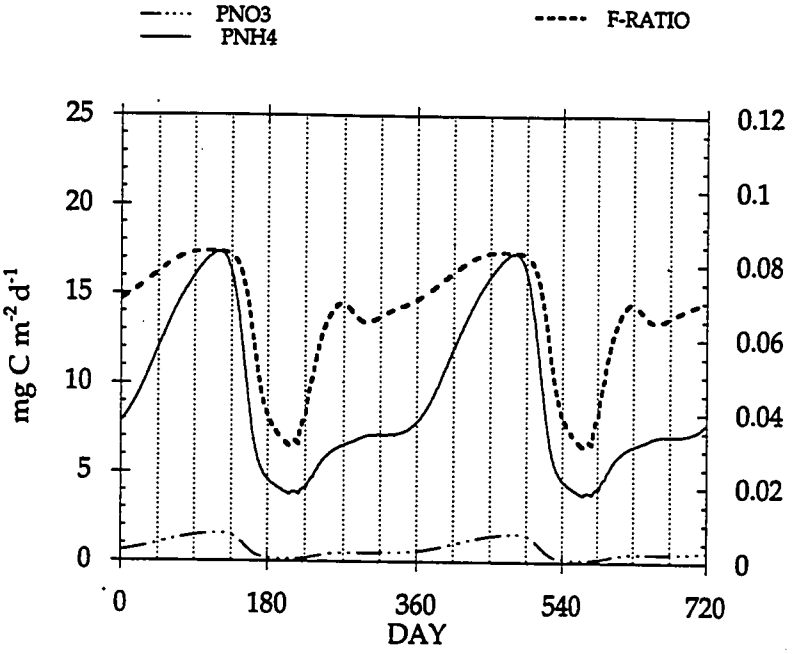

PHYTO (µmol C l-1)
BACTERIA (µmol C l-1)
ZOOP (µmol C l-1)
0.25
0.2
0.15
0.1
0.05
0
0.05
0.04
0.03
0.02
0.01
0
0 100 200 300 400 500 600 700
DAY

Figure I-8. Model results from increased bacterial efficiency (Experiment #2): phytoplankton production integrated over the euphotic zone (upper 50 m) using nitrate (PNO3; thin dotted line) and ammonium (PNH4, solid line) in mg C m^{-2}, where F-ratio (heavy dashed line) is PNO3/(PNO3 + PNH4).


PNO3
PNH4
F-RATIO
25
20
15
10
5
0
0.12
0.1
0.08
0.06
0.04
0.02
0
mg C m-2 d-1
0
180
360
540
720
DAY

grazer step. This interesting result is sensitive to the extent of hydrolysis of POM by bacteria, but may be important in some parts of the ocean. As in the standard model, phytoplankton production and biomass increased in the Spring (Fig I-9 a and b). Once the threshold concentration was reached (~ Day 100), however, all of the "excess" biomass (phytoplankton concentration - P_{min}) is shunted into POM and sinks. (Note: phytoplankton concentration never dropped to P_{min} on our time-series plots because we averaged the entire 50 m water column.) The high production of POM which resulted from phytoplankton sinking contributed to a higher DOM pool (via hydrolysis) and more bacterial biomass (Fig. I-9c; Day 100). Increased remineralization rates also increased nutrient concentrations and phytoplankton production (Fig I-9a). As a result, even zooplankton biomass increased (Fig I-9b). This outcome was almost certainly sensitive to the sinking rate of POM, the depth of the model ocean, and vertical mixing rates.

The fourth sensitivity experiment decreased the fraction of grazer uptake lost to respiration, excretion, and fecal pellets by a factor of two. As with the bacteria in Experiment #2, this increased zooplankton incorporation efficiency and biomass. Our model was highly sensitive to this parameter. Variations with time become much stronger (Fig. I-10a) and zooplankton biomass can, for a short time, be two orders of magnitude higher than in the standard model (Fig. I-10b). This strong episodicity occurs because nutrients are not limiting phytoplankton growth and, thus, both phytoplankton and zooplankton operate at or near their maximum growth rates. Since phytoplankton have doubling times faster than zooplankton, they pace ahead of the grazers just enough to allow exponential growth. With the increase in food concentration, however, the zooplankton soon caught up to and

ultimately decimated the phytoplankton population. Such a high zooplankton biomass contributed significantly to nutrient pools, even with high incorporation efficiency. All of this adds to the nutrient pool and stimulates a second phytoplankton bloom (Fig. I-10a and b; ~ Day 150).

Interestingly, some instabilities also existed in this version of the model, even though it ran at equilibrium for over 300 y; slight differences occurred in the seasonal patterns of biomass and production from one year to the next (compare the two summer production or f-ratio curves at ~ Day 190 and ~ Day 550; Fig. I-10a).

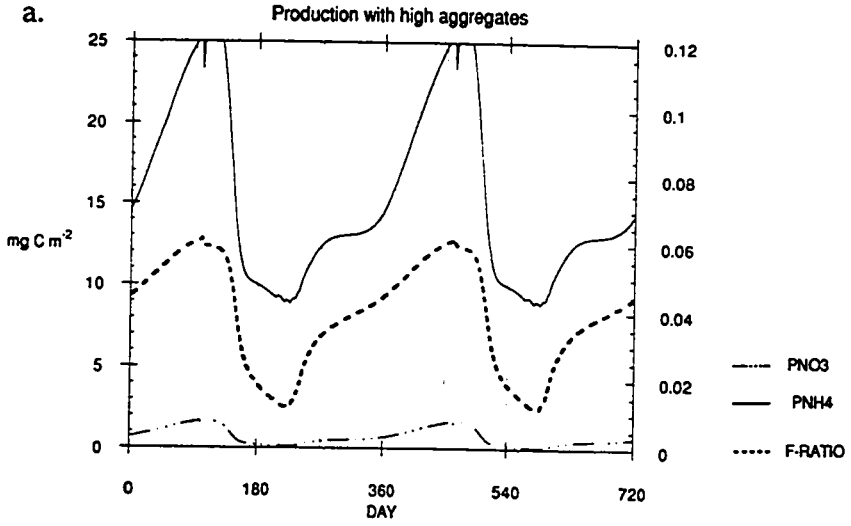
With this strong result, we compared the standard seasonal sea-to-air carbon flux calculated by the standard model (where CO_2 fluxes between the surface ocean and the atmosphere mostly as a function of temperature) to that which resulted from the high-efficiency grazer run. As we hypothesized, episodic biological processes lead to episodic reduction of the summertime efflux of CO_2 from the ocean to the atmosphere (Fig. I-11). This sensitivity to a single parameter may be the first indication of how potential non-linearitiesin the biological system can impact physical and chemical processes in the ocean.

Perturbation experiment

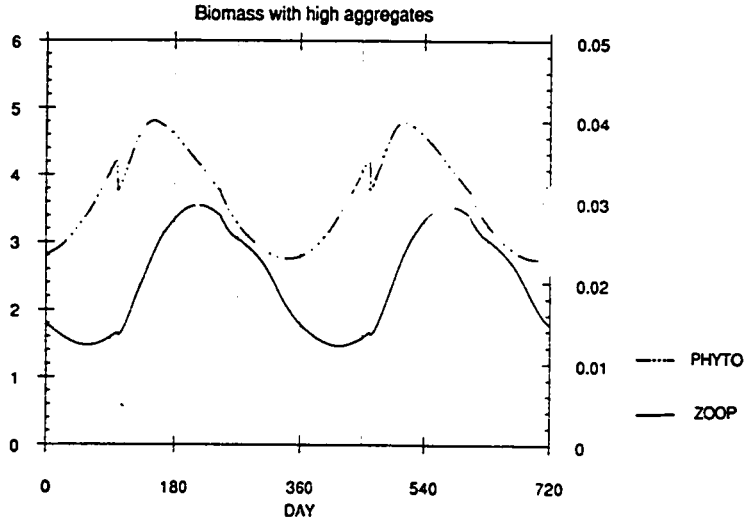
To see if perturbations in the seasonal uptake of CO_2 could affect the longer-term net uptake of a transient increase in CO_2, we ran both the standard model and the high-efficiency grazer model to equilibrium, then added a one-percent-per-year increase in atmospheric pCO_2. The resulting profiles of the transient CO_2 taken up in the two models show little difference after 100 y (Fig. I-12). If anything, the standard model shows a slightly greater

Figure I-9. Model results from aggregation effect (Experiment #3): a) phytoplankton production integrated over the euphotic zone (upper 50 m) using nitrate (PNO3; thin dotted line) and ammonium (PNH4; solid line) in mg C m^{-2}, where F-ratio (heavy dashed line) is PNO3/(PNO3 + PNH4); b) phytoplankton (dotted line) and zooplankton (solid line) biomass averaged over upper 50 m in µmol C l^{-1}; c) bacterial biomass (solid line) averaged over upper 50 m in µmol C l^{-1}.

a.


Production with high aggregates
mg C m-2
25
20
15
10
5
0
0.12
0.1
0.08
0.06
0.04
0.02
0
0
180
360
540
720
DAY
PNO3
PNH4
F-RATIO

b.


Biomass with high aggregates
6
5
4
3
2
1
0
0.05
0.04
0.03
0.02
0.01
0
0
180
360
540
720
DAY
PHYTO
ZOOP

c.

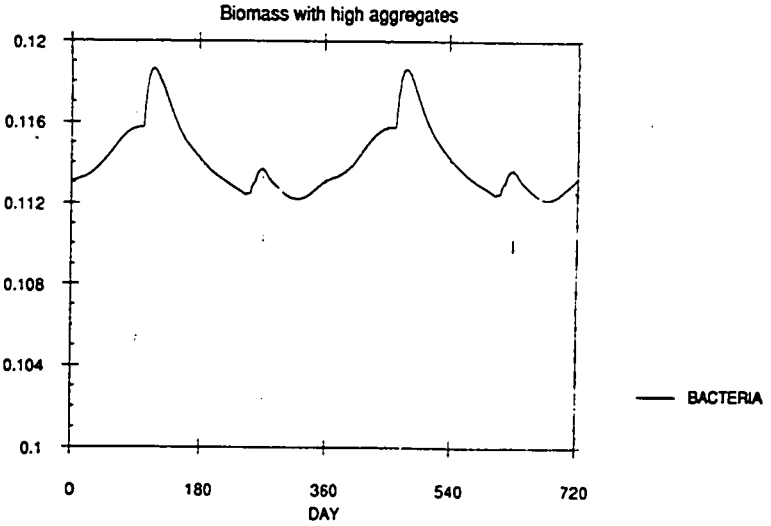
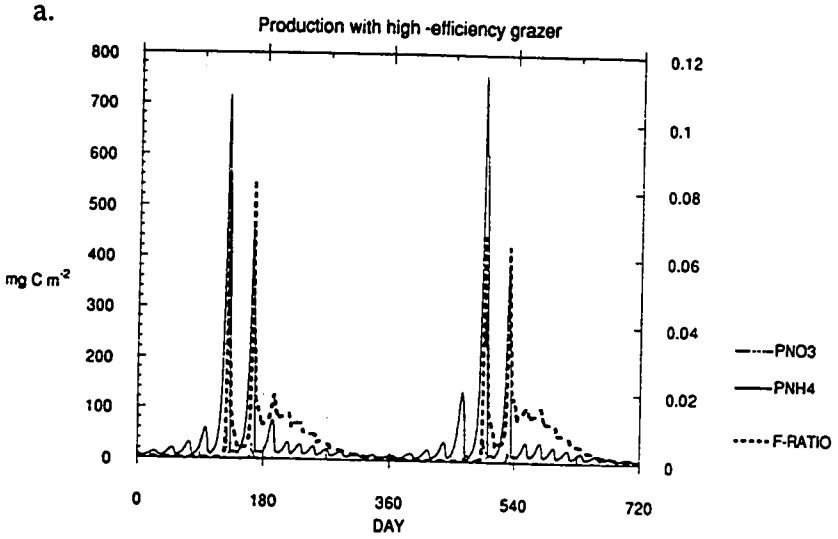
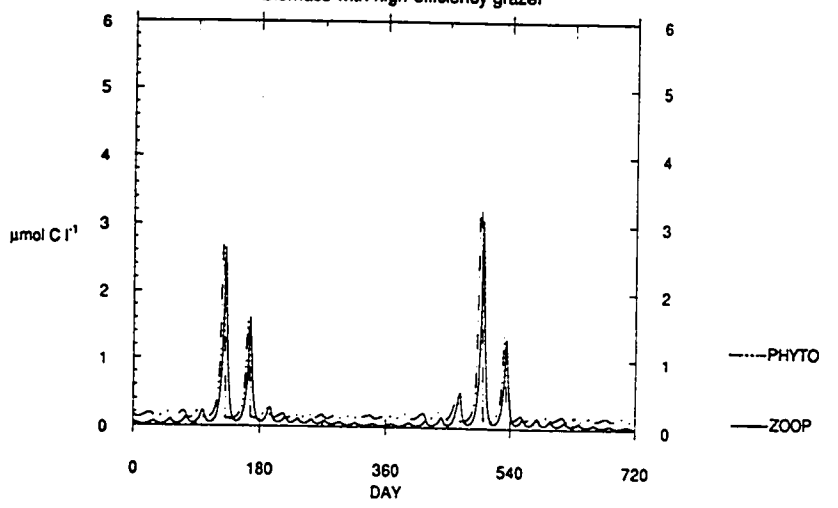

Biomass with high aggregates
0.12
0.116
0.112
0.108
0.104
0.1
0
180
360
540
720
DAY
BACTERIA

Figure I-10. Model results from increased zooplankton efficiency (Experiment #4): a) phytoplankton production integrated over the euphotic zone (upper 50 m) using nitrate (PNO3; thin dotted line) and ammonium (PNH4; solid line) in mg C m^{-2}, where F-ratio (heavy dashed line) is PNO3/(PNO3 + PNH4); b) phytoplankton and zooplankton biomass averaged over upper 50 m in µmol C l^{-1}; and c) bacterial biomass (dotted line) integrated over upper 50 m in µmol C l^{-1}.

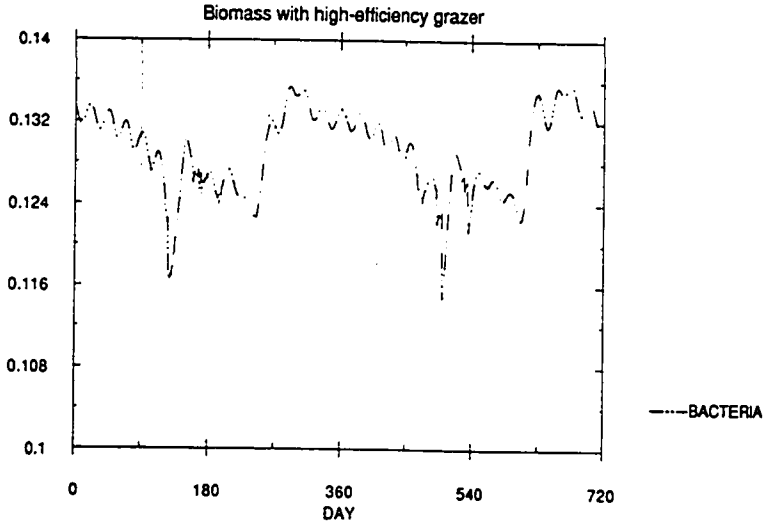
a.


Production with high -efficiency grazer
800
700
600
500
400
300
200
100
0
mg C m-2
0.12
0.1
0.08
0.06
0.04
0.02
0
0
180
360
540
720
DAY
PNO3
PNH4
F-RATIO

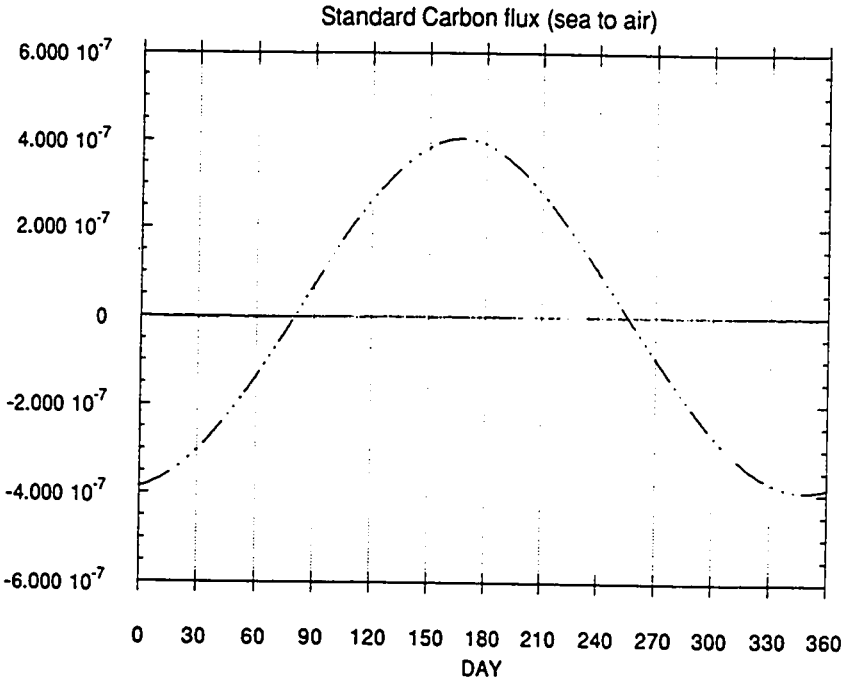
b.


Biomass with high-efficiency grazer
6
5
4
3
2
1
0
µmol C l-1
0
180
360
540
720
DAY
PHYTO
ZOOP

c.


Biomass with high-efficiency grazer
0.14
0.132
0.124
0.116
0.108
0.1
0
180
360
540
720
DAY
BACTERIA

a.



b.

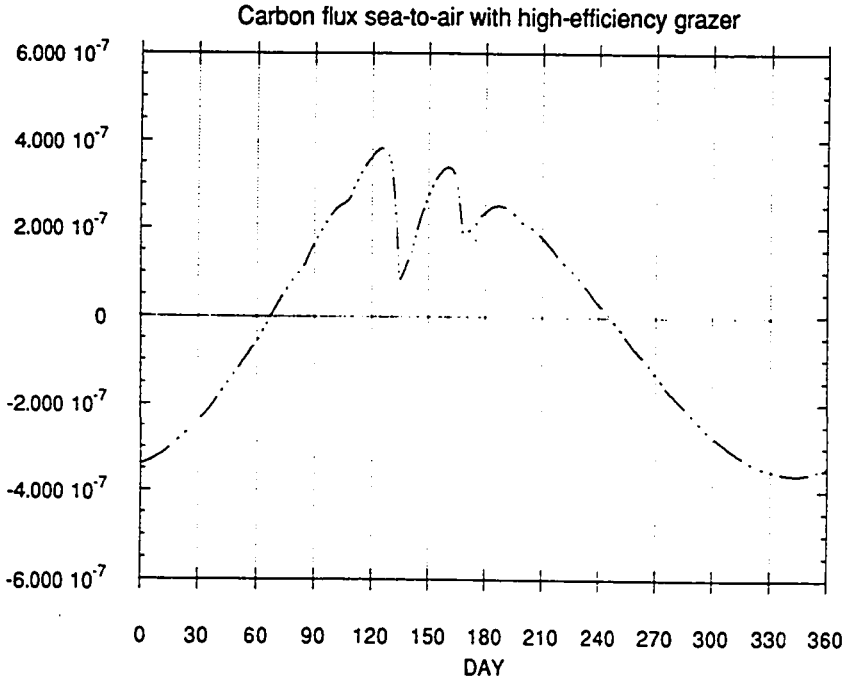


Figure I-11. Comparison of model results for annual carbon flux (mol C d^{-1}), where positive values indicate flux from ocean to atmosphere: a) Standard model; and b) Experiment #4.

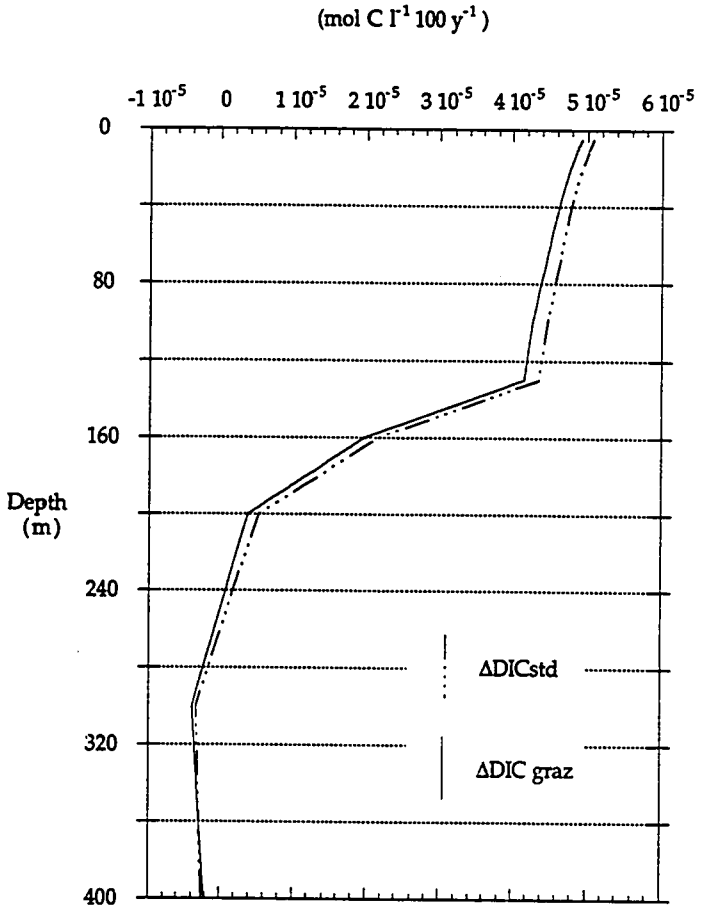


Figure I-12. Model-generated oceanic uptake of atmospheric pCO_2 increase of one-percent-per-year as a function of depth (m), where ΔDIC (dotted line) indicates the perturbation response (excess transient CO_2 taken up) by standard model (mol C l^{-1} 100 y^{-1}), and ΔDIC graz (solid line) is the perturbation response of the Experiment #4 model (mol C l^{-1} 100 y^{-1}).

uptake. Given this result, we must conclude that episodic events in our model (patterned after the subarctic Pacific) have no long term impact on the uptake of the transient CO_2 signal. As recounted below, flaws or limitations of this overall preliminary exercise, however, leave the question open.

Conclusions and future plans

Our initial attempt to investigate the influence of marine biology on oceanic CO_2 uptake, using a more complex biological scheme than most geochemical models, showed that nonlinear biological responses influence short-term but not long-term oceanic uptake of CO_2. The model showed sensitivity to some changes in the partitioning of carbon and rates of recycling. More work is needed to completely understand these sensitivities. Episodic events do show strong impacts on the flux of CO_2 between the ocean and the atmosphere. This result should be kept in mind when measurements of pCO_2 are made by oceanographers over short time scales. Even small changes in biological community structure can impact the short-term flux of CO_2 to and from the ocean.

Our hypothesized change in the long-term (100-y) behavior of oceanic CO_2 uptake and the anthropogenic transient due to biology was not detected. This result suggests that biological processes may not influence long term anthropogenic carbon uptake. Absence of evidence, however, does not indicate evidence of absence; there are several alternative explanations, particularly in light of certain problems with the initial model: 1) No long term sinks exist in the model; carbon mixes down to the bottom of the one-dimensional ocean and then mixes up again. With such a simple ocean, having no advection or even local sediment sinks, there was in fact no place

for carbon to be sequestered. 2) The diffusion coefficients used in the model were too low; although steady state was observed in the surface ocean after 300 y, the deep ocean may not have been at equilibrium. There was no penetration of the 100-y carbon transient below about 300 m (see Fig. I-12); this might have been improved by increased diffusive exchange. 3) This is not a high-latitude ecosystem model; significant carbon export is not expected in the sub-arctic Pacific. We need to better constrain some of the parameters to better represent unique export-related aspects of the Arctic, both hydrographical and biological. 4) The ecosystem part of the model is oversimplified in the areas we most expect to have an effect, particularly zooplankton. 5) With only a small number of sensitivity tests, we do not have a complete picture of the nonlinear behavior of the model; feedbacks between changes in more than one parameter could affect the short-term carbon flux enough to change long-term carbon uptake to a level we can resolve. The most obvious improvements, therefore, would be to add a sink term (either through episodic advection or sedimentation), increase the diffusion coefficients, improve the biological model with aspects of high latitude biota highlighted in other chapters of this dissertation, and run a more complete sensitivity analysis.

Given the observed model sensitivity to grazer parameters and the apparent importance of bacterivory observed in the NEW Polynya (Ch. 3-4), a major improvement to the biological model would be to separate bacterivores from herbivores so that the microbial loop can function distinctly from the herbivorous food web. Recent developments suggest that protozoa, and not bacteria, may be responsible for much of the remineralization that occurs in the ocean (Berman, 1991; Caron, 1991). Add this effect to their apparent

control over bacterial activity in some high-latitude environments, and the importance of protozoa cannot be underestimated.

For my postdoctoral research at the University of Georgia (with Drs. R. G. Wiegert and L. R. Pomeroy), I plan to become more familiar with ecosystem modeling techniques used by ecologists to better represent and analyze the role of biological complexity. I want to use these techniques to improve the existing model, initially making it more diagnostic in order to determine the acceptable level of ecosystem simplicity that could be incorporated into more complex ocean-atmosphere models. The combination of traditional oceanographic modeling techniques with those learned from ecologists may provide unique insight. Once a reasonable sink term and protozoa are added to the model, my goals are, first, to continue to perform sensitivity analyses and test for the impact of episodic and non-steady-state biological processes on short-term (days to seasons) as well as long-term (annual to decadal) carbon inventories and, second, to examine how the structure of an ecosystem can influence the flux of carbon (e.g., Boyd and Newton, 1995; see also, for example, the discussion in Legendre and Rassoulzadegan, 1994, on herbivorous food webs versus microbial loops). Ultimately, the improved model could be used to predict the perturbation response of high-latitude ecosystems to increased anthropogenic carbon and ocean temperature. The results should prove useful to global carbon cycle modelers interested in how sensitive results are to the parameterization of marine biological processes.

II. The Northeast Water Polynya as an Atmospheric CO_2 Sink: A Seasonal Rectification Hypothesis*

* This chapter published as Yager *et al.*, (1995).

Abstract

During the multidisciplinary 'NEW92' cruise of the United States Coast Guard Cutter (USCGC) *Polar Sea* to the recurrent Northeast Water (NEW) Polynya (77-81°N, 6-17°W; July-August 1992), total dissolved inorganic carbon and total alkalinity in the water column were measured with high precision to determine the quantitative impact of biological processes on the regional air-sea flux of carbon. Biological processes depleted the total inorganic carbon of summer surface waters by up to 2 mol C m^{-2} or about 3%. On a regional basis this depletion correlated with depth-integrated values of chlorophyll *a*, particulate organic carbon, and the inorganic nitrogen deficit. Replacement of this carbon through exchange with the atmosphere was slow owing to the low wind speeds during the month of the cruise, although model calculations indicate that the depletion could be replenished by a few weeks of strong winds before ice forms in the autumn. These measurements and observations allowed formulation of a new hypothesis whereby seasonally ice-covered regions like the NEW Polynya promote a unique biologically and physically mediated "rectification" of the typical (ice free, low latitude) seasonal cycle of air-sea CO_2 flux. The resulting carbon sink is consistent with other productivity estimates and represents an export of biologically cycled carbon either to local sediments or offshore. If this scenario

is representative of seasonally ice-covered Arctic shelves, then the rectification process could provide a small, negative feedback to excess atmospheric CO_2.

