A. PROJECT SUMMARY

Reductions in sea ice and increases in terrestrial inputs to the coastal Arctic have profound implications for productivity, ecosystem structure, and carbon fluxes in this region. For the Arctic coastal region in particular, ecosystem shifts could be considerable if sea-ice changes lead to increased water-column production and reduced benthic production. Such a restructuring would ultimately alter the pathways and magnitude of energy transfer to upper trophic levels such as fish, sea birds and marine mammals, and impact the people dependant on those resources. Our challenge today is to transform such generalizations with mechanistic and detailed understanding of specific ecosystems and their components to allow assessment of past, present, and future variation. One important component lacking detailed understanding is the role of microorganisms in Arctic marine ecosystems. The importance of microbes to the productivity and carbon fluxes of low latitude marine ecosystems has been established. In contrast to the extensive research in temperate marine systems, much less is known about marine microorganisms in the Arctic. We do know that bioavailable nitrogen is key to the productivity of the coastal Arctic, yet how it gets partitioned to microbial autotrophs and heterotrophs is entirely undetermined. Such unknowns prevent us from predicting with any confidence the impact of climate change on food webs and basic biogeochemical processes in the coastal Arctic.

Intellectual merit: This project brings together three experienced investigators to jointly investigate the microbiological controls on the productivity of a coastal Arctic ecosystem. In the waters near Barrow, Alaska and with international collaborations of opportunity, we hypothesize that the balance of autotrophy and heterotrophy (net community production) in the Arctic is regulated by seasonally variable competition for nitrate between autotrophic and heterotrophic microorganisms. Although nitrate assimilation by bacteria has been observed in other marine environments, its impact on present and future Arctic productivity and carbon flux is potentially unique for several reasons. First, high-latitudes experience long periods of darkness that shut down photosynthesis relative to respiration, likely giving heterotrophs a competitive edge and potentially reducing mineral nitrogen available to the photosynthesizers in the spring. Second, because large amounts of carbon-rich but nitrogen-poor terrestrial dissolved organic matter are delivered to the arctic, microbes are replete with organic carbon, but require other sources of nitrogen for their metabolic needs. Third, the shelf-dominated Arctic Ocean has tight benthic-pelagic coupling and high rates of denitrification but no significant nitrogen fixation, further limiting nitrogen relative to other oceans. Finally, climate change in the Arctic will increase the flow of rivers and the amount of terrestrial carbon contributed to the Arctic Ocean, but the seasonal dark-light cycle will not change. Future projections of increased Arctic productivity depend on a supply of new nitrogen to autotrophs. The coastal Arctic is where the battle for nitrogen will likely be most intense in the future.

The **broader impacts** of this project include 1) broad dissemination of results via scientific literature and public outreach; 2) training and mentoring of new researchers: two graduate and at least six undergraduate students; 3) training and mentoring of high school science students; 4) K12 curriculum development, 5) climate change implications and societal relevance; 6) research important to the lives of Alaskan Inupiat Eskimos; 7) encouragement for participation in research science by underrepresented groups, especially minority undergraduates from the state of Georgia; 8) historical data mining and synthesis; and 9) establishment of new research infrastructure with a coastal monitoring legacy for the local community.

P. Yager (sole PI). OCE 97-53170/98-96334 - POWRE: An Arctic Ocean time-series of dissolved inorganic carbon; $85,000; 10-1-1997 to 9-31-2000. (FSU, transferred to UGA in '98). This project was part of a collaborative effort to evaluate seasonal patterns of biological production and respiration in the central Arctic Ocean. The grant provided instrumentation to measure dissolved inorganic carbon (DIC) and alkalinity, the opportunity to collect weekly depth profiles and experimental samples from the yearlong SHEBA field station (October 1997-1998) in the Canadian Basin, and support for simultaneous springtime measurements "upstream" in the Chukchi Sea, where a suite of biogeochemical and microbial inventories and rates were measured (data are available from the CDIAC database). Outcomes include: 9 papers presented at national meetings, 5 invited seminars given, 3 peer-reviewed papers published (*Yager et al. 2001*; *Miller et al. 2002*; *Connelly et al. 2006*), and 4 female students (2 M.S., 2 Ph.D.) partially supported or trained (one gave a research presentation to the Barrow Arctic Science Consortium in 2001) Additionally, 8 undergraduates participated in sample analysis including 1 African-American and 1 Hispanic female, and 2 African-American women participated in the UGA Summer Undergraduate Research Program.

D. Bronk OPP-9530732–New and regenerated production in the Southern Ocean: Ross Sea Studies (USJGOFS); $84,404; 7-1-1996 to 6-30-1999. B. Cochlan (PI, SFSU) and D. Bronk (Co-PI, UGA). The broad continental shelf of the Ross Sea is characterized by relatively high biomass with large phytoplankton blooms in the austral spring-summer. The objectives of this study were to obtain quantitative estimates of plankton nitrogen (N) nutrition and to understand the factors that control the magnitude and variability of primary production and vertical flux from the euphotic zone. Four cruises were taken to the Ross Sea as part of this project. Outcomes include: 5 papers presented at national meetings, and 3 peer-reviewed publications (*Cochlan & Bronk 2001*; *2003*; *Cochlan et al. 2002*), and 1 undergraduate senior thesis. **Additionally, OCE-0752490–Combining flow cytometry and stable isotope techniques: A method to measure phytoplankton- and bacteria-specific nitrogen and carbon uptake; $232,237; 5-2008 to 4-2011. D. Bronk (PI, VIMS) and M. Lomas (co-PI, BIOS).** This grant is funding the refinement and application of the flow cytometry sorting method to quantify N and carbon uptake rates by phytoplankton and bacteria. Two manuscripts have been submitted (*Bradley & Bronk submitted*; *Bradley et al. submitted*; available on Bronk's webpage.

M.E. Frischer (Co-PI). OPP-00-83381 - Biocomplexity: Bio-feedback basis of self organization in planktonic ecosystems using *Phaeocystis* as a model complex adaptive ecosystem; $2,300,000; 12-1-2000 to 12-1-2005; PIs: P.G. Verity, M.E. Frischer, M.E. Hay, B.C. Patten. Based on the premise that *Phaeocystis* is a model organism for the study of biocomplexity we asked: how do chemical, biological, and self-organizational mechanisms interact with life-cycle transformations of *Phaeocystis* to mediate ecosystem patterns of trophic structure, biodiversity, and energy flow? Research was focused on several aspects that project modeling activities indicated were critical gate valves in the ecology of *Phaeocystis* ecosystems: life cycle transformations, grazing, viruses, *in situ* assessment, development of molecular tools (including many of the gene-specific assays to be used in the proposed project, see marked (*) references in citation section), and the initial stages in colony formation. Project results show that *Phaeocystis* is so ecologically successful because it has a unique set of behavioral and physiological capabilities: gelatinous morphology, heteromorphic life cycle, size changes, and allelochemistry. Outcomes include: 46 papers presented at national and international meetings, >40 published manuscripts, several submitted or in review, 4 postdoctoral students, 2 Ph.D. students graduated, 1 M.S. student in development, and numerous undergraduate interns trained. Most importantly relative to the proposed project, the influence of the type and ratio of available nutrients was identified as a critical data gap of relevance towards predicting the response of Arctic coastal pelagic food webs to changing climate and thus serves to motivate the proposed research.

