PROJECT FINAL TECHNICAL REPORT

MOLECULAR APPROACHES FOR *IN SITU* IDENTIFCIATION OF NITRATE UTILIZATION BY MARINE BACTERIA AND PHYTOPLANKTON

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Executive Summary

In response to the US Department of Energy's solicitation for the Biotechnology Investigations – Ocean Margins Program we were awarded three separate projects spanning the period from November 1997 through November 2006. The project was further extended to November 2007 with a no cost extension.

Traditionally, the importance of inorganic nitrogen (N) for the nutrition and growth of marine phytoplankton has been recognized, while inorganic N utilization by bacteria has received less attention. Likewise, organic N has been thought to be important for heterotrophic organisms but not for phytoplankton. However, accumulating evidence suggests that bacteria compete with phytoplankton for nitrate (NO_3^-) and other N species. The consequences of this competition may have a profound effect on the flux of N, and therefore carbon (C), in ocean margins. Because it has been difficult to differentiate between N uptake by heterotrophic bacterioplankton versus autotrophic phytoplankton, the processes that control N utilization, and the consequences of these competitive interactions, have traditionally been difficult to study. Significant bacterial utilization of DIN may have a profound effect on the flux of N and C in the water column because sinks for dissolved N that do not incorporate inorganic C represent mechanisms that reduce the atmospheric CO_2 drawdown via the "biological pump" and limit the flux of POC from the euphotic zone.

The overall scientific objective of this project was to develop a novel set of molecular (genetic)-enabled tools that could be utilized to estimate key ocean margin biogeochemical rates that link the carbon and nitrogen cycles.

It was also a primary objective of this project to develop a meaningful and sustainable relationship between the Skidaway Institute of Oceanography (SkIO) and Savannah State University (SSU) with the long-term goal of enhancing the diversity of the future STEM workforce, particularly the representation of African Americans in the Marine Sciences and Biotechnology. SSU is a Historically Black University with programs in Marine Sciences, Environmental Sciences and Biotechnology. Specific partnership objectives during this project included enhancing curricular offerings in the marine science in both the undergraduate and graduate programs at SSU, the provision of undergraduate research internship opportunities for SSU students, the institutionalization of the SSU/SkIO collaborative responsibility for the new Masters of Marine Science program at SSU and the acquisition of additional research/education funds from state and federal sources. Significant efforts to recruit, retain, and support graduate students within the scientific context of this study were also made.

During the first phase of this project (1997 – 2000) we pursued the hypothesis that the assimilatory nitrate reductase genes from autotrophic cyanobacteria and heterotrophic bacteria have conserved regions with sufficient group-specific divergence to facilitate a molecular, PCR-based approach to identifying the abundance, distribution, and activity of particular nitrate assimilating organisms and groups of organisms. Activities involved developing global sequence databases of *nas*A (heterotrophic bacteria) and *nar*B (cyanobacteria and eukaryote) gene diversity and developing PCR-based techniques to explore the expression of these genes in ocean margin environments. Validation of this approach was accomplished using laboratory

and field incubation studies where both molecular and more classical ^{15}N radiotracer studies were performed simultaneously. In support of the partnership objectives a significant undergraduate research internship program was established between SkIO and SSU and several courses were developed and offered at SSU by BI-OMP investigators.

During the second project phase (2001 – 2003) we focused on the development of *nas*A group-specific primer sets suitable for use in qPCR and qRT-PCR assays. These new assays were utilized in concert with several laboratory and field studies that allowed levels of gene abundance and expression to be directly compared to estimated N-uptake rates by bacteria and phytoplankton. The results of these studies supported the overall hypothesis that real time molecular measurements of *nas*A gene abundance and expression can be used as a proxy for estimated bacterial NO_3 uptake rates. In support of the project partnership goals, the undergraduate research intership program was continued, curriculum development was continued and sustained and a proposal was developed and submitted to the University System of Georgia for a new Masters program in Marine Sciences at SSU.

During the third project phase (2004 – 2007) research efforts focused on identifying ecologically relevant properties of *nas*A gene expression in representative *nas*A-containing bacteria representative of the genetic diversity of this gene. The results of these studies indicated that phylogenetic variation in the *nas*A gene sequence is not correlated with overall NO_3 uptake rates and thus it is not possible to extrapolate from diversity studies the potential for bacterial NO_3 assimilation. Efforts also focused on the conceptual integration of bacterial NO_3^- utilization patterns into Ocean Carbon cycling Models (OCMs). The results of these preliminary modeling studies confirmed the hypothesis that bacterial competition with phytoplankton for NO_3 may have profound impacts on global carbon cycling. In support of research/education partnership goals several significant breakthroughs occurred. The SSU Masters program in Marine Sciences was approved by the University of Georgia System and the program was initiated in 2004. The first class began to graduate in 2007. Additional funding to support the SkIO/SSU partnership was acquired from the US National Science Foundation and the US Department of Education. Although no new state funds were approved, the success at a national level of the SSU Masters Program precipitated the administration of SSU to designate the program as its flagship graduate program.

