



FINAL REPORT FOR AWARD # 0425583

GA Tech Res Corp - GIT

Collaborative Research: Biology and Ecology of Newly Discovered Diazotrophs in the Open Ocean

Participant Individuals:

Technician, programmer(s) : Poneh Davoodi

Graduate student(s) : Carrie Holl; Jason Landrum

Undergraduate student(s) : Alex Engelman; Kathy Ghanouni

Technician, programmer(s) : Samantha Allen

Graduate student(s) : Jan Drexel

Undergraduate student(s) : Connie Rich; Camila Santiago

Graduate student(s) : Rachel Horak

Undergraduate student(s) : Beth van Gessel; Gauthami Penakalapati; Meera Gujjar; Mary Crumley

Senior personnel(s) : Annalisa Bracco

Undergraduate student(s) : Rachel Sedlack; Carolyn Hernandez

Participants' Detail

Partner Organizations:

University of California-Santa Cruz: Collaborative Research

Jon Zehr is a co-PI on this project. His lab group handles the molecular characterization of diazotroph diversity and activity (mRNA expression).

Institut fuer Ostseeforschung Warnemuend: Collaborative Research; Personnel Exchanges

I was invited to take part in a research cruise to the South China Sea aboard the F/S Sonne.

This gave me an opportunity to make N₂-fixation measurements there in April 2006.

A graduate student from the IOW, Deniz Bombar, took part in our first cruise to the Tropical Atlantic as a guest member of my research group.

Institute of Oceanography, Nha Trang: Collaborative Research; Personnel Exchanges

I took part in a research cruise to the South China sea in collaboration with Vietnamese and German scientists.

Woods Hole Oceanographic Institution: Collaborative Research; Personnel Exchanges

Mak Saito, John Waterbury, Eric Webb, and members of their lab groups took part in our cruise to the Tropical Atlantic (June–July 2006).

University of Southern California: Collaborative Research; Personnel Exchanges

A graduate student (Jill Sohm) from Doug Capone's lab took part in our cruise to the Tropical Atlantic.

University of Miami Rosenstiel School of Marine&Atmospheric Sci: Collaborative Research; Personnel Exchanges

Members of Alex Worden's group took part in our cruise to the Tropical Atlantic (June–July 2006).

Netherlands Institute of Ecology, Roy. Neth. Acad. Arts&Sci.: Personnel Exchanges

Lucas Stal of NIOO took part in our recent cruise to the Tropical Atlantic (June–July 2006). Unfortunately, problems with contaminated acetylene prevented him from carrying out the high-sensitivity acetylene reduction assays that he had planned for the cruise.

IRD-Marseille: Collaborative Research; Personnel Exchanges

Dr. Isabelle Bieggala of the IRD-Marseille took part in our cruise to the SW Pacific in March–April 2007. She focused on microscopical characterization of diazotrophs during this cruise and we anticipate future collaborations with her.

Massachusetts Institute of Technology: Collaborative Research; Personnel Exchanges

Dr. Ed Boyle took part in our cruise to the SW Pacific in March–April 2007. He collected samples for trace metal characterization during this cruise.

Oregon State University: Collaborative Research; Personnel Exchanges

Dr. Angel White from Ricardo Letelier's lab took part in our cruise to the SW Pacific in March–April 2007. Angel used an optical profiler to characterize the distribution of CDOM and pigments in the upper water column.

Other collaborators:

Dave Karl (University of Hawaii) has been involved in data analysis and manuscript preparation based on work done at HOT.

Maren Voss (IOW) has shared her data from the Tropical Atlantic to help us in preparing for our first cruise (summer 2006). One of her students (Deniz Bombar) took part in our cruise to the tropical Atlantic

Lucas Stal took part in our tropical Atlantic cruise to measure N₂-fixation using a high-sensitivity photoacoustic system. Contamination problems prevented him from actually carrying out these measurements.

Mak Saito took part in our tropical Atlantic cruise to measure Fe and other metals in the water column. One technician (Tyler Goepfert) and a student from his group were aboard on the first leg, and one student (Whitney Krey) was aboard for the 2nd leg.

John Waterbury took part on one leg of our Tropical Atlantic cruise and one leg of our cruise to the SW Pacific.