DOES COMPETITION FOR NITROGEN BETWEEN AUTOTROPHS AND HETEROTROPHS CONTROL CARBON FLUXES IN THE WESTERN COASTAL ARCTIC?

I. Introduction

The Arctic is changing (*ACIA 2005; IPCC 2007*). Clear trends reveal an air temperature increase with consequent reductions in the volume and extent of sea ice (*Richter-Menge et al. 2006*). Associated changes in albedo result in a positive feedback on warming trends, suggesting these changes will accelerate. Transformations on land impact coastal hydrology and biogeochemistry through increased river discharge, rising sea level, thawing of permafrost, and coastal erosion. These physical changes are expected to impact marine biota and biogeochemistry through changes in the timing and magnitude of production cycles, shifts in species distributions, risk to marine species dependent on sea-ice, and increased UV exposure. For the Arctic continental shelf in particular (~30% of the Arctic Ocean), ecosystem shifts could be considerable if changes lead to increased water-column production and reduced benthic production (e.g. *Grebmeier et al. 2006*). Such a restructuring will ultimately alter the pathways and magnitude of energy transfer to fish, sea birds and marine mammals, and impact the people dependant on those resources. Our challenge today is to transform such generalizations with a mechanistic understanding of specific ecosystems and their components to assess future change.

One important component lacking detailed understanding is the role of microorganisms in Arctic coastal ecosystems. Microbial communities, including phytoplankton, protists, prokaryotes (bacteria and Archaea, herein referred to as bacteria), fungi and viruses are the most abundant and taxonomically and genetically diverse group of organisms in the biosphere. Microbes dominate the biological biomass, production, and remineralization in marine systems, while large organisms and upper trophic levels primarily respond to rather than set the level of productivity. Microbes are also the major producers and consumers of CO_2 and other greenhouse gases. The Chukchi and Beaufort continental shelves act as major carbon (C) pumps to the deep Arctic Ocean, with the highest net community production occurring near Barrow, Alaska (1-3 gC m^{-2} d^{-1}; *Bates et al. 2005, Bates 2006*) and microbiota are major contributors (*Kaltin & Anderson, 2005*). Microbes can also be important sentinels of environmental change, because alterations in the structure and biomass of microbial communities can herald changes in pathways of nutrient and energy transfer in food webs. The role of microbes in the Arctic and other perennially cold oceans has been debated since Sorokin first proposed inhibition of microbial activity by low temperature (see review by *Karl 1993*). More recent study, however, has shown that extreme environments harbor unique, highly adapted microorganisms. Though cold-loving (psychrophilic) bacteria have been actively studied for more than 70 years, many questions about marine microorganisms in the Arctic remain unanswered – a serious impediment to predicting the impact of climate change on Arctic food webs and biogeochemical cycles.

In "Frontiers in Polar Biology in the Genomic Era" (*NRC 2003*), the National Academy of Science's Polar Research Board reported that key questions on polar ecosystem biology could be addressed by genomics and other new technologies. Two of these questions are: 1) what types of microorganisms are present in polar aquatic ecosystems and what roles do they play in ecosystem processes? and 2) what is the relationship between the composition and biogeochemical function of polar microbial communities? Creative use of new molecular approaches, such as we propose here, is expected to help make progress on these fronts. The Arctic Natural Sciences program supports projects that "emphasize understanding the adaptation of organisms to the unique aspects of the Arctic environment" and "ocean science projects that advance knowledge of the processes of the Arctic Ocean and adjacent seas and their interactions with their boundaries." In this proposal we aim

to achieve both goals using a hypothesis-driven fundamental research approach at a time in history when improved mechanistic understanding is critical to projections of future change.

II. Objectives and Hypotheses

The objective of this project is ***to investigate the microbial controls on the productivity of a coastal Arctic ecosystem by focusing on the competition between autotrophs and heterotrophs for nitrogen (N)***. In the winter and summer waters near Barrow, Alaska, supplemented with complementary international research opportunities, we propose to measure *in situ* concentrations of key microbial and biogeochemical constituents, relevant uptake activities, and expression of key N cycling genes to address the following hypotheses:

H1: The balance of autotrophy and heterotrophy in the Arctic is regulated both temporally and spatially by nitrate (NO_3^-) and light.

- Phytoplankton outcompete heterotrophic bacteria for NO_3^- during the well-lit spring and summer conditions.
- Heterotrophic bacterial uptake of NO_3^- will be greater during the dark winter and under sea ice than in open water or well-lit summer conditions when bacteria are replete with phytoplankton-derived DON.
- NO_3^-, combined with terrestrial DOC derived from riverine and groundwater flow, may provide the means for bacterial growth and respiration during the dark winter.

H2: Microbial community structure will vary according to the seasonal light cycle and the sources of available N. The observed lag in bacterial response to phytoplankton growth is due to a community shift.

- Bacterial community composition will vary significantly between winter and summer.
- The composition and dynamics of bacterial communities will correspond to concentrations and sources of N.
- The expression of key N-cycling genes, N-uptake and regeneration, and DON uptake kinetics will vary with season and community composition.

Although NO_3^- assimilation by bacteria is observed in other marine environments (*Kirchman & Wheeler 1998; Kirchman 2000; Allen et al. 2005*), its impact on present and future Arctic productivity is potentially unique for several reasons. First, the high-latitudes experience long periods of darkness that shut down photosynthesis relative to respiration. Thus, the microbial heterotrophs have a significant opportunity to impact NO_3^- levels without competition from autotrophs during a large part of the year. Second, unlike the Antarctic, the Arctic receives more than twice as much riverine input than any other ocean (~10% of the global discharge to ~5% of the global area and ~1.5% of global ocean volume; *Aagard & Carmack 1989*), which delivers large amounts of terrestrial DOM derived from the tundra that is rich in C but relatively low in N (*Amon 2004*). Thus, unlike the Antarctic, the coastal microbes in the Arctic are replete with dissolved organic C (DOC), but require other sources of N for their metabolic needs. Third, the Arctic Ocean is dominated by continental shelves (25% of the worlds shelf area in 5% of the world ocean). Tight benthic-pelagic coupling is common, denitrification rates are high (*Devol et al. 1997*), and N fixation appears to be insignificant (*Codispoti et al. 2001; 2005*). Thus, relative to other coastal oceans, N is particularly limiting during the summer (*Codispoti et al. 2005*). Finally, climate change in the Arctic is expected to increase the flow of rivers (*Peterson et al. 2003*) and the amount of terrestrial DOC contributed to the Arctic Ocean (*Benner et al. 2004; 2005*), but the seasonal dark-light cycle will not change. Future projections of increased Arctic productivity (e.g. *Walsh et al. 2004*) depend on a supply of N to the autotrophs. The coastal Arctic is where the battle for N will likely be most intense in the future.

Response to reviewers. The 2007 ANS panel was supportive of the project and encouraged resubmission after addressing a few specific issues. Reviewers and Panel recommended that we 1) focus measurements to better address specific questions and improve our quantitative assessments of the data, 2) justify our description of the coastal Arctic as N-limited, 3) better justify selection of our field site, and 4) clarify our work plan. In response, we have focused our experimental design, added some data from the region, include more information about our field site and included additional sampling opportunities, and strengthened links to modeling efforts. The objective of the project is not to fully describe the seasonal succession of N-cycling, but to test specific hypotheses concerning N utilization by Arctic autotrophic and heterotrophic microorganisms.