Science Objectives

The overall scientific objective of this project was to develop a novel set of molecular (genetic)-enabled tools that could be utilized to estimate key ocean margin biogeochemical rates that link the carbon and nitrogen cycles. Specific science objectives during the final project period focused largely on I) the completion of analysis and synthesis of the efforts of the previous two project years, II) the initiation of experimental studies utilizing bacterial isolates with genetically distinct sequences of *nas*A genes to determine the ecologically significance, if any, of genetic diversity of *nas*A containing bacterial communities, and III) investigations of the diversity of *ure*C genes associated with coastal and estuarine bacterial communities. A fourth objective was the continuation of the development of molecular (nucleic acid) and flow cytometry-based technology for field (ocean) application.

Partnership Objectives

Specific partnership objectives during this project included enhancing curricular offerings in the marine science in both the undergraduate and graduate programs at SSU, the provision of undergraduate research internship opportunities for SSU students, the institutionalization of the SSU/SkIO collaborative responsibility for the new masters of marine science program at SSU, and the acquisition of additional research/education funds from state and federal sources. Significant efforts to recruit, retain, and support graduate students within the scientific context of this study were also made.

I. Summary of Research Objectives, Findings and Implications.

Diversity and Ecological Significance of the *nas*A gene (Phase I – 1997-2000).

During the first phase of this project we pursued the hypothesis that the assimilatory nitrate reductase genes from autotrophic cyanobacteria and heterotrophic bacteria have conserved regions with sufficient group-specific divergence to facilitate a molecular, PCR-based approach to identifying the abundance, distribution, and activity of particular nitrate assimilating organisms and groups of organisms. Activities involved developing global sequence databases of *nas*A (heterotrophic bacteria) and *nar*B (cyanobacteria and eukaryote) gene diversity and developing PCR-based techniques to explore the expression of these genes in ocean margin environments. Validation of this approach was accomplished using laboratory and field incubation studies where both molecular and more classical ^{15}N radiotracer studies were performed simultaneously.

During this project phase we expanded our knowledge of *nas*A gene diversity from one sequence to over 500 (Figure 1). PCR primer sets were developed that enabled the specific amplification of an ~800 bp fragment of the heterotrophic bacteria assimilatory nitrate reductase gene (*nas*A) that was uniquely differentiated from the cyanobacteria and eukaryotic assimilatory nitrate reductase gene (*nar*B).

