Nitrogen cycling in East Matagorda Bay

Mark J. McCarthy and Wayne S. Gardner
(Appendix by Dmitri Sobolev)

University of Texas Marine Science Institute
750 Channel View Drive
Port Aransas, Texas 78373

Final report submitted to:

The Texas Water Development Board

Contract # 2001-483-387

October 2002

1. Introduction.

As a part of continuing efforts to investigate nitrogen (N) cycling in Texas bays and estuaries, denitrification, N fixation, dissimilatory nitrate reduction to ammonium (DNRA), and sediment oxygen demand (SOD) were measured in East Matagorda Bay, Texas. East Matagorda Bay is located between Matagorda and Galveston Bays and has little direct freshwater inflow (Figure 1). The Colorado River flows directly to the Gulf of Mexico, and its delta separates Matagorda and East Matagorda Bays. East Matagorda Bay is oriented in a southwest-northeast direction for ~20 miles with a width of ~4 miles. The bay is enclosed on all sides, and the only access is via the Intracoastal Waterway. Few studies have focused on this shallow (maximum depth <2m), muddy bay, but a recent survey examined the effect of freshwater inflow on macrobenthos abundance (Montagna 2001).

Estuarine phytoplankton production can be limited by N availability, and sediments often are an important source of dissolved inorganic N (DIN = $NH_4^+ + NO_2^- + NO_3^-$). Denitrification transforms combined N to gaseous forms (N_2 or N_2O; Seitzinger 1988, 1990). These end-products represent unavailable nutrient sources to estuarine producers (e.g., phytoplankton, microphytobenthos, and bacteria; Howarth et al. 1988) with the exception of N-fixing organisms. Thus, denitrification may drive systems toward N limitation (Seitzinger 1990). However, DNRA conserves N in a form that is available to organisms and may help explain the persistence of Texas brown tide (TBT) in south Texas bays. The TBT organism (*Aureomonas lagunensis*) can use NH_4^+ or NO_2^- but not NO_3^- as a N source (DeYoe and Suttle 1994). The relative partitioning between denitrification and DNRA can determine the degree of available N conservation in coastal systems (Tobias et al. 2001)

The purposes of this study were to measure nutrient fluxes, denitrification, N fixation, evaluate the relative importance of NO_3^- reduction pathways (denitrification and DNRA) in N cycling, and examine environmental conditions influencing N cycling in East Matagorda Bay. A membrane inlet mass spectrometer (MIMS) for dissolved gas measurement and high performance liquid chromatography (HPLC) system for NH_4^+ isotope measurements were used to quantify denitrification, N fixation, DNRA, and SOD. Six sampling trips to East Matagorda Bay were made in June, July, and October 2001 and January, April, and July 2002. Here, we report water column measurements and incubation experiment results.

2. Study area and methods

Two stations in East Matagorda Bay were selected to measure water column characteristics (temperature, salinity, chlorophyll *a*, and dissolved oxygen using a Hydrolab® multiprobe) and conduct sediment core incubation experiments. These stations (EMB-A and EMB-F) were selected to coincide with stations examined in Montagna (2001) and are located at 28° 39.000' N, 95° 56.000' W and 28° 44.000' N, 95° 43.500' W, respectively (Figure 1). EMB-A is located in the southwest part of the bay and represents a higher salinity area in East Matagorda Bay, and EMB-F is located in the northeast part of the bay nearest the freshwater input (Montagna 2001). The macrobenthos survey found that EMB-F had the lowest biomass and abundance with these parameters increasing in a southwest direction (Montagna 2001).

Bottom water at each station was collected for nutrient analysis and sediment core incubation. Undisturbed sediment cores (7.6 cm diameter, 30 cm length; 3 per station) with bottom water were collected from a boat using a coring device equipped with a PVC pipe handle. Within 4 hours of collection, cores were transported to the laboratory, and a flow-through plunger with Teflon inlet and outlet tubes was installed over each sediment core (Lavrentyev et

al. 2000). Flow-through chambers consisted of an aerated intake water vessel, Teflon flow tubes, peristaltic pump, temperature-controlled incubation bath, and sample collection vessels. Sediment cores were placed in the incubation bath at *in situ* temperature, and bottom water from the site was passed continuously over the core surface at 1.2 ml min^{-1}. The cores were covered with aluminum foil to prevent light effects. Water column depth over the sediment was maintained at about 5 cm to give a water volume of ca. 570 ml in each core. After one day of incubation to allow steady-state conditions to develop, triplicate samples of inflow and outflow water were collected daily for dissolved gas analysis. Outflow samples also were collected for analysis of DIN compounds and ortho-phosphate (o-PO_4) via Lachat QuikChem 8000 FIA.

Addition experiments with $^{15}NO_3^-$ were conducted to provide insights about the fate of NO_3^- at the sediment-water interface. After the second day of sampling, inflow water was enriched with $^{15}NO_3^-$ (~100 μM final concentration) and concentrations of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, and $^{15}NH_4^+$ were measured in inflow and outflow waters. Three different masses of N_2 gas were produced by denitrification ($^{28}N_2$ from $^{14}NO_3^-$, $^{30}N_2$ from $^{15}NO_3^-$, and $^{29}N_2$ from $^{14}NO_3^-$ and $^{15}NO_3^-$; Nielson 1992). Dissolved N_2, O_2, and Ar were measured with MIMS using methods modified from Kana et al. 1994 (An et al. 2001a). Concentration and atom % ^{15}N for NH_4^+ were determined by HPLC (Gardner et al. 1995). Sediment flux of each compound was calculated based on the concentration difference between inflow and outflow water, flow rate, and cross-sectional area (Lavrentyev et al. 2000).

During the June and October 2001 sampling events, water column isotope dilution experiments were conducted by adding $^{15}NH_4^+$ to bay water in light and dark bottles incubated at *in situ* temperature and light conditions for ~24 hours. The amount of the $^{15}NH_4^+$ spike was 8 μM in June 2001 and 16 μM in October 2001. Samples were collected at 14 and 24 hours (June)

and 15, 19, and 22 hours (October) to determine total NH_4^+ concentration and atom % 15N using HPLC (Gardner et al. 1995). Ammonium regeneration and uptake rates were calculated from these data using the Blackburn/Caperon model (Blackburn 1979; Caperon et al. 1979). Samples also were collected for microbial food web analysis in 125 mL plastic bottles with acid Lugol's and formalin preservatives.

3. Results and Discussion

3-1. Environmental characteristics

Table 1 shows the results of water column Hydrolab measurements. The Hydrolab was sent to the manufacturer for repairs and was unavailable for the April 2002 sampling, so temperature and salinity were measured using a standard mercury thermometer and refractometer, respectively. Mean water depth at EMB-A and EMB-F were 1.5 and 1.1 m, respectively. Bottom water was oxygenated at both stations due to wind-driven mixing and shallow water depth. Mean DO was higher at EMB-F (5.44 ± 0.27 mg L^{-1}) than EMB-A (4.86 ± 0.18), and both stations exhibited slight decreases in DO with depth. Temperature differences between stations and bottom and surface water were minimal.

Bottom water salinity ranged from 15.1 to 29.5 ‰ at EMB-A and 18.5 to 27.7 ‰ at EMB-F. Average salinity in East Matagorda Bay (20.8 ‰) was higher than Sabine Lake (9 ‰; An et al. 2001b) and Galveston Bay (15 ‰; An and Joye 2001) and lower than Laguna Madre/Baffin Bay (30 ‰; An and Gardner 2000). Average salinity differences between EMB-A and EMB-F were ~3.5 ‰ with EMB-F having higher salinity at all times except July 2002. Note that significant rainfall events occurred in late June/early July 2002, and south Texas endured extensive flooding. However, the salinities at both stations during this time were at or near their

highest levels supporting the idea that East Matagorda Bay does not receive direct freshwater inflows. There may be a lag period between rain events and lower salinity in the bay with the lag period being longer if the rainfall occurs over inland areas versus coastal areas. For the July 2002 sampling after the large rain event, the salinity at EMB-F was lower than EMB-A (the only time this occurred), and bottom water salinity at EMB-F was higher than surface water salinity suggesting that freshwater inflow occurs from Caney Creek and other small creeks near the northeast corner of the bay (Montagna 2001). Lowest salinities at each station were observed in January 2002, and highest salinities occurred in July 2001 for EMB-F and July 2002 for EMB-A. Salinities at EMB-A did not vary with depth, but higher bottom water salinities were observed at EMB-F during July 2001 (25.4 ‰ at the surface vs. 27.7 ‰ at 0.9m), January 2002 (17.9 ‰ vs. 21.9 ‰), and July 2002 (23.5 ‰ vs. 24.3 ‰).

Absolute mean chlorophyll *a* concentration was higher at EMB-F (14.3 $\mu g\ L^{-1}$) than EMB-A (12.4 $\mu g\ L^{-1}$), but standard error bars overlapped between the two stations. Interestingly, chlorophyll *a* concentration was higher in bottom versus surface water at both stations, and this difference was significant at EMB-F (Figure 2). Light inhibition of primary production in surface water may explain this difference, especially given the high turbidity in East Matagorda Bay. Since East Matagorda Bay is very shallow and well-mixed, light inhibition is expected to be a factor only near the surface. Bottom water should receive light only after it is scattered by the high turbidity, thus reducing the effects of light inhibition at the bottom. With the exception of the January 2002 sampling, Secchi depths were less than 30cm (Table 1). Benthic primary production plays an important role in Galveston Bay (An and Joye 2001). Given the proximity of East Matagorda Bay to Galveston Bay and the observed higher

chlorophyll concentrations in bottom versus surface waters, benthic primary production may be important in East Matagorda Bay.

Nutrient concentrations were low at both stations (Table 2). There were no significant differences between the two sites except EMB-A (1.35 ± 0.20 μM) had higher mean o-PO_4 concentrations than EMB-F (0.80 ± 0.23 μM). Mean NO_3^- concentration was higher at EMB-F (2.11 ± 0.39 μM) than EMB-A (1.55 ± 0.30 μM) if the data from June 2001 is omitted (the only time NO_3^- concentration was <0.5 μM and NO_3^- was higher at EMB-A). Ammonium was observed at either station only in July 2001 and July 2002.

3-2. Water column NH_4^+ regeneration and uptake and microbial food web

Figure 3 summarizes water column NH_4^+ regeneration and uptake rates at EMB-A and EMB-F. Regeneration and uptake rates in light bottles were higher than dark bottles in June and October 2001. Light regeneration and uptake were higher at EMB-A versus EMB-F. Dark regeneration at EMB-F was higher than EMB-A. On average, NH_4^+ regeneration and uptake rates in East Matagorda Bay are similar to other Texas coastal systems (Table 3a; Figure 4).

Water samples were collected in June 2001 from both sites to measure microbial food web structure (Matt First and Peter Lavrentyev, University of Akron, unpublished data). Microzooplankton (MZP) biomass was 60 and 45.6 μg C L^{-1} for EMB-A and EMB-F, respectively (Table 3b). These values are similar to those measured in Corpus Christi Bay (~20 - 60 μg C L^{-1}), upper Laguna Madre (~75 μg C L^{-1}), and Nueces River (~40 μg C L^{-1}). MZP biomass in East Matagorda Bay was higher than lower Laguna Madre (~18 μg C L^{-1}), Gulf of Mexico (<10 μg C L^{-1}) and Nueces Bay (~15 μg C L^{-1}).

MZP biomass was dominated by dinoflagellates at EMB-A (>90%) and EMB-F (~75%). Nueces River and Nueces Bay also are dominated by dinoflagellates, but Corpus Christi Bay, Laguna Madre, and the Gulf of Mexico are dominated by ciliates. A site with higher MZP biomass is expected to have higher regeneration rates. This generalization holds with respect to the sites in East Matagorda Bay, but upper Laguna Madre had the highest MZP biomass, yet regeneration rates were lower than those at EMB-A.

Picoplankton biomass was higher at EMB-F (~800 μg C L^{-1}) than EMB-A (~400 μg C L^{-1}) and dominated by bacteria (>90%) at both sites (Table 3b). Picoplankton biomass in East Matagorda Bay was lower than the Nueces River (~1300 μg C L^{-1}), similar to upper Laguna Madre (~600 μg C L^{-1}), and higher than Nueces Bay (~300 μg C L^{-1}), Corpus Christi Bay (~200 μg C L^{-1}), lower Laguna Madre (~100 μg C L^{-1}), and the Gulf of Mexico (<100 μg C L^{-1}). Bacterial domination of this size-fraction was observed in all south Texas systems examined. Bacteria dominated systems are expected to have higher dark regeneration rates, and EMB-F had higher bacteria biomass and dark regeneration rates than EMB-A.

3-3. Sediment-water interface nutrient flux

Sediments in East Matagorda Bay are nutrient sources for primary producers in overlying water. Table 4 gives sediment nutrient fluxes for each sampling event. Table 5 summarizes these rates to encompass the entire study period for the two sites. Average o-PO_4 efflux rates were 4.0 ± 1.5 and 3.4 ± 1.1 μmol P m^{-2} h^{-1} for EMB-A and EMB-F, respectively (Figure 5). After $^{15}NO_3^-$ addition, o-PO_4 efflux decreased, but not significantly. The NO_2^- efflux difference between EMB-A (2.4 ± 0.9 μmol N m^{-2} h^{-1}) and

EMB-F (5.3 ± 0.9 μmol N m^{-2} h^{-1}) was significant, and $^{15}NO_3^-$ addition resulted in a 3-fold increase in NO_2^- efflux from EMB-A (8.9 ± 1.1 μmol N m^{-2} h^{-1}) and EMB-F (16.0 ± 1.7 μmol N m^{-2} h^{-1}) sediments. Average NO_3^- efflux before $^{15}NO_3^-$ addition was 8.4 ± 3.1 μmol N m^{-2} h^{-1} at EMB-A and 6.0 ± 1.0 μmol N m^{-2} h^{-1} at EMB-F. As expected, $^{15}NO_3^-$ addition resulted in large NO_3^- influx rates from the overlying water. Sediments in East Matagorda Bay also are a source of NH_4^+, and efflux rates were 27.8 ± 9.8 and 44.6 ± 13.8 μmol N m^{-2} h^{-1} for EMB-A and EMB-F, respectively, before $^{15}NO_3^-$ addition. These rates increased after $^{15}NO_3^-$ addition, but not significantly. The finding that these sediments are a nutrient source may explain why bottom water chlorophyll concentrations were higher than surface water concentrations and support the idea that benthic primary production may be important in East Matagorda Bay.