III. Scientific background

The balance between autotrophy and heterotrophy. Heterotrophic microorganisms in low latitude systems play essential roles in the marine food web (*Pomeroy 1974, Azam et al. 1983*) as: 1) decomposers of non-living organic matter and thus nutrient regenerators for the traditional herbivorous food web; 2) transport vectors linking "unavailable" detritus and DOC to higher trophic levels; and 3) the primary contributors to respiration and metabolism in seawater (*Williams et al. 1981*). Heterotrophic prokaryotes in these systems consume roughly 50% of primary production and are largely responsible for the retention of C and other elements in the upper surface layer. Because of this retention and recycling, only a small fraction of primary production escapes mineralization and is available for higher trophic levels or sinks to the benthos. In contrast, the present day coastal Arctic, seems to have a more efficient flux to the benthos (*Peterson & Curtis, 1980*).

What limits bacterial productivity in the Arctic? Biomass of heterotrophic microbes in Arctic surface waters, including bacterioplankton and heterotrophic protists, vary in response to seasonal changes in phytoplankton stocks. For example, in the Chukchi Sea, surface mixed layer concentrations of bacteria start out low in the spring (0.3-0.4 x 10^9 L^{-1}), increase over the course of the bloom (up to 0.9 x 10^9 L^{-1}; *Yager et al. 2001*), and are highest in late summer (*Steward et al. 1996, Hodges et al. 2005*). Depth profiles during the summer generally show high abundance near the surface correlating with high particulate organic C (POC) and N (PON) or chlorophyll *a* (chl *a*) concentrations (*Hodges et al. 2005*), but subsurface peaks are also common offshore *(Lovejoy et al. 2002)* down to 100 m (*Steward et al. 1996*). Although these features are also seen in low-latitude oceans, other field data comparing autotrophic to heterotrophic microbial biomass and activity indicate that polar marine ecosystems may be unique. Bacterial abundance is generally lower for a given concentration of chl *a* (*Karl et al. 1991, Smith et al. 1995, Ducklow & Yager 2007*) when compared to other marine ecosystems (*Cole et al. 1988*). The greatest degree of uncoupling occurs when chl *a* concentrations are maximum (*Karl 1993*). Despite predicted growth limitations, bacterial growth rates in cold regions are similar to those in temperate zones (*Cota et al. 1996, Rivkin et al. 1996, Yager 1996, Rich et al. 1997, Carlson et al. 1998, Ducklow et al. 1998, Rivkin & Anderson 2000, Kirchman et al. 2005*). If growth rates for Arctic microbes are similar to those in low latitude oceans, it raises questions about why prokaryotic biomass appears lower and why activity of heterotrophic prokaryotes may be less able to respond to large blooms of phytoplankton in the Arctic than elsewhere. *We hypothesize that large seasonal changes in the quality of available organic matter accompanied by competition with autotrophs for limited N are responsible.*

Nitrogen cycling in the coastal Arctic. New production is defined as autotrophic production fueled by N originating outside the euphotic zone, including NO_3^- from the deep ocean, N_2-fixation, and riverine and atmospheric inputs, while regenerated production is fueled by local N-sources, NH_4^+, urea, and DON derived from biological processes occurring within the euphotic zone (*Dugdale & Goering 1967*). In the open ocean, export equal to new production occurs mainly

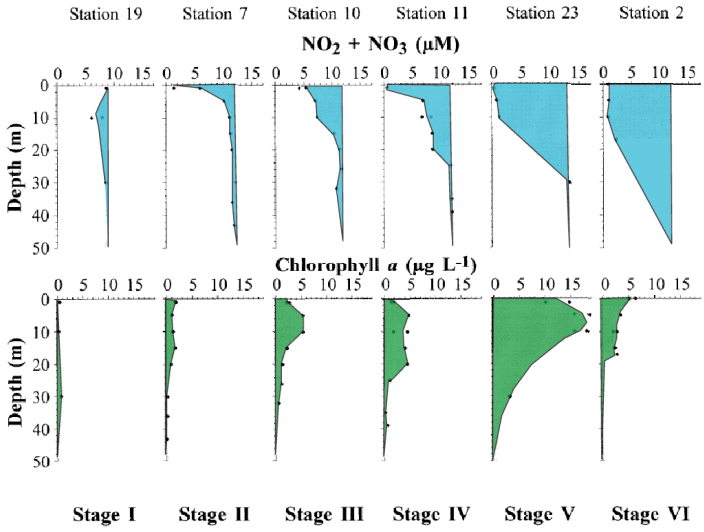

Fig. 1. Surface nitrate + nitrite depletion (top, blue) and chlorophyll a buildup (bottom, green) during a phytoplankton bloom in late May and early June in the Chukchi Sea. From *Yager et al. 2001.*

through the sinking of particles, but other losses to fish catches and seabird guano, for example, are included. On the Arctic continental shelf, which resembles an estuary, new N comes from inflowing rivers and Pacific or Atlantic Ocean waters. In the Chukchi Sea in late May, surface NO_3^- inventories are about 10 µM until the spring bloom builds up enough phytoplankton biomass to deplete it (Fig. 1; *Yager et al., 2001*). By mid-June, surface NO_3^- concentrations are less than 1 µM. New production is available for export to the deep, but more importantly supports the large influx of migratory animals (seabirds, fish, whales, etc.) that feed in the Arctic during the summer months and return south for the winter. Local subsistence hunting and fishing also represent an export.

Traditionally, new production is equated with NO_3^- uptake and regenerated production with NH_4^+ uptake, but this is an oversimplification (e.g. *Bronk et al. 1994*). We now know that nitrification occurs within the lower euphotic zone resulting in 'regenerated' NO_3^- (*Ward 1987, Ward et al. 1989; Eppley et al. 1990*); that rates of N_2 fixation are substantially higher than previously thought, though considered extremely low in low temperature marine environments (*Capone et al. 1997, 1998; Karl et al. 1997; Paerl and Zehr 2000; Zehr et al. 2001*); that atmospheric inputs of N can be large (*Owens et al. 1992; Cornell et al. 1995; Seitzinger & Sanders 1999; Aneja et al. 2001; Valigura et al. 2001*); and that uptake of DON substrates such as urea (e.g. *Lomas et al. 2002*), dissolved free and combined amino acids (DFAA, e.g. *Glibert et al. 1991*; DCAA, e.g. *Jørgensen et al. 1993*), and humics (*See et al. 2006*) often contribute substantially to phytoplankton N nutrition and so can result in underestimates of regenerated production if not quantified (*Antia et al. 1991; Bronk et al. 1994; Lomas et al. 2002*). We also know that heterotrophic bacteria supplement their DON consumption with NH_4^+ *(Wheeler & Kirchman 1986; Kirchman 2000; Rodrigues & Williams 2002)* and NO_3^- *(Kirchman & Wheeler 1998; Kirchman 2000; Allen et al. 2002)*. These findings highlight the complexity of marine processes involved in NO_3^- uptake, requiring thorough study of all N forms to identify sources of production.