Alex Worden's group took part in our Tropical Atlantic cruise (3 participants on leg 1, 2 participants on leg 2). One member of her lab took part in the first leg of our cruise to the SW Pacific.

A student from Doug Capone's group (Jill Sohm) took part in our cruise to the Tropical Atlantic.

Isabelle Biegala (IRD-Marseille) took part in our cruise to the SW Pacific.

Ed Boyle (MIT) took part in our cruise to the SW Pacific.

Cecile Rousseaux, a student from the University of Western Australia, took part in our cruise to the SW Pacific.

Annalisa Bracco (GT) is now working with us in characterizing the mesoscale circulation field during cruise KM0703.

Activities and findings:

Research and Education Activities:

The first project year was spent in lab experiments on the controls on diazotrophy (Carrie Holl), continued analysis of samples from previous field efforts, and ongoing preparation of manuscripts based on earlier work. We also spent a great deal of time beginning to organize for our first cruise to the Tropical Atlantic in June-July 2006.

Our major effort in year 2 centered on our cruise to the Tropical Atlantic on the R/V Seward Johnson. The cruise was quite successful and allowed us to sample across a broad stretch of the Tropical Atlantic. We carried out five major experiments designed to test the controls on N₂-fixation as well as a series of vertical profiles of activity measurements across the basin. I have attached a copy of my report from that cruise, which provides additional details on our activities at sea.

Our major effort in Year 3 centered on a cruise to the SW Pacific in March-April 2007. We had a very successful cruise and sampled waters in the Coral Sea and in the tropics eastward to 170W (around Tonga and Niue). We sampled 26 stations in all and collected samples of POM and zooplankton for stable isotope analysis and carried out rate measurements of N₂-fixation at each station. I have appended our cruise report from KM0703 to the report from SJ0609.

Our primary effort in Year 4 has been to process the experimental (tracer) and natural abundance stable isotope samples from our two cruises. We have made significant progress with our tracer samples (ca. 70% completed overall) but have been greatly slowed overall by instrumental down-time caused by electronic problems in the mass spectrometer (optima) control and amplifier systems. Beginning in late 2007, we were forced to cannibalize an older system for parts that are no longer available from the manufacturer in order to keep our primary system running. We are now able to run samples, but our throughput has been reduced by about 30-40% because of

the need for
close monitoring of analytical runs in progress.

We are now (July 2008) working through our backlog of natural abundance
samples
(zooplankton and particles) and wrapping up our analysis of tracer samples.

In parallel
with our analytical efforts, we have begun collaborating with a physical
oceanographer to
explore the role of mesoscale variability in controlling diazotroph activity.
We are well
along in synthesizing our rate measurements with physical data.

Year 5:

We made some improvements to our analytical procedures, which allowed
us to analyze
smaller samples than previously. As a result, we are now reanalyzing
subsamples of
material from stations where we could not previously get usable results.

Findings:

Carrie Holl's work has demonstrated that nitrate has a variable effect
in suppressing N₂-
fixation by Trichodesmium, which can take up nitrate and fix N₂ simultaneously
in the
light.
Carrie also developed an isotopic index to the contribution of diazotrophy
to the food web
of
the Gulf of Mexico.

Continued analysis of our data on N₂-fixation by unicells has shown that
these organisms
make a major contribution to the N budget of the Tropical Pacific and
other waters.

Year 2:

We are just beginning to work up our samples from cruise SJ0609. Qualitatively,
we were
surprised and intrigued to find Trichodesmium all the way down to the
equator. We are
awaiting molecular results, but anticipate that other diazotrophs were
also present and
active at low latitudes in the Atlantic.

Year 3:

We just completed a major cruise and are gearing up to continue our analysis
of samples
from SJ0609. Samples collected on cruise KM0703 are still en route back
to Atlanta and
will enter the queue as soon as they arrive. During the last cruise,
we sampled diverse

waters, including a major bloom of *Trichodesmium* and a station with extremely high abundances of *Crocospaera*-type unicells at depth.

Year 4:

We have made significant progress in analyzing samples from our rate experiments.

Our data from the SW Pacific are especially interesting, with strong spatial heterogeneity and extremely high rates of N₂-fixation at our southernmost station (30S).