Introduction

High-latitude oceans are believed to influence atmospheric CO_2 levels on timescales of hundreds to thousands of years owing to high and variable biological activity and extensive exchange with deep-ocean inorganic carbon (Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984). On a seasonal timescale, biological processes in these regions can cause significant depletions in surface water pCO_2, influencing the local air-sea exchange of carbon (Codispoti *et al.*, 1982, 1986; Takahashi *et al.*, 1993). Within the Arctic, most biological production occurs over seasonally ice-free continental shelves (Subba Rao and Platt, 1984), for which polynyas (ice-free waters within ice-covered regions) are under consideration as model systems (NEWater Investigators, 1993).

In the coastal Arctic Ocean, summer is characterized by continuous sunlight and nutrient-bearing waters derived from the ice-covered Arctic basin. Photosynthetic production rates P can therefore be high (Smith *et al.*, 1991). At the same time, summertime respiration rates R are believed to be low, in part due to the high incorporation efficiencies of local cold-tolerant microbial communities (Deming and Yager, 1992; Yager and Deming, 1993; W. Ritzrau and J.W. Deming, unpublished manuscript; Chapter 4) and high P/R ratios for phytoplankton at low temperature (Smith and Sakshaug, 1990). Continental shelf ecosystems have been considered as either balanced between primary production and respiration or as net heterotrophic (i.e., net

sources for atmospheric CO_2; Smith and Hollibaugh, 1993), in part due to the flux of terrestrial production from rivers but also because wintertime respiratory processes and upwelling of carbon-rich deep waters typically cause supersaturated levels of pCO_2 in surface waters. The potential for high P/R ratios in the Arctic distinguishes the region and its carbon cycles from temperate latitudes.

Total dissolved inorganic carbon and total alkalinity were measured in the Northeast Water (NEW) Polynya during the United States Coast Guard Cutter (USCGC) *Polar Sea* cruise (77-81°N, 6-17°W; July-August 1992) in order to determine the quantitative impact of biological processes on the regional air-sea flux of carbon. Detection of a significant impact allowed formulation of an annual carbon cycling hypothesis that would establish the polynya as a net sink for atmospheric CO_2. We refer to this hypothesized cancellation of one half of the typical air-sea exchange cycle of an ice-free ocean as "rectification."

Methods

Sampling technique and analysis

Seawater samples from 10 stations in the NEW Polynya region (Figure II-1, Table II-1) were collected for onshore analyses of total dissolved inorganic carbon C_T and total alkalinity A_T. Selected stations corresponded with those where biological processes were being monitored intensively; they were not intended to give complete temporal or spatial coverage. Subsamples for CO_2 parameters were taken, stored, and analyzed according to standard protocols

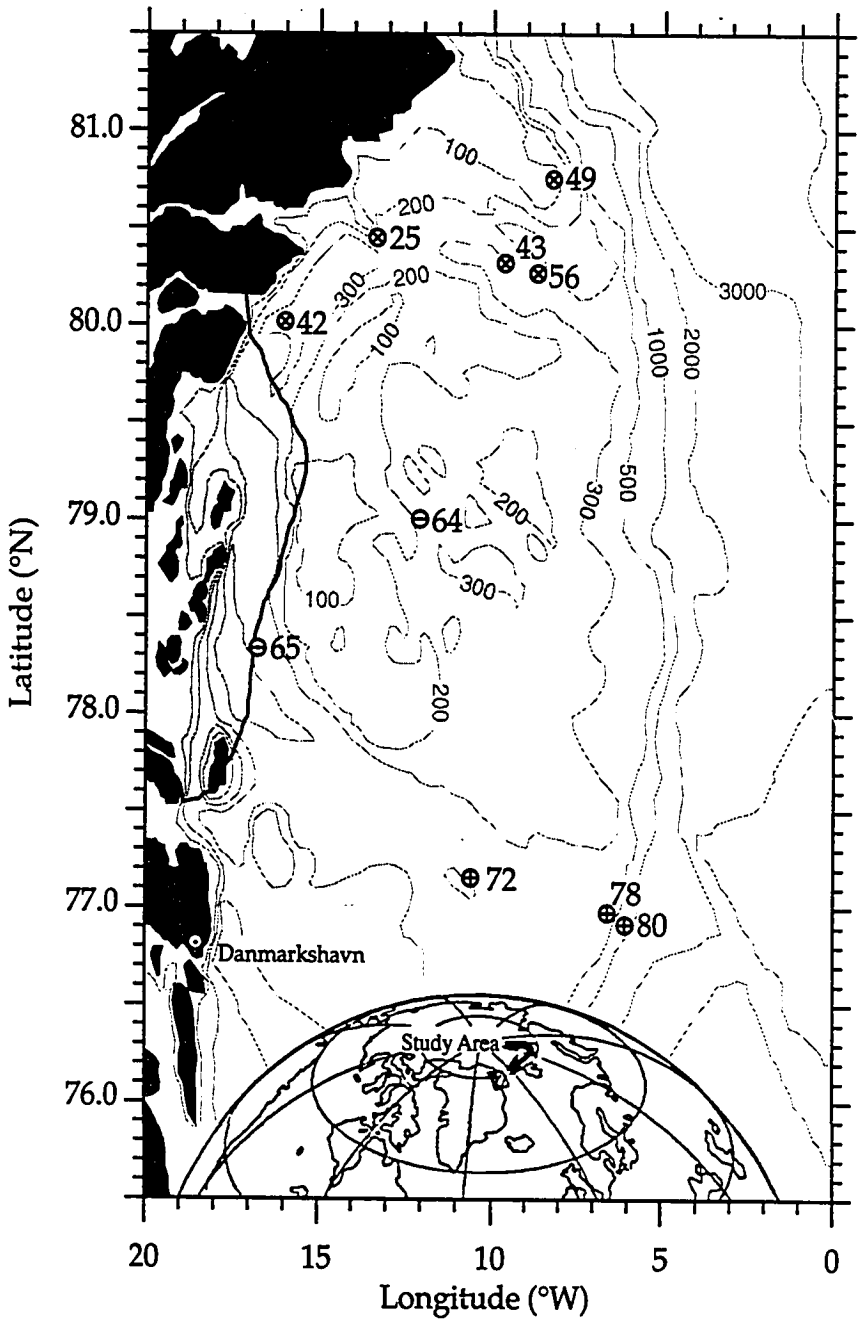


Figure II-1. Topographic map of the study site showing sampling locations: main polynya (≤ 5/10 ice coverage) and Westwind (or "northern") Trough region (circled x's); central region (theta symbol); and Belgica (or "southern") Trough region (circled pluses). See Table II-1 for individual station information.

TABLE II-1. NEW Polynya Stations with Associated Inorganic Carbon Data

Region	Station[a]	Location	Ice Cover[b] (tenths)	B (mol C m^{-2})[c]	Surface pCO_2 (µatm)[d]
	25a	(80°27', -13°20')	0	0.63	250
POLYNYA	25b	(80°27', -13°23')	0	1.6	222
and	42	(80°02', -16°00')	1	0.42	-
NORTHERN	43a	(80°19', -09°40')	1-5	1.3	214
TROUGH	43b	(80°19', -09°40')	8	0.92	217
REGION	49	(80°45', -08°14')	8	0.92	167
	56	(80°16', -08°40')	2	0.32	273
			average:	*0.87*	*224*
CENTRAL	64	(79°01', -12°03')	7.5	0.16	204
REGION	65	(78°19', -16°46')	6	0.053	279
			average:	*0.11*	*242*
	72a	(77°08', -10°33')	5	0.92	188
SOUTHERN	72b	(77°10', -10°32')	7	0.71	204
REGION[e]	78	(76°59', -06°35')	8	1.2	191
	80	(76°55', -06°04')	6	1.0	207
			average:	*0.96*	*198*

[a] a and b indicate return samplings at a given station within a 48 hour period.

[b] Observed shipboard at the hour of sampling.

[c] Biologically mediated inorganic carbon deficit (*B*) calculated from mass balance (see text); one standard deviation equals ± 0.1 mol C m^{-2}, based on Monte Carlo simulation (see text).

[d] Calculated from measured C_T and A_T (see text); one standard deviation equals ± 6.2 µatm, based on calculated values for twelve pairs of duplicate samples.

[e] Integrates to 50 m only (see Figure 2*a*).

(Dickson and Goyet, 1991) to minimize storage effects. C_T and salinity S were measured in November 1992 by a coulometric titration analyzer fitted with a SeaBird® conductivity sensor (Johnson *et al.*, 1987, 1993). Measurements (2188.95 ± 1.06 μmol kg^{-1}, number of samples n = 5) of Certified Reference Material (Dickson, 1990) compared favorably to the expected value (2188.70 μmol kg^{-1}). A_T was measured on the samples in February 1993 using a closed-cell potentiometric titration system (Bradshaw *et al*, 1981; Brewer *et al.*, 1986; Dyrssen, 1965). Precision for C_T and A_T was determined to be ± 1.0 μmol kg^{-1} and 3.6 μeq kg^{-1}, respectively, using 12 pairs of duplicate samples (Dickson and Goyet, 1991). A_T, C_T, and the equilibrium constants of Goyet and Poisson (1989) were used with an iterative Newton-Raphson numerical method (Press *et al.*, 1989) to solve for carbonate alkalinity A_C and pCO_2 (Peng *et al.*, 1987). Precision estimates calculated similarly for A_C and pCO_2 are ± 2.8 μeq kg^{-1} and 6.2 μatm, respectively. Debate over choice of equilibrium constants continues to this day. Those of Goyet and Poisson (1989) were considered to be the most accurate for low temperatures at the time we used them (C. Goyet, personal communication). Sensitivity of our results to these constants should not affect our overall conclusions.

Calculation of biological impact on C_T

Using a Lagrangian mass balance approach for C_T (modified from Broecker and Peng, 1992) at each sampled depth in the Polar Water (PW) mass (0-70 m) in the polynya, we assumed that all changes in C_T during a water parcel's transit through the polynya region were attributable to dilution D by sea ice melt or rivers, calcium carbonate precipitation or dissolution (+ or - P, respectively, which effectively includes any removal or addition of alkalinity

not accounted for by dilution), atmospheric gas exchange due to warming G_w or diffusion G_d, and net biological processes B:

$$C_{To} = C_{Ts} - D - P - G_w - G_d - B \qquad (1)$$

where subscript o stands for observed values and s for source water values. Splitting the gas exchange term into two parts is somewhat artificial since the two processes are certainly linked. Our purpose was to try to distinguish between outgassing due to solubility and dissociation changes caused by warming (which we could account for explicitly; see below) and mechanically driven diffusive gas exchange.

Mixing between the PW and the deeper Arctic Intermediate Water (AIW) is considered negligible because of the strong density gradient (Bourke *et al.*, 1987; Schneider and Budéus, 1994). We solved for D based on changes in salinity, P based on changes in nitrate-corrected A_C, and G_w based on changes in pCO_2 (using the empirical k = 4.23% °C^{-1}; Chipman *et al.*, 1993) and K_H (according to the constants of Goyet and Poisson, 1989) as a function of temperature T (thereby accounting for the effects of temperature on pCO_2 solubility and all apparent dissociation constants). The remaining difference between source and observed values must be due to G_d and B. Thus for each profile and depth (z),

$$G_d + B_{(z)} = C_{T_s} - \left[C_{T_s}\left(\frac{S_s - S_{o(z)}}{S_s}\right)\right] - \left(\frac{1}{2}\left\{\frac{S_{o(z)}}{S_s}\left[A_c + (NO_3)\right]_s - \left[A_c + (NO_3)\right]_{o(z)}\right\}\right)$$

$$- \left[K_{H_{o(z)}} pCO_{2_s} exp^{k(T_{o(z)} - T_s)} - K_{H_{o(z)}} pCO_{2_s}\right] - C_{T_{o(z)}} \qquad (2)$$

We used C_{Ts} = 2110 μmol kg^{-1}, S_s = 32.276, A_{Cs} = 2178 μeq kg^{-1}, NO_{3s} = 6 μmol kg^{-1} (or 8 μmol kg^{-1} for four southern stations where East Greenland Current (EGC) influence is greater), pCO_{2s} = 315 μatm, and T_s = -1.7°C as source water values representing PW at 70 m (Wallace *et al.*, 1995a). Use of these baseline source values required several assumptions. (1) The water column was vertically well mixed in winter. (2) The values at 70 m remained unchanged since winter. (3) Nitrate depletion occurred after meltwater dilution. The first assumption is supported by observations of well-mixed surface waters during the FS *Polarstern* ARKTIS expedition (ARK IX leg 2) in spring of 1993 (Budéus and Schneider, 1994). The second is supported by salinity-scaled C_T - A_T diagrams of the area (see below) and by the observation that the base of the PW (~70 m) was at the maximal extent of the observed 1% light level. This level approximates the compensation depth where biological activity has no net effect on inorganic carbon (Sverdrup, 1953). Moreover, microheterotrophic respiration rates were very low in intermediate waters below the euphotic zone (W. Ritzrau and J.W. Deming, unpublished manuscript) which had a mean depth of 31 ± 12 m in the polynya. Nitrogen depletion (e.g., by ice algae) may have occurred before dilution of surface layers by ice meltwater. In this event the larger removal of nitrate would affect alkalinity and increase the contribution of precipitation to the total loss of C_T by less than 1% in nearly all cases.

Mean wind speeds in the polynya during the month of the cruise were low (3.9 ± 1.5 m s^{-1} at 16 m above sea level), resulting in low gas transfer velocities (Erickson, 1993; Liss and Merlivat, 1986) and therefore negligible diffusive gas exchange, regardless of any carbon gradient between the surface ocean and the atmosphere (see below). By setting G_d equal to zero, we solved

for $B_{(z)}$, the depth-specific inorganic carbon deficit due to biological processes. Integrating over the entire PW mass gave the biologically mediated C_T deficit B for each station. A Monte Carlo-type error analysis was performed for B. The calculation was repeated 1000 times, using randomly generated values for each parameter from a Gaussian distribution with its own mean and standard deviation (Press *et al.*, 1989). Mean values in the analysis were either source water values (as above) or average PW values. Standard deviations for each were estimated from measurements on 12 pairs of duplicate samples (Dickson and Goyet, 1991).

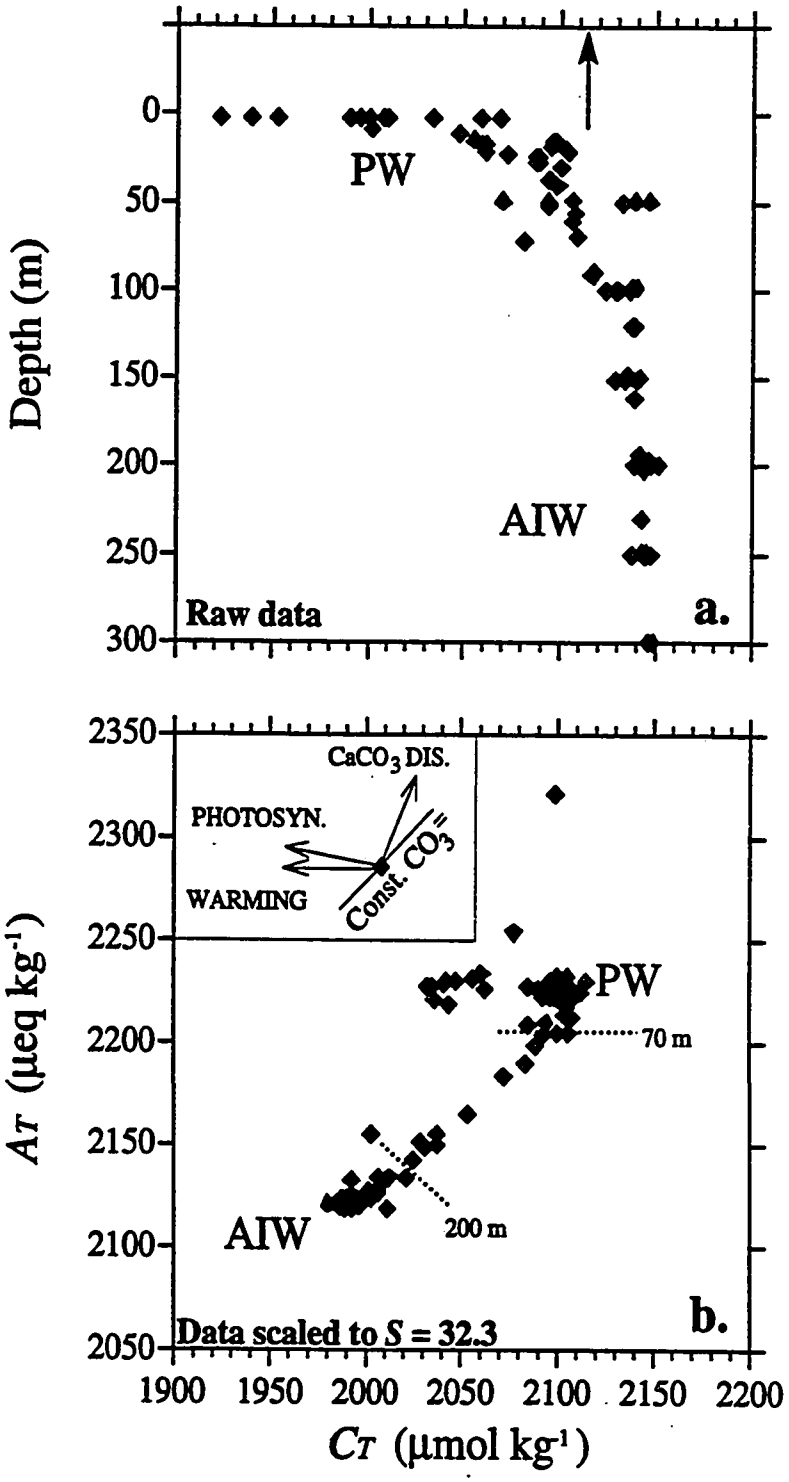
Results and discussion

Physical and chemical processes

Most stations showed a significant reduction of total dissolved inorganic carbon C_T in the surface waters (Figure II-2a). Dilution by meltwater was the dominant physical mechanism, responsible for an average of 36% of the total apparent C_T deficit in the polynya (up to 100% at low-productivity stations in the ice-covered central region). Surface (0-70 m) A_T correlated positively with S ($\alpha < 0.01$, data not shown) in this environment where freshwater input produced surface waters with salinities as low as 22.

Calcium carbonate precipitation P accounted for an average of 12% of the total C_T deficit in the polynya. In the surface waters of some stations (notably station 49 in the far northeastern part of the region), however, dissolution of $CaCO_3$ contributed excess carbon up to 61% of the measured difference between observed values and source C_T values. Excess alkalinity due to dissolution can be observed on a plot of salinity-scaled C_T versus A_T

Figure II-2. (a) Total dissolved inorganic carbon C_T depth profiles for all stations sampled showing surface depletion. Polar Water (PW) is shallower than 70 m; Arctic Intermediate Water (AIW) is below 70 m. Arrow denotes baseline C_T value for PW. Three data points richer than C_T baseline at 50 m were from the Belgica Trough region where the PW layer was shallower. (b) C_T scaled to a salinity of 32.3 and compared to similarly scaled total alkalinity A_T, essentially removing the effects of dilution. PW samples cluster in the top right corner and AIW samples in the bottom left. Sample depth is indicated by dotted lines. Data to the left and above the PW endpoint at C_T ~ 2110 μmol kg^{-1} and A_T ~ 2223 μeq kg^{-1} show the combined influence of photosynthesis, $CaCO_3$ dissolution or precipitation, and warming (inset after *Broecker and Peng*, 1989). The data point with the highest A_T, showing the greatest influence of $CaCO_3$ dissolution, is from the most northeasterly station (49).


Depth (m)
0
50
100
150
200
250
300
PW
AIW
Raw data
a.
2350
2300
2250
2200
2150
2100
2050
$CaCO_3$ DIS.
PHOTOSYN.
WARMING
Const. $CO_3^{=}$
PW
70 m
AIW
200 m
Data scaled to $S = 32.3$
b.
A_T (μeq kg^{-1})
1900
1950
2000
2050
2100
2150
2200
C_T (μmol kg^{-1})

(Figure II-2b) when D is essentially removed. The extrapolated freshwater alkalinity end-member (y intercept of the S versus A_T plot) was low (57 ± 170 μeq kg^{-1}) compared with the 1400 μeq kg^{-1} end-member observed in the nearby EGC (Anderson and Dyrssen, 1981), an excess which has been attributed to alkaline riverine input to the Arctic Ocean. The polynya value can be explained by the local melting of sea ice which contributes up to 112 μeq kg^{-1} in the EGC region (Tan *et al.*, 1983).

Near-surface waters (0-10 m) in the polynya exhibited temperatures as high as 3°C (Wallace *et al.*, 1995a), indicating a warming of up to 5°C from initial wintertime conditions (i.e., the freezing point of seawater, approximately -1.8°C). Surface pCO_2 values, however, were never found to be supersaturated (average surface pCO_2 = 218 ± 34; see Table II-1). In the most extreme case, warming could have caused only minor oversaturations of pCO_2; source waters from 70 m (pCO_2 ~ 315 μatm), if somehow warmed up to 3°C without any dilution or biological uptake of C_T, would give a maximal pCO_2 of 385 μatm. The average pCO_2 that would be achieved by warming all PW samples to 3°C was 323 ± 62 μatm. Losses of carbon due to warming had a negligible effect on C_T.

Biological depletion

After correcting for the effects of these physical-chemical processes, C_T remained depleted in the surface waters compared to the assumed source water. The range determined for the biologically mediated inorganic carbon deficit B over the entire region was 0.05 - 1.6 mol C m^{-2} (± 0.1; standard deviation based on results of Monte Carlo simulation), with low values found in the ice-covered central region and higher values seen in both the

open waters of the northern trough (also known as the Westwind Trough) and the more ice-covered southern trough (or Belgica Trough) region influenced by the EGC (Table II-1). Alternative calculations of B assuming predilution depletion of nitrate were lower by less than 1% in nearly all cases. On average, biological processes account for more than half of the depleted inorganic carbon in the polynya and as much as 97% at some stations.

Independent confirmation of biological removal of C_T was provided by comparing B to other biologically relevant parameters on a regional basis. Significant correlations were found between B and integrated chlorophyll a ($\alpha < 0.01$, $n = 11$; data not shown), integrated particulate organic carbon (POC)($\alpha < 0.05$, $n = 10$; see Figure II-3a), and the dissolved inorganic nitrogen (DIN) deficit (ΔDIN; $\alpha < 0.05$, $n = 13$, see Figure II-3b). Errors on the estimated slopes of each reduced major axis (Sokal and Rohlf, 1981) were large, not because of measurement error but probably just because of high variability between stations (see below). This degree of variability agrees with general observations of productivity in the Arctic (Codispoti *et al.*, 1991) and the observed "mosaic" of primary productivity in the polynya (NEWater Investigators, 1993), suggesting that the polynya was heterogeneous in both space and time. At each station, however, the biologically mediated C_T deficit B provides a local, time-integrated, unambiguous signature of net organic carbon production.

Despite the high variability between stations and resulting high error estimates on the derived slopes of each reduced major axis (expressed as ± 95% confidence limits below) the regional relationships between B and other biologically important parameters provide some insight to carbon cycling

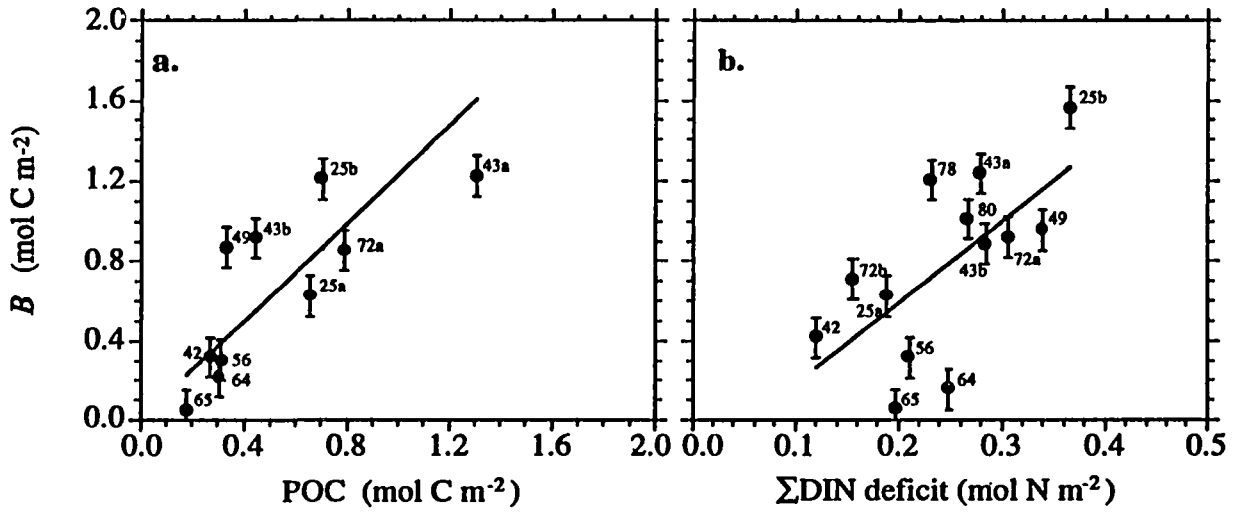


Figure II-3. Biologically mediated C_T deficit B (mol C m^{-2}) as a function of (a) integrated particulate organic carbon (POC) (mol C m^{-2}; $r^2 = 0.598$) and (b) salinity-scaled dissolved inorganic nitrogen deficit (ΔDIN) (mol N m^{-2}; $r^2 = 0.434$). Error bars denote 1 standard deviation for B based on Monte Carlo simulation (see text). Solid lines result from geometric mean regression. Numbers adjacent to points indicate stations (see Table II-1). Values for B in Figure 3a are sometimes integrated to shallower depths than in Figure 3b to match POC measurements.

within the polynya. Ratios of *B* to chlorophyll (114 ± 57 g C g chl a^{-1}, based on geometric mean regression of integrated values; Sokal and Rohlf, 1981) were high compared to typical cellular carbon-to-chlorophyll ratios (30, on average for temperate phytoplankton; 25 for vigorously growing phytoplankton, 60 for phytoplankton in nitrate-depleted water; Antia *et al.*, 1963). Phytoplankton carbon-to-chlorophyll ratios from subsurface chlorophyll maxima averaged about 26 (± 16 g C g chl a^{-1}) but increased to values as high as 300 in the near-surface waters (B. Booth, personal communication, 1994). Polar phytoplankton often exhibit carbon-to-chlorophyll ratios higher than temperate phytoplankton. Typical summertime polar algae carbon-to-chlorophyll range from 20 to 200, depending on species composition, irradiance, day length, mixed-layer depth, and temperature (Sakshaug, 1989; Smith and Sakshaug, 1990). The *B*-to-integrated-chlorophyll ratios measured in the polynya may suggest that the late-summer depleted inorganic carbon is in living cells near the surface that have adapted to long day lengths, high light levels, and low temperatures. Alternatively, they may suggest some loss of the chlorophyll relative to depleted inorganic carbon. Mechanisms for the latter include degradation within the POC pool (death of algal cells), particulate carbon export from the euphotic zone (e.g., by sinking), or carbon conversion to other organic pools, such as dissolved organic carbon (DOC) (via UV photolysis or extracellular enzymes; see Vetter and Deming, 1995), nonliving POC, or other biomass.