Quantifying autotrophic versus heterotrophic nitrogen uptake. Surprisingly, relatively little is known about the interaction between phytoplankton and heterotrophic bacteria when exploiting shared N resources. The relationship between DON and dissolved inorganic N (DIN, NH_4^+ and NO_3^-) consumption by heterotrophic bacteria and its effect on phytoplankton N nutrition are unresolved pieces of the marine N cycle – components that are particularly important in polar regions. Competition between phytoplankton and bacteria for DIN in the Arctic is likely to be intense because of regional N-limitation (*Berger & Naumov 2000; Dale & Prego 2003; Strom et al. 2006; Tremblay et al 2006*) likely peaking in late summer when surface water DIN concentrations are minimal and phytoplankton productivity and biomass is N-limited (*Rysgaard et al. 1999, Codispoti et al. 2005; Dittmar & Kattner 2003*), although this may not be the case trans-Arctic (*Kristiansen et al. 1994*).

Nitrogen fixing bacteria are not important in the Arctic (*Codispoti et al. 2001; 2005*) and the N** values from the Western Arctic shelf and slope are consistently lower than 3 (confirming the dominance of denitrification; *Codispoti et al. 2005*). In the 50-m deep shelf waters offshore from Barrow, Shelf Basin Interactions project data indicates that NO_3^- in the surface waters clearly goes to zero while phosphate and silicate remain (*Codispoti et al. 2005*), confirming that organic N does not alleviate N-limitation. Under such a scenario, increased bacterial DIN use during the winter may exacerbate phytoplankton N limitation in the spring, diminish primary productivity or biomass accumulation (e.g. *Joint et al. 2002*), or exert a selective pressure favoring phytoplankton taxa that can either compete effectively with bacteria for DIN or use available DON (*Kirchman 2000*).

Methods limitations. Though important, separating phytoplankton from bacterial N use is difficult. The most commonly used technique is size-fractionation using filters that target the size difference between the two groups (e.g. *Wheeler & Kirchman 1986; Allen et al. 2002*). Glass fiber filters are typically used for measuring phytoplankton N uptake (i.e. Whatman GF/F filters, 0.7 μm nominal pore size). Numerous studies have shown, however, that GF/F filters retain a variable but significant fraction (generally over 50%) of the bacterial community (e.g. *Lee & Fuhrman 1987; Lee et al. 1995; Gasol & Morán 1999*). In most published work where new production was estimated with ^{15}N, the uptake rates used represented autotrophic and some unknown amount of heterotrophic uptake, skewing calculations for such parameters as *f*-ratios. Alternatively, some researchers have used inhibitors to distinguish between prokaryotic and eukaryotic N assimilation (e.g. *Middelburg & Nieuwenhuize 2000; Veuger et al. 2004; Fouilland et al. 2007*), but these inhibitors vary in effectiveness and specificity (*Oremland & Capone 1988; Lee et al. 1992*). The recent development of molecular techniques that identify the presence and expression of N assimilation genes in various microbial groups is promising *(Allen et al. 2001; Zehr & Ward 2002; Fan et al. 2003)*; however, these results cannot provide estimates of N uptake rates. Thus, none of these approaches can accurately quantify phytoplankton-specific or bacteria-specific N use in marine ecosystems.

A newer approach to quantifying autotroph or heterotroph specific uptake is to physically separate phytoplankton and heterotrophic bacteria by flow-cytometry. Using the sorting capabilities of a flow cytometer, microorganisms of interest can be isolated based on specific cellular properties, such as size or pigment autofluorescence. *Paau et al. (1979)* were the first to separate algal cells from bacteria using such an approach, and others have done similarly in order to quantify primary production (*Li 1994*), bacterial activity (*Servais et al. 1999*), phytoplankton growth rates *(Pel et al. 2004)* and N assimilation (*Lipschultz 1995; Zubkov & Tarran 2005*) on a cellular scale. Recently, we combined the flow cytometric sorting technique with mass spectrometry to separate autotrophic from heterotrophic ^{15}N uptake in Chesapeake Bay (*Bradley 2008; Bradley & Bronk submitted; Bradley et al. submitted*). In these studies, we found that including heterotrophic biomass on the uptake filter resulted in large overestimates of autotrophic uptake depending on the substrate. The ability to separate autotrophic from heterotrophic N uptake is an important ***breakthrough*** in the estimation of new and regenerated production. *[Reviewers expressed concern that the ^{15}N flow cytometry method we propose is unproven. We note that D. Bronk received an NSF grant to apply the new flow cytometer method (see Prior Support). Her collaborator in the development of the method (Mike Lomas) has also recently published a paper using the approach (Casey et al. 2007) and there are two other papers using the method submitted (Bradley and Bronk submitted; Bradley et al. submitted). We will also complement our new method with a traditional size fractionation for comparison.]*

Bacterial nitrogen uptake. In a recent review, bacteria account for 16% of all NO_3^- uptake in marine and freshwaters (*Kirchman 2000*). Using the flow cytometric approach in Chesapeake Bay, we directly measured a significant amount of bacterial NO_3^- uptake (*Bradley & Bronk submitted*).

Data collected by our group from a variety of ocean margin environments, including Arctic continental shelves, support Kirchman's general conclusions (*Allen et al. 2002*). Interestingly, compared to the relative importance of bacterial NO_3^- uptake in lower latitudes, the importance of bacterial DIN uptake in the Arctic appears to be *more significant*, with NO_3^- accounting for a greater fraction of bacterial N demand than NH_4^+. In the Eastern Barents Sea, NO_3^- accounted for a larger fraction of bacterial N demand in 11 out of 15 samples collected along a transect from northern Norway into the ice during a summer cruise (*Allen et al. in prep*). Similar observations were recently reported in Baffin Bay (*Fouilland et al. 2007*) and recent analysis of Shelf Basin Interactions project data from the Chukchi and Beaufort Seas also suggest the importance of NO_3^- to bacteria (*Yager & Kirchman in prep*). Molecular surveys targeting bacterial assimilatory NO_3^- reductase genes (*nas*A/*nar*B) suggest that the genetic potential for bacterial utilization of NO_3^- is ubiquitous with Arctic communities possessing several genetically distinct clades that appear to be rare in other environments (*Allen et al. 2001*); the ecological significance of this observation is unclear. A partial least squares regression analysis between the diversity of nasA genes and a suite of biogeochemical parameters indicated that the concentration of NO_3^- was the best predictor of *nas*A gene diversity (see Fig. 3 in *Allen et al. 2005*). These observations suggest that NO_3^- availability influences the composition of bacterial communities and may determine whether NO_3^- is used by phytoplankton or bacteria. Understanding relative uptake affinities of the two groups is critical for the coastal Arctic.

During the late springtime, heterotrophic bacterial kinetic uptake of DON shifts in response to the algal bloom. During a bloom sequence (Fig. 1), *Yager et al*. (2001) also observed a downshift in affinity for amino acids while at the same time measuring an upshift in maximum uptake velocities. This change from apparent oligotrophic to eutrophic adaptation corresponded with a shift in bacterial community structure (*Yager et al. 2001*), perhaps explaining the lag behind the algal bloom.

Just as heterotrophs have not traditionally been regarded as important utilizers of NO_3^-, autotrophs have not been considered to be important utilizers of DON. Evidence continues to accumulate, however, that suggests DON is an important source of N for phytoplankton, and possibly C as well via mixotrophy (*Caron 2000; Lewitus et al. 1999;* reviewed in *Bronk 2002* and *Bronk et al. 2007*). While much of the DON pool consists of unidentified, refractory compounds, some identified compounds have been shown to play an important role in phytoplankton nutrition. Urea, for example, contributes significantly to total measured N uptake in many areas (reviewed in *Bronk 2002*). In general, urea uptake rates are highest in estuarine waters and lowest in oceanic waters; however, the contribution of urea to total uptake appears highest in coastal waters. The question is ***to what degree urea uptake contributes to autotrophic versus heterotrophic nutrition.*** The answer could have profound impacts on C cycling and flux in the ocean.