3-4. Seasonal variations of denitrification, DNRA, and SOD

Table 6a summarizes cumulative sediment-water interface process rates measured during this study. Tables 7a and 7b give average sediment-water interface process rates for each sampling event in 2001 and 2002, respectively. For SOD and Net Δ N_2, **(b)** denotes rates ***before*** $^{15}NO_3^-$ addition, and **(a)** denotes rates ***after*** the addition. DNRA, N fixation (NF), the ratio of ^{15}N denitrification to ^{14}N denitrification [DNF(15:14)], and total denitrification [DNF(tot)] are measured only after $^{15}NO_3^-$ addition. Missing data from the 2001 sampling events were due to MIMS software malfunction.

Over the course of this study (six sampling events in about one calendar year), net N_2 flux in East Matagorda Bay was not different from zero at either station, before or after $^{15}NO_3^-$ addition (Table 6a). Net N_2 gas consumption, indicative of N fixation, was observed in summer

and winter, but net N_2 production (denitrification) was observed in fall (October 2001) and spring (April 2002; Figure 6). Mean denitrification rates were higher in October 2001 (90 μmol N_2 m^{-2} h^{-1}) versus April 2002 (4.3 μmol N_2 m^{-2} h^{-1}) and higher at EMB-A (56 μmol N_2 m^{-2} h^{-1}) versus EMB-F (38 μmol N_2 m^{-2} h^{-1}) before $^{15}NO_3^-$ addition. Net N_2 flux in East Matagorda Bay was not correlated to bottom water temperature, salinity, or dissolved O_2 (Figures 7 and 8). However, N_2 flux was correlated loosely with chlorophyll *a* concentration in bottom water (Figure 8). The loose correlation was positive for EMB-A ($r^2 = 45$; $p = 0.22$) but slightly negative for EMB-F ($r^2 = 0.46$; $p = 0.21$).

Table 6b compares denitrification, DNRA, and salinity between East Matagorda Bay and other recently evaluated systems. Denitrification rates in East Matagorda Bay in fall (90 μmol N_2 m^{-2} h^{-1}) are comparable to those reported in other Texas estuaries [Sabine Lake (49 μmol N_2 m^{-2} h^{-1}; An et al. 2001b), Laguna Madre/Baffin Bay (0-265 μmol N_2 m^{-2} h^{-1}; An and Gardner 2000), and Galveston Bay (85 μmol N_2 m^{-2} h^{-1}; An and Joye 2001)]. Denitrification may be inhibited by high sulfide concentrations produced during sulfate reduction, especially in areas with high salinity (An et al. 2001a). Compared to high salinity and *expected* high sulfate reduction in Laguna Madre/Baffin Bay and low salinity and *expected* sulfate reduction in Sabine Lake, East Matagorda Bay should, and does, fall between with respect to denitrification. Molecular biology studies conducted in our laboratory suggest that N-transformation processes in East Matagorda Bay do not conform to the traditional paradigm of N being oxidized to NO_3^- before denitrification can occur. A portion of the N_2 flux seems to derive from a source other than NO_3^-. There is evidence that two separate and distinct populations of denitrifying bacteria derive N from different N pools. Experimental evidence for this possibility is detailed in the attached appendix.

Similarly, East Matagorda Bay would be expected to fall between Sabine Lake and Laguna Madre/Baffin Bay with respect to DNRA since high salinity and sulfate reduction enhances this NO_3^- reduction pathway (An and Gardner 2002). DNRA rates were 4.2 ± 0.7 and 3.0 ± 0.9 μmol N m^{-2} h^{-1} for EMB-A and EMB-F, respectively (Table 6a). As expected, DNRA rates were lower than those from Laguna Madre/Baffin Bay (12.1 – 78.6 μmol N m^{-2} h^{-1}) and higher than Sabine Lake (not different from zero; Table 6b). Highest DNRA rates were 6.0 μmol N m^{-2} h^{-1} for EMB-A during April 2002 and 6.9 μmol N m^{-2} h^{-1} for EMB-F during October 2001. Minimum DNRA rates were observed during June 2001 for EMB-A (1.6 μmol N m^{-2} h^{-1}) and EMB-F (0.4 μmol N m^{-2} h^{-1}). DNRA was not correlated with bottom water temperature or dissolved O_2 (Figures 9 and 10). Further, DNRA at EMB-F was not correlated to bottom water salinity or chlorophyll *a*. However, DNRA at EMB-A was correlated loosely with bottom water salinity ($r^2 = 0.42$; $p = 0.16$) and chlorophyll *a* ($r^2 = 0.74$; $p = 0.06$).

Negative numbers in Tables 6a and 7 for Net Δ N_2 (b) suggest that N fixation exceeded denitrification in some or all samples. N_2 flux into the sediment, presumably from N fixation, was higher in summer (41 μmol N_2 m^{-2} h^{-1}) than winter (30 μmol N_2 m^{-2} h^{-1}) and higher at EMB-A (52 μmol N_2 m^{-2} h^{-1}) than EMB-F (25 μmol N_2 m^{-2} h^{-1}). N fixation measured after $^{15}NO_3^-$ addition using the An et al. (2001a) calculations was positive only in July 2002 (4.2 and 12.6 μmol N_2 m^{-2} h^{-1} at EMB-A and EMB-F, respectively).

Highest SOD values were observed in July 2002 (Table 7) for both stations before (935 μmol O_2 m^{-2} h^{-1} and 600 μmol O_2 m^{-2} h^{-1} for EMB-A and EMB-F, respectively) and after (1440 μmol O_2 m^{-2} h^{-1} and 1140 μmol O_2 m^{-2} h^{-1} for EMB-A and EMB-F, respectively) $^{15}NO_3^-$ addition. Minimum SOD for both stations (353 and 530 μmol O_2 m^{-2} h^{-1} for EMB-A and EMB-F, respectively) was observed in January 2002. In general, SOD was higher at EMB-A versus

EMB-F, but the difference was not significant. Addition of $^{15}NO_3^-$ resulted in an 82% increase in SOD at EMB-A and 63% at EMB-F. In areas with high benthic primary production, such as Galveston Bay and, possibly, East Matagorda Bay, SOD should be considered a "net O_2 change" rather than a characteristic of remineralization activity (An and Joye 2001). SOD was not correlated with bottom water dissolved O_2 or chlorophyll *a* (data not shown). Weak correlations were observed between SOD at EMB-A and net N_2 flux ($r^2 = 0.52$; $p = 0.28$), DNRA ($r^2 = 0.56$; $p = 0.25$), and bottom water temperature ($r^2 = 0.41$; $p = 0.36$). SOD at EMB-F was not correlated with net N_2 flux or bottom water salinity, but a weak correlation was observed between SOD at EMB-F and bottom water temperature ($r^2 = 0.51$; $p = 0.28$). Stronger correlations were observed between SOD at EMB-A and bottom water salinity ($r^2 = 0.89$; $p = 0.06$) and SOD at EMB-F and DNRA ($r^2 = 0.84$; $p = 0.08$).

In summary, N fixation exceeded denitrification in East Matagorda Bay during the summer and winter dry seasons (when organic matter input from surface water runoff should be low), but denitrification exceeded N fixation in the spring and fall wet seasons (assuming high organic matter input from surface water runoff). This relationship may suggest that organic matter limitation leads to lower denitrification rates in dry seasons. East Matagorda Bay does not receive direct freshwater inputs, so most terrestrial organic matter input would occur during the wet seasons. However, rainfall along the Texas coast decreases from northeast to southwest, so the area around East Matagorda Bay receives considerably more rainfall than Laguna Madre/Baffin Bay, which also does not receive direct freshwater inputs and exhibits a similar seasonal denitrification trend (An and Gardner 2000). Low organic carbon concentrations can limit heterotrophic activity, even under favorable environmental conditions. Since denitrification

is a heterotrophic process, the data is consistent with the idea that low organic matter availability may limit denitrification rates (Koike and Sørensen 1988, Cornwell et al 1999).

4. Conclusions

1. N fixation exceeded denitrification in East Matagorda Bay in summer and winter, but denitrification exceeded N fixation in spring and fall.
2. Net N_2 fluxes ranged from –154 to 106 μmol N_2 m^{-2} h^{-1} during June 2001 to July 2002.
3. Potential DNRA rates were 0.4 – 6.9 μmol N m^{-2} h^{-1}.
4. Denitrification in spring and fall and DNRA rates for East Matagorda Bay fell between those from Sabine Lake (low salinity) and Laguna Madre/Baffin Bay (hypersaline).

5. Acknowledgements

This project was supported by the Texas Water Development Board (TWDB; Contract #2001-483-387. The authors wish to thank Dr. David Brock, TWDB, for project management. Dr. Dmitri Sobolev conducted molecular analyses and authored the Appendix. Jaime Haberer provided laboratory assistance with running nutrient samples. Dr. Soonmo An, Kyungnam University, Korea, assisted with preparation of the report. Dr. Peter Lavrentyev and Matt First, University of Akron, OH, provided microbial food web data and field assistance in June 2001. The authors also acknowledge the assistance of the UTMSI boat crew for their assistance with field operations: Captains Noe Cantu, Hayden Abel, John Turany, and Stan Dignum.

6. References

An S, Gardner WS, Kana TM (2001a) Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. Appl Env Micro 67:1171-1178.

An S, Gardner WS (2000) Nitrogen cycling in Laguna Madre and Baffin Bay. Final Report to Texas Water Development Board. 45pp.

An S, Gardner WS (2002) Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). Mar Ecol Prog Ser. 237:41-50.

An S, Joye SB (2001) Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. Limnol Oceanogr 46(1):62-74.

An S, McCarthy MJ, Gardner WS (2001b) Nitrogen cycling in Sabine Lake. Final Report to Texas Water Development Board. 24pp.

Blackburn TH (1979) Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N-NH_4^+ dilution technique. Appl Env Microbio. 37(4):760-765.

Caperon J, Schell D, Hirota J, Laws E (1979) Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. Mar Biol 54:33-40.

Cornwell JC, Kemp WM, Kana TM (1999) Denitrification in coastal ecosystems: methods, environmental controls and ecosystem level controls, a review. Aquat Ecol 33:41-54.

DeYoe HR, Suttle CA (1994) The inability of the Texas brown tide alga to use nitrate and the role of nitrogen in the initiation of a persistent bloom of this organism. J. Phycol. 30:800-806.

Gardner WS, Bootsma HA, Evans C, St. John PA (1995) Improved chromatographic analysis of ^{15}N: ^{14}N ratios in ammonium or nitrate for isotope addition experiments. Mar Chem 48:271-382.

Howarth RW, Marino R, Lane J (1988) Nitrogen fixation in freshwater, estuarine and marine ecosystems. I. Rates and Importance. Limnol Oceanogr 33:669- 687.

Kana TM, Darkangelo C, Hunt MD, Oldham JB, Bennett GE, Cornwell JC (1994) Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Anal Chem 66(23):4166-4170.

Koike I, Sørensen, J (1988) Nitrate Reduction and Denitrification in Marine Sediments, In Blackburn, T. H. and Sørensen, J. (eds.), Nitrogen Cycling in Coastal Marine Environments. Wiley.

Lavrentyev P, Gardner WS, Yang L (2000) Effects of the Zebra mussel on microbial composition and nitrogen dynamics at the sediment-water interface in Saginaw Bay, Lake Huron. Aquat Microb Ecol 21:187-194.

Montagna PA (2001) Effect of freshwater inflow on macrobenthos productivity in minor bay and river-dominated estuaries. Final report to the Texas Water Development Board/University of Texas Marine Science Institute Technical Report TR/01-002. 59pp.

Nielson LP (1992) Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microb Ecol 86:357-362.

Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystem: Ecological and geochemical significance. Limnol Oceanogr 33:702-724.

Seitzinger SP (1990) Denitrification in aquatic sediments. 301-322. *In* Revsbech, N.P. and Sørensen, J. [eds.], Denitrification in soil and sediment. FEMS Symposium No. 56. Plenum Press.

Tobias CR, Anderson IC, Canuel AC, Macko SA (2001) Nitrogen cycling through a fringing marsh-aquifer ecotone. Mar Ecol Prog Ser 210:25-39.

