

# **Nitrogen cycling in Laguna Madre and Baffin Bay**

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## 1. Introduction.

Estuarine phytoplankton production can be limited by N availability (Ryther & Dunstan 1971, Hecky & Kilham 1988) and sediments often serve as an important source of dissolved inorganic N ( $\text{DIN} = \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$ ). Denitrification is the only biological process that transforms combined N to gaseous forms ( $\text{N}_2$  or  $\text{N}_2\text{O}$ ; Seitzinger 1988, 1990). These gaseous end-products represent unavailable nutrient sources to most estuarine producers (e.g., phytoplankton and bacteria; Howarth et al. 1988). Benthic denitrification has proven to be a significant sink for combined N in systems where detailed studies of denitrification have been completed. This process may drive systems toward N limitation (Seitzinger 1990). The purposes of this study were to evaluate the importance of denitrification in nitrogen cycling and to examine environmental conditions influencing nitrogen cycling in Laguna Madre and Baffin Bay. A membrane inlet mass spectrometer (MIMS) system for dissolved gas measurement and a high performance liquid chromatography (HPLC) system for ammonium isotope measurement, that are available at the University of Texas Marine Science Institute, allowed us to measure dissimilatory nitrate reduction to ammonium (DNRA) as another major nitrate reduction pathway in addition to denitrification.

The relative partitioning between these two major pathways of nitrate reduction helps determine the degree of conservation of available nitrogen in shallow estuaries (Tiedje et al. 1982, Sørensen 1987, Jørgensen 1989, Patrick et al. 1996). When nitrate is consumed by denitrification, "total available" nitrogen is decreased because the final product of denitrification ( $\text{N}_2$  gas) is less available for biological production than either ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ) (Howarth et al. 1988). On the other hand, if the nitrate is used during DNRA, nitrogen is

conserved in a form that is available to organisms (Koike and Hattori 1978, Patrick et al. 1996, Jørgensen 1989). Although the occurrence of DNRA has been demonstrated in marine sediments (e.g. Koike and Hattori 1978, and Sørensen 1978), the ecological significance of the process is not well understood (Sørensen 1987, Cornwell 1999). Rates for this process can sometimes be as high as those for denitrification in shallow estuaries and tidal flat sediments (Kasper 1983, Koike and Hattori 1987, Jørgensen 1987, Rysgaard et al. 1996, Bolnin et al 1998).

Laguna Madre/Baffin Bay is the largest estuary in Texas but receives no major river discharges. Despite the lack of known nutrient inputs, this region is nutrient replete and has suffered from a long lasting bloom of the Texas Brown Tide. This monospecific algal bloom of *Aureomonas lagunensis* started in January 1989 and continued for about a decade (Buskey et al. 1998). It caused a decline in seagrasses and benthic fauna (Dunton 1994, Montagna and Kalke 1995). The persistence of an algal bloom in this area is unusual and the cause of the bloom is not fully understood. *Aureomonas lagunensis* has the unique characteristic of being able to use  $\text{NH}_4^+$  or  $\text{NO}_2^-$  but not  $\text{NO}_3^-$  as a nitrogen source (DeYoe and Suttle 1994). We hypothesize that *Aureomonas lagunensis* can out compete other autotrophs in this system because of its reliance on reduced N forms. The DNRA mechanism, by supplying  $\text{NH}_4^+$ , could help explain the success of Texas Brown Tide.

One reason that the DNRA process is not fully understood in coastal systems is that methods to measure DNRA rates at the sediment-water interface have been inconvenient. The use of  $\text{NO}_3^-$  with isotopically heavy N ( $^{15}\text{NO}_3^-$ ) as a tracer and measurement of  $^{15}\text{NH}_4^+$  production rates (for DNRA) have required extensive equipment and efforts (Sørensen 1987, Jørgensen 1989, Binnerup et al. 1992, Bonin et al. 1998). Although the acetylene inhibition

technique for denitrification measurements is relatively simple and sensitive, some problems are associated with the technique including its inhibition of coupled nitrification-denitrification (Knowles 1990). Membrane inlet mass spectrometry (MIMS) allows accurate and simple measurement of N<sub>2</sub>/Ar changes caused by denitrification (Kana et al. 1994). In this study, we expanded the capability of this technique by measuring different isotopic forms of N<sub>2</sub> gas (<sup>29</sup>N<sub>2</sub>=<sup>14</sup>N + <sup>15</sup>N and <sup>30</sup>N<sub>2</sub>=<sup>15</sup>N+<sup>15</sup>N) relative to Ar. The modified setup allowed us to conduct mechanistic experiments involving <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition. By adding the tracer, the denitrification measurements estimated from the N<sub>2</sub>/Ar ratio can be verified with an isotope pairing technique (Nielson 1992). Also the simultaneous measurement of nitrogen fixation and denitrification is feasible (An and Gardner unpublished data). Using the modified MIMS system and a HPLC technique to measure the <sup>15</sup>NH<sub>4</sub><sup>+</sup> composition in water samples (Gardner et al. 1995), we were able to measure the two major nitrate reduction processes (denitrification and potential DNRA) in Laguna Madre/Baffin bay with a flow-through sediment incubation chamber (Kana et al. 1994, Lavrentyev et al. 2000).

Here, we report the results of sediment incubation experiments conducted in April, August and December 1999 and April 2000. The potential interaction between the fate of NH<sub>4</sub><sup>+</sup> and the presence of sulfide, which occurs in the study region is discussed.

## 2 Study area and method

Laguna Madre/ Baffin Bay is a shallow, semi-enclosed estuary located in the southeastern part of the Texas coast. Water exchange with the Gulf of Mexico is limited in Laguna Madre/ Baffin Bay; it is a typical negative estuary where freshwater input is less than evaporation (water residence time = 1 year). The salinity is often more than 40 ppt and can vary annually up to 60 ppt (Buskey et al. 1998).

Four stations in Baffin Bay and Laguna Madre were selected to measure water column characteristics (temperature, salinity, and dissolved oxygen using a Hydrolab<sup>®</sup>) and conduct sediment core incubation experiments (Fig. 1). Station B24 located in Baffin Bay represents the deepest region of the bay. Station B6 is shallow and the salinity of the overlying water at that station was higher than at B24. The sediment type in Station B24 includes fine clay whereas Station B6 has a higher sand content (see Results). The water depths in Laguna Madre stations were 0.8 - 0.9 m and did not show spatial variability. Laguna Madre stations were populated with *Thalassia testudinum* (300-600 shoots m<sup>-2</sup>; Lee and Dunton 1999). During our sampling period, salinity was lower than had been observed earlier (Buskey et al. 1998) and did not exceed 40 ppt (Table 1).

Baffin Bay (B6 and B24) and Laguna Madre (L155 and L189) stations were visited in Apr-99, Aug-99, Dec-99 and Apr-00. Benthic chambers were used to measure *in situ* fluxes of O<sub>2</sub>, N<sub>2</sub>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>. Initial water samples for nutrient and dissolved gas measurements were taken after the chambers were assembled. Middle samples were

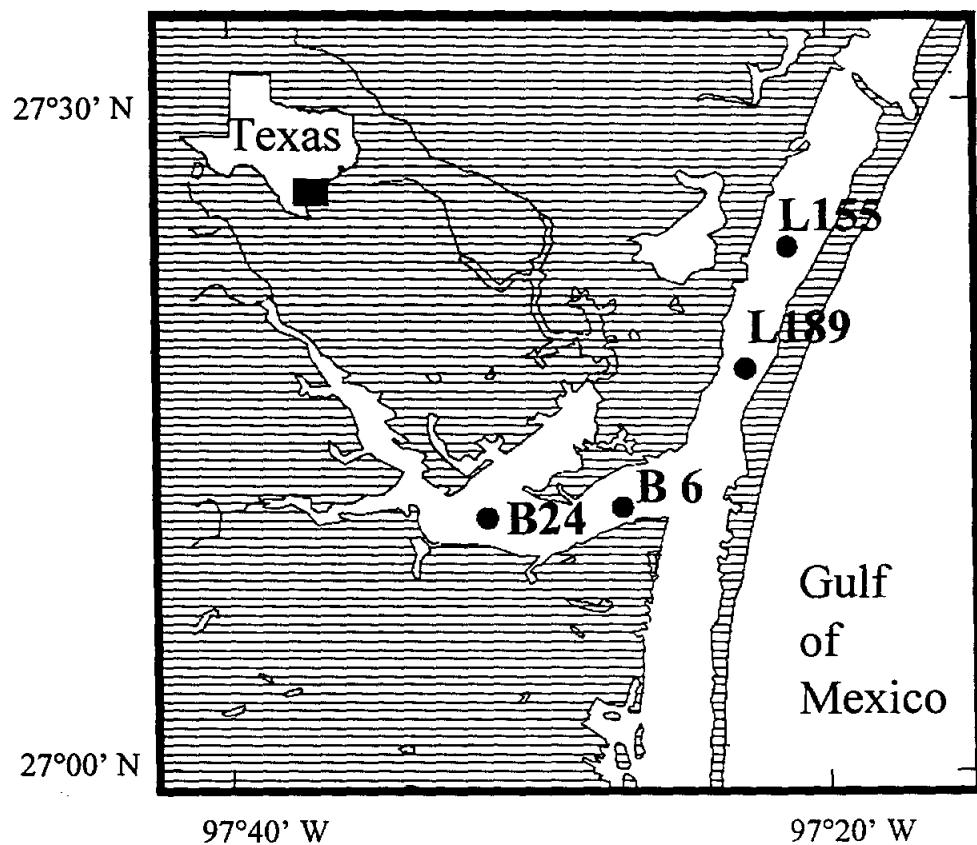


Figure 1. Map showing the sampling stations in Baffin Bay (B6, B24) and Laguna Madre (L155, L189).

taken 2-3 hours after the initial sample to evaluate the linearity of oxygen demand and N<sub>2</sub> gas evolution (denitrification) rate with time. After about 24 h of incubation, final samples were taken from the chambers. Successful benthic chamber measurements were obtained at all stations in Aug-99 and at Laguna Madre stations (L155 and L189) in Dec-99.

Undisturbed sediment cores (12 cm diameter, 30 cm length; 4 per station) with bottom water were collected using scuba gear. Within 3 hours of collection, the 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). The flow-through chamber setup consisted of an intake water vessel, Teflon flow tubes, a peristaltic pump, a temperature-controlled incubation bath, and a sample collection vessel. The sediment cores were placed in the incubation bath set at *in situ* temperature and bottom water from the site was passed continuously over the core surface at a rate of 1.2 ml min<sup>-1</sup>. One half of the cores were incubated under dim light (~ 30 µE m<sup>-2</sup> sec<sup>-1</sup>) whereas the others were covered with aluminum foil. The dim light condition did not cause significant light effects so data from the two treatments were combined. Water column depth over the sediment was maintained at about 5 cm to give a water volume of ca. 570 ml in each core. Triplicate samples of feed water and outlet water were collected at intervals for dissolved gas analysis after one day of incubation, to allow steady state conditions to develop. Water samples were also collected for analysis of dissolved inorganic nitrogen compounds (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup>).

