FINAL TECHNICAL REPORT
THE ECOLOGY AND GENOMICS OF CO_2 FIXATIION IN OCEANIC RIVER PLUMES
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Abstract

Oceanic river plumes represent some of the most productive environments on Earth. As major conduits for freshwater and nutrients into the coastal ocean, their impact on water column ecosystems extend for up to a thousand km into oligotrophic oceans. Upon entry into the oceans rivers are tremendous sources of CO_2 and dissolved inorganic carbon (DIC). Yet owing to increased light transmissivity from sediment deposition coupled with the influx of nutrients, dramatic CO_2 drawdown occurs, and plumes rapidly become sinks for CO_2. Using state-of-the-art gene expression technology, we have examined the molecular biodiversity of CO_2 fixation in the Mississippi River Plume (MRP; two research cruises) and the Orinoco River Plume (ORP; one cruise). When the MRP extends far into the Gulf because of entrainment with the Loop Current, MRP production (carbon fixation) can account for up to 41% of the surface production in the Gulf of Mexico. Nearer-shore plume stations ("high plume," salinity$\leq$ 32 ppt) had tremendous CO_2 drawdown that was correlated to heterokont (principally diatom) carbon fixation gene expression. The principal form of nitrogen for this production based upon ^{15}N studies was urea, believed to be from anthropogenic origin (fertilizer) from the MRP watershed. Intermediate plume environments (salinity 34 ppt) were characterized by high levels of *Synechococcuus* carbon fixation that was fueled by regenerated ammonium. Non-plume stations were characterized by high light *Prochlorococcus* carbon fixation gene expression that was positively correlated with dissolved CO_2 concentrations. Although data from the ORP cruise is still being analyzed, some similarities and striking differences were found between the ORP and MRP. High levels of heterokont carbon fixation gene expression that correlated with CO_2 drawdown were observed in the high plume, yet the magnitude of this phenomenon was far below that of the MRP, most likely due to the lower levels of anthropogenic nutrient input. The offshore ORP was characterized by haptophyte and in places *Prochlorococcus* carbon fixation gene expression in surface water, with greater heterokont rbcL RNA at SCM depths. MODIS satellite chlorophyll-a data implied a plume of high chlorophyll water far into the eastern Caribbean, yet field observations did not support this, most likely because of high levels of colored dissolved organic matter (cDOM) in the ORP. The presence of pelagic nitrogen fixers (*Trichodesmium* and cyanobacterial diatom endosymbionts) most likely provided N for the offshore MRP production. The results underscore the importance of oceanic river plumes as sinks for CO_2 and the need for their incorporation in global carbon models as well as estimates of CO_2 sequestration.

PUBLICATIONS RESULTING FROM THE AWARD AND BRIEF SUMMARY

Publications

1. Wawrik, B. , J.H. Paul, and F.R. Tabita. 2002. Real Time PCR Quantification of *rbcL* (Ribulose bisphosphate carboxylase/oxygenase) mRNA in diatoms and pelagophytes . Applied and Environmental Microbiology 68:3771-3779.

This paper describes the development of a quantitative PCR assay for the detection of diatom Rubisco and pelagophyte gene expression. This tool was further developed and used in future river plume studies.

2. Wawrik, B., J.H. Paul, L. Campbell, D. Griffin, L. Houchin, A. Fuentes-Ortega, and F. Muller-Karger. 2003. Vertical Structure of the Phytoplankton Community associated with a Coastal Plume in the Gulf of Mexico. Mar. Ecol. Progr. Ser. 251:87-101

These are the results of our first MRP study (1999 cruise aboard the R/V Pelican) and investigated an offshore plume environment, first noting the elevated production in the surface waters. This offshore plume station was dominated by *Synechcoccus* in the surface and prasinophytes at the subsurface chlorophyll maximum (SCM).

3. Kerkhof, L., J. Corredor, J. Lopez, J. Paul, D. Bronk, and J. Cherrier. 2003. Experiment explores inter-calibration of biogeochemical flux and nucleic acid measurements. Eos 84:167-168

This note describes the Geochemical Rate /RNA Integration Study (GRIST) that took place inn Tuckerton, NJ in 2002.