IV. Research Approach

Study sites. Because of its unique combination of year-round access to the coastal Arctic Ocean and strong scientific support system (Barrow Arctic Science Consortium; see Hastings letter of support), we propose to make our primary winter and summer measurements from Barrow, Alaska. At 71°N, Barrow receives 24-hour sunlight between May 10 and August 2, and is in 24-h darkness between November 18 and January 24. Less than 1 km from shore, shelf depths exceed 10m, and significantly deeper waters (>100 m) are not far away. Twice each year (January and July) for two years, working from Barrow, we will use either small boat or skidoo to travel offshore to sample seawater. We anticipate having access to surface waters of 10-20 m depth within a mile of

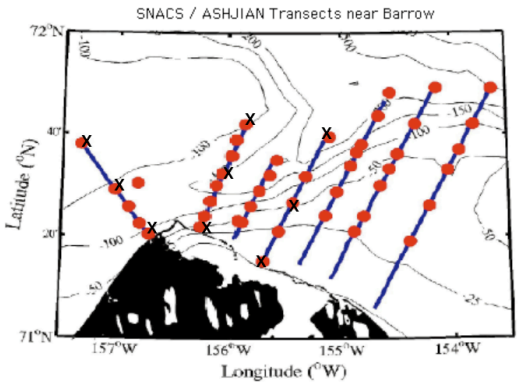

Fig. 2. C. Ashjian and colleagues were recently able to deploy from a small boat a CTD/Rosette system along transects (blue lines with red station markers) reaching from the shallow nearshore region to the shelf break. During our summer fieldwork, we propose to sample select stations along *three* of these same transects (three stations each, labeled with an "X"). Map from *Ashjian et al. 2004*.

the town of Barrow. We plan to sample biological and biogeochemical inventories along *three* offshore transects, with 3-5 depths that sample through the surface mixed layer and into the subsurface layer, accessing both the eastward coastal and the offshore westward currents (*Weingartner 2006*). More extensive rate measurements and incubation studies will be made at selected sites and depths. The rationale for the transects is to sample the microbial community response to the cross-shelf and depth gradients in DIN availability. Nearshore stations will be N-limited throughout the water column in the summer. Offshore stations may have significant NO_3^- below summer stratification. As part of SNACS (Study of the Northern Alaska Coastal System), C. Ashjian and colleagues have recently completed summer research near Barrow, using small (43') boats to investigate environmental controls on zooplankton populations (Fig. 2). They will have nutrient profiles offshore, which will help guide our study. During the summer, we will coordinate with native Inupiat subsistence whalers (Barrow Whaling Captain Association; see Brower letter of support). In the winter, safe travel over the ice by foot or snow machine, as far out as the nearshore lead, will offer access to the ocean using an ice auger. We will not be able to sample far offshore during winter, but gradients will be weaker due to mixing.

Barrow offers a prime coastal Arctic research environment and good opportunity for collaborations. It is the only US Arctic research site with science support staff and facilities during the dark winter. Further, it has a rich history of research. Benthic denitrification rates were measured during both winter and summer at Barrow (Devol *et al.* 1997) and during the spring and summer expeditions of the nearby Shelf Basin Interactions project (Codispoti *et al.* 2005). D. Kirchman and M. Cottrell were funded by NSF to investigate NH_4^+ and methane oxidation by chemoautotrophs in the waters near Barrow. Their results from winter 2008, summer 2008, and winter 2009 will illuminate our measurements. Other SNACS research in the Barrow region by W. Oechel, on interconnections between terrestrial, atmospheric, and ocean systems, and by C.-L. Ping, on coastal erosion, will offer additional context for our measurements. Combined, these data will allow a more complete understanding of the local N cycle.

Fieldwork: Testing H_1: Regulation of net autotrophy and heterotrophy in the Arctic. To address the hypothesis that net community production in the Arctic is regulated temporally and spatially by NO_3^- and light availability, we propose to sample a suite of parameters in the summer and winter that will allow us to constrain nutrients, C inventories, net community production, dark respiration, and N uptake and regeneration rates in shelf waters near Barrow, Alaska. This information will be synthesized in coordination with measures of bacterial and plankton community composition and with genetic diversity and expression of critical bacterial N-cycling genes.

We propose to organize and carry out two 14-day expeditions to Barrow (January and July) during each of two years (2009-2010 and 2010-2011) to determine the activities and relative N uptake of autotrophs and heterotrophic microorganisms during the two seasonal end-members. January is a time of full ice cover and darkness. July experiences full sunlight along with the start of

sea-ice break up and open water. These two seasons allow us to sample the end points of activities for both autotrophs and heterotrophs. During each sampling period we will also measure inorganic nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4, SiO_4), organic N (urea, amino acids, humic-N, DON, PON), C inventories (total dissolved inorganic C, total alkalinity (to help calculate net community production), DOC, humic-C, POC), the composition of microbial heterotrophic and autotrophic communities, and the diversity, abundance and expression of critical bacterial functional N-assimilation genes (assimilatory NO_3^- reductase [*nas*A/*nar*B and urease [*ure*C]). The use of these genes as appropriate, specific, and sensitive indicators of *in situ* bacterial uptake of NO_3^- and urea have been investigated in our laboratories. For example, in two separate studies we compared *nas*A gene expression and NO_3^- uptake rates in mixed natural communities and in culture studies using genetically distinct *nas*A gene containing Arctic bacterial isolates. In both studies a robust relationship between gene expression and uptake rates was observed (Fig 3 – *Added in response to reviewer comments*). Similar studies are underway with *ure*C, and preliminary results suggest that *ure*C gene expression is a good proxy for bacterial urea utilization (not shown). Concurrent with these inventory measures, we will estimate a suite of microbial rate processes including kinetic rates of uptake for NO_3^-, NH_4^+, urea, and amino acids by phytoplankton and bacteria (using both a size fractionation and a flow cytometry approach), net community production, dark respiration, and primary productivity (see method section for details).

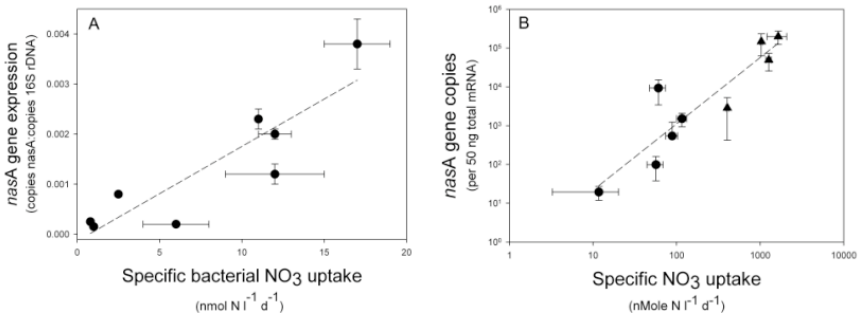

Fig. 3. Relationship between *nas*A gene expression and the specific uptake rate of NO_3^-. for (A) *Marinobacter* spp. in the Skidaway River estuary (r^2 = 0.79). (B) Arctic isolates (r^2 = 0.82). Specific NO_3^-uptake was estimated by ^{15}N tracer studies and gene expression by quantitative RT-PCR.