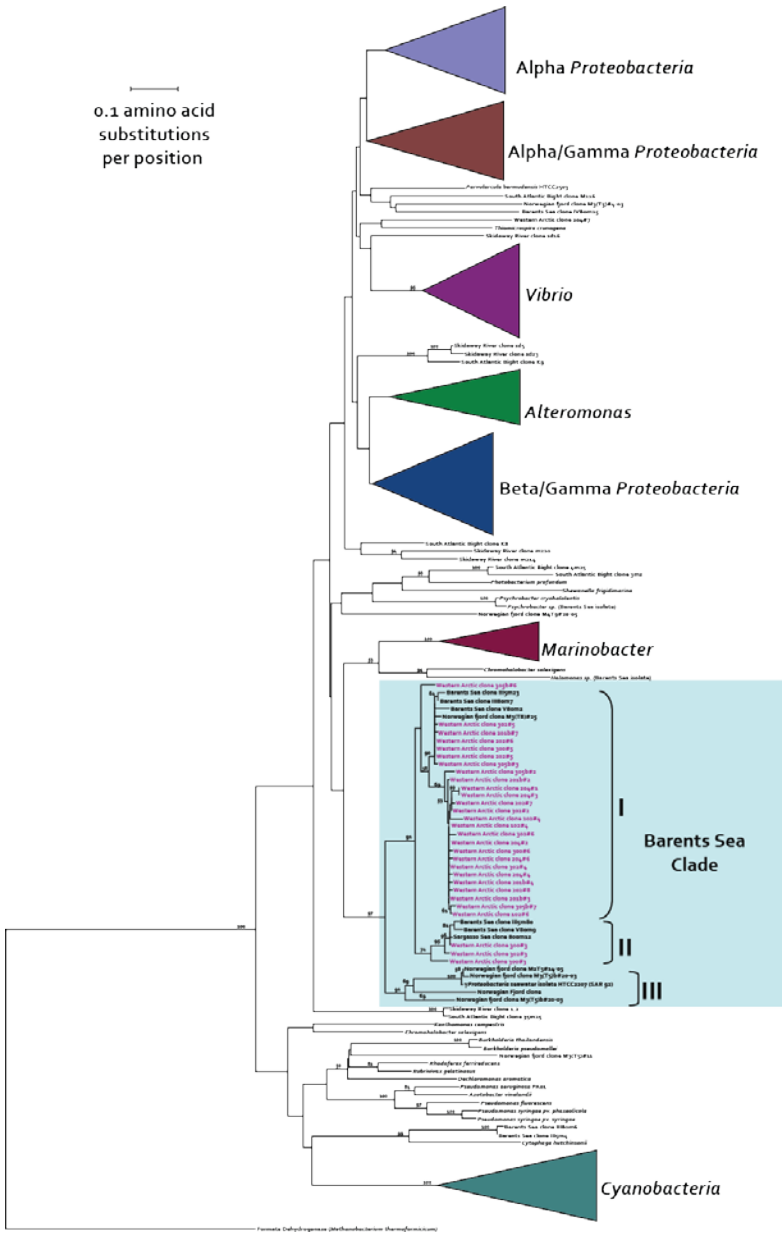

Figure 1. Global phylogenetic diversity of the bacterial assimilatory nitrate reductase gene (*nas*A). Phylogenetic tree constructed from over 500 *nas*A sequences collected from ocean margin regions around world. Phylogenetic reconstructions based on inferred amino acid sequences. Globally, the most commonly retrieved *nas*A sequences belong to the Barents Sea Clade for which we have no cultured representatives.

These studies indicate that the ability of heterotrophic bacteria to utilize nitrate is ubiquitous in the world's ocean margins (Figure 2). Thus, there exists the potential for direct competition between autotrophic phytoplankton and heterotrophic bacteria for nitrate. Because nitrate limits net primary production in most oceanic regions, the competition between heterotrophs and autotrophs for nitrate has profound implications for global carbon cycling.

Figure 2. Global distribution of *nas*A gene and gene transcripts in the world's oceans. *nas*A genes and gene transcripts were detected in all locations where they were investigated.

Merging Molecular and Biogeochemical Measurements (Phase II – 2001-2003).

During the second project phase we focused on the development of *nas*A group-specific primer sets suitable for use in qPCR and qRT-PCR assays. These new assays were utilized in concert with several laboratory and field studies that allowed levels of gene abundance and expression to be directly compared to estimated N-uptake rates by bacteria and phytoplankton.

Group specific primer sets for three major clades of *nas*A containing bacteria were designed, developed, validated and utilized in several sets of laboratory and field studies. Culture-based studies were conducted to investigate the regulation of *nas*A gene expression. In these studies it was observed that some groups of *nas*A-containing bacteria could regulate gene expression in response to the availability of more reduced species of N while others constitutively expressed the *nas*A gene (Figure 3). Field studies in subtropical estuaries, the South Atlantic Bight, the mid-Atlantic Bight, the North Atlantic Ocean and the Barents Sea revealed a high correlation between *nas*A gene abundance, expression and NO_3^- uptake rates estimated from ^{15}N tracer studies (Figure 4).

