Final Report: DOE-Biological Ocean Margins Program. Active Microbes Responding to Inputs from the Orinoco River Plume.

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OBJECTIVES

The overall goal of the proposed work is to identify the active members of the heterotrophic community involved in C and N cycling in the perimeter of the Orinoco River Plume (ORP), assess their spatial distribution, quantify their metabolic activity, and correlate these parameters to plume properties such as salinity, organic matter content and phytoplankton biomass.

Our specific objectives are:

1) To measure the respiratory response of the microbial loop will be assessed along the ORP gradient by monitoring electron transport activity (ETS).
2) To determine the active microbes in the ORP by analyzing high molecular weight ribosomes as a template with 16S and 18S rRNA gene primers and molecular fingerprinting techniques.
3) To analyze the bulk ^{15}N uptake of nitrate and ammonium in various size classes of the particulate fraction in ORP waters.
4) To assess bacterial members that are incorporating $^{15}NO3-$ or $^{15}NH4+$ using stable isotope probing.
5) To map active heterotrophic bacteria using different classes of ^{13}C-labeled compounds in the ORP.

The original proposal contemplated implementation of two oceanographic expeditions along the axis of the Orinoco River plume in order to address these research questions. Unfortunately, early termination of the program on the part of DOE necessitated curtailing the field effort. With authorization of the program, manager, funds remaining were redirected towards research on internal tides in the Mona Passage and inorganic carbon cycling on coral reefs. Both these efforts resulted in publications here listed.

Data presented below was collected during a single research expedition (denoted Oripex VIII) aboard LUMCON's R/V PELICAN in September 2007. A copy of the cruise report submitted to the Department of State as required for operations in foreign waters is included in Appendix I.

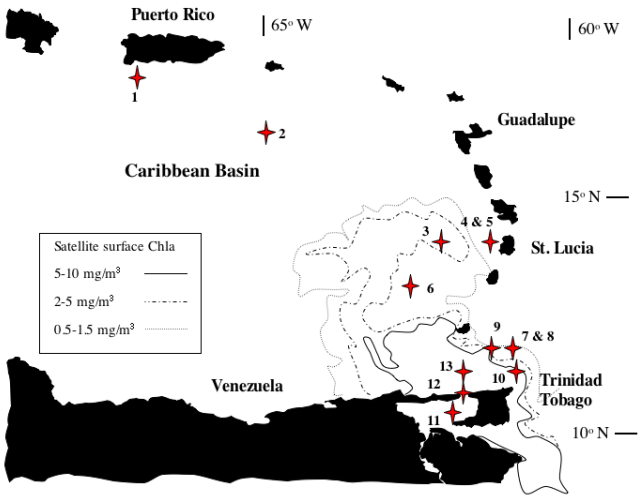


Figure 1. Map of collection sites on first leg of OriPEx VIII cruise aboard the R/V Pelican, in September 2007. Stations are indicated in red.

Members of the Corredor/Morell labs from the University of Puerto Rico, Mayaguez and the Kerkhof lab from Rutgers participated in an oceanographic cruise collecting samples along the Orinoco River Plume, sampling at 13 stations within the Caribbean

basin based upon near real-time satellite imagery (coastwatch.noaa.gov; see Figure 1). Water was collected by CTD at 4 depths (two depths within the plume, one depth below, and one at the deep chlorophyll maximum) from each sampling site (Figure 2), except in the shallow regions at the mouth of the Orinoco River. For each sample, water was collected for activity measurements or microbial biomass from 4 liters was concentrated onto 0.2 µm SUPOR filters, frozen and shipped at liquid nitrogen temperatures, then placed at –80^{o} C prior to laboratory extraction. Duplicates from a select set of stations and depths were also collected.

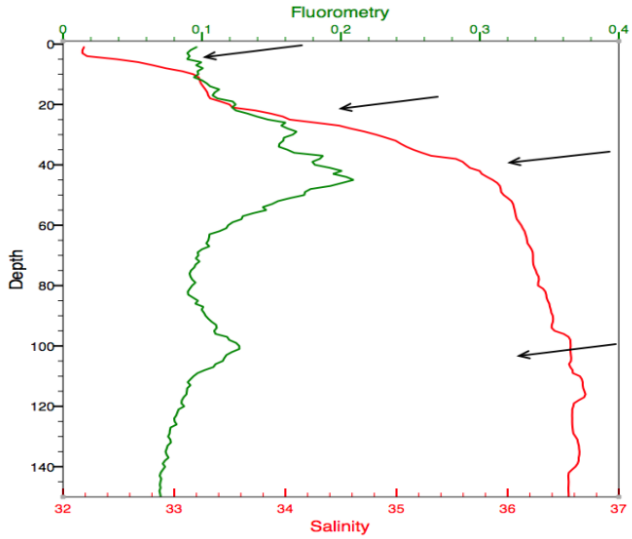


Figure 2. Depth profile of fluorometry and salinity with locations of typical samples indicated by the arrows.