Nitrate addition experiments with <sup>15</sup>NO<sub>3</sub><sup>-</sup> were conducted during Aug-99, Dec-99 and Apr-00 to provide insights about the fate of nitrate at the sediment water interface. After the first or second day of sampling, the feed water was enriched with <sup>15</sup>NO<sub>3</sub><sup>-</sup> and the concentration of

$^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ ,  $^{30}\text{N}_2$ , and  $^{15}\text{NH}_4^+$  was measured in inflow and outflow waters. Three different masses of nitrogen gas were produced by denitrification ( $^{28}\text{N}_2$  from  $^{14}\text{NO}_3^-$ ,  $^{30}\text{N}_2$  from  $^{15}\text{NO}_3^-$ , and  $^{29}\text{N}_2$  from  $^{14}\text{NO}_3^-$  and  $^{15}\text{NO}_3^-$ ; Nielson 1992). Dissolved  $\text{N}_2$ ,  $\text{O}_2$ , and  $\text{Ar}$  were measured by membrane inlet mass spectrometry (MIMS) using methods modified from Kana et al. 1994. By using the membrane inlet system and  $\text{Ar}$  as an internal standard, errors normally associated with dissolved gas extraction and atmospheric contamination are reduced and the measurement procedure with the MIMS system is fast, simple and precise (Kana et al. 1994). We modified the method of Kana et al. (1994) to measure dissolved  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  in addition to  $\text{Ar}$ ,  $\text{O}_2$ , and  $^{28}\text{N}_2$ . The  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  concentrations were obtained from the "excess" atomic mass unit (AMU) 29 and 30 signals, respectively, caused by the conversion of added  $^{15}\text{NO}_3^-$ . The ratio between  $^{29}\text{N}_2$  and  $^{28}\text{N}_2$  is 0.00632 (0.00316 x 2) in natural samples, considering that the natural abundance of  $^{15}\text{N}$  is 0.316 % (Lide 1992). The relationship between  $^{29}\text{N}_2$  and  $^{28}\text{N}_2$  was obtained from 30 ppt sea water held at different temperatures (21°C and 30°C ; Fig 2 a). This relationship was used to determine the excess AMU 29 in a water sample. The feed water did not have the excess AMU 29 resulting from  $^{29}\text{N}_2$  while outflow water had excess AMU 29 indicating the presence of  $^{29}\text{N}_2$  produced during denitrification (Fig 2 a). The excess AMU 29 signal was converted to excess  $^{29}\text{N}_2$  concentration by comparing results with those from standard water (30 ppt, 21°C and 30°C). The  $\text{NO}^+$  ions (AMU 30) formed from  $\text{N}_2$  and  $\text{O}^+$  inside the mass spectrometer produced a linear relationship between AMU 30 and  $(\text{AMU}28 \times \text{AMU}32)^{0.5}$  (Jensen et al. 1996). Jensen et al. (1996) suggested that a linear relationship occurs between AMU 30 and  $(\text{AMU}28 \times \text{AMU}32)$  but we found that AMU 30 exhibited a better regression with  $(\text{AMU}28 \times \text{AMU}32)^{0.5}$  than with  $(\text{AMU}28 \times \text{AMU}32)$  (Fig 2 b). It is reasonable to assume that  $\text{NO}^+$  has a linear relationship with  $(\text{N}_2\text{O}_2)^{0.5}$  rather than with  $\text{N}_2\text{O}_2$ . For example, if oxygen (O) and nitrogen (N)

(a) AMU 28 vs. AMU 29

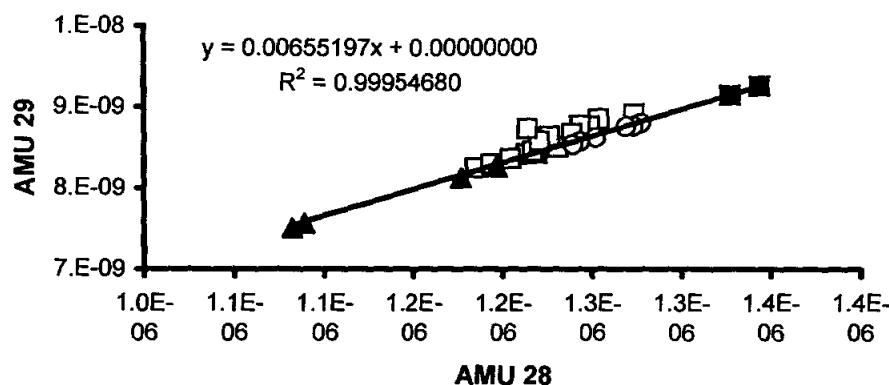
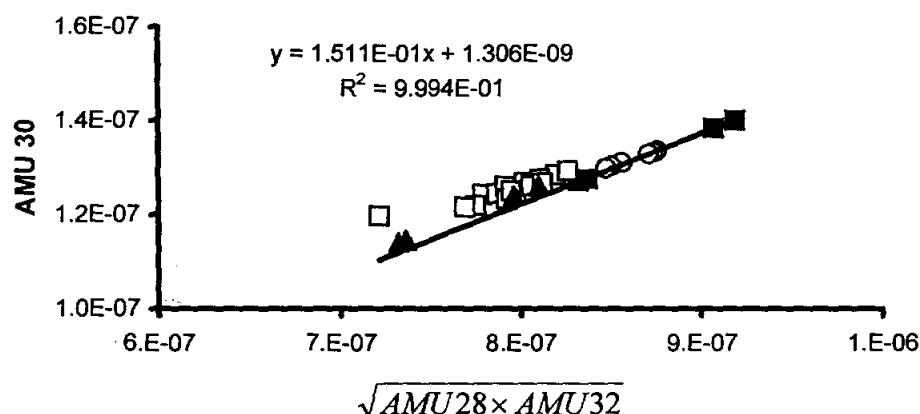
(b)  $\sqrt{AMU28 \times AMU32}$  vs. AMU-30

Figure 2. Relationship between different atomic mass unit (AMU) signals in liquid samples measured with quadrupole mass spectrometer. The solid symbol represents standard water ( ■ : 30 ppt, 21 °C, ▲ : 30 ppt, 30 °C) hollow symbols represents samples ( □ : outflow water, ○ : inflow water). The line represents the regression among the standard water. (a) AMU-28 versus AMU-29 signal (b)  $\sqrt{AMU28 \times AMU32}$  versus AMU 30 signal. The signal represents partial pressure of the particular gas (mbar) in the mass spectrometer.

atoms are combined randomly as N<sub>2</sub>, NO, and O<sub>2</sub>, and the proportions O and N are p and q, respectively, then the proportions of the three products should show a binomial distribution (N<sub>2</sub>=p<sup>2</sup>, NO=2pq, O<sub>2</sub>=q<sup>2</sup>) and account for the observed square root relationship of N<sub>2</sub>O<sub>2</sub> with NO. This relationship was used to determine the excess AMU 30 in the water samples. The excess AMU 30 was converted to excess <sup>30</sup>N<sub>2</sub> concentration by comparing values with those of standard water. The concentration and atom % <sup>15</sup>N for NH<sub>4</sub><sup>+</sup> were determined in the water samples by HPLC (Gardner et al. 1995). Sediment flux of each compound was calculated based on the concentration difference between feed water and outflow water, flow rate, and cross-sectional area (Lavrentyev et al. 2000)

### **3. Results and discussion**

#### **3-1 Environmental characteristics**

Table 1 shows the results of Hydrolab measurements. Laguna Madre stations had higher salinity than the Baffin Bay stations. Salinity was high during Aug-99 and Apr-00 and low during Apr-99 and Dec-99. The salinity in Laguna Madre and Baffin Bay did not show a reverse relationship with local precipitation (Fig. 3). The salinity appeared to increase with local precipitation. Evaporation rather than the precipitation may be responsible for salinity fluctuation in this area. Alternatively, the time lag between precipitation and low salinity in the bay may have caused the pattern shown in Fig 3. For example, the low precipitation in early 1999 may have caused the high salinity of Aug-99. Likewise, the low salinity in Dec-99 may have been caused by high precipitation in Aug-99. Surface water dissolved oxygen (DO) concentration was at saturation for the existing temperature and salinity. Bottom water was

Table 1. Environmental variables in Laguna Madre and Baffin Bay.  
NA: data not available.

	B6	Apr-99	Aug-99	Dec-99	Apr-00	B24			
						Apr-99	Aug-99	Dec-99	Apr-00
<b>Location</b>									
Longitude	97.25	39.9				97.33	11.1		
Latitude	27.16	46.7				27.15	47.6		
Water depth (m)		2.2				1.8			
Sand content (%; > 64 µm)		33.9				5.2			
<b>Salinity (ppt)</b>									
Surface	22.55	35.23	30.4	35.08		22.67	31.6	25.7	31.1
Bottom	23.34	19.2	30.5	26.56		21.11	24.11	25.7	25.03
<b>Temperature (°C)</b>									
Surface	26.22	31.06	16.21	26.07		25.98	30.62	15.8	25.86
Bottom	26.17	30.75	16.19	26.08		25.95	30.36	15.75	25.86
<b>Dissolved oxygen (mg/L)</b>									
Surface	6.12	5.77	7.66	5.72		5.78	NA	6.95	5.77
Bottom	4.37	1.35	7.09	5.76		5.87	NA	5.61	5.73

	L155	Apr-99	Aug-99	Dec-99	Apr-00	L189			
						Apr-99	Aug-99	Dec-99	Apr-00
<b>Location</b>									
Longitude	97.21	25.1				97.23	17.7		
Latitude	27.24	34.2				27.20	53.1		
Water depth (m)		0.8				0.9			
Sand content (%; > 64 µm)		72.4				12.1			
<b>Salinity (ppt)</b>									
Surface	32.76	38.07	31.9	34.08		30.99	37.18	30.5	34.41
Bottom	32.81	38.14	32.1	34.32		31.05	37.34	31.4	34.32
<b>Temperature (°C)</b>									
Surface	26.86	30.18	16	21.21		26.43	29.54	15.64	20.77
Bottom	26.87	30.22	15.59	20.53		26.43	29.56	15.79	20.75
<b>Dissolved oxygen (mg/L)</b>									
Surface	6.5	5.35	6.58	7.57		6.58	4.66	6.76	6.89
Bottom	6.39	5.32	5.88	6.65		6.35	4.53	6.33	6.61