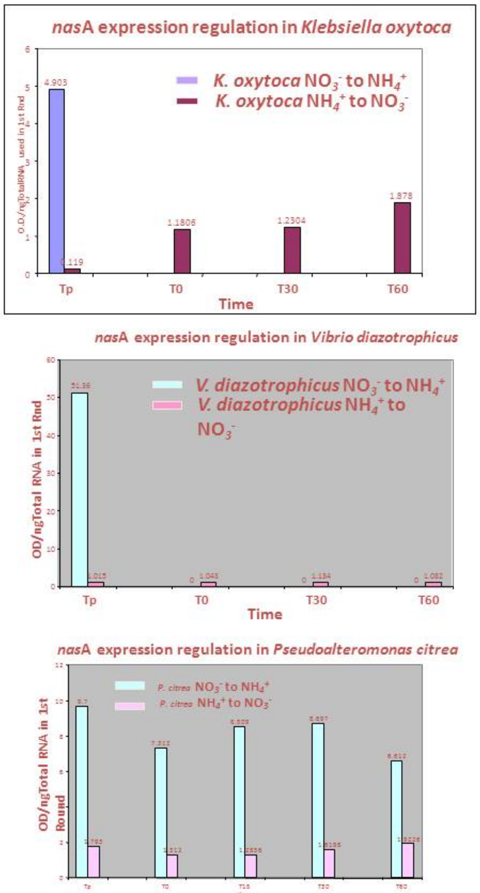

Figure 3. Regulation of the *nas*A gene in 3 different *nas*A containing bacteria. Cultures were either grown in media containing NO_3 or NH_4 and then washed and switched to media containing the alternate N source. *K. oxytoca* exhibited a classical regulation pattern with the *nas*A gene expressed in the presence of NO_3 and repressed when NH_4 was present. Alternatively, *V. diazotrophicus* expressed the *nas*A gene when NO_3 was present but removal of NH_4 did not result in activated expression repressed cultures. Alternatively, *nas*A was constitutively expressed in *P. citrea*

These studies confirm the ubiquity of heterotrophic bacteria with the ability to utilize NO_3^- as a primary source of N and support the hypothesis that biogeochemical rates of NO_3^- utilization by heterotrophic bacteria can be estimated based on molecular measurements of *nas*A gene expression and gene abundance. Further, these studies suggest that in especially in regions where bacterial are not carbon limited, nitrate uptake can be significant. However, studies conducted in large (11 m^3) mesocosms in a Norwegian fjord that simulated a spring phytoplankton bloom, indicated that phytoplankton were always able to outcompete heterotrophic bacteria for NO_3^-.

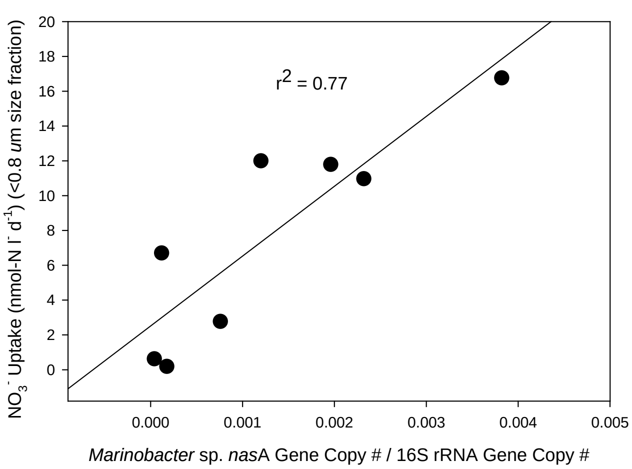

Figure 4. Relationship between NO_3 uptake and normalized *nas*A gene abundance in bacterial communities from the South Atlantic Bight.

Ecological Significance of *nas*A Gene Diversity and Incorporation in Basin Scale Ocean Carbon Cycle Models (Phase III – 2004-2007).

During the final project phase efforts focused on identifying ecologically relevant properties of *nas*A gene expression in representative *nas*A-containing bacteria representative of the genetic diversity of this gene. Efforts also focused on the conceptual integration of bacterial NO_3^- utilization patterns into Ocean Carbon cycling Models (OCMs).

To explore the ecological significance of *nas*A gene diversity, laboratory-based studies utilizing axenic cultures of *nas*A containing bacteria examined growth-rate, yields and *nas*A gene expression under a variety of growth conditions. These studies revealed considerable variability associated with these parameters. However, this variability seemed to be independent of the variability of the *nas*A gene sequence. Disappointedly, this finding suggests that it will be difficult to predict the response of bacterial nitrate utilization based on the composition of *nas*A assemblage of a bacterial community alone.

To extrapolate the conceptual findings of this study to the global scale, we integrated the ability of heterotrophic bacteria to compete with phytoplankton for nitrate. Although insufficient data on many rate processes made it difficult to realistically implement the bacterial/phytoplankton competitive dynamic, *in silico* experiments suggest profound implications of such a competition. For example, shunting 10% of available nitrate from phytoplankton to heterotrophic bacteria resulted in significant reduction in the ability of the ocean to drawdown CO_2 and export carbon out of the mixed layer (Figure 5).