Testing H_2: Bacterial community structure and gene expression will differ between summer and winter, light availability, and N sources. Microbial community structure may be the key to variability observed in both temperate and cold marine ecosystems. The types of bacteria present in a community can significantly impact local microbial activity and biogeochemical cycling. *Riemann et al.* (2000) used mesocosms to demonstrate that marine bacterial community composition could change dramatically in response to short term (1-2 d) changes in a phytoplankton bloom. This same type of succession may be at work in the Arctic. In the past, phenotypic characterizations of cultivable bacteria were used to describe bacterial community structure and metabolic capabilities. In what is now a textbook (e.g. *Atlas & Bartha 1987*) case for microbial community succession, *Kaneko et al.* (1977; 1979) used this "numerical taxonomy" technique to characterize bacterial types isolated from coastal waters of the Beaufort Sea *near Barrow* (part of the Outer Continental Shelf Environmental Assessment Program). They demonstrated changes in cultivable bacterial phenotypes according to season, geography, and in response to algal blooms. Isolated strains were either psychrophilic or psychrotolerant and tended to differ significantly from those found in other marine environments. While the conclusions that can be drawn about *in situ* communities from cultivable populations are limited (*Ferguson et al. 1984*), predictions can be made regarding a high potential for genetic diversity in the Arctic and about successional responses to strong seasonality. Biogeographic analyses of sub-surface Central Arctic Ocean bacterial communities confirm the potential for high

diversity (*Ferrari & Hollibaugh 1999*). With modern molecular techniques, we will return to the Arctic and test these ideas. We propose to monitor seasonal changes in microbial community structure by using 454 Tag sequencing of coastal water column samples using Bacterial, Archaeal, and Eukaryotic primers and also by identifying active subgroups of the community using microautoradiography and fluorescent in situ hybridization (MAR-FISH; see for example review by Okabe et al., 2004). We expect to find some groups that can be classified according to their activities: winter or summer specialists, NO_3^- or DON specialists, and generalists. We will also monitor expression of NO_3^- assimilation and urea assimilation genes by RT-PCR using the same primer sets used for assessing the diversity of these genes.

Comparative studies in the eastern Arctic. Several additional field opportunities are available via international collaborations that will provide the opportunity to test our hypotheses and broaden the relevancy of observations made in Barrow to other Arctic coastal environments for relatively little added cost. In particular, we will take advantage of new laboratory and field opportunities at Ny Ålesund, Svalbard (78°55'N, 11°56'E') and apply for European Union (EU) funding support through a newly funded Seventh Framework Programme MESOAQUA. The Svalbard site is one of the only other Arctic laboratories in the world that provide logistical support for winter sampling. Specifically, working in cooperation with Frede Thingstad and Jens Nejstgaard at the University of Bergen (see letters of support), we will investigate processes similar to those described above in nearshore waters and in experimental mesocosm enclosures (1 m^3) and compare them to those made at Barrow. In the mesocosms at Svalbard, we will determine the effect of N additions (NO_3 & DON as humics) on the competitive outcomes between heterotrophic bacteria and autotrophs. The Norwegian research group is currently investigating C and phosphorous cycling in microbial communities and the impact of dynamic and changing nutrient stochiometry on Arctic pelagic food webs. Recently, working at Ny Ålesund, *Thingstad et al*. (2008) reported the counter intuitive observation that the addition of labile C reduced phytoplankton activity and biomass and hypothesized that this was due to the competitive advantage of bacteria over phytoplankton for inorganic N and/or other mineral nutrients. Specific investigation of N-cycling processes, however, is beyond the scope of their current research and expertise, and thus they are eager to collaborate with us (see support letters from Nejstgaard and Thingstad). If our proposal to MESOAQUA is successful, they would provide logistical support including travel, laboratory, and marine operations fees including the use of the small research vessel Teisten for up to 4 US scientists working in conjunction with EU partners.

Reviewers wondered about the representative nature of Barrow. If we are funded, we will aim to take advantage of other trans-Arctic sampling opportunities. The Norwegian group (above) has proposed several research cruises in the northern Norwegian Sea to the polar front and they have invited us to participate if they are funded. We have also been invited to collaborate with Andrey Sazhin at the P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences, and participate in his ongoing studies of the winter microbial communities in the White Sea (see Sazhin letter of support). This is an especially unique sampling opportunity since there is so little logistical access to this site for Western researchers. Finally, we have been in contact with a group of benthic scientists working in Kotzebue Sound (led by L. Clough). Logistics there are much more difficult than Barrow, but if both groups are funded, we would try to work together. Kotzebue Sound is interesting because it receives a great deal of terrestrial and riverine input during spring and summer.

Modeling. Of critical interest is how our measurements can elucidate potentially changing community production and remineralization rates in the coastal Arctic. Without a large-scale multi-year field program, the best way to do this is through modeling efforts. The organizing hypothesis of

this project is *that the net community production in Arctic marine systems is controlled by a competition between bacteria and phytoplankton for NO_3^-*. Ultimately, we would like to investigate the idea that warmer temperatures, increased river runoff, and larger ice-free areas associated with climate warming will lead to intensified competition between autotrophs and heterotrophic bacteria for mineral N in the Arctic and thereby alter the annual balance between these processes. To explore this broad hypothesis would require a modeling effort outside the scope of the proposed work plan. However, the data generated in the field and experimental research outlined above will generate crucial rate and process data required for developing realistic models of high latitude coastal ecosystems (*Walsh et al. 2001; 2004*). For example, in our (Frischer & Bronk) ongoing studies of continental shelf N–cycling, numerical modeling efforts suggest that competition for NO_3^- between autotrophs and heterotrophs can have a profound influence on CO_2 and POC export to the atmosphere and benthos, respectively (*Frischer et al. in prep*). Insufficient data are available to realistically constrain these models, however, particularly with respect to understanding regulation of heterotrophic and autotrophic activities. Further, there has been much recent interest in resource competition (e.g., Follows et al. 2007) and Yager and her UGA colleague Adrian Burd have been working with a simple model of competition for DON between several bacterial phenotypes that leads to community shifts as resources change. Output from these models will be useful in interpreting measurements, for example in understanding community rates in terms of individual group rates. We envision a future collaborative proposal that would incorporate our data into such models (see letter of support from A. Burd). Our data would also be of specific benefit to modeling efforts through our collaboration with the Norwegian Polar Aquatic Microbial Ecology (PAME) program. The leader of this effort, F. Thingstad, has expressed a strong interest to collaborate with us because their work lacks an N component (see Thingstad letter).

V. Analytical Methods

1. Nutrient and organic inventories (Bronk) will be quantified using methods routinely used in our labs. Concentrations of NH_4^+ and dissolved primary amines will be measured manually (*Grasshoff et al. 1999*), NO_3^-, NO_2^-, SiO_4 and PO_4 will be measured using a Lachat autoanalyzer, DON will be measured using persulfate oxidation (*Valderamma 1981*), DOC will be measured on a Shimadzu TOC-5000, and POC and PON will be measured on the mass spectrometer (below). Humic-N and C will be measured by measuring the TDN and DOC concentration of a sample before and after passage through DAX-8 resin as outlined in *See & Bronk (2005)*.