The overall relationship between *B* and integrated POC (operationally includes living and nonliving particulate organic carbon) had a slope near unity (1.2 ± 0.63, Figure II-3a; geometric mean regression; Sokal and Rohlf, 1984), suggesting POC as the dominant sink for *B*, although given the error on

the slope, we cannot rule out that part of B could also have been exported, converted to DOC, or shunted to higher trophic levels by the time of sampling.

The geometric mean regression of the biologically mediated C_T deficit ($\Delta DIC_B = B$) on the dissolved inorganic nitrogen deficit (ΔDIN) in the upper water column (Figure II-3b, $\alpha < 0.05$) gave a $\Delta DIC_B : \Delta DIN$ slope of 6.2 ($\pm$ 3.1). Wallace *et al.* (1995c) calculate the C : N ratio in a way that does not presume a preformed NO_3 concentration and come up with a very similar value (7.13 $\pm$ 1.53). The ratio also agrees well with the average particulate organic carbon to nitrogen ratio (POC : PON) for the same polynya stations (7.5 $\pm$ 3.2), the classical "Redfield" elemental ratio of 6.6 (error bars unknown; Redfield *et al.*, 1963), and the North Atlantic thermocline $\Delta C_T : \Delta NO_3$ ratios (5.5 $\pm$ 0.32; Takahashi *et al.*, 1985). The observed $\Delta DIC_B : \Delta DIN$ ratios in the polynya do differ significantly from the higher ratios of 11 - 14 ($\pm$ 1.7 - 3.1; Sambrotto *et al.*, 1993) reported for other high-latitude regions. The latter ratios have been interpreted to reflect complex nutrient cycling (Toggweiler, 1993), perhaps driven by zooplankton grazers (Banse, 1994). The low $\Delta DIC_B : \Delta DIN$ values at some stations in the polynya resemble that found in protein and bacteria (~4 ; Klapper, 1977; Porter, 1946) and in healthy phytoplankton cells (~3 for vigorous growth; Banse, 1974) and may have implications for a reduced microbial loop (Pomeroy and Wiebe, 1988), consistent with reduced macrograzer populations in the polynya (NEWater Investigators, 1993; Ashjian *et al.*, 1995) and for consequent export of high-quality (N rich) food to the benthos (Ambrose and Renaud, 1995).

To compare this estimate of biological removal of inorganic carbon to production calculations based on short-term incubations, B must be scaled to

a biologically relevant timescale (which may be different for each station). Assuming a constant value of 60 days for production, we estimate an average net production rate for the Northern Trough and polynya region of 14 ± 7.4 mmol C m^{-2} d^{-1} (maximal rate ~26 mmol C m^{-2} d^{-1}). This rate compares well with total primary production observed at the same stations using ^{14}C bicarbonate incubations (average = 21 ± 13 mmol C m^{-2} d^{-1}). Assuming that *B* approximates export (see discussion below) gives a median *e* ratio (export : total production) of 0.58, which also compares favorably to directly measured surface *f* ratios (new : total production) of 0.69 (± 0.16) determined by $^{15}NO_3$ and $^{15}NH_4$ incubations (Smith, 1995). *B* also compares well with the calculations of particle production estimates based on gradients in suspended particulate matter (31.6 mmol C m^{-2} d^{-1}; Smith *et al.*, 1995) and particle export based on ^{234}Th budgets (13 mmol C m^{-2} d^{-1} in 1992 Northern Trough and polynya stations; Cochran *et al.*, 1995). Our range falls below the estimate of export production based on along-trough nitrate gradients in the polynya (41.4 ± 16.1 mmol C m^{-2} d^{-1} ; Wallace *et al.*, 1995c). Selection of station-specific time periods over which to scale *B* (chosen based on forthcoming knowledge of hydrography) should improve the correspondence and resolve questions of production versus export.

Air-sea gas exchange and pCO_2

Surface waters of this region were undersaturated (average pCO_2 = 218 ± 34 µatm, *n* = 12; see Table II-1) relative to the atmosphere (pCO_2 ~ 345 µatm). On the basis of the Liss and Merlivat (1986) formulation of the gas transfer velocity, the average wind speed during the cruise, the average polynya air-sea gradient (ΔpCO_2 = 127 µatm), and CO_2 solubility (Weiss, 1974), the average

late summer air-sea flux was ~1.3 mmol C m^{-2} d^{-1}. This low flux was attributable to the low wind speeds experienced during the summer and validates the assumption of setting G_d equal to zero. Any exchange which may have occurred would have caused an underestimate of B.

In order for biological depletion of inorganic carbon in the NEW Polynya to be replenished locally from the atmosphere, a period of increased wind shear must occur before ice forms in the autumn. For most stations a stormy period of a few weeks would be sufficient to draw enough CO_2 from the atmosphere to replace most of the depleted carbon (Figure II-4). The actual number of days depends on which gas transfer velocity formulation is used. A complementary calculation based on buoyancy (Nelson *et al.*, 1989) suggests that these same winds would also mix the upper 70 m. Both of these calculations were based on simplified approximations and require further refinement. Data from a nearby weather station at Danmarkshavn (Figure II-5; see Figure II-1 for location) suggest that such wind events are not unreasonable, though the observed winds in September 1991 would not have completely removed all of the deficit seen in 1992 at all stations using the conservative Liss and Merlivat (1986) formulation of the air-sea gas exchange velocity. Erickson (1993) recently showed that the air-sea gas transfer velocity could be a factor of 2 greater than the Liss and Merlivat (1986) formulation at wind velocities in the range of 10-15 m s^{-1} when there is an air-sea temperature gradient such as that commonly seen in the Arctic. Use of this model would allow the Danmarkshavn wind data to replenish the depleted carbon at all stations. Additional wind data are necessary to determine the extent and interannual variability of autumn storm conditions in the polynya region. A partial test of the likelihood of complete replenishment is the

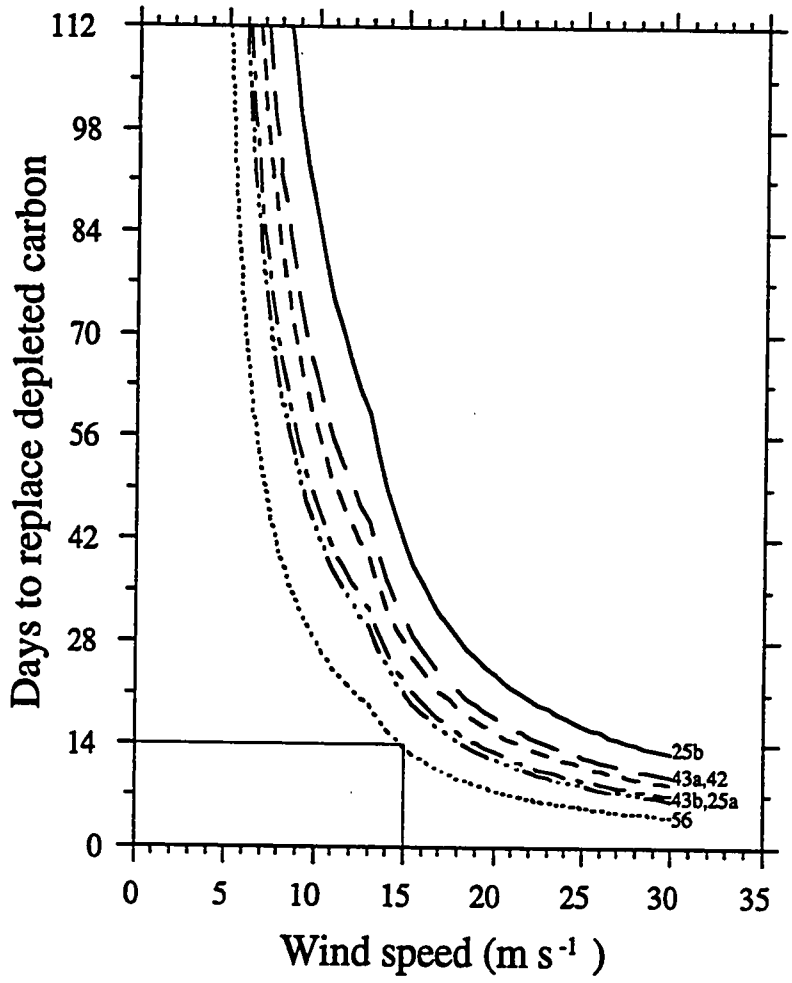


Figure II-4. Modeled results of the time required to replenish the biologically mediated C_T deficit B by air-sea gas exchange as a function of wind speed, using the most conservative Liss and Merlivat (1986) formulation of the air-sea gas exchange velocity, observed pCO_2 air-sea gradients, calculated biologically mediated carbon deficits, and in situ temperatures to calculate flux. For example, 14 days of winds at 15 m s^{-1} are required to replenish B at station 56. The six stations shown were in the polynya and Northern Trough regions (see Table II-1). Using the formulation of Erickson (1993) would shorten the required windy period by a factor of about 2 owing to enhanced turbulent mixing by a strong air-sea temperature differential.

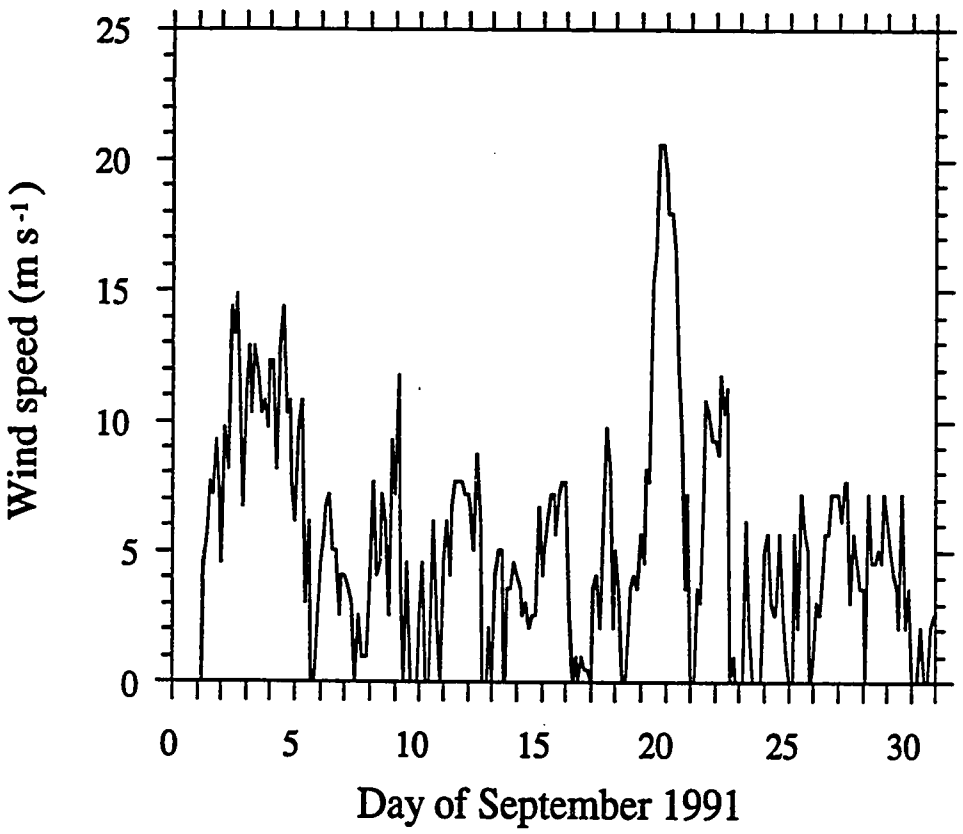


Figure II-5. Wind speed data from nearby Danmarkshavn weather station (on the peninsula just west of the southern trough; see Figure II-1) during September 1991. Measurements were recorded every 3 hours. A week of 10-15 m s^{-1} wind speeds plus several days of up to 20 m s^{-1} wind speeds should replenish most of the depleted inorganic carbon in the surface waters of the polynya.

degree to which the observed gases at 70 m are in equilibrium with the atmosphere. Late summer oxygen data from deep Polar Water suggest that the late fall mixed layer closely approached equilibrium with the atmosphere (O_2 saturation at 50-100 m = 95-100%; Wallace *et al.*, 1995c). The inorganic carbon data reported here (average pCO_2 at 50-100 m = 318 ± 17), however, do not support as complete an equilibrium. This test assumes that the 70-m water found in the polynya during summer was ventilated in the area during the previous autumn/winter. The validity of this assumption awaits a better understanding of the overall residence time of PW in the polynya region.

Rectification Hypothesis

The hypothesis

Our conceptual model for annual carbon cycling in the polynya (Figure II-6) depicts a net influx of CO_2 across the air-sea interface. Phytoplankton transfer significant inorganic carbon C_T to organic carbon (OC on Figure II-6) pools by photosynthesis during the summer. Although air-sea exchange is limited during the calm Arctic summer, replenishment could be achieved by a few weeks of strong winds before ice formation in the autumn (see Figure II-4; less time required if the Erickson (1993) model is used for gas transfer velocity). From the end of the autumn through the long (dark) winter when respiration must exceed carbon fixation, ice provides a barrier to the release of inorganic carbon to the atmosphere (Poisson and Chen, 1987). Subsequent, extensive springtime blooms of ice algal communities in this region (observed by myself and others aboard the FS *Polarstern* in May of 1993) provide a mechanism for initial removal of accumulated respiratory carbon

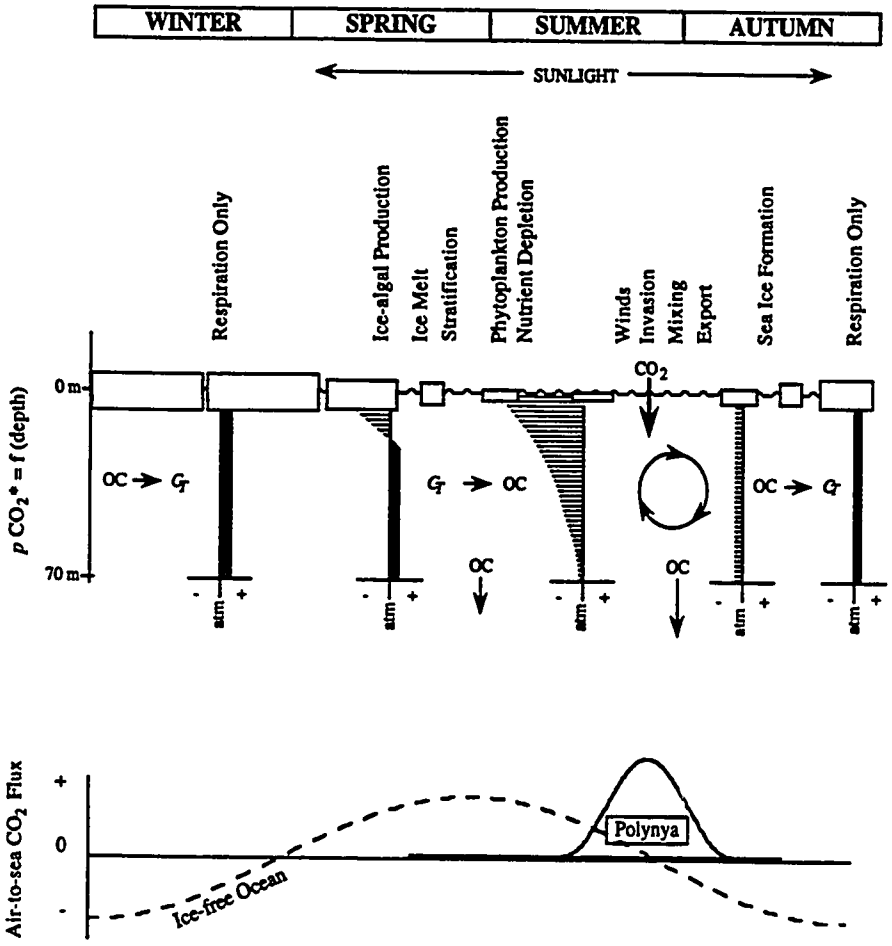


Figure II-6. Proposed annual cycle of biological and physical processes affecting CO_2 cycling in regions with seasonal sea ice (see text); top time line indicates vertical profiles of potential pCO_2 (asterisk indicates as if at 1 atm), supersaturated (solid) or undersaturated (striped) relative to the atmosphere; bottom time line compares the typical seasonal cycle for air-sea gas exchange in an ice-free ocean (dashed line, schematic only, actual timing may vary) to the proposed "rectification" scenario for polynyas or other seasonally ice-free Arctic shelves (solid line).

in near-surface layers before the ice melts (quantitative estimates of the amount of carbon removed by this effect are forthcoming). In addition, once the ice melts, meltwater creates a thin, near-surface, highly stratified layer which isolates most of the PW from air-sea gas exchange. In this scenario there is no time during the polynya's annual cycle when CO_2 can escape to the atmosphere. We refer to this cancellation of one half of the typical air-sea exchange cycle of an ice-free ocean as "rectification." With this annual cycle of events the polynya becomes a net sink for atmospheric carbon.

The application of this hypothesis to the polynya or any other Arctic region requires better understanding of the local and remote ventilation of the surface water. If there is significant flow of water into the polynya sometime during the annual cycle, then the hypothesized CO_2 replenishment by autumn storms could occur via replacement from nonpolynya waters instead. Source values observed at 70 m should reflect the properties of these waters. Forthcoming understanding of hydrography of this region will ultimately resolve the issue. If significant flow through the polynya region does occur, then the fate and timing of the outflowing, C_T-depleted polynya water will determine the effectiveness of the rectification process. We fully expect that as more data become available for the NEW Polynya and other Arctic regions, the hypothesis will be tested rigorously. Information required for a thorough test of the hypothesis includes seasonal pCO_2 data for the polynya or Arctic shelves in general, more accurate measurements or calculations of air-sea gas exchange (Erickson, 1993; Liss and Merlivat, 1986), improved wind data showing the extent of interannual spatial and temporal variability (late summer 1992 appeared to be much less windy than late summer 1993), and a more complete understanding of the hydrography of the

region, specifically the extent of flushing of the PW and the degree of mixing in the autumn.

Implications of the hypothesis

If we assume an interannual steady state and the replenishment of depleted inorganic carbon by the atmosphere, then export of an equivalent amount of carbon (organic or inorganic) to either the local sediments or the adjacent deep ocean is required. The annual net carbon export from the polynya is given by the annual air-sea flux of carbon which based on the rectification scenario is determined by the summertime carbon deficit attributable to biological cycling *B* set over the entire year, 0.32 - 1.6 mol C m^{-2} yr^{-1} (± 0.1, range now reflects polynya values only). This estimate of carbon export from an Arctic shelf ecosystem is consistent with previous estimates of carbon fluxes from shelves to the central Arctic Ocean based on transient tracer and oxygen data from the Arctic Ocean interior (0.8 - 2.8 mol C m^{-2} yr^{-1}; Wallace *et al.*, 1987) and is somewhat lower than those based on short-term incubations (0.39 - 8.6 mol C m^{-2} yr^{-1}; (Subba Rao and Platt, 1984) and an inorganic carbon budget calculated for the Arctic Ocean (2.1 - 5.4 mol C m^{-2} yr^{-1}; Anderson *et al.*, 1990).

If this level of export is extended to other seasonally open Arctic Shelf waters (total area = 3.4 x 10^{12} m^2; Walsh *et al.*, 1989), then up to 6 x 10^{12} mol C yr^{-1} (0.07 Gt C yr^{-1}) can be transferred from the atmosphere to the ocean by this process alone, assuming productivity in other arctic shelves is no higher than it was in the polynya. If we include the Antarctic, the total area of seasonally ice-covered seas is 25 million km^2, or 7% of the world's oceans (Niebauer and Alexander, 1989), which increases the total export to 4 x 10^{13}

mol C yr^{-1} (0.5 Gt C yr^{-1}). This quantity, though small relative to global primary production (~10^{15} mol C yr^{-1}), depends on the area of seasonal sea ice, according to our proposed scenario. The aerial extent of seasonal sea ice coverage appears to be very sensitive to atmospheric CO_2 levels. In a model by Wilson and Mitchell (1987), the areal extent of Arctic seasonal sea ice (ocean covered by ice in the winter but ice free in the summer) increases by 235% with a doubled CO_2 model scenario. If we take the total Arctic export number above (6×10^{12} mol C yr^{-1}) and increase it by 235%, we get 1.41×10^{13} mol C yr^{-1}, which is about 9% of the hypothesized missing oceanic sink of anthropogenic CO_2 (i.e., 2 Gt C yr^{-1} or 1.6×10^{14} mol C yr^{-1}; Broecker *et al.*, 1979; Quay *et al.*, 1992). Interestingly, the Wilson and Mitchell (1987) model shows the areal extent of seasonal sea ice decreasing in the Antarctic by 206%, but the net global change is still +127%. If we take the global area and increase it by 127%, we get 3.2×10^{13} m^2 of seasonally ice-covered seas, or an addition of 7×10^{12} m^2. If the rectification scenario can be extended to this increased area, then the additional uptake would be up to 1×10^{13} mol C yr-1 which is 7% of the hypothesized net oceanic uptake of anthropogenic CO_2. This effect would be amplified if higher production rates as measured in other Arctic waters pertained (Subba Rao and Platt, 1984) or if productivity increased in the Arctic Ocean due to global warming as hypothesized (Codispoti *et al.*, 1991; Walsh, 1989), although wind fields and hydrography in the Arctic Ocean might also affect the rectification scenario differently than in the polynya region.

The physical-biological interactions we have outlined for this environment provide a potential negative feedback to global CO_2 increases via their response to the predicted temperature increase and resultant changes in ice cover.

Conclusions

During a 1-month summertime study in the NEW Polynya off the northeast coast of Greenland, biological activity was found to have reduced significantly the total dissolved inorganic carbon inventory of the surface waters of most stations. This deficit correlated well on a regional basis with other biological parameters, such as integrated chlorophyll, integrated particulate organic carbon, and the dissolved inorganic nitrogen deficit. It was also consistent with other measurements of productivity in the polynya. Placing this deficit in the context of the annual sequence of events likely to occur in the polynya region allowed us to hypothesize a "rectification" of the typical air-sea CO_2 flux. This rectification would be unique to seasonally ice-covered regions. As a result, up to 2 mol C m^{-2} may be exported annually from the NEW Polynya. The possibility of off-shelf export and deepwater formation nearby may amplify the significance of this mechanism for carbon dioxide removal from the atmosphere to the deep ocean. By extrapolating this seasonal rectification cycle to other Arctic shelves and assuming an increased area of seasonal sea ice as postulated due to climate change, a negative feedback to increased atmospheric CO_2 has been identified via the interaction of biological and physical processes at high latitudes.

III. Pelagic Microbial Activity in the Northeast Water Polynya: Testing for Temperature and Substrate Interactions Using a Kinetic Approach.

Abstract

Previous research in high-latitude oceans has sometimes revealed a decoupling between phytoplankton and bacterial processes which could enhance vertical fluxes significant to global carbon cycling. A disproportionately negative effect of low temperature on the bacteria has been offered as a possible explanation. Pure cultures of marine bacteria have shown an enhanced substrate requirement at low temperatures, suggesting that cold-water bacteria may underutilize substrate pulses and thus enhance carbon export. To test these hypotheses, we used ^{14}C-amino acids and a kinetic approach with natural populations of pelagic Arctic bacteria during the summertime 1992 and 1993 expeditions to the Northeast Water Polynya. We also obtained and examined other data from the study region, including organic matter and chlorophyll *a* concentrations, bacterial abundance, size, and potential growth rates over a range of temperatures, to assist interpretation of the kinetics data. Results analyzed using multivariate ANOVA showed that added substrate concentration had a significant effect on utilization rates at all stations, but only some stations in the polynya showed a significant temperature effect. Interactions between temperature and substrate were detected only rarely. Values computed for the dimensionless ratio (P^*) of cell-specific diffusive flux to maximum uptake velocity suggested that the bacteria were not diffusion limited in this sub-zero environment, although this conclusion was sensitive to assumptions about

the percentage of metabolically active cells. Contrary to prediction, measurements of specific affinity (a^0_A, *sensu* Button), maximum utilization rate (V_{max}), and half-saturation concentration (K_t) revealed mixed responses to temperature depending on station location, with extremely psychrophilic behavior observed at stations most likely to be influenced by direct Arctic ocean outflow. Unusually large cells were also observed and argued to be consistent with both low substrate concentration and grazing pressures on these organisms. The observed heterogeneity in sensitivity to temperature, within a relatively small and well-defined ecosystem such as the polynya, implies the need for improved understanding of phenotypic and genotypic microbial diversity of psychrophiles before we can generalize or predict the role of temperature in high-latitude carbon export.

Introduction

For a seasonally ice-covered marine ecosystem to function as a sink for atmospheric carbon, net autotrophy must exceed net heterotrophy during the times when surface waters are free of ice and open to air-sea gas exchange (Yager *et al.*, 1995). Of interest are the mechanisms by which such a condition may occur and whether or not these mechanisms are sensitive to warming. Pomeroy and co-workers have consistently recorded a de-coupling between phytoplankton and bacterioplankton activity in the high-latitude environments they have examined (Pomeroy and Deibel, 1986; Pomeroy *et al.*, 1990; Pomeroy and Wiebe, 1988; Pomeroy and Wiebe, 1993), suggesting that polar waters may provide more carbon and energy to higher trophic levels because bacteria are disproportionately affected by low temperature compared to autotrophs. Depending on the activities of the higher trophic

levels, this hypothesis can also be invoked to explain why polar oceans may function as efficient biological pumps (Ashjian *et al.*, 1995). The possibility that the situation could change with predicted warming at high latitudes (Hansen *et al.*, 1984) and cause a feedback to atmospheric carbon levels has stimulated much research in these regions (Smith *et al.*, 1990).

Further research by Pomeroy and co-workers suggests that the bacterial limitation occurs at the level of substrate uptake. In the field, they observe a connection between in situ temperature and required food concentrations for growth (Pomeroy *et al.*, 1991); from studies of laboratory cultures they conclude that mesophilic (moderate temperature-loving) and psychrotolerant (cold-tolerant) bacterial isolates from cold environments have enhanced substrate requirements at low temperatures (Wiebe *et al.*, 1992; 1993) but grow equally well on any substrate concentration if warmed. The response of psychrophiles (cold-loving bacteria) appears more complicated (Pomeroy *et al.*, 1995), motivating the need to better understand the phenotypic and genotypic diversity of indigenous polar microbes.

Independent tests of the decoupling hypothesis have yielded mixed results, undoubtedly due in part to the potential for very different types of microbial communities to exist in each test environment (Baross and Morita, 1978 ; DeLille *et al.*, 1988), the myriad of techniques used to measure activity, and the range in temporal and spatial scales over which the activity has been measured. Also unclear is the importance of grazing pressure on the bacteria (Billen and Becquevort, 1991; Kuparinen and Bjørnsen, 1992a; Moisan *et al.*, 1991; Putt *et al.*, 1991) and the potential response of bacterivores to low temperature (Choi and Peters, 1992).

A common method for assessing substrate dependence of bacteria is to use phytoplankton biomass (e.g. chlorophyll *a* concentration) or production as a proxy for bacterial substrate. This assumes a direct coupling between the availability of utilizable dissolved organic matter (UDOM) for the bacteria and its ultimate source, primary production. Most of the temperate ocean seems to satisfy this assumption reasonably well, as exemplified by a positive linear relationship between chlorophyll *a* concentration and bacterial abundance and also between net primary production and bacterial production (Ducklow and Carlson, 1992). There are indications, however, that not all polar environments follow the same pattern as the temperate ocean or as each other.