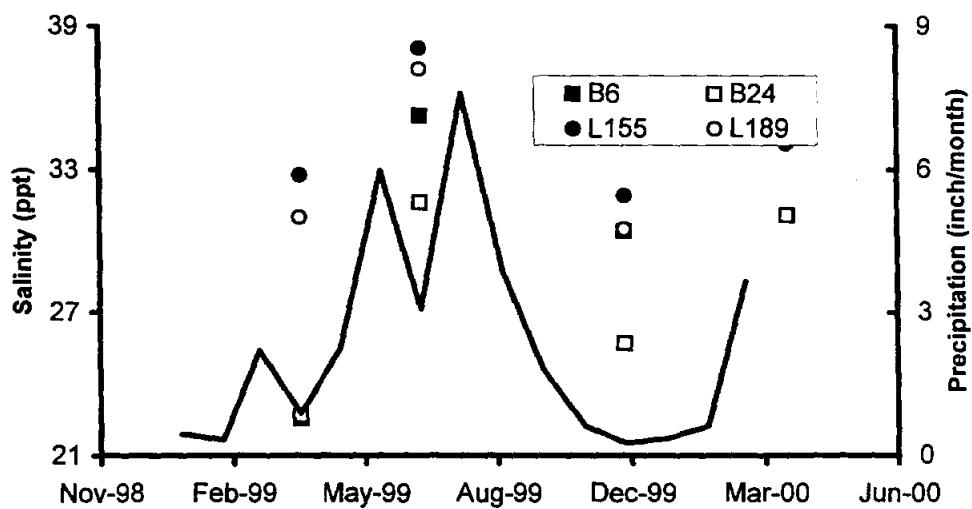


Figure 3. Salinity variations in Laguna Madre and Baffin Bay and local precipitation recorded in Corpus Christi, Texas. Line shows precipitation and symbol shows salinity.

oxygenated at most stations due to wind-driven mixing, but oxygen concentrations were low in Baffin Bay stations during Aug-99, perhaps because of ground water intrusion (low salinity, low oxygen and high nitrate water) rather than due to increased sediment oxygen demand (Personal comm. D. Brock).

### **3-2. Seasonal variations of sediment oxygen demand and denitrification rates**

Denitrification rates in Baffin Bay ( $0\text{-}230 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ) and Laguna Madre ( $0\text{-}530 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ) were in the range of those reported for other coastal marine environments (Seitzinger 1988, Herbert 1999). The annual average denitrification rate ( $70 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ) was lower than those for other Texas estuaries such as Galveston Bay ( $\sim 170 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ; using a direct  $\text{N}_2$  flux method; An and Joye in press). The apparent  $\text{N}_2/\text{Ar}$  ratios tend to decrease with  $\text{O}_2$  increases since the  $\text{O}^-$  ion scavenges  $\text{N}_2$  during the formation of  $\text{NO}^-$  inside the MIMS system (Kana personal communication). Since the  $\text{O}_2$  concentrations of outflow waters in our flowthrough system were lower than those of inflow water, the denitrification would be overestimated by the  $\text{NO}^-$  effect. The denitrification rates reported here were not corrected for that effect. However, the overestimation of denitrification rate should be less than 3% because  $\text{O}_2$  concentration of outflow water was maintained at over 80 % of that for inflow water during sediment incubation (calculation using Kana's calibration curve).

Our measurements were comparable to denitrification rates measured in Galveston Bay with the  $\text{N}_2$  flux method after gas purging ( $0\text{-}94 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ; Zimmerman and Benner 1994). They also resembled denitrification measurements using the gas purging  $\text{N}_2$  flux method in Texas estuaries ( $8\text{-}140$  and  $10\text{-}70 \mu\text{g atom N m}^{-2}\text{h}^{-1}$  for Nueces and Guadalupe estuary, respectively; Yoon and Benner 1992). However, the gas purging denitrification method may

have underestimated actual denitrification rates for those sites because of the extended incubation time required to remove atmospheric N<sub>2</sub> (see reviews in Cornwell 1999, An and Joye 1997). We conclude that denitrification rates in our study region are at the low end of the range of those occurring in coastal environments.

Tables 2 and 3 show the sediment oxygen demand (SOD) and denitrification rates in Baffin Bay and Laguna Madre. The denitrification and SOD rates of the flowthrough systems reported in Tables 2 and 3 are values measured before the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition. Since the denitrification rates shown in Table 2 were estimated from "net" N<sub>2</sub>/Ar change, total denitrification rates would be higher if nitrogen fixation is important. The negative numbers suggest that nitrogen fixation rates exceeds denitrification rates in some samples (Table 2). Both SOD and denitrification rates showed maximum values in Apr-99 (1491  $\mu\text{mole O}_2 \text{ m}^{-2} \text{ h}^{-1}$  and 257  $\mu\text{g atom N m}^{-2} \text{ h}^{-1}$ , respectively; Table 2, 3). The minimum SOD (520  $\mu\text{mole O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) was observed in Dec-99. In Apr-00, SOD tended to increase relative to Dec-99 while denitrification rates remained low. Both denitrification rates and SOD were higher at L189 than at other stations in Apr-99. Denitrification rates were more than two times higher than at other stations. In other sampling seasons, however, denitrification rates in L189 were similar to rates at other stations (Table 2,3).

The seasonal variations of denitrification rates and SOD were compared with water temperature fluctuations in Baffin Bay/ Laguna Madre (Fig. 4). Neither SOD nor denitrification rates followed temperature fluctuations. Sediment oxygen demand decreased from 1491  $\mu\text{mole O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in Apr-99 to 1013  $\mu\text{mole O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in Aug-99 whereas denitrification rates decreased

Table 2. Denitrification rates ( $\mu\text{g atom N m}^{-2} \text{ h}^{-1}$ ) measured using intact core flowthrough systems and benthic chambers. Denitrification rates were estimated from the  $\text{N}_2:\text{Ar}$  ratio changes between inflow and outflow (flowthrough) and between initial and final (benthic chamber) water samples.

Triplicate sequential (flowthrough) and replicate (benthic chamber) water samples were used for the  $\text{N}_2$  concentration measurements in the MIMS system. The precision (CV) of MIMS measurement was < 0.05%. SE: standard error between two core samples. NA: measurement not available.

Samples	Station	B6	B24	L155	L189	Grand Mean
<b>Apr-99</b>						
Flowthrough						
Light 1		224	47	133	302	
Light 2		35	70	137	705	
Light Mean (SE)		130 (94)	58 (12)	134 (2)	504 (202)	206
Dark 1		287	254	494	975	
Dark 2		168	102	37	137	
Dark Mean (SE)		228 (60)	178 (76)	266 (228)	554 (416)	306
<b>Mean</b>		<b>180</b>	<b>118</b>	<b>200</b>	<b>530</b>	<b>257</b>
<b>Aug-99</b>						
Flowthrough						
Light 1		44	64	130	20	
Light 2		60	72	40	50	
Light Mean (SE)		50 (8)	68 (4)	84 (46)	34 (16)	60
Dark 1		152	-10	-6	14	
Dark 2		78	20	52	24	
Dark Mean (SE)		114 (38)	4 (14)	24 (28)	20 (4)	40
<b>Mean</b>		<b>82</b>	<b>36</b>	<b>54</b>	<b>28</b>	<b>50</b>
Benthic chamber						
Light		58	40	NA	40	46
Dark		120	82	NA	108	104
<b>Mean</b>		<b>89</b>	<b>61</b>	<b>NA</b>	<b>74</b>	<b>75</b>

Table 2. Continue

Station Samples	B6	B24	L155	L189	Grand Mean
<b>Dec-99</b>					
Flowthrough					
Light 1	14	-2	-14	-9	
Light 2	-41	-34	-16	-41	
Light Mean (SE)	-14 (28)	-18 (16)	-15 (5)	-13 (16)	-9
Dark 1	-82	113	-43	-5	
Dark 2	16	6	-23	-9	
Dark Mean (SE)	-32 (48)	60 (54)	-32 (10)	-6 (2)	-2
<b>Mean</b>	<b>-23</b>	<b>20</b>	<b>-28</b>	<b>-16</b>	<b>-12</b>
Benthic chamber					
Light	NA	NA	NA	9.2	
Dark	NA	NA	NA	15.5	
<b>Mean</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>12</b>	
<b>Apr-99</b>					
Flowthrough					
Light 1	-24	46	-37	-32	
Light 2	-39	-53	-31	27	
Light Mean (SE)	-32 (8)	-4 (50)	-34 (4)	-2 (30)	-18
Dark 1	17	-49	119	-8	
Dark 2	-24	-43	-22	-13	
Dark Mean (SE)	-4 (20)	-46 (2)	48 (70)	-10 (3)	2
<b>Mean</b>	<b>-18</b>	<b>-24</b>	<b>8</b>	<b>-6</b>	<b>-10</b>

Table 3. Sediment oxygen demands ( $\mu\text{mole O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) measured using intact core flowthrough systems and benthic chambers. Sediment oxygen demands were estimated from changes in  $\text{O}_2$  concentration between inflow and outflow (flowthrough) and between initial and final (benthic chamber) water samples. Triplicate sequential (flowthrough) and replicate (benthic chamber) water samples were used for the  $\text{O}_2$  concentration measurements in the MIMS system. The precision (CV) of MIMS measurement was < 0.05 %. SE: standard error between two core samples. NA: measurement not available.

Samples	Station	B6	B24	L155	L189	Grand Mean
<b>Apr-99</b>						
Flowthrough						
Light 1		800	892	2200	1725	
Light 2		1171	1104	1300	1246	
Light Mean (SE)		985 (185)	996 (105)	1751 (450)	1484 (239)	1304
Dark 1		1758	1879	938	2396	
Dark 2		1546	1621	1292	1996	
Dark Mean (SE)		1652 (107)	1750 (129)	1115 (176)	2195 (199)	1678
<b>Mean</b>		<b>1319</b>	<b>1373</b>	<b>1433</b>	<b>1840</b>	<b>1491</b>
<b>Aug-99</b>						
Flowthrough						
Light 1		1217	1321	1697	581	
Light 2		NA	1241	1099	998	
Light Mean (SE)		1217(NA)	1281 (40)	1398 (299)	789 (208)	1171
Dark 1		919	833	527	681	
Dark 2		965	1222	833	808	
Dark Mean (SE)		942 (23)	1027 (195)	680 (153)	744 (63)	863
<b>Mean</b>		<b>1095</b>	<b>1154</b>	<b>1039</b>	<b>767</b>	<b>1013</b>
Benthic chamber						
Light		242	402	NA	149	264
Dark		516	780	NA	708	668
<b>Mean</b>		<b>379</b>	<b>591</b>	<b>NA</b>	<b>429</b>	<b>466</b>