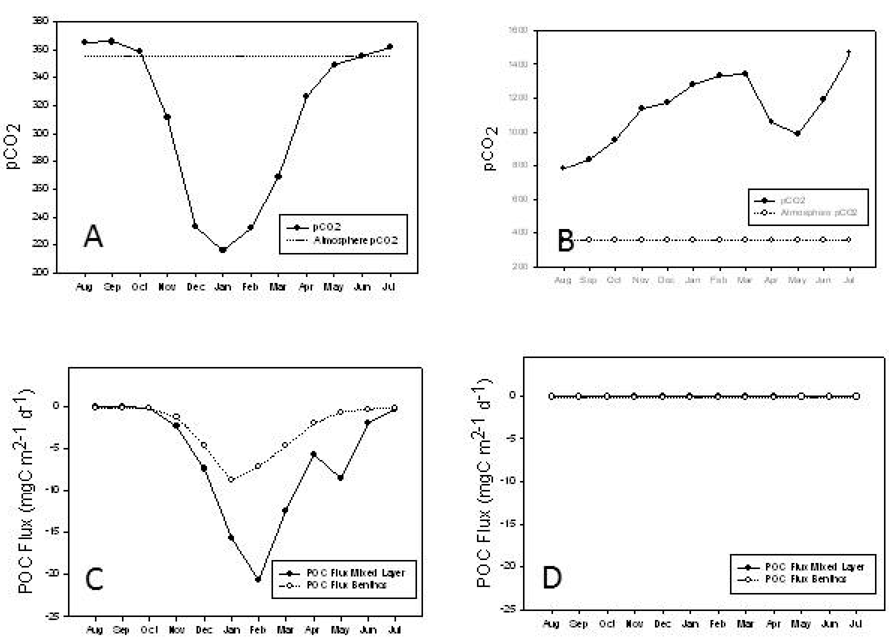

Figure 5. Model outputs of pCO_2 and POC flux when bacteria are not allowed to utilize NO_3 for growth (A & C) and w2hen 10% of NO_3 is shunted to stimulate bacterial growth (B & D). Simulations were run using a Basin Scale OCM model for the Southern Ocean (after Walsh et al 2001).

This same model treatment also suggested that production at the top of the food web could be significantly impacted. However, it should be noted that significantly more information and experimental studies are required to confirm these findings.

II. Summary of Partnership Activities

An integral priority of the DOE BI-OMP program was to foster research/education-based partnerships between research institutions and minority serving institutions. Accordingly, this program involved a substantial partnership between the Skidaway Institute of Oceanography and Savannah State University (SSU). Savannah State University is a Historically Black University (HBCU) with both undergraduate and graduate programs in Marine Sciences. The BI-OMP program supported these programs and contributed to the development of a new Masters program in Marine Sciences at SSU. The other research partner institutions including the University of Georgia (UGA), Rensselaer Polytechnic Institute (RPI) (project phase I) and the Virginia Institute of Marine Sciences (VIMS) (Phase II & III) supported this partnership. Additionally, during the final phase of the project Dr. Melissa Booth who had been a postdoctoral researcher with a joint appointment at SkIO and SSU accepted a faculty position at Roanoke College (RC). At Roanoke, Dr. Booth continued both her research and education engagement in the project during the final 3 years of the project.

The partnership with SSU involved three primary components: 1) Undergraduate and graduate support and mentorship, 2) Curricular enhancement and 3) Development of a joint graduate program (masters level) in marine science at SSU. The highlights and accomplishments of these project components are summarized below.

Student Support and Mentorship

Over the course of this BI-OMP project 24 undergraduate students were either directly or indirectly were supported and mentored through the BI-OMP program. The majority of these students were African American and have, since the completion of the program, matriculated from SSU and pursued careers in STEM areas. Each student research internship was awarded on a competitive basis. Award competitions considered academic achievement and letters of recommendation but awards were heavily weighted to students who expressed and could demonstrate real motivation to be involved. These less tangible attributes were evaluated based on an essay prepared by the student, letters or recommendation and mentor evaluations after a day-long orientation program held in association with each round of application. A critical component of the research mentorship program involved a careful mentor/student matching process.

In addition to the undergraduate research opportunities afforded by this project, this program supported 6 Masters students, 4 Doctoral students and 2 Postdoctoral fellows.

Curriculum Enhancement at SSU

A primary goal of this BI OMP project was the enhancement of the curriculum in Marine Sciences at Savannah State University. To enhance the SSU program in Marine Sciences BI-OMP investigators at SkIO developed and taught courses at SSU. Over the course of the BI-OMP program 4 courses were developed and offered at SSU by BI-OMP investigators. Courses included: Coastal Oceanography (MSCI 5202), Marine Microbial Ecology (MSCI 7801), Analytical Techniques in Seawater, Soils, and Sediment (MSCI 6550), and Current Issues in Oceanography (MSCI 4501/6501). In addition, specialized courses were offered in Molecular Ecology and Bioinformatics as Directed Research courses to support the research training of graduate students directly involved in BI-OMP research.