We employed 3 methods to determine activity of the microbial community in response to the ORP, electron transport system measurements (ETS), ribosomal RNA profiling, and stable isotope probing using ^{13}C labeled "carrier" technology (Gallagher et al. 2005). The ETS measurements were performed by members of the Morell lab. Electron transport system (ETS) determinations were carried out according to Kenner and Ahmed (1975) using a modification of the tetrazolium reduction technique proposed by Packard (1971). The highest ETS values for stations #12 (Gulf of Paria), #9 (SSW of Grenada) and #13 (Dragon's Mouth). ETS measurements displayed an inverse relationship with salinity and a positive relationship with the vertical attenuation coefficient (Kd; Fig.3). This finding suggests the highest microbial community activity was associated with the nearshore, colored surface waters of the ORP harboring the highest organic matter content.

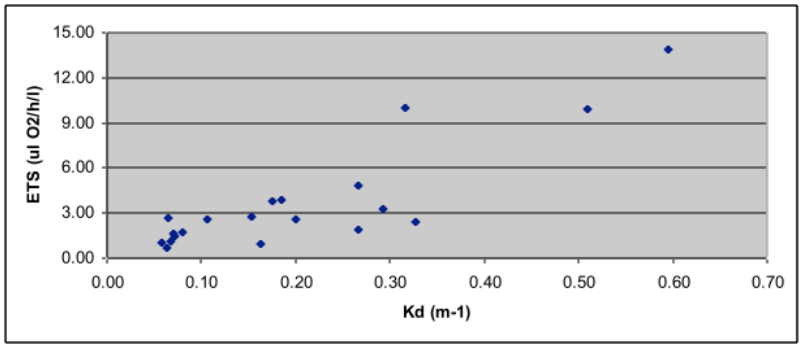


Figure 3. Relationship between electron transport system (ETS) and vertical attenuation coefficient (Kd) for cruise OriPEx VIII.

Ribosome profiling and stable isotope probing with 3 complex classes of carbon compounds and 2 forms of inorganic nitrogen at stations 3, 6, 9, and 12; representing different chlorophyll *a* containing regions of the ORP were performed by the collaborating team of the Kerkhof laboratory and is the subject of a separate report.

Manuscripts submitted:

Ferraro, C., Corredor, J., Morell, J., McGuinness, L. and L. J. Kerkhof. Monitoring Active

Microorganisms in the Orinoco River Plume by TRFLP Profiling of High Molecular Weight Ribosomes. Submitted Molecular Ecology

McGuinness, L., J.E. Corredor, J.M. Morell, and L.J. Kerkhof. Stable Isotope Probing using ^{13}C and ^{15}N in the Orinoco River Plume. Submitted to Plos One.

Ramón López, José M. López, Julio Morell, Jorge E. Corredor and Carlos E. Del Castillo. In Review. Influence of the Orinoco River on the primary production of eastern Caribbean surface waters. J. Geophys. Res. Oceans.

Other Miscellaneous Publications:

Corredor, J. 2008. Las Olas Internas del Canal de La Mona. La Regata Año 11 Número 2.

Corredor, J. 2008. Development and Propagation of Internal Waves in the Mona Passage. Sea Technology. October 2008.

Presented posters/papers supported by the project:

2011

1. Brocco, B. A.,Morell, J., Corredor, J., López, J. M., Antoun, H., Modulation of The Planktonic Heterotrophic Activity in the Eastern Caribbean Sea by the Orinoco River Plume ASLO Aquatic Sciences Meeting, San Juan Puerto Rico February 2011.