4. Wawrik, B. John H. Paul, Deborah A. Bronk, David John, Mike Gray. 2004. High rates of ammonium recycling drive phytoplankton productivity in the offshore Mississippi River plume found in the oligotrophic Gulf of Mexico. Aquatic Microbial Ecology 35:175-184.

This paper describes our first plume axis sampling effort of an offshore MRP and integrated study of ^{15}N uptake study (FG Walton Smith cruise in 2001). The offshore plume was fueled by regenerated ammonium and CO_2 fixation was attributed to *Synechococcus* populations.

5. Wawrik, B. and J.H. Paul. 2004. Phytoplankton community structure and productivity along the axis of the Mississippi River Plume. Aquat. Microb. Ecol. 35:185-196

This is the companion paper to the previous, and shows the diversity of the transcriptionally active phytoplankton involved in carbon fixation. Diatoms were prevalent at the inshore plume, being replaced by *Synechococcus* offshore. This paper included the first estimates of total plume contribution to total GOM production.

6. Jorge E. Corredor, Boris Wawrik, John H. Paul, Lee Kerkhof, José M. López and Angel Dieppa. 2004. The Geochemical Rate/RNA Integration Study (Grist): I. Rubisco Transcription And Photosynthetic Capacity Of Planktonic Photoautotrophs. Applied and Environmental Microbiology 70: 5459-5468

This paper describes the Rubisco gene expression part of the GRIST study. Strong diel periodicity in C fixation and Rubisco gene expression was observed. The best correlation occurred with diatom/pelagophyte rbcL gene expression as measured by real time PCR. Clone libraries of transcriptionally-active rbcL genes included diatoms, prymnesiophytes, and unicellular green algae.

7. John, D.E., B. Wawrik, J.H. Paul, and F. R. Tabita. 2006. Gene diversity and organization in *rbcL*-containing genome fragments from uncultivated *Synechococcus* in the Gulf of Mexico. Marine Ecol. Progr. Ser. 316:23-33

A bacterial artificial chromosome (BAC) library derived from the FG Walton Smith MRP cruise was screened for rbcL-containing BACs. Eight were found, and all of them from pheycoerythrin-containing *Synechococcus.* The synteny of the carbon fixation genes was highly conserved, although genes outside this cluster were variable, and suggested gene transfer events with *Prochlorococcus.*

8. John, D.E. and J.H. Paul. 2007. Enhanced quantitative reverse transcription PCR assays for measurement of ribulose 1,5 bisphosphate carboxylase/oxygenase gene expression from marine phytoplankton. Marine Biotechnology 9:747-759.

Quantitative PCR assays were developed for rbcL from diatoms/pelagophytes,, haptophytes, PE-containing *Synechococcus,* and high light *Prochlorococcus.* Results of application of these assays to depth profiles of rbcL gene expression inside and outside the MRP are reported. These revealed the dramatic restriction of rbcL gene expression to plume surface waters, dominated by heterokonts (diatoms), and peaks of RNA levels at SCM depths outside the plume.

9. D.E. John, Z.A. Wang, X. Liu, R.H. Byrne, J.E. Corredor, J.M. López, A. Cabrera, D.A. Bronk, and J.H. Paul. 2007. Carbon fixation gene (RuBisCO) transcripts and CO_2 flux in the Mississippi River plume The ISME Journal 1(6): 517-531

The crowning paper of the MRP work describes Rubisco gene expression studies as they relate to CO_2 flux measurements during a cruise aboard the R/V Pelican in 2005. This paper shows an inverse correlation between heterokont (principally diatoms) Rubisco expression and DIC concentrations, showing that diatoms can be productive even once they have greatly reduced dissolved CO_2 concentrations. The reverse of this was observed for high light *Prochlorococcus*, with Rubisco expression positively correlated to CO_2 levels.

10. D.E. John, Z.A. Wang, X. Liu, R.H. Byrne, J.E. Corredor, J.M. López, A. Cabrera, D.A. Bronk, and J.H. Paul. 2007. Carbon fixation gene (RuBisCO) transcripts and CO_2 flux in the Mississippi River plume The ISME Journal 1(6): 517-531

This manuscript shows the relationship of rubisco gene expression, N uptake, and CO_2 drawdown in the ORP.