Table 3. Continue

Station	B6	B24	L155	L189	Grand				
Samples					Mean				
<b>Dec-99</b>									
Flowthrough									
Light 1	394	510	605	592					
Light 2	404	652	692	319					
Light Mean (SE)	339 (5)	580 (71)	648 (43)	455 (136)	521				
Dark 1	398	559	263	609					
Dark 2	538	721	601	468					
Dark Mean (SE)	467 (70)	639 (81)	431 (168)	538 (70)	519				
<b>Mean</b>	<b>403</b>	<b>610</b>	<b>540</b>	<b>497</b>	<b>520</b>				
Benthic chamber									
Light	NA	NA	NA	198					
Dark	NA	NA	NA	302					
<b>Mean</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>250</b>					
<b>Apr-99</b>									
Flowthrough									
Light 1	476	618	659	677					
Light 2	336	341	459	959					
Light Mean (SE)	406 (70)	480 (138)	559 (100)	818 (141)	566				
Dark 1	811	658	806	779					
Dark 2	562	402	1292	1257					
Dark Mean (SE)	686 (125)	530 (128)	1049 (243)	1018 (239)	821				
<b>Mean</b>	<b>546</b>	<b>505</b>	<b>804</b>	<b>918</b>	<b>693</b>				

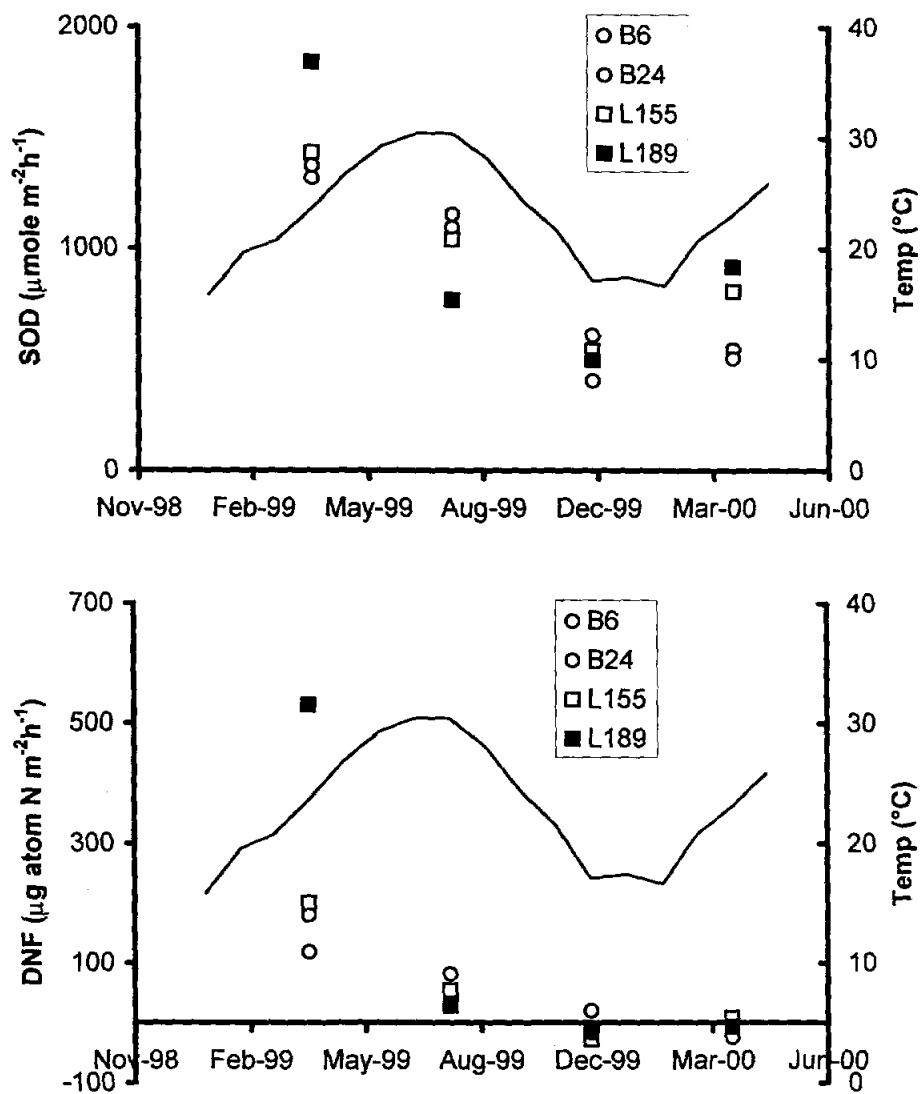


Figure 4. Seasonal variation of sediment oxygen demand (SOD) and denitrification rates (DNF) in Baffin Bay and Laguna Madre stations. The solid line shows water temperature fluctuation (monthly average).

128 to 25  $\mu\text{g atom N m}^{-2} \text{ h}^{-1}$  over the same interval. The low SOD in Aug-99 may have resulted from organic matter limitation. Low concentrations of available organic carbon can limit heterotrophic activity even under favorable temperature conditions. Since denitrification is a heterotrophic process, low organic matter availability could also limit denitrification rates (Koike and Sørensen 1988, Cornwell et al 1999). Denitrification rates showed an exponential relationship with SOD (Fig. 5), which also suggested that organic matter may limit denitrification. The organic matter supply to Laguna Madre/ Baffin Bay sediment might be related to brown tide and seagrass biomass fluctuation. During Apr-99, observed inorganic nitrogen ( $\text{N}_2 + \text{NH}_4^+ + \text{NO}_3^-$ ) flux was higher than the regenerated nitrogen flux calculated from sediment oxygen demand assuming the Redfield ratio ( $\text{O}_2:\text{N}=6; 153\%$ ; Table 4), indicating the remineralization of fresh organic matter with a low C/N ratio at this time. The ratio decreased in the subsequent sampling season and showed a low value in Apr-00 (88%) suggesting the remineralization of more refractory (high C/N) organic matter. The water column chlorophyll concentrations in Laguna Madre and Baffin Bay were high in early 1999 and continually decreased until Dec-99 (Villareal unpublished data). The chlorophyll concentrations tended to recover in Jan-00 and may have caused the higher SOD in Apr-00 (Fig 4). However, denitrification rates in Apr-00 remained low. The decrease in plankton biomass may have been responsible for the decreased SOD and denitrification rates, and increased C/N ratio observed after Apr-99.

In addition to organic matter limitation, the low denitrification rates in summertime compared to Apr-99 may have been caused by sulfide inhibition (Joye and Hallibaugh 1995, Gould and McCready 1982, Jensen and Cox 1992). Anoxic regeneration would increase as oxygen is consumed by respiration of organic materials. In a saline estuary like Laguna

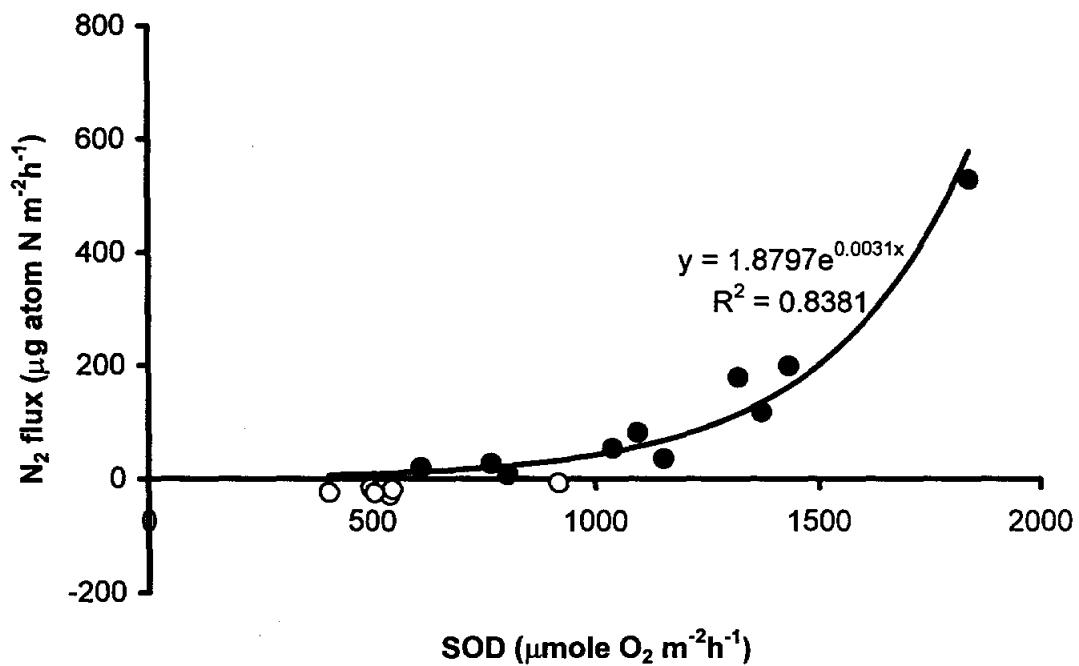


Figure 5. Relationship between sediment oxygen demand and denitrification rates (N<sub>2</sub> flux) in Baffin Bay and Laguna Madre sediment. Only positive N<sub>2</sub> flux was used for the regression because negative values would be caused by N-fixation.

Table 4. Comparison between regenerated nitrogen calculated based on sediment oxygen demand and observed inorganic nitrogen flux.

		N regenerated <sup>a</sup> ( $\mu\text{mol m}^{-2}\text{h}^{-1}$ )	N observed <sup>b</sup> ( $\mu\text{mol m}^{-2}\text{h}^{-1}$ )	Proportion (%)
Apr-99	B6	220	311	142
	B24	229	261	114
	L155	239	208	87
	L189	307	822	268
	<b>AVG</b>			<b>153</b>
Aug-99	B6	183	329	181
	B24	192	179	93
	L155	173	54	31
	L189	128	100	78
	<b>AVG</b>			<b>96</b>
Dec-99	B6	67	72	107
	B24	102	144	141
	L155	90	31	34
	L189	83	115	139
	<b>AVG</b>			<b>105</b>
Apr-00	B6	91	197	216
	B24	84	-19	-22 <sup>c</sup>
	L155	134	34	26
	L189	153	33	21
	<b>AVG</b>			<b>88</b>

a: calculated from observed O<sub>2</sub> flux assuming Redfield ratio (O<sub>2</sub>:N=6).

b: summation of observed N<sub>2</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> flux

c: excluded in average calculation

Madre/Baffin Bay (salinity during this study = 25~38 ppt), sulfate reduction is a major anoxic process and sulfide (the product of sulfate reduction) concentrations in porewater may increase under low oxygen conditions. Sulfide is toxic to many sediment bacterial processes including nitrification and denitrification (Joye and Hallibaugh 1995, Gould and McCready 1982, Jensen and Cox 1992). Due to the shallow water depth, the water column was well mixed, even in Aug-99 (Table 1), but the depth of oxygen penetration in sediment may have decreased during summer at times when conditions were stable. Although seasonal data for porewater sulfide concentrations are not available for our study area, high porewater sulfide concentrations (102-160  $\mu\text{M}$  in July 1996) occur in this region (Lee and Dunton unpublished). Finally, salinity-induced inhibition of denitrifying activity by physiological effects other than sulfide inhibition may have contributed to the low denitrification activity in Baffin Bay and Laguna Madre as was suggested for a Danish estuary (Rysgaard et al. 1999).