These high rates occurred at stations dominated by Group A cyanobacteria (data from the Zehr group). We are currently exploring the physical context and history of the waters we sampled in collaboration with Annalisa Bracco, but our preliminary workup indicates that these extremely high rates are associated with an anticyclonic eddy.

Further north, we have found elevated rates of N₂-fixation near Fiji in a *Crocospaera* 'bloom'. This feature was notable because it was confined to a rather narrow lens of relatively fresh water at depth.

Reanalysis of samples previously unmeasurable because of size constraints has allowed us to confirm that the very high activity in the SW Pacific extends through at least the upper 50-70 m of the water column. We are working with Annalisa Bracco to characterize the flow fields associated with these extremely high rates of activity. We continue to work with Jon Zehr's group to relate activity measurements to the organisms involved. To date, we have strong evidence that Group A unicellular cyanobacteria are responsible for the high rates.

Training and Development:

Two graduate students and two undergraduates gained experience in stable isotope biogeochemistry and general oceanography while taking part in this project.

In year 2, two graduate students and two undergraduates gained experience in the lab and at sea during our 6 week cruise to the Tropical Atlantic.

In year 3, three graduate students and three undergraduates worked on this project either in the lab or at sea.

In year 4, two graduate students and four undergraduates worked on the lab-based analysis of samples collected for this project. Both graduate students took part in national meetings.

In year 5, two graduate students and three undergraduates worked on the lab-based analysis of samples collected at sea. One graduate student (Jason Landrum) completed his PhD and the other (Rachel Horak) is on track to finish within the year.

Outreach Activities:

I incorporate our findings into my lectures in introductory biology (Biology 1510), which has an enrollment of over 400 students each Fall term. I also meet with students individually, including high school and elementary students from the local public schools.

I also incorporate our findings on N₂-fixation into lectures in introductory Ecology (Biology 2335) and Biological Oceanography (Biology 4221).

Rachel Horak, a graduate student in my lab, has done outreach and educational work through the Georgia Aquarium, sharing our work and our findings with the general public.

In Summer 2008, my lab hosted a high school teacher and two high school students as guest researchers through the Georgia Tech GIFT (Georgia Intern- Fellowships for Teachers) program.

In Summer 2009, my lab hosted a high school teacher and four high school students as guest researchers through the Georgia Tech GIFT Program. In addition, a GT undergraduate interested in secondary school teaching joined my lab for the summer and worked with the high school group through the new Tech to Teaching program.

Journal Publications:

Montoya, J P, Holl, C.M., Zehr, J.P., Hansen, A., Villareal, T.A., Capone, D.G., "High rates of N₂-fixation by unicellular diazotrophs in the oligotrophic Pacific", *Nature*, vol. 430, (2004), p. 1027., " " Published

Campbell, L, E.J. Carpenter, J.P. Montoya, A.B. Kustka, D.G. Capone, "Picoplankton community structure within and outside a Trichodesmium bloom in the southwestern Pacific Ocean", *Vie et Milieu*, vol. 55, (2005), p. 185., " " Published

Capone, D.G., J.A. Burns, J.P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A.F. Michaels,

and E.J. Carpenter, "Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropics and subtropical North Atlantic Ocean", *Global Biogeochemical Cycles*, vol. 19, (2005), p. doi:10.1029/2004GB002543, " " Published

Holl, C.M. & J.P. Montoya, "Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (Cyanophyta)", *Journal of Phycology*, vol. 41, (2005), p. 1178., " " Published

Krauk, J.M., T.A. Villareal, J.A. Sohm, J.P. Montoya, and D.G. Capone, "Plasticity of N:P ratios in laboratory and field populations of *Trichodesmium* spp.", *Aquatic Microbial Ecology*, vol. 72, (2006), p. 243., " " Published

Burns, J.A., Zehr, J.P., Montoya, J.P., Kustka, A.B., and Capone, D. G., "Effect of EDTA additions on natural *Trichodesmium* spp. (CYANOPHYTA) populations", *Journal of Phycology*, vol. 42, (2006), p. 900., " " Published

Wilson, C., Villareal, T.A., Maximenko, N., Bograd, S.J., Montoya, J.P., Schoenbaechler, C.A., "Biological and physical forcings of late summer chlorophyll blooms at 30 degrees N in the oligotrophic Pacific", *Journal of Marine Research*, vol. 69, (2008), p. 164., " " Published

