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Nitrogen Cycling and Bacterial Production

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Acknowledgements

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I. Bacterial Abundance and Production

Introduction

Heterotrophic bacteria are the major decomposers of dissolved and particulate organic matter in aquatic ecosystems. Bacterial activity influences most biogeochemical cycles and can affect the overall productivity of ecosystems through the regulation of nutrient regeneration and the production of bacterial biomass. Estimates of bacterial carbon production typically range between 10 and 50% of phytoplankton production on an areal basis (Azam et al. 1983; Cole et al. 1988). Bacteria therefore process a large percentage of phytoplankton production and may serve as an important link in aquatic food webs.

Bacterial abundance and production were measured over various spatial and temporal scales in the Guadalupe and Nueces Estuaries. Relationships among bacterial abundance, bacterial production, salinity and temperature are investigated. Carbon and nitrogen flow through bacterioplankton is estimated, and the potential role of bacteria in estuarine food webs is examined.

Materials and Methods

Sampling Locations Water samples were collected from four stations (A, B, C, D) in the Guadalupe Estuary. All stations were located in San Antonio Bay. The location of these sampling sites can be found on the maps located at the beginning of this report. The mean depths at Stations A, B, C and D were 1.25 m, 1.9 m, 1.9 m and 1.75 m respectively. These stations were sampled during January, April and July of 1987 when salinities were very low (0-5‰) due to high freshwater runoff, and during July 1988 when salinities had increased (10-25‰) owing to drier conditions. Water samples were collected from four stations (A, B, C, D) in the Nueces Estuary, including both Nueces and Corpus Christi Bays.

The location of these sampling sites can also be found on the maps located at the beginning of this report. The mean depths at Stations A, B, C and D were 1.2 m, 1.9 m, 3.66 m and 2.2 m respectively. These stations were sampled during October 1987 and February, May and July 1988. Strong salinity gradients were not present during the study period, and the total range of salinities at these stations on these dates was about 24 to 37 ‰. All data reported are for surface water samples which were collected in acid-washed polycarbonate bottles unless otherwise stated.

Bacterial Abundance Bacteria in water samples were fixed with a formalin solution buffered with sodium cacodylate (final concentration was 3% formalin and 0.1 M cacodylate). Each fixed sample was stained with an acridine orange solution (final concentration 0.025%) for 5 minutes and filtered through a 0.2 µm pore-size black Nuclepore filter. Bacterial cells retained on the filter were counted using the epifluorescence microscopy technique (Hobbie et al. 1977) and a Zeiss Universal Microscope.

Bacterial Biomass Water samples prepared for epifluorescence microscopy were also used to measure bacterial biovolumes for estimation of bacterial biomass (Fuhrman 1981; Benner et al. 1988). Cells were photographed on Kodak Ektachrome (200 ASA) 35-mm film. The dimensions of ~50 bacteria from each water sample were measured by projecting the slides on a wall screen overlaid with tracing paper. Cells were outlined on the tracing paper, and biovolumes were calculated from measurements of cell length and width using the formula:

$$\text{Biovolume} = \pi/4 \cdot W^2 \, (L-W/3)$$

where W is the measured cell width and L the measured cell length (L=W for cocci). The accuracy of our measurement technique was checked with fluorescent latex beads with a diameter (0.434 µm, Duke Scientific) similar to those of naturally occurring bacteria. Carbon content of bacteria was estimated with the conversion factor 2.2×10^{-7} µg C μm^3 (Bratbak and Dundas 1984).

Bacterial Production Rates of incorporation of [^{3}H-methyl]-thymidine were measured and converted to rates of bacterial production by standard procedures (Fuhrman and Azam 1982). [^{3}H]Thymidine was added to 10 ml samples at a final concentration of 10 or 20 nM. Samples were incubated in a flowing-water bath at the *in situ* temperature for 0.75 to 2h. After incubation samples were chilled for 5 min in an ice bath and filtered through 0.22 µm pore-size Millipore filters using an Hoeffer Scientific filtration apparatus. Filter towers were chilled before each use. A 5 ml portion of 5% trichloroacetic acid (TCA) was added to the filter tower, and the filters were extracted for 5 min. The filters were rinsed with several mls of 5% TCA and placed in glass scintillation vials with 2 ml 5% TCA. Vials were heated to 95°C for 30 min and then chilled in an ice bath for 10 min. The chilled samples were filtered through 0.22 µm pore-size Millipore filters, washed with 1 ml of 5% TCA and collected directly into scintillation vials. The collected fraction consists primarily of hydrolyzed nucleic acids (DNA and RNA), whereas proteins are retained on the filter. Liquid scintillation cocktail (Research Products International) was added to the vials for radioassay using a Beckman LSC 5500 spectrometer.

Time course experiments were conducted in October 1987 and August 1988 to determine if thymidine incorporation rates were linear during the course of the incubation. In October incorporation rates were linear for at least 3 h and in August rates were linear for at least 1.5 h. Thymidine dilution curve experiments were conducted in February and August with thymidine concentrations ranging from 2.5 to 40 nM. Saturation or maximal incorporation rates were obtained with 10 nM or less of thymidine on both dates.

Conversion Factor Experiments Experiments were conducted in February at Station A in Nueces Bay and Station C in Corpus Christi Bay to determine the number of bacteria produced per mole

of thymidine incorporated into nucleic acids. Water samples (1 l) were filtered through 1.0 μm pore-size Nuclepore filters to remove bacteriovores and incubated in the dark for 24 h. Aliquots of water were withdrawn at approximately 4 h intervals for determination of bacterial abundance and incorporation of $[^3H]$thymidine. Conversion factors were calculated with slight modifications of the method of Kirchman et al. (1982). The equation is:

$$\text{Conversion Factor} = \mu\ (N/v)$$

where μ is the slope of natural logarithm of bacterial abundance versus time (i.e. specific growth rate), N is the average number of cells measured during exponential growth, and v is the average number of moles of thymidine incorporated during exponential growth.

Results and Discusion

Bacterial Abundance Bacterial abundance was measured in two bay-estuaries during 1987-88. In Guadalupe estuary bacterial populations ranged from 0.76 to 14.60 x 10^9 cells l^{-1} (Table 1). Bacterial abundance was lowest in winter and highest during the summer, but bacterial abundance was not significantly ($P = 0.05$) correlated with temperature. There was a strong correlation ($r = 0.79$; $P < 0.001$) between bacterial abundance and salinity, with greater abundances at higher salinities. Bacterial populations in the Nueces estuary, including both Nueces and Corpus Christi Bays, ranged from 1.23 to 8.20 x 10^9 cells l^{-1} (Table 2). Bacterial populations were typically highest during the summer and lowest during the winter. Bacterial abundance in these bays was significantly correlated with both temperature ($r = 0.67$; $P = 0.01$) and salinity ($r = 0.57$; $P = 0.05$). Salinities were much higher in Nueces Estuary (range 24-37%) than in Guadalupe Estuary (range 0-25%.).

The range of values for bacterial abundance in these Texas

estuaries were similar to those measured by the same methodology in a variety of other estuaries (see Coffin and Sharp 1987 and references therein). Several investigators have reported a strong positive relationship between temperature and bacterial abundance in other estuaries (Vaatanen 1980, Wilson and Stevenson 1980, Wright and Coffin 1983, Coffin and Sharp 1987). There does not appear, however, to be a precedent for a strong relationship between bacterial abundance and salinity in most estuaries. Albright (1983) reported highest bacterial numbers in the upper estuary of several British Columbia rivers, Wright and Coffin (1983) reported highest bacterial numbers in the middle of a salt-marsh estuary in Massachusetts, and Coffin and Sharp (1987) reported highest bacterial numbers in the lower Delaware estuary.

Bacterial Production Rates of [^{3}H]thymidine incorporation into macromolecules (cold TCA extracted filters) were measured in Guadalupe Estuary during sampling dates in 1987. Rates of [^{3}H]thymidine incorporation into a nucleic acid fraction were measured in Guadalupe Estuary during July 1988 and at all sampling dates in Nueces Estuary. Rates of bacterial production were estimated from incorporation rates of thymidine using empirically-derived conversion factors. Conversion factor experiments were conducted in Nueces and Corpus Christi Bay waters, but no conversion factor experiments were conducted in San Antonio Bay waters. The conversion factor derived from experiments with Nueces and Corpus Christi Bay waters were used for data collected in San Antonio Bay (Guadalupe Estuary). Calculated conversion factors were similar in Nueces and Corpus Christi Bays (1.60 and 1.86 x 10^{18} cells mol^{-1} thymidine), so an average of the two values was used for all calculations of bacterial production. Conversion factors typically do not vary by more than a factor of 3 in estuarine systems (Rieman et al. 1987), and the factor used in this study (1.73 x 10^{18} cells mol^{-1} thymidine incorporated) is relatively conservative.

Rates of bacterial production in Guadalupe Estuary ranged from 4.45 to 61.7 x 10^7 cells l^{-1} h^{-1} (Table 3). During January, April and July 1987, rates of bacterial production increased with increasing distance from the inflow of the Guadalupe River. This trend was reversed during July 1988 when salinities were much higher throughout the estuary. Overall, there was no significant linear relationship between production and salinity ($r = 0.03$; $P > 0.05$). There was a strong linear relationship ($r = 0.82$; $P < 0.001$) between rates of bacterial production and temperature. Bacterial turnover times (cell abundance/cell production) ranged from 5 h in July 1987 to 76 h in April 1987 (Table 4). These turnover times are very short indicating that bacterial growth was rapid.

Rates of bacterial production in Nueces Estuary ranged from 1.13 to 12.3 x 10^7 cells l^{-1} d^{-1} (Table 5). On average, rates of bacterial production in Nueces Estuary were 5-fold lower than rates in Guadalupe Estuary. As in Guadalupe Estuary, bacterial production was significantly correlated with temperature ($r = 0.73$; $P < 0.01$) but not with salinity ($r = 0.25$). Turnover times of bacterial populations ranged from 21 to 210 h with the longest turnover times occurring during the colder winter months (Table 6).

Additional studies of bacterial production in Nueces Estuary were conducted throughout the study period to investigate small-scale spatial and temporal variations. The primary purpose of these experiments was to investigate the validity of extrapolating our limited number of bacterial production estimates over the entire water column for 24 h periods. In October 1987 we sampled three sites (surface water samples 10 m apart) at each station. We found that within station variability was minimal and that the differences we observed between stations in Nueces Bay and Corpus Christi Bay were significant. In February 1988 we investigated the diel variability in rates of production. Surface

water samples were collected every 6 h beginning at noon at Stations A and D. Rates of production at both stations were invariable during 24 h periods.

Day-to-day variability was investigated during May 1988. Surface water samples were collected from all stations on May 9 and on May 13. At Stations A, B and C daily variation in production rates differed by factors of 1.3 to 1.4, but at Station D production rates were 2-fold higher on May 9. Thus, day-to-day variability can be significant indicating that sampling once a season was probably not sufficient to determine an accurate picture of seasonal variations. In July 1988 production rates in surface and bottom water were compared at all stations. No significant variation was observed at Stations A, B and C. The water column was stratified at Station D on this date with a lens of high-salinity (38‰), low-oxygen water at the bottom. Rates of production at Station D were 1.7-fold higher in bottom water than in surface water. It therefore appears that surface water measurements of bacterial production typically give reasonable approximations of production throughout the water column in these shallow, well-mixed estuaries.

Bacterial volumes were measured for all surface water samples from Nueces Estuary. Average biovolumes at each sampling location at each date ranged from 0.044 to 0.284 μm^3 with higher values from summer and fall samples (Table 7). Cells were typically smaller during February and May. No obvious relationship between biovolumes and sample location was observed. The average volume of bacterial cells from all sample locations and all sample dates was 0.096 μm^3. These biovolume measurements are slightly larger than measurements of cells from Delaware Bay which ranged from 0.019 to 0.142 μm^3 (Coffin and Sharp 1987).

Rates of bacterial production are often most useful when expressed in units of carbon on an areal basis. These values can be compared to measurements of primary production, and the

relative importance of bacteria to carbon and nutrient flow and their potential significance in food webs can be evaluated. Bacterial carbon production estimates were calculated using cell production data and the measured biovolumes of cells from Nueces Estuary (Table 7). The conversion factor 2.2 x 10^{-7} µg C μm^{-3} (Bratbak and Dundas 1984) was used to convert biovolume data to units of carbon. In Nueces Estuary, bacterial biomass and carbon production were lowest during February and May and were typically highest during July (Figure 1). Bacterial carbon production measurements in Nueces Estuary are similar to estimates from other estuaries (Table 8). Cell biovolume measurements were not made in Guadalupe Estuary samples so we used the average size (0.096 μm^3) of Nueces Estuary bacteria to estimate bacterial biomass and carbon production (Figure 2). Bacterial biomass in Guadalupe Estuary was similar to biomass in Nueces Estuary. Bacterial carbon production in Guadalupe Estuary, however, was about 4-fold higher and appears to be high relative to other estuaries (Table 8).

In Nueces Estuary bacterial carbon production ranges from 1 to 30% of primary production with an average near 10% (see Dean Stockwell's section for primary production estimates). On an areal basis bacterial production typically averages 30% of primary production in the marine systems investigated to date (Cole et al. 1988). Bacteria are obviously processing a large percentage of primary production in Nueces Estuary and they may contribute significantly to food webs, but they do not appear to be as quantitatively important here as they are in many other marine environments. The situation is quite different, however, in Guadalupe Estuary where annual bacterial production averaged 130 µg C m^{-2} d^{-1}. Bacterial production averaged 36% of primary production in Guadalupe estuary. Nutrient and energy flow through bacteria are obviously of greater quantitative significance in Guadalupe Estuary relative to Nueces Estuary.

Comparisons of bacterial production to phytoplankton production are not meant to imply that bacteria derive all their nutritional requirements for growth from phytoplankton. Additional nutrient sources, such as marsh and seagrass detritus (POM and DOM) and terrestrially-derived riverine inputs, could be important for fueling bacterial production in these estuaries. The data presented herein do not provide specific information on the sources of organic matter fueling bacterial production, but the higher rates of bacterial production in Guadalupe Estuary given similar phytoplankton production in the two estuaries suggest that additional nutrient sources are relatively more important to bacteria in Guadalupe Estuary or that bacteria in Guadalupe estuary more efficiently process phytoplankton-derived nutrients. The former explanation seems more likely as we do not know of any theoretical basis for the latter.

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Table 1. Bacterial abundance (10^9 cells $liter^{-1}$) in Guadalupe estuary surface waters.

Station	Jan. 1987	Apr. 1987	July 1987	July 1988
A	1.23 ± 0.53	2.74 ± 1.07	1.58 ± 0.49	14.10 ± 0.28
B	0.76 ± 0.22	3.94 ± 0.99	1.76 ± 0.40	14.60 ± 0.21
C	2.39 ± 0.40	6.96 ± 1.53	5.56 ± 1.26	10.20 ± 0.92
D	2.19 ± 0.40	8.73 ± 1.45	4.15 ± 0.91	ND

Table 2. Bacterial abundance (10^9 cells liter^{-1}) in Nueces estuary surface waters.

Station	Oct. 1987	Feb. 1988	May 1988	July 1988
A	3.71 ± 0.45	2.75 ± 0.26	5.45 ± 1.05	4.88 ± 0.40
B	4.04 ± 0.38	3.00 ± 0.35	3.13 ± 0.48	6.54 ± 0.39
C	2.15 ± 0.20	1.98 ± 0.35	1.52 ± 0.25	6.36 ± 0.01
D	2.29 ± 0.95	1.39 ± 0.23	1.23 ± 0.15	8.20 ± 0.06

Table 3. Bacterial production (10^7 cells l^{-1} h^{-1}) in Guadalupe estuary surface waters.

Station	Jan. 1987	Apr. 1987	July 1987	July 1988
A	5.19	4.45	29.06	49.28
B	6.90	5.17	30.40	38.92
C	13.32	23.28	59.57	22.99
D	18.53	18.68	61.66	ND

Table 4. Bacterial turnover times (h) in Guadalupe estuary surface waters.

Station	Jan. 1987	Apr. 1987	July 1987	July 1988
A	24	62	5	29
B	11	76	6	38
C	18	30	9	44
D	12	47	7	ND

Table 5. Bacterial production (10^7 cells l^{-1} h^{-1}) in Nueces estuary surface waters.

Station	Oct. 1987	Feb. 1988	May 1988	July 1988
A	12.70	1.98	5.56	7.20
B	8.27	1.43	4.50	9.63
C	4.04	1.33	2.67	5.90
D	4.10	1.13	6.96	12.30

Table 6. Bacterial turnover times (h) in Nueces estuary surface waters.