2008

1. Fuentes-Figueroa, D., Morell, J. M, **Corredor, J. E.,** Otero, E., Gonzalez, J. G., Variation of Planktonic Community Structure Along the Orinoco River Plume. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

2. **Corredor, J E**, Morell, J M, López, J M, Cabrera, A, Community Composition, Photosynthetic Capacity, Diazotroph Abundance and Nitrogenase Activity Of Phototrophic Plankton In The Orinoco River Plume. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

3. Morell, J M, **Corredor, J E**, López, J M, Brocco, B, Fuentes, D, Antoun, H, López, R, Cabrera, A, Méndez, M, Major River Plumes In The Tropical Ocean: Physical and Biogeochemical Expression. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

4. Brocco, Morell, J M, **Corredor, J E**, Lopez, J, Influences of The Orinoco River Plume In The Balance Between Plankton Photosynthesis And Respiration In The Caribbean Sea. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

5. Cullison, S E, DeGrandpre, M D, Langdon, C, Corredor, J E, Establishing Natural Variation in pH and pCO2 on a Coral Reef Using High Temporal Resolution Autonomous Sensors. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

6. Muñoz-Hincapié, M F, Morell, J**, Corredor**, **J**, Respiratory Rates At The Caribbean Time Series Station (Cats). ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

7. **L. J. Kerkhof,** Jorge E. Corredor , Julio Morell , and Lora McGuinness; Stable Isotope Probing of Active Bacteria in the Orinoco River Plume using ^{13}C and ^{15}N Substrates. ISME General Meeting. Cairns, Australia, Aug 18-22, 2008.

8. C.A. Fraser, **J.E. Corredor**, J.M. Morell, L. McGuinness, L.J. Kerkhof. Active Microbes in the Orinoco River Plume. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008.

9. L. McGuinness, **J.E. Corredor,** J.M. Morell, L.J. Kerkhof. Stable Isotope Probing using ^{13}C and ^{15}N in the Orinoco River Plume. ASLO General Meeting. Orlando, FL, Mar 2-6, 2008

2007

1. **Corredor, J.E.** J.M. Morell, J.M. López, A. Cabrera and H. Antoun. Who Put Out The Light? Caribbean Deep Phytoplankton Community Response To Orinoco River Plume Intrusion. ASLO Aquatic Sciences Meeting, Feb 5-9, 2007

M.Sc. Thesis Supported at UPRM Department of Marine Sciences:

Fuentes-Figueroa, Dihalia. 2007. Variation of planktonic community structure along the Orinoco River Plume.
Antoun-Kučerova, Helena. 2009. Mesoscale Forcing, Phytoplankton Community Structure and Size Class Distribution in the Caribbean.
Brocco-Jaime, Belitza A. 2010. Influence of the Orinoco River Plume on the Balance Between Plankton Primary Production and Respiration in the Caribbean Sea

Ph.D. Dissertations Supported at UPRM Department of Marine Sciences:

López-Rosado, Ramón. 2008. Photosynthetic Efficiency of Phototrophic Plankton and Bio-Optical Variability as Influenced By Mesoscale Processes in The Eastern Caribbean Basin.

APPENDIX I

Cruise Report

Orinoco River Plume Productivity Experiment (ORIPEX) 2006

University of Puerto Rico
University of South Florida
College of William and Mary
Rutgers University

R/V PELICAN

September 18 - October 02, 2006

INTRODUCTION

The Orinoco River Plume Productivity Experiment (OriPEx) was carried out as a joint research cruise aboard R/V PELICAN addressing the goals of two US Department of Energy funded projects: "Active Microbes Responding to Inputs from the Orinoco River Plume" (AM) and "Ecology and Genomics of CO_2 Fixation in Major River Plumes" (E&G). Both projects are targeted towards understanding the role of large river plume microbial communities in carbon cycling but, while AM focuses on the heterotrophic component of the plankton, E&G focuses on the autotrophic (photosynthetic) component. This complementarity allowed for a comprehensive assessment of the activity of communities involved in biological fixation of inorganic carbon and those involved in its release. The AM component was executed during legs 1 and 2 of the cruise along the salinity gradient of the Orinoco River plume (ORP) from the island of Puerto Rico in the NW Caribbean Basin south to the Gulf of Paria at the river mouth. Research goals for the AM component of the cruise were to identify the members of the heterotrophic community active in C and N recycling in the perimeter of the Orinoco River Plume (ORP) in the Caribbean basin, to assess their spatial distribution, to quantify their metabolic activity, and to correlate these parameters to plume properties such as salinity, optical diffuse attenuation, organic matter content and phytoplankton biomass. A multi-pronged approach investigating ribosome fingerprinting, bromodeoxyuridine (a thymidine analogue) incorporation, ^{15}N uptake experiments, stable isotope probing, oxygen untilization and overall electron transport activity (ETS) was used to elucidate the response of the microbial community to terrestrial input and to identify the active bacterial/ phytoplankton/microzooplankton in the ORP.