Table 1. Water quality characteristics of East Matagorda Bay

Sampling Date	Station	Depth meters	Temp. °C	Salinity ppt	Diss. O_2 mg/L	Chl a µg/L	Secchi cm
19-Jun-01	EMB-A	0.52	29.14	17.94	4.73	6.5	13
		1.18	29.10	17.96	4.61	7.2	
		1.23	29.12	17.94	4.65	7.0	
		1.24	29.13	18.02	4.66	7.0	
	EMB-F	0.63	29.91	19.68	5.23	15.9	10
		0.94	29.88	19.63	5.07	17.1	
		1.01	29.49	19.61	4.90	17.9	
30-Jul-01	EMB-A	0.46	29.60	22.27	3.83	14.3	7.6
		0.83	29.59	22.25	3.76	14.9	
		1.38	29.54	22.23	3.71	16.6	
		1.45	29.51	22.21	3.67	20.9	
	EMB-F	0.40	30.24	25.44	4.19	9.4	15.2
		0.84	30.22	27.73	3.70	12.2	
		0.88	30.27	27.67	3.94	11.0	
3-Oct-01	EMB-A	0.55	23.12	17.12	4.81	21.3	22.9
		0.81	23.11	17.14	4.79	20.4	
		0.88	23.11	17.08	4.77	22.3	
		1.40	23.09	16.97	4.73	20.2	
		1.57	23.09	17.07	4.79	22.4	
	EMB-F	0.47	23.67	18.61	4.89	7.1	15.2
		0.67	23.67	18.57	4.80	7.9	
		0.88	23.65	18.56	4.78	7.4	
		1.07	23.65	18.55	4.73	7.8	
		1.16	23.64	18.53	4.70	9.3	
14-Jan-02	EMB-A	0.58	14.29	15.13	5.72	2.6	91.4
		0.86	14.29	15.04	5.63	2.5	
		1.10	14.28	15.10	5.68	2.4	
		1.37	14.28	15.10	5.69	2.7	
	EMB-F	0.46	14.24	17.86	6.06	7.9	45.7
		0.69	14.24	17.82	6.05	8.9	
		0.96	14.45	21.73	5.87	10.8	
		1.03	14.50	21.94	5.54	23.3	
1-Apr-02	EMB-A		23.00	25.00			22.9
	EMB-F		23.00	25.50			15.2
8-Jul-02	EMB-A	0.37	30.38	29.62	20.00	10.2	15.2
		1.36	30.37	29.61	6.19	11.5	
		1.67	30.37	29.45	5.98	14.7	
	EMB-F	0.43	30.50	23.50	7.86	16.1	7.6
		0.59	30.49	23.60	7.55	26.9	
		0.80	30.48	23.61	7.41	30.0	
		1.18	30.37	24.29	6.05	25.1	

Table 2. Nutrient concentrations in East Matagorda Bay
N/D = not detected (detection limit ~ 0.01 μM)

Sampling Date	Station	o-PO_4 μM	NH_{4+} μM	NO_2^- μM	NO_3^- μM
19-Jun-01	EMB-A	1.40	N/D	0.17	0.27
	EMB-F	0.57	N/D	0.05	0.13
30-Jul-01	EMB-A	1.22	2.38	0.19	2.06
	EMB-F	0.57	1.61	0.17	2.72
3-Oct-01	EMB-A	2.06	N/D	0.33	1.62
	EMB-F	1.03	N/D	0.16	1.76
14-Jan-02	EMB-A	0.95	N/D	0.40	1.31
	EMB-F	0.51	N/D	0.41	1.73
1-Apr-02	EMB-A	0.76	N/D	0.41	2.22
	EMB-F	0.30	N/D	0.33	3.26
8-Jul-02	EMB-A	1.71	1.51	0.02	0.53
	EMB-F	1.83	1.23	0.14	1.07

Table 3a. Comparison of light (L) and dark (D) NH_4^+ regeneration and uptake rates from various south Texas coastal systems. CCB = Corpus Christi Bay; ULM = upper Laguna Madre; LLM = lower Laguna Madre; BB = Baffin Bay; GOM = Gulf of Mexico; NR = Nueces River; NB = Nueces Bay; EMB = East Matagorda Bay; n = number of measurements

System	n	Reg (L)	Reg (D)	Uptake (L)	Uptake (D)
CCB	7	0.139	0.093	0.471	0.204
SE		0.047	0.018	0.086	0.088
ULM	4	0.225	0.253	2.379	1.931
SE		0.070	0.051	1.177	1.074
LLM	3	0.229	0.158	1.372	1.052
SE		0.121	0.098	0.842	0.575
BB	6	0.322	0.276	2.168	1.564
SE		0.121	0.073	0.515	0.304
GOM	4	0.021	0.021	0.038	0.014
SE		0.007	0.005	0.013	0.002
NR	3	0.335	0.206	1.288	0.577
SE		0.008	0.038	0.509	0.292
NB	2	0.098	0.160	0.640	0.156
SE		0.020	0.085	0.112	0.071
EMB-A	2	0.493	0.150	1.053	0.232
SE		0.122	0.023	0.322	0.095
EMB-F	2	0.306	0.213	0.759	0.236
SE		0.051	0.015	0.083	0.058

Table 3b. Microbial food web components in East Matagorda Bay versus other south Texas systems. MZP = microzooplankton (i.e. dinoflagellates and ciliates); Pico = picoplankton (i.e. bacteria and picophytoplankton); abundances in μg C L-1.

System	MZP	Pico
EMB-A	60	400
EMB-F	45.6	800
CCB	20-60	200
ULM	75	600
LLM	18	100
GOM	<10	<100
NR	40	1300
NB	15	300

Table 4. Sediment nutrient fluxes (μmol m^{-2} h^{-1}) before and after $^{15}NO_3^-$ addition. Negative values indicate flux into sediment.

Date	Station	Time	o-PO_4	NH_4^+	NO_2^-	NO_3^-
19-Jun-01	EMB-A	before	5.32	30.52	5.26	13.91
		se	0.61	26.96	0.77	8.89
		after	-0.24	38.35	7.70	-130.35
		se	1.14	16.12	0.66	54.76
	EMB-F	before	1.27	32.40	7.96	6.67
		se	2.33	32.98	5.26	5.71
		after	3.97	51.44	9.92	-161.09
		se	0.95	12.25	0.98	92.32
30-Jul-01	EMB-A	before	-1.30	0.00	2.38	1.32
		se	2.09	0.00	2.33	0.11
		after	7.64	58.13	13.65	-102.10
		se	7.72	5.03	1.71	72.67
	EMB-F	before	-1.51	0.00	3.31	4.34
		se	0.40	0.00	1.88	1.32
		after	4.39	29.83	14.02	41.05
		se	5.57	8.42	4.50	45.21
3-Oct-01	EMB-A	before	11.72	13.30	-0.95	5.87
		se	6.32	12.48	5.24	1.01
		after	2.72	29.77	4.50	-68.35
		se	2.75	1.61	0.43	28.41
	EMB-F	before	5.98	48.61	2.96	2.28
		se	7.14	39.03	1.75	2.17
		after	-1.66	62.10	18.22	-146.22
		se	1.49	20.71	4.59	112.29
14-Jan-02	EMB-A	before	-1.77	0.57	-0.66	-2.65
		se	2.62	0.57	3.04	5.77
		after	-0.51	-2.86	3.10	-109.76
		se	2.24	13.40	0.70	17.32
	EMB-F	before	7.41	29.88	2.70	8.04
		se	5.29	5.65	0.58	4.23
		after	-2.38	22.76	12.41	-155.02
		se	2.24	18.00	2.78	35.32
1-Apr-02	EMB-A	before	3.94	30.91	1.30	4.29
		se	1.14	12.08	1.03	2.49
		after	2.06	60.85	10.16	-98.08
		se	0.40	7.41	2.12	22.91
	EMB-F	before	0.24	19.84	3.99	3.25
		se	0.24	3.11	0.61	3.31
		after	1.08	47.12	12.04	-59.75
		se	0.26	2.86	0.77	37.75
8-Jul-02	EMB-A	before	6.32	91.18	7.06	27.43
		se	2.09	7.42	2.35	7.27
		after	1.39	55.63	14.45	-158.48
		se	1.27	8.87	0.30	23.96
	EMB-F	before	6.85	136.62	10.69	11.69
		se	1.09	29.30	4.87	2.65
		after	4.00	61.07	29.31	-27.51
		se	1.06	37.74	4.32	37.21

Table 5. Cumulative sediment nutrient fluxes (μmol m^{-2} h^{-1}) before and after $^{15}NO_3^-$ addition

Station	Time	o-PO_4	NH_4^+	NO_2^-	NO_3^-
EMB-A	before	4.04	27.75	2.40	8.36
	se	1.46	9.80	0.93	3.13
	after	2.18	39.98	8.93	-111.19
	se	0.72	5.89	1.13	7.43
EMB-F	before	3.37	44.56	5.27	6.04
	se	1.10	13.82	0.95	1.01
	after	1.57	45.72	15.99	-84.76
	se	0.73	3.94	1.72	20.08

Table 6a. Cumulative sediment-water interface process rates
SOD = sediment O_2 demand; DNRA = dissimilatory NO_3^- reduction to NH_4^+; NF = N_2 fixation; DNF = denitrification

Station	Process	Units	Average	SE
EMB-A	SOD(b)	μmol O_2 m^{-2} h^{-1}	607.5	128.9
	SOD(a)	μmol O_2 m^{-2} h^{-1}	1105.0	168.5
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-2}	-16.0	34.0
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-2}	-15.3	42.9
	DNRA	μmol N m^{-2} h^{-3}	4.2	0.7
	NF	μmol N_2 m^{-2} h^{-2}	-14.8	10.1
	DNF(15:14)	μmol N_2 m^{-2} h^{-2}	31.8	13.2
	DNF(tot)	μmol N_2 m^{-2} h^{-2}	21.3	4.9
EMB-F	SOD(b)	μmol O_2 m^{-2} h^{-1}	556.0	15.1
	SOD(a)	μmol O_2 m^{-2} h^{-1}	908.9	115.3
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-2}	-3.9	16.2
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-2}	-19.5	47.6
	DNRA	μmol N m^{-2} h^{-3}	3.0	0.9
	NF	μmol N_2 m^{-2} h^{-2}	-7.7	10.2
	DNF(15:14)	μmol N_2 m^{-2} h^{-2}	60.2	34.7
	DNF(tot)	μmol N_2 m^{-2} h^{-2}	23.7	8.4

Table 6b. Relationship between salinity, denitrification, and DNRA in some south Texas systems. Salinity in ppt; DNF = denitrification in μmol N_2 m^{-2} h^{-1}; DNRA = dissimilatory NO_3^- reduction to NH_4^+ in μmol N m^{-2} h^{-1}. N/D = no data.

System	Salinity	DNF	DNRA
East Matagorda Bay	21	90	3.6
Sabine Lake	9	49	0
Galveston Bay	15	85	N/D
Laguna Madre/Baffin Bay	30	0-265	12-79

Table 7a. Sediment-water interface process rates (2001)
SOD = sediment O_2 demand; DNRA = dissimilatory NO_3^- reduction to NH_4^+; NF = N_2 fixation; DNF = denitrification

Station	Process	Units	19-Jun-01	SE	30-Jul-01	SE	3-Oct-01	SE
EMB-A	SOD(b)	μmol O_2 m^{-2} h^{-1}			459.4	45.8		
	SOD(a)	μmol O_2 m^{-2} h^{-1}						
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-1}	-154.3	104.5	-18.4	7.0	105.5	11.4
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-1}	-115.5	5.6	-175.6	16.6	90.9	25.3
	DNRA	μmol N m^{-2} h^{-1}	1.6	1.2	4.7	0.8	5.4	2.6
	NF	μmol N_2 m^{-2} h^{-1}						
	DNF(15:14)	μmol N_2 m^{-2} h^{-1}						
	DNF(tot)	μmol N_2 m^{-2} h^{-1}						
EMB-F	SOD(b)	μmol O_2 m^{-2} h^{-1}			548.5	10.3		
	SOD(a)	μmol O_2 m^{-2} h^{-1}						
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-1}	-31.5	134.1	-16.0	5.9	73.4	15.8
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-1}	-97.0	13.9	-217.2	15.0	103.0	53.9
	DNRA	μmol N m^{-2} h^{-1}	0.4	0.3	2.0	0.5	6.9	1.3
	NF	μmol N_2 m^{-2} h^{-1}						
	DNF(15:14)	μmol N_2 m^{-2} h^{-1}						
	DNF(tot)	μmol N_2 m^{-2} h^{-1}						

Table 7b. Sediment-water interface process rates (2002)

Station	Process	Units	14-Jan-02	SE	1-Apr-02	SE	8-Jul-02	SE
EMB-A	SOD(b)	μmol O_2 m^{-2} h^{-1}	352.8	22.2	683.2	56.3	934.6	7.5
	SOD(a)	μmol O_2 m^{-2} h^{-1}	894.6	41.2	982.3	40.9	1438.1	4.2
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-1}	-29.6	11.2	7.0	5.2	-6.2	1.5
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-1}	42.4	8.5	41.5	5.6	24.5	3.2
	DNRA	μmol N m^{-2} h^{-1}	2.3	1.0	6.0	1.9	5.4	5.1
	NF	μmol N_2 m^{-2} h^{-1}	-30.3	7.0	-18.3	3.4	4.2	2.8
	DNF(15:14)	μmol N_2 m^{-2} h^{-1}	33.5	11.0	53.8	9.4	8.1	0.6
	DNF(tot)	μmol N_2 m^{-2} h^{-1}	12.1	2.2	23.2	4.3	28.7	2.6
EMB-F	SOD(b)	μmol O_2 m^{-2} h^{-1}	529.9	47.1	545.9	3.5	599.5	16.3
	SOD(a)	μmol O_2 m^{-2} h^{-1}	804.1	29.1	783.4	63.8	1139.2	111.1
	Net Δ N_2(b)	μmol N_2 m^{-2} h^{-1}	-30.0	10.1	1.5	1.7	-21.0	9.4
	Net Δ N_2(a)	μmol N_2 m^{-2} h^{-1}	33.1	5.5	33.4	5.9	27.6	9.9
	DNRA	μmol N m^{-2} h^{-1}	2.3	1.1	2.6	1.7	4.1	3.1
	NF	μmol N_2 m^{-2} h^{-1}	-15.8	4.4	-19.8	4.0	12.6	5.3
	DNF(15:14)	μmol N_2 m^{-2} h^{-1}	21.3	4.7	29.9	5.3	129.3	99.9
	DNF(tot)	μmol N_2 m^{-2} h^{-1}	17.3	1.4	13.6	2.0	40.3	7.6