Station	Oct. 1987	Feb. 1988	May 1988	July 1988
A	29	139	87	67
B	49	210	62	68
C	53	149	21	108
D	56	123	46	67

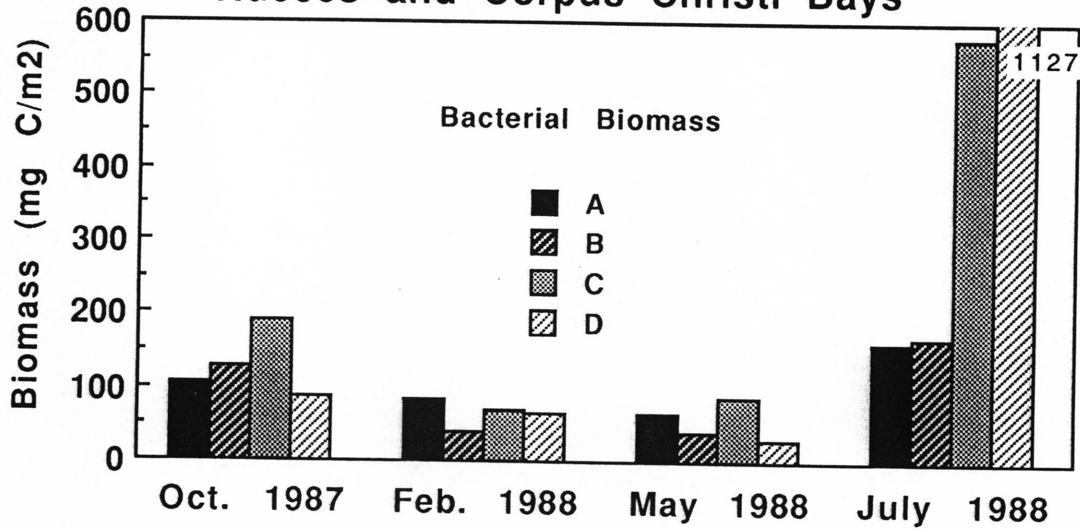
Table 7. Bacterial biovolume (μm^3) in Nueces estuary surface waters.

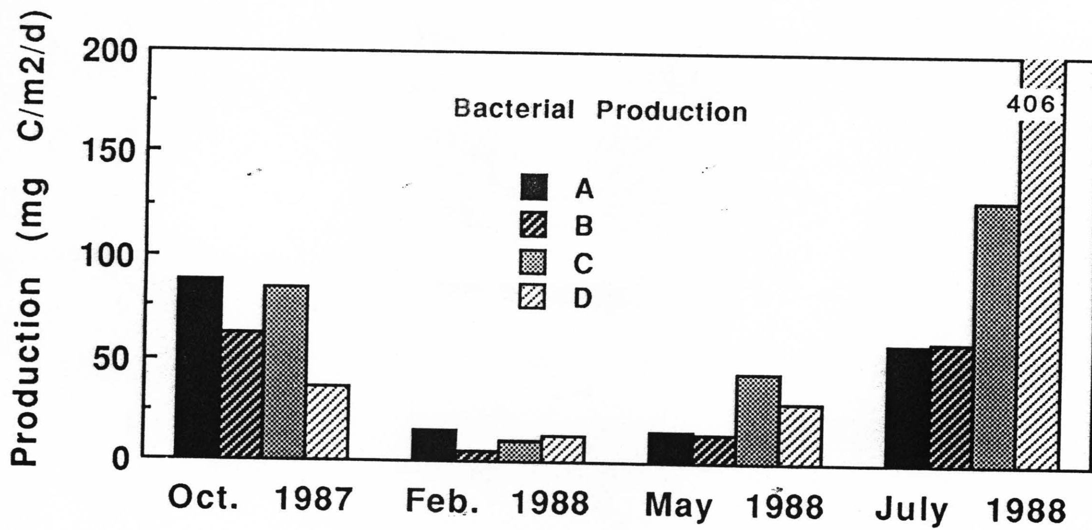
Station	Oct. 1987	Feb. 1988	May 1988	July 1988
A	0.109 ± 0.093	0.117 ± 0.071	0.045 ± 0.031	0.127 ± 0.057
B	0.110 ± 0.071	0.046 ± 0.042	0.045 ± 0.033	0.091 ± 0.097
C	0.109 ± 0.066	0.044 ± 0.028	0.070 ± 0.054	0.113 ± 0.097
D	0.078 ± 0.051	0.096 ± 0.059	0.049 ± 0.037	0.284 ± 0.407

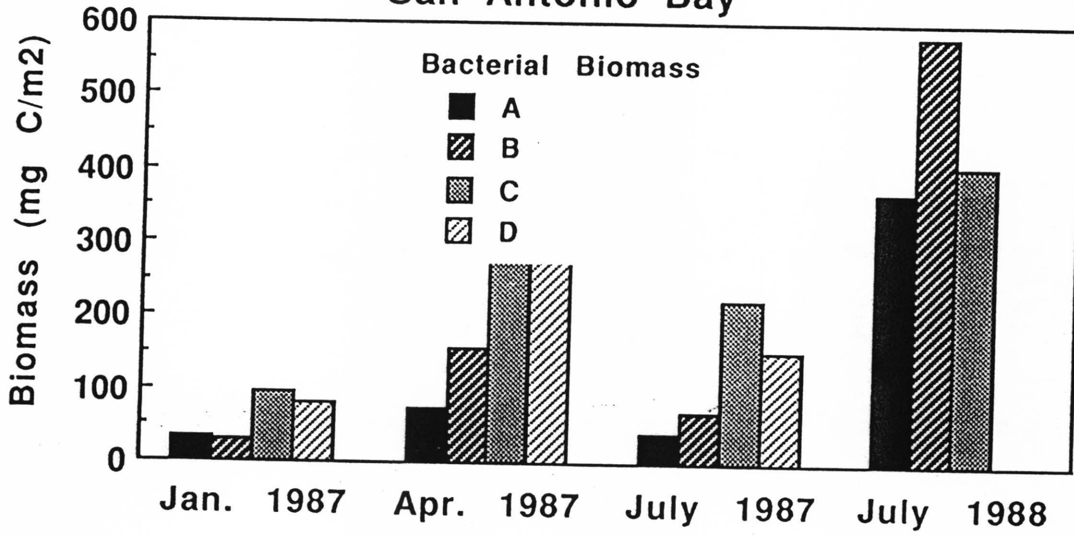
Table. 8 Comparison of bacterial production estimates in a variety of estuarine environments (ranges, means, or both are given).

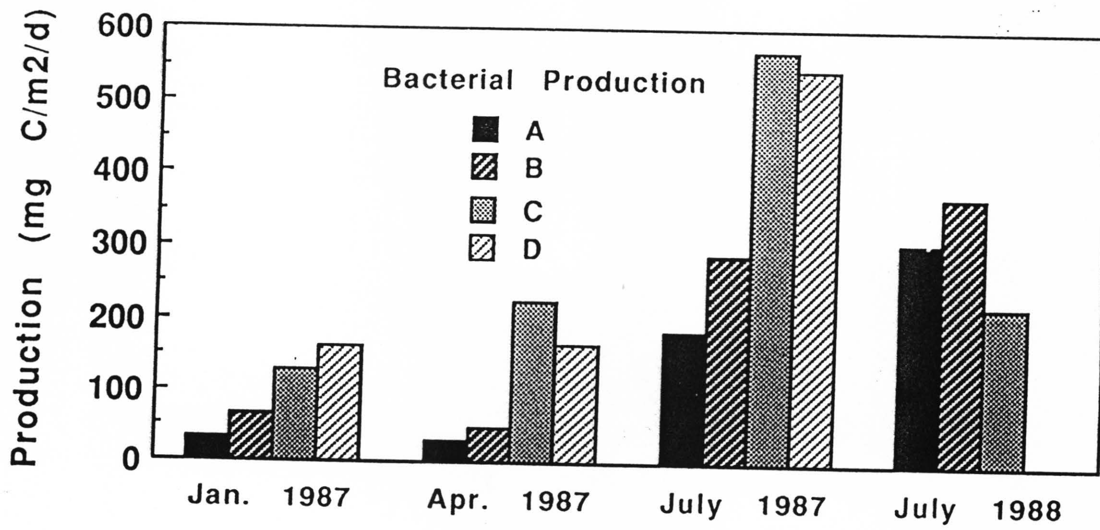
Site	Production ($\mu g\ C\ l^{-1}\ d^{-1}$)	Reference
Delaware Bay	0 - 152 (24)	Coffin and Sharp 1987
Parker River, MA	4 - 409	Wright and Coffin 1984
Chesapeake Bay	12 - 72	Ducklow 1985
York River, VA	7 - 75	Ducklow 1982
James River, VA	74 - 426	E.T. Koepfler per. com.
Guadalupe Estuary	22 - 311 (130)	This study
Nueces Estuary	3 - 184 (34)	This study

Nueces and Corpus Christi Bays






San Antonio Bay
Bacterial Biomass
A
B
C
D
Biomass (mg C/m2)
600
500
400
300
200
100
0
Jan. 1987
Apr. 1987
July 1987
July 1988


Bacterial Production
A
B
C
D
Production (mg C/m2/d)
600
500
400
300
200
100
0
Jan. 1987
Apr. 1987
July 1987
July 1988

II. Ammonium Regeneration and Utilization in the Nueces Estuary, Texas

Introduction

Nitrogen is one of the most important nutrients which regulate primary production in open oceans as well as in coastal waters (Dugdale and Goering, 1967; Ryther and Dunstan 1971). Ammonium is preferentially utilized by marine phytoplankton, even when concentrations of other forms of nitrogenous nutrients are much higher (Walsh and Dugdale, 1971; McCarthy et al., 1977). Ammonium that is regenerated in the water column supplies a significant portion of the nitrogen demand of planktonic organisms in many marine environments (Harrison, 1978; Hopkinson et al., 1987). Ammonium is regenerated in the water column by the heterotrophic processes of a variety of planktonic organisms ranging from macrozooplankton to heterotrophic bacteria. Size-fractionation studies have been used to estimate the contribution of each group of planktonic organisms to ammonium regeneration. These experiments consistently have indicated that heterotrophic microplankton (< 100 µm) provide the major fraction of regenerated ammonium in the water column (Harrison, 1978; Caperon et al.,1979; Glibert, 1982; Hopkinsonet al., 1987). Goldman et al.(1985) reported that ammonium excretion by microflagellates was quantitatively more important than bacterial remineralization of ammonium during laboratory experiments. Likewise, Harrison (1978) and Glibert (1982) concluded from field experiments that ammonium regeneration by nanoplankton (1 - 35 µm size fraction) was much greater than by bacteria (< 1.0 µm size fraction).

Ammonium is utilized by nonphotosynthetic microorganisms as well as by photoautotrophs (Eppley et al., 1977). Wheeler and Kirchman (1986) reported that 78 % of total ammonium uptake was by procaryotes in Georgia coastal waters and a significant portion

of this was due to utilization by heterotrophic bacteria. Harrison and Wood (1988) recently observed, however, that heterotrophic utilization of ammonium was less important than photoautotrophic utilization in the water column over Georges Bank near Nova Scotia. Thus, it appears that heterotrophic utilization of ammonium can have quite variable impacts on ammonium cycling in different environments.

During the past two years, we have been studying nitrogen cycling in the Nueces Estuary on the south Texas Gulf coast. One aspect of these studies has been to estimate the relative contributions of bacteria, nanoplankton and larger plankton in ammonium utilization and regeneration. Gentle gravity filtration through Nitex screening (20 µm mesh opening) or glass fiber filters (1.5 µm nominal pore size) was used to obtain specific size classes of planktonic organisms. In this report we present results from these studies and studies of the diel fluctuations in ammonium turnover.

Materials and Methods

Study area

Nueces Estuary is one of seven major estuaries along the Texas coast. Nueces Estuary is shallow, mostly less than 5 m deep with the exception of the ship channel which reaches a maximum depth of 15 m. The estuary includes Corpus Christi Bay, Nueces Bay and Oso Bay. The southern portion of the estuary adjoins with the upper Laguna Madre and the northeastern portion adjoins with Redfish Bay. Nueces River, which flows into Nueces Bay, provides most of the freshwater input to the estuary. The estuary is separated from the Gulf of Mexico by Mustang Island. Water exchange with the Gulf of Mexico occurs through a jettied inlet (Aransas Pass) at Port Aransas.

Determination of ammonium regeneration and utilization rates

Ammonium regeneration and utilization rates were determined

using a $^{15}N-NH_4^+$ dilution method (Blackburn, 1979; Caperon et al.,1979). At the beginning of experiments, 0.2 μg-atoms N of $^{15}N-NH_4Cl$ (99 % ^{15}N, KOR Isotopes) was added to two liters of estuarine water in a polycarbonate bottle immediately after collection. The bottle was gently mixed, and approximately 700 ml of seawater was filtered through Whatman GF/F filters for determination of the initial concentration of ammonium and the initial 15N/14N ratio of ammonium. The remaining seawater was incubated for two hours by submerging the bottle at the depth where seawater was sampled. Incubation was stopped by filtration through GF/F filters. Filtered seawater samples were kept frozen until analyzed.

Ammonium was recovered from the filtered seawater samples by steam distillation. Prior to distillation, the ammonium concentration of the seawater sample was determined from the triplicate subsamples (Solozano, 1969). Immediately prior to steam distillation, NaOH (2 ml of 1.0 N) was added to 500 ml of the sample to raise the pH to about 10. If the total NH_4-N in 500 ml of the seawater sample was less than 1 μg-atom N, $^{14}N-NH_4Cl$ was added as a carrier. About 70 - 75 % of the ammonium in seawater was routinely recovered from 100 ml of distillate which was collected in a flask containing 10 ml of 0.1 N H_2SO_4. The pH of the distillate was raised (> 10) by adding 2 ml of 50 % NaOH. The flask was immediately sealed with a cap in which a wick (a piece of GF/C filter) soaked with 15 μl of 0.5N H_2SO_4 was attached. The flask was shaken at 100 RPM for 24 hours, and the wick was dried at 50°C for 24 hours in a clean glass vacuum desicator containing phosphorus pentoxide. The 15N/14N ratio of recovered ammonium was determined by a modified Dumas method (Fiedler and Proksch, 1975) as follows. Wicks were cut into several strips and inserted into Pyrex tubes (4 mm ID, 150 mm Length) sealed at one end and containing 0.3 g of a mixture (1:1, w/w) of previously heated CaO

(at 900°C) and CuO (at 550°C). Air was evacuated from the tube (10^{-4} Torr) using a vacuum line (Japan Spectroscopic Co., Model OPS-10M), and the tube was sealed with a propane torch under vacuum. The sealed tubing was heated at 550°C for 5 hours to convert ammonium to nitrogen gas. The 15N/14N ratio of the sample was determined using an emission spectrometer (Japan Spectroscopic Co., Model N-150).

Prefiltration of seawater for the size-fractionation study

Surface estuarine water was sampled with an acid-washed clean bucket at noon and at midnight. Samples from each station were divided into three size fractions before addition of $^{15}N-NH_4Cl$. The three size fractions consisted of water that was unfiltered, water that was passed through a 20 µm mesh-opening Nitex screen and water that passed through Whatman 934-AH microfiber filters (1.5 µm nominal pore size). All samples were gravity filtered. We obtained 2 liters of each size fraction in less than 30 minutes by using at least three sets of the filtration units simultaneously.

Determination of bacterial abundance and chlorophyll a

Seawater samples were fixed with a formalin solution buffered with sodium cacodylate (final concentration was 3 % formalin and 0.1 M cacodylate, pH 7.5). Each fixed sample was stained with an acridine orange solution (final concentration, 0.025 %) for 5 minutes and filtered through a 0.2 µm black Nuclepore filter. Bacterial cells retained on the membrane filter were counted using the epifluorescence microscopy technique (Hobbie et al., 1977) with a Zeiss Universal Microscope. Chlorophyll a concentrations were determined fluorometrically on acetone extracts (Holm-Hansen et al.,1965).

Results and Discussion

During the study period, the ammonium concentration of seawater was always higher in the upper estuary station (Station A), and generally declined with distance from the river mouth

(Table 1). The highest ammonium concentrations were observed in May (average, 2.75 μg-atoms N l^{-1}) and the lowest was measured in July (average, 0.38 μg-atoms N l^{-1}) in the study area. The range of ammonium regeneration rates during the study period was 0.031 - 0.393 μg-atoms N $l^{-1}h^{-1}$ (Fig.1a). Regeneration rates were highest in October (average, 0.226 μg-atoms N $l^{-1}h^{-1}$) and lowest in July (average, 0.053 μg-atoms N $l^{-1}h^{-1}$). Rates of ammonium regeneration were higher in the mid (Station C) and lower estuary (Station D) than in the the upper estuary station (Station A) during October and February (Fig.1a). During May and July, however, the highest rates were observed in the upper estuary (Station A). The lowest regeneration rates were consistently observed in mid Nueces Bay (Station B).