The E&G component was executed during the 2nd leg of the cruise between T&T and PR. The goal of this component was to characterize phototrophic carbon (C) fixation and the

genomic composition and gene activity of photosynthetically active phytoplankton in the ORP with a view to establishing whether this (and other major plumes) constitute significant C sinks. Our research plan was designed to address the following questions:

1. Do major river plumes outgas or take up CO2?

By accurately measuring all forms of DIC in the plume, CO2 above and windspeed, an estimate of ΔpCO2 can be made.

2. What groups of phytoplankton are ultimately responsible for DIC uptake in plumes?

To be answered by a combination of size fractionation and ^{14}C incorporation studies as well as rbcL gene expression analysis using clade-specific probes and Real Time PCR.. rbcL libraries will be constructed from mRNA obtained from both MRP and Amazon/Orinoco plume environments and compared.

3. What forms of nitrogen are fueling this DIC uptake?

Regenerated ammonium seems to be a major nitrogen source in the ORP. Through size fractionation studies, the uptake of ^{15}N nitrate, ammonium, urea, and amino acids were measured to characterize partitioning between phototrophic types for the nitrogen utilized (ie. patterns of nitrogen uptake between large diatoms and autotrophic picoplankton [*Synechococcus*]).

Previous work in the ORP indicated that substantial changes in optical properties and community composition occur in this salinity range. Moreover, recent work in the Amazon River plume (Subramaniam, Capone personal communication) indicates that highest production rates and phytoplankton biomass are to be found in “mesotrophic areas” along ocean/river plume fronts well offshore from the plume source waters in the salinity range 31-34. Such environments are depleted in N but provided with excess P and N-fixing organisms prevail, particularly

diatom/*Richelia* associations but also *Trichodesmium*. Such environments may experience increased DON fluxes and consequently enhanced microbial activity. We devoted particular attention to finding and characterizing such environments.

OPERATIONS AREA AND SCHEDULE

Operations took place in the eastern Caribbean Basin in an area roughly bounded by the coordinate 10 to 18^{o} N, 60-67^{o} W. Leg 1 of the cruise, Puerto Rico to Trinidad & Tobago, (AM) was implemented between September 18 and 23, 2006. Thirteen stations were occupied. Leg 2, Trinidad & Tobago to Puerto Rico, (AM and E&G) was implemented September 26 – October 2, 2006. Eight stations were occupied of which one (station 4) was a diel study in which 6 sampling points were taken alongside a Lagrangian drifter deployed within the plume over the course of a day (first at 06:57; last at 22:40). Station locations for both cruises appear in figure 1.

PRELIMINARY FINDINGS

The ORP was at the apex of its spread during the time of the cruise. The low salinity/high chlorophyll plume was first encountered at 15^{o} N 62.75^{o} W a distance of 260 nautical miles from the Dragon's Mouth at the Gulf of Paria. Low salinity high chlorophyll waters were apparent outside the island arc in the Atlantic Ocean only to the south of the island of Grenada. Satellite ocean color imagery at this time depicted the plume of high chlorophyll water extending inside the Antillean island arc as far north as the island of Guadeloupe at 16^{o} N with sharp boundaries to the east along the inside of the island arc and to the west along about 63.5 (see below).