2. Net community production (Yager). Net community production and dark community respiration will be determined by measuring changes in total DIC following light and dark incubation (e.g. *Carlson et al. 1999*) and by mass balance calculations of DIC and alkalinity in the field (*Bates et al. 2005*). We use a SOMMA/coulometer (*Johnson et al. 1993; DOE 1997; Cooley & Yager 2006*; precision ~0.5 μmol/kg) and an automated open-cell alkalinity titrator (*Cooley & Yager 2006*).

3. Primary productivity (Bronk and Frischer) in field samples will be estimated using ^{13}C-labeled bicarbonate as previously described (*Dauchez et al. 1995, Legendre & Gosselin 1997*). Labeled bicarbonate will be added to all incubations with ^{15}N-labeled NH_4^+ and NO_3^- such that N and C uptake rates will be measured on the same mass spec filter. Estimating primary production by ^{13}C instead of ^{14}C methods will reduce considerably the amount of sample processing at the field site.

4. Nitrogen uptake (Bronk). Duplicate uptake incubations will be performed with inorganic ^{15}N (NH_4^+, NO_3^-, NO_2^-) and organic ^{15}N (urea and an amino acid mixture) tracers following *Cochlan & Bronk (2001)*. Incubation times will vary with season (12 to 24 hours), and all incubations will be done under *in situ* light and temperature. We will use a new flow cytometetry/mass spectrometry approach to estimate N uptake rates of either autotrophic or

heterotrophic cells (*Lipschultz 1995; Casey et al. 2007; Bradley & Bronk submitted*). Following incubation, phytoplankton cells will be sorted based on their chl *a* autofluorescence using a Beckman-Coulter EPICS Altra flow cytometer (VIMS). Cells will be collected into polypropylene tubes and then filtered onto GF/F filters; sort purity will be assessed using FCM re-analysis of sorted samples. To obtain sufficient N mass for analysis 1 to 2 µg N of ammonium sulfate carrier is routinely added to the pelletized filters containing sorted cells. Bacterial uptake of the tracer is calculated by difference between uptake by the whole sample and uptake by the sorted phytoplankton. *A reviewer questioned whether the approach has the sensitivity to measure uptake in the Arctic. We note that we have alraedy successfully used the approach in a Norwegian fjord (Bradley 2008; Bradley et al. in prep). In response to this concern, however, we have added a traditional size fractionation approach to assure that we obtained meaningful data if the flow cytometric approach lacks the required sensitivity.* For the size fractionation approach, uptake will be measured on cells collected on a >0.8 to 5.0µm silver filter (i.e. phytoplankton fraction); the filter used will be based on microscopic analysis of the sample. The filtrate will then be passed through a 0.2µm silver filter (i.e. bacterial fraction; *Allen et al. 2002; Sanderson et al. 2008*). The degree that we achieved a separation between phytoplankton and bacteria will be quantified microscopically. Rates of regeneration of NH_4^+ and NO_3^- will be measured with the solid phase extractions technique (*Dudek et al. 1986*) and the denitrifier method (*Sigman et al. 2001*) respectively; NH_4^+ and NO_3^- uptake rates will be corrected for isotope dilution. All ^{15}N and ^{13}C samples will be run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler in D. Bronk's lab.

5. Composition of phytoplankton communities (FCM: Bronk; Microscopy: Sazhin, see letter of support). We will use flow cytometry to rapidly determine the community composition and abundance of phytoplankton groups in our field studies. Samples will be collected, preserved with 1% paraformaldehyde (*Marie et al. 1999*) and frozen in liquid N until the size and fluorescence characteristics of the particles can be measured flow cytometrically (see Facilities).

6. Composition of bacterial communities (Yager). The richness and phylogenetic clustering of bacterial and archaeal communities will be targeted with high-throughput 454-sequencing techniques (Sogin et al. 2006). Fluorescent in situ hybridization (FISH; Pernthaler et al. 2002) will be used to quantify abundant populations over variable spatial scales after such populations have been identified by 16S rRNA sequencing. We will link activity with bacterial community structure using microautoradiography (following uptake of ^{14}C-labeled DON) in combination with fluorescent in situ hybridization. The MAR-FISH method is well-developed, although it has not been used for kinetic studies with increasing concentrations of radiolabelled substrate to link substrate affinity with specific groups. We will endeavor during Y1 to develop that application as part of this project.

7. Genetic diversity, abundance, and expression of key bacterial N-cycling genes (Frischer). The diversity of *nas*A and *ure*C genes will be estimated by sequence analysis of PCR amplified functional gene fragments using degenerate nested PCR primers sets targeting conserved regions of the *nas*A (*Allen et al. 2001*) and *ure*C gene (*Brofft et al. 2006, Brofft et al. submitted*). Pooled purified DNA samples from each expedition to Barrow (8) and representative collections from Svalbard mesocosms (5), and White Sea (1) will be used to asses the phylogenetic diversity as described above for 16S rDNA clone collections. A total of 14 *nas*A and *ure*C tagged clone libraries will be constructed and sequenced. Although this does not guarantee that the diversity of these genes in Arctic waters will be described fully, preliminary examinations in subtropical and subarctic environments suggest that this level of coverage will allow us to detect meaningful seasonal patterns and geographical differences with respect to these genes (*Allen et al. 2002, Brofft et al. in prep*). PCR

primers and amplification conditions have all been described previously and are routinely accomplished at the Skidaway Institute of Oceanography (*Frischer et al. 2006, Allen et al. 2005, Brofft et al. submitted*). Expression of *nas*A and *ure*C genes will assayed routinely by quantitative RT-PCR using the same primer sets utilized for assessing the diversity of these genes. Gene expression will be calibrated with NO_3^- and urea specific uptake rates, such that the activity of NO_3^- and urea utilizing bacterial communities can be estimated without incubation bias and in more samples then would be possible by the proposed incubation studies alone. We used a similar strategy to quantify population and expression dynamics of these genes in subarctic environments (*Brofft et al. 2006* (*ure*C); *Wafula et a.l 2007* (*nas*A).

8. Data analyses and synthesis. Non-linear curve fits can be used on rate data collected as a function of increasing substrate (DIN or DON) to determine substrate affinities and maximum uptake velocities (Yager and Deming 1999). We will use multivariate statistical methods (e.g. PCA, Factor analysis) to determine relationships between measured variables (Yager has experience with this method in the Amazon plume; *Subramaniam et al. 2008*). MDS or other clustering techniques will be used to identify stations with similar plankton community structure. Clusters can then be compared to principal components of physical and chemical variables to elucidate potential mechanisms (see *Hodges et al. 2005*). While our observations should provide the basis for future time series, we also propose to link our data to any ongoing measurements made at Barrow. Thus, we will collect/synthesize data that could be used to both test models and inform interpretations of the data, leading to better predictive skill for future climate effects.

VI. Overall Project Schedule and Work Plan

We propose four two-week periods of fieldwork in Barrow during the dark winter and light summer periods, starting in January of 2010 and continuing to July of 2011 (see Hastings letter, although we note that he budgeted us for 4 rather than 2 trips each year). If funded, we would also apply for EU support for international travel and logistical support to join the efforts at the MesoAqua site in Svalbard. We would hope to work there during 2010 and 2011.