Coastal Oceanography (MSCI 5202) is a 4 credit team-taught introductory graduate course that serves as part of the core graduate curriculum of the joint SSU-SkIO masters program in Marine Science at Savannah State University. This program was developed jointly by SSU and SkIO faculty and began formally in 2004 with approval from the USG Board of Regents. In addition to DOE support, funding from NSF through the CIRE (Collaboration to Integrate Research and Education) program, and the Department of Education Title III (Part F) program to strengthen minority-serving institutions. No funding for this initiative was provided by the University System of Georgia. MSCI 5202 was first taught by SkIO faculty in Fall 2004, every following spring thereafter until 2009, and again in the Fall of 2010 - 2012. Class sizes have ranged from 2-13 students per semester. Verity was the course lead from 2004-2009 and Frischer assumed leadership from 2010 until the present. The current structure of the course involves 4 modules taught by Blanton, Sanders or Brandes, Alexander, and Frischer with various guest lectures by other SkIO faculty throughout the semester. Most SkIO faculty have contributed at some level to this course over its history.

Marine Microbial Ecology (MSCI 7802) is a senior graduate level 3 credit seminar format course. The course has been offered twice by Frischer, first in the Fall of 2003 (6 students) and again in the Fall of 2005 (8 students). This course served specifically to support the BI-OMP research goals and thereby, in addition to enhancing curricular offerings at SSU enhanced BI-OMP- specific research activities.

Analytical Techniques in Seawater, Soils, and Sediment (MSCI 6550). This is a senior level 3 credit methods course. The course has been offered twice by K. Maruya. First in the Fall of 2004 (8 students) and again in the Fall of 2005 (3 students).

Current Issues in Oceanography (MSCI 4501/6501). This was a capstone undergraduate course cross listed as a graduate course. The course was conceived by Frischer and Verity as a component of the DOE BI-OMP program and developed by project postdocs Melissa Booth and Jennifer Brofft (Bailey). The course was offered during the Fall semesters 1999-2006 when the BI-OMP program concluded. The course fulfilled one of the specific objectives of the BI-OMP program, and resulted in one publication (Gilligan et al 2007 Journal of Geosciences Education 55: 531-540).

In addition to new BI-OMP sponsored courses at SSU, during the third project phase Dr. Booth developed and offered an undergraduate course in environmental microbiology which emphases marine systems and the incorporation of molecular ecology.

Collaborative SSU/SkIO Masters Program

A long term objective of this BI OMP program was to support the establishment of a collaboratively administrated and taught Masters degree program in Marine Sciences at SSU. The establishment of this program is viewed as the primary mechanism that will allow the continuation of the collaborative relationship between SSU and SkIO beyond specific DOE funding. During the second phase of this project a proposal for the new degree program was developed and received approval from the University System of Georgia. The program accepted its first students in the Fall of 2002 and began graduating students in 2004. The program is having a significant impact on diversifying the marine sciences workforce. For example, between 2004 – 2007 a total of 35 MS degrees were granted. On a national basis, 33% of all masters degrees in marine sciences earned by African Americans were by students in the SSU/SkIO program. SSU MS graduates also have been retained at a relatively high rate. Since its inception 20% of SSU MS graduates have continued in PhD programs and 47% of the graduates are employed in STEM areas.

III. OUTPUT

Publications

1. Bradley, P.B., D.A. Bronk, M.E. Frischer, J.E. Brofft, J.H. Braxton, M.G. Booth, M.P. Sanderson, and L.J. Kerkhof. (2010). Inorganic and organic nitrogen uptake by phytoplankton and heterotrophic bacteria in the stratified Mid-Atlantic Bight. *Estuarine, Coastal and Shelf Science.* 88: 429-441.

2. Bradley, P.B., M.P. Sanderson, J.C. Nejstgaard, A.F. Sazhin, M.E. Frischer, L.M. Killberg-Thoreson, P.G. Verity, L. Campbell, and D.A. Bronk. (2010). Nitrogen uptake by phytoplankton and bacteria during an induced Phaeocystis pouchetii bloom, measured using size fractionation and flow cytometeric sorting. *Aquatic Microbial Ecology*. 61: 89-104.

3. Bradley, P. B., M. Lomas, and D. A. Bronk. (2010). Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay measured using a flow cytometric approach. *Estuaries and Coasts*. 33: 971-984.

4. Sanderson, M.P., D.A. Bronk, J.C. Nejstgaard, P.G. Verity, A.F. Sazhin, and M.E. Frischer. (2008). Phytoplankton and bacterial uptake of inorganic and organic nitrogen during an induced bloom of *Phaeocystis pouchetii*. Aquatic Microbial Ecology. 51: 153-168.

5. Gilligan, M.R., P.G. Verity, C.B. Cook, S.B. Cook, M.G. Booth, and M.E. Frischer. (2007). Building a diverse and innovative ocean workforce through collaboration and partnerships that integrate research and education: HBCUs and marine laboratories. *Journal of Geosciences Education*. 55: 531-540.