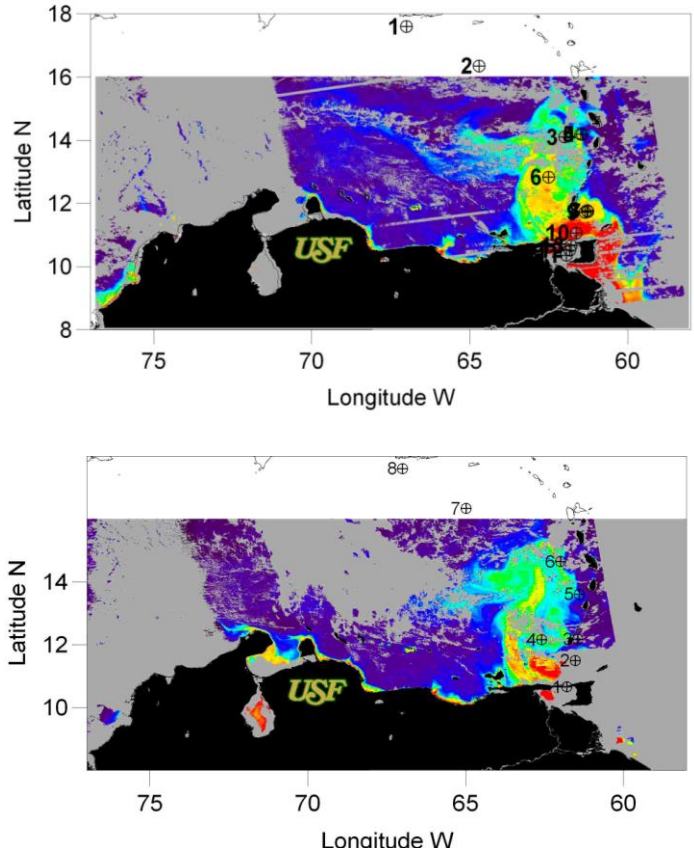


Figure 1. Top - Leg 1 station positions on ocean color image for 09/19/06. Bottom - Leg 2 station positions on ocean color image for 09/26/06.

Surface salinity varied between a low of 11.5 within the Gulf of Paria and a maximum of 35 outside the influence of the plume. No significant correlation was found between salinity and chlorophyll. Plots of surface salinity versus latitude for both legs of the cruise are presented in below.

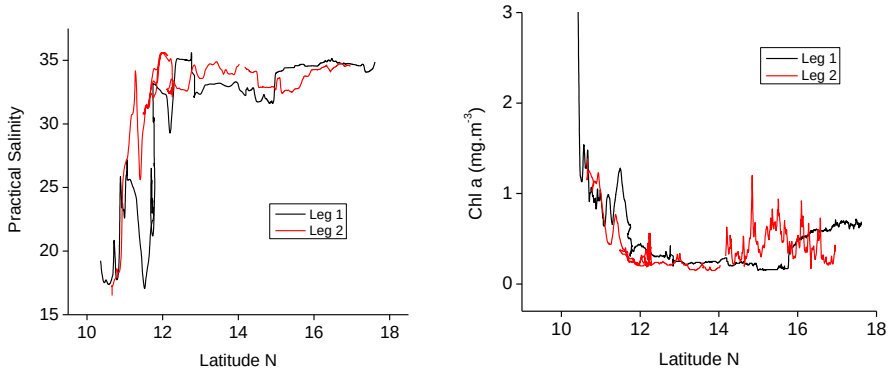


Figure 2. Surface salinity (left) and chlorophyll content (right) along the cruise tracks.

Salinity profiles obtained by CTD/rosette cast show that plume influence extends at most to a depth of ca 40 m. However, the largest salinity gradients are found at depths less that 20 m. Analogous results were found for phytoplankton Chl a and diffuse light attenuation (Kd) with high Chl a and Kd near the river source in low salinity surface waters.

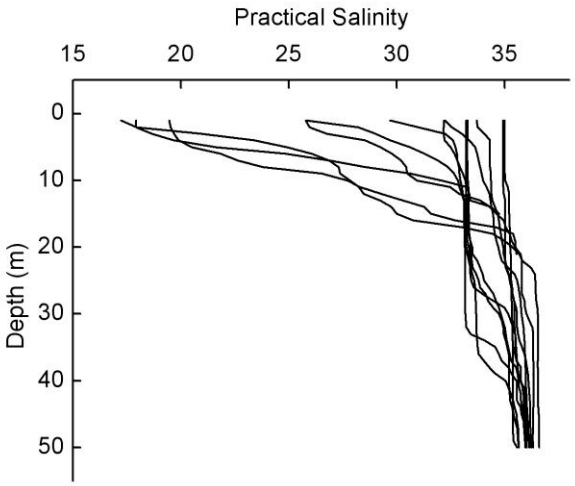
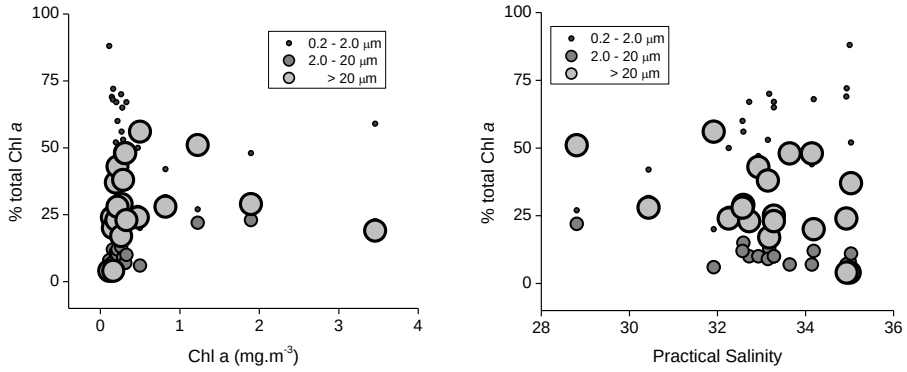