In keeping with the temperate model, some polar regions do show a significant positive correlation between phytoplankton production and bacterial production (Kuparinen and Bjørnsen, 1992b; Li and Dickie, 1987; Sullivan *et al.*, 1990). Some sea-ice microbial communities also show good correspondence between the temperature ranges for maximal growth rate of both autotrophs and heterotrophs (Kottmeier and Sullivan, 1988) and a good correlation between the timing of bacterial growth and that of ice-algal production (Smith *et al.*, 1989).

In other polar regions, including the Northeast Water (NEW) Polynya where we worked, the coupling between bacteria and phytoplankton is either different from the temperate standard (Karl *et al.*, 1991; Rivkin *et al.*, 1991; Smith *et al.*, 1995) or missing altogether (Billen and Becquevort, 1991). In these cases, the mechanism explaining poor or absent coupling may not be a disproportionate response to low temperature but rather a temporal or spatial disconnect between phytoplankton production and the generation of UDOM

for the bacteria (Billen, 1990), although such a lag is not always observed (Thingstad and Martinussen, 1991). For example, the generation of UDOM may be delayed if cells are not "leaky" (e.g. Hellebust, 1965), if there are few herbivores present to generate UDOM by sloppy feeding or incomplete digestion (e.g. Fuhrman, 1987), or if cells aggregate or colonize and sink rapidly from the surface waters (Wassman *et al.*, 1990). Also, contrary to prediction, limitations on bacterial abundance in these cases appear at higher chlorophyll *a* concentrations ($\geq$ 2.5 µg Chl *a* l^{-1}; Karl *et al.*, 1991) rather than at lower chlorophyll *a* concentrations where the pure culture data suggest it should be.

In questioning the biological basis for disproportionate temperature effects at low temperatures, Jumars *et al.* (1993) analyzed the diffusive flux of dissolved organic matter (DOM) to free-living osmotrophs over a range of cell sizes. They predicted that an enhanced substrate requirement by bacteria-sized cells at low temperature may be due simply to diffusional effects, since low temperature can reduce the diffusive flux of substrate to the cell surface (through its effect on the seawater viscosity and the diffusion coefficient). Bacteria can maximize this diffusive flux in two ways: increase cell size (since flux increases linearly with cell radius) to a point where the marginal gains of being larger exceed the costs (Jumars, 1993) or maintain the maximum gradient in substrate between cell surface and external medium by keeping cell-surface concentration at zero. Diffusion limitation occurs when the cell is able to maintain surface concentration at zero, but the magnitude of the diffusive flux to the cell is less than the rate of substrate transport across the cell membrane. The extent of diffusion limitation is described by a dimensionless ratio of these two rates (P*; Pasciak and Gavis, 1974) which can

also be considered a mass Biot number (e.g. a ratio of two mass transport rates; (Carberry, 1976).

In the absence of diffusion limitation, the ability to take up substrate depends on the membrane transport systems of the cell and will thus rely on transporter density and capacity as described by kinetic parameters of specific affinity, a^0_A , and maximum uptake velocity, V_{max} (Button, 1985). Specific affinity is perhaps the most direct way to test for substrate limitation since it directly reflects the responsiveness of cells to small increases in substrate concentration.

In this study, we used a kinetic approach with ^{14}C-amino acids to test for temperature effects on a^0_A, V_{max}, and P^*, as determined for natural populations of pelagic bacteria living in the moderately productive, low temperature Arctic coastal environment of the NEW Polynya. To assist in interpreting the kinetics results, we also examined concentrations of natural substrates, chlorophyll *a*, and bacteria throughout the study area, and determined median cell size and temperature optima for potential growth rates at selected stations. As "the science that relates microbial growth rates to the nutrient concentrations on which they depend" (Button, 1985), kinetics can be divided into a two step process: the concentration-limited utilization of nutrients and the efficiency with which those nutrients translate into growth (Button, 1985). We address the first process in this chapter (Yager and Deming, in prep); discussion of growth efficiency in this environment will follow (Chapter 4; Yager and Deming, in prep.).

Methods

Sample collection

The Northeast Water (NEW) Polynya on the continental shelf of Northeast Greenland (Fig. III-1) was the site of a major international and multi-disciplinary study during the summers of 1992 and 1993 (NEWater Investigators, 1993; Overland *et al.*, 1995; Hirche and Deming, in press). Samples were collected during both cruises of the USCGC *Polar Sea* (July 15 - August 15, 1992; July 18 - August 18, 1993) and one leg of the FV *Polarstern* cruise in 1993 (ARKIX/2, May 20-June 29). Unless otherwise stated, water samples were recovered in Niskin bottles from near-surface fluorescence maxima, as measured by a SeaTech fluorometer attached to the CTD, from a depth range of 0 to 60 m (Fig. III-1; Table III-1). Samples were removed carefully to sterile polyethylene containers immediately upon arrival on deck and stored in a -1°C waterbath. All manipulations with live samples, prior to experimental incubations in temperature-controlled waterbaths, were carried out using ice/seawater baths to keep samples at -1°C at all times. This effort was assisted by performing all operations (except open-container radioisotope handling on board *Polarstern*) inside refrigerated (0-2°C) laboratory vans. Water samples were not prefiltered prior to incubation so as not to introduce artifacts in DOM concentrations (Carlson *et al.*, 1996).

Substrate availability

Subsamples were taken for dissolved free amino acids (DFAA), dissolved organic carbon (DOC), and total organic carbon (TOC). DFAA concentrations were measured using high-performance liquid

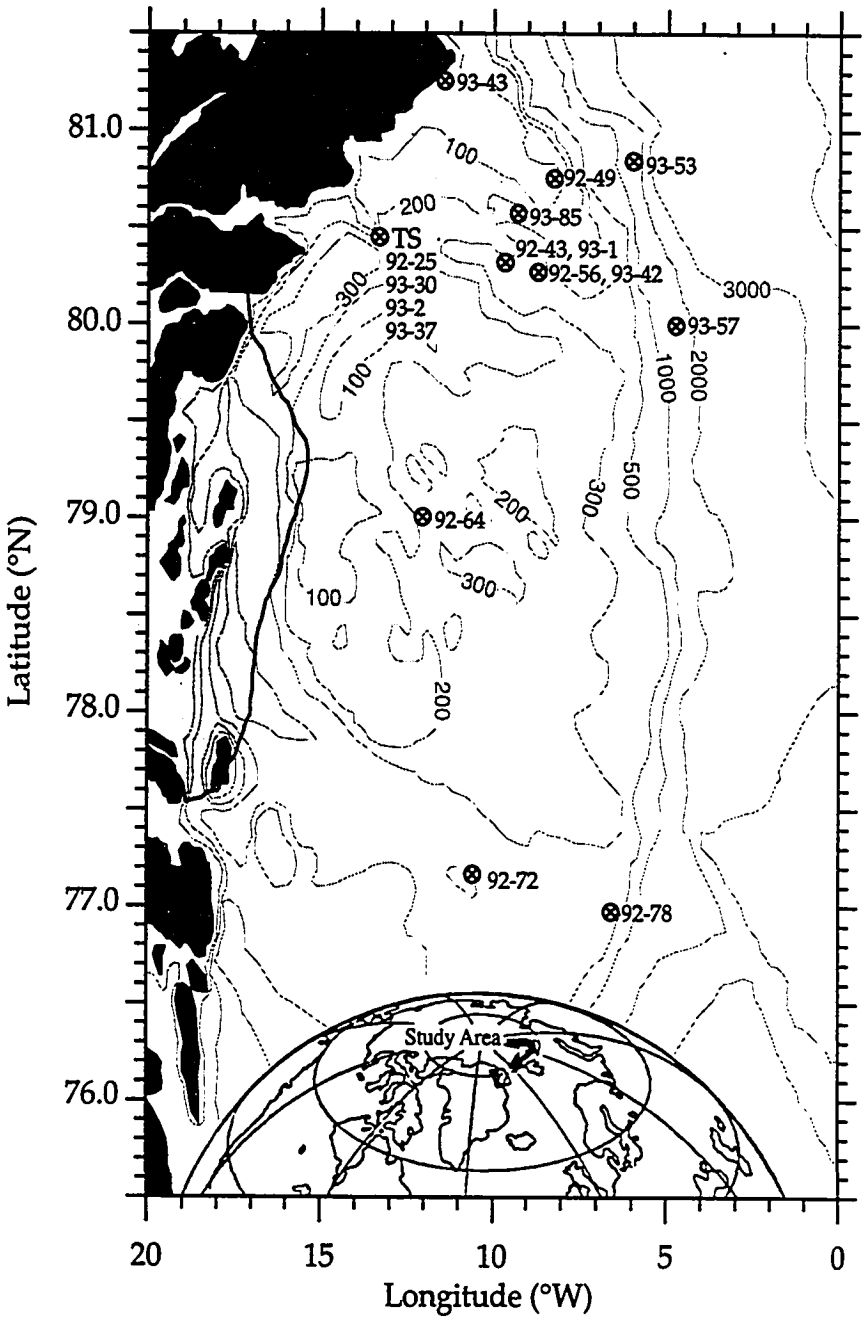


Figure III-1. Map of the Northeast Water Polynya with station locations for microbiological experiments. Depth contours in meters. Station numbers from each cruise are shown preceded by the last two digits of the year they were sampled. Several locations, including the time series location (TS), were sampled multiple times during the two field seasons (see Table III-1 for individual station information).

Table III-1: NEW Polynya station information for microbiological samples.

Station	Date sampled	Latitude (N.)	Longitude (W.)	Sample depth (m)	BNLID[a]	In situ Temp. (°C)	Salinity	DFAA[b] (nM)	Bacteria x 10^7 l^{-1} (± 1SE)[c]	FDC[d] (% ± 1SE)[c]
92-25A[e]	7/26/92	80° 26.56'	13° 19.89'	16	394	0.266	32.316	67	5.01 (1.59)	1.30 (0.57)
92-25B[e]	7/28/92	80° 27.15'	13° 23.04'	25	476	-1.087	32.322	86	6.91 (0.33)	2.63 (0.48)
92-43A	7/31/92	80° 18.71'	9° 41.87'	38	733	-1.477	32.334	31	9.75 (1.21)	2.93 (0.77)
92-43B	8/1/92	80° 17.80'	9° 25.55'	17	804	-1.308	31.770	47	6.30 (2.95)	1.56 (0.65)
92-49	8/5/92	80° 46.20'	8° 10.86'	50	880	-1.344	31.901	63	5.15 (0.70)	2.83 (0.32)
92-56	8/7/92	80° 15.82'	8° 40.02'	16	985	-1.390	31.578	143	3.93 (1.10)	1.98 (1.09)
92-64	8/9/92	79° 01:34'	12° 02.98'	27	1115	-1.552	31.997	-	5.15 (0.80)	1.45 (1.32)
92-72	8/12/92	77° 08.23'	10° 32.84'	252	1257	+1.317	34.870	ND	2.63 (0.34)	1.37 (1.87)
92-78	8/14/92	76° 59.28'	6° 34.53'	230	1375	+1.441	34.884	ND	4.12 (0.27)	1.34 (0.70)
93-30[e]	5/30/93	80° 26.34'	13° 38.66'	0[f]	NA	-1.700	32.310	-	-	-
93-43	6/5/93	81° 15.00'	11° 30.00'	26	NA	-1.570	31.580	[108 : 6.4]	-	-
93-85	6/14/93	80° 35.00'	9° 22.00'	58	NA	-1.620	32.060	[94 : 5.12]	-	-
93-1	7/23/93	80° 19.38'	9° 45.36'	20	3024	-1.404	32.313	-	6.72[g]	1.61[g]
93-2[e]	7/23/93	80° 26.40'	13° 20.58'	12	3047	-1.194	32.237	-	6.36[g]	2.27[g]
93-37[e]	7/30/93	80° 26.70'	13° 19.98'	14	3638	-1.621	32.252	-	5.50	4.26
93-42	8/1/93	80° 16.14'	8° 57.00'	23	3808	-1.331	32.289	-	8.36	4.09
93-53	8/3/93	80° 49.86'	6° 00.06'	14	3988	-1.148	31.208	-	11.3[g]	3.35[g]
93-57	8/4/93	79° 58.68'	4° 44.16'	13	4084	-1.330	31.032	-	17.3	3.26

a. Brookhaven National Laboratory Identification bottle number, used throughout both Polar Sea cruises. NA = not applicable: samples collected aboard FV Polarstern, no ID# associated with bottle.
b. DFAA concentrations except for [ ] values at *Polarstern* stations which are DOC:DON values in µM; ND = not detected; - = samples lost.
c. Standard errors calculated from counts on up to three replicate samples in 1992. Standard errors not available for 1993; n = 1.
d. FDC is Frequency of Dividing Cells, or cells showing fractional septa, as seen under epifluorescence microscope and Acridine orange stain.
e. Time-series location; A and B refer to repeated visits to same location.
f. Sample collected using tripod-mounted Niskin bottle from just under ice after drilling.
g. Bacterial count made from sample taken by different Niskin bottle at approximately the same depth, time, and location.

chromatography (HPLC; Cowie and Hedges, 1992) on seawater samples that had been filtered (0.2 µm cellulose acetate; Corning), acidified using 100 µl double-distilled HCl, and then stored refrigerated until processed in Seattle. TOC (unfiltered) and DOC (filtered as above) samples were collected into muffled glass scintillation vials (20 ml), acidified with 50 µl H_3PO_4 (in 1992) or 50 µl double distilled HCl (in 1993), capped with Teflon septa which had been rinsed with acetone, and then frozen for processing in Seattle using Shimadzu TOC-5000 Total Organic Carbon Analyzer. Chlorophyll *a* concentrations from the 1992 and 1993 Polar Sea cruises are available in data reports (Wallace *et al.*, 1995a; 1995b), accessible via the National Technical Information Service (U.S. Dept. of Commerce) or the NSDIC database and summarized for 1992 in the literature (Smith *et al.*, 1995).

Bacterial abundance and cell size

Subsamples were fixed with 2% formaldehyde and kept cold and in the dark until processed in Seattle. Samples were filtered onto Irgalan-black 0.2-µm Nuclepore filters, stained with both DAPI (0.002%) and Acridine Orange (AO; 1%), and counted using epifluorescence microscopy (Relexans *et al.*, in press; this approach uses AO to count and DAPI to confirm). The frequency of dividing cells was also noted during counting.

Cell size was determined for 20 samples from the times-series station (80° 26'N, 13° 20'W, Sta. 92-25 and 93-37) by measuring with calipers the length and width of at least 100 photographed cells stained with AO and projected onto a flat white surface (Lee and Fuhrman, 1987). A cross-calibration was done as above using fluorescent 5-µm microspheres. Biovolumes were calculated assuming cells were either coccoid [$^4/_3 \pi r^3$,

where r is radius) or rod-shaped $(\pi(W/2)^2 L) + 4/3\pi(W/2)^3$, where W is the width and L is the length of the cell). Cell sizes for both rods and cocci measured from a single sample exhibited distinctly non-normal frequency distributions, so medians, rather than means were computed. The cell volume for each sample was determined by combining the frequency-weighted median volumes of cocci and rods. The 20 medians were normally distributed, so from them we could compute a mean (average) cell size for all samples.

Encounter probabilities between bacteria and potential predators were estimated with coagulation theory according to Jumars (1993), using second-order rate coefficients (K_{ij}) and contact efficiencies (E_{ij}) for neutrally buoyant, spherical particles of diameter d_i (bacteria as prey) and d_j (predator). The mechanisms included in the calculation were 1) Brownian motion without settling and 2) turbulent shear for particles smaller than the Kolmogorov scale (Hill and Nowell, 1990). We assumed a typical turbulent kinetic energy dissipation rate (ε) of 0.01 $cm^2\ s^{-3}$ (or 10^{-6} W kg^{-1}; see review by Yamazaki and Osborn, 1988) and used a typical in situ temperature (-1.5°C) and salinity (32‰) from the polynya region to compute dynamic (μ) and kinematic (ν) viscosity (Jumars *et al.*, 1993).

Potential growth rates

Potential growth rates were determined by measuring changes in the numbers of bacterial cells in organically enriched samples incubated over an 8-d time-course experiment (method modified from Deming, 1985). Two-ml aliquots were distributed into 4-ml sterile plastic test tubes, enriched with 30 mg C l^{-1} yeast extract (DIFCO; final concentration 0.01% by weight), and

incubated in the dark in temperature-controlled water baths at -1.5, 2, 5, 10, or 20°C. Additional replicates of the in situ temperature (-1.5°C) incubations were augmented with 0.002% nalidixic acid, with or without addition of 0.025% yeast extract (Kogure *et al.*, 1979), to assess for percent active bacteria. All samples were fixed using 2% formaldehyde after incubation for 0, 2, 4, or 8 d and kept refrigerated in the dark until processing. All samples were stained and counted using epifluorescence microscopy, as described above.

Kinetics

To test the temperature sensitivity of substrate uptake kinetics, we measured the utilization of a mixture of uniformly (all carbons) ^{14}C-labeled amino acids purchased from New England Nuclear (NEC-445E, L-[^{14}C(U)] amino acid mixture, 3.80 μmol mCi^{-1} or 218 μg C mCi^{-1}) using a method modified from Deming (1993), based on the original method by Wright and Hobbie (1966). The prepared mixture of amino acids we used compares favorably ($r = 0.83$, $P < 0.01$) on a mole % basis with amino acid compositions of coastal marine phytoplankton (Cowie and Hedges, 1992b). The added concentrations ranged from tracer to saturation levels: 0.5, 1, 2, 5, 10, 20, 50, or 100 nM (0.03 to 6 μg C l^{-1}). The range of incubation temperatures (-1.5 to 5°C) included and extended slightly beyond that to which organisms were typically exposed in the surface waters of the polynya (range of -1.8 to 3.5 °C in 1992, Wallace *et al.*, 1995a; although occasionally the top 2 m warmed to as much as 6.4°C in 1993; Wallace *et al.*, 1995b).

Each experiment involved 18 treatments (six substrate concentrations and three temperatures) with either two or three replicates for each. The rates for each replicate were determined by either time-course (up to seven time

points including a time zero) or end-point experiments. Time-course experiments were carried out early in the first cruise and then occasionally throughout to determine the best time to use for endpoint experiments. Subsamples were taken by 20-ml increments using a sterilized dispenser (Brinkman Instruments, Inc.; 05-03-040-7) for individual incubations in 50-ml, acid-washed, autoclaved, borosilicate glass serum bottles (Kimble, 61000G-50). Each sample received a small volume (0.54 ml) of sterile stock solution of mixed amino acids to obtain the desired final concentration. Samples were capped with gray-butyl stoppers (Kimble, 73828-21) and aluminum crimps (Kimble, 73820-20) which allowed for submerged incubation in waterbaths at -1.5, -1, 0, 2, 3.5, or 5 °C; the average in situ temperature for the depths sampled was -1.2°C ± 0.20 (± 2x standard error; 95% confidence limit). Samples were incubated in the dark, for 3 to 24 h, but typically about 12 h for endpoint experiments.

At the end of each incubation (including time-zero and 2% formaldehyde-killed controls), inorganic carbon was extracted using a method modified from (Hobbie and Crawford, 1969). Bottle caps were replaced with sterile rubber septa (Kontes, 882310-0000) equipped with center wells (Kontes, 882320-0000) containing a fluted, 2- x 5-cm piece of Whatman No. 1 filter paper "wick" saturated with 0.2 ml of the CO_2-absorber, phenethylamine (PEA; Sigma Chemical). Control experiments showed no significant CO_2 was lost during cap replacement. Once wick and well were in place and sealed with rubber cement, incubations were stopped by injecting 0.4 ml of 4 N H_2SO_4 into the liquid sample to reduce pH to < 2. Samples were kept in the dark and gently shaken on a shaker table (ca. 100 rpm) for at least 1 h (or for 12 h without shaking when the shaker table malfunctioned; controls showed no

difference between these two treatments) to collect the respired $^{14}CO_2$ onto the wick. Following the extraction of $^{14}CO_2$ from the seawater, the Whatman filter paper was transferred to a scintillation vial. CO_2 capture efficiency was determined by adding $NaH^{14}CO_3$ to a series of 0.2-µm-filtered, sterile seawater samples, incubating over the same range of temperatures for 12 h in the dark, and extracting CO_2 as above. The 86.5% efficiency we measured (± 5.86; 95% confidence interval based on n = 22 control samples) agrees with the 86.54% efficiency reported by (Thompson and Hamilton, 1974) for a similar technique. Extraction efficiency was independent of CO_2 concentration and temperature (data not shown).

The ^{14}C-labeled cells from the remaining seawater sample were collected onto a 0.2-µm Millipore nitrocellulose filter by gentle vacuum filtration. The sample was followed by a 40-ml rinse of 0.2-µm filtered seawater of the same salinity. The filters were transferred to a scintillation vial; all filters were allowed to air-dry before scintillation cocktail (Ecolume) was added. Counts were performed 12 h after addition of cocktail on Beckman scintillation counters, using standard curves and H# corrections to account for quench. Total utilization was determined by adding together the radiolabeled carbon respired (on the Whatman filter) and incorporated (on the Millipore filter). Note that the term "uptake" is not used to describe our results because acidification and filtration would not allow collection of intracellular dissolved pools of substrate not yet incorporated into macromolecules (Griffiths *et al.*, 1974a) or the substrate loosely bound to cell membranes (Griffiths *et al.*, 1974b). As such, the technique we used provides a conservative estimate of substrate uptake.

Data analysis

All statistical analyses were performed using SYSTAT 5.2.1 for the Macintosh (SYSTAT, 1992). Multivariate analyses were done using fully factorial M(ANOVA) with total utilization rate *(v)* as the dependent variable and incubation temperature *(T)* and added substrate concentration *(A)* as factors (Wilkinson, 1975; 1980). Non-linear surface fits were done using distance-weighted least-squares technique (McLain, 1974).

Non-linear curve fits to the hyperbolic model equation were done initially with the SIMPLEX estimation method (O'Neill, 1971; Griffiths, 1985). The model equation we used (see Appendix for derivation) assumes steady state at the cell surface and combines cell-specific diffusive flux (J_D) and cell-specific uptake (v_S) as a function of substrate concentration far from the cell surface (C_∞), cell radius (r), and the diffusion coefficient (D), to solve for cell-specific maximum uptake velocity (V^S_{max}) and the half-saturation concentration (K_t):

$$v^S = \frac{V^S_{\max}}{\left\{1+\left(\frac{2*K_t}{C_\infty - K_t - \frac{V^S_{\max}}{4\pi r D} + \sqrt{\left(\frac{V^S_{\max}}{4\pi r D} + K_t - C_\infty\right)^2 + 4C_\infty K_t}}\right)\right\}} \quad (1)$$

We computed D for an amino acid with a "radius" of 10 Å (Solomons, 1988) at the experimental temperature and station salinity for each treatment (Jumars *et al.*, 1993), and used a cell radius equal to 0.5 µm (which would

equal the median cell radius if all cells had been spherical; see below). Since we did not have measures of UDOM concentration for every station and current measures of DOM do not really reflect bioavailable UDOM anyway (Karl, 1986), we substituted added substrate concentration, A, for C_∞ instead of $S + A$, where S is the bioavailable concentration of amino acids. As such, our estimation of the half-saturation concentration equals the true half-saturation coefficient minus the bioavailable substrate concentration ($K_t - S$) rather than a true K_t . Though the result of this assumption has no effect on the calculation of V_{max}, it means we can only compare $K_t - S$ values within a single station and not across all stations. Since the primary goal of this study was to examine treatment (e.g. temperature) effects at individual stations, relative values are sufficient for our purposes here.

To examine diffusion limitation, we computed the dimensionless ratio, or mass Biot number, P^* (Pasciak and Gavis, 1974) from the curve fit output:

$$P^* = \frac{4\pi\, r\, D\, K_t}{V^S_{\max}} \qquad (2)$$

For each of the above calculations, we had to scale our measured community utilization rates by the number of bacteria per liter of sample to input v^S. This assumes 100% active cells (see discussion) and may underestimate activity per cell if a smaller percentage is active. The complex equation above simplifies to the more common

$$v = \frac{V_{\max}\, C_\infty}{K_t + C_\infty} \qquad (3)$$

and becomes independent of cell number when $V^S_{max} << 4 \pi r_o D K_t$ (or $P^* >> 1$).

Standard errors were computed by estimating the Hessian (second derivative) matrix at the end of the SIMPLEX iteration (O'Neill, 1971; Griffiths, 1985). In most cases, the complexity and redundancy of Eq. (1) would not allow a positive definite solution of the matrix and, consequently, standard errors were not computable. In cases where $P^* > 1$, therefore, we solved the simpler Eq. (3) to estimate standard error. In all such cases, estimates of V_{max} and K_t were within less than 5% of values computed from Eq. (1).

Specific affinity (a^0_A) is defined as the initial slope of the substrate (C_∞) versus cell specific utilization (v^S) curve (Button, 1994). This parameter represents the instantaneous community response to an increase in substrate concentration. Again, since we cannot know S with any certainty and were primarily interested in relative affinity as a function of temperature, we computed a^0_A as the slope of the added substrate A versus utilization rate v^S curve. If S is large, this assumption may have caused us to underestimate a^0_A. Also, since we did not know the percent active cells, our estimates of cell-specific affinities may be underestimates if a significant percentage of the directly counted bacteria are inactive. Such underestimation would not affect within-station comparisons, however.

We calculated a^0_A by performing a sequence of linear regressions (Sokal and Rohlf, 1981) on the data from each experiment, starting with lowest concentrations and adding data points for higher added substrate concentrations until the slope started to decrease with saturation. At stations

with noisy data, this process could be a bit subjective. Our strategy was to choose the maximum slope attained while also including the most data points and getting the best fit (r^2). Assuming a perfect hyperbolic model, one can also calculate $a^0_A = V_{max} / K_t$. Because such a calculation compounds potentially high errors associated with V_{max} and K_t estimations, we found that we obtained less error with the initial slope regressions and could thus detect differences due to temperature that we could not have detected statistically using V_{max} and K_t. In most cases, the two methods produced comparable values for a^0_A; only the magnitude of the estimated error differed.

Comparisons between computed values for a^0_A and V_{max} were done using planned pairwise T-tests (Sokal and Rohlf, 1981), comparing values from one temperature to values at the next warmest temperature.

Results

Substrate availability

Total dissolved free amino acids (DFAA) concentrations at the fluorescence-maximum ranged from 33 to 153 nM (approximately 2.5 to 13 μg l^{-1}) in 1992 (Table III-1; 1993 samples fouled) and were fairly low compared to temperate oceans (15 - 80 μg l^{-1}; as reviewed by Andrews and Williams, 1971). As is often reported for marine waters (e.g. Hubberten *et al.*, 1994), the individual amino acids that dominated the composition were aspartic acid, serine, glycine, and methionine, with glutamic acid, tyrosine, alanine, histidine, and valine sometimes detected (data not shown). No significant correlation was observed between chlorophyll *a* concentration and DFAA for

the samples measured ($r = 0.25$, $P > 0.1$). Unfortunately, organic carbon samples were contaminated and the data were not useful. Data collected by others aboard *Polarstern* overlap with our sampling depths and suggest that DOC and DON concentrations in some of our 1993 samples ranged from 88 to 194 µM (or 1 to 2 mg C l^{-1}) and 5.1 to 6.4 µM (or 61 to 77 µg N l^{-1}), respectively (A. Skoog, personal communication). Of note are the very high DOC:DON ratios (14 - 34) of the DOM pool since they may indicate low lability of DOM in the polynya waters.