Zehr, J.P., Montoya, J.P., Hewson, I., Mondragon, E., Short, C., Hansen, A., Jenkins, B.D., Church, M.J., Karl, D.M., "Nitrogenase gene expression and N₂ fixation in the North Pacific Subtropical Gyre", *Limnology and Oceanography*, vol. 52, (2007), p. 169., " " Published

Holl, C.M. and Montoya, J.P., "Diazotrophic growth of the marine cyanobacterium, *Trichodesmium* IMS101 in continuous culture: Effects of growth rate on N₂-fixation rate, biomass, and C:N:P stoichiometry", *Journal of Phycology*, vol. , (2008), p. ., " " Accepted

Holl, C.M., Waite, A.M., Pesant, S., Thompson, P., Montoya, J.P., "Unicellular diazotrophy as a source of nitrogen to Leeuwin Current coastal eddies", *Deep-Sea Research I*, vol. 54, (2007), p. 1045., " " Published

Montoya, J.P., M. Voss, and D.G. Capone, "Spatial variation in N₂-fixation rate and diazotroph activity in the Tropical Atlantic", *Biogeosciences*, vol. 4, (2007), p. 396., " " Published

Holl, C.M., T.A. Villareal, C.D. Payne, T.D. Clayton, C. Hart, J.P. Montoya, "Trichodesmium in the western Gulf of Mexico: ¹⁵N₂-fixation and natural abundance stable isotope evidence", *Limnology and Oceanography*, vol. 52, (2007), p. 2249., " " Published

Waite, AM; Muhling, BA; Holl, CM; Beckley, LE; Montoya, JP; Strzelecki, J; Thompson, PA; Pesant, S, "Food web structure in two counter-rotating eddies based on delta N-15 and delta C-13 isotopic analyses", *DEEP-SEA RESEARCH PART II-TOPICAL STUDIES IN OCEANOGRAPHY*, vol. 54, (2007), p. 1055., "10.1016/j.dsr2.2006.12.01 " Published

Holl, CM; Montoya, JP, "Diazotrophic growth of the marine cyanobacterium *Trichodesmium* IMS101 in continuous culture: Effects of growth rate on N₂-fixation rate, biomass, and C : N : P stoichiometry", *JOURNAL OF PHYCOLOGY*, vol. 44, (2008), p. 929., "10.1111/j.1529-8817.2008.00534. " Published

Paerl, RW; Foster, RA; Jenkins, BD; Montoya, JP; Zehr, JP, "Phylogenetic diversity of cyanobacterial *narB* genes from various marine habitats", *ENVIRONMENTAL MICROBIOLOGY*, vol. 10, (2008), p. 3377., "10.1111/j.1462-2920.2008.01741. " Published

Naqvi, SWA; Voss, M; Montoya, JP, "Recent advances in the biogeochemistry of nitrogen in the ocean", *BIOGEOSCIENCES*, vol. 5, (2008), p. 1033., " " Published

Subramaniam, A; Yager, PL; Carpenter, EJ; Mahaffey, C; Bjorkman, K; Cooley, S; Kustka, AB; Montoya, JP; Sanudo-Wilhelmy, SA; Shipe, R; Capone, DG, "Amazon River enhances diazotrophy

and carbon sequestration in the tropical North Atlantic Ocean", *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, vol. 105, (2008), p. 10460., "10.1073/pnas.071027910 " Published

Hewson, I; Poretsky, RS; Beinart, RA; White, AE; Shi, T; Bench, SR; Moisander, PH; Paerl, RW; Tripp, HJ; Montoya, JP; Moran, MA; Zehr, JP, "In situ transcriptomic analysis of the globally important keystone N-2-fixing taxon *Crocospaera watsonii*", *ISME JOURNAL*, vol. 3, (2009), p. 618., "10.1038/ismej.2009. " Published

Hannides, CCS; Landry, MR; Benitez-Nelson, CR; Styles, RM; Montoya, JP; Karl, DM, "Export stoichiometry and migrant-mediated flux of phosphorus in the North Pacific Subtropical Gyre", *DEEP-SEA RESEARCH PART I-OCEANOGRAPHIC RESEARCH PAPERS*, vol. 56, (2009), p. 73., "10.1016/j.dsr.2008.08.00 " Published