11.David E. John, Jorge E. Corredor, Jose M. Lopez, Alvaro Cabrera, and John H. Paul.2012 Diel Patterns of Phytoplankton Photosynthesis Parameters and Carbon Fixation Gene Transcripts in the Mississippi and Orinoco River Plumes. Hydrobiologia 679:155–173

This seminal paper of the project l compares the plumes.

12. David E. John, Brian L. Zielinski, and John H. Paul.2009. Creation of a pilot Meta-transcriptome library from eukaryotic plankton of a eutrophic bay (Tampa Bay, Florida) 7:249-259

This paper describes the development of technology to make eukaryotic metatranscriptomes.

Presentations

Paul, J.H. and B. Wawrik. 2002. From mRNA to satellites: A view of the Mississippi River Plume in the Gulf of Mexico. ASLO Ocean Sciences Meeting, Honolulu, Hawaii, 11-15 February 2002. AbsOS22I-04.

B.Wawrik, J. Paul, and M. Gray. 2002. ntcA mRNA levels as an indicator of picocyanobacterial nitrogen status in the Gulf of Mexico. ASLO Ocean Sciences Meeting, Honolulu, Hawaii, 11-15 February 2002. AbsOS41H-08.

B. Wawrik, J.H. Paul, L. Houchin, D. Griffin, and A. Fuentes-Ortega. 2000. rbcL Expression in a low-salinity plume feature in the Gulf of Mexico. 100th General meeting of the American Society for Microbiology, Los Angeles, CA. Abs. N-73.

B. Wawrik and J. Paul. 2000. rbcL gene expression and molecular diversity of phytoplankton communities in coastal high chlorophyll plumes in the Gulf of Mexico. American Society of Limnology and Oceanography, Aquatic Sciences Meeting, Copenhagen, DK, abs. SS08-23P

B. Wawrik and J.H. Paul. 2001. Diatom rbcL (ribulose bisphosphate carboxylase large subunit) gene expression by real time PCR. 101st General Meeting of the American Society for Microbiology, Orlando, FL May 20-24. Abs Q147

Wawrik, B., J.H. Paul, and L. Campbell. 2001. Sequence analysis of transcriptionally-active carbon fixation genes indicates near-surface and sub-surface clades of Phrochlorococcus in the Gulf of Mexico. ASLO 2001 Meeting, Albuquerque, New Mexico, Feb. 12-16. Aquatic Sciences

John, D.E., B. Wawrik, and J.H. Paul. 2004. Finding a needle in a "BAC-stack": The search for *rbcL* genomic fragments I the ocean. 104th General Meeting of the American Society for Microbiology, May 23-27, New Orleans, LA Abs H-186

John, D., B. Wawrik, and J.H. Paul. 2005. Uncovering diversity in the rbcL genomic environment of marine *Synechococcus.* ASLO 2005 Aquatic Sciences Meeting, Feb. 20-25, Salt Lake City, UT

J.H. Paul. 2005. From Satellites to Genomes: Significance of Oceanic River Plumes. National Council for Science in the Environment Meeting on Forecasting Environmental Change. February 3-5, Washington, DC

J.H. Paul. 2005. From Gene Expression to Ecological Processes-the *rbcL* story. ASLO Ocean Science Meeting, February 21, Salt Lake City, UT

Witte, B.H., D. John, J.H. Paul, B.Wawrik, and F.R. Tabita.2005. Functional *Synechococcus* RubisCO from an oceanic metagenomic library. 105th General Meeting of the American Society for Microbiology, Atlanta, GA abs.N-035

John, D.E., B. Witte, S.S. Patterson, X. Liu,Z. Wang, R.H. Byrne, J.M. Lopez, A. Cabrera, J. Corredor, F.R. Tabita, and J.H. Paul. 2006. RbcL expression and CO2 Flux in the Mississippi River Plume-A first Look. ASLO, TOS, AGU Ocean Sciences Meeting, Feb. 20-24, Honolulu, Abs.OS44G-05

John, D.E., B. Zielinski, D.A. Bronk, R.H. Byrne, J.E. Corredor, and J.H. Paul. 2008. Quantification and cloning of carbon-fixation (Rubisco) mRNA transcripts from the Orinoco River Plume and Eastern Caribbean Sea. ASLO AGU TOS Ocean Sciences Meeting, Orlando, FL, March 3-7