Bacterial abundance

Bacterial abundance data for all depths and stations sampled are available in published data reports (Wallace *et al.*, 1995a; 1995b) accessible via the National Technical Information Service (U.S. Dept. of Commerce) or the NSDIC data base and are summarized for 1992 in the literature (Smith *et al.*, 1995). In 1993, bacterial abundance within the euphotic zone was fairly low and averaged $1.66 \pm 0.13 \times 10^8$ liter^{-1} (± 2 x standard deviation, equals 95% confidence interval) with the maximal value at 7.92×10^8 liter^{-1}. Bacterial abundance correlated positively with chlorophyll concentration in 1992 ($r^2 = 0.19$, $p < 0.01$; Smith *et al.*, 1995) and also in 1993 ($r^2 = 0.14$, $n = 315$, $p < 0.05$, Fig. III-2), suggesting a coupling between bacteria and phytoplankton, but the regression coefficients ($r^2 = 0.14 - 0.19$) are small compared to most other regions ($r^2 = 0.75$, Cole *et al.*, 1988; see also Ducklow and Carlson, 1992), indicating that a high percentage (>80%) of the variability in bacterial abundance is not explained by chlorophyll at the sampled spatial and temporal scales. The slope of the logarithmic relationship is less than one (0.21 in 1992, Smith *et al.*, 1995; 0.24 in 1993, Fig. III-2), and may also be

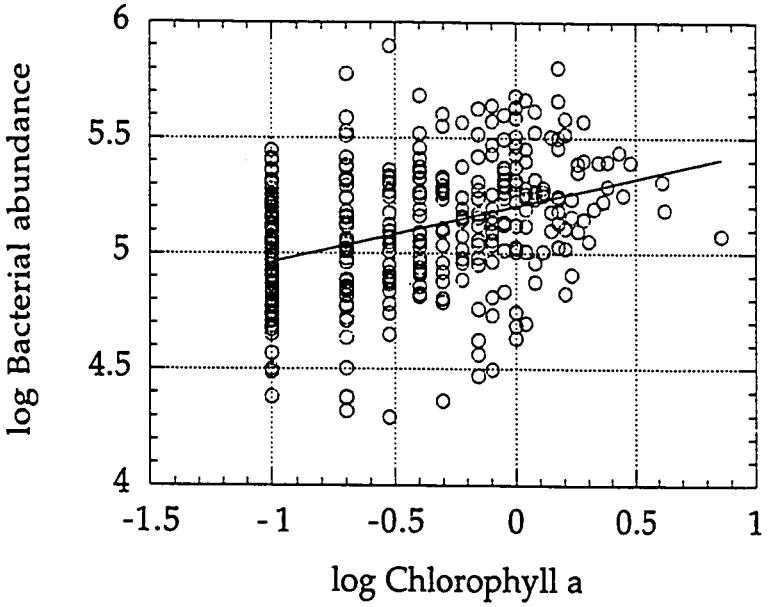


Figure III-2. Plot of $\log_{10}$ chlorophyll *a* concentration ($\mu g\ kg^{-1}$) versus $\log_{10}$ bacterial abundance (cells ml^{-1}) for 1993 *Polar Sea* cruise. Regression line using standard linear regression has slope of 0.24, y-intercept of 5.2, n = 315, and $r^2 = 0.14$. Geometric mean (Type II) regression gives a slope of 0.64.

shallower than in other regions (0.53; Cole *et al.*, 1988), i.e., bacterial abundance does not increase to as great an extent at high chlorophyll concentrations, indicating either strong grazing pressure (Pace and Cole, 1994) or, alternatively, earlier saturation at the higher chlorophyll concentrations. The y-intercept values, perhaps indicative of overwintering stocks of bacteria present before phytoplankton bloom, are not significantly different from temperate ocean values (5.97; Cole *et al.*, 1988) or each other, but they tended to be slightly higher in 1992 (6.84; Smith *et al.*, 1995) and lower in 1993 (5.20, Fig. III-2). Note that all of these regressions were done using standard linear regression (Type I) for literature comparison. Geometric mean (Type II) regressions would be more accurate in terms of a predictive relationship, since there is error associated with the chlorophyll measurements. If geometric mean regressions are performed, the slope increases by dividing by the correlation coefficient, r (Sokal and Rohlf, 1981). For 1992, the slope more than doubles to 0.48 (Smith *et al.*, 1995) and for 1993, to 0.64. When r is large, the difference is minor (e.g., the slope for all oceans determined by Cole *et al.*, 1988, increases from 0.52 to 0.60), but when r is small, as in the case of the polynya, the difference is striking. The Type II regression puts the polynya chlorophyll-bacteria relationship in a range more comparable to the rest of the ocean.

Bacterial size

Bacteria were unusually large and their populations dominated by rod-, crescent-, and spiral-shaped cells (72 ± 6.5 % of total), rather than the small cocci which typically dominate in the marine environment (Ferguson and Rublee, 1975). The average cell volume in the euphotic zone (computed from

the median cell size measured for each of 20 samples) was 0.525 ± 0.09 μm^3, with the maximum observed to be 0.835 μm^3. Notably, this large cell size corresponds well with a prey diameter that would produce the fewest encounters with non-swimming, 5- to 100-μm diameter predators at in situ temperature and salinity (Fig. III-3).

To convert biovolume to biomass, we used 133 fg C μm^{-3} reported by Simon and Azam (1989) for "average marine bacteria" at 0.4 μm^3 (their largest size category). This factor decreases non-linearly with increasing size, and may overestimate slightly (< 10%) the amount of carbon per volume in the large polynya bacteria.

Potential growth rates

Growth studies showed that all of the pelagic communities we measured in 1993 were dominated by psychrophiles when given enriched levels of organic nutrients. Maximum specific growth rates were as high as 1.96 (± 0.14) d^{-1} at in situ temperature and 2.85 (± 0.10) d^{-1} with warming (Fig. III-4). The optimal growth temperature ranged from 2°C to 10°C depending on the station. Station 93-42 even showed a bimodal psychrophilic response (Fig. III-4D). Experiments at the time-series station (Figs. III-4B and III-4C), one week apart from each other, produced similar growth curves. For comparison, the temperature response measured on a slurry of material from a short-term floating sediment trap deployed near the time-series station (Fig. III-1) showed a more psychrotolerant community with growth rates increasing to a maximum at 20°C (Fig. III-4F).

Nalidixic acid treatments with substrate showed no differences from those without enrichment, and did not exhibit the typically elongated cells we

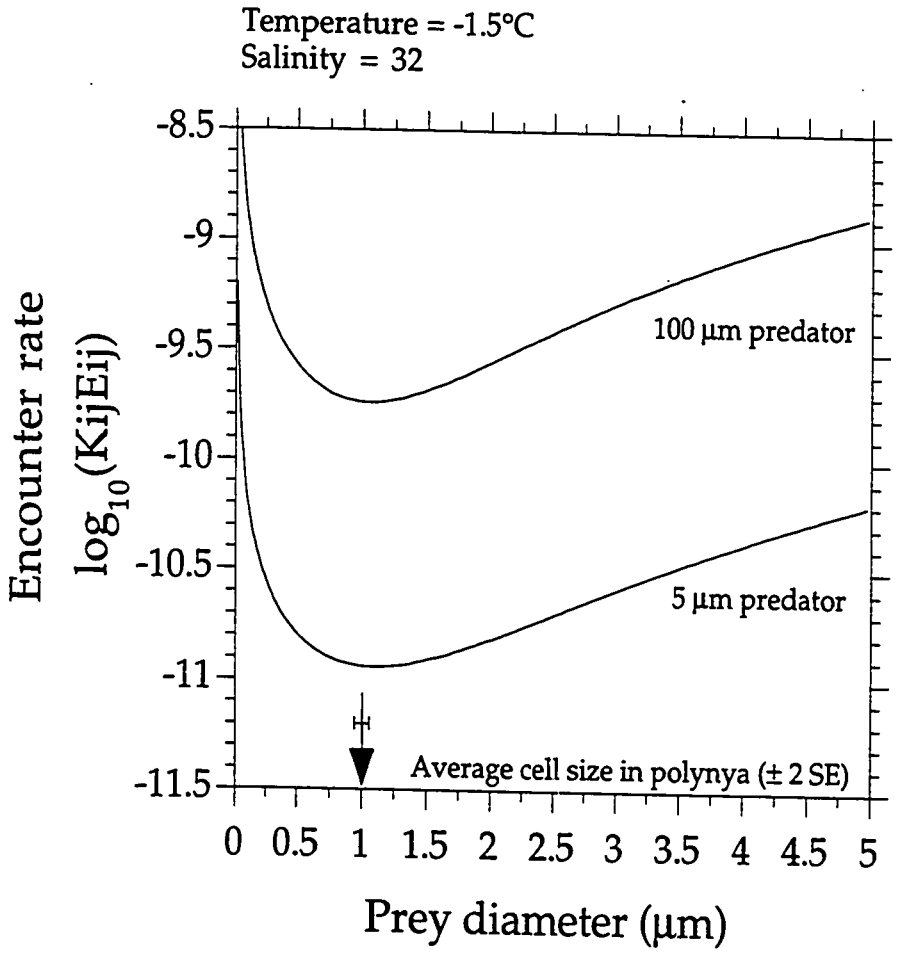
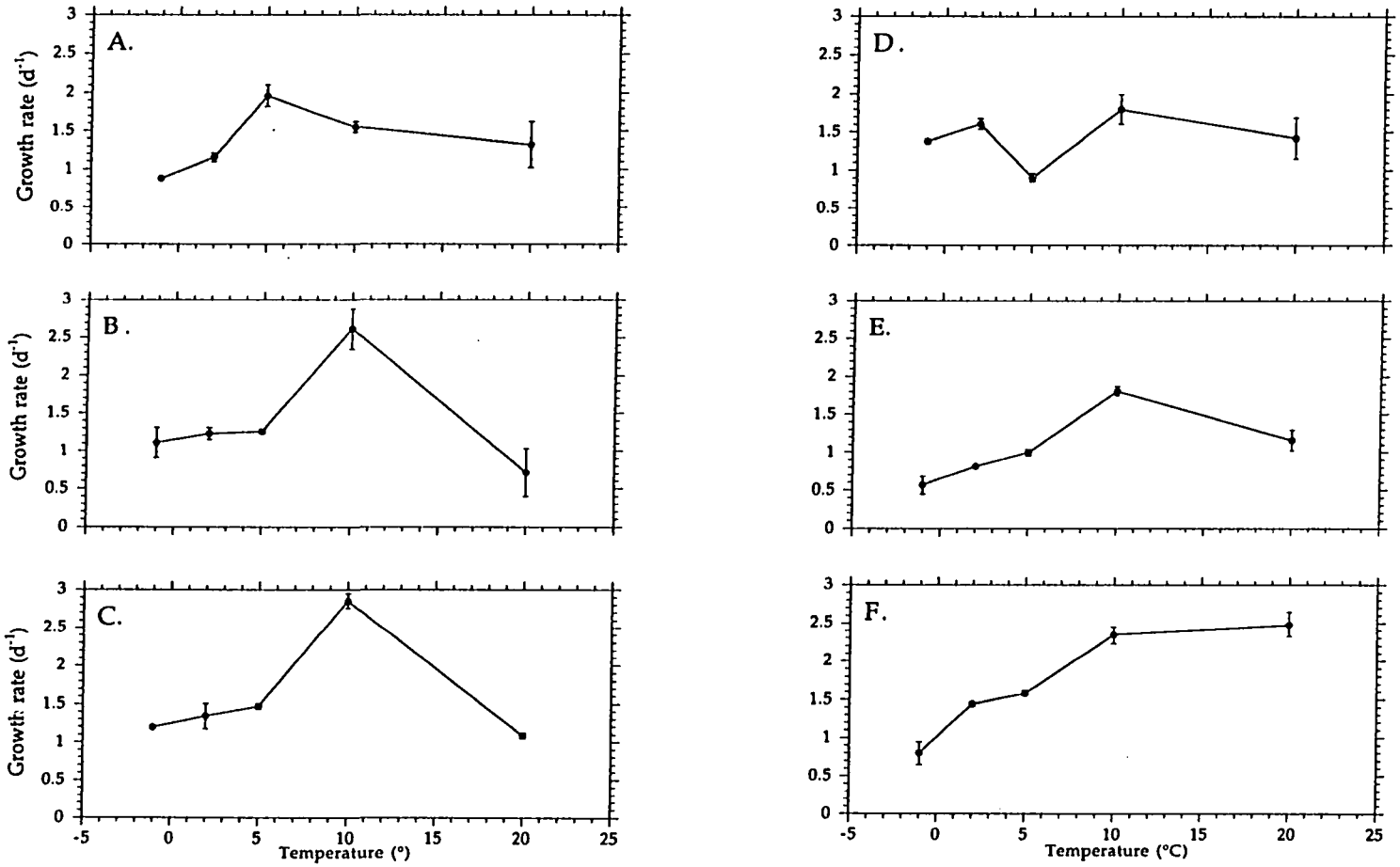


Figure III-3. Encounter rate (scaled to particle concentration; includes Brownian motion and turbulent shear coagulation mechanisms) as a function of prey diameter for two different predator sizes (see text or Jumars, 1993, for further explanation). Note that the shape and position of the curve is not very sensitive to predator size. To calculate actual encounter rate, multiply $\log_{10}(K_{ij}E_{ij})$ times the concentrations of predators and prey. Arrow indicates the average cell diameter (± 2 standard errors) computed from the cell volumes of 20 samples from the time series location (see text).

Figure III-4. Potential bacterial growth rates from samples supplemented with 0.01% yeast extract as a function of incubation temperature. Error bars indicate ± 2 standard errors on estimate of growth rate. Seawater samples were from depths of maximum fluorescence: a) Sta. 93-1; b) 93-2; c) 93-37; d) 93-42; and e) 93-53 (see Table III-1). Growth rates were also measured on a particle slurry (f) recovered from a short-term floating sediment trap deployed at the base of the euphotic zone (~ 50 m) near the time-series station, 93-37.


A.
B.
C.
D.
E.
F.
Growth rate (d-1)
Temperature (°)
Temperature (°C)

expected. Either polynya bacteria are resistant to Nalidixic acid or, more likely, incubation conditions were not optimal for detecting enlarged cells. The method has not been applied before to sub-zero waters to our knowledge; our experiments represent incomplete tests of the method as we did not verify optimal substrate type or concentration for this environment, but rather used the published method directly. In any case, the percentage of active cells was not accessible using this method.

Utilization

Utilization rates at near-surface fluorescence maximum depths showed linearity in the time-course experiments up to about 16 h (Fig. III-5) at subzero temperatures, even at the highest concentrations of added substrate. An end point of 12 h or less, therefore, was used for most subsequent end-point experiments. From the observed linearity, we expected that a 12-h incubation would be short enough to avoided changes in community structure during our experiment (see, for example, discussion in Jumars *et al.*, 1993); in samples where we observed the relatively high specific growth rates described above, however, community shifts may have occurred. In the experiments with deep, warmer water samples (92-72, 92-78; see Table III-1) utilization was not detected.

Multivariate ANOVA was used to analyze the kinetics experiments for added substrate and temperature effects. In all nine experiments analyzed (Table III-2), added substrate concentration had a significant effect on utilization rates (with $P_{substrate} < 0.001$ at all but one station, where $P_{substrate}$ was 0.022). Temperature had a significant effect at six of the nine stations, but had a combined effect with substrate concentration at only two of the nine

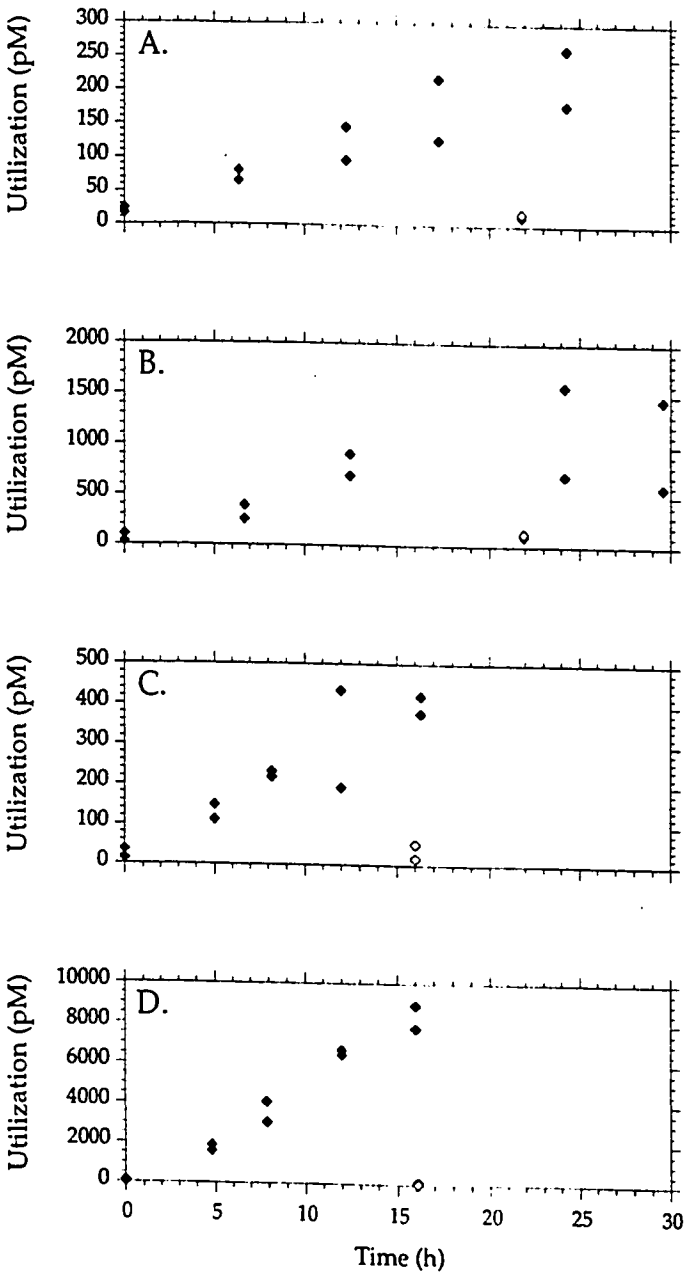


Figure III-5. Utilization of ^{14}C-amino acid mixture (pmol l^{-1}; solid diamonds) as function of time (h) for seawater samples from station 92-25A (a and b) and 92-43A (c and d) with 1 nM (a and c) and 100 nM (b and d) substrate additions. Open diamonds are 2%-formaldehyde killed controls.

TABLE III-2: Results of M(ANOVA) test for effects on utilization rate.

Station	n	R^2	$P_{[A]}$ Added Substrate	P_T Temperature	P [A] x T
92-25B	36	0.893	<0.001**	0.003**	0.157
92-43B	36	0.736	<0.001**	0.390	0.620
92-49	36	0.883	<0.001**	0.002**	0.195
92-56	35	0.571	0.022**	0.798	0.920
92-64	36	0.912	<0.001**	0.002**	0.039*
93-30	50	0.964	<0.001**	<0.001**	<0.001**
93-43	52	0.810	<0.001**	<0.001**	0.545
93-85	100	0.818	<0.001**	<0.001**	0.289
93-2	90	0.714	<0.001**	0.651	0.930

** Indicates > 99% confidence.

* Indicates > 95% confidence.

stations. On average, added substrate concentration and temperature explained 81 ± 12 % of the variance seen in these experiments. A notable endmember is Station 92-56 which exhibited the lowest r^2 and $P_{substrate}$ values; this station also had the highest measured DFAA concentration.

Kinetics

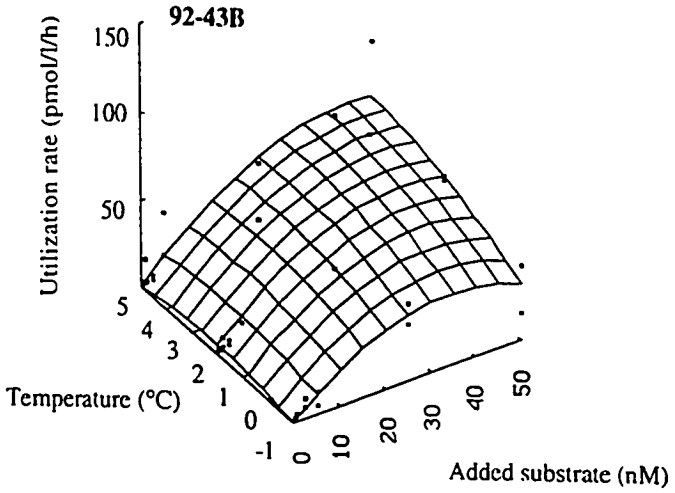
Kinetic curves for the amino acid mixture appeared to follow a standard rectangular hyperbolic model for single substrate-enzyme interactions. Most of the stations showed rapid increase in utilization rates at low added concentrations and saturation at higher concentrations (Fig. III-6). With this result, we felt confident assuming the Michaelis-Menton uptake model and calculating a^0_A, V_{max}, and K_t for each station.

Specific affinity. Values for a^0_A ranged from 1.23 to 12.8 x 10^{-3} h^{-1} at sub-zero temperatures (Table III-3). Button (1994) reports specific affinity scaled to cell biomass per media volume (liter g-cells^{-1} h^{-1}). To convert our data to these units, we used bacterial abundance from each station and assumed a cell mass of 116 fg cell^{-1} (0.525 μm^3 x 133 fg C μm^{-3} x 0.56 fg C fg dry weight^{-1}; Simon and Azam, 1989). Results ranged from 307 to 2340 liter g-cells^{-1} h^{-1}, or up to 4290 liter g-cells^{-1} h^{-1} when warmed to 2°C. These values overlap with and even exceed the high end of values reported by Button (1994) for pure cultures of oligotrophs.

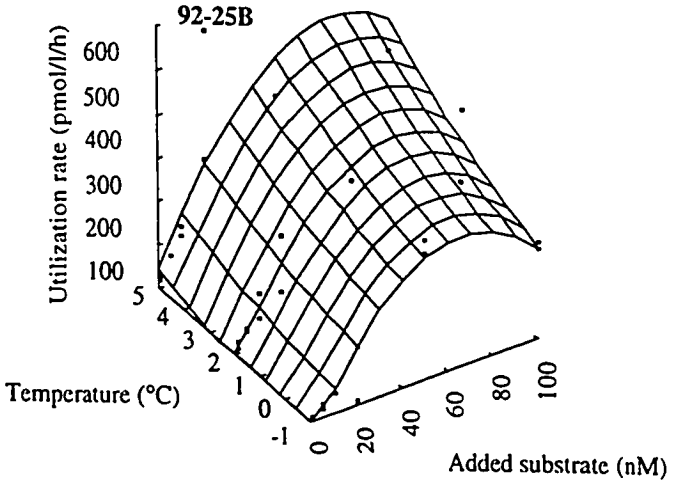
The response of a^0_A to temperature was not uniform across all the stations in the polynya (Table III-3). Four stations confirmed the Pomeroy hypothesis of increased affinity for substrate at warmer temperatures. Three of the four experiments that showed this response were from the time-series

Figure III-6: Distance-weighted least-squares surface fits (tension = 1) of utilization rate (pmol l^{-1} h^{-1}) as a function of added substrate concentration (nM) and temperature (°C) for selected stations chosen to exemplify three behaviors described in text according to statistical information in Table III-2. a) substrate alone had a significant effect (Station 92-43B); b) substrate and temperature had significant effects (Station 92-25B); c) substrate, temperature, and an interaction between substrate and temperature, had significant effects (Station 92-64).

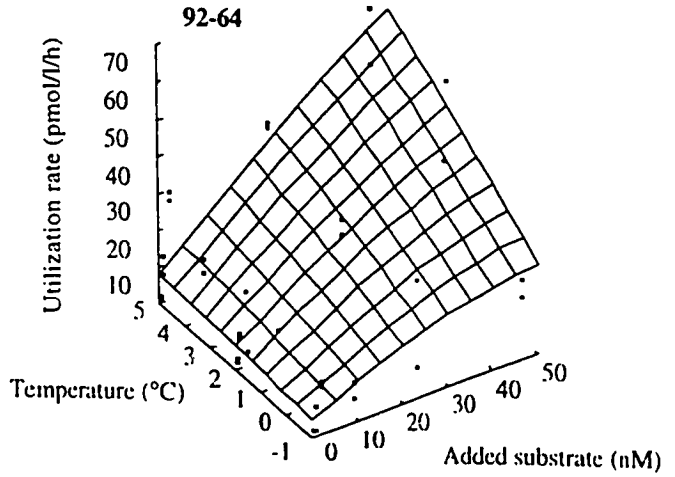
A.


92-43B
Utilization rate (pmol/l/h)
150
100
50
5
4
3
2
1
0
-1
Temperature (°C)
0
10
20
30
40
50
Added substrate (nM)

B.


92-25B
Utilization rate (pmol/l/h)
600
500
400
300
200
100
5
4
3
2
1
0
-1
Temperature (°C)
0
20
40
60
80
100
Added substrate (nM)

C.


92-64
Utilization rate (pmol/l/h)
70
60
50
40
30
20
10
5
4
3
2
1
0
-1
Temperature (°C)
0
10
20
30
40
50
Added substrate (nM)

location in the central part of the polynya region. Two stations from the northeastern region showed strong psychrophilic behavior, with maximum affinities at either -1 or 2 °C. Three stations showed a bimodal response, with higher affinities at subzero temperatures decreasing at intermediate temperatures and then increasing again with warming. This result suggests either two distinct microbial communities or a shift in uptake mechanisms used by a single population.

Community V_{max} values ranged from 20 to 400 pmol l^{-1} h^{-1} (or 1.2 to 22 ng C l^{-1} hr^{-1}) at sub-zero temperatures and were as high as 500 pM h^{-1} when incubated at 5°C (Table III-4; please note that the ordering of stations is different from in Table III-3). Community V_{max} did not correlate with bacterial abundance ($r = 0.063$, $p > 0.1$), highlighting the weakness of the assumption that 100% of the directly counted bacteria were active. Thus, we do not present cell-specific V^S_{max} here, but rather community V_{max} values. For comparison to other published data sets, however, if we do assume 100% active cells, V^S_{max} ranged from 0.4 to 5.3 amol $cell^{-1}$ h^{-1} (or 0.35 to 10 mg g-$cell^{-1}$ h^{-1}, if we assume 116 fg $cell^{-1}$; Simon and Azam, 1989).

Temperature effects on V_{max} were mixed (Table III-4). At four of the stations, including the two visits to the time-series station, there was a monotonic increase in V_{max} as incubation temperature warmed. At three of the stations there was a step up in V_{max} upon warming from sub-zero to 0 or 2°C, but no significant increase after that. One station to the northeast showed extreme psychrophilic behavior with a maximum at 2°C. One station (93-85) showed no difference in V_{max} due to temperature.

K_t - S, our estimation of the half-saturation concentration, ranged from 2.2 to 82.5 nM (or 0.3 to 10 μg liter^{-1}) at sub-zero temperatures and tended to have high associated standard errors (Table III-4). Computed values for an oligotrophic capacity index ($\log_{10}(a^0_A/K_t)$; Button, 1994) were 8.58 ± 0.71 (n = 9) at sub-zero temperatures, not significantly different from the value (9.0) described for organisms well adapted for survival in the pelagic ocean. This index only drops to 7.84 ± 0.44, if we try to compensate for our assumption that $K_t \approx K_t - S$ by letting S = 10 μg liter^{-1}, an upper end of most of the amino acid concentrations measured, although this calculation does not account for any potential increase in a^0_A had we incorporated S in the estimation.