Ammonium utilization rates ranged from 0.067 to 0.663 μg-atoms N $l^{-1}h^{-1}$ during the study period (Fig.1b). Utilization rates were highest in May(average, 0.412 μg-atoms N $l^{-1}h^{-1}$) and lowest in July (average, 0.154 μg-atoms N $l^{-1}h^{-1}$) during the study period. Rates of ammonium utilization were typically higher in the lower estuary (Stations C,D) in October and February but were lower at these stations in May and July. The rates of ammonium regeneration and utilization in Nueces Estuary are within the range of those reported for other estuarine and coastal waters (Caperon et al.,1979; Glibert et al.,1982; Paasche and Kristiansen,1982; Hopkinson et al.,1987).

Contributions of different size fractions of planktonic organisms to ammonium turnover were studied in February and May. Planktonic organisms less than 20 μm in size accounted for 42 - 92% (average, 76 %) of total ammonium regeneration and 72 - 97 % (average, 85 %) of total ammonium utilization (Figs.2a,b,c,d). These results are consistent with other reports (Harrison,1978; Caperon et al.,1979; Glibert,1982; Hopkinson et al.,1987). Of the three different size fractions of planktonic organisms (> 20 μm, 1.5 - 20 μm, < 1.5 um), the 1.5 - 20 μm fraction generally

appeared to be most important for both ammonium regeneration and utilization. The contribution of this size fraction to total ammonium regeneration ranged 36 to 67% (average, 55%) with no obvious temporal or spatial pattern (Figs.2a,2c). Planktonic organisms larger than 20 μm (presumably macro and micro-zooplankton) contributed 4 - 29% (average, 24%) of total regeneration with the exception of very high contribution (58%) during the night at Station A in February. Macro- and microzooplankton contributed more to ammonium regeneration in the upper estuary than in the lower estuary.

The bacterial size fraction (< 1.5 μm) accounted for 6 - 33% (average, 22%) of total ammonium regeneration during all sampling periods. During February, bacteria contributed much more to ammonium regeneration in the lower estuary (> 30%) than in the upper estuary (6%). During May, bacterial contribution to ammonium regeneration was similar in the upper and lower estuary (20% in daytime, 29% in night). In Chesapeake Bay, bacterial size fraction (< 1 μm) was responsible for 10 - 40% of ammonium regeneration (Glibert, 1982). Harrison (1978) observed that 39% of ammonium regeneration by < 1 μm fraction in seawater of Scripps pier.

We assume that heterotrophic bacteria were responsible for most, if not all, of the ammonium regeneration in the < 1.5 μm fractions because most heterotrophic microprotozoa (including microflagellates and ciliates) are in the size range of 2 - 20 μm (Sherr and Sheer, 1983). In some samples the contribution of bacteria to ammonium regeneration may be underestimated because bacterial abundance in the < 1.5 μm fraction was less than 50 % of the total abundance on some occasions (Table 3). This underestimation could be more serious at Station A in February when 30% or less of the bacteria in unfiltered estuarine water were found in the < 1.5 μm size fraction. This may be responsible for the relatively low ammonium regeneration in the bacterial size

fraction at that time (only 6% of total). It cannot be ruled out, however, that some regeneration in this fraction may be due to microflagellates(Johannes, 1965; Fuhrman and McManus, 1984; Cynar et al., 1985).

Chlorophyll a concentration was always higher in the upper estuary (Station A) than in the lower estuary (Station D) (Table 2). Phaeopigment concentration also showed the same pattern. Chlorophyll a concentration ranged from 1.68 - 14.12 µg l^{-1} and phaeopigment concentration ranged from 1.28 - 6.28 µg l^{-1} during the study period. The size fractionation study showed that most of chlorophyll a (59 - 92 % of total) and phaeopigment (67 - 94 % of total) were found in 1.5 - 20 µm fractions.

In accordance with chlorophyll a concentation, 1.5 - 20 µm size fraction (nanoplankton) accounted for most of the ammonium utilization (28 - 85 %, average 67 %). Netphytoplankton (> 20 um) contributed 3 - 28 % (average, 15 %) of total ammonium utilization. Picoplankton (< 1.5 µm) were responsible for 5 - 44% (average,18 %) of total ammonium utilization. Results from our study indicates that nanophytoplankton are generally the primary consumers of ammonium in Nueces Estuary. Glibert (1982) observed that the 1 - 10 µm size fraction was responsible for most (75 - 87%) of the ammonium uptake in two out of three stations in Chesapeake Bay. Wheeler and Kirchman (1986), however, observed that bacteria (< 1 µm) were the major ammonium utilizers in Georgia coastal waters. In our study area, picoplankton (< 1.5 µm) appeared to be the most important ammonium consumers (44 % of total) during only one night sampling period at the lower bay station (Station D).

Nitrogen requirements of heterotrophic bacteria were calculated from bacterial production data. According to our calculation (on the assumption that C/N atomic ratio of bacteria = 5) the nitrogen requirements of heterotrophic bacteria ranged from 0.21 to 0.30 mg-atoms N m^{-2} day^{-1} (Table 4). However, ammonium

utilization by the picoplankton fraction ranged from 1.06 to 3.77 mg-atoms N m^{-2} day^{-1}, which is much higher than the total bacterial nitrogen demand. Moreover, amino acids and other organic compounds are believed to be important nitrogen sources for heterotrophic bacteria. Therefore, it is likely that a significant portion of the ammonium utilized by the < 1.5 µm size fractions was due to microorganisms other than heterotophic bacteria. Nitrifying bacteria could have utilized some of the ammonium, but the proportion of ammonium utilized by nitrifying bacteria in seawater was usually negligible (Selmer, 1988). Chlorophyll a analyses indicated 2 - 16 % of total content resided in the $<$ 1.5 µm size fractions (Table 2), suggesting that photoautotrophs were responsible for most of the ammonium utilization in this size fraction. Cyanobacteria were observed in several water samples and they may have been responsible for a significant portion of ammonium utilized by the < 1.5 µm size fraction. Substantial decreases in ammonium utilization during nighttime as compared to during daytime (Figs. 2b,2d) supports this hypothesis.

A highly significant correlation ($r = 0.65$, $p < 0.001$) was observed between ammonium utilization rates and chlorophyll a concentrations. Ammonium regeneration rates were also significantly correlated to chlorophyll a concentration ($r = 0.55$, $p < 0.01$), but there was no significant relationship between phaeopigment concentrations and ammonium regeneration or utilization. This implies that ammonium regeneration is more tightly linked to phytoplankton biomass than to detritus in Nueces Estuary.

The rates of ammonium utilization were usually higher during the day than during night regardless of the size fractions investigated (Figs.2b,2d). For intact water, ammonium utilization rates during the night reached as much as 25 - 81 % of daytime rates. Dugdale and Goering (1967) observed dark ammonium uptake was 25 - 60 % of light uptake in the open sea, and Hanson and

Robertson (1988) reported that 70 - 75 % of daytime ammonium utilization occurred in the absence of sunlight in coastal waters off the southeastern United States. Planktonic organisms larger than 1.5 µm utilized 56 - 95 % of ammonium during night (Figs. 2b,2d).

Rates of ammonium regeneration in intact waters were lower during the night than during the day (36 - 74 % of daytime rates) with one exception. At Station A in February, the rate of ammonium regeneration during the night was 2-fold higher than during the day (Figs.2a). In a study of ammonium dynamics in Swedish coastal waters, Selmers (1988) found that ammonium regeneration rates were by 2.3 - 7 fold higher between midnight and midday than between midday and midnight in March, but no significant diel variation was observed in September. In the present study, diel varaitions in ammonium regeneration and utilization rates were similar in unfiltered seawater and 20 µm screened seawater. Diel variations in the picoplankton fractions were greater than those in unfiltered seawater.

Ammonium regeneration and utilization were not in balance in the water column of Nueces Estuary. Utilization rates usually exceeded regeneration rates in this estuary (U/R > 1) (Fig.4). Similar observations were reported from various coastal waters (Caperon et al.,1979 ; Paasche and Kristiansen,1982 ; La Roche,1983), but Harrison (1978), Glibert (1982) and Hanson and Robertson (1988) observed that, in general, regeneration rates were greater than or equal to utilization rates in the open ocean and some other coastal waters. In Nueces Estuary, the U/R ratios were consistently higher in the upper estuary at Stations A, B than in the lower estuary at Stations C, D (Fig.3). This trend indicates that a larger fraction of the ammonium utilized in the water column of the upper estuary was supplied by benthic regeneration or fluvial input than the lower estuary. Water depths in the upper estuary averaged 1.3 m and in the lower

estuary averaged 2.9 m.

During the night, the U/R ratios were lower than during the day for all size fractions in both the upper and lower estuary (Fig.4). Ammonium regeneration in the intact water appeared to be greater than utilization (U/R < 1.0) at nighttime in the lower estuary (Station D) because of a decline in utilization. It is notable that, for picoplankton fractions, ammonium utilization rates exceeded regeneration rates in the upper estuary (U/R > 1.0), but regeneration rates exceeded utilization rates in the lower estuary (U/R < 1.0) regardless of diel variations (Fig.4). Glibert (1982) observed that ammonium regeneration in some cases exceeded ammonium uptake by 2 - 10 fold at first light of the day, but uptake rates either equalled or exceeded regeneration rates at midday in the Sargasso Sea.

Filtrations for the size fractionation study were carried out prior to incubation with ^{15}N-ammonium. Larger heterotrophic plankton were therefore excluded in picoplankton fraction (< 1.5 µm). Larger heterotrophic plankton (e.g. zooplankton and protozoans) usually release urea and amino acids (Smith, 1978; Andersson et al.,1985) which are often quickly remineralized to ammonium by heterotrophic bacteria. If such reactions were tightly coupled, bacterial ammonium regeneration obtained by the size fractionation study may be underestimated. Moreover, Glibert (1982) and Selmer (1988) speculated that the ammonium regeneration rate obtained by the $^{15}N-NH_4^+$ dilution method could be higher than the actual rates because large heterotrophic organisms like zooplankton may be stressed during the experimental procedure. If ammonium regeneration in unfiltered samples were overestimated because of stress to zooplankton, the contributions of bacteria to total ammonium regeneration could be underestimated.

Primary production was measured during the same period in May as our measurements of ammonium cycling were made. Phytoplankton nitrogen demand was calculated from phytoplankton production using

the Redfield ratio (C/N atomic ratio = 6.97). The calculated nitrogen demand ranged from 12.7 to 34.4 mg-atoms N m^{-2} day^{-1} in May (Table 5). Thus, ammonium regeneration in the water column could supply 6 - 87 % of the nitrogen demand of phytoplankton in Nueces Estuary during that period. Ammonium utilization in the water column was much less than phytoplankton nitrogen demand in the study area with exception in the upper estuary (Station A). This indicates that other nitrogeneous nutrients, such as nitrate, may be important nitrogen sources to phytoplankton in Nueces Estuary.

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Table 1. Ammonium concentrations in surface waters of the Nueces Estuary during the study period.

Unit; µg-atom N/l

Station	October	February	May	July
St. A	0.70	4.36	7.43	0.45
St. B	0.42	1.76	2.67	0.45
St. C	0.36	0.64	0.34	0.41
St. D	0.47	0.61	0.60	0.19

Table 2. Chlorophyll a and phaeopigment concentration in fractionated seawater.

Nueces Estuary

Month	Time	Station	Chlorophyll a (µg/l) > 20µm	1.5 - 20 µm	< 1.5 µm	Total	Phaeopigment (µg/l) > 20 µm	1.5 - 20 µm	< 1.5 µm	Total
Feb.	Day	St. A	1.75 (20%)	6.53 (75%)	0.45 (5%)	8.73 (100%)	0.98 (16%)	5.00 (79%)	0.30 (5%)	6.28 (100%)
		St. D	0.04 (2%)	1.37 (82%)	0.27 (16%)	1.68 (100%)	0.01 (1%)	0.99 (77%)	0.28 (22%)	1.28 (100%)
	Night	St. A	2.25 (23%)	7.16 (74%)	0.23 (3%)	9.64 (100%)	0.19 (3%)	6.67 (94%)	0.21 (3%)	7.07 (100%)
		St. D	0.31 (12%)	1.79 (73%)	0.38 (15%)	2.48 (100%)	0.25 (10%)	1.74 (73%)	0.40 (17%)	2.39 (100%)
May	Day	St. A	0.30 (2%)	12.98 (92%)	0.84 (6%)	14.12 (100%)	0.03 (1%)	2.60 (80%)	0.61 (19%)	3.24 (100%)
		St. D	3.36 (37%)	5.43 (59%)	0.34 (4%)	9.13 (100%)	0.17 (8%)	1.76 (82%)	0.22 (10%)	2.15 (100%)
	Night	St. A	0.06 (1%)	8.27 (94%)	0.45 (5%)	8.78 (100%)	0.26 (5%)	4.45 (87%)	0.39 (8%)	5.10(100%)
		St. D	2.01 (36%)	3.33 (61%)	0.18 (3%)	5.52 (100%)	0.59 (27%)	1.46 (67%)	0.13 (6%)	2.18 (100%)

- Seawater was filtered through 20 µm Nitex or Whatman 934-AH (pore size 1.5 µm) glass microfiber filters by gravity for size fractionation of planktonic organisms.
- Percentages shown in parentheses indicate the concentration of chlorophyll a relative to unfiltered seawater.

Table 3. Bacterial abundance in fractionated seawater.

Nueces Estuary

Month	Time	Station	Bacterial abundance (10^6 cells/ml) > 20µm	1.5 - 20 µm	< 1.5 µm	Total
Feb.	Day	St. A	0.17 (5%)	2.27 (65%)	1.03 (30%)	3.47 (100%)
		St. D	0.03 (2%)	0.01 (1%)	1.59 (97%)	1.63 (100%)
	Night	St. A	0.23 (6%)	2.59 (67%)	1.04 (27%)	3.86 (100%)
		St. D	0.18 (7%)	1.12 (41%)	1.40 (52%)	2.70 (100%)
May	Day	St. A	0.07 (1%)	1.67 (25%)	4.86 (74%)	6.60 (100%)
		St. D	0.02 (1%)	0.61 (24%)	1.92 (75%)	2.50 (100%)
	Night	St. A	0.66 (10%)	2.50 (38%)	3.42 (52%)	6.58 (100%)
		St. D	0.05 (2%)	0.65 (28 %)	1.65 (70%)	2.35 (100%)

* Seawater was filtered through 20 µm Nitex or Whatman 934-AH (pore size 1.5 µm) glass microfiber filters by gravity for size fractionation of planktonic organisms.
* Percent of total indicates the percent of bacterial number relative to unfiltered seawater

Table 4. Comparison of bacterial nitrogen demands with ammonium utilizations by picoplankton.

Unit ; (mg-atom N/m2/day)

	February		May	
	Station A	Station D	Station A	Station D
Bacterial N-demand	0.24	0.21	0.30	0.25
NH4 utilization by picoplankton (<1.5 μm)	1.06	2.85	3.77	1.21

* Bacterial nitrogen demand was calculated from bacterial production assuming that C/N atomic ratio of bacteria is 5.
*Ammonium utilization by picoplankton was obtained from the size fractionation study using a 15N-NH4 dilution method.

Table 5. Contribution of ammonium regeneration in the water column to the phytoplankton nitrogen demand.

Nueces Estuary

Station	(D) Phytoplankton N-Demand (mg-atom N/m2/day)	(R) NH4 regeneration in the water column (mg-atom N/m2/day)	NH4 utilization in the water column (mg-atom N/m2/day)	R/D *100 (%)
A	12.67	10.97	19.09	86.6
B	28.04	1.72	2.92	6.1
C	34.39	17.83	16.65	51.9
D	23.89	11.13	13.07	46.6

* Phytoplankton nitrogen demand was calculated from phytoplankton production using the Redfield ratio (C/N atomic ratio = 6.97).
* Phytoplanktonproduction data was provided by Dr. Dean Stockwell.
* All field experiments were conducted in Nueces Estuary in May , 1988.

Figure 1. Ammonium regeneration (a) and utilization (b) in surface waters of the Nueces Estuary.