Figure 3. Salinity profiles of representative stations along the latitudinal gradient depicting the depth of influence of the plume.

Phytoplankton size class distribution

Phytoplankton size class distribution was determined by sequential filtration into 3 size classes, 02. – 2.0 μm, 2.0 – 20 μm and >20 μm, representing respectively the nano-, pico- and micro-plankton fractions. Size class distribution responded strongly to the total amount of chlorophyll and to the environmental parameters salinity and optical diffuse attenuation (K_D). Nanoplankton abundance decreased proportionally with increases in total chlorophyll and with decreases in salinity and K_D. The pico- and micro-plankton fractions exhibited opposite behavior. The ORP exhibits low salinity and high K_D and chlorophyll content, so the phytoplankton community of the plume is characterized by populations of large cell species while the oceanic high salinity waters of the Caribbean favor the picoplankton component. As the Orinoco River originates at the southeastern extreme of the Caribbean Basin, this distribution is also apparent along the latitudinal gradient (figure 3).



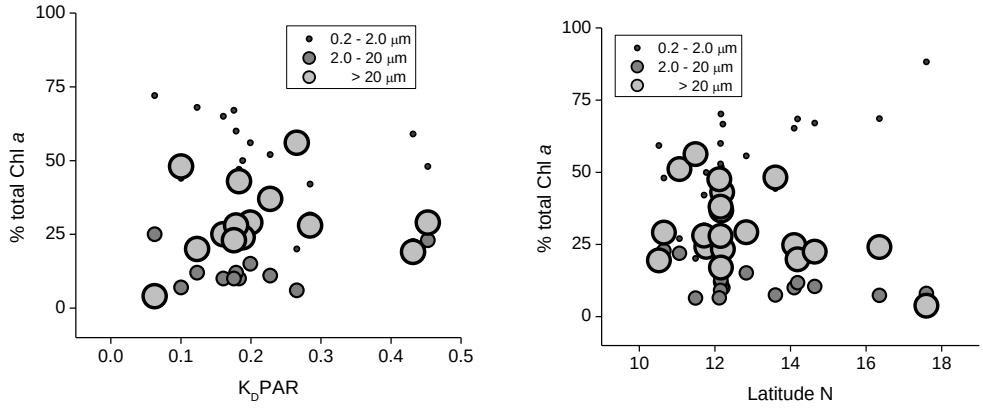
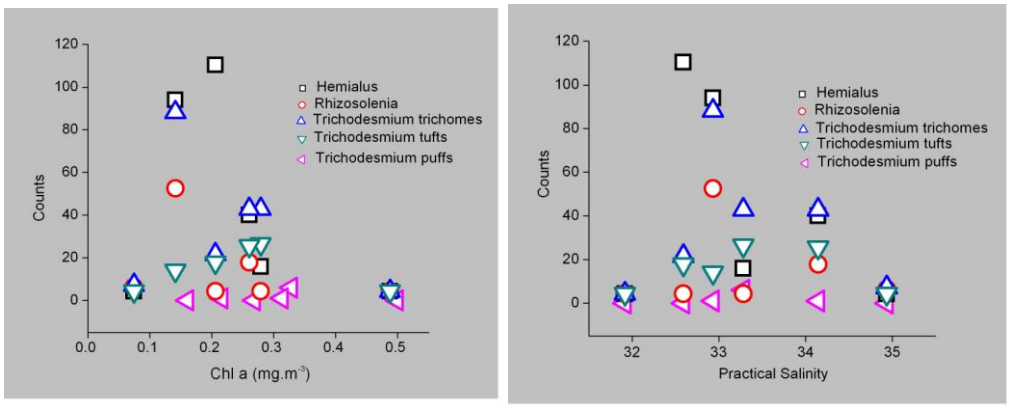


Figure 3. Size class distribution in relation to total chlorophyll content, salinity, diffuse attenuation and latitude.