Diffusion limitation

For each experimental treatment we determined P^*, a dimensionless ratio that suggests diffusion limitation if it drops below about 2 (Pasciak and Gavis, 1974). If we assumed that 100% of the bacteria were active, P^* values ranged from 6 to 127 at in situ temperatures (Table III-3), indicating no diffusion limitation. If we calculate P^* as a function of percent active cells, diffusion limitation at sub-zero temperature begins when the percent active drops below 15% (Fig. III-7). Field measurements from Kiel Bight suggest anywhere from 2 to 60% of directly counted cells are actively taking up radiolabeled substrate (Meyer-Reil, 1978). Our cell morphology observations indicated that over 70% of the bacteria in the polynya were rod-, spiral-, or crescent-shaped. The dominance of small, apparently inactive (Morita, 1985), cocci expected in typical oligotrophic environments (Dawes, 1985; Deming and Baross, 1994) was not observed in the polynya and suggests that most of the cells were probably active. To completely address this question, a good

TABLE III-3: Specific affinity as a function of incubation temperature (T).

Station	T (°C)	n	Specific affinity[a] ($\pm s_b$)[b] (liter g-cells^{-1} h^{-1})		Q_{10}[c] Step	Q_{10}[c] Avg.
Monotonic increase in affinity: $(a^oA)_1 < (a^oA)_2 < (a^oA)_3$ [d]						
92-25B	-1	10	748	(82.7)		
	2	8	984	(245)	2.49	
	5	8	2490	(569)	22.2	7.44
93-30	-1.5	6	919	(290)		
	0	6	1880	(63.2)	114	
	3.5	6	2270	(151)	1.75	6.13
93-85	-1.5	26	640	(70.1)		
	0	22	1240	(161)	82.8	
	3.5	22	1550	(202)	1.87	5.84
93-2	-1.5	12	301	(37.8)		
	0	12	399	(41.9)	6.36	
	2	12	539	(64.2)	4.57	5.26
Bimodal - intermediate minima in affinity: $(a^oA)_1 > (a^oA)_2 < (a^oA)_3$ [d]						
92-43B	-1	6	307	(81.5)		
	2	8	197	(48.5)	0.23	
	5	8	422	(172)	12.72	1.70
92-64	-1	6	909	(171)		
	2	6	659	(470)	0.34	
	5	6	2040	(166)	42.8	3.83
93-43	-1.5	9	684	(128)		
	0	9	435	(85.7)	0.05	
	3.5	9	1135	(217)	15.5	2.76
Step down in affinity: $(a^oA)_1 > (a^oA)_2 = (a^oA)_3$ [d]						
92-56	-1	8	2350	(316)		
	2	7	1010	(104)	0.06	
	5	8	1130	(175)	1.45	0.29
Intermediate maximum (extreme psychrophilic): $(a^oA)_1 < (a^oA)_2 > (a^oA)_3$ [d]						
92-49	-1	8	1910	(258)		
	2	8	4290	(570)	14.8	
	5	8	2830	(897)	0.25	1.92

[a] Specific affinity (liter g-cells^{-1} h^{-1}) unit conversion assumes 100% active cells, cell counts from Table III-1, 116 fg cell^{-1} (Simon and Azam, 1989), and $C_\infty \approx A$. (see text).

[b] s_b = 1 standard error of the specific affinity slope estimate (Sokal and Rohlf, 1981).

[c] Step $Q_{10} = ((a^oA)_{n+1} / (a^oA)_n)^{(10/(T_{n+1}-T_n))}$. Avg. $Q_{10} = (a^oA)_3/(a^oA)_1)^{(10/(T_3-T_1))}$.

[d] Determined by one-tailed, planned pairwise T-tests for equality of samples with unequal variance, $\alpha \leq 0.05$, where $(a^oA)_1$ is a^oA at the lowest, $(a^oA)_2$ the intermediate, and $(a^oA)_3$ the warmest temperature.

TABLE III-4: Vmax and Kt-S as function of incubation temperature (T).

Station	T (°C)	V_{max} (pM h^{-1})	(ASE)[a]	Q_{10}[b] Step	Avg.	K_t - S[c] (nM)	(ASE)[a]	P*[d]
Monotonic increase in V_{max}: $V_1 < V_2 < V_3$ [e]								
92-25B	-1	379	(109)			39.9	(26.6)	17.7
	2	418	(75.5)	1.39		25.3	(12.5)	11.4
	5	511	(117)	1.94	1.64	16.4	(12.1)	6.76
92-43B	-1	42.1	(9.2)			10.1	(6.55)	54.0
	2	86.1	(30.3)	10.9		15.2	(14.1)	44.6
	5	175	(337)[f]	10.7	10.8	81.2	(231)	127
92-49	-1	128	(19.6)			6.93	(3.80)	7.41
	2	240	(28.0)	8.32		2.92	(1.62)	1.88
	5	333	(58.4)	2.95	4.95	9.42	(5.71)	4.87
93-2	-1.5	128	(58.1)			52.5	(40.4)	59.8
	0	199	(131)	18.9		62.2	(67.6)	48.5
	2	318	(382)[g]	10.5	13.5	120	(229)	63.5
Step up in V_{max}: $V_1 < V_2 = V_3$ [e]								
92-64	-1	21.4	(3.90)			2.20	(1.59)	14.5
	2	54.4	(30.0)	22.4		12.8	(23.7)	36.7
	5	51.3	(4.71)	0.82	4.29	2.71	(1.07)	9.34
93-30	-1.5	52.3	(8.04)			24.0	(10.2)	17.5
	0	101	(28.8)	81.9		74.8	(42.0)	29.6
	3.5	119	(12.7)	1.58	5.16	32.1	(8.43)	12.3
93-43	-1.5	29.1	(5.10)			23.1	(11.4)	30.1
	0	39.2	(7.02)	7.36		30.4	(13.7)	31.2
	3.5	40.3	(4.88)	1.08	1.92	9.11	(4.01)	10.4
No change in V_{max}: $V_1 = V_2 = V_3$ [e]								
93-85	-1.5	159	(41.3)			82.5	(40.5)	20.1
	0	149	(27.6)	0.67		52.4	(21.6)	14.3
	3.5	150	(22.7)	1.02	0.9	33.7	(13.2)	10.4
Intermediate maximum V_{max} (Extreme psychrophilic): $V_1 < V_2 > V_3$ [e]								
92-56	-1	78.3	(18.3)			5.70	(4.76)	6.03
	2	267	(100)	59.4		22.4	(22.3)	7.97
	5	33.8	(8.15)	0.001	0.25	2.90	(2.36)	8.76

[a] Associated Standard Error (SYSTAT, 1992).

[b] Step $Q_{10} = (V_{n+1}/V_n)^{(10/(T_{n+1} - T_n))}$. Average (Avg.) $Q_{10} = V_3/V_1)^{(10/(T_3-T_1)}$.

[c] Curve fit made to added concentration [A], not total [S + A], half-saturation value is K_t-S.

[d] P* is the ratio of diffusive flux to the cell and cell-specific maximum uptake rate (see text); Assume 100% active cells. P* ≤ 2 indicates diffusion-limitation (Pasciak and Gavis, 1974).

[e] Determined by one-tailed, planned pairwise T-tests for equality of samples (variances assumed unequal), where V_1 is V_{max} of lowest, V_2, intermediate, and V_3, warmest temp.

[f] High associated standard error forces $\alpha > 0.05$, but monotonic trend still inferred.

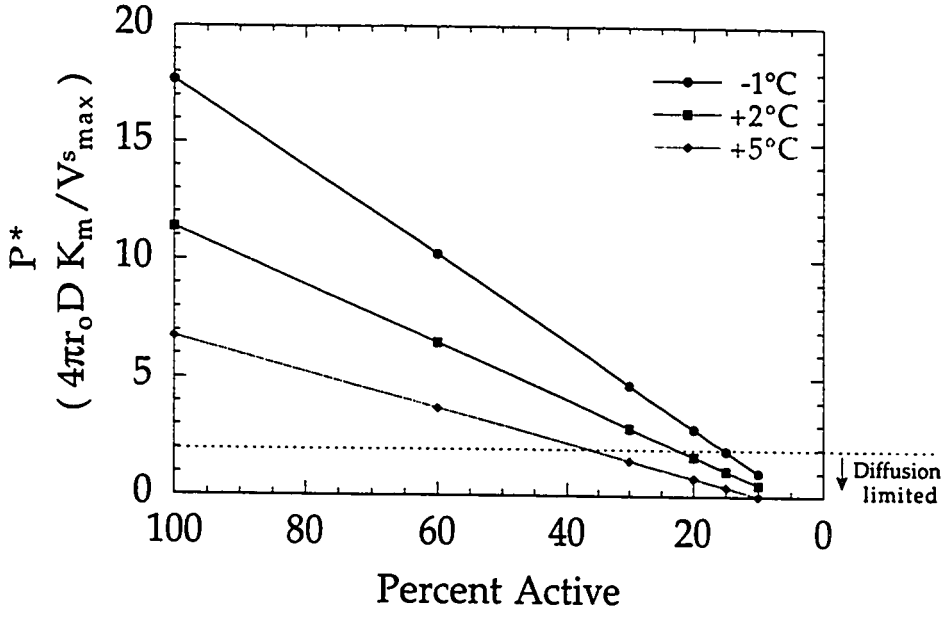


Figure III-7. Dimensionless ratio *(P*)* of cell-specific diffusive flux to cell-specific maximum utilization rate (as determined from measured values for community V_{max} and direct counts of bacterial abundance) as a function of the percentage of active cells assumed (data from Station 25B). Results generated by repeated non-linear curve fits to same data set using different concentrations of active cells. Diffusion limitation (as defined by P* < 2; dotted line) at sub-zero temperatures begins when active cells drop below about 15% of direct counts.

measure of the percent active cells in a high-latitude environment is required. Also, if the dominant food source for microbes were larger molecules than we modeled (see below), diffusion rates could be slower and cells diffusion-limited in situ.

Discussion

In polar oceans, heterotrophic pelagic bacteria must cope with a highly seasonal fresh food supply but permanently cold temperatures. The most competitive organism in such an environment would be expected to have constitutive, high-affinity transport systems but metabolic rates sensitive to available substrate concentrations. Combining the pure culture data of Wiebe *et al.* (1992, 1993) with the kinetic model of Button (1985; 1991; 1994), we expected to find a strong temperature sensitivity in substrate affinity (or in growth efficiencies; Chapter 4), with affinity increasing with warming, as long as the cells were not diffusion-limited. We also expected V_{max}, which reflects the density of membrane transporters as well as the most rate-limiting step in cytoplasmic enzymatic processing, to change little with temperature, with Q_{10} (reaction rate quotient for 10°C: $\log Q_{10} = \log\{V_2/V_1\}\{10/T_2\text{-}T_1\}$; Thimann, 1963) ranging between the diffusional value of 1.4 (Jumars *et al.*, 1993) and the physiological value of about 2.

Temperature effects on a^0_A and V_{max} followed our predictions at only some of the stations we studied. The time-series station exhibited consistently increasing values with warming for both V_{max} and a^0_A and a population that showed optimum potential growth at 10°C. This station is in the central, mostly open-water region and may have received greater cumulative radiative flux by the time of our sampling (Minnett, 1995)

compared to other stations. Strong psychrophilic behavior was observed at other stations, particularly those in the far northeast corner of the study region influenced more by Arctic Ocean source waters (Wallace *et al.*, 1995c). Although the variability we observed likely reflects naturally occurring variability in the system, some of the variability in the data may have been caused by unknown differential presence of grazers, since we did not prefilter the samples. We opted not to prefilter, however, because of the potentially dramatic effect filtration can have on the DOM available to bacteria (e.g. Carlson *et al.*, 1996).

In our experiments, we looked only at microbial utilization of low molecular weight, N-rich organic matter. Implicit in these experiments, therefore, is the assumption that microbes will use this type of substrate preferentially over other, less labile or higher molecular weight organic material. Since bacteria are known to be capable of diauxic growth (as originally proposed by Monod, 1942), they can be expected to regulate uptake processes so as to utilize the better substrate first; i.e., as the NEW Polynya bacteria encounter amino acids, they will prefer them to other less labile material. By using a mixture of amino acids that mimics the composition of phytoplankton or zooplankton exudate, we expected our results to reflect "reality" better than if we had used a single substrate. Nevertheless, if the bacteria in the polynya are not regularly exposed to such a labile food supply, we measured their behavioral response to high quality food pulses and not some "average" condition. Since our goal was a comparative one (temperature and substrate concentration effects on utilization), we can still draw conclusions about the sensitivity of this microbial response.

Generalizing the results to the overall carbon cycle of the polynya should await a better understanding of controls on DOM production in the region.

Specific affinities were high compared to those reported by Button (1994) and seem to indicate that the bacteria in the polynya were ready to take up whatever labile substrate diffused towards them. This responsiveness is also confirmed by high oligotrophic capacities. Polynya bacteria certainly appear ready, at least at some stations, to respond quickly to increases in substrate availability.

Polynya V_{max} values (ranging from 21 to 510 pmol l^{-1} h^{-1}) compared favorably to some of those measured by other investigators in coastal Arctic waters (1.4 - 770 pmol l^{-1} h^{-1} for glutamic acid in Alaskan waters; Griffiths et al., 1978). Our rates also compare well with those reported by Baross and Morita (1978) for glutamic acid utilization in Arctic (6.8 - 1160 pmol l^{-1} h^{-1}; Morita *et al.*, 1977) and Antarctic seawater (7.5 - 591 pmol l^{-1} h^{-1}; Morita, 1975). Pomeroy *et al.* (1990) did not calculate V_{max}, but they added sub-saturating (at least according to those we observed in this study) concentrations of substrate (34 nM) to an Arctic Ocean water sample from 30 m depth and measured utilization of ^{14}C-glutamic acid over an order of magnitude greater (2670 pmol l^{-1} hr^{-1}) than most of our maximum rates.

The result that utilization did not correlate with bacterial abundance has been reported before for polar (Antarctic) environments (Karl *et al.*, 1991; Hanson *et al.*, 1983) and may indicate either a high degree of metabolic diversity of cells related to phenotypic or genotypic community structure or that a large and variable percentage of the cells were inactive. If grazing was important in the polynya (see Chapter 4 for data suggesting that it was), the

half-life of an inactive cell should have been fairly short, supporting the former rather than the latter hypothesis.

The larger than normal cell size is indeed consistent with significant grazing pressure since the cell diameter that provides the minimum encounter rate with non-swimming prey at these temperature and salinities is around 1 μm (see Fig III-3), which gives a spherical volume of about 0.524 μm^3, precisely the average cell size measured in the polynya (0.525 μm^3). This correspondence may be merely coincidence, especially since the coagulation model we used does not account for non-spherical or motile "particles." Incorporating grazer morphology and behavior, and also the efficiency with which encounter translates into ingestion, can increase or decrease the optimum prey size for minimal encounter (Shimeta, 1993; Shimeta *et al.*, 1995). Until we know more about the bacterivores from the polynya, however, accounting for such complexity is not possible in our analysis. The most we can infer is that the result from the simple coagulation model is consistent with interpretations of the chlorophyll *a* versus bacterial abundance comparison (Fig. III-2 and Smith *et al.*, 1995): both approaches support the hypothesis that the bacteria are under significant grazing pressure. (As discussed in Chapter 4, results of directly measuring bacterivory also agree with this conclusion.)

On the basis of previous research, we expected to measure a single response to temperature by the polynya bacteria. The null hypothesis was that specific affinity would increase with warming; we expected to collect data that would either support this hypothesis or disprove it. In fact we have some stations that support the hypothesis and others that do not. It is clear that the polynya region supports a complex microbial community with a

range of responses to temperature. Since the collection of our data, Pomeroy and Wiebe have experimented with pure cultures of psychrophiles and reached much the same conclusion (Pomeroy *et al.*, 1995). Their research with pure cultures confirms that we can no longer simplify the temperature and substrate responses of psychrophiles with a single type of behavior. Prediction now becomes more difficult, unless we can obtain a better idea of community structure at any given point in time and space and what determines it.

The larger question is what will happen over longer-term warming of Arctic water masses? Will a permanent environmental temperature shift cause a behavioral shift by the existing community, as we observed in our short-term experiments, or will a change in temperature favor one part of the community over the other and generate a shift in community structure? We might have predicted the former had we only observed one behavioral response to warming. However, our detection of several different behaviors favors the latter scenario, in which case a closer examination phenotypic and genotypic microbial diversity (and related controls) is warranted.

Conclusions

Pelagic bacteria in the NEW Polynya exhibited very high affinity for mixed amino acids and appeared to be well-adapted to living at low temperatures. In the NEW Polynya region, phytoplankton productivity and biomass has been described as a mosaic in terms of spatial and temporal heterogeneity (Smith, 1995). In the case of the bacterioplankton, we observed a similar heterogeneity with respect to temperature responses, reflecting perhaps large variations in community structure linked to primary

productivity, hydrography, or both. Temperature limitation seems to occur only sometimes, and then in the main polynya regions where residence times of water masses may be longer (Top, 1994), and surface waters have the potential to warm more later in the season. More psychrophilic behavior seems to occur in the northeast quadrant, perhaps reflecting more influence from the Arctic outflow, including increased nutrient flux, primary production, and herbivory. For the remineralization role of pelagic bacteria to be modeled accurately, and insightful prediction of carbon fluxes in the Arctic reached, a better understanding of microbial community structure and what controls it must be achieved. Our work suggests that more knowledge of the behavior of psychrophilic bacteria is warranted and that grazing pressure on bacteria cannot be ignored in either ecosystem or carbon-flux models.

IV. Pelagic microbial efficiency in the Northeast Water Polynya: Implications for inorganic carbon cycling and the biological pump.

Abstract

The microbial fate of carbon in the Northeast Water (NEW) Polynya (77-81°N, 6-17°W) during summertime 1992 and 1993 was investigated using a mass balance approach involving a diverse suite of measurements. We measured microbial utilization of ^{14}C-labeled organic substrates and followed carbon through anabolic (incorporation) and catabolic (respiration) pathways. Incorporation efficiencies (incorporation relative to total utilization) were obtained for mixed amino acids, leucine, glutamic acid, and glucose. Unusually high efficiencies were observed for mixed amino acids (86 ± 5 %) and leucine (>95%), suggesting that high-latitude microbes may tend to reserve rather than quickly metabolize nitrogen-rich or labile food supplies. Bacterial nucleic acid and protein production were measured using ^{3}H-thymidine and ^{14}C-leucine incorporation. Unbalanced growth with preference for biomass building over cell division was observed, depending on our assumption of genome size. This result corresponds with the observations of unusually large cells and low to moderate bacterial abundance in these waters. Bacterivory, measured by the dilution technique, was patchy and ranged from 25 to >100 % of bacterial growth when detected by this method, suggesting that these highly efficient bacteria provide a link to higher trophic levels. Bacterial specific growth rates (0.74 - 3.0 d^{-1}), at in situ

temperatures (-1.5°C) obtained using the dilution technique, also agreed with potential bacterial growth rates measured using 8-d enrichment experiments. Our results suggest some survival strategies adopted by these high-latitude microbes and allow us to estimate the role of pelagic microbes in the overall carbon flux of the NEW polynya region; they concur with undersaturated inorganic carbon concentrations observed in the polynya surface waters and suggest a highly efficient biological pump in the NEW Polynya surface waters.

Introduction

One of the most resilient paradigms in oceanography is the idea of the biological pump, the mechanism by which organisms facilitate carbon removal from the atmosphere to the deep ocean. The efficiency of the biological pump at high latitudes influences atmospheric carbon concentrations over glacial-interglacial time periods (Knox and McElroy, 1984) and is of vital importance in understanding the linkages between ocean productivity, ocean chemistry, and atmospheric carbon dioxide (Berger *et al.*, 1989). Coastal oceans are both more productive and better at sequestering carbon than open-ocean ecosystems, perhaps due to their more episodic nature (Berger *et al.*, 1989) and thus more efficient biological pump. Episodicity is especially critical in high-latitude ecosystems where spatial and temporal heterogeneity are the rule (e.g., Smith *et al.*, 1995). The multi-disciplinary NEW Polynya project (NEWater Investigators, 1993; Overland, 1995; Hirche and Deming, in press) provided a unique opportunity for us to investigate the efficiency of the biological pump in a region which combines both coastal and high-latitude characteristics.

The NEW Polynya is a seasonally recurrent opening in the permanent ice of the coastal Arctic (Fig. IV-1). It typically starts to open in May, and closes by late September to mid-October (Böhm *et al.*, 1995). During the 24-h sunlight of the boreal summer, reduced snow and ice cover allow deeper light penetration which enables first ice algae and then phytoplankton to bloom. Polynyas are being considered as model environments for seasonally ice-covered Arctic continental shelves. The proposed biological pump for the NEW Polynya region (Deming *et al.*, 1995) shows how it functions as a carbon shunt locally to the shelf benthos and regionally to the slope and Greenland Sea basin. The timing of biological activity with respect to sea-ice coverage is quite important, however, in controlling the magnitude and direction of carbon flux in seasonally ice-covered oceans like the polynya (Yager *et al.*, 1995).

In coastal Arctic regions, where carbon export may not follow typical steady-state assumptions, the efficiency of the biological pump may depend on the role of the microbial loop (Chapter 1); i.e., whether microbes operate as a carbon link between dissolved organic matter (DOM) and higher trophic levels (e.g., Azam *et al.*, 1983) or simply respire the available organic carbon, returning primary production to the inorganic pool, and allowing little carbon transfer to higher trophic levels (e.g., Ducklow *et al.*, 1986).

The behavior of the microbial loop in high-latitude regions is of particular interest (Karl, 1993; Pomeroy and Wiebe, 1993) because sub-zero *in situ* temperatures seem to constrain some, though not all (Chapter 3), marine bacteria to utilizing organic matter only when it is available in high concentrations (Pomeroy and Deibel, 1986; Pomeroy *et al.*, 1990; 1991; Wiebe *et al.*, 1992; 1993). Organic matter concentrations in high-latitude seas that

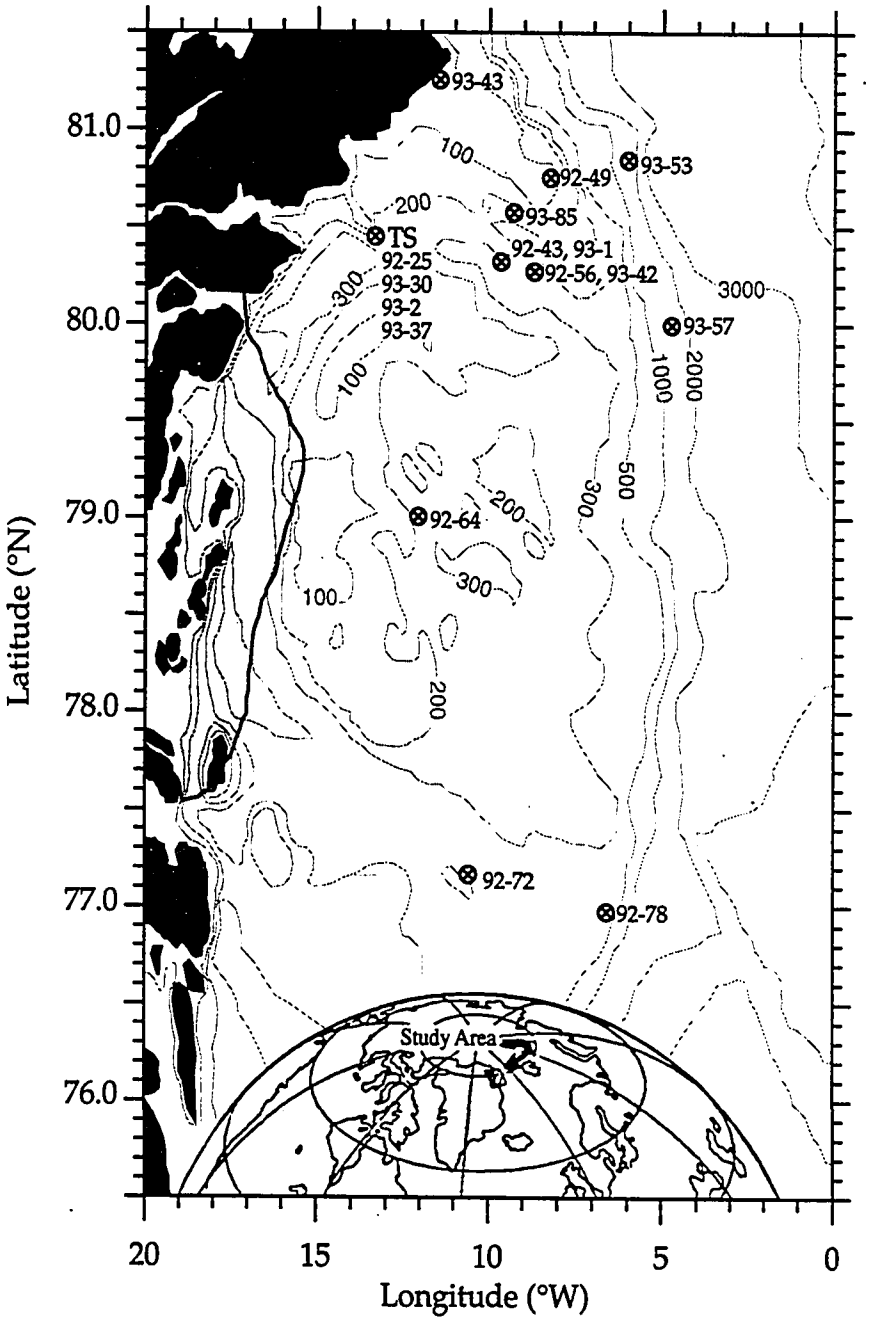


Figure IV-1. Map of NEW Polynya with microbiological stations. Depth contours in meters.

remain below this threshold presumably escape microbial remineralization and contribute to a more efficient biological pump. An alternative mechanism for minimizing the respiration of carbon is for some high-latitude bacteria to respond to low concentrations of organic matter, but utilize it very efficiently, acting as a biomass shunt or "link" to higher trophic levels. By storing carbon in the organic form during critical times of the annual cycle, high-latitude seas may enhance their function as atmospheric carbon sinks (Chapters 1 and 2). Whether by one or both of these mechanisms, minimized summertime respiration contributes to the seasonal model for air-sea gas exchange hypothesized for the NEW Polynya region (Yager *et al.*, 1995). Understanding the sensitivity of this storage behavior to predicted high-latitude warming (Hansen *et al.*, 1984) may be critical to predicting potential biogenic feedbacks to anthropogenic perturbation.

The first hypothesis described above, that high-latitude bacteria are unable to access low concentrations of organic matter at sub-zero temperatures, was supported by only some microbial community experiments in the NEW Polynya (Chapter 3). Other stations, particularly those that may be more strongly influenced by recent Arctic Ocean outflow (e.g., 92-49 and 92-56), show no such limitation (Chapter 3; Table III-3), yet still exhibit significant biologically mediated inorganic carbon deficits during the summer (Chapter 2; Table II-1). This chapter will address the latter part of the second hypothesis described above, that psychrophilic bacteria contribute to the biological pump in high-latitude seas by efficiently incorporating or storing DOM as biomass and acting as a link to higher trophic levels.

The efficiency with which bacteria and other microorganisms utilize their food supply depends on several factors. Low carbon to nitrogen (C/N)

ratios of utilizable substrates have been shown to increase gross growth efficiency of some bacteria (Goldman *et al.*, 1987; Linley and Newell, 1984). Limited nitrogen supplies, however, can also contribute to increased storage and cell size in some bacteria (Kragelund and Nybroe, 1994; Ramsay *et al.*, 1992), which may resemble high growth efficiency depending on the measurement method used. Lower temperatures may also contribute to higher efficiency by reducing respiratory rates relative to growth (Bjørnsen, 1986; Christian and Wiebe, 1974) or inducing higher enzyme production (Reichardt, 1991) in cold-adapted microbes. We found deep-sea benthic microbial populations at high latitudes to show increased incorporation efficiency (Deming and Yager, 1992) at cold (sub-zero) temperatures relative to temperate deep-sea counterparts (at 2°C), when identical measurement methods were used. The apparent trend of decreased respiration at cold temperatures motivated us to investigate the respiration-temperature relationship more explicitly in the permanently cold (≤ 5°C) pelagic zone of the NEW Polynya.