In Dec-99 and Apr-00, denitrification rates were not significantly different from zero and fluxes of  $\text{N}_2$  into the sediment due to nitrogen fixation were apparent. The fluxes of  $\text{N}_2$  (-2 to -53  $\mu\text{g atom N m}^{-2} \text{h}^{-1}$ ) may provide a conservative estimate of net nitrogen fixation in this area because our incubations were done under dark or low light conditions (Joye and Pearl 1994). The measured rates are at the low end of the range reported for other seagrass beds. Typically, seagrass beds have higher nitrogen fixation rates (2-500  $\mu\text{g atom N m}^{-2} \text{h}^{-1}$ ) than uncolonized areas (0.04-10  $\mu\text{g atom N m}^{-2} \text{h}^{-1}$ ; Herbert 1999).

### **3-3. Spatial variability of sediment oxygen demand and denitrification rates**

Spatial trends of SOD and denitrification rates were not obvious in Laguna Madre/ Baffin Bay. Both SOD and denitrification rates were significantly higher in

Table 5. Level of significance ( $p$ ) in a t-test against equal average  $N_2$  and  $O_2$  flux assumption between two stations ( $n=4$ ). Significant difference ( $p \leq 0.05$ ) were marked as bold.

$N_2$ flux			$O_2$ flux					
Apr-99			Apr-99					
	B24	L155	L189		B24	L155	L189	
B6	0.21	0.42	0.09		B6	0.43	0.37	0.08
B24		0.25	0.06		B24		0.43	0.1
L155			0.09		L155			0.15
Aug-99			Aug-99					
	B24	L155	L189		B24	L155	L189	
B6	0.09	0.22	<b>0.04</b>		B6	0.21	0.49	<b>0.04</b>
B24		0.38	0.31		B24		0.34	<b>0.02</b>
L155			0.21		L155			0.18
Dec-99			Dec-99					
	B24	L155	L189		B24	L155	L189	
B6	0.15	0.49	0.39		B6	<b>0.01</b>	0.17	0.21
B24		0.13	0.17		B24		0.27	0.11
L155			0.24		L155			0.36
Apr-99			Apr-99					
	B24	L155	L189		B24	L155	L189	
B6	0.39	0.28	0.27		B6	0.41	0.15	<b>0.05</b>
B24		0.25	0.26		B24		0.09	<b>0.02</b>
L155			0.37		L155			0.31

Laguna Madre than in Baffin Bay in Apr-99 (t-test,  $p<0.05$ ). Sediment oxygen demand was significantly higher in Laguna Madre than in Baffin Bay in Apr-00 ( $p<0.01$ ) but denitrification rates were not significantly different ( $p>0.05$ ). The average  $N_2$  fluxes in each station are not significantly different ( $p>0.05$ ) except between B6 and L189 in Aug-99 (Table 5). The SOD in L189 was significantly higher than B6 and B24 in Aug-99 and Apr-00. Except for these incidents, the average fluxes between each station were not significantly different among sampling dates (Table 5). We expected higher remineralization activity in Laguna Madre due to the organic matter input from seagrass biomass, which is absent in Baffin Bay, but the trend was not apparent. If the brown tide biomass is a major organic matter provider in Laguna Madre and Baffin Bay sediment, the relative effect of seagrass would be lessened.

### 3-4 Total nitrogen load and denitrification

A 20-year average for total nitrogen load calculated from freshwater inputs into Baffin Bay was  $1.27 \times 10^6$  g atom N month $^{-1}$  (Brock unpublished data), which is less than 1/100 of that for Galveston Bay, Texas ( $168 \times 10^6$  g atom N month $^{-1}$ , Brock 1995). The average areal total nitrogen input in Baffin Bay is  $9 \mu\text{g atom N m}^{-2}\text{h}^{-1}$  (assuming that the total area of Baffin Bay=193 km $^2$ , Diener 1975), which is much less than the average denitrification rate in Baffin Bay ( $46 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ). This rough calculation confirms that the nitrogen loading via freshwater input plays a minor role in Baffin Bay. Other possible nitrogen sources include water exchange with Laguna Madre and air deposition. Groundwater intrusion could be another source of nitrogen into the area. Nitrogen fixation is already accounted for in our study because the denitrification rate was

estimated from net N<sub>2</sub> change. However, nitrogen fixation in algal mats around the shore and marsh area may be high and important as a nitrogen source in this area and should be investigated.

### **3-5 Nitrate (<sup>15</sup>NO<sub>3</sub><sup>-</sup>) addition experiments**

The inflow water of the flow-through system was enriched with ~100 μM of <sup>15</sup>NO<sub>3</sub><sup>-</sup> and the production of <sup>29+30</sup>N<sub>2</sub> and <sup>15</sup>NH<sub>4</sub><sup>+</sup> in each sediment core were monitored to examine the potential fate of nitrate at each site. The effect of NO<sub>3</sub><sup>-</sup> addition on other processes (SOD and denitrification) is evaluated by comparing the rates before and after the addition. The production rates of two products (<sup>29+30</sup>N<sub>2</sub> and <sup>15</sup>NH<sub>4</sub><sup>+</sup>) are compared with NO<sub>3</sub><sup>-</sup> flux to elucidate the partitioning of two nitrate reduction processes (denitrification and DNRA). Finally, possible reasons for the different total recovery of added <sup>15</sup>NO<sub>3</sub><sup>-</sup> in each sampling site are discussed.

#### **3-5-1 Variations of fluxes after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition**

The sediment oxygen demand tended to increase after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition but the difference was not significant at most stations (Fig. 6; p>0.05). In Aug-99, the SOD in B6 decreased from 1154±109 μmole O<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> before the addition to 432±52 μmole O<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition. In December, fluxes were monitored for 3 days in Laguna Madre (Fig. 6 d) and for 6 days in Baffin Bay (Fig. 6 c). Sediment oxygen demand increased significantly in Baffin Bay stations (B6, B24) on Day 6 (4 days after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition) relative to Day 3 (first day after the addition; Fig. 6 c; p<0.05). In Laguna Madre, SOD (1st day after the addition) was

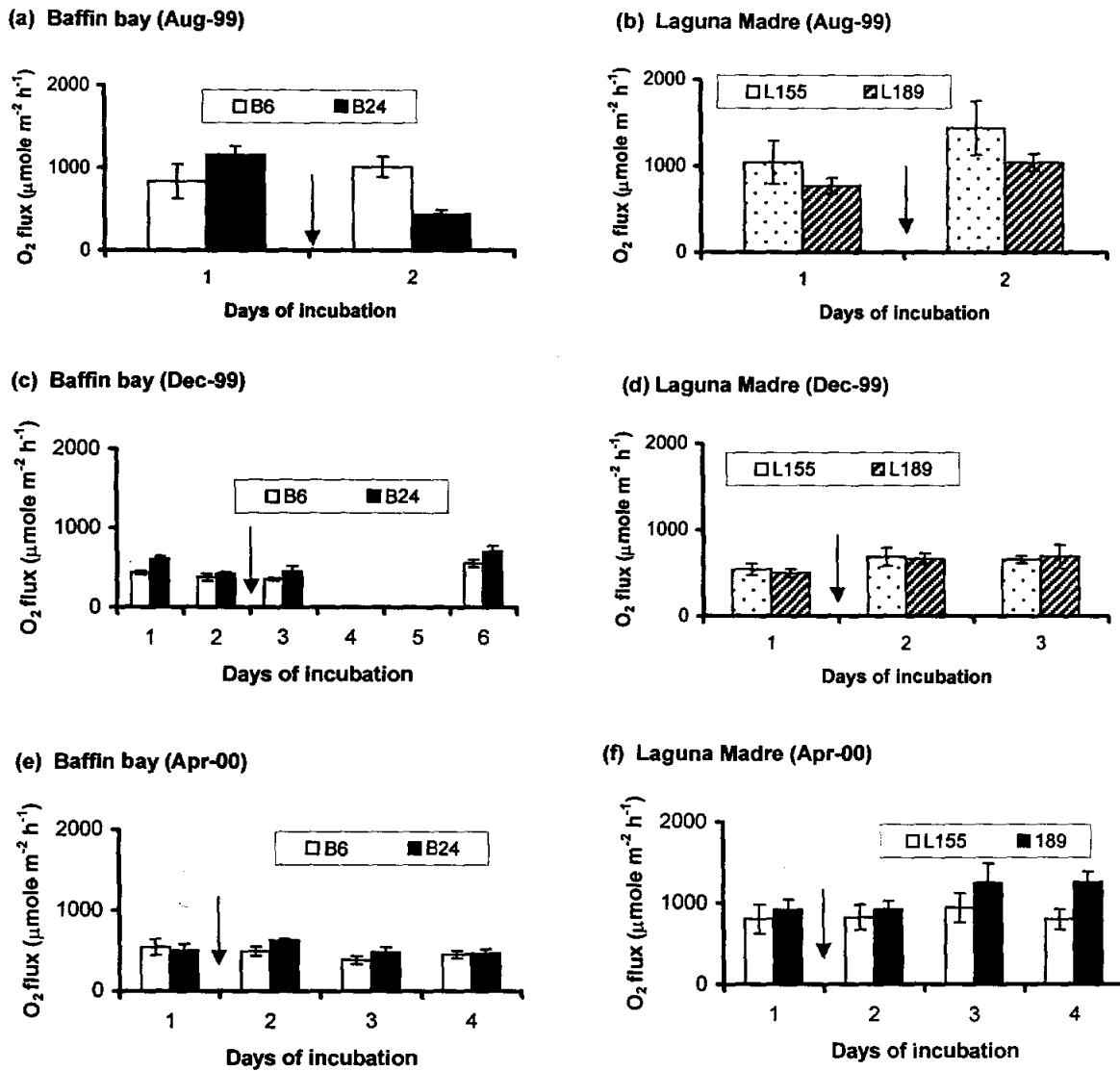


Figure 6. Sediment oxygen demand ( $\mu\text{mole O}_2 \text{ m}^{-2} \text{h}^{-1}$ ) before and after  $^{15}\text{NO}_3^-$  additions. Arrows show the times of additions. Each bar represents an average flux and each line represents  $\pm$  one SE among 4 sediment cores.