Diazotroph abundance and activity

Distribution of microplanktonic autotrophic diazotrophs, those members of the larger size class of the photosynthetic phytoplankton community capable of fixing molecular nitrogen, conformed well to the distribution observed in the Amazon River Plume by Subramaniam et al. Near surface abundances of *Trichodesmium, Rhizosolenia* and *Hemiaulus* were low at low and at high salinities but increased substantially in the intermediate salinity range of 32-34 (figure 4). Nitrogenase activity, correlated to the capacity for nitrogen fixation, exhibited a similar pattern with maximum rates in this salinity range (figure 4).



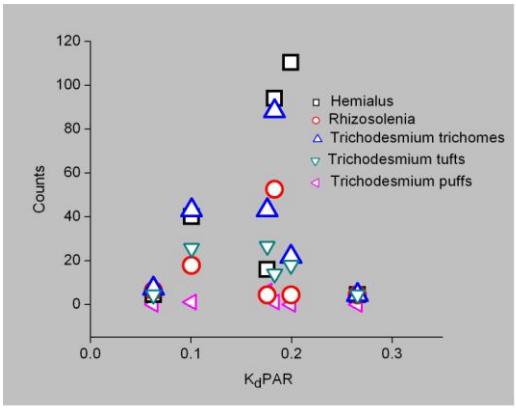


Figure 4. Relationship between abundance of microplanktonic autotrophic diazotrophs and total chlorophyll, salinity and diffuse attenuation.

Although few data points were collected, nitrogenase activity conformed well to the distribution pattern of diazotrophic microplankton with higher rates observed in the salinity range 32-34 (figure 5). These observations further corroborate the arguments of Subramaniam et al. for significant rates of nitrogen fixation in the far-field of major tropical river plumes.

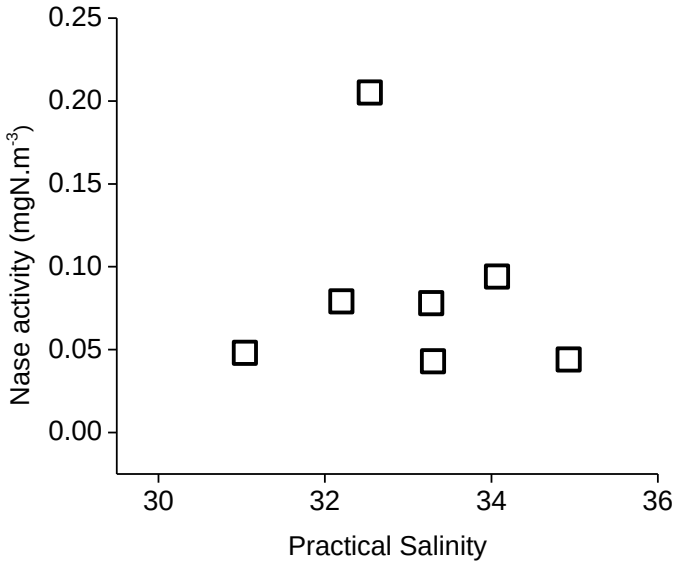


Figure 5. Relationship between nitrogenase activity and salinity.

ETS Activity

We measured ETS activity as INT reduction of cell-free extracts in the presence of exogenous substrate. We also measured oxygen consumption in darkened BOD bottles using the Winkler assay. We find an overall strong correlation between ETS activity and respiration (figure 6).

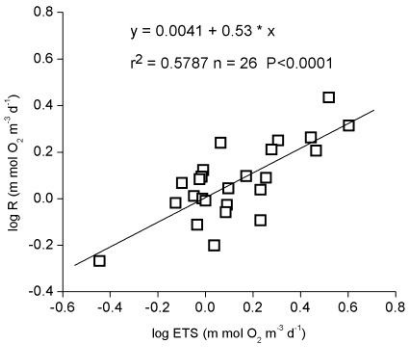


Figure 6. Relationship between ETS activity and microbial respiration rates.