Methods

During the 1992 and 1993 cruises of the USCGC *Polar Sea* (July 15 - August 15, 1992; July 18 - August 18, 1993) and FV *Polarstern* (ARK IX/2 May 20 - June 29, 1993) to the continental shelf of northeast Greenland (Fig. IV-1), our goals were to determine the microbial fate of carbon in the surface waters of the NEW Polynya and link it to the inorganic carbon cycle. Fig. IV-2 shows a schematic representation of the carbon pools and fluxes we considered, along with the techniques used to measure them.

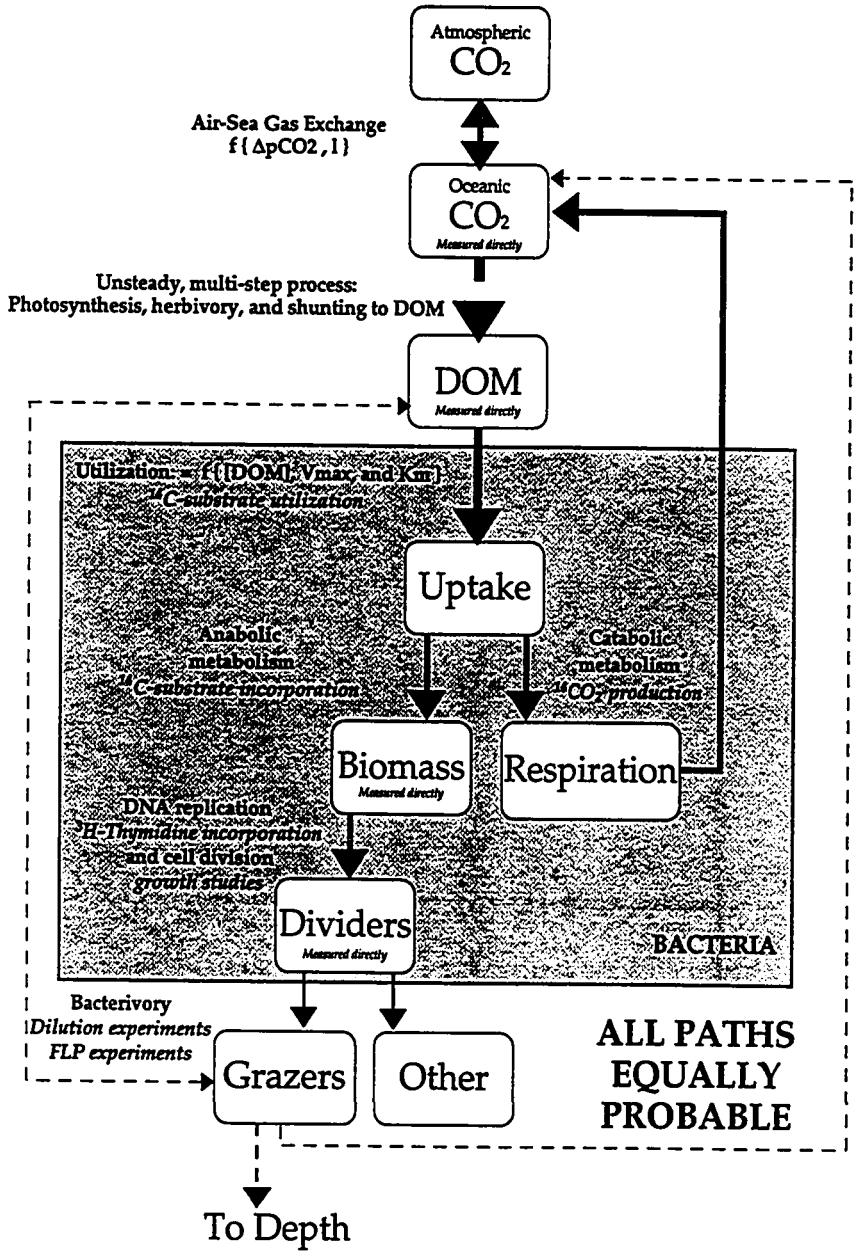


Figure IV-2. Mass-balance flow chart for polynya bacteria with methods used to measure illustrated stocks and fluxes; all fluxes equally probable.

Seawater samples from the NEW Polynya were collected and subsampled as described in Chapter 3. DOM utilization was measured using a method modified from (Deming, 1993), originally developed by Wright and Hobbie (1966), using uniformly ^{14}C-labeled substrates purchased from New England Nuclear (NEC-445E L-[^{14}C(U)] amino acid mixture, 3.80 µmol mCi^{-1} or 218 µg C mCi^{-1}; NEC-279E L-[^{14}C(U)] leucine, 314.8 mCi $mmol^{-1}$; NEC-290EL-[^{14}C(U)] glutamic acid, 261.6 mCi $mmol^{-1}$; NEC-042X D-[^{14}C(U)] glucose, 298.0 mCi $mmol^{-1}$). The prepared mixture of amino acids compared favorably ($r = 0.83$, $P < 0.01$) on a mole % basis with amino acid compositions of coastal marine phytoplankton (Cowie and Hedges, 1992). Substrates were added to final concentrations of 0.5, 1, 2, 5, 10, 20, 50, or 100 nM. Samples were incubated in the dark, in temperature-controlled water baths (-1.5, 0, 2, 3.5, or 5°C) for time-course or end-point experiments ranging from 3 to 24 h; the average in situ temperature for the fluorescence-maximum depths sampled was -1.2°C ± 0.66. At the end of each incubation (including time-zero and 2% formaldehyde-killed controls), incorporation (^{14}C incorporated to macromolecules captured on a 0.2-µm Millipore filter) and respiration (determined as $^{14}CO_2$ extracted from seawater) were determined as described in Chapter 3. Incorporation efficiency (IE) was defined as the fraction of ^{14}C incorporation relative to total utilization (^{14}C-incorporation plus ^{14}C-respiration). Standard curves and quench corrections for both data types making up the IE ratio were examined intensively to account for any differential effects on the ratio value. Quenching by the CO_2-trapping PEA on the Whatman filter paper was greater than quenching by the Millipore filter, so respired counts-per-minute (CPM) needed to be corrected to disintegration-per-minute (DPM) to a greater extent (x 1.08) than incorporated CPM to DPM

(x 1.04). Because we were looking for an enhancement in efficiency, we processed the data conservatively so as to minimize IE (preferring Type I to Type II error, Sokal and Rohlf, 1981). Any enhancement seen is, therefore, an underestimate.

Bacterial biomass production was measured by the incorporation of ^{14}C-radiolabeled leucine (New England Nuclear: NEC-279E L-[^{14}C(U)] leucine, 314.8 mCi $mmol^{-1}$) into cold-TCA insoluble material (Kirchman, 1993). Substrate was added to 20-ml seawater samples to a final concentration of 15 nM. Samples were capped and incubated in the dark, in temperature-controlled water baths at -1 or -1.5°C (approximate in situ temperature) for time-course experiments ranging from 3 to 12 h. At the end of each incubation (including time-zero and 2% formaldehyde-killed controls) activity was stopped using 0.1 ml of 5 N NaOH. Samples were stored in the dark at 2°C for up to three months, as the ship returned to Seattle. Samples were processed by first adding 20 ml 10% ice-cold TCA to the serum bottle, then gently filtering the sample through a 0.2-µm nitrocellulose filter to collect the precipitate. The filter was then rinsed twice with 20 ml of ice-cold 5% TCA and once with 20 ml of distilled water. Filters were transferred to scintillation vials and counted using Ecolume scintillation cocktail. Conversion from incorporated leucine to biomass production assumes no extracellular leucine, and uses 13.7 g protein g leucine $^{-1}$ and 0.88 g C g $protein^{-1}$ (53.3 fg C and 60.6 fg protein per 0.4 μm^3 cell; Simon and Azam, 1989).

Bacterial DNA production was measured using ^{3}H-radiolabeled thymidine incorporation into the cold TCA-, chloroform-phenol-, and cold ethanol-insoluble fractions (Wicks and Robarts, 1987). Substrate (New

England Nuclear: NET-027Z [Methyl-^{3}H]-thymidine, 82.3 Ci mmol^{-1}) was added to 20-ml seawater samples to a final concentration of 15 nM. Samples were treated in a manner identical to the bacterial biomass production samples described above except for an additional rinse of 5 ml of phenol-chloroform (50% w/v) followed by two rinses of 5 ml of ice-cold 80% ethanol. Conversion from incorporated thymidine to biomass was made using 2×10^{18} cells mol thymidine^{-1} and 10 fg C cell^{-1} (Ducklow and Carlson, 1992). These values range in the ocean from 1×10^{17} to 60×10^{18} cells mole^{-1} and 2 to 28 fg C cell^{-1}, respectively (Ducklow and Carlson, 1992), and thus the interpretation of our results will be quite sensitive to the choice we make for these conversion factors (see discussion).

We measured bacterivory and bacterial growth rate using the dilution method (Tremaine and Mills, 1987). One-liter samples were pre-filtered through a 200-µm Nitex mesh and then diluted to 100, 75, 50, and 25% of original concentration using gently-filtered (0.2-µm) seawater from the same station and depth. Filtration was done using the lowest possible setting of a vacuum pump and frequent filters changes, yet some DOC enrichment may have still occurred due to cell breakage under vacuum. Time-zero samples were taken and then each bottle incubated for 24 h in the on-deck water table while screened to 5% ambient sunlight (bath temperature typically ranged from -1 to 0°C). After incubation, samples were fixed and counted for bacterial abundance, as described in Chapter 3, to determine bacterial growth rate as a function of dilution factor. Grazing rate is taken from the slope of this relationship; bacterial growth rate in the absence of grazers is taken as the y-intercept. This technique is more commonly applied to measure herbivory by macrozooplankton, but can also be used to measure bacterivory (Tremaine

and Mills, 1987, E. Lessard, personal communication). Subsamples for chlorophyll *a* were taken from the same incubations simultaneously to determine rates of protozoan herbivory (Sime-Ngando *et al.*, manuscript in preparation).

Results

14C-labeled substrates were taken up and respired at detectable levels for all substrate concentrations and incubation temperatures used for samples from the depths of maximum fluorescence. The efficiency at which cells incorporated substrate into biomass was remarkably consistent across all stations in the polynya except for those stations sampled aboard Polarstern earlier in the Spring of 1993 where IE tended to be lower.

Since IE measurements can be very sensitive to the technique used (Bjørnsen, 1986), particularly to the extent of radiolabel that enters the cell (and is only then capable of being respired) during incubation, we first examined the effects of incubation time and strength of the radioactive signal. Incorporation efficiency of mixed amino acids was not a significant function of incubation time ($P > 0.1$) over the range we measured (4-16 h). Nor was it significantly sensitive to the strength of the signal as measured by pmol substrate utilized per liter of sample (i.e., the rate measurement before being scaled by time of incubation; $P > 0.1$). At very low signal strengths (< 500 pmol liter^{-1}), however, there was more noise in the measurement: IE for mixed amino acids ranged from 30 to 100%, although the average value (82%) for all 1992 stations at low signal strength was not different from that measured at up to 9000 pmol liter^{-1}. These results provide some confidence that our estimates are valid and interpretable.

For 10 nM substrate additions incubated at in situ temperature over a total of eight stations in 1993, the uniformity of IE as a function of substrate type was remarkably high (Fig. IV-3). In general, leucine was used extremely efficiently, ranging from 90 to 100%. Mixed amino acids were used slightly less efficiently, averaging 82 ± 5%. The efficiency on glucose averaged 68 ± 4%, while glutamic acid, which may go directly into the respiratory cycle, averaged 41 ± 5%.

Multivariate ANOVA was used, as with utilization rate in Chapter 3, to test for effects of temperature and substrate on incorporation efficiency. Overall, temperature and substrate explained an average of 71.6 ± 11.8% of the variation in incorporation efficiency (i.e., the average $r^2 = 0.716 \pm 0.118$; $n = 9$). In all but one of the nine stations analyzed, substrate had a significant effect ($P < 0.05$) on IE (Table IV-1). Station 92-56 was the exception; as we have already discussed in Chapter 3, this was the station with the highest DFAA concentrations. Again, high in situ substrate concentrations appear to dampen any substrate effects.

Temperature had a significant ($P < 0.05$) effect on IE in only three of the nine experiments (Table IV-1), two of which were visits to the time-series location. Of note is the fact that these two experiments (Stations 92-25B and 93-30) also exhibited temperature effects on utilization rate (Table III-2). Combined effects of temperature with substrate were found at two of the nine stations. The heavily ice-covered station over the central bank (92-64) was the one station that showed an interaction between temperature and substrate concentration for both IE and utilization rate.

Examining the three-dimensional surface fits to IE as a function of temperature and substrate concentration, we detected significant non-

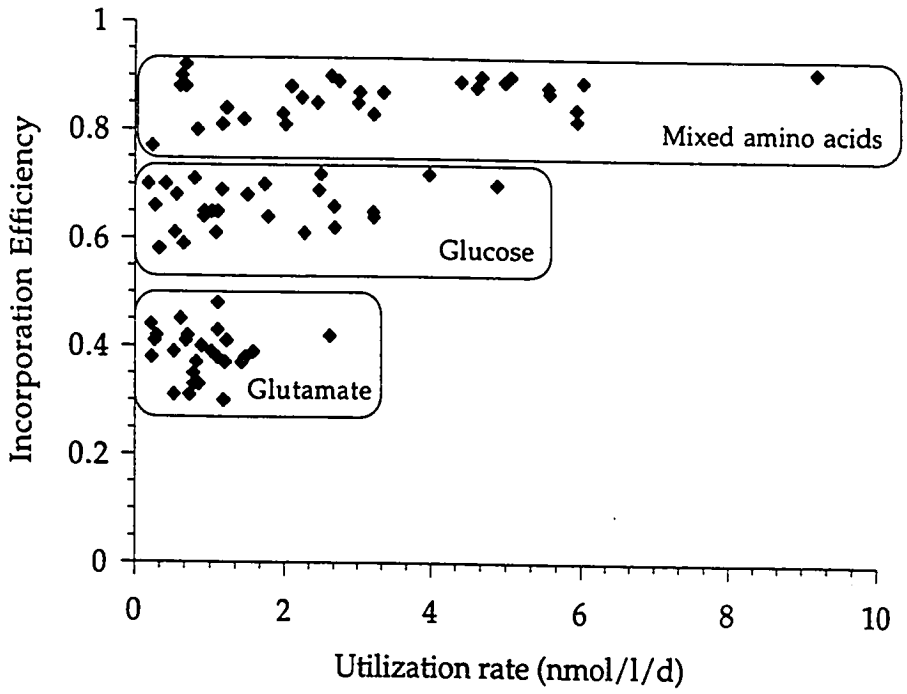


Figure IV-3. Incorporation efficiency versus utilization rate for seawater samples with 10 nM added mixed amino acids, glucose, or glutamic acid, incubated at -1.5°C.

TABLE IV-1: M(ANOVA) results for Incorporation Efficiency

Station	n	R^2	P Added Substrate	P Temperature	P [A] x T
92-25B	35	0.802	0.007**	<0.001**	0.258
92-43B	36	0.784	0.013**	0.004**	0.022**
92-49	36	0.683	0.001**	0.829	0.736
92-56	35	0.555	0.664	0.096	0.313
92-64	36	0.888	<0.001**	0.147	0.035*
93-30	50	0.755	<0.001**	0.013**	0.406
93-43	52	0.627	<0.001**	0.736	0.331
93-85	54	0.553	0.002**	0.074	0.210
93-2	53	0.801	<0.001**	0.344	0.664

* Indicates significance at ≥ 95% confidence.

** Indicates significance at ≥ 99% confidence.

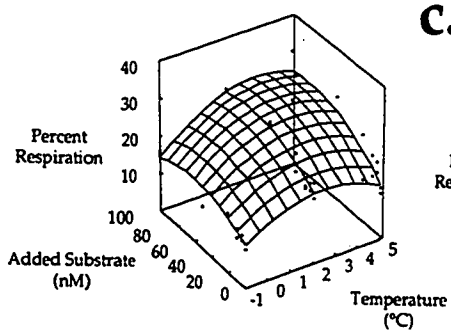
linearity in the surfaces. For improved visualization, we plotted respiration efficiency (RE % = 100 - IE%) for each station (Figure IV-4). Incorporation efficiencies tended to drop with higher substrate concentrations, perhaps indicating luxury consumption or less tendency for storage.

Bacterial protein production measured using leucine (BP-LEU) ranged from 0 to 12 μg C l^{-1} d^{-1} (with the median rate at 0.54 μg C l^{-1} d^{-1} for 1992 stations and 2.61 μg C l^{-1} d^{-1} for 1993). This production equaled, on average, about 20-25% of primary production rates for the same depth and station (Figure IV-5; the slope of the linear regression was 0.20 ± 0.04 for all stations, although the r^2 value, 0.08, was not significant). There were a few stations where primary production was low and bacterial production high (92-25B and 93-86), and vice versa (particularly at the time series location in 1993: 93-2, 93-17, and 93-37), indicating that the two processes were often decoupled in the polynya region.

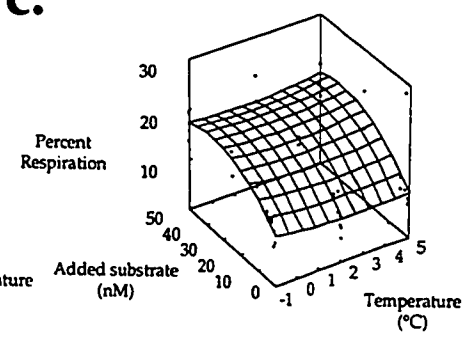
Bacterial production using thymidine incorporation into DNA (BP-TDR) ranged from 0 to 9.0 μg C l^{-1} d^{-1} (with the median rate for 1992 at 0.23, and for 1993, 1.63 μg C l^{-1} d^{-1}), if we assumed the oceanic median genome size (2×10^{18} cells mol thymidine^{-1}; Ducklow and Carlson, 1992) and applied the large cell biovolume found in the polynya (Chapter 3; 0.525 μm^3) with the cell carbon to biovolume ratio determined by Simon and Azam (1989; ~ 60 fg C cell^{-1} for a cell size greater than 0.4 μm^3). Compared to the BP-LEU estimates, which do not require a cell biomass to biovolume assumption, BP-TDR rates were about the same or lower (BP-LEU : BP-TDR ratio had a median of 1.56 and ranged from 0.03 to 81) and exceeded the BP-LEU rates at only a few stations (Fig. IV-6). The estimated BP-TDR rates were significantly reduced if we instead used a theoretical genome size (ca. 0.5×10^{18} cells mol thymidine^{-1})

Figure IV-4. Three-dimensional surface plots of percent respiration (R = 1 - IE) versus temperature (T°C) and added substrate concentration ([A]): a) Station 92-25B; b) Station 92-43B; c) Station 92-49; and d) Station 92-56.

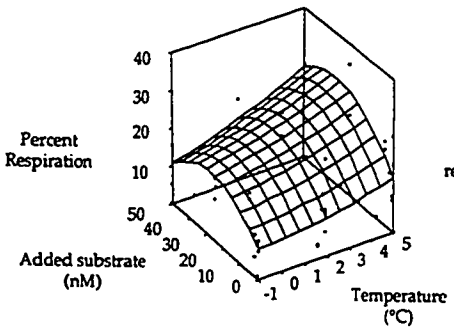
a.


Percent
Respiration
40
30
20
10
100
80
60
40
20
0
Added Substrate
(nM)
-1 0 1 2 3 4 5
Temperature
(°C)

c.


Percent
Respiration
30
20
10
50
40
30
20
10
0
Added substrate
(nM)
-1 0 1 2 3 4 5
Temperature
(°C)

b.


Percent
Respiration
40
30
20
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Added substrate
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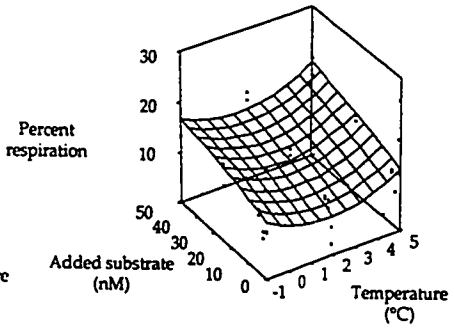
Temperature
(°C)

d.


Percent
respiration
30
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Added substrate
(nM)
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Temperature
(°C)

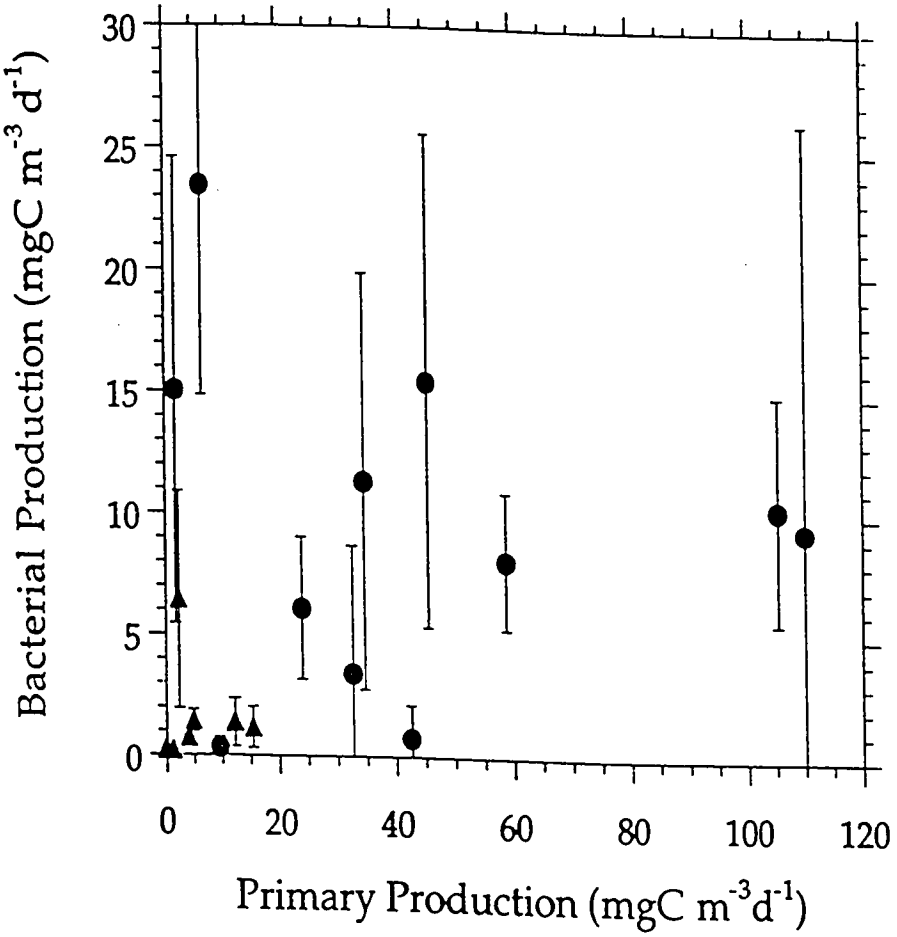

Figure IV-5. Bacterial production measured by ^{14}C-leucine incorporation into TCA insoluble biomass (mg C m^{-3} d^{-1}) as a function of primary production (mg C m^{-3} d^{-1}; data courtesy of W. O. Smith) in the euphotic zone. Circles indicate stations measured in 1993, and triangles in 1992. Error bars indicate one standard error on slope (rate) estimate from time course or endpoint experiments.

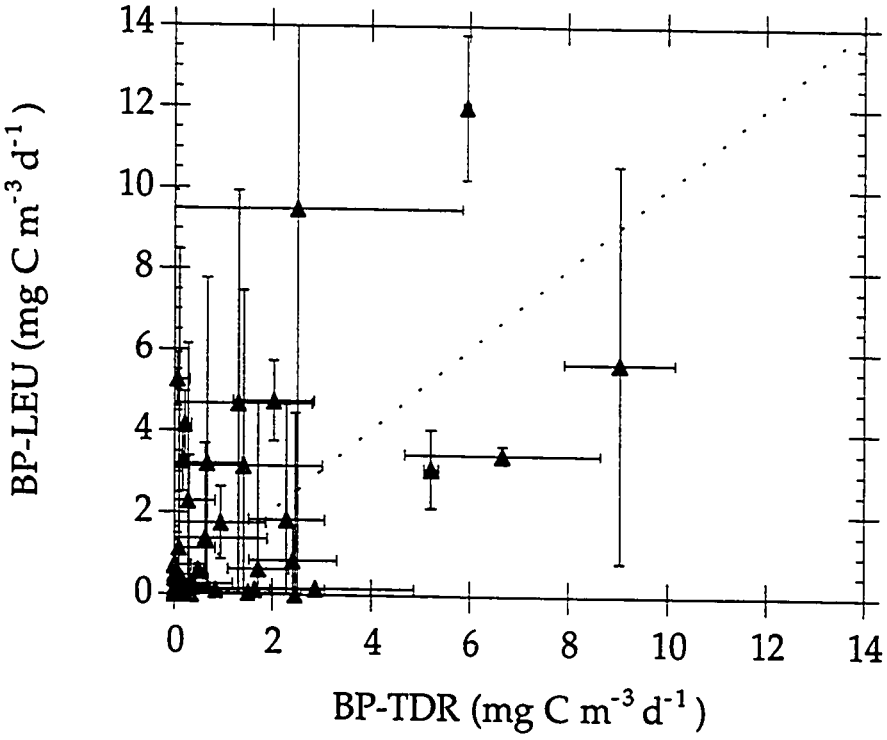

Figure IV-6. Bacterial production estimated by ^{3}H-thymidine incorporation to DNA (mg C m^{-3} d^{-1}) versus bacterial production estimated by ^{14}C-leucine incorporation into protein (mg C m^{-3} d^{-1}). Error bars indicate one standard error on slope (rate) estimates from time course or endpoint experiments.

or a more typical cell biomass (e.g., 10 fg C cell-1; Ducklow and Carlson, 1992). In both cases, the production estimates would drop below those determined by leucine incorporation and indicate "unbalanced" growth (Kirchman *et al.*, 1986; see Discussion).

Bacterivory ranged from 0 to 3.7 d^{-1} (using specific growth rates; up to 5 $\mu g\ C\ l^{-1}\ d^{-1}$ if we convert to carbon) and in general kept pace (Fig. IV-7) with bacterial growth estimated using the y-intercept of the dilution curve (theoretical growth rate in the absence of grazers). These bacterial growth rates, estimated using the dilution technique, compared reasonably well to the potential growth rates described in Chapter 3 and to the bacterial production rates described above.

If we subdivide our *Polarstern* cruise stations into the two ecosystem types suggested by Pesant *et al.*, (1995; using the size-fractionated phytoplankton biomass and production to divide the polynya stations into those ecosystems dominated by large diatoms, Type I, and those dominated by small autotrophic flagellates, Type V), we find that bacterivory tends to equal or exceed bacterial growth at Type V stations (n=5) but fall short of growth at Type I stations (n = 4; Fig. IV-8). According to Legendre and LeFevre (1995), these ecosystem types indicate the local importance of the microbial loop, with Type I reflecting an herbivorous food web and Type V reflecting a microbial food web or loop. Our data confirms this idea in that a stronger link between bacterial growth and bacterivory occurs where autotrophic flagellates dominate. In an herbivorous food web, bacteria are active, but apparently not as strongly controlled by microzooplankton grazers.