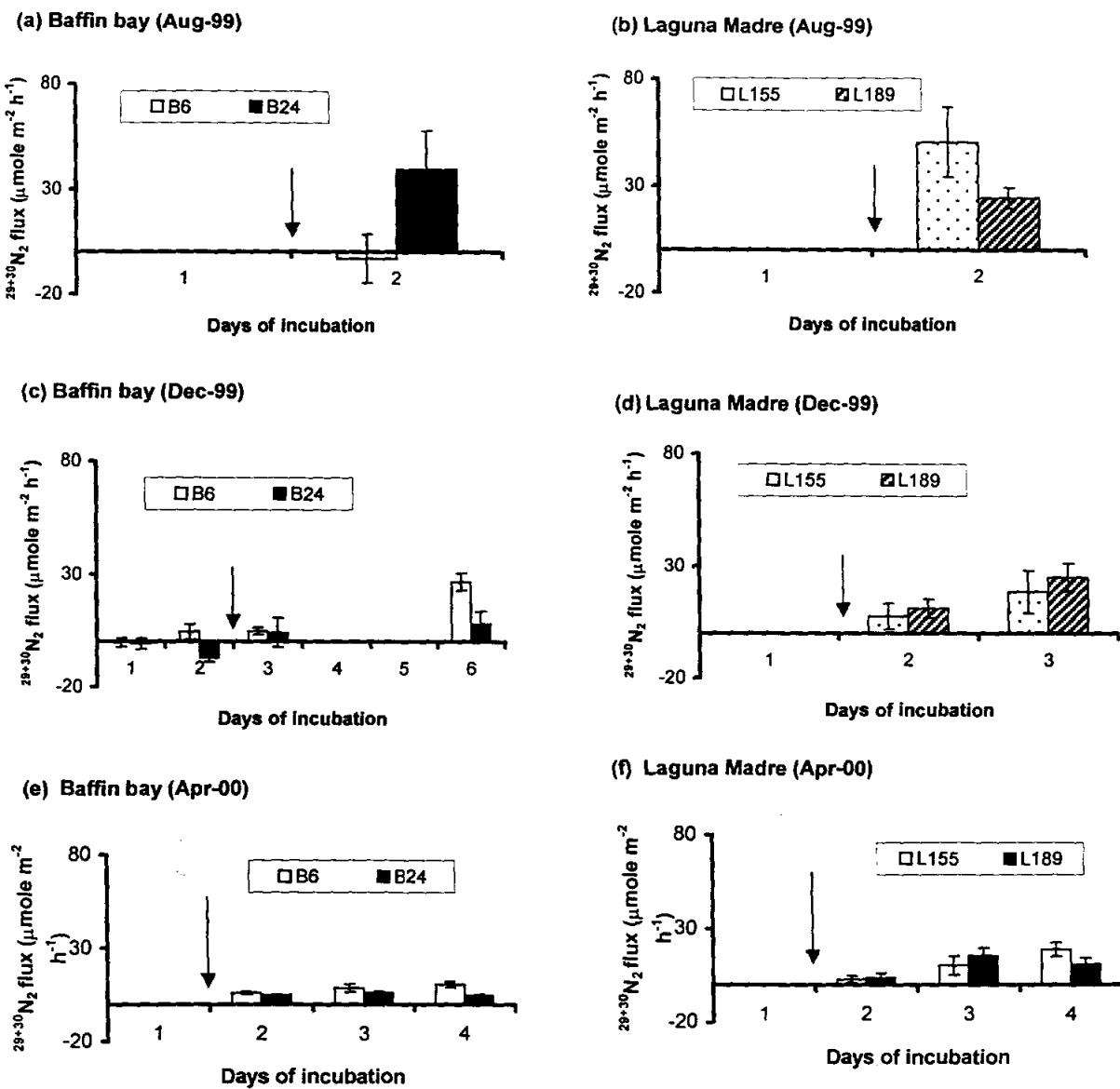


Figure 7. Denitrification rate of  $^{15}\text{NO}_3^-$  ( $^{29}\text{N}_2$  plus  $^{30}\text{N}_2$  production) before and after  $^{15}\text{NO}_3^-$  additions. Arrows show the time of additions. Each bar represents an average flux and each line represents  $\pm$  one SE among 4 sediment cores.

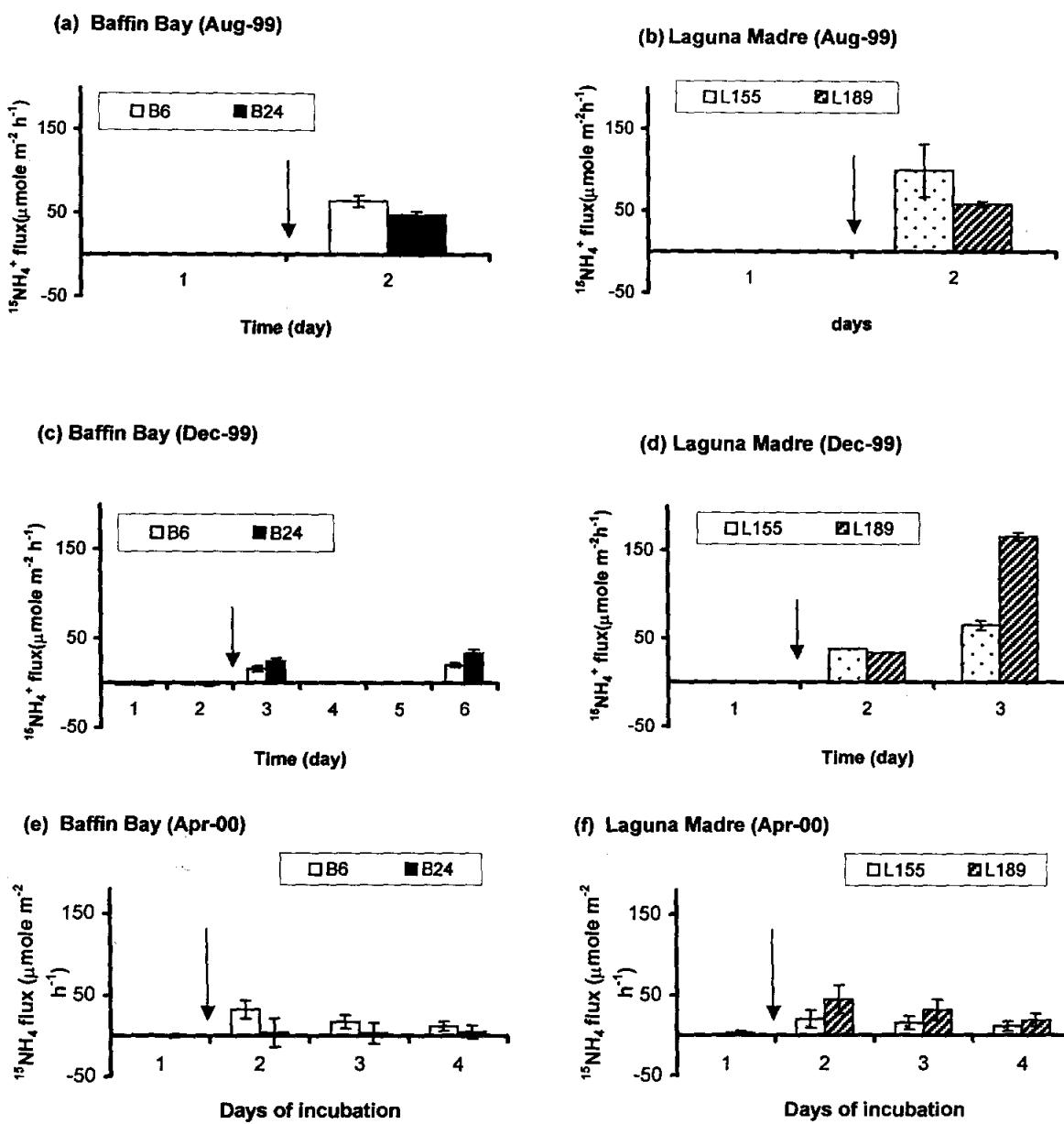


Figure 8. Heavy ammonium ( $^{15}\text{NH}_4^+$ ) flux before and after  $^{15}\text{NO}_3^-$  additions. Arrows show the time of additions. Each bar represents an average flux and each line represents  $\pm$  one SE among 4 sediment cores.

not significantly different on Day 2 from that on Day 3 (Fig. 6 d;  $p>0.05$ ). In Apr-00, SOD did not vary significantly after the addition (Fig. 6 e f;  $p>0.05$ )

Figures 7 and Figure 8 show the heavy N<sub>2</sub> gas (<sup>29+30</sup>N<sub>2</sub>) flux produced during <sup>15</sup>NO<sub>3</sub><sup>-</sup>-based denitrification and the <sup>15</sup>NH<sub>4</sub><sup>+</sup> flux produced from <sup>15</sup>NO<sub>3</sub><sup>-</sup> via DNRA. As expected, neither process was measurable before the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions. After the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions, fluxes were 6.9-37.6 and 12.1-76.8  $\mu\text{g atom N m}^{-2}\text{h}^{-1}$  for <sup>29+30</sup>N<sub>2</sub> and <sup>15</sup>NH<sub>4</sub><sup>+</sup>, respectively (Figure 7, 8 c d, Table 6). Production of <sup>15</sup>N products sometimes increased with time after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions (Figure 7, 8 c d). In station B6, the N<sub>2</sub> gas (<sup>29+30</sup>N<sub>2</sub>) flux increased significantly on Day 6 compared to Day 3 (Figure 7 c). In Laguna Madre stations (L155 and L189), <sup>15</sup>NH<sub>4</sub><sup>+</sup> flux increased significantly on Day 3 compared to Day 2 of the incubation (Fig. 8 d). Except for these incidents, the increases in fluxes over time were not significant (Figure 4, 5 c d).

Flux changes in the different forms of N<sub>2</sub> gas and in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> before and after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions are presented in Table 6. The denitrification rates based on the <sup>14</sup>NO<sub>3</sub><sup>-</sup> (<sup>28</sup>N<sub>2</sub> production; before <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions) were higher in Aug-99 than in Dec-99 and Apr-00. Heavy nitrogen gas (<sup>29+30</sup>N<sub>2</sub>) flux also decreased from Aug-99 to Apr-00.

### 3-5-2. Partitioning of added <sup>15</sup>NO<sub>3</sub><sup>-</sup> to denitrification and DNRA

In Aug-99 and Apr-00, “total” denitrification activities (<sup>28</sup>N<sub>2</sub> plus <sup>29+30</sup>N<sub>2</sub>) did not increase after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions. In December, the <sup>29+30</sup>N<sub>2</sub> fluxes after the additions were comparable to the fluxes in August but the “total” denitrification rates increased after the additions. The <sup>15</sup>NH<sub>4</sub><sup>+</sup> fluxes after the additions ranged from 12.1 to 78.6  $\mu\text{g atom N m}^{-2}\text{h}^{-1}$  (Table 6). Laguna Madre had higher <sup>15</sup>NH<sub>4</sub><sup>+</sup> fluxes than Baffin Bay. The <sup>15</sup>NH<sub>4</sub><sup>+</sup> fluxes after the additions had the highest value in Aug-99 and decreased afterward as was observed

Table 6. Nitrogen gas ( $^{28}\text{N}_2$  and  $^{29+30}\text{N}_2$ ),  $\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  flux ( $\mu\text{g atom N m}^{-2} \text{ h}^{-1}$ )

during sediment incubation experiments in Aug-99, Dec-99 and Apr-00. Average fluxes and standard errors (parenthesis) among replicate cores ( $n=16\sim32$ ) were presented before and after the  $^{15}\text{NO}_3^-$  additions.