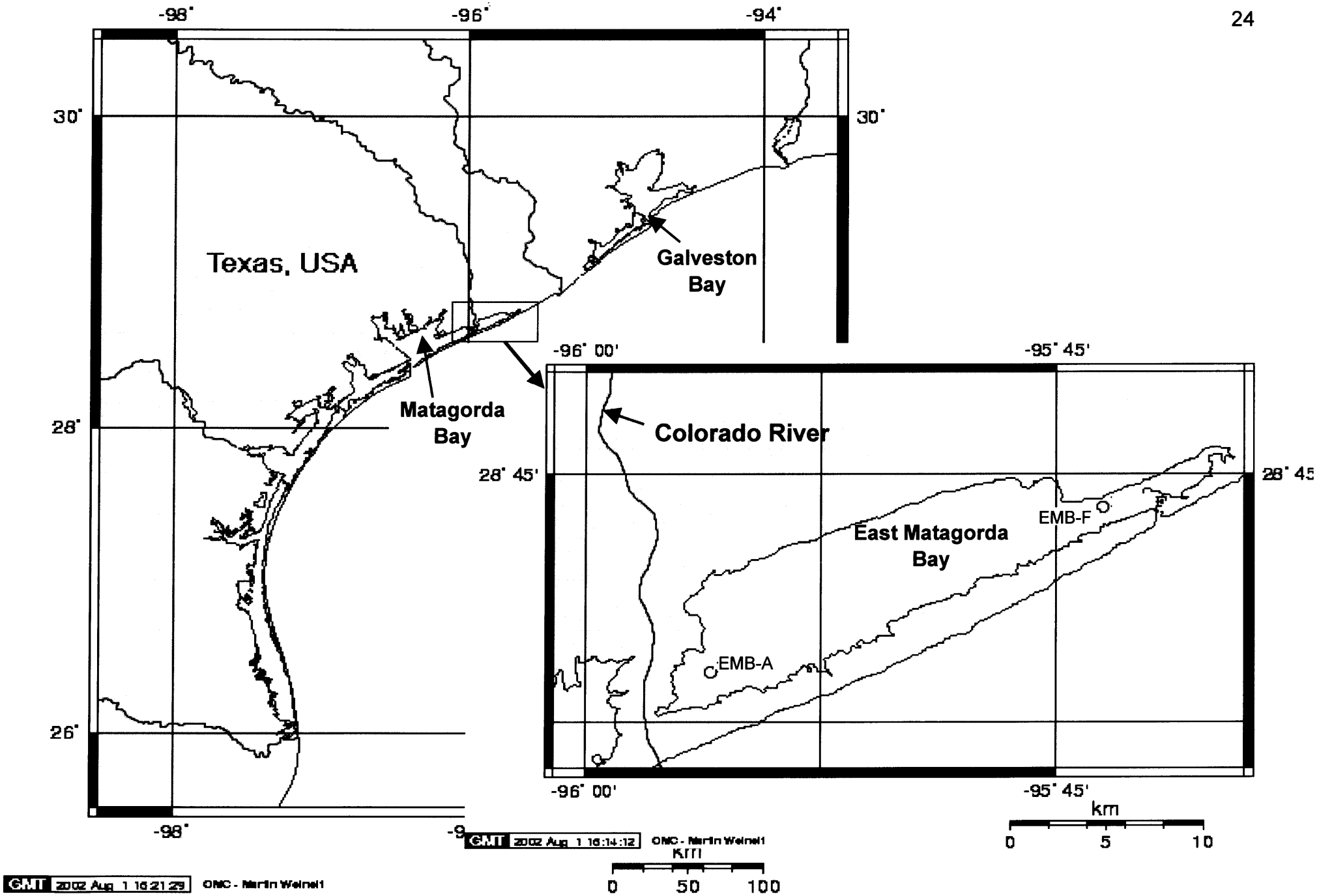


Figure 1. Location of sampling sites in East Matagorda Bay

Figure 2. Surface and bottom water chlorophyll *a* concentrations

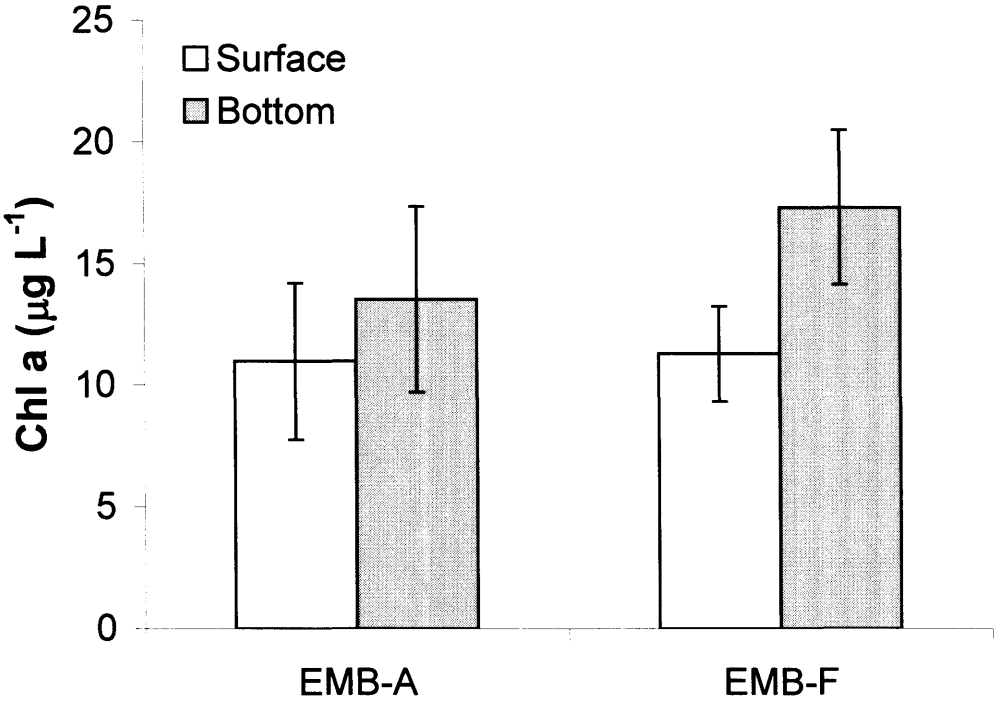


Figure 3. Ammonium regeneration (d) and uptake (I) rates (μmol N L^{-1} h^{-1}) in East Matagorda Bay

Sampling Date	Station	Light/Dark	d	SE	I	SE
19-Jun-01	EMB-A	L	0.371	0.004	0.731	0.004
		D	0.127	0.016	0.137	0.022
	EMB-F	L	0.356	0.008	0.676	0.054
		D	0.228	0.022	0.294	0.016
3-Oct-01	EMB-A	L	0.614	0.182	1.374	0.217
		D	0.173	0.007	0.327	0.010
	EMB-F	L	0.255	0.023	0.841	0.016
		D	0.197	0.007	0.178	0.012

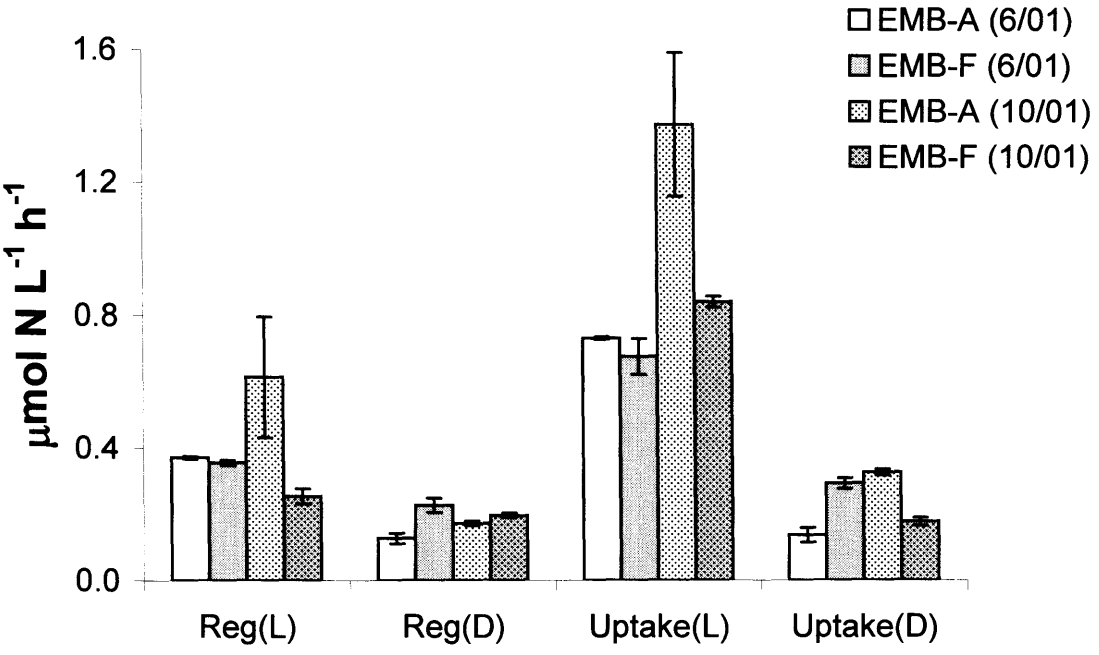


Figure 4. Ammonium regeneration and uptake in East Matagorda Bay compared to other Texas coastal systems (CCB = Corpus Christi Bay; ULM = Upper Laguna Madre; LLM = Lower Laguna Madre; BB = Baffin Bay; GOM = Gulf of Mexico; NR = Nueces River; NB = Nueces Bay)

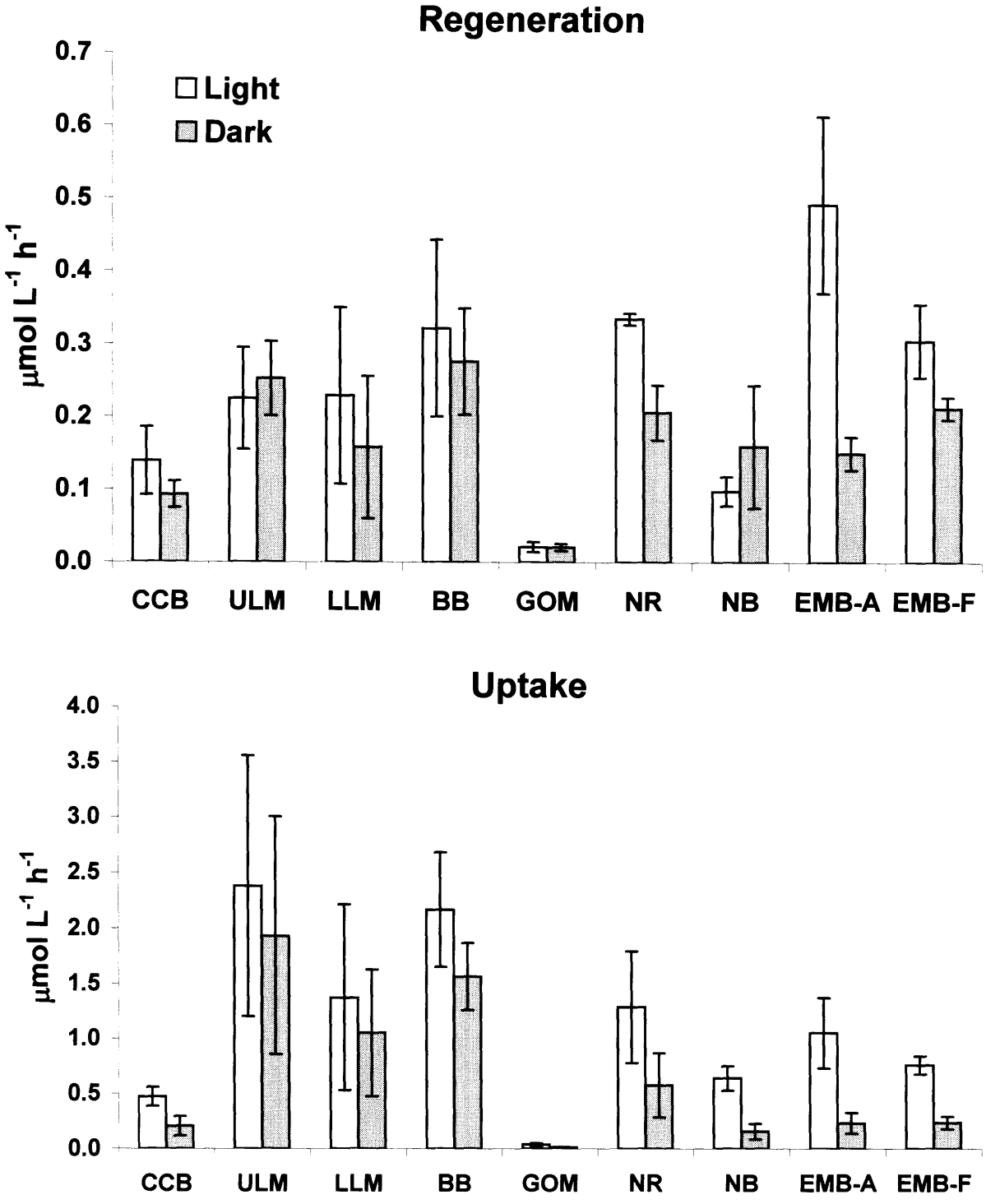


Figure 5. Sediment nutrient fluxes in East Matagorda Bay before and after $^{15}NO_3^-$ addition

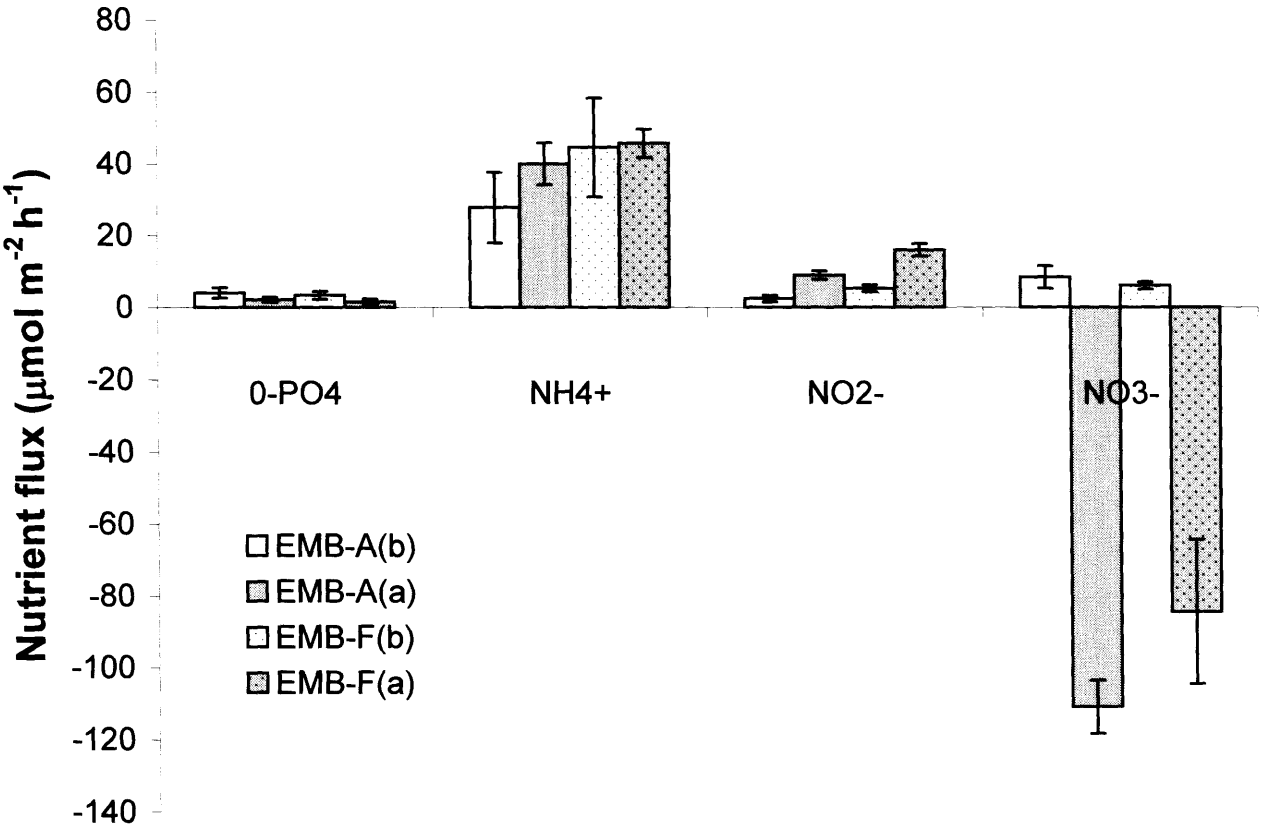


Figure 6. Net N_2 flux in East Matagorda Bay

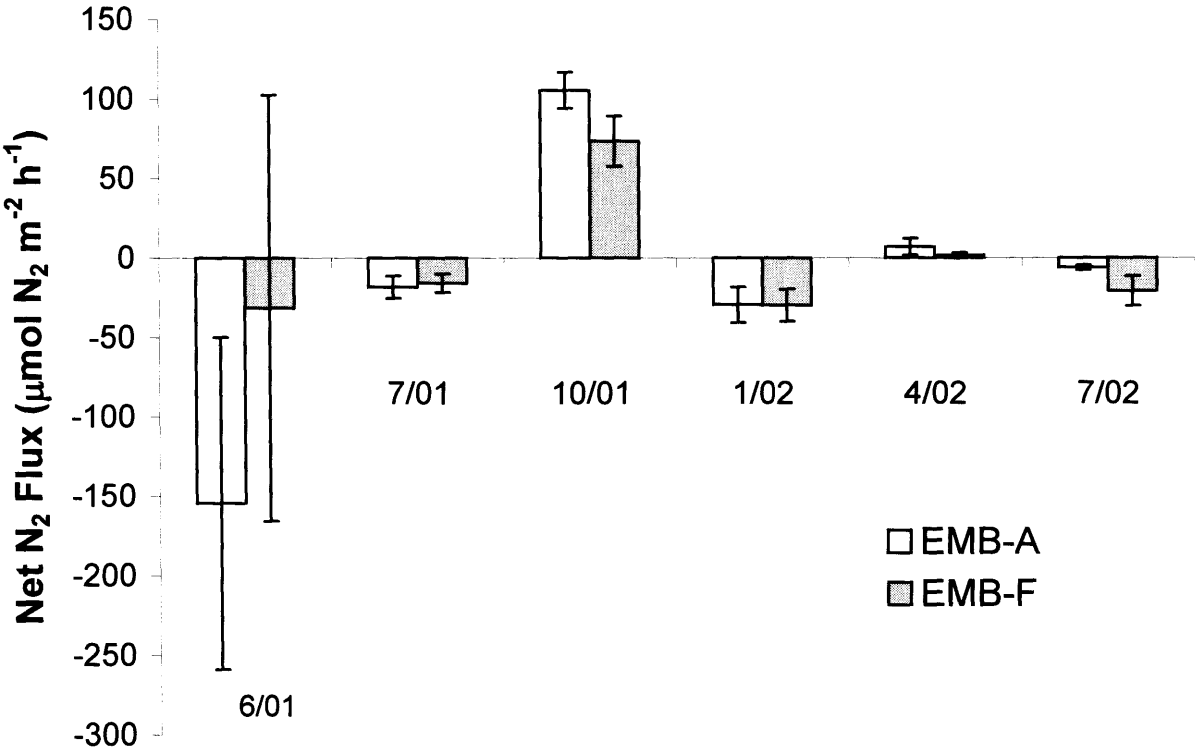
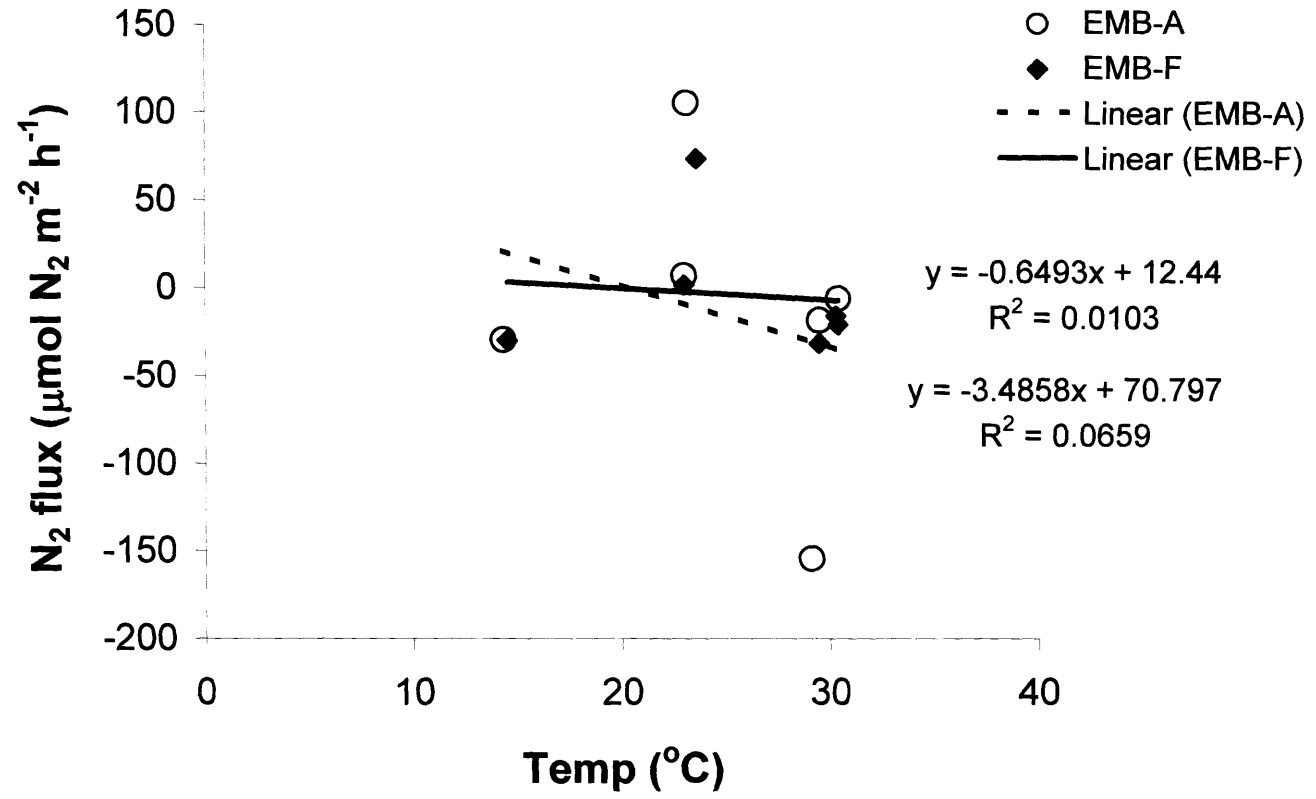


Figure 7. N_2 Flux vs. temperature and salinity



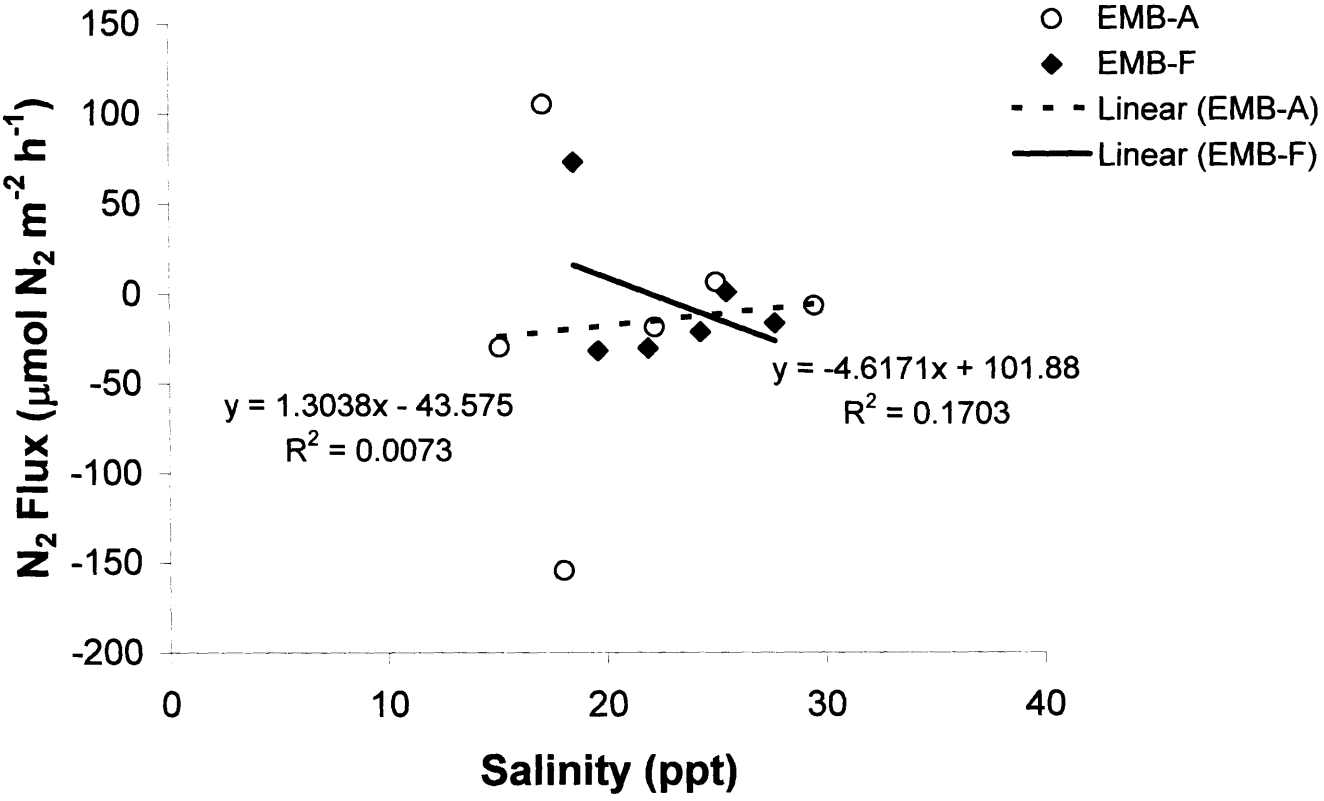
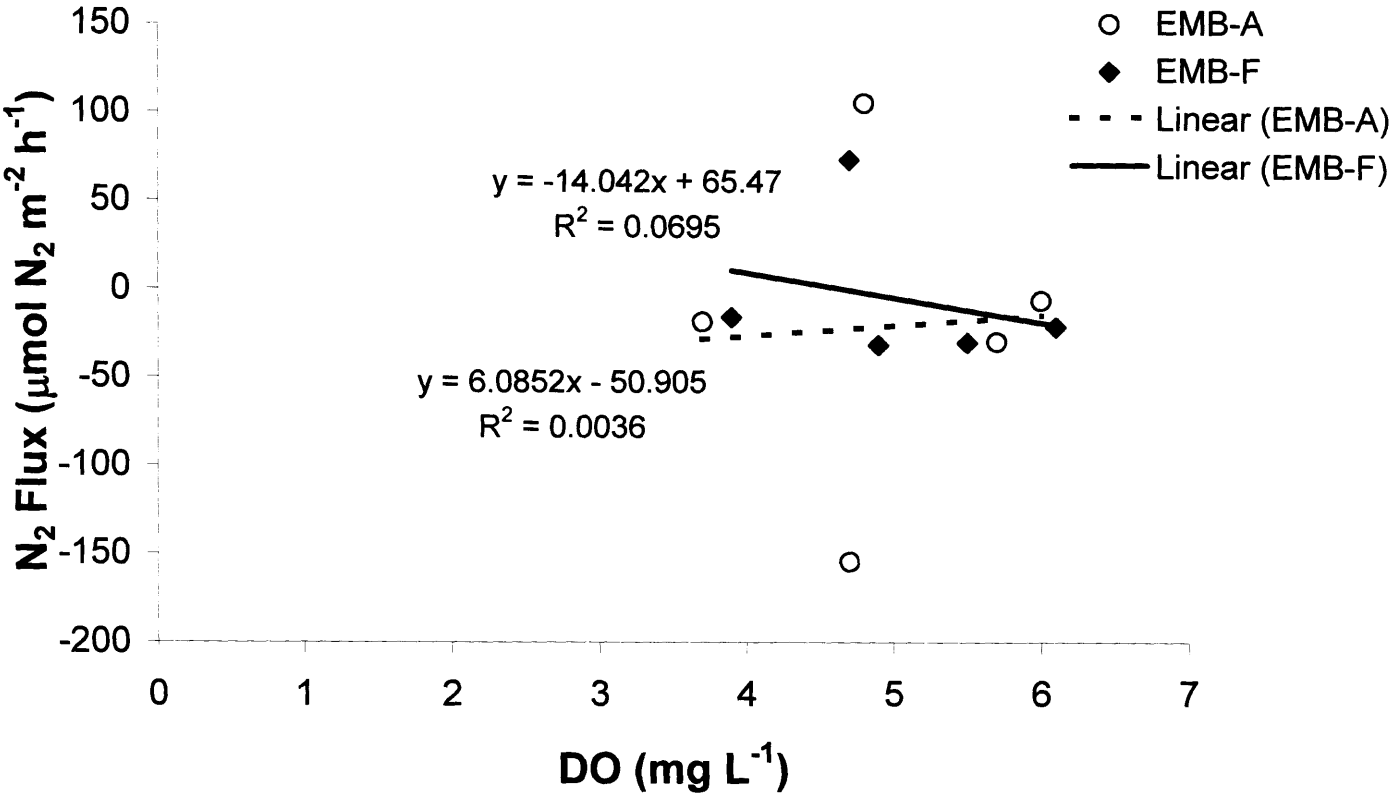


Figure 8. N_2 flux vs. dissolved O_2 and chlorophyll



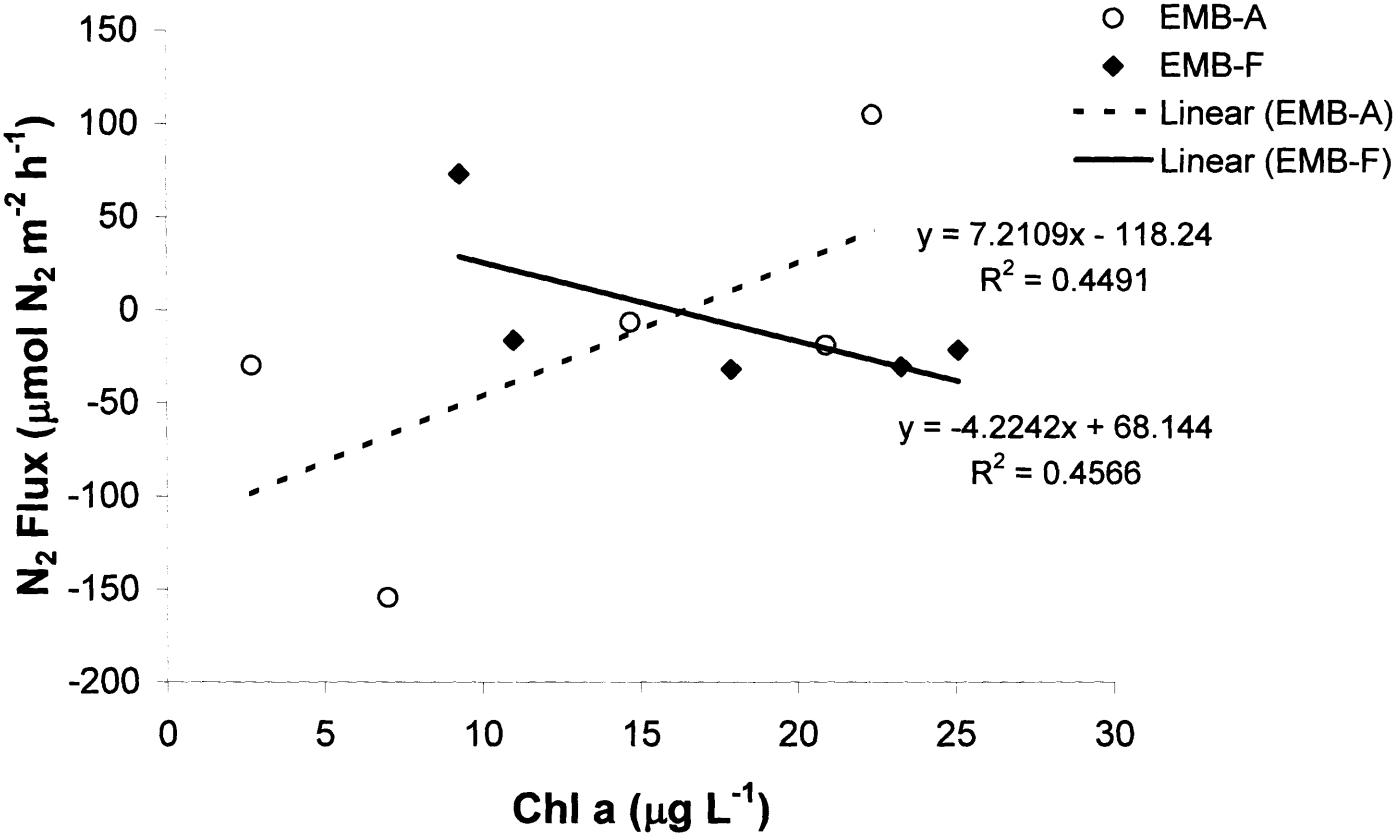
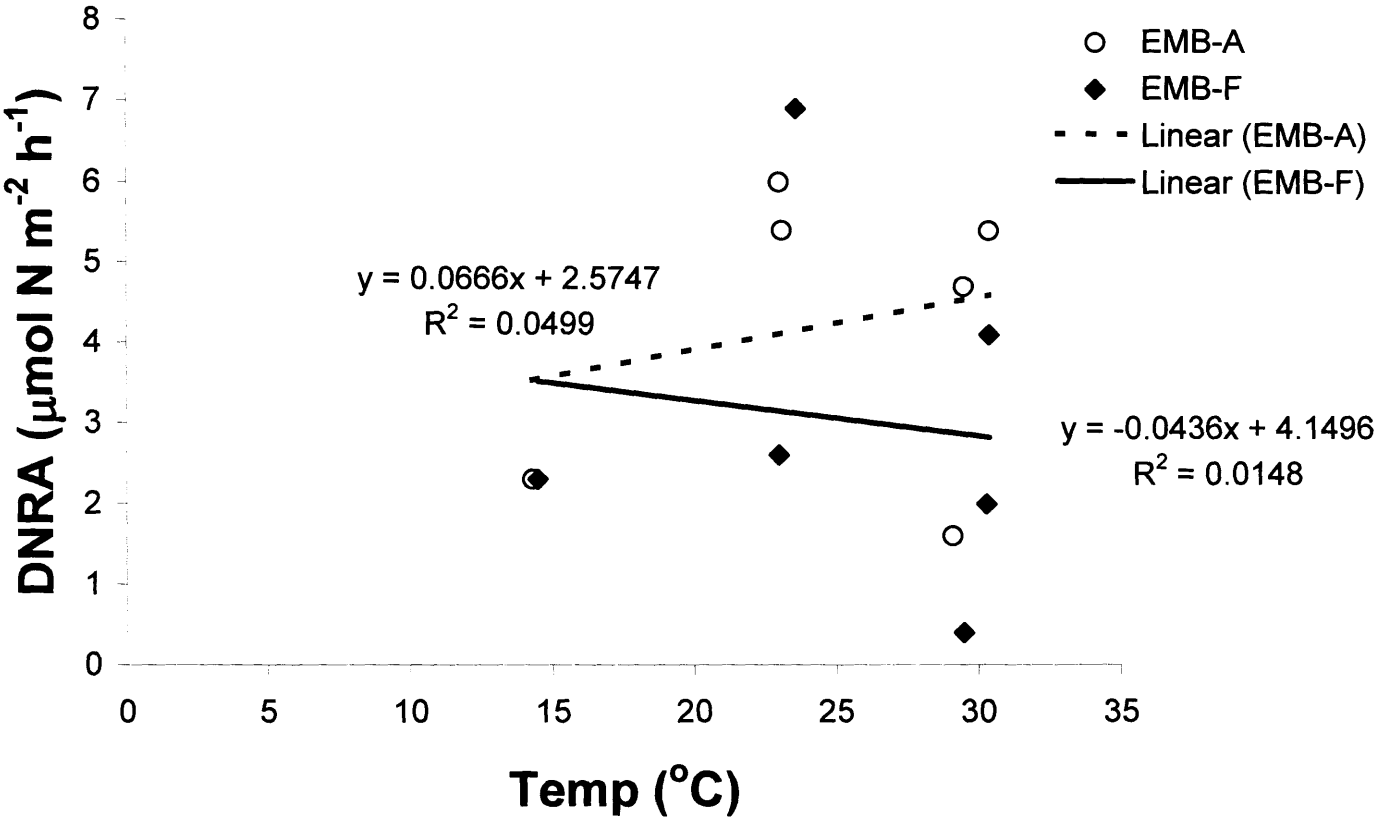


Figure 9. DNRA vs. temperature and salinity



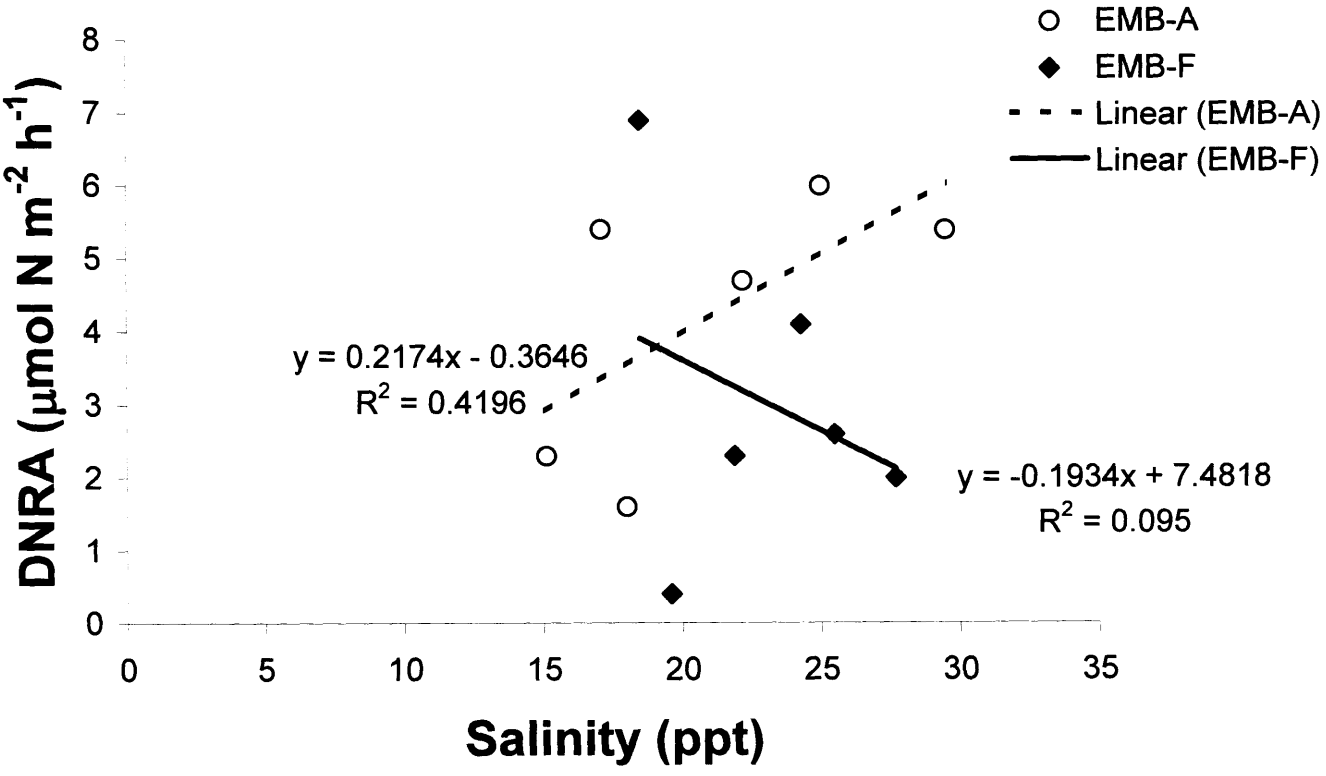
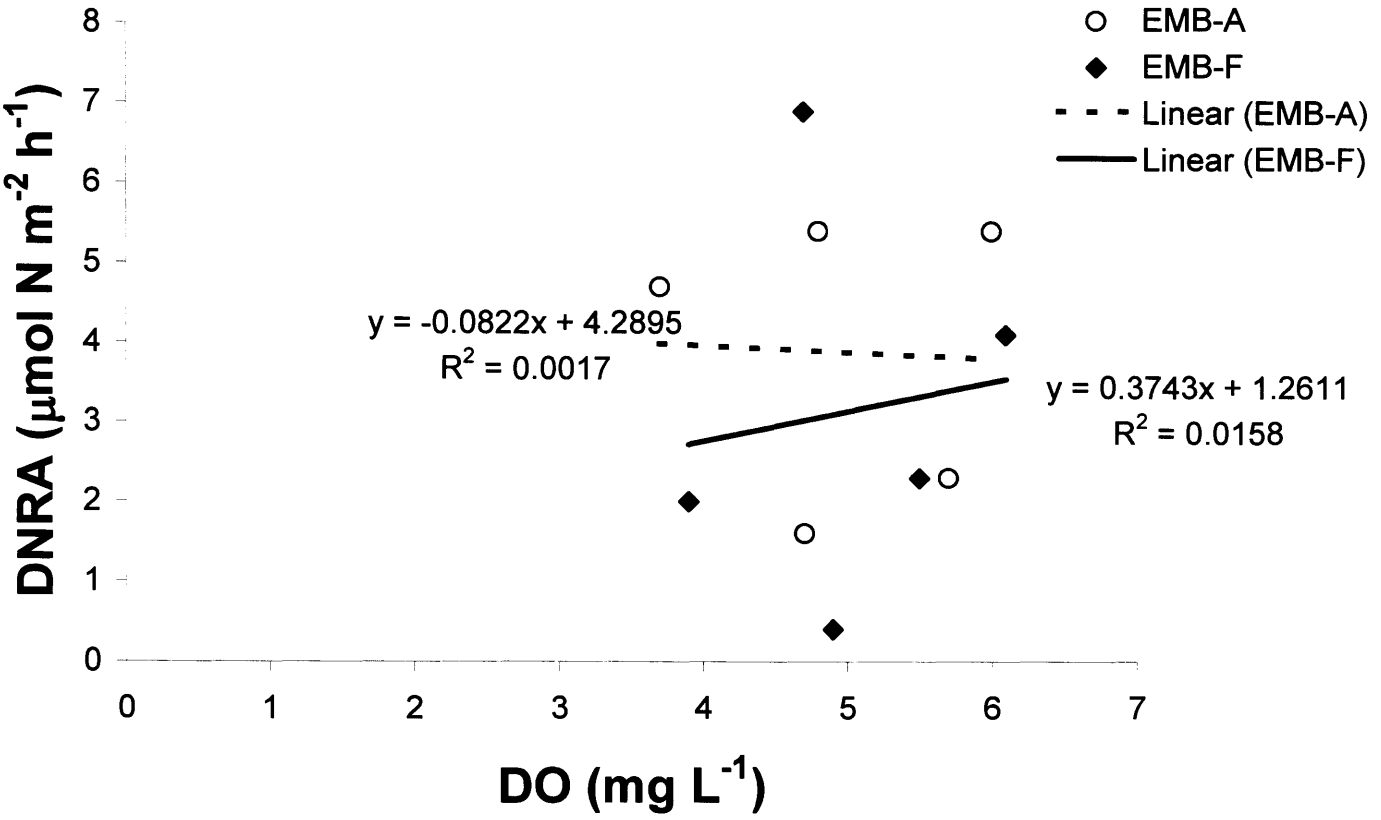
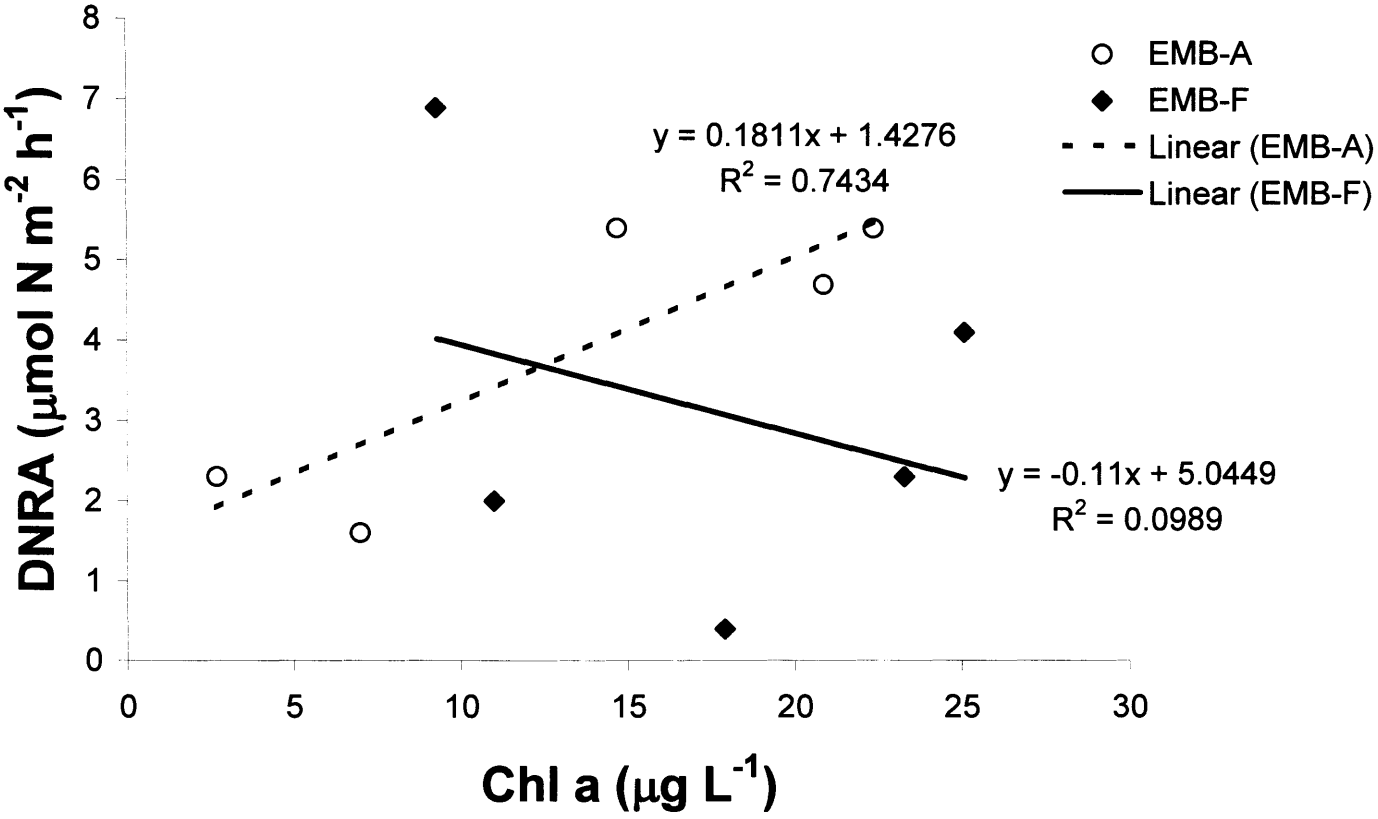


Figure 10. DNRA vs. dissolved O_2 and chlorophyll





Appendix: Gene analysis of denitrifiers in East Matagorda Bay (Dmitri Sobolev)

Introduction

Genes involved in the denitrification process are well studied and sequences of genes *nar*, *nirK*, *nirS*, *nor*, and *nos* (encoding NO_3^- reductase, copper-containing NO_2^- reductase, heme-containing NO_2^- reductase, nitric oxide reductase, and nitrous oxide reductase, respectively) are known (Bothe et al. 2000). Thus, a system with high potential for denitrification will exhibit high copy numbers of *nar*, *nirS* and *nirK*, *nor*, and *nos* genes. Molecular tools appear to be the only technique by which the abundance and diversity of bacteria involved in the N cycle can be assessed. Traditional culture methods account only for a small minority of organisms present in the environment. Culture-based enumeration (e.g., most probable number determination) of organisms from a significant number of samples is labor-intensive and does not offer insight into the diversity of organisms of interest. In contrast, molecular methods offer quick and easy ways to estimate the gene diversity (and, by extension, the organism diversity), via polymerase chain reaction (PCR), followed by denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993).

Materials and Methods

Core collection and incubation. Shallow marine sediment cores were collected at site EMB-A in East Matagorda Bay as described in the main text. Cores were incubated at *in situ* temperature in a flow-through setup as described previously. After 48 hours of equilibration, N compounds were added to separate tanks feeding triplicate core sets as

follows: $^{15}NO_3^-$ addition (designated NO3) received ~100 μmol L^{-1} $K^{15}NO_3^-$ (^{15}N content 98 atom %) and 20 μmol L^{-1} $^{14}NH_4Cl$; $^{15}NH_4^+$ addition (NH4) received ~100 μmol L^{-1} $K^{14}NO_3^-$ and 20 μmol L^{-1} $^{15}NH_4Cl$ (^{15}N content 99 atom %). Thus, except for the isotopic composition of the N compounds, both treatments were identical.

Chemical analyses.

Ammonium concentration and isotopic composition was determined via HPLC (Gardner et al. 1995), and isotope dilution calculations for the NH_4^+ release and uptake were performed in a manner similar to Blackburn (1979).

Model considerations. A theoretical model was constructed describing the ratio between N_2 fluxes of different isotopic composition (i.e., $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$), assuming a single population of bacteria acting upon a single pool of NO_3^-, with perfect mixing of added $^{15}NO_3^-$ and native $^{14}NO_3^-$. While the details of the model shall be reported elsewhere (Sobolev et al. in prep), the predicted $^{28}N_2$ flux (F_{28t}) is calculated from the $^{29}N_2$ (F_{29}) and $^{30}N_2$ (F_{30}) fluxes as follows:

$$F_{28t} = (F_{29})^2/4F_{30} \qquad (1).$$

The positive difference between observed $^{28}N_2$ flux and F_{28t} flux calculated from Equation 1 ("excess $^{28}N_2$ flux") was attributed to a ^{14}N-rich pool feeding N_2 generation processes, independently of the ^{15}N-rich NO_3^- pool. Since the observed $^{28}N_2$ flux takes into account $^{28}N_2$/Ar ratio, the possibility of contamination with atmospheric $^{28}N_2$ was ruled out.

DNA extractions and molecular analyses. Sediment cores designated for molecular analyses were sectioned in the lab at one cm intervals. Sections were placed into sterile 50 mL centrifuge tubes, frozen in liquid N_2, and stored at –20°C. DNA was extracted

from ca. 0.5 g of sediments by use of UltraClean Soil DNA extraction kit (MoBio) and purified with a GeneClean Spin kit (Qbiogene). A gene fragment encoding copper-containing NO_3^- reductase (*nirK*) was pre-amplified with nirK1F and nirK5R primers according to the procedure described by Braker et al. (2000). The resulting amplification product was re-amplified with primers nirK1F and nirK3R (Braker et al. 2000), the former having a GC-clamp attached at the 5' end (Muyzer et al. 1993). DGGE analysis was performed as described in Jackson et al. (1998).

Results.

Before $^{15}NO_3^-$ addition, N_2 flux out of the cores was represented by "excess" $^{28}N_2$, which is probably a model artifact. Upon addition of $^{15}NO_3^-$, the excess flux represented ~46% of total N_2 flux (Fig. 1A). A semi-quantitative PCR/DGGE analysis of the ~ 200 bp fragment of the gene encoding copper-containing NO_3^- reductase (*nirK*) has shown that a single form of the gene dominated the *nirK* pool, with a number of secondary forms appearing in some samples (Fig. 2A).

Discussion.

There was a large discrepancy between the $^{28}N_2$ flux predicted from $^{29}N_2$ and $^{30}N_2$ fluxes (F_{28t}) and $^{28}N_2$ flux measured directly via MIMS. Therefore, one or both of the following conditions exists: (i) a great degree of isotope fractionation occurring within the samples, or (ii) more than one N pool feeding N_2 flux through ***separate and distinct*** bacterial populations. Option (i) does not merit serious consideration under our conditions, as it would require discriminating tens of μmol of N per m^{-2} per h^{-1}. Isotope addition techniques are a tool for demonstrating option (ii). Since the isotopic composition of an N_2 molecule is not defined until the point of N_2O formation, the first two-N compound in

denitrification (Richardson and Watmough 1999), it is assumed that the isotope composition of the NO_x pool (substrate for nitric oxide reductase, an enzyme forming nitrous oxide) is identical to the isotope composition of the source NO_3^- pool (since denitrification generally does not imply interorganism transfer and/or environmental release of N compounds). Therefore, isotope introduced before the formation of nitrous oxide (i.e., NO_3^-, NO_2^-, nitric oxides) within the denitrification process will have no effect upon the model. However, if two distinct populations separately derive two-N compounds (N_2O and/or N_2) from two different N pools (rich in ^{15}N and ^{14}N, respectively), with only limited cross-flow of N compounds between the two populations, the resulting N_2 will be enriched in $^{28}N_2$ compared to the model.

Since up to 46% of the N_2 generated within the sediment cores in the $^{15}NO_3^-$ addition treatment does not derive from the ^{15}N-spiked NO_3^- pool, as calculated via excess $^{28}N_2$ formation, the likely explanation for this phenomenon is that a certain population of microorganisms generates N_2 by drawing N compounds from a ^{14}N-rich pool (e.g., NH_4^+), perhaps through nitrification-denitrification coupling at the oxic-anoxic boundary. But, NH_4^+ in the overlying water seemed to contribute little to this process (less than 2% of total N_2 flux was represented by ^{15}N in the $^{15}NH_4^+$ addition treatment) indicating that the flow of N from overlying water NH_4^+ to N_2 is not sufficient to explain the excess $^{28}N_2$ flux observed in the $^{15}NO_3^-$ addition experiment. It could be suggested that coupled nitrification-denitrification that feeds the $^{28}N_2$ flux in the $^{15}NO_3^-$ addition experiment is driven by high N remineralization in the surficial sediments, which increases NH_4^+ concentration and causes a net diffusional $^{14}NH_4^+$ flux from sediments to the overlying water. Intensive N remineralization appears to be a plausible explanation

since isotope dilution calculations on the NH_4^+ pool suggest an NH_4^+ release rate of up to 126 μmol m^{-2} h^{-1} in the $^{15}NH_4^+$ addition experiment (data not shown). With net upward NH_4^+ flux and the assumption that $^{14}NH_4^+$ and $^{15}NH_4^+$ do not diffuse independently, the only real-time possibility for $^{15}NH_4^+$ from the overlying water to enter the postulated nitrification-denitrification coupling zone within the sediments is against-the-gradient diffusional mixing, a minor to negligible process. However, there is no similar impediment to NO_3^- since sediments are considered a NO_3^- sink due to denitrification.

An issue that needs to be addressed is why $^{15}NO_3^-$ entering the sediments does not affect the $^{28}N_2$ flux. If the bacterial sub-population that generates $^{28}N_2$ were capable of using environmental NO_3^-, no $^{28}N_2$ excess with respect to $^{29}N_2/^{30}N_2$ would be observed, as the isotope pairing situation described by the single-pool, single-population model above would apply. A suggestion could be made that denitrifying bacteria within the postulated symbiotic community that couples denitrification and nitrification are better adapted to accepting NO_3^- from the nitrifying community partners. But, there could be N flux between the N transformation pathway linked to the postulated symbiotic community and the generally accepted pathway, and such a flux could not be quantified under our model since it is designed to measure only the degree to which those pathways are separated. A more reaching and intriguing possibility is that the exchange between the nitrifying and denitrifying partners occurs at the *NO_3^-* level, thus completely eliminating the step of NO_2^- to NO_3^- conversion and at least one interorganism N transfer (from NH_4^+ oxidizing bacteria to NO_2^- oxidizing). Obviously, if the latter concept is true, disturbance of the isotopic composition of the NO_3^- pool will have no direct effect upon this community. This notion is further supported by the fact that the sediment cores were

a net source of NO_2^-, before and after isotope addition, suggesting that some NO_2^- has an endogenous origin, coming from N compounds present in the sediments, with some NO_2^- diverted into denitrification.

It is possible that some of the excess $^{28}N_2$ could be generated by autotrophic nitrifying bacteria. The capacity of these organisms to carry out NH_4^+ oxidation to NO_2^-, followed by the reduction of NO_2^- to N_2 and/or NO and N_2O has been demonstrated (Bock et al. 1995). It is worth noting that genes for copper-containing NO_2^- reductase, potentially one of the key enzymes in the latter process, has been found in NH_4^+-oxidizing bacteria (Casciotti and Ward 2001) and was recovered from East Matagorda Bay sediment samples. Since production of reduced N compounds by nitrifying bacteria requires reduced O_2 concentrations (Philips et al. 2002), we hypothesize that a peculiar form of the *nirK* gene identified at ~ 25 mm sediment depth is involved (Fig. 2A). Further work of identifying the source of the gene via cloning/sequencing is planned.

Our findings in this experiment suggest that N transformation processes in East Matagorda Bay sediments do not conform to established concepts (unlike Corpus Christi Bay, where no excess $^{28}N_2$ flux was measured – data not shown). A significant part of the N_2 flux seems to derive from a source other than NO_3^-. So, a hypothesis could be that N loss to the atmosphere is greater in East Matagorda Bay than expected from conventional models, resulting in decreased primary production. Furthermore, two separate pathways of N_2 generation may be under different regulation, necessitating modeling them separately. Clearly, a more detailed study of these processes is needed.

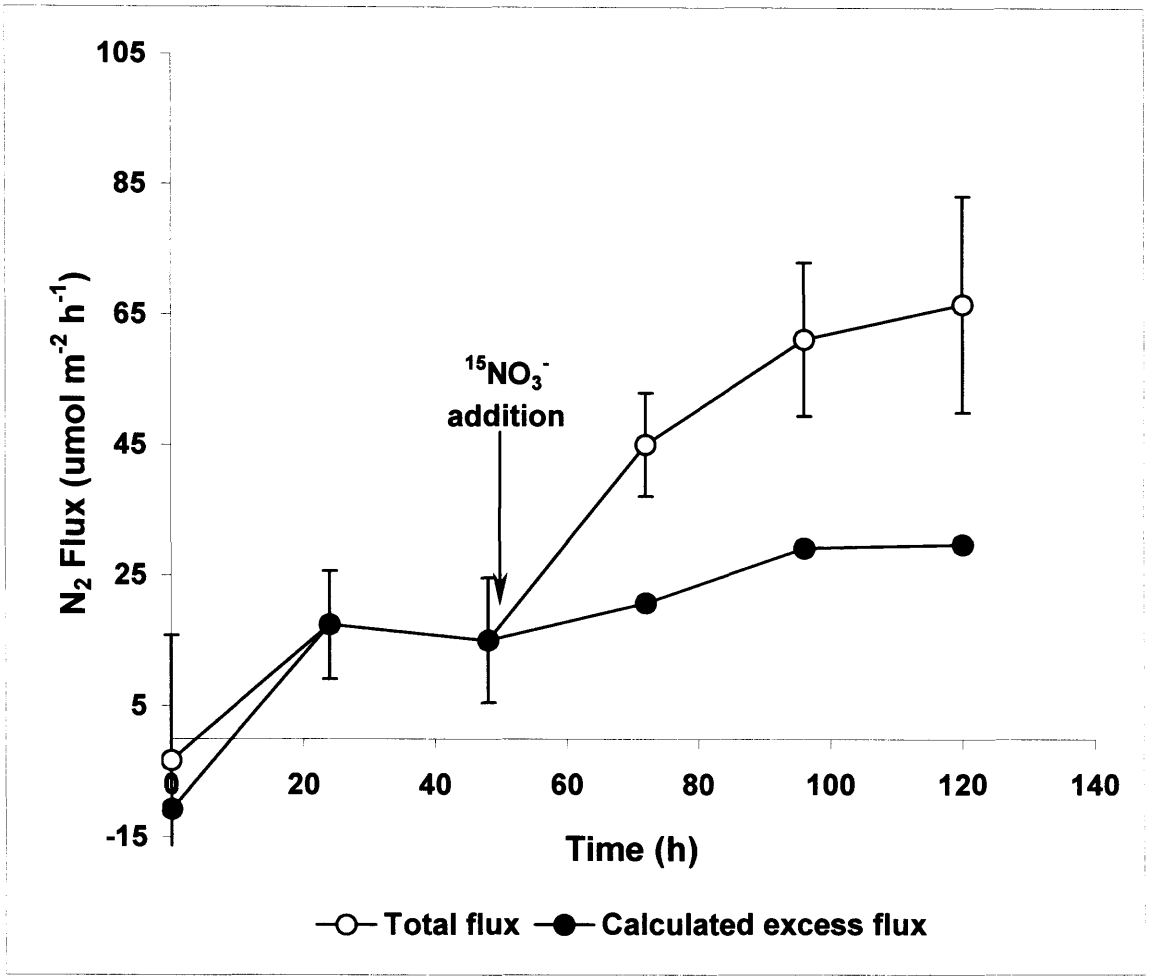


Figure 1A. Total observed and calculated $^{28}N_2$ excess fluxes in East Matagorda Bay sediment samples. Note that the excess flux not derived from $^{15}NO_3^-$-spiked NO_3^- pool represents over 40% of the total N_2 flux, suggesting that a significant portion of the N_2 does not derive from the NO_3^- pool.

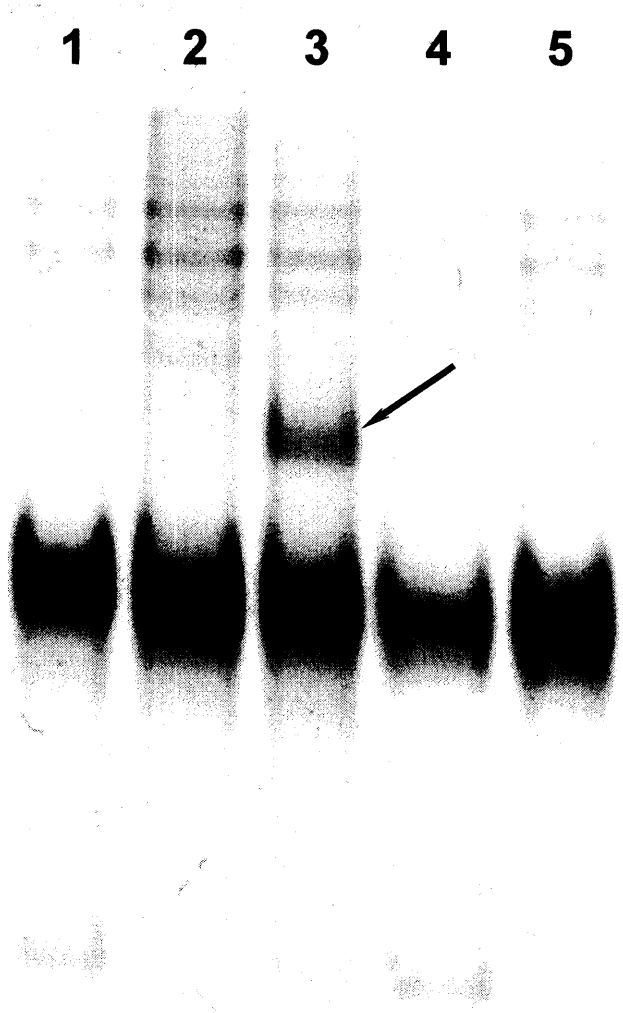


Figure 2A. DGGE gel of the *nirK* gene fragment. Lanes 1, 2, 3, 4, and 5 represent depth horizons 0-10; 10-20; 20-30; 30-40, and 40-50 mm, respectively. Arrow indicates form of *nirK* gene hypothesized to be involved in excess $^{28}N_2$ production.

References

Blackburn, T. H. 1979. Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N-NH_4^+ dilution technique. Appl. Environ. Microbiol. **37:** 760-765.

Bock, E., I. Schmidt, R. Stuven, and D. Zart. 1995. Nitrogen loss caused by nitrifying *Nitrosomonas* cells using ammonia or hydrogen as electron donors and nitrite as electron acceptor. Arch. Microbiol. **163**.

Bothe, H., G. Jost, M. Schloter, B. B. Ward, and K.-P. Witzel. 2000. Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiol. Rev. **24:** 673-690.

Braker, G., J. Zhou, L. Wu, A. H. Devol, and J. M. Tiedje. 2000. Nitrite reductase genes (*nirK* and *nirS*) as functional markers to investigate diversity of denitrifying bacteria in pacific northwest marine sediment communities. Appl. Environ. Microbiol. **66:** 2096-2104.

Casciotti, K. L., and B. B. Ward. 2001. Dissimilatory nitrite reductase genes from autotrophic ammonia-oxidizing bacteria. Appl. Environ. Microbiol. **67:** 2213-2221.

Gardner, W. S., H. A. Bootsma, C. Evans, and P. A. St. John. 1995. Improved chromatographic analysis of ^{15}N:^{14}N ratios in ammonium or nitrate for isotope addition experiments. Marine Chem. **48:** 271-282.

Jackson, C. R., E. E. Roden, and P. F. Churchill. 1998. Changes in bacterial species composition in enrichment cultures with various dilutions of inoculum as monitored by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **64:** 5046-5048.

Muyzer, G., E. C. D. Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:** 695-700.

Philips, S., S. Wyffels, R. Sprengers, and W. Verstraete. 2002. Oxygen-limited autotrophic nitrification/denitrification by ammonia oxidisers enables upward motion towards more favourable conditions. Appl. Microbiol. Biotechnol. **59:** 557-566.

Richardson, D. J., and N. J. Watmough. 1999. Inorganic nitrogen metabolism in bacteria. Curr. Opin. Chem. Biol. **3:** 207-219.

Sobolev, D., M. McCarthy, and W. S. Gardner. 2003. Probability-based approach to nitrogen isotope transformations: a case for two distinct sub-populations of denitrifying bacteria in the marine sediments. In prep.

e. It appears that there is other data collected from the sediment cores or shared from Montagna's study that is not included in tables. Please include all records of all field and laboratory measurements made, at least as an appendix.
f. The authors say that additional data on water column processes is forthcoming. It would be pertinent to examine the data from core fluxes, water column processes, and water column concentrations, for correlations.