6. Jacobsen, A., A. Larsen, J. Martinez-Martinez, M.E. Frischer, P.G. Verity. (2007). Susceptibility of colonies and colonial cells of Phaeocystis pouchetii (Haptophyta) to viral infection. *Aquatic Microbial Ecology*. 48: 105-112.

7. Larsen, J.B., M.E. Frischer, K.W. Ockelmann, L.J. Rasmussen, and B.W. Hansen (2007). Temporal occurrence of planktotrophic bivalve larvae identified morphologically and by single step nexted multiplex PCR (SSNM-PCR). *J. Plankton Research*. 29: 423-436.

8. S.J. Whipple, B.C. Patten, P.G. Verity, M.E. Frischer, J.D. Long, J.C. Nejstgaard, J.T. Anderson, A. Jacobsen, A. Larsen, J. Martinez-Martinez, and S. Borrett. (2007). Gaining integrated understanding of *Phaeocystis* spp. (Prymnesiophyceae) through model-driven laboratory and mesocosm studies. *Biogeochemistry*. 83: 293-309.

9. Sazhin, A.F., L.F. Artigas, J.C. Nejstgaard, M.E. Frischer (2007). Colonization of *Phaeocystis* species by pinnate diatoms and other protists: An important contribution to colony biomass. *Biogeochemistry*. 83: 137-145.

10. Troedsson, C., M.E. Frischer, J.C. Nejstgaard, and E.M. Thompson. (2006). Molecular Quantification of Ingestion Rates and House Particle Trapping in the Appendicularian, Oikopleura dioica. *Limnology and Oceanography*. 52: 416-427.

11. Nejstgaard, J.C., M.E. Frischer, , P.G. Verity, J.T. Anderson, A. Jacobsen, M.J. Zirbel, A. Larsen, J. Martinez, A.F. Sazhin, T. Walters, S.J. Whipple, S. Borett, B.C. Patten, J.D. Long. (2006). Temporal patterns in planktonic food web development in mesocosms with added nutrients and Phaeocystis. *Marine Ecology Progress Series*. 321: 99-121.

12. Frischer, M.E. and P.G. Verity. (2006). Alternatives to Coliform Bacteria As Indicators of Human Impact on Coastal Ecosystems. In: Implications of Land Use Change to Coastal Ecosystems Challenges to Effective Resource Management (eds) G.S. Kleppel, M.R. DeVoe, and M. Rawson. Springer Verlag. pp. 253-273.

13. Allen, A.E., M.G. Booth, P.G. Verity, and M.E. Frischer. (2005). Influence of nitrate availability on the distribution and abundance of heterotrophic bacterial nitrate assimilation genes in the Barents Sea during summer. Aquatic Microbial Ecology. 39: 247-255.

14. Lee, R.F. and M.E. Frischer (2004). Where Have the Blue Crabs Gone: Evidence for a Drought Induced Epidemic of a Protozoan Disease. *The American Scientist*. 92: 547-553.

15. Nejstgaard, J.C., M.E. Frischer, C.L. Raule, T. Gruebel, K.E. Kohlberg, and P.G. Verity. (2003). A new approach to an old problem: molecular detection of algal prey in copepod guts and faecal pellets. Limnology and Oceanography: Methods. 1: 29-38.

16. Kerkhof, L., Corredor, J., Lopez, J., Paul, J. Bronk, D., Cherrier, J. 2003. Experiment explores inter-calibration of biogeochemical flux and nucleic acid measurements. EOS. 84: 167. *non-peer reviewed.

17. Allen, A.E, Howard-Jones, M.H., Booth, M.G., Frischer, M.E., Verity, P.G., Bronk, D.A., Sanderson, M.P. (2002) Importance of heterotrophic bacterial assimilation of ammonium and nitrate in the Barents Sea during summer. *Journal of Marine Systems*. 38: 93-108.

18. Verity, P.G., P. Wassmann, M.E. Frischer, M.H. Howard-Jones, and A.E. Allen. (2002). Grazing of primary production by microzooplankton in the Barents Sea during early summer. *Journal of Marine Systems*. 38: 109-123.

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20. Howard-Jones, M.H., V.D. Ballard, A.E. Allen, M.E. Frischer, and P.G. Verity. (2002). Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer. *Journal of Marine Systems*. 38: 77-91.

21. Allen, A.E., M.G. Booth, M.E. Frischer, P.G. Verity, J.P. Zehr, and S. Zani. (2001). Diversity of assimilatory nitrate reductase genes in heterotrophic bacteria and their detection in the marine environment. *Appl. Environ. Microbiol*. 67:5343-5348.