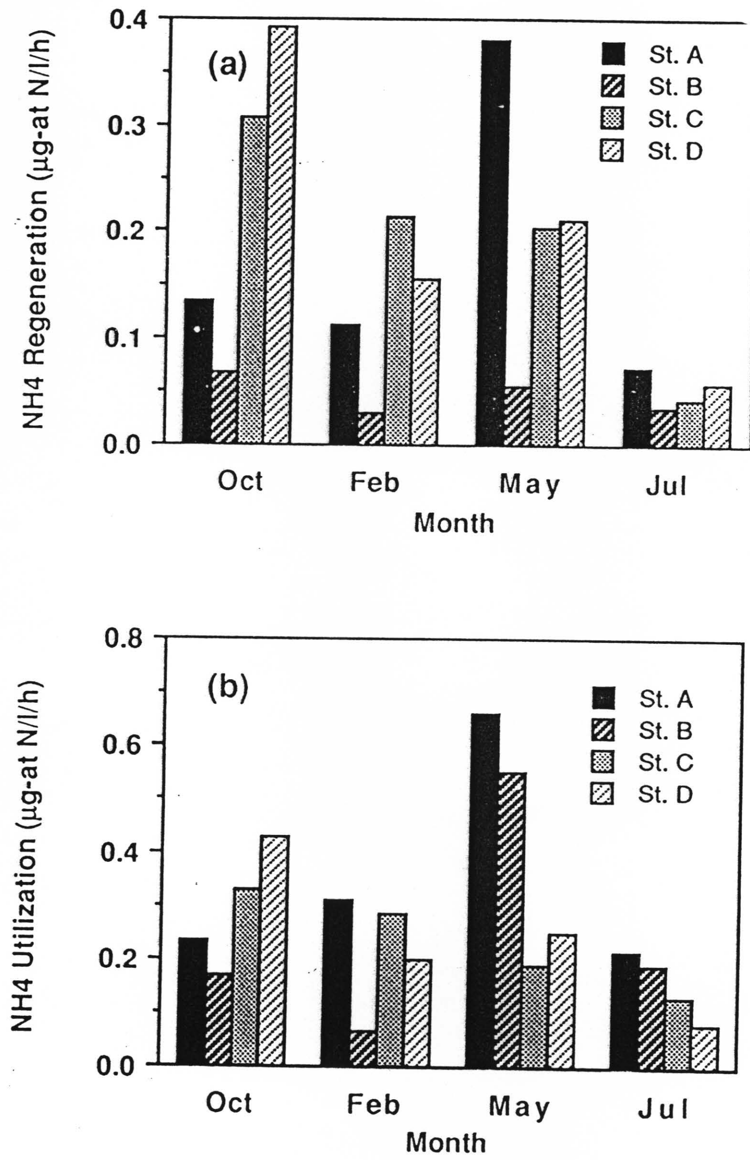


Figure 2a. Ammonium regeneration rate by different size fractions of planktonic organisms

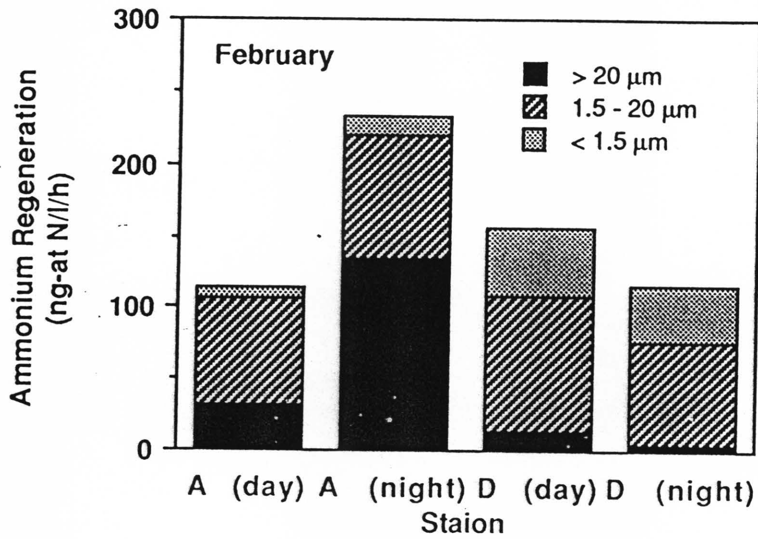


Figure 2b. Ammonium utilization rates by different size fractions of planktonic organisms

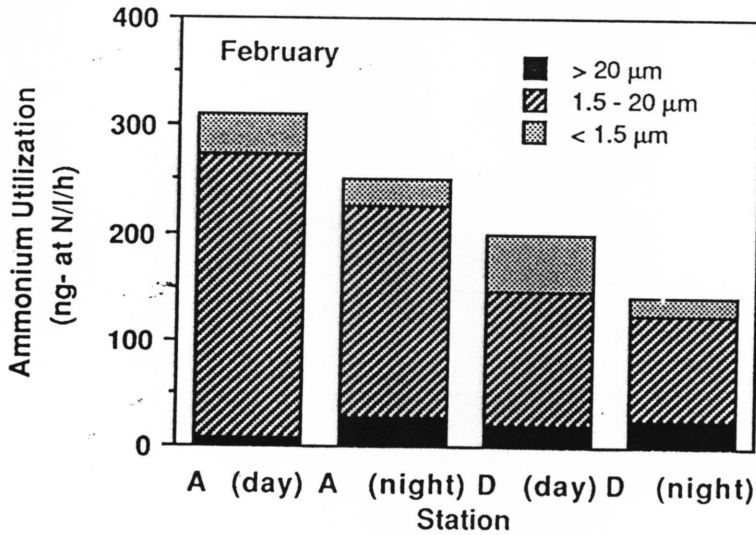


Figure 2c. Ammonium regeneration rates by different size fractions of planktonic organisms

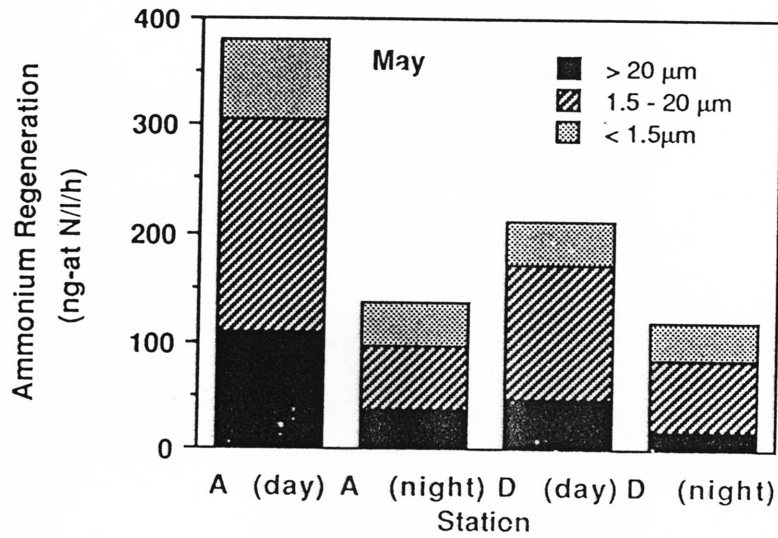


Figure 2d. Ammonium utilization rates by different size fractions of planktonic organisms

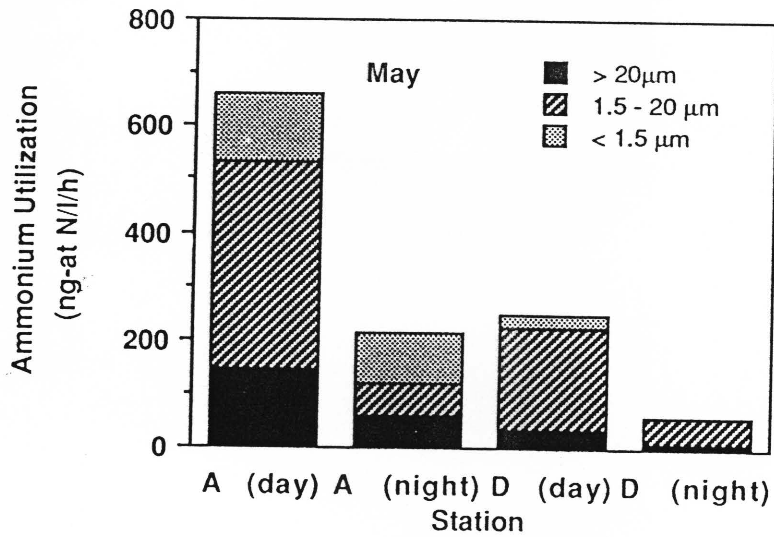


Figure 3. Ratio of ammonium utilization (U) to regeneration (R) in Nueces Estuary.

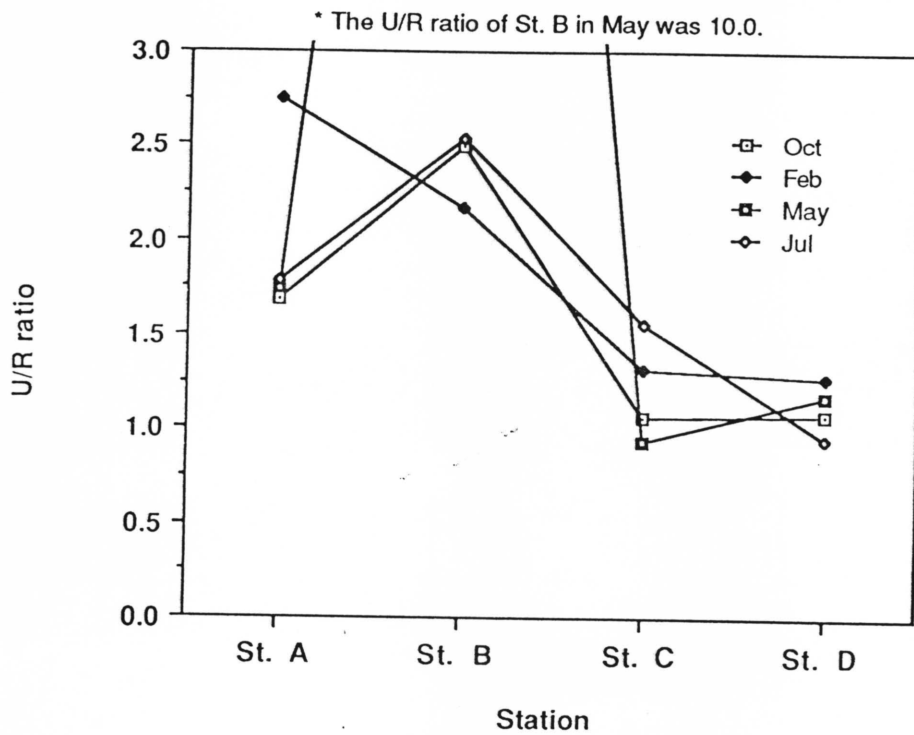
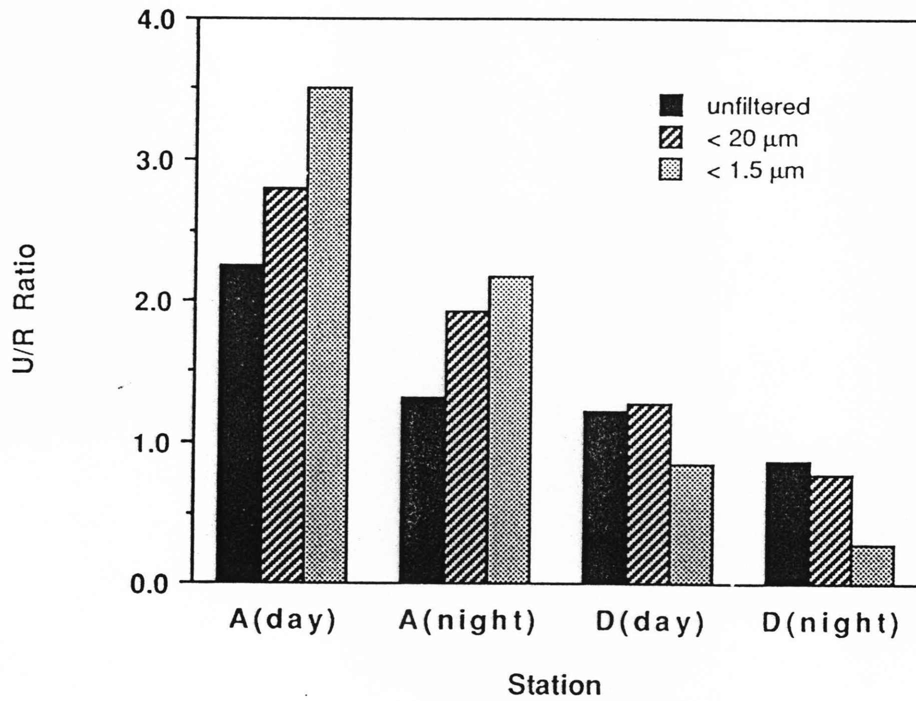


Figure 4. Ratio of ammonium utilization to regeneration (U/R) of size fractionated seawater.



III. Comparison of Benthic Ammonium Flux with Ammonium Regeneration in the Water Column of Nueces and Guadalupe Estuaries, Texas

Introduction

In estuarine and coastal marine environments, nitrogenous nutrients utilized by primary producers are generally supplied by fluvial input, benthic flux and rapid regeneration in the water column. Numerous studies have investigated the benthic flux of nitrogen from coastal and estuarine sediments (e.g. Klump and Martens,1981; Callender and Hammond, 1982; Fisher et al.,1982; Boynton and Kemp, 1985; Williams et al.,1985; Flint et al.,1986). According to these studies, nitrogen fluxes from sediments meet a substantial portion of the nitrogen demand in overlying waters, and ammonium generally comprises the major fraction of regenerated nitrogen in sediments. Ammonium is also a preferred nitrogen source by phytoplankton compared to other forms of nitrogenous nutrients (McCarthy et al.,1977; Walsh and Dugdale, 1979).

Ammonium fluxes across the sediment-water interface are usually estimated from changes in ammonium concentration in benthic chambers which are deployed at the sediment-water interface for several hours (Smith et al.,1972; Fisher et al.,1982; Callender and Hammond,1982; Boynton and Kemp,1985; Williams and Gill,1985; Flint et al.,1986). Flux estimates are based on the assumption that ammonium regeneration and utilization in chamber water itself are either negligible or in balance (Fisher et al.,1982). If rates of ammonium regeneration and utilization in the chamber water are significantly out of balance, it could lead to a significant error in the estimation of benthic ammonium flux. Few reports, however, have accounted for this potential problem.

Ammonium regeneration in the water column also provides a significant portion of the nitrogen required for primary

production (Harrison,1978; Hopkinson et al.,1987). However, to our knowledge, no reports have directly compared ammonium regeneration in the water column with concurrently occurring benthic ammonium flux in shallow estuarine environments. In this study, we measured ammonium fluxes using benthic chambers and, simultaneously, we determined rates of ammonium regeneration and utilization in the water column. The relative importance of water column and benthic ammonium regeneration are compared in two Texas estuaries. Actual benthic ammonium fluxes are calculated taking water column processes into account. Turnover times of the ammonium pools in sediments and in the water column are also given in this paper.

Materials and Methods

Study areas

Field experiments were conducted during the summer of 1988 in two Texas estuaries, the Nueces Estuary and the Guadalupe Estuary. The Nueces River provides the major riverine freshwater input into Nueces Estuary, and the San Antonio and Guadalupe Rivers are the main sources of freshwater inflow to the Guadalupe Estuary. The Guadalupe Estuary receives a 5-fold larger (36 year average, 1941 - 1976) freshwater inflow compared to the Nueces Estuary. The Nueces Estuary includes Nueces Bay, Corpus Christi Bay and Oso Bay. In Nueces Estuary, two stations (Station NA and NB) were in Nueces Bay which adjoins with the Nueces River, and the other two stations were in Corpus Christi Bay (Station NC and ND). In Guadalupe Estuary, all stations (Station SA, SB and SC) were located in San Antonio Bay. During the study period (summer, 1988) water temperature ranged from 28 - 32°C in both estuaries. No salinity gradient was apparent in the Nueces Estuary (36 - 44 ppt), but a salinity gradient ranging from 8 to 26 ppt existed in the Guadalupe Estuary.

Analytical menthods

Ammonium concentrations of all samples were determined colorimetrically using a spectrophotometer (Solorzano, 1969). Chlorophyll a was determined fluorometrically on acetone extracts (Holm-Hansen et al.,1965). Ammonium pool sizes in the upper 10 cm of sediments were calculated from the porosity of sediments and the ammonium concentration of sediment pore water. Sediments were dried for two weeks at 40°C for porosity measurements. Sediment cores (ID 6.5 cm, height 25 cm) were collected with minimal disturbance using a hand-driven core sampler. Sediment cores were sectioned at 1 cm intervals over the top 10 cm. Each section was put into a sterile plastic petri dish which was then tightly sealed with black electrical tape to avoid any loss of pore water. Triplicate samples were frozen immediately and were kept frozen until analyzed. Pore water was extracted from each sediment section by centrifugation (5000 g).