Data management: By its very nature, the research we propose is interdisciplinary and interdependent. We therefore anticipate that all data collected will be available to all participants immediately following initial quality control and quality assurance processing by individual investigators. We would establish a limited-access website at UGA during the first year to ease the sharing of data between investigators. At the same time we expect, however, that all investigators will respect intellectual ownership of specific hypotheses and lines of scientific inquiry, particularly in cases where students are involved. We expect that all data collected will be posted to public-access scientific databases as required by OPP-NSF within one year of collection.

Science management. Key to the success of this project will be seamless communication and data exchange. 1) *Verbal communication*: We will have monthly conference calls between PIs to keep abreast of all activities. 2) ***Web-based communications****:* Yager will set up a blog at UGA for the project describing activities and allowing questions and discussion between students, PIs, and other interested parties. 3) ***Face to face meetings***: Once each year, the entire group will meet for two days before or after an international meeting (such as AGU) for data presentations, progress reports and project planning. 4) ***Links to other research groups****:* PIs will attend annual discipline meetings. At least 1 PI will attend OCB meetings each year to summarize findings to the ocean carbon community. We will post to SOLAS and IMBER newsletters. The planned research is highly conducive to joint publications, and workshops described above would thus facilitate paper writing and illustrate the scientific process for graduate and undergraduate students. We would also plan to

gather as a group at the 2011 Gordon Research Conference for Polar Marine Science (for which Yager will be the chair). That meeting would provide an excellent opportunity to coordinate with our international colleagues and introduce our students to a high-level scientific exchange.

VII. Education and Outreach Activities

This project would train at least one graduate student and six undergraduate students in field and laboratory methods; we would make a concerted effort to attract women and minorities. In specific, the **Summer Undergraduate Research Program** at UGA recruits minority undergraduates from Georgia to careers in research science; Yager will be an active participant (see Johnson letter). We also plan to interact significantly with the local **Barrow community**. These plans include an introductory presentation (coordinated by BASC) on our plans and interests. We are particularly sensitive to the history of radioactive isotope use near Barrow and would like to make sure that the local community is comfortable with our research. We also plan to communicate with the local subsistence hunter population for two reasons - to coordinate our sampling with them and to learn about the changes going on in their world. The rich oral history of the native Alaskans may offer insight to the long-term changes going on within the marine ecosystem that we would otherwise be unable to assess. We also propose to work with the children of Barrow to develop a monitoring legacy that they can own (see details below). Further, we will offer a Schoolyard Saturday presentation near the beginning and end of the project to relay our results. Finally, we propose to build on an established relationship between Yager and a recent PolarTrec teacher, Ms. Delores Garay from Houston, Texas. They traveled to the Antarctic together in 2007-2008 and have been actively developing climate-change and marine science curriculum for elementary and middle school science students. For this project (see attached letter), Garay will apply for supplemental funds to join us in Barrow, participate in the science, and develop a "bi-polar" component for her ocean and climate change curriculum modules.

VIII. Significance and Broader Impacts

Arguably more than any other ecosystem, the Arctic is responding more quickly and dramatically to planetary warming trends. Although it is generally recognized that ongoing changes will affect Arctic coastal ecosystem processes with consequences ultimately influencing higher marine trophic levels and the people dependant on these resources, a detailed understanding of these processes is lacking. This lack of knowledge is particularly acute when one considers the role and response of microorganisms. Climate change will very likely enhance the competition for N in the coastal Arctic as it reduces sea ice and increases terrestrial organic inputs, without affecting the annual light cycle. These changes will reduce the efficiency of the biological pump and the flux of C to the benthos.

While it is unlikely that we will detect climate-driven ecosystem change within a 3-year study, it is imperative that we first make a start at defining what the ecosystem is doing now by collecting a standardized data set against which comparisons can be made in the future. We also plan to make an effort to look both backward and forward to place our results into the climate change framework. To place our data into a temporal framework, we will include:

1) Retrospective analyses. We propose to investigate past microbial activities in the region by mining historical data sets. In the 1960's Bill and Josephine Boyd made some microbial measurements in the waters near Barrow (e.g. Boyd and Boyd 1963). During the late 1970's and early 1980's, the Minerals Management Service (e.g. Bureau of Land Management) sponsored the Outer Continental Shelf Environmental Assessment Program (OCSEAP) to assess the impact of oil and gas development on the Alaskan outer continental shelf, including the Beaufort Sea. Microbiologists (i.e. Rita Horner, Ron Atlas, Dick Morita, Richard Griffiths) were part of an intense

5-7 year multi-disciplinary investigation. Their data are mostly locked up in the gray literature of quarterly and annual reports. Rita Horner kindly provided us with an extensive bibliography and a short review she wrote in 1986. The OCSEAP documents are available at the University of Alaska Fairbanks library. As part of Dr. Yager's first trip to Barrow, she would include a few days in Fairbanks to access these publications. Efforts will be made to repeat key methodologies where possible for the sake of historical comparison (e.g. comparing numerical taxonomy to sequencing).

2) Nutrient monitoring legacy. Part of our outreach program will involve members of the Barrow community, likely Barrow High School students (see Buckley letter of support), in monitoring their local waters into the future. We have included in our budget a Trilogy Turner Fluorometer with modules designed to easily measure chl *a*, CDOM, phosphate, and silicate concentrations of seawater. We will train teachers and students on nutrient sample collection and instrument operation, collect replicate samples for cross-calibration within our own laboratories, and assist the students with data interpretation. We expect to leave the instrument at Barrow Arctic Science Consortium **(**BASC) and maintain an intellectual connection with the students and the data generated beyond the duration of this proposal. We will also provide advanced nutrient analytical training to several Barrow HS teachers so that they can increase their N analytical capabilities. The objective of this activity is to increase the ability of Barrow citizens to conduct their own monitoring, education, and research programs that will persist well beyond the lifetime of this project. We will invite a minimum of two teachers to our labs and provide training in nutrient, microbiology, and microbiological analytical techniques in a hands-on setting. Funds to support these activities will be requested separately from the NSF "Research Experience for Teachers" supplement opportunity (NSF 07-039).

Summary: The primary objective of this project is to address the hypothesis *that a critical limit on net community production in the Arctic is regulated by the competition between microbial autotrophs and heterotrophs for new N*. Specifically, we will use newly developed techniques to investigate the hypothesis that during dark periods and under the ice, heterotrophic bacteria maximize production and their activity by the assimilation of NO_3^-, thereby reducing the standing stock of mineral N and limiting new autotrophic production when light becomes available. The result of this temporally separated competition limits C fixation, export, sequestration, and transfer to higher trophic levels in Arctic coastal systems. It also affects both autotrophic and heterotrophic community structure, with ramifications extending up the food web. Because ongoing changes in Arctic ecosystems are expected to intensify N-limitation without affecting irradiance, a consequence of climate change will likely be an adjustment to the competitive balance for N, and a resultant change in the structure and production of Arctic coastal systems. The data and insight gained through this project will be critical for the development of predictive climate change models and via our ongoing domestic and international collaborations we will insure that the data produced will be effectively utilized for these purposes. Furthermore, because the competition for NO_3^- and other N species between phytoplankton and bacteria is a fundamental biological process with far reaching implication to both the N and C cycles, insights gained through this project will be globally applicable. This collaborative project will also promote the goals and intentions of the International Polar Year by training new Arctic scientists, establishing long-lasting ties with resident Arctic human communities, building and strengthening international collaborations, and leaving a legacy of data and infrastructure.

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