22. Howard-Jones, M.H., M.E. Frischer, and P.G. Verity (2001). Determining the physiological status of individual bacteria cells. In. J.H. Paul (ed.) Methods in microbiology volume 30: marine microbiology. Pages 175-206. Academic Press Ltd, London.

23. Frischer, M.E., J.M. Danforth, M.A. Newton Healy, and F. M. Saunders. (2000). Whole cell versus total RNA extraction for the analysis of microbial community structure using 16S rRNA targeted oligonucleotide probes in saltmarsh sediments. 66:3037-3043. *Appl. Environ. Microbiol*

24. Williams, S.C., Y. Hong, D.C.A. Danavall, M.H. Howard-Jones, D. Gibson, M.E. Frischer, and P.G. Verity. (1998). Distinguishing between living and nonliving bacteria: evaluation of the vital stain propidium iodide and the combined use with molecular probes in aquatic samples. *J. Microbiol. Methods*. 32:225-236.

Students

Undergraduate Research Interns

2005 – 2009	LaGina Fraizer, Savannah State University
2006 – 2007	Meade McGuire, Roanoke University
2006-2007	Brianna Schoen, Roanoke University
2006-2007	Dayton Gordley, Roanoke University
2006-2007	Lora Greene, Roanoke University
2006	Annette Holzle, Fachuschul Manneheim, Germany
2006	Tatjana Schneidt, Fachuschul Manneheim, Germany
2004 – 2005	Kenya Crawford, Savannah State University
2004 – 2005	Bobby Settle, Savannah State University
2004 – 2005	Kenya Crawford, Savannah State University
2003 – 2005	Brian Williams, Savannah State University
2003 – 2004	Robert Settel, Savannah State University
2002 – 2004	John Braxton, Savannah State University
2002 – 2003	Juan Aragon, Armstrong Atlantic University
2000 – 2002	Heidi Hendrikson, Savannah State University
2000 – 2002	Sandra Pagen, Savannah State University
2000 – 2002	Christina Archer, Savannah State University
2000 – 2002	Tara Foy, Armstrong Atlantic State University
1999 – 2000	Roxanne Bower, Dalhousie University, Halifax, Canada
1998 – 2001	Victoria Ballard, Savannah State University
1999 - 2000	Barbara Webb, Savannah State University
1999 – 2000	Deangelo Stuart, Savannah State University
1999 – 2000	Cindy Janus, Savannah State University
1999 – 2000	Ms. Lourdes Pennill, Savannah State University

Graduate Students

Doctorate

1997 – 2002	Michelle Howard-Jones. Development of the Vital Stain and Probe Method to Distinguish the Physiologic Status of Individual Bacteria in the Marine Environment. (Co-advisors, P.G. Verity and T. Christina; Degree granted from Georgia Institute of Technology). Ph.D. Awarded 2002.
1998 – 2002	Andrew Allen. Distribution and Diversity of Marine Bacteria Capable of Utilizing Nitrate. (Co-advisors, P.G. Verity and D. Bronk; Degree granted from the University of Georiga). Ph.D. Awarded 2002.
2001-2008	Paul Bradley. Cross-system comparison of nitrogen utilization patterns in phytoplankton and heterotrophic bacteria. (Advisor, Deborah Bronk, VIMS). Ph.D. awarded 2008.
2004-2011	Lynn Killberg-Thoreson. Role of organic nitrogen uptake in harmful algal blooms. (Advisor, Deborah Bronk, VIMS). Ph.D. awarded 2011.

Masters Students

2005 – 2008	Denis Wafula, Savannah State University
2003 – 2006	Megan Singleton, Savannah State University
2002 – 2005	Whitney Palefsky, Savannah State University
1999 – 2001	Teressa Fu, Georgia Institute of Technology
1997 – 2000	Michele Healy, Georgia Institute of Technology
1997-1999	Nikolle Reyes, Georgia Institute of Technology

Postdoctoral Fellows

2002 – 2007	Jennifer Brofft, Ph.D. Genetic Expression of Assimilatory Nitrate Reductase in Marine Bacteria. Collaborative Education Programs with Savannah State Univeristy. Development of "Current Topics of Oceanographic" Course (Co-advisors, P.G. Verity and M.G. Gilligan)
1998 – 2003	Melissa Booth, Ph.D. Genetic Expression of Assimilatory Nitrate Reductase in Marine Bacteria. Collaborative Education Programs with Savannah State Univeristy. Development of "Current Topics of Oceanographic" Course (Co-advisors, P.G. Verity and M.G. Gilligan)