A negative flux denotes a flux into the sediment. The percent of recovery for  $^{29+30}\text{N}_2$  and  $^{15}\text{NH}_4^+$  was calculated assuming that all the  $\text{NO}_3^-$  flux consisted of  $^{15}\text{NO}_3^-$ .

		$^{28}\text{N}_2$ flux	$\text{NO}_3^-$ flux	$^{29+30}\text{N}_2$ flux		$^{15}\text{NH}_4^+$ flux		% $^{15}\text{NO}_3^-$ recovery as $\text{N}_2 + \text{NH}_4^+$
				flux	% of $\text{NO}_3^-$ flux	flux	% of $\text{NO}_3^-$ flux	
Aug-99	Baffin Bay	Before	67.8 (15.8)	0.74 (1.3)	0 (0)	0 (0)	15.0	19.9
		After	47.5 (6.5)	-370 (103)	18.2 (21.3)	55.4 (8.3)		
	Laguna Madre	Before	41.3 (14.3)	0.23	-0.12 (0.04)	0	61.4	90.8
		After	35.3 (20.3)	-128 (79)	37.6 (13)	78.6 (20)		
Dec-99	Baffin Bay	Before	-2.3 (9.3)	1.2 (0.3)	-1.05 (2.7)	-2.5 (0.37)	16.0	23.3
		After	3.1(10.5)	-146 (4.3)	10.7 (5.4)	23.3 (3.8)		
	Laguna Madre	Before	-19.9 (5.2)	0.13 (0.04)	0.34 (9.2)	-0.7 (0.9)	74.8	90.1
		After	-9.0 (8.8)	-101.7 (33)	15.5 (9.9)	76.1 (18)		
Apr-00	Baffin Bay	Before	-10 (6.1)	-0.3 (0.2)	0.01 (0.00)	0.63 (0.8)		
		After	-17 (6.8)	-96 (38)	6.9 (0.9)	12.1 (3.5)	12.6	19.8
	Laguna Madre	Before	0.2 (9.2)	1.4 (0.39)	0.16 (0.03)	2 (1.2)		
		After	-10 (6.8)	-47 (13)	10.2 (2.5)	23.6 (7.7)	50.2	71.9

in  $^{29+30}\text{N}_2$  fluxes. In Dec-99, a small apparent negative  $^{15}\text{NH}_4^+$  fluxes were observed before the additions, but they were not significantly different from zero and can be attributed to analytical variability. Before the additions, small  $\text{NO}_3^-$  fluxes out of the sediment were observed at all stations. After the additions,  $\text{NO}_3^-$  fluxes into the sediment were high (up to  $370 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ). Baffin Bay sediment consumed more  $\text{NO}_3^-$  (higher negative  $\text{NO}_3^-$  flux) than Laguna Madre after the additions.

Since  $\text{NO}_3^-$  fluxes were small before the additions, all  $\text{NO}_3^-$  fluxes after the additions were assumed to be  $^{15}\text{NO}_3^-$  for comparison with  $^{29+30}\text{N}_2$  and  $^{15}\text{NH}_4^+$  fluxes. Denitrification consumed 5-29 % of the added  $^{15}\text{NO}_3^-$  while DNRA accounted for 13 – 75 % of the added  $^{15}\text{NO}_3^-$  (Table 6). Both the  $^{29+30}\text{N}_2$  and  $^{15}\text{NH}_4^+$  fluxes were smaller in Baffin Bay than in Laguna Madre, whereas the  $\text{NO}_3^-$  fluxes into the sediment were higher in Baffin Bay. As a result, the percentage recovery of added  $^{15}\text{NO}_3^-$  was much higher in Laguna Madre than in Baffin Bay. The recovery of  $^{15}\text{NO}_3^-$  as  $^{29+30}\text{N}_2$  and  $^{15}\text{NH}_4^+$  did not show marked seasonal differences. For example,  $^{15}\text{NH}_4^+$  flux remained almost the same in three seasons. The total recovery of added  $^{15}\text{NO}_3^-$  as  $^{29+30}\text{N}_2$  and  $^{15}\text{NH}_4^+$  ranged from 20 to 91 % but seasonal differences were not noticeable. In general, denitrification rates were not enhanced significantly by  $^{15}\text{NO}_3^-$  additions. This result was surprising because, in many estuaries, denitrification is limited by  $\text{NO}_3^-$  availability (Koike and Sørensen 1988, Cornwell et al. 1999). Instead, a portion of the added  $^{15}\text{NO}_3^-$  was converted to  $^{15}\text{NH}_4^+$ . The  $^{15}\text{NH}_4^+$  production rates were comparable to those measured in Mangoku-Urs, Simoda Bay and Toyo Bay, Japan ( $5-228 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ; assuming 0-3 cm depth integration and  $2.3 \text{ g cm}^{-3}$  bulk sediment density; Koike and Hattori 1978) even though the  $^{15}\text{NO}_3^-$  enrichment level varied (~15-30  $\mu\text{M}$  and ~100  $\mu\text{M}$  in Koike and Hattori [1978] and this study, respectively) and the experimental setup was different. Higher production rates (800-3200  $\mu\text{g}$

atom N m<sup>-2</sup>h<sup>-1</sup>) were observed in Norsminde Fjord, Denmark with a high <sup>15</sup>NO<sub>3</sub><sup>-</sup> enrichment (~700 μM; assuming 0-4 cm depth integration; Jørgensen 1989). Our rates were much lower than those from sediment slurry incubations (~ 2300 μg atom N m<sup>-2</sup>h<sup>-1</sup>; assuming 0-12 cm depth integration; Sørensen 1978) with a similar <sup>15</sup>NO<sub>3</sub><sup>-</sup> incubation enrichment (~100 μM). Reduced conditions favor DNRA over denitrification, and slurry experiments tend to have higher rates of DNRA in partitioning the added <sup>15</sup>NO<sub>3</sub><sup>-</sup> compared to intact core experiments (Kasper 1983). In our study, DNRA rates were higher than denitrification rates even though intact cores were used for incubation and O<sub>2</sub> concentration in the water column was maintained at more than 80 % of saturation. Thus, it seems unlikely that this high DNRA partitioning over denitrification resulted from highly-reduced conditions in the sediments as suggested by Kasper (1983).

We do not believe that the high partitioning of NO<sub>3</sub><sup>-</sup> to DNRA was the result of <sup>15</sup>NO<sub>3</sub><sup>-</sup> enrichment. The enrichment of NO<sub>3</sub><sup>-</sup> should not only stimulate both denitrification and DNRA but could be expected to favor denitrification over DNRA (Sørensen 1987). Also, since DNRA bacteria have higher Km (100 to 500 μM NO<sub>3</sub><sup>-</sup>) than denitrifiers (5 to 10 μM NO<sub>3</sub><sup>-</sup>; Jørgensen 1987), the enrichment level in our study (~100 μM) should favor denitrification rather than DNRA if the level of enrichment affects the partitioning.

Low denitrification rates and high DNRA activity were observed during summer in a French coastal lagoon (Rysgaard et al. 1996). Sulfate reduction rates were high at this time of the year and the low denitrification rates were attributed to inhibited nitrification. The high DNRA activity was attributed to the presence of sulfate reducing bacteria, which have DNRA capacity as a secondary metabolism (Rysgaard et al. 1996). This finding agrees with observations of King and Nedwell (1985) and Jørgensen (1989). DNRA may be favored in

environments where  $\text{NO}_3^-$  availability is variable because DNRA bacteria have constitutive enzymes (Jørgensen 1989).

Chemolithoautotrophic bacteria that use reduced sulfur compounds as an electron donor and reduce  $\text{NO}_3^-$  to  $\text{NH}_4^+$  have been reported (Schedel and Truper 1980), but the ecological significance of the process is not known (Sørensen 1987). Ammonium production coincided with  $\text{NO}_3^-$  and  $\text{H}_2\text{S}$  depletion in sediment slurry experiments enriched with  $\text{NO}_3^-$  and different forms of sulfur compounds, (Brundet and Garcia-Gil 1986). Whereas denitrification was incomplete (final product was  $\text{N}_2\text{O}$  instead of  $\text{N}_2$ ), DNRA occurred in the presence of high sulfide concentrations; in contrast, denitrification was the major  $\text{NO}_3^-$  sink when sulfide concentrations were low (Brundet and Garcia-Gil 1986). Abundant populations of  $\text{NO}_3^-$ -storing sulfur bacteria of genus *Thioploca*, *Beggiatoa* and *Thiomargarita* have been reported in sediments of coastal upwelling regions (Schultz et al. 1999). A chemolithotrophic coupling of  $\text{NO}_3^-$  and sulfide through  $\text{NO}_3^-$ -storing sulfur bacteria may be a widespread feature of coastal sediment (Schultz et al. 1999).

Along with the high sulfide concentrations that occur in this region (Lee and Dunton unpublished), our data support the idea that sulfide could influence DNRA at both of our sampling sites. Being a negative estuary where evaporation exceeds precipitation, salinity in Laguna Madre/Baffin Bay is higher than in most estuaries and sulfate reduction may be a major organic matter degradation process. The higher DNRA activity observed in Laguna Madre than in Baffin Bay (Table 6) may result from high sulfide reduction activity fueled by seagrass detritus (Rysgaard 1996). Figure 9 shows the proposed influence of sulfide on nitrogen transformations in this system. High sulfide concentrations may inhibit nitrification and keep  $\text{NO}_3^-$  availability at a low level. Since denitrification is also inhibited by sulfide,  $\text{NO}_3^-$  that is

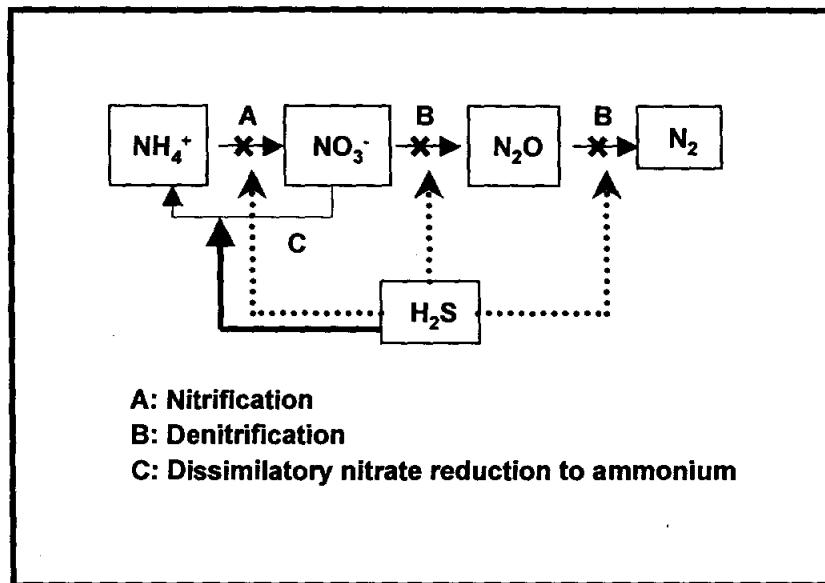


Figure 9. Proposed relationship between sulfide and nitrogen transformations. Solid arrow represents positive effect and broken arrow represent negative effect. Modified from Brunet and Garcia-Gil (1996).

present will be available for DNRA rather than denitrification. Inhibition of denitrification can explain the enhanced DNRA that we observed, even though the Km for DNRA bacteria is higher than for denitirifiers and the  $^{15}\text{NO}_3^-$  enrichment level was relatively low. Thus, whereas high sulfide concentrations inhibit nitrification and denitrification, the sulfide may fuel DNRA by providing an electron donor. The high SOD, high DNRA and low denitrification rates in Laguna Madre compared to Baffin Bay support this hypothesis.