Landrum, JP; Montoya JP, "Organic matter processing by the shrimp *Palaemonetes* sp.: Isotopic and elemental effects", *Journal of Experimental Marine Biology and Ecology*, vol. , (2009), p. ., " " Submitted

Book(s) of other one-time publications(s):

Montoya JP, "Natural abundance of ^{15}N in marine planktonic ecosystems" , bibl. Blackwell, (2007). *Book Published*

of Collection: Lajtha K and Michener R, "Stable Isotopes in Ecology and Environmental Science, 2nd edition"

Zehr JP & Montoya JP, "Measuring N_2 Fixation in the Field" , bibl. Elsevier, Amsterdam, (2007). *Book Published*

of Collection: Bothe H, Ferguson S, & Newton WE, "Biology of the Nitrogen Cycle"

Montoya JP, "Nitrogen stable isotopes in marine environments" , bibl. Blackwell, (). *Book Accepted*
of Collection: Capone DG, Carpenter EJ, Mulholland MR, "Nitrogen in the Marine Environment"

Montoya, J.P. Voss, M, "Nitrogen cycling in anoxic waters: Isotopic signatures of nitrogen transformations in the Arabian Sea Oxygen Minimum Zone" , bibl. NATO Science Series IV: Earth and Environmental Sciences, v64, Springer, Dordrecht, Netherlands., (2006). Published
of Collection: Neretin, L.N., "In Past and Present Water Column Anoxia"

Other Specific Products:

Contributions:

Contributions within Discipline:

Our major finding has been that small diazotrophs make an important contribution to the new N budget of oligotrophic waters in the Pacific. This unanticipated result requires a reevaluation of the marine nitrogen budget.

Our continuing work on the controls on N_2 -fixation are helping us understand the factors that control diazotroph activity in the open ocean.

Our two cruises have given us an unprecedented opportunity to evaluate the spatial extent and controls on N₂-fixation in oligotrophic waters. Our data set will be the most comprehensive to date. We have transmitted all of our hydrographic and nutrient data to NODC and have begun discussions with BCO-DMO about submission of these and other data. We are still vetting and organizing our stable isotope measurements, which form important parts of two PhD theses and one undergraduate senior thesis. As these data are finalized, we will transmit them to the BCO-DMO database.

We have discovered significant variability in diazotroph activity and impact, and have found very high rates of N₂-fixation in association with an anticyclonic eddy in the South Pacific. Mesoscale variation in N₂-fixation activity may be a critical component of the upper ocean N budget that has been missed in previous sampling efforts.

Contributions to Other Disciplines:

Our discovery that unicellular diazotrophs are making a large contribution of new nitrogen to the oligotrophic Pacific has important implications for our understanding of the global C cycle since new production is a critical part of the biological pump moving carbon from the atmosphere into the ocean interior. Our finding of localized, but extremely high rates of N₂-fixation in a mesoscale eddy in the SW Pacific also has important implications for oceanic N budgets and estimates of new production supported by diazotrophy.

Contributions to Education and Human Resources:

Two PhD students (Carrie Holl and Jason Landrum) completed their dissertations with partial support from this grant. Carrie Holl is now a staff scientist at the Oceanic Institute in Hawaii. Jason Landrum is entering a new program at GT for scientists interested in International Relations, where he expects to work on Science Policy.

Another PhD student (Rachel Horak) gained valuable experience at sea as part of this project. One MS student (Jan Drexel) and ten undergraduates have received significant training and

lab experience in connection with this project.

One undergraduate (Mary Crumley) is working up the stable isotope data from KM0703 as her senior honors thesis. Although Mary did not have the opportunity to go to sea, she has learned a great deal of oceanography and isotope biogeochemistry through this project.

Contributions to Resources for Science and Technology:

Our findings have been incorporated into teaching materials used in courses in Introductory Biology, Oceanography, and Biological Oceanography at Georgia Tech.

As noted above, our hydrographic and nutrient data have been transmitted to NODC (Accession nos. 0059071 and 0059113). We are still vetting and assembling our stable isotope data, which form parts of the dissertations of two PhD students and one senior thesis project. I have initiated a discussion of data transfer to BCO-DMO and will submit our isotope measurements as we finalize these data sets.

Conference Proceedings:

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