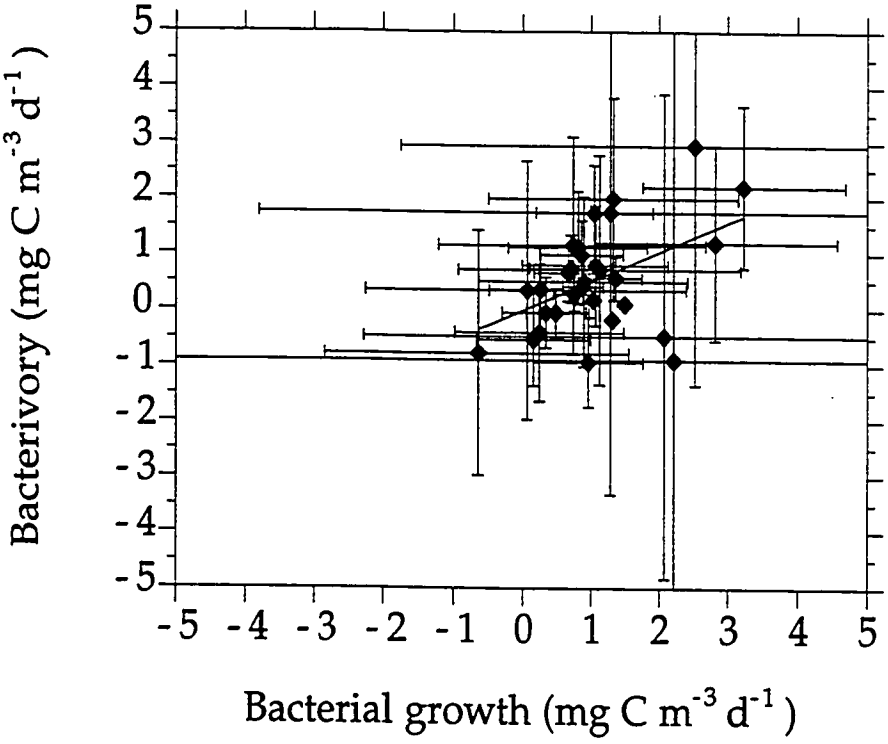

Figure IV-7. Grazing rate (g_b, mg C m^{-3} d^{-1}; estimated by the slope) as a function of bacterial growth in the absence of grazers (μ, mg C m^{-3} d^{-1}; estimated by the y-intercept) determined from the dilution versus bacterial growth relationship. Conversion of growth rate from units of d^{-1} to mg C m^{-3} d^{-1} assumes 60 fg C $cell^{-1}$ (see text) and uses the measured bacterial abundance for each station (see Table III-1).

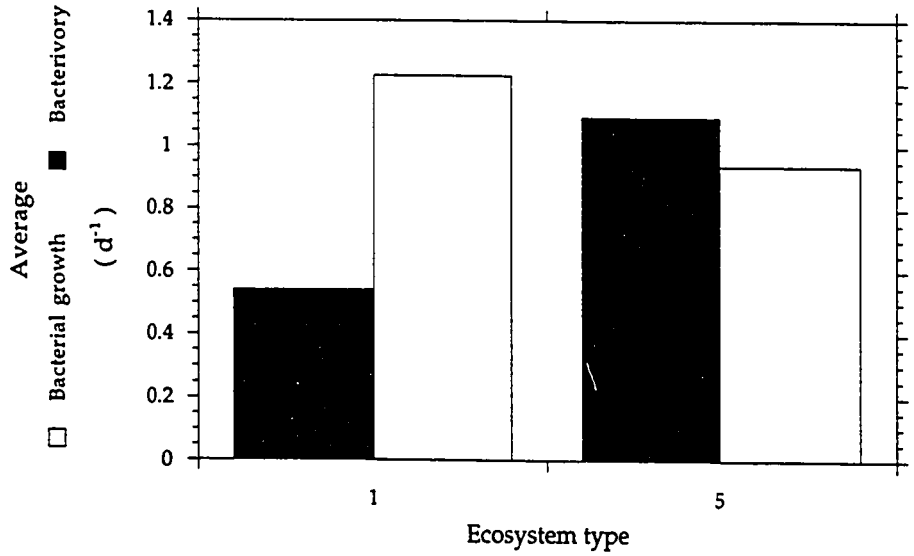

Figure IV-8. Average bacterial growth and bacterivory for *Polarstern* 1993 stations subdivided into Type I ecosystems (diatom-dominated; n = 5) and Type V ecosystems (autotrophic flagellate-dominated; n = 4). Under conditions favoring the microbial loop, bacterivory tends to dominate bacterial growth. (Because of the small number of stations, however, error bars are ± >100%; we can only infer a trend.)

Discussion

Taken together, this suite of results points to the conclusion that bacteria in the NEW Polynya were taking up dissolved organics as they became available, using them efficiently with minimal respiration, and passing the carbon and energy to higher trophic levels (Fig. IV-9). Some of these carbon transfers may also be sensitive to warming and could feedback to perturbations of the inorganic carbon cycle.

Why do these results appear so different from the paradigm that the microbial loop is inactive in polar waters? One explanation may be that unique strategies adopted by high-latitude bacteria are underestimated by traditional methods. Previous attempts to measure bacterial activity at high latitudes have often relied solely on measurements of respiration (O_2 utilization or CO_2 production) or on thymidine incorporation into DNA. According to our data, Arctic bacteria may not expend much of their limited energy on respiration and bacterial production measured with thymidine incorporation may be underestimated if standard conversion factors are used (see below).

Why the metabolic partitioning of organic matter by polar bacteria should be different from temperate organisms is not entirely clear, unless we assume that oligotrophic strategies are especially successful in the polar environment. If there is a link between psychrophily and oligotrophy in the marine environment (Zobell, 1946), it should be detected most clearly in polar environments. High efficiencies may be related to the episodic nature of the polar food source (and resultant adaptations associated with oligotrophy, e.g., storage) or to community structure (i.e., if psychrophiles are more efficient

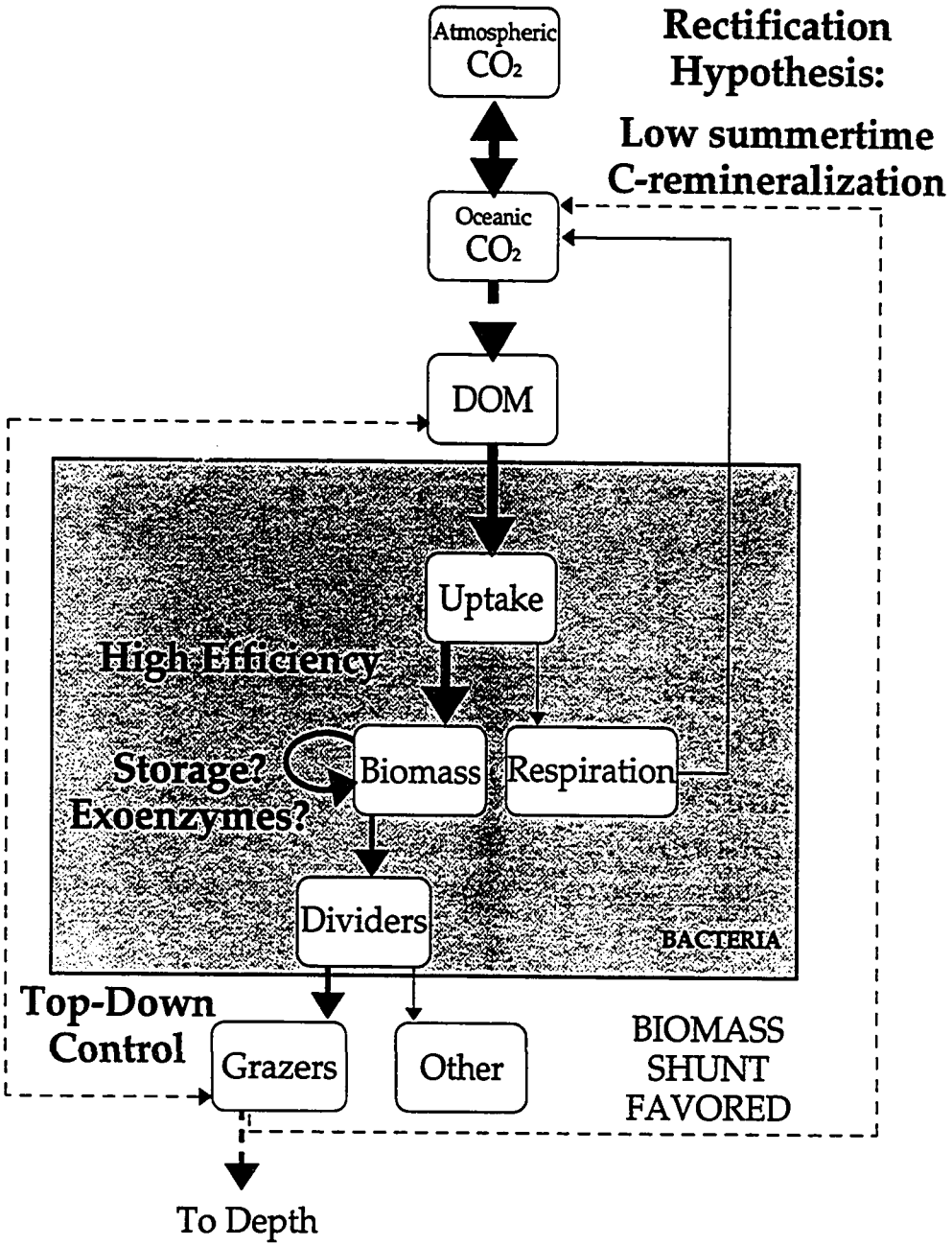

Figure IV-9. Mass-balance figure redrawn, showing overall conclusion from this study that the biomass shunt is favored in the NEW Polynya over carbon respiration; arrows are proportional.

than psychrotolerant or mesophilic bacteria, the dominance of psychrophiles under some incubation conditions might be reflected in our observations). High polar efficiency as a strategy for survival is further supported by recent data from the Alaskan Arctic showing that high-latitude methane oxidation is also highly efficient (~60%, E. McLaughlin, personal communication). High incorporation efficiencies and unbalanced growth may also be a response to grazing pressure if larger cell size does provide more of a refuge from predation (Chapter 3).

There is some discussion in the literature about the validity of using radiolabeled substrates to measure growth efficiency (see for example, the review by Pomeroy and Wiebe, 1993). Bacterial incorporation of 14C-labeled substrates appears to be more efficient than that observed on a more "natural" mix of dissolved organic matter (e.g., Linley and Newell, 1984) or than that determined by the ratio of net biomass production relative to the sum of respiration (CO_2) and net biomass production (e.g., Bjørnsen, 1986). Bacteria gain more benefit from taking up an amino acid than a less labile organic molecule, and may therefore utilize it differently (e.g., more efficiently). Since bacteria are known to be capable of diauxic growth (as originally proposed by Monod, 1942), they can be expected to regulate their uptake processes so as to utilize the better substrate first when confronted with resources of diverse quality; i.e., as NEW Polynya bacteria encounter amino acids, they will prefer them to other less labile material. By using a mixture of amino acids that mimics the composition of phytoplankton or zooplankton exudate, we expected our results to reflect "reality" better than if we had used a single substrate. Nevertheless, if the bacteria in the polynya are not regularly exposed to such a labile food supply, we measured their behavioral

responses to high quality food pulses and not some "average" condition. Since our goal was a comparative one (temperature and substrate concentration effects on efficiency), we can still draw conclusions about the sensitivity of this microbial response. Generalizing our results to the overall carbon cycle of the polynya should await better understanding of controls on DOM production in the region.

Interpretation of bacterial production data is also challenging. Because of the unusually large cell sizes in the polynya, particularly the fact that a high percentage (72 ± 6.5%) of cells were rod-, spiral-, or crescent-shaped (Chapter 3), rather than tiny cocci, the choice of conversion factors becomes a dilemma. The thymidine technique works best in oligotrophic environments when cells are uniformly small and contain a single genome (J. Baross, pers. comm.). Once cells become large and active, the genome-to-carbon ratio can fluctuate a great deal and make interpretation difficult. Data from other arctic environments, where measurements were made frequently throughout the year (Rivkin, 1994), indicate that conversion factors can vary several orders of magnitude depending on the season. Given the problems with conversion, we cannot be certain that bacterial growth in the polynya was either balanced or unbalanced. The appearance of unbalanced growth, however, indicates a microbial community out of steady state, a finding that supports the idea that high-latitude bacteria may have unusual survival strategies for their extremely heterogeneous environment.

Under conditions of nitrogen limitation, bacteria store carbon energy in the form of long-side-chain polyhydroxyalkanoate (PHA; Ramsay *et al.*, 1992). Temperature (Huijberts *et al.*, 1992) and unsteady food supply (Pagni *et al.*,

1992) can also influence PHA production. Carbon storage due to these or other factors may explain our observations of high incorporation efficiency.

Extracellular enzyme production may also increase at low temperatures (Reichardt, 1991). If such enzymes were produced from the radiolabeled substrate added in our experiments and also exhibited the high binding affinities to particle surfaces predicted from modeling exercises (Vetter and Deming, 1996), they may have been retained on the 0.2-μm filter and thus contributed to the measures of high incorporation efficiency.

From this study, pelagic bacteria in the polynya appear to be highly responsive to pulses of labile organic matter (Chapter 3) and very efficient at retaining it in the organic carbon pool, releasing only a small fraction of it to respiration (this chapter). Bacterial biomass does not accumulate, however, because the cells are, by all measures (Fig. III-3 and Fig. IV-7), living under intense grazing pressure. An obvious question arises: given that bacteria have minimized respiration in this and perhaps other polar environments, do the bacterivores play a more dominant role than their prey in carbon and nitrogen remineralization? The only study of the growth efficiency of polar heterotrophic flagellates, of which I am aware, is that by Choi and Peters (1992). In this study using cultured organisms, IE was high at subzero temperatures (60-70%) and decreased with warming, much as we observed in some of our experiments in the polynya. If these flagellate results can be extrapolated to other grazers in polar waters, then highly efficient trophic links may continue up the food web, providing an ecosystem response that contributes as a whole to an efficient biological pump.

Conclusions

We found active and responsive microbial populations at the fluorescence maxima of the NEW Polynya surface waters. High incorporation efficiencies on labile organic matter suggest that polynya bacteria reserve rather than quickly metabolize (catabolize) favored food sources and may have evolved survival strategies for dealing with episodic food availability, nitrogen limitation, and grazer control. Incorporation efficiencies tended to drop with higher substrate concentrations, perhaps indicating luxury consumption. When sensitive to temperature, IE showed a varied response, showing a decrease with warming at some stations, but highly non-linear and non-monotonic responses at others. Unbalanced growth was observed (subject to choice of conversion factors), with a tendency towards biomass building consistent with the large cell size observed. This behavior may reflect a storage response to episodic food supply or a refuge response to grazing pressure given the role of size in determining predator-prey encounter efficiency. Bacterivory was patchy but accounted for 25- >100% of bacterial growth when detected by the dilution method, suggesting that bacteria provide a link to higher trophic levels when there is sufficient food available for bacterial growth. This link may be enhanced in those communities dominated by the microbial loop. We suggest that the biological pump functions with high efficiency in polar environments not because the microbial community is inactivated by low temperature, but rather because active psychrophilic microbial communities minimize summertime respiration of labile organic carbon as a survival strategy in the face of an episodic food supply and significant grazing pressure.

V. Overall Conclusions and Future Research Plans

The fundamental conclusion derived several times from this research project is that mechanisms exist by which high-latitude marine biota may feedback to global climate change via their effects on the inorganic carbon cycle. These mechanisms include 1) non-steady state behavior in the temporally and spatially heterogeneous environment of the high-latitude seas; 2) interactions between marine biota and their physical environment (particularly with respect to timing) which influence the extent to which ice-cover can act as a wintertime lid to outgassing of CO_2; and 3) temperature and food source effects on microbes which can influence the strength and efficiency of the biological carbon pump.

In Chapter 1, the sensitivity of local air-sea carbon flux to small changes in a single biological parameter was shown using a food web model linked to a simple ocean and atmosphere. Field data collected for Chapter 2 from the Northeast Water (NEW) Polynya confirmed that there were indeed significant biologically mediated inorganic carbon deficits in the summertime surface waters. This drawdown, put into the context of the seasonal cycle of ice-cover and wind speed, suggests that because of the marine biota, there is no time during the year when seasonally ice-covered seas can be a source of CO_2 to the atmosphere. Due to this effect, these regions likely function as efficient biological pumps, sequestering carbon from the atmosphere. Because of strong cloud and ice-albedo feedbacks at high latitudes, the temperature sensitivity of the polar regions is acute and the global area of seasonally ice-covered seas is expected to vary with natural and anthropogenically driven climate change. If the results we found in our study area can be applied to

these other regions, a small but significant negative feedback to climate change can be identified.

The above described scenario depends on low respiration rates during the productive summer season. These low respiration rates have been attributed historically to a disproportionate shut-down of the microbial remineralizers at sub-zero temperatures compared to the primary producers in polar regions. The mechanism for this shut down was thought to be an inability on the part of bacteria to acquire food in the form of low concentrations of organic matter, either because of the physiological challenges of this process at low temperature, or because diffusion rates of dissolved material were reduced significantly by the temperature effect on seawater density and viscosity. This study has shown that neither explanation is correct for all microbial communities sampled in the Northeast Water Polynya. In fact, there are indications that the microbial communities living in these regions are dominated by psychrophilic (cold-loving) bacteria and that, for some, acquisition of dissolved organic matter is fastest at sub-zero temperatures compared to just a few degrees warmer (Chapter 3).

If the microbial communities are active, then why are the respiration rates so low in this region? One possibility supported by our data is that the active bacteria are not respiring much of the carbon they use, preferring to store it in response to either an episodic food supply or heavy grazing pressure. Since grazing pressure was indeed heavy, particularly at those stations where microbial loop processes were most developed, the biomass stored in the bacteria is likely transferred to the bacterivores rather than returned to inorganic form by significant respiration. Thus, an active and

responsive bacterial community can still contribute to an efficient biological carbon pump at high latitudes.

Still, we are left with the question of what will happen when the Arctic warms up. Will the one-way sink remain intact as the area of seasonally ice-covered regions increases and thus help ameliorate climate change forces? Will the warmer surface waters shift the microbial remineralization response, perhaps negating the rectification? Since we observed more than one response to temperature from communities sampled across a fairly small region, we are as yet unable to predict the final outcome.

Future research on this question of prediction requires two approaches, a theoretical and an experimental one. As described at the end of Chapter 1, many questions remain to be addressed using modeling, in particular the question of how the biologically mediated carbon flux can influence long term sequestration of carbon from the atmosphere, specifically for the case of anthropogenically enhanced concentrations of CO_2. For the case of increasing temperature, we need to be able to predict which of the potential biotic feedbacks will dominate. This can be accomplished best through focused modeling exercises.

To determine the extent of phenotypic and genotypic diversity of microorganisms in polar seas requires an experimental approach. In order to better predict the response of microbial communities to warming, we need to know the players, how each responds to temperature, and what controls their activity with respect to other organisms in the same area. Molecular methods used to identify and distinguish in situ microorganisms genotypically, followed by laboratory work with pure cultures to distinguish ecologically relevant phenotypes, will be very helpful in this quest.

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APPENDIX 1: DERIVATION OF EQUATION III-1

Start with the Michaelis-Menton uptake equation which describes utilization rate, v, as a function of substrate concentration at the cell surface, C_o :

$$v = \frac{V_{max} \cdot C_o}{K_t + C_o} \tag{1}$$

and assuming steady state at the cell membrane, set the diffusive flux to the cell equal to the kinetic uptake of substrate by the cell:

$$v = \frac{V_{max} \cdot C_o}{K_m + C_o} = J_D = 4\pi \cdot r_o \cdot D \cdot (C_\infty - C_o) \tag{2}$$

Rearrange to put in the form of a quadratic equation to solve:

$$C_o^2 + \left[\frac{V_{max}}{4\pi \cdot r_o D} + K_m - C_\infty\right] C_o - C_\infty K_m = 0 \tag{3}$$

and then solve for C_o:

$$C_o = \frac{1}{2}\left[C_\infty - K_m - \frac{V_{max}}{4\pi \cdot r_o D} \pm \sqrt{\left(\frac{V_{max}}{4\pi \cdot r_o D} + K_m - C_\infty\right)^2 + 4C_\infty K_m}\right] \tag{4}$$

Substitute Eq. 4 into Eq. 1 to obtain a complete, albeit complex, equation to which the data can be fit:

$$v = \frac{V_{max}}{\left\{1 + \left(\frac{2 * K_m}{C_\infty - K_m - \frac{V_{max}}{4\pi r_0 D} + \sqrt{\left(\frac{V_{max}}{4\pi r_0 D} + K_m - C_\infty\right)^2 + 4C_\infty K_m}}\right)\right\}} \tag{5}$$

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1985-1988 — University of Washington, School of Oceanography, Seattle, Washington. Master of Science. Advisor: A. R. M. Nowell. Masters Thesis: *Enhanced Deposition to Pits: the Effect of Microtopography on Food Sources for Benthic Organisms.*

1981-1985 — Brown University, Department of Geology, Providence, Rhode Island. Bachelor of Science, Sigma Xi. Advisor: W. L. Prell. Undergraduate Thesis: *Sediment Distribution in Hundred Acre Cove and its Use as a Key to Estuarine Circulation and Sediment Transport.*

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1992, 1993 — *Research practicum* (DOE fellowship program), Brookhaven National Laboratory, Ocean and Atmospheric Sciences Division, Department of Applied Science, Upton, New York. Instruction and sample analysis using SOMMA total inorganic carbon analyzer (Dr. D. W. R. Wallace and K. Johnson) and closed-cell potentiometric titration alkalinity system (Dr. C. Goyet, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts).

1991-1992 — Institute of Ecology and Department of Microbiology, University of Georgia, Athens, Georgia. Courses in *Microbial Ecology, Microbiology,* and *Biochemistry.* Drs. Hodson, Pomeroy, Moran, Wiebe, Whitman, Shimkets, Wiegel, Dailey, and others.

1991 Friday Harbor Marine Laboratories, University of Washington, Friday Harbor, Washington. Summer course: *Climate and the Marine Biosphere.* Dr. R. H. Gammon and others.

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1984 S.E.P.M. Short Course, Geological Society of America, Providence, Rhode Island. Course: *Mechanics of Sediment Movement.* Drs. G. V. Middleton and J. B. Southard.

1984 Department of Geology, Brown University, Providence, Rhode Island. Micropaleontology Short Course in *Benthic Foraminifera.* Dr. W. A. Berggren.

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PROFESSIONAL EXPERIENCE

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1988 Teaching Assistant for Dr. A. Duxbury, *Introductory Oceanography;* lecture and laboratory.

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1986 Teaching Assistant for Drs. A. R. M. Nowell and P. A. Jumars. Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington. *Dynamics at the Sediment-Water Interface,* lecture, laboratory, and fieldwork.

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1991-1995 Department of Energy, Graduate Fellowship for Global Change Research.

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1992, 1993	Research cruises to Northeast Water (NEW) Polynya (77-81°N, 6-17°W), aboard Coast Guard icebreaker USCGC *Polar Sea,* July - August 1992, 1993. Temperature and substrate effects on pelagic microbial utilization and efficiency, inorganic carbon depletion associated with biological activity, part of an interdisciplinary research team studying carbon cycling in Arctic polynyas.
1993	Research cruise to NEW Polynya, aboard German icebreaker FS *Polarstern,* May 16 - June 24. Collaboration with Canadian research team studying microbial loop processes in Arctic polynyas, part of International NEW project.
1991	Research cruise to Santa Catalina Basin, California, aboard RV *Atlantis II* and DSRV *Alvin*. Effects of whale carcass eutrophication on deep-sea benthic microbial community.
1990	Research cruise to Norwegian Sea, aboard German research vessel, FS *Meteor*. Effects of pressure and temperature on deep-sea benthic microbial processes. Collaborated with Drs. G. Graf and L. A. Meyer-Reil.
1987, 1988	Hydrodynamics Laboratory, Friday Harbor Laboratories, San Juan Island, Washington. Masters Thesis research: Experiments using race-track, straight-through, and annular flumes to study effect of biogenic microtopography on deposition of particles.
1987	Research Platform in Santa Catalina Basin, California. Using Remote Underwater Manipulator *(RUM II)*. Deep-sea biological-sedimentary interactions, deployment of benthic pit mimic.
1987	Research cruise to Santa Catalina Basin, California, aboard RV *Atlantis II* and DSRV *Alvin*. Deep-sea biological-sedimentary interactions, deployment of benthic pit mimic.
1986	Research cruise to California continental shelf, aboard RV *Thomas G. Thompson*. STRESS project: examined sediment transport and storm effects on continental shelf and slope with side-scan sonar and box-coring.
1985	Fieldwork using canoe and motorized raft. Hundred Acre Cove, Barrington, Rhode Island. Undergraduate Research Project: collected estuarine benthic samples using grabs and corers for sediment analysis and hydrodynamics study.
1984	Intertidal fieldwork. False Bay, San Juan Island, Washington. Effect of biogenic roughness height and density on local erosion and deposition.

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Yager, P. L., D. W. R. Wallace, K. M. Johnson, W. O. Smith Jr., P. J. Minnett, and J. W. Deming (1995). The Northeast Water Polynya as an atmospheric CO_2 sink; a seasonal rectification hypothesis. *Journal of Geophysical Research, 100*: 4389-4398.

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Deming, J. W. and P. L. Yager (1992). Natural bacterial assemblages in deep-sea sediments: towards a global view. *In:* G. T. Rowe and V. Pariente (eds.), *Deep-Sea Food Chains and the Global Carbon Cycle.* Kluwer Academic Publishers, Netherlands, pp. 11-27.

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Yager, P. L., and J. W. Deming (1996). Microbial activity in the Northeast Water Polynya: testing for temperature and substrate effects using a kinetic approach. 1996 Ocean Sciences Meeting, American Geophysical Union / American Society of Limnology and Oceanography, San Diego, February 1996.

Yager, P. L., J. W. Deming, T. Sime-Ngando, and K. Juniper (1995). Pelagic microbial activity in the Northeast Water Polynya: Implications for the inorganic carbon cycle. International Northeast Water Polynya Symposium, Helsingør, Denmark, May 1995.

Yager, P. L. and J. W. Deming (1993). Collaborative research in the Northeast Water Polynya: temperature and substrate effects on pelagic bacterial kinetics. Annual meeting, The Oceanography Society, Seattle, April 1993.

Yager, P. L., D. W. R. Wallace, and K. M. Johnson (1993). Collaborative research on the Northeast Water Polynya: inorganic carbon parameters and uptake of atmospheric CO_2. Annual meeting, The Oceanography Society, Seattle, April 1993.

Yager, P. L. and J. W. Deming (1993). Collaborative research on the Northeast Water polynya (U.S. NEW 1992): Pelagic Microbial Dynamics. International Workshop on Arctic Polynyas, Seattle, January 1993.

Deming, J. W. and P. L. Yager (1991). Benthic bacterial populations in the Greenland Sea corridor: response to increased carbon flux and temperature. Arctic Ocean-Atmosphere-Ice Interactions in Global Change. Fall meeting, American Geophysical Union, San Francisco, December 1991.

Yager, P. L., A. R. M. Nowell, and P. A. Jumars (1989). Enhanced deposition to pits: the effect of microtopography on food sources for deposit feeders. Annual meeting, North American Benthological Society, Guelph, Ontario, May 1989.