Benthic ammonium flux

Two transparent chambers (polycarbonate, 19 cm height, 11.17 liter volume) were set carefully by scuba divers at the sediment-water interface with minimal disturbance of sediments. The chambers were positioned for at least 20 minutes prior to sealing to let the chamber water equilibrate with outside water through the openings of the chamber. The incubation was started by withdrawing a water sample for the initial ammonium concentration and stoppering the openings of the chamber. At one hour intervals scuba divers sampled the chamber water with syringes which were installed previously on the side of chamber. Samples were were kept frozen until analyzed. Changes in ammonium concentration in the chamber were plotted against time to obtain the measured ammonium flux rate. Ammonium concentrations in the chamber water was also dependent on ammonium regeneration and utilization in the overlying water inside of the chamber. Therefore, the bottom water was carefully collected at < 20 cm above the sediment-water

interface by a scuba diver for determination of ammonium regeneration and utilization rates. The rate of change of ammonium concentration in the chamber water due to the actual ammonium flux from sediments was calculated using the following equation:

$$\text{Actual Flux} = \text{Measured Flux} - R + U$$

where the measured flux is the rate of change in ammonium concentration in the chamber water, R is the ammonium regeneration rate in the bottom water, and U is ammonium utilization rate in the bottom water.

Determination of rates of ammonium regeneration and utilization

The same methods described in the previous section were used for determination of ammonium utilization and regeneration rates. The water column regeneration and utilization rates were obtained by integration of the whole water column using surface and bottom water data.

Results and Discussion

During the experimental period, ammonium concentrations ranged from 0.19 to 0.61 µg-at N $l^{-1}h^{-1}$ in the Nueces Estuary and 0.22 to 0.78 µg-atoms N $l^{-1}h^{-1}$ in the Guadalupe Estuary. Ammonium concentrations in the Nueces Estuary were similar at Stations NA,NB and NC, but were significantly lower at Station ND (Fig. 1a). In Guadalupe Estuary, ammonium concentrations in the upper estuary station were similar to those in the lower estuary station, but bottom water concentrations were much higher than surface water concentration at all stations.

The rates of ammonium regeneration in the study areas ranged 0.036 to 0.179 µg-atoms N $l^{-1}h^{-1}$ with no large differences between two estuaries (Fig.2a). This is within the range of ammonium regeneration rates reported in other coastal waters

(Caperon et al.,1979; Wheeler and Kirchman, 1986; Selmer,1988), but is lower than ammonium regeneration rates in the Chesapeake Bay (Glibert et al., 1982). Rates of ammonium regeneration were up to 3.9 fold higher in bottom water than in surface water for both Nueces and Guadalupe Estuary.

Results from our previous studies in Nueces Estuary and other reports (Harrison,1978; Glibert,1982; Hopkinson et al.,1987) indicate that microheterotrophs (< 35 µm size fraction) are the major ammonium producers in seawater. Figure 1c shows that concentrations of phaeopigments were always higher in bottom water than in surface water. It is therefore possible that microhetrotrophs associated with detritus were more active in bottom water, resulting in more ammonium regeneration. Zooplankton abundance in bottom water is generally higher than in surface water during daylight hours even in shallow water (Banse,1964). Therefore, it can not be ruled out that a higher density of zooplankton in bottom waters may have been responsible for higher regeneration rates of ammonium. It is also possible that benthic organisms present in the bottom water may have contributed to ammonium regeneration.

Rates of ammonium utilization were in the range of 0.087 - 0.365 µg-atoms N $l^{-1}h^{-1}$ in the study areas (Fig. 2b). These rates are comparable to or higher than those reported in other coastal areas (Harrison,1978 ; Paasche and Kristiansen,1982; La Roche,1983; Wheeler and Kirchman, 1986; Selmer,1988), but are lower than those in the Chesapeake Bay (Glibert et al.,1982). In the Nueces Estuary, rates of ammonium utilization in bottom water were slightly higher than in surface water during the study period. Ammonium utilization rates in bottom water in the Guadalupe Estuary were, however, much higher than in surface water. Ammonium utilization rates in Nueces Estuary were significantly correlated with chlorophyll a concentration ($r = 0.63$, $p < 0.05$), but when Guadalupe Estuary data were included,

the overall correlation was not significant. However, there was a strong correlation between ammonium concentration and ammonium utilization rate in the study areas ($r = 0.90$, $p < 0.01$). Therefore, higher rates of ammonium utilization in bottom water of the Guadalupe Estuary may have been related to higher concentrations of ammonium in bottom water. Ammonium consumption through nitrification is usually negligible in the water column (Selmer, 1988). However, it is possible that nitrification activity in bottom water (we collected bottom water at < 20 cm above from the sediment-water interface) was considerable, resulting in higher rates of ammonium utilization than in surface water.

The measured benthic ammonium flux (*i.e.* the change in ammonium concentration in chamber water during a certain period of time) is compared with the actual ammonium flux (water column regeneration and utilization accounted for) in Table 1. Measured benthic fluxes were within 20 % of the actual flux, ranging from 87 - 119 % of the actual flux. The measured flux was usually lower than the actual flux because ammonium utilization in bottom water usually exceeded ammonium regeneration in the study areas. However, ammonium regeneration in bottom water was higher than utilization at one station (Station ND) in Nueces Estuary, resulting in a higher value for the measured flux than the actual flux. Therefore, the benthic ammonium fluxes obtained only from the chamber experiments can be either overestimates or underestimates, depending on the balance between ammonium regeneration and utilization in the chamber water. We did not, however, find a substantial difference between the measured flux and the actual flux in these estuaries. It is notable that the rate of increase in ammonium concentration in the chamber water due to the actual flux across the sediment-water interface was 0.16 μg-atoms N $l^{-1}h^{-1}$, while the rate of ammonium regeneration in the bottom water was 0.14 μg-atoms N $l^{-1}h^{-1}$ at this station

(Station ND) in Nueces Estuary (Table 1). This indicates that about half of the ammonium in chamber water was not derived from sediments at this station.

The actual ammonium flux from sediments ranged from 0.74 to 5.68 mg-atoms N $m^{-2}day^{-1}$ (average 3.36 mg-atoms N $m^{-2}day^{-1}$) in Nueces Estuary (Table 1). These values are similar to the annual average (2.9 mg-atoms N $m^{-2}day^{-1}$) previously obtained by Flint and Kalke (1985) in the same area. The actual benthic flux in Guadalupe Estuary ranged from 4.51 to 11.02 mg-atoms N $m^{-2}day^{-1}$ (average, 7.47 mg-atoms N $m^{-2}day^{-1}$), which is higher than in Nueces Estuary. Ammonium fluxes in these estuaries are generally comparable to those reported for other estuaries (Table 3). Both in Nueces and Guadalupe Estuary, the actual ammonium flux generally declined with distance from the river mouth.

To our knowledge, there have been no reports which directly addressed the comparison of two major sources of nitrogen (benthic flux and water column regeneration) in shallow estuaries. Benthic ammonium fluxes in these Texas estuaries are compared with the ammonium regeneration in the water column in Table 2. In Nueces Estuary, the benthic ammonium flux exceeded the water column regeneration in the upper estuary stations (Stations NA and NB), but ammonium regeneration in the water column was much greater than the benthic ammonium flux at the lower estuary stations (Stations NC and ND). In Guadalupe Estuary, the benthic ammonium flux was larger than the water column regeneration at the station near the river mouth (Station SA) as observed in Nueces Estuary. At the mid estuary station (Station B) and the lower estuary station (Station C), benthic regeneration was about equal to or slightly less than the water column regeneration. In both estuaries, benthic ammonium flux generally decreased with distance from the river mouth, while the importance of water column ammonium regeneration increased in deeper or lower estuary stations.

In the water column, ammonium utilization(U) usually exceeded ammonium regeneration(R) in both estuaries (U/R > 1) (Figs.3a,3b). This indicates that ammonium regenerated in the water column is not enough to supply the ammount of ammonium consumed in the water column. The ammonium deficit in the water column could be met by benthic flux and/or ammonium supply by freshwater inflow. The U/R ratios in the upper estuary were high (> 1.5) in both estuaries, and generally declined in lower estuary stations (Figs.3a,3b). In Nueces Estuary, ammonium utilized in the water column was 83 - 114 % of total ammonium regeneration (*i.e.* the benthic flux plus the water column regeneration) (Fig.3a). However, in Guadalupe Estuary, ammonium utilized in the water column was only 47 - 81 % of total ammonium regeneration (Fig.3b). This indicates that total ammonium regeneration and utilization were closely in balance in the Nueces Estuary, but not in the Guadalupe Estuary, especially at the upper estuary station (Station SA). The lowest U/T ratio (U/T = 0.47) was found at that station owing to the very high benthic ammonium flux (Table 2). Bottom water samples at Station A had the highest ammonium concentration measured during this study (Fig. 1a).

Phytoplankton nitrogen demand is generally calculated from phytoplankton production using the Redfield ratio (C/N atomic ratio = 6.97) (Redfield et al, 1963). The contributions of benthic ammonium flux and ammonium regeneneration in the water column to phytoplankton nitrogen demand in the two estuaries are given in Table 2. In Nueces Estuary, the total ammonium regenerated in the estuary (the benthic flux plus the water column regeneration) supplies an average of 70 % (15 - 150 %) of the phytoplankton nitrogen demand (Table 2). Total ammonium regenerated in the upper estuary (Nueces Bay) satisfied more than 100 % of the nitrogen requirements of phytoplankton in the overlying water. However, in the mid to lower estuary (Station NC and ND in Corpus Christi Bay) total regenerated ammonium supplied

less than 20 % of the nitrogen requirements of phytoplankton. Total ammonium regeneration and utilization were generally in balance in Nueces Estuary (U/T ~ 1.0) (Fig.3a), indicating that there were no significant external ammonium sources. This implies that other nitrogenous nutrients, such as nitrate, were more important than ammonium to phytoplankton production in the lower Nueces Estuary. The sources of other nitrogenous nutrients are unknown, but may be derived from precipitation and anthropogenic inputs.

In Guadalupe Estuary, the total regenerated ammonium supplied 40 - 165 % of the nitrogen demand of phytoplankton (Table 2). Unlike Nueces Estuary, the nitrogen demand of phytoplankton in the upper estuary station was not fully met by the total regenerated ammonium in this estuary. This suggests that phytoplankton production at the upper Guadalupe Estuary station is dependent upon nitrogen inputs from the river.

Heterotrophic bacteria also require nitrogen for their growth, and Wheeler and Kirchman (1986) reported that bacterial ammonium utilization can be significant in coastal waters. Bacterial nitrogen demand calculated from bacterial production (see Chapter 2 of this section) was 1.0 - 7.5 mg-atoms N $m^{-2}day^{-1}$ in Nueces Estuary. This amounts to 3 - 21 % of phytoplankton nitrogen demand. In Guadalupe Estuary, bacterial nitrogen demand was greater than phytoplankton nitrogen demand by 2 - 3 fold. According to the estimation of Wheeler and Kirchman (1986), ammonium accounted for 20 - 60 % of the summed ammonium plus amino acid utilization by bacteria. During the study period, if bacteria obtain 40 % of their nitrogen requirement from ammonium, bacteria could consume the ammount of ammonium which was almost equal to phytoplankton nitrogen demand in Guadalupe Estuary. Considerable bacterial utilization of ammonium may have caused no significant relationship between ammonium utilization rate and chlorophyll a concentration in Guadalupe Estuary.

The ammonium concentration in sediment pore waters generally increased with increasing sediment depth at Stations SA and SB, but the pattern was reversed at Station SC (Fig.4a). Porosity of sediments ranged from 0.61 to 0.82 in Guadalupe Estuary. Porosity was lower at Station C than at Stations A and B (Fig.4b). Ammonium turnover times in sediments and the water column were calculated on the assumption that the ammonium pools both in sediments and overlying waters were in steady state. In addition to diffusive flux of ammonium into the water column from sediments, ammonium could be utilized by micoorgnisms such as heterotrophic bacteria, nitrifying bacteria and benthic algae. Therefore, the calculated turnover time of ammonium in sediments is maximal. Ammonium turnover times in the water column of Nueces Estuary (1.7 - 2.6 hours) were similar to those in Guadalupe Estuary (1.7 - 2.6 hours) (Table 4). Ammonium turnover times in the upper 10 cm of sediments in Guadalupe Estuary were 45.4 - 81.6 hours (average 59.3 hours). On the basis of these calculations, ammonium turnover times in the water column were 27 times faster than those in the upper 10 cm sediments. However, ammonium pool sizes in pore waters of the upper 10 cm sediments were about 12 - 30-fold larger than those in the water column.

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Table 1. Comparison of the actual benthic flux with the measured benthic flux in Nueces and Guadalupe Estuaries.

	Station	Δ[NH4]/h in the chamber water (μg-atom N/l/h)	NH4 regeneration in the bottom water (μg-atom N/l/h)	NH4 utilization in the bottom water (μg-atom N/l/h)	Measured benthic flux (M) (mg-atom N/m2/d)	Actual benthic flux (A) (mg-atom N/m2/d)	M/A*100 (%)
Nueces Estuary	NA	0.83	0.18	0.24	3.80	4.07	93.4
	NB	1.12	0.14	0.26	5.12	5.68	90.1
	NC	0.63	0.13	0.15	2.88	2.93	98.3
	ND	0.19	0.14	0.11	0.88	0.74	119.2
Guadalupe Estuary	SA	2.18	0.12	0.37	9.92	11.02	90.0
	SB	1.33	0.15	0.33	6.08	6.89	88.2
	SC	0.86	0.09	0.22	3.93	4.51	87.1

Table 2. Contribution of the benthic ammonium flux and the water column ammonium regeneration to the nitrogen demand in the water column.

Location	Station	Depth (m)	(B)Benthic flux	(W)Water column regeneration	(T)Total regeneration	Water column utilization	(P) Phytoplankton N - demand	B/P*100 (%)	W/P*100 (%)	T/P*100 (%)
Nueces	NA	1.2	4.07	3.65	7.72	6.52	5.25	77.5	69.5	147.0
Estuary	NB	1.3	5.68	2.77	8.45	7.04	8.35	68.0	33.2	101.2
	NC	3.7	2.93	7.76	10.69	12.18	71.61	4.1	10.8	14.9
	ND	2.2	0.74	5.17	5.91	4.95	35.65	2.1	14.5	16.6
Guadalupe	SA	1.3	11.02	2.80	13.82	6.49	35.00	31.5	8.0	39.5
Estuary	SB	1.9	6.89	8.82	15.71	11.67	9.52	72.4	92.6	165.0
	SC	1.9	4.51	4.11	8.62	6.95	15.49	29.1	26.5	55.6

* Units are in mg-atom N/m2/day , otherwise designated

* T = B + W

* Phytoplankton N demand was calculated using the Redfield ratio (C/N atomic ratio = 6.97). Phytoplankton production data were provided by Dr. Dean Stockwell.

Table 3. Percent of phytoplankton nitrogen demand supplied by benthic ammonium flux and (or) water column ammonium regeneration in estuarine and coastal waters.

Region	Benthic flux (mg-atom N/m2/day)	Benthic flux/ Phytoplankton N-demand (%)	Water column regeneration (mg-atom N/m2/day)	Water column regeneration/ phytoplankton N-demand (%)	References
Narragansett Bay (RI, USA)	11	35			Furnas et al.,1976
Buzzards Bay (MA,USA)	1.9	74			Rowe et al.,1975
Long Island Sound (USA)	0.1 - 8.0	20 - 25			Aller & Benninger, 1981
Great South Bay (NY,USA)	2 - 62	30			Dietz,1981
Potomac River Estuary (USA)	-4.8 - 26.0	35			Callender & Hammond, 1982
North Carolina estuaries (NC,USA)	0 - 5.4	35			Fisher et al.,1982
Chesapeaske Bay (USA)	0.9 - 13.5	13 - 40			Boynton & Kemp, 1985
Nueces Estuary (TX,USA)	2.9	60			Flint & Kalke,1985
Bedford Basin (Canada)				13 - 16	La Roche, 1983
Davies Reef (Australia)				31 - 313	Hopkinson et al.,1987
Nueces Estuary (TX,USA)	0.7 - 5.7 (3.4)	2 - 78 (38)	2.8 - 7.8 (4.8)	11 - 70 (32)	This paper
Guadalupe Estuary (TX,USA)	4.5 - 11.0 (7.5)	29 - 72 (44)	2.8 - 8.8 (5.2)	8 - 93 (42)	This paper

Table 4. Ammonium turnover times in the water column and in pore waters of the upper 10 cm sediments.

	Station	NH4 pool (mg-atom N/m2)	Benthic NH4 flux (sediment) or NH4 utilization rate (water column) (mg-atom N/m2/hour)	Turnover time (hour)
Water column	NA	0.52	0.27	1.9
	NB	0.69	0.29	2.4
	NC	1.37	0.53	2.6
	ND	0.35	0.21	1.7
	SA	0.63	0.26	2.4
	SB	0.77	0.45	1.7
	SC	0.78	0.30	2.6
Sediment (upper 10 cm)	SA	20.88	0.46	45.4
	SB	23.44	0.29	81.6
	SC	9.57	0.19	50.9

* Turnover Time = NH4 pool / benthic flux (for sediment) or NH4 utilization rate (for water column).
* NH4 pool size in the upper 10 cm sediment was calculated using porosity of sediments and NH4 concentration in sediment pore water.

Figure 1a. Ammonium concentration in Nueces and Guadalupe Estuaries.

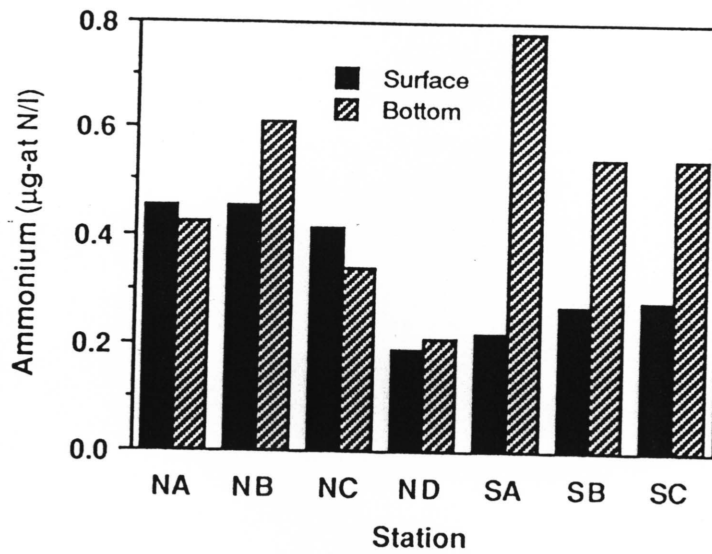


Figure 1b. Chlorophyll a concentration in Nueces and Guadalupe Estuaries.

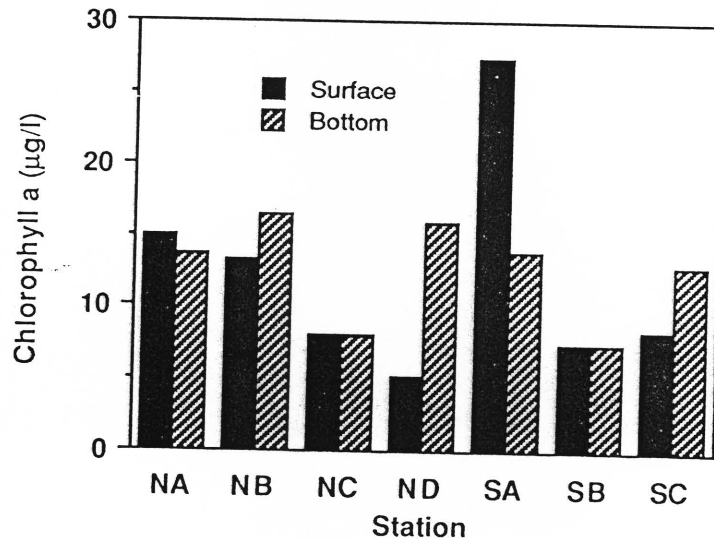


Figure 1c. Phaeopigment concentration in Nueces and Guadalupe Estuaries.

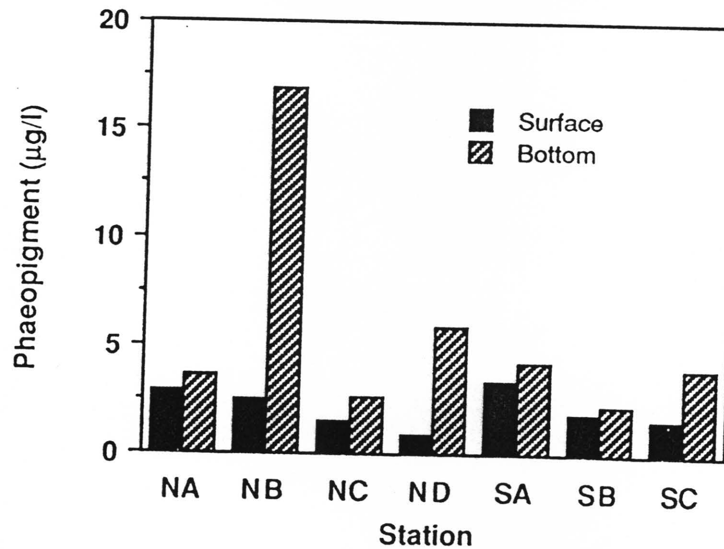


Figure 2. Ammonium regeneration rates (a) and utilization rates (b) in Nueces and Guadalupe Estuaries.

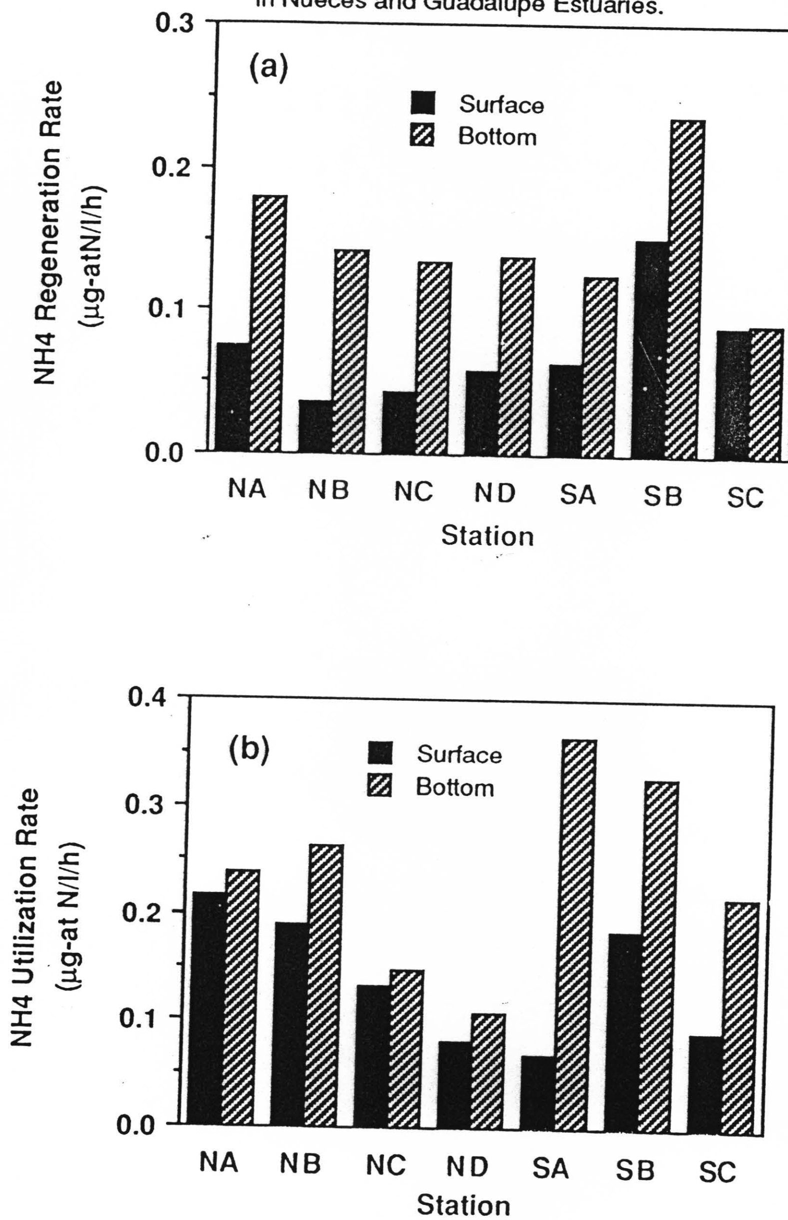
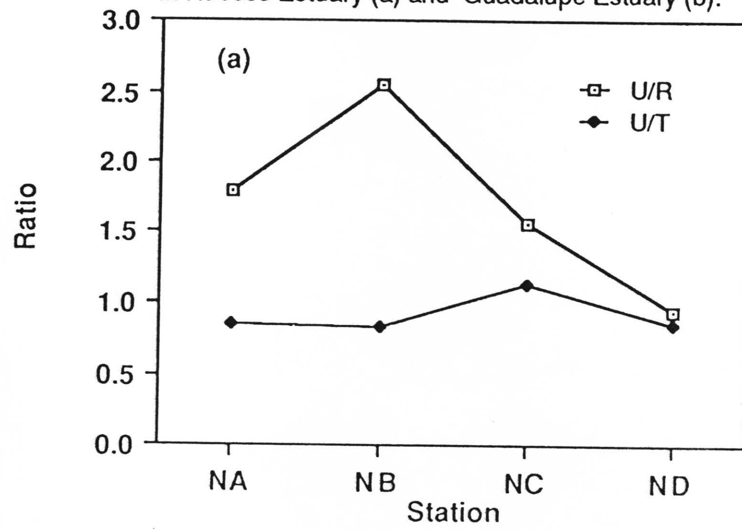


Figure 3. Ratios of ammonium utilization to regeneration (U/R) in the water column and ratios of ammonium utilization to total ammonium regeneration (U/T) In Nueces Estuary (a) and Guadalupe Estuary (b).



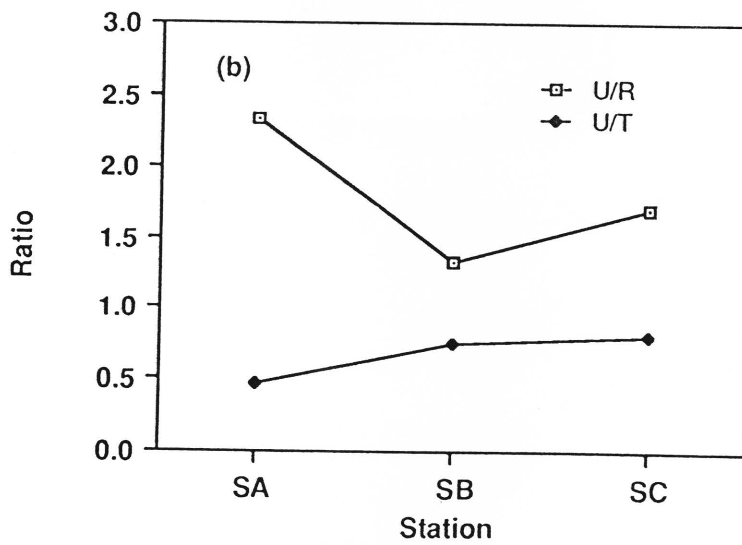
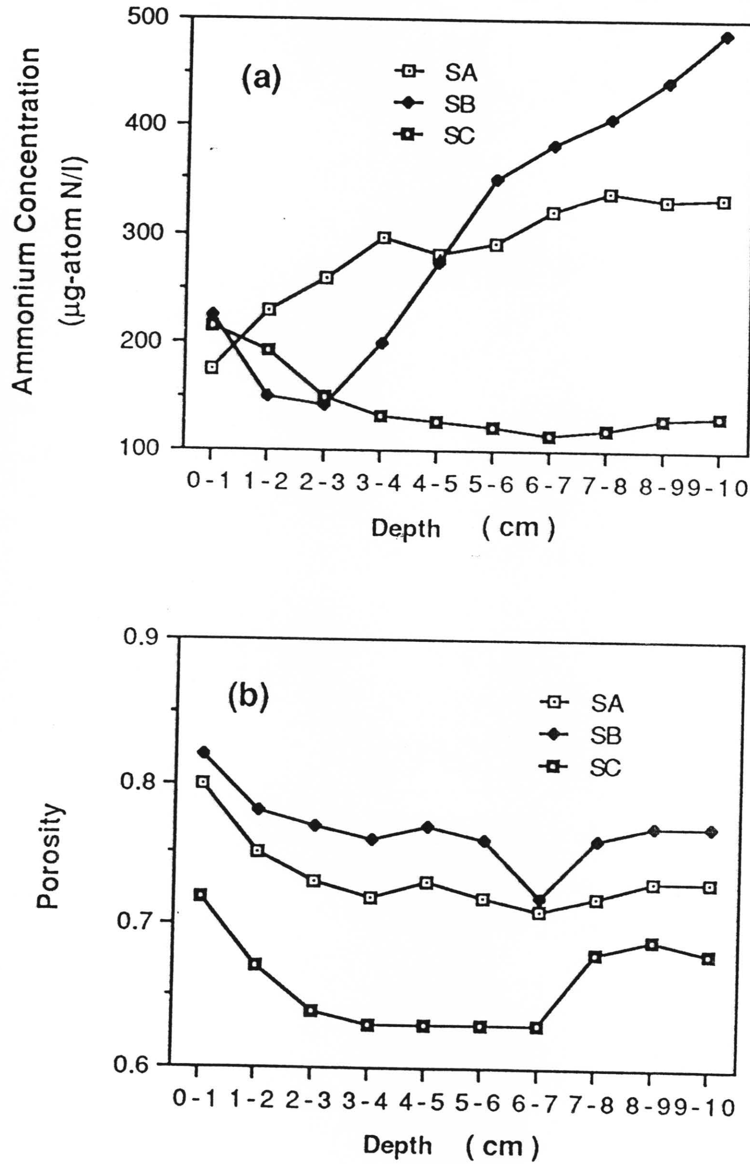


Figure 4. Ammonium profiles (a) and porosity (b) of sediments in the Guadalupe Estuary.



IV. Denitrification Rates in Sediments of Nueces and Guadalupe Estuaries, Texas

Introduction

Estuaries are often sites of high productivity due in large part to the input of terrestrially-derived nutrients (see Deegan et al. 1986 and references therein). Of these nutrients, nitrogen is of primary importance for maintaining high productivity, and thus the transformations and fates of nitrogenous compounds are of great interest. There are four potentially major pathways for the removal or sink of fixed nitrogen in estuaries: 1) transport to coastal waters, 2) removal by fisheries activity, 3) burial in sediments, 4) loss to the atmosphere as molecular nitrogen resulting from denitrification.

Denitrification is reported to remove a significant amount of fixed nitrogen which enters estuaries (Seitzinger, 1988), and in some cases, denitrification may control coastal primary productivity (Nixon et al., 1981). In Norsminde Fjord Estuary (Denmark), about 25 % of the external nitrate input is removed by denitrification (Jørgensen and Sørgensen, 1988). Smith et al.(1985) reported that the amount of nitrogen removed via denitrification was about 50 % of the riverine nitrate entering Four League Bay, Louisiana. In Ochlockonee Bay (Florida), about 54 % of the river input of dissolved inorganic nitrogen is removed by denitrification (Seitzinger, 1987).

Denitrification results from anaerobic respiration by heterotrophic bacteria that utilize nitrate as a terminal electron acceptor in the absence of molecular oxygen. Denitrification occurs mainly in sediments rather than in the overlying water column due to the lower oxygen level and much higher availability of nitrate and organic substates in sediments (Goering and

Dugdale, 1966; Chan and Campbell, 1980). Thus, denitrification rates are affected by the nitrate concentration (Smith et al., 1985; Jørgensen and Sørensen, 1988), oxygen concentration (Anderson et al., 1984; Jørgensen and Sørensen, 1988), and organic matter content in sediments (Jensen et al., 1988).

Because of the ecological and geochemical importance of denitrification, there have been numerous studies of denitrification in many coastal marine sediments (Billen,1978; Sørensen 1978; Nishio et al. 1982; Kaspar,1983; Jenkins and Kemp, 1984; Sietzinger et al. 1984; Andersen et al., 1984; Smith et al. 1985; Slater and Capone, 1987; Seitzinger et al., 1987; Jensen et al., 1988; Jørgensen, 1989). However, there have been few reports of seasonal variations in denitrification rates in estuarine environments. Seitzinger et al. (1984) observed that seasonal variations in denitrification rates were not very marked in the Narragansett Bay. However, denitrification rates during late spring were 4-fold higher than those during late winter in Ochlockonee Bay (Seitzinger, 1987). Jørgensen (1989) also reported that the denitrification capacity exhibited the peak in the late spring for Norsminde Fjord estuary in Denmark.

During the past two years, we have been studying nitrogen cycling in Nueces and Guadalupe Estuaries on the south Texas Gulf coast. The size of these two estuaries are similar, but freshwater inflow to Guadalupe Estuary is about five times more than that to Nueces Estuary. In this report, we present results of seasonal and spatial variations of denitrification rates and evaluate the singificance of denitrification to nitrogen cycling in these two estuarine areas.

Materials and Methods

Study areas

Sediment core samples for the denitrification measurements were collected in two Texas estuaries, the Nueces Estuary and the

Guadalupe Estuary. The Nueces River is the main source of freshwater inflow into Nueces Estuary, and the San Antonio and Guadalupe Rivers provide the major freshwater input into the Guadalupe Estuary. The Guadalupe Estuary typically receives a 5-fold larger freshwater inflow than the Nueces Estuary. In Nueces Estuary, two stations (Stations NA and NB) were in Nueces Bay which adjoins with Nueces River, and two stations were in Corpus Christi Bay (Stations NC and ND), which is the major bay system in the Nueces Estuary. In Guadalupe Estuary, all stations (Stations SA, SB and SC) were located in San Antonio Bay. Samples from Nueces Estuary were collected in August, January and May, and samples from Guadalupe Estuary were collected in October, January and May.

Sediment collection and incubation

Sediment cores (7.6 cm ID, 25 cm deep) were collected using SCUBA or a hand-operated coring device from a boat. Sediment cores were transferred to gas-tight incubation chambers of the same inner diameter. Chambers have two sampling ports which are sealed with butyl rubber stoppers and screw caps with 9 mm openings (Bellco Glass Inc.). A gas sampling port is located near the top of the chamber, and a water sampling port is located just above the sediment-water interface in the chamber. The depth of sediments in the chamber was approximately 7 cm. The bottom of the chamber was sealed with a butyl rubber stopper held in place with a plexiglass frame. The volume of the water-phase was about 180 - 190 ml and the volume of the gas-phase was approximately 60 - 70 ml. After sealing the ports with butyl rubber stoppers, the water and air phases of each chamber were flushed with a N_2-free gas mixture (80 % He and 20 % O_2) for one hour through the sampling ports (the lower port was the inlet and the upper one was the outlet) using syringe needles. The butyl rubber stoppers in the sampling ports were changed after each sampling.

Killed controls received 10 ml of formalin (37 % formaldehyde) that was added to the water phase of the chamber. When determination of ammonium and nitrate plus nitrite in the water phase was necessary $HgCl_2$ was used for the killed controls (the final concentration of $HgCl_2$ in the water phase was approximately 200 µM). For aerobic killed controls, the water and gas phases were flushed by a N_2-free gas (mixture, He 80 % and O2 20 %). For anaerobic killed controls, the water and gas phases were flushed with helium.

Chambers were incubated by submerging the bottom part of the chamber (about 1/3 of the chamber) in a water bath in the dark. During the incubation period, chambers were shaken (70 RPM) continuously or intermittently for one hour every 24 hours. When shaken intermittently, chambers were shaken immediately before gas sampling to equilibrate the gas phase with the water phase.

Before sampling from the gas phase of the chamber, a gas-tight syringe (250 µl) was flushed 6 times with N_2-free helium. After flushing, the syringe was filled with helium which was then ejected to the air to prevent contamination with atmospheric N_2 while the syringe needle was inserted into the gas sampling port on the chamber. The gas sampling port of the chamber was also flushed vigorously with helium to reduce the possibility of N_2 contamination during sampling. In order to prevent contamination of the gas sample in the syringe with atmospheric N_2 before injection into the gas chromatograph, a 200 µl sample was taken and 100 µl of the sample was ejected to the air during transfer to the injection port of the gas chromatograph. The injection port was also flushed vigorously with helium. Using the above procedure, we could not detect any contamination by atmospheric N_2 during sampling. After gas sampling, water was sampled from chambers for inorganic nitrogen determinations. Chamber water was

then replaced with water collected from the sampling site, and the next incubation was started repeating the previous procedure.

To check for contamination with atmospheric N_2 during incubation, distilled water or seawater were poisoned with formalin (3 % final concentration) and added to the chambers until the volume of the gas phase was same as in chambers with sediments. After the water and gas phases were flushed with a helium and oxygen gas mixture, the chambers were incubated in the water bath in the dark as described previously. Gas samples were analyzed every 20 hours over a three day incubation period.

Fluxes of ammonium and nitrate plus nitrite from sediments

Fluxes of ammonium and nitrate plus nitrite from sediments to the overlying water were determined from measurements of net increases in the concentrations of these nutrients. Parallel incubations with estuarine waters collected from the sampling stations were used to measure the net change in ammonium and nitrate plus nitrite concentrations in the water column.

Analytical methods

Gas samples (100 µl) were analyzed using a gas chromatograph (Model 8500, Carle Instruments Inc.) equipped with a thermal conductivity detector. Gases were separated using a stainless steel column (3 m x 3mm) packed with molecular sieve 5A (70/80 mesh size) and helium as the carrier gas (20 ml min^{-1}). The concentrations of N_2 and O_2 were calculated from peak areas using a C-R4A Shimadzu integrator. Denitrification rates were calculated by subtracting the N_2 gas concentration in the killed control chamber from that in the live chamber.

Colorimetric methods were used for determination of ammonium (Solozano, 1969) and nitrate concentrations (Strickland and Parson, 1972) using either a spectrophotometer (Beckmann Model 24)

or autoanalyzer (Technicon II). Redox potentials (Eh) of sediments were measured using a platinum electrode.

Results

Sediment characteristics of Nueces and Guadalupe Estuaries

Redox potentials of surface sediments usually showed positive values throughout the study period, and negative values began to appear 2 to 4 cm below the sediment-water interface (Table 1a). During winter (January), redox potentials of sediments were higher compared to other seasons. We observed negative values of redox potentials even in very surface sediments at Station ND in Nueces Estuary during the summer. We also observed that the bottom water of the Station ND had low concentrations of dissolved oxygen during that period. Sediments from Station ND had positive redox potentials at every depth, however, during the winter and spring time.

Sediments from Station SB in Guadalupe Estuary had the highest content of organic matter (14 - 15 %) of all study sites (Table 1b). Sediments from the lower estuary stations had lower percentages of organic matter in both Nueces and Guadalupe Estuaries. Sediments at all stations were muddy, except Station ND of Nueces Estuary where sediments contained fine sand. Sediments at Station ND had the lowest amount of organic matter (2 - 4 %) among the two estuaries.

Sediments of Station NA in Nueces Estuary were very soft and had the highest content of water (the upper 10 cm of sediments averaged 85 %(V/V) water), while the sediments of Station ND had the lowest water content (53 %) among all stations in the study areas (Table 1c).

Denitrification rates of sediments

Fluxes of nitrogen gas from the formalin killed controls

declined over the first 9 days of incubation and were stable at 30 to 40 μ mol m^{-2} h^{-1} after 12 days (Fig. 1). Higher fluxes of nitrogen gas during the initial incubation period were probably due to the flux of nitrogen gas that was initially dissolved in sediment pore waters. This trend is consistent with the observation by Seitzinger et al. (1980) who recommended that flux measurements be made after the initial degassing period. In the present study, denitrification rates were measured after the first 9 days of incubation. After this initial degassing period increases in nitrogen gas were linear in both live and control chambers (Fig. 2).

We were interested in determining whether the flux of nitrogen gas measured in killed controls resulted from continued pore water exchange or if atmospheric nitrogen was entering the chambers during incubation. To test for these possibilities chambers were incubated with formalin-killed water samples. A steady flux of 36.3 ± 1.5 μmol m^{-2} h^{-1} (n = 8,95 % confidence interval) of nitrogen gas was measured in all chambers. These rates of nitrogen gas flux were similar to the rates (30 to 40 μmol m^{-2} h^{-1}) measured in killed control chambers with sediments, indicating that the gas flux after 9 days of incubation results primarily from the diffusion of atmospheric nitrogen gas during incubation rather than exchange of pore water nitrogen. We also found that there was no significant difference ($p > 0.1$, $n = 36$) in flux of nitrogen gas between aerobic killed controls and anaerobic killed controls. Killed controls were run at all stations for each sampling period, and nitrogen gas flux rates measured in the controls were subtracted from rates measured in live chambers.

Denitrification rates ranged from 4.0 to 71.1 μmol m^{-2} h^{-1} in Nueces Estuary and from 4.6 to 34.7 μmol m^{-2} h^{-1} in Guadalupe Estuary (Table 2). In general, upper estuary stations exhibited higher denitrification rates than lower estuary stations. The

highest denitrification rate was found at the upper station (Station NA) of Nueces Estuary during summer, and the lowest rate was found at the lower station (Station ND) of Nueces Estuary during winter. Rates of denitrification in Guadalupe Estuary were within the range of those in Nueces Estuary.

During the study period, salinities ranged from 32 to 44 ppt in Nueces Estuary and 11 to 29 ppt in Guadalupe Estuary. Salinity ranges in the two estuaries reflect differences in freshwater inflow. The average freshwater inflow (the mean of 35 years) is 1.6×10^6 m^3 per day in Nueces Estuary and 6.1×10^6 m^3 per day in Guadalupe Estuary. We found no significant correlation between salinity and rates of denitrification.

Oxygen consumption rates

Total oxygen consumption (chemical plus biological) rates in Nueces Estuary sediments ranged from 176 to 409 µmol m^{-2} h^{-1} except for an exceptionally high rate (818 µmol m^{-2} h^{-1}) at Station ND in the summer (Table 2). As mentioned earlier, the bottom water of Station ND had low dissolved oxygen concentrations when the sediments were collected, and the redox potential of surface sediments was -236 mV (Table 1) indicating very reducing conditions. Therefore, the rate we measured under relatively high dissolved oxygen concentrations was probably the potential rate rather than the actual rate. The range of total oxygen consumption rates of Guadalupe Estuary (208 - 550 µmol m^{-2} h^{-1}) was similar to that observed in Nueces Estuary. Oxygen consumption rates at the upper estuary stations were generally higher than at the lower estuary stations for both Nueces and Guadalupe Estuaries as was observed for denitrification rates.

We consistently observed that oxygen was continuously consumed in the killed controls after 9 days of incubation. Oxygen consumption in killed controls was most likely due to chemical oxidation of reduced inorganic compounds in sediments. To further

investigate the potential chemical oxygen consumption in sediments, incubations containing 4% formalin and 1 or 2 cm sections of sediment cores were mixed vigorously after 15 h of incubation to ensure penetration of formalin throughout the sediments. Oxygen consumption was very high (5 mmol O_2 m^{-2} h^{-1}) immediately following mixing indicating a very high potential chemical oxygen consumption in the sediments (Fig. 3). Chemical oxygen consumption was 40 - 73 % of total oxygen consumption in the study areas (Table 3). Dale (1978) reported that chemical oxygen consumption was 61 - 77 % of total oxygen consumption in coastal marine sediments of western Norway. Hargrave (1972) also observed that 10 to 65 % of total oxygen consumption was due to chemical oxygen consumption in lake sediments.

Nitrification rates

Nitrification rates in sediments were calçulated assuming that nitrate which was either consumed during denitrification or released into the water phase of the chamber was continuously provided by nitrification in the sediments. The above assumption seems reasonable because almost no nitrate (plus nitrite) was released into the water phase of the chambers when sediments were poisoned (Table 4). Using $^{15}NO_3^-$ and $^{15}NH_4^+$, Jenkins and Kemp (1984) observed that denitrification was closely coupled with nitrification in sediments. Gardner et al.(1987) also reported that the overlying water did not serve as a significant net source of nitrate for denitrification in sediments. On the basis of this assumption, the calculated nitrification rates in sediments ranged from 17.2 - 140.2 µg-atom N m^{-2} h^{-1} in Nueces Estuary and 40.5 - 79.4 µg-atom N m^{-2} h^{-1} in Guadalupe Estuary during the spring (Table 4). During the fall, nitrification rates in Guadalupe Estuary ranged from 48.9 to 78.1 µg-atom N m_2^{-1} h^{-1}. The spatial variation of nitrification activity was very large in Nueces

Estuary. The nitrification rate at the upper estuary station (Station NA) was about 8-fold higher than at the lower station (Station ND) of Nueces Estuary.There was, however, only a 2-fold range in the rates of nitrification at stations in the Guadalupe Estuary.

The contribution of nitrification to total and biological oxygen consumption in sediments is shown in the Table 3. Nitrification was responsible for 42 - 62 % of the biological oxygen consumption (17 - 41 % of total oxygen consumption) in the study areas except for Station NA where nitrification accounted for as much as 154 % of the biological oxygen consumption (68% of total consumption). This suggests that the calculated rates of nitrification in sediments at Station NA are overestimated. The contribution of nitrification to total oxygen consumption in Nueces and Guadalupe Estuaries was similar to that in Narragansett Bay (Seitzinger 1984) and in Ochlocknee Bay (Seitzinger 1987), and was within the range which was observed by Cooper (1984) in streams.

Seasonal variation

Seasonal variations in denitrification rates and total oxygen consumption rates appeared to be dominated by temperature. The average denitrification rate in Nueces Estuary (the average of all stations) was about 6 times higher in the summer and 3 times higher in the spring than in the winter (Table 5). In Guadalupe Estuary, the average denitrification rate during the fall was similar to the average rate during the spring, but was 1.6-fold higher than during the winter. In Nueces Estuary, the total oxygen consumption rate (the average of all stations) was 2.5- and 1.3-fold higher during the summer and the spring than during the winter. In Guadalupe Estuary, the average total oxygen consumption rate during the fall was similar to that during spring, but about 2-fold higher than that during the winter.

There was no substantial difference in denitrification rates (the average of all stations) between Nueces Estuary and Guadalupe Estuary during the winter and the spring (Table 3). The total oxygen consumption rate in Guadalupe Estuary was higher than in Nueces Estuary during the spring, but rates were similar in these estuaries during the winter.

Fluxes of ammonium, nitrate and nitrite

Ammonium fluxes across the sediment-water interface wererelatively constant in cores from the mid and lower estuary stations during the 24 d incubation period, but ammonium fluxes decreased over time in cores from the upper estuary stations (Fig.4a and 4b). Ammonium fluxes were much greater in the upper estuary cores. The fluxes of nitrate plus nitrite during the experimental period were relatively low and stable compared to ammonium fluxes (Figure 4c and 4d). With the exception of ammonium at the upper estuary stations, fluxes of inorganic nitrogen remained fairly constant for the duration of the experiments indicating that a relatively stable nitrate supply was available to denitrifiers over the course of the experiments.

Ammonium fluxes from sediment cores ranged from 10.8 to 101.5 µg-atom N m^{-2} h^{-1} (or 0.3 to 2.4 mg-atom N m^{-2} d^{-1}) in the study areas during the fall and the spring (Table 4). This flux range is similar to what we observed during short-term (2 h) *in situ* benthic chamber experiments in the summer (0.74 - 11.02 mg-atom N m-2 d-1) (Chapter III). As results obtained from the *in situ* benthic chamber experiment (see Chapter III), the ammonium flux showed the highest value in the upper estuary and decreased with the distance from the river mouth in both Nueces and Guadalupe Estuaries during the spring (Table 4). The flux of nitrate plus nitrite exhibited a similar pattern. The fluxes of nitrate plus nitrite were much lower than ammonium fluxes in the study areas. There were no measurable fluxes of nitrate plus and nitrite in the

lower estuary stations (Stations ND and SC) for both estuaries. The flux of nitrogen gas (from denitrification) accounted for 31 to 41 % of the total nitrogen flux (N_2 + NH_4^+ + NO_3^- plus NO_2^-) in Nueces Estuary and 20 to 59 % in Guadalupe Estuary.

Ammonium fluxes in $HgCl_2$-killed controls appeared to be much higher than those in live chambers (Table 4). However, fluxes of nitrate plus nitrite were either very small or not detectable in the killed controls.

Carbon mineralization by denitrifiers

We calculated carbon mineralization by aerobic heterotophs to compare with that by denitrifiers. For this calculation, oxygen consumed by aerobic heterorophs was obtained by subtracting both chemical oxygen consumption and oxygen consumption by nitrification from total oxygen consumption. In Table 4, we calculated the nitrification rate by summing the denitrification rate and the flux of nitrate plus and nitrite from sediments. However, we did not measure the flux of nitrate plus nitrite in all experiments. The fluxes of nitate plus nitrite from sediments were usually much lower than the denitrification rates in the study areas (Table 4). In experiments where nitrate and nitrite fluxes were not measured we assumed that the nitrification rate was equal to the denitrification rate for the calculation of oxygen consumption during nitrification. Thus, oxygen consumption by nitrifiers may have been slightly underestimated and oxygen consumption by heterotrophs slightly overestimated.

An average oxidation state of carbon in carbohydrates was used for calculation of carbon mineralization rates by aerobic heterotrophs and denitrifiers. The carbon mineralization rate by aerobic heterotrophs ranged from 41 to 56 μg-atom C m^{-2} h^{-1} in Nueces Estuary and 52 to 131 μg-atom C m^{-2} h^{-1} in Guadalupe Estuary (Table 6). Carbon mineralization by denitrifiers was calculated assuming that 2.4 moles of nitrogen gas is produced by

denitrifiers during the mineralization of 1 g-atom of carbon (Gottschalk, 1979). The carbon mineralization rate by denitrifiers ranged from 22 to 73 μg-atom C m^{-2} h^{-1} in Nueces Estuary, and from 41 to 131 μg-atom C m^{-2} h^{-1} in Guadalupe Estuary. The carbon mineralized by denitrifiers was 40 to 179 % of that which was mineralized by aerobic heterotrophs in the study areas.

Discussion

Three direct methods have been used to estimate denitrification rates in sediments: 1) the acetylene blockage method, 2) the measurement of $^{15}N_2$ after addition of $^{15}NO_3^-$, 3) the measurement of nitrogen gas production. Each method has relative advantages and disadvantages, but we chose the later method because it measures actual rather than potential rates (see Seitzinger 1988). There are, however, two potential disadvantages of this method: 1) relatively long incubation periods are required, 2) contamination by atmospheric N_2 during incubation and sampling. We observed that in most incubations the fluxes of ammonium, nitrate and nitrite remained fairly constant over the course of incubations (24 d) suggesting that rates of ammonification and nitrification were not changing dramatically, suggesting that denitrification rates were also not changing substantially during incubation. Likewise, Seitzinger (1982) also observed that nutrient fluxes remained fairly constant for the duration of the incubation period.

The potential for contamination of incubations with atmospheric N_2 is high, but with careful techniques we found that contamination during sampling was avoidable. As observed by Seitzinger et al.(1980), we found that most pore water N_2 was released from sediment cores during the first 9 days of incubation, indicating that denitrification measurements should be

made after this period. In the present study various types of killed controls were used to monitor for atmospheric N_2 contamination during incubation. To our knowledge this approach has not been used before, and it indicated that contamination with atmospheric N_2 was significant but, for the most part, invariable among chambers and incubation conditions. Therefore, all data presented herein for denitrification rates were corrected for atmospheric N_2 contamination during incubation. Another important advantage of having killed controls is that biological and chemical oxygen consumption can be differentiated. Chemical oxygen consumption was found to be similar in magnitude to biological oxygen consumption, and this has very important implications when using oxygen consumption as a measure of total aerobic carbon mineralization in ecosystems.

Denitrification rates reported for marine coastal areas are summerized in Table 7. As discussed earlier, there may be differences in measurements due to variations in the methods used to estimate denitrification rates, but data are presented here for relative comparison. The denitrification rates we measured in Nueces and Guadalupe Estuaries fall within the ranges measured in other systems. Denitrification rates in Ochlockonee Bay in Florida (Seitzinger 1987) and Narragansett Bay in Rhode Island (Seitzinger et al. 1984) were determined by the direct measurement of nitrogen gas. Denitrification rates in Nueces and Guadalupe Estuaries were within the ranges reported for those two bays (Table 7), but the highest denitrification rates in Nueces and Guadalupe Estuaries were much less than those in Ochlockonee and Narragansett Bays.

Significant spatial differences in denitrification and oxygen consumption rates were observed in both Nueces and Guadalupe Estuaries. Spatial differences were more pronounced in Nueces Estuary, but in both estuaries rates of denitrification and oxygen

consumption were highest in the upper estuary. Two characteristics of the sediments in these estuaries, water content (porosity) and organic matter content, appear to influence the rates of these microbial processes. The water content of sediments may influence denitrification and oxygen consumption by affecting the diffusion of dissolved oxygen into sediments. Organic matter serves as the carbon and energy source for both denitrifiers and aerobic heterotrophs.

Nitrification is an aerobic process that can be limited by oxygen penetration into sediments (Grundmanis and Murray 1977). Nitrification can be an important process affecting denitrification rates in sediments because most of the nitrate used by denitrifiers is supplied by sedimentary nitrifiers (Jenkins and Kemp 1984). Sediments at the uppermost station in Nueces estuary had the highest water content and the highest denitrification rates of all stations, whereas sediments at the lowermost station in Nueces Estuary had the lowest water content, denitrification rates, organic matter content and oxygen consumption rates. Both the organic matter content of sediments and the oxygen consumption rates in Guadalupe Estuary were typically higher than those in Nueces Estuary.

Strong seasonal variations in denitrification and oxygen consumption rates were also observed in Nueces and Guadalupe Estuaries. Spring and summer denitrification rates were 2- to 6-fold higher than winter rates, and spring and summer oxygen consumption rates were about 2.5-fold higher than winter rates. Seitzinger et al.(1984) observed no marked seasonal variation in denitrification rates for Narragansett Bay. However, the highest denitrification rates in Ochlockonee Bay in Florida were reported during the late spring (Seitzinger,1987). Likewise, Jørgensen (1989) found the highest denitrification rates in Norsminde Fjord during the spring. Seasonal variations in denitrification rates in the present study appeared to be primarily influenced by

temperature.

A partial budget of nitrogen cycling in Nueces and Guadalupe Estuaries is presented in Table 8. The amount of nitrogen removed from the Guadalupe Estuary by denitrification was equivalent to about 43 % of total dissolved nitrogen (NO_3^- + NO_2^- + NH_4^+ + dissolved organic nitrogen) or 73 % of the dissolved inorganic nitrogen (DIN) entering the estuary from riverine inflow. This value is high relative to values (40 - 50 % of DIN) found in several other estuaries where such data exist (Seitzinger, 1988).

In Nueces Estuary, the riverine input of DIN (NO_3^- + NH_4^+) was much lower (about 39-fold) than the nitrogen removed by denitrification, indicating that additional nitrogen sources are required. We do not have data for riverine inputs of DON but it is unlikely that this could account for much of the nitrogen deficit in the budget. Nitrogen fixation may supply some portion of the nitrogen required in the system, but fixation generally accounts for only a minor percentage of nitrogen inputs to estuaries (Howarth et al. 1988). Atmospheric deposition, primarily as precipitation, may be an important nitrogen source as well as anthropogenic inputs. We have no data for inputs from precipitation, and we have very incomplete data for anthropogenic inputs. Oppenheimer et al.(1975) estimated that point source effluents, as well as urban and agricultural runoff, provided approximately 181 kg-atom N d^{-1}. The current anthropogenic nitrogen input into the estuary is probably much higher and must be a major nitrogen source to the estuary.

In the Guadalupe Estuary, the estimated nitrogen supply (riverine input + total regenerated ammonium) can meet the phytoplankton nitrogen demand (Table 8). In Nueces Estuary, however, riverine inputs of DIN and ammonium regeneration in the water and sediments satisfy only 60 % of the phytoplankton nitrogen demand. These data also strongly suggest that unaccounted for inputs to this estuary provide a significant

fraction of the total nitrogen budget.

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Table 1. Sediment charateristics of Nueces and Guadalupe Estuaries

(a) Eh (mv)

Station	NA			NB			NC			ND			SA			SB			SC		
	Aug	Jan	May	Aug	Jan	May	Aug	Jan	May	Aug	Jan	May	Oct	Jan	May	Oct	Jan	May	Oct	Jan	May
Depth(cm)																					
surface	336	19	10	200	na	na	13	70	90	-236	46	226	352	71	121	188	31	2	na	125	35
2	-64	-27	-188	-92	na	na	-221	-85	20	-362	20	179	259	7	-142	-109	20	-230	na	91	-275
4	-95	-78	-240	-95	na	na	-202	-7	-64	-340	42	191	-117	-12	-173	-87	-96	-140	na	97	-225
6	-115	-64	-263	-86	na	na	-152	-78	-102	-312	40	na	-104	-30	-227	-77	-45	-250	na	104	-240
8	-80	-69	-279	-93	na	na	-216	-165	-137	-350	50	na	-47	-100	-176	-101	-94	-245	na	-40	-220
10	-111	-330	-297	-135	na	na	-221	-223	-115	-365	140	na	-61	-129	-195	-107	-97	-282	na	-104	-201

* na = not available.

(b) % of organic matter(ignition loss at 550 C for 4 hours) and general description of sediments.

Station	NA	NC	ND	SA	SB	SC
Depth(cm)						
0 - 1	11.4	na	4.4	9.9	15.3	8.5
1 - 2	11.0	na	2.8	11.2	14.1	8.4
2 - 3	12.1	na	2.1	11.5	14.2	9.2
3 - 4	12.8	na	2.0	11.3	13.8	8.1
4 - 5	13.9	na	2.3	14.1	15.5	10.4
descrition	soft mud	hard mud	fine sand with mud	mud	mud with shell fish	mud

* Samples were collected in December 1988.

(c) Water content of sediments (% of total volume, annual average)

Station	NA	NC	ND	SA	SB	SC
Depth(cm)						
0 -1	91	na	73	74	84	71
1 - 2	78	na	50	72	79	68
2 - 3	89	na	48	69	79	64
3 - 4	90	na	49	75	78	62
4 - 5	86	na	44	75	78	60
5 - 6	84	na	49	68	76	58
6 - 7	86	na	52	75	67	57
7 - 8	81	na	48	75	76	66
8 - 9	79	na	61	78	77	67
9 - 10	87	na	na	78	76	na

* na = not available

Table 2. Denitrification rates and oxygen consumption rates in Nueces and Guadalupe Estuaries.

Location	Station	Month	Salinity (ppt)	N2 production (μ mol/m2/h	SD	O2 consumption (μ mol/m2/h)	SD
Nueces Estuary	NA	August	38	71.1	10.6	395	176
		January	35	11.7	7.9	230	77
		May	36	61.5	43.7	409	116
	NB	August	36	50.7	3.8	264	158
		January		na		na	
		May		na		na	
	NC	August	36	53.9	11.9	250	182
		January	33	11.3	3.5	186	73
		May	32	17.6	7.5	197	26
	ND	August	44	43.1	12.1	818	40
		January	33	4.0	0.5	177	86
		May	35	8.6	2.2	176	70
Guadalupe Estuary	SA	October	15	30.3	5.8	550	105
		January	22	22.5	10.5	283	46
		May	11	14.2	5.9	487	258
	SB	October	22	na		na	
		January	19	15.4	9.1	211	68
		May	21	34.7	5.7	485	177
	SC	October	29	16.8	9.2	416	72
		January	23	4.6	4.7	208	78
		May	25	21.1	12.8	423	243

*na = not available

*Incubation temperatures were: 30 C (August), 24 C (October), 16 C (January) and 23 C (May).

Table 3. Comparison of biological and chemical oxygen consumption in sediments, and the significance of oxygen consumption due to nitrification. (May, 1989)

Station	Biological oxygen consumption (BOC) (% of total)	Chemical oxygen consumption (% of total)	*Oxygen consumption due to nitrification (% of total)	*Oxygen consumption due to nitrification (% of BOC)
NA	45	55	68	154
NB	60	40	41	62
NC	47	53	20	42
SA	36	64	17	46
SB	27	73	33	54
SC	36	64	20	56

*Oxygen consumption due to nitrification was calculated on the basis of the following overall reaction: $NH_3 + 2 O_2 \rightarrow HNO_3 + H_2O$
Nitrite was considered as nitrate for the calculation.

Table 4. The benthic fluxes of ammonium, nitrate + nitrite, nitrogen gas (denitrification), and the calculated nitification rates in sediments of Nueces and Guadalupe Estuaries.

Month	Station	NH4	NO3 + N02	N2	Total N flux	Calculated nitirification rates
			(µg-atom N/m2/h)			
October,1988	SA	56.6 (42 %)	17.5 (13 %)	60.6 (45 %)	134.7 (100 %)	78.1
	SB	101.5	0.4	na	na	na
	SC	47.8 (49 %)	15.3 (16 %)	33.6 (35 %)	96.7 (100 %)	48.9
	SA(KC)	191.8	0.1	* 0	191.9	
	SB(KC)	344.3	ND	* 0	344.3	
	SC(KC)	54.5	ND	* 0	54.5	
May, 1989	NA	98.2 (41 %)	17.2 (7%)	123 (52 %)	238.4 (100%)	140.2
	NC	16.6 (31 %)	1.4 (3 %)	35.2 (66 %)	53.2 (100%)	36.6
	ND	8.4 (33 %)	ND (0 %)	17.2 (67 %)	25.6 (100 %)	17.2
	SA	58.7 (59 %)	12.1 (12 %)	28.4 (29 %)	99.2 (100 %)	40.5
	SB	43.7 (36 %)	10 (8 %)	69.4 (56 %)	123.1 (100%)	79.4
	SC	10.8 (20 %)	ND (0 %)	42.2 (80 %)	53 (100 %)	42.2

* The percentage shown in the parenthesis indicates % of total nitrogen flux from sediments.
*na = not available
*ND = Not detectable
*(KC) ; Sediments were poisoned by HgCl2 (killed control)
*O; We assumed that the denitrification rate was zero.
*Calculated nitrification rate = denitrification rate + flux of nitrate plus nitrite

Table 5. Seasonal variations in rates of denitrification and oxygen consumption in Nueces and Guadalupe Estuaries. Mean values of three stations are shown.

Location	Month	Temperature (C)	Denitrification rate (μ mol/m2/h)	Oxygen consumption (m mol/m2/h)
Nueces Estuary	August	30	56.0	0.49
	January	16	9.0	0.20
	May	23	29.2	0.26
Guadalupe Estuary	October	24	*23.2	0.50
	January	16	14.2	0.23
	May	23	23.3	0.47

* The mean of two stations (Stations A and C) is shown.

Table 6. Comparison of carbon mineralized in sediments by aerobic heterotrophs with that by denitrifiers. (Average rates of all stations are given)

Location	Month	*C-mineralized by aerobic heterotrophs (µg-atom C/m2/h)	**C-mineralized by denitrifiers (µg-atom C/m2/h)
Nueces Estuary	August	na	na
	January	56.3	22.3
	May	40.8	73.0
Guadalupe Estuary	October	131.6***	52.5***
	January	52.0	35.3
	May	122.6	58.6

na = not available

*C-mineralized by aerobic heterotrophs was calculated assuming that one mole of O_2 is consumed to mineralize 1g-atom of carbon.
O_2 consumed by aerobic heterotrophs = Total O_2 consumption - Chemical O_2 consumption - O_2 consumption due to nitrification
O_2 consumption by nitrifiers was calculated assuming that two moles of O_2 are consumed in the oxidation of one g-atom of nitrogen.
We assumed that the nitrification rate was equal to the denitrification rate.

**C-mineralized by denitrifiers was calculated assuming that 2.4 moles of nitrogen gas are produced during the mineralization of 6 g-atom C .

***The average of two stations (Station SA and SC) is given.

Table 7. Denitrification rates in coastal marine sediments.

Locations	Rate (µg-atom N/m2/h)	Method	Reference
Randers Fjord (Denmark)	41	acetylene blockage	Sørensen 1978
Tokyo Bay (Japan)	8 - 16	[15-N] NO3 addition	Nishio et al. 1982
Tama Estuary (Japan)	138 - 394	[15-N] NO3 addition	Nishio et al. 1982
Odawa Bay (Japan)	0.1 - 19.6	[15-N] NO3 addition	Nishio et al. 1982
San Francisco (California)	0.8 - 1.2	acetylene blockage	Oremland et al. 1984
Patuxent River estuary (Chesapeake Bay tributary)	77 - 89	[15-N] NO3 addition	Jenkins and Kemp 1984
Narragansett Bay (Rhode Island)	10 - 115	direct measurement of N2 gas	Seitzinger et al. 1984
South Island west coast (New Zealand)	0.8 - 77	acetylene blockage	Kaspar et al. 1985
Four League Bay (Louisiana)	6.9 - 8.5	acetylene blockage	Smith at al. 1985
Ochlockonee Bay (Florida)	0 - 210	direct measurement of N2 gas	Seitzinger 1987
Aarhus Bight (Denmark)	<2.1 - 41.7	acetylene blockage	Jensen et al. 1988
Norsminde fjord (Denmark)	83 - 417	acetylene blockage	Jørgensen and Sørensen 1988
Nueces Estuary (Texas)	4.0 - 71.1	direct measurement of N2 gas	This study
Guadalupe Estuary (Texas)	4.6 - 34.7	direct measurement of N2 gas	This study

Table 8. A partial budget of nitrogen cycling in