### 3-5-3 Recovery of added $^{15}\text{NO}_3^-$

The recovery of  $^{15}\text{NO}_3^-$  was much higher in Laguna Madre than in Baffin Bay (Table 6). The  $^{15}\text{NO}_3^-$  flux into the sediment was higher in Baffin Bay whereas  $^{15}\text{NH}_4^+$  and  $^{29+30}\text{N}_2$  fluxes were lower than in Laguna Madre. The  $^{15}\text{NH}_4^+$  flux difference was more distinctive than that for  $^{29+30}\text{N}_2$  flux. One possible explanation is different sediment types between the two sites. Even though the sand content ( $>64 \mu\text{m}$ ) was not much different between the two sites (Table 1), the fine clay content may be high and reduce light penetration, and seagrass production in Baffin Bay (Lee and Dunton 1999). If that is the case, the fine particles in Baffin Bay would have a large surface area relative to the volume and may provide  $\text{NH}_4^+$  binding sites. If a large portion of  $^{15}\text{NH}_4^+$  is fixed in silicate lattices of mineral particles (Jørgensen 1987), measured  $^{15}\text{NH}_4^+$  flux could be less than the conversion rate from  $^{15}\text{NO}_3^-$  into  $^{15}\text{NH}_4^+$  until a steady state is reached. The high recovery of  $^{15}\text{N}$  in Laguna Madre might also be caused by a high seagrass biomass. The presence of seagrass increases the availability of labile organic matter and  $\text{O}_2$  (Lee and Dunton 1999). The abundance and community structure of microbes in the seagrass bed may be different than those in regions without seagrass. For example, the bacteria responsible for the  $^{15}\text{NH}_4^+$  production in Laguna Madre may be sulfate reducers, which have  $\text{NO}_3^-$  reduction

capability as a second metabolism (Rysgaard 1996). Nitrate reduction to ammonium by sulfate reducing bacteria was the main pathway of  $\text{NO}_3^-$  reduction in a seagrass (*Zostera noltii*) dominated tidal basin in Southern France (Bassin d'Arcachon; Rysgaard 1996). The density of sulfate-reducing bacteria may be low in Baffin Bay and other microbes such as  $\text{NO}_3^-$ -storing sulfur bacteria might be responsible for the  $^{15}\text{NH}_4^+$  production (Schultz et al. 1999). The presence of nitrate storing bacteria could help account for the low recovery of  $^{15}\text{N}$  from  $^{15}\text{NO}_3^-$  that was observed in Baffin Bay. Although we did not confirm the importance of ground water intrusion in Baffin Bay, low-salinity, low  $\text{O}_2$ , and high  $\text{NO}_3^-$  bottom water was observed at Station B6 during August (Table 1). The low  $\text{O}_2$  and high  $\text{NO}_3^-$  environment is similar to the habitat of  $\text{NO}_3^-$ -storing sulfur bacteria in upwelling region (Schultz et al. 1999). Although *Thalassia testudinum* in Laguna Madre prefers  $\text{NH}_4^+$  as a nitrogen source, the seagrass can also use  $\text{NO}_3^-$  (Lee and Dunton 1999). The  $^{15}\text{NO}_3^-$  assimilation by seagrass and subsequent regeneration to  $^{15}\text{NH}_4^+$  is an extra pathway of  $^{15}\text{NH}_4^+$  production in Laguna Madre, which is absent in Baffin Bay. However, the pathway should take longer than the direct conversion of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  by DNRA and the contribution of this pathway to the high  $^{15}\text{NO}_3^-$  recovery in Laguna Madre is not certain.

Our average recovery of 56 % was similar to the results in Norsminde Fjord, Denmark, where more than 50 % of the added  $^{15}\text{NO}_3^-$  was recovered from intact core incubations (Jørgensen 1989). Suggested reasons for incomplete recovery include  $^{15}\text{NH}_4^+$  fixation into the nonexchangeable  $\text{NH}_4^+$  pool, production of  $^{15}\text{NO}$ , and incorporation to dissolved or particulate organic matter. The  $^{15}\text{N}$  content in organic matter was not measured in our study. It is reasonable that a loss to organic N production could account for some of the lost  $^{15}\text{NO}_3^-$ , even though the proportion may be small (Jørgensen 1989; 2-4 %). However, the proportion of

DNRA (recovered as  $^{15}\text{NH}_4^+$ ) was higher (42 %) on our study than values reported by Jørgensen (1989; 22%). Recovery via denitrification in our study was 14 %, which is lower than the Jørgensen (24%; 1989) or Kaspar values (70-95%; 1983).

#### 4. Nitrogen cycling and organic matter input

Differences in SOD and denitrification rates were not obvious between Baffin Bay and Laguna Madre, but temporal variations were distinctive. In the  $^{15}\text{NO}_3^-$  addition experiments, the Laguna Madre had high  $^{15}\text{NO}_3^-$  based denitrification rates, high DNRA and high recovery of added  $^{15}\text{NO}_3^-$  compared to Baffin Bay. The seasonal trend in the enrichment experiments showed the same pattern as SOD and denitrification rates.

The distinctive temporal pattern and minimum spatial variability may have been caused by the organic matter supply changes that affected both Baffin Bay and Laguna Madre. The decline of Texas Brown Tide could be an example. On the other hand, Laguna Madre and Baffin Bay responded differently to the nitrate additions, which may have resulted from the high sulfate reduction fueled by seagrass biomass in Laguna Madre.

Sulfide-inhibited denitrification and sulfide-induced DNRA would contribute to the preservation of nitrogen in Laguna Madre/ Baffin Bay. The preserved nitrogen might sustain abundant biota in this region when other major nutrient inputs are absent. It may also explain apparent phosphate limitation of primary production observed in the region (personal comm. J. Cotner, Univ. of Minn.). The high potential for DNRA in this region suggests that the dominant form of inorganic nitrogen available to phytoplankton is  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$ . The Texas brown tide organism, *Aureomonas lagunensis*, which can use  $\text{NH}_4^+$  or  $\text{NO}_2^-$ , but cannot use  $\text{NO}_3^-$

as a nitrogen source, could out compete other algae in this  $\text{NH}_4^+$  replete environment. It is reasonable that the persistency of the Brown Tide algal bloom in this area could have been enhanced by DNRA activity.

## 5. Conclusions

1. The denitrification rates measured in Laguna Madre/ Baffin Bay were  $0\text{--}257 \mu\text{g atom N m}^{-2}\text{h}^{-1}$  during the study period of Apr-99 to Apr-00.
2. Denitrification rates were highest in Apr-99 and decreased in subsequent measurements. The spatial variability was small.
3. Denitrification rates did not follow water temperature fluctuations and appeared to be limited by organic matter supply into the sediment.
4. Potential rates of dissimilatory nitrate reduction to ammonium were high ( $12.1\text{--}78.6 \mu\text{g atom N m}^{-2}\text{h}^{-1}$ ) and comparable or higher than denitrification rates.

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**ATTACHMENT 1**  
**TEXAS WATER DEVELOPMENT BOARD**  
Review of the Draft Final Report: Contract No. 99-483-278  
University of Texas Marine Science Institute,  
"Nitrogen Cycling in Laguna Madre and Baffin Bay"

The report "Nitrogen Cycling in Laguna Madre and Baffin Bay" meets or surpasses all contractual requirements in the Scope of Work. The report was exceptionally well written. Results and discussion cover the specific objectives of the project and provide additional insights, which will be helpful in preparing recommendations for freshwater inflow needs of the Laguna Madre system. Data from the project (parts I and II) have been received by the contract manager in the form of excel spreadsheets.

The following are technical comments and recommendations for Dr. Gardner's and Dr. An's consideration.

1. The cover sheet should include the report date and the TWDB contract number.
2. Page 2: The word "since" has a temporal denotation; "because" is a better word and preserves the meaning of the sentence.
3. Page 3: Delete the word "other" from "decline in seagrass and other benthic fauna" in the middle of the second paragraph because seagrass is flora not fauna. Also with reference to this same sentence, did Montagna and Kalke (1995) address the effects of brown tide on seagrass and benthic fauna, or just benthic fauna? The sentence reads as though M&K 1995 draws conclusions regarding both.
4. Page 11, Figure 3: The precipitation line is so fine that it can be lost when xeroxed. The figure would benefit from a thicker line size.
5. Page 13: A few sentences have only one space after the period (see middle paragraph). Also, reword first sentence of last paragraph; it is unclear. For example, "...comparable to our measurements in spite of problems with the gas purging denitrification method caused by lengthy incubation periods..."
6. Page 15: A parenthetical note where Table 2 is introduced in the text or an explanatory line in the heading for Table 2 explaining the possible significance of negative values would prime the reader to look for and accept those entries.
7. Pages 15, 18, and 31: Table heading line spacing is irregular; some lines appear to be 1.5 spaced while others are single-spaced. Also on page 18, seasonal means are missing standard errors; perhaps "Mean (SE)" should be changed to "Mean".
8. Page 17: In the header to Table 3, last line, using the "less than" symbol might avoid confusion over whether precision was good or less than good.
9. Page 23: The name Rysgaard is used a number of times in the manuscript and is sometimes misspelled (see page 33). Rysgaard may be spelled with an "ä".

10. Page 25: The last sentence of 3-3 may be worded too strongly. The authors might consider whether it would be more appropriate to end the statement as "...the relative effect of seagrass would be lessened." This is just a suggestion.
11. See comment for page 2; "Since" is used in second to last sentence. Suggested rewording: "Nitrogen fixation is already accounted for in the study because the denitrification rate was estimated from N<sub>2</sub> change."
12. Page 33: Jorgensen is probably spelled with an "ø".
13. Page 36, section 3-5-3, line 6: There appears to be text missing between "Baffin Bay" and "considering that seagrass density...".
14. Page 39: The first bulleted conclusion is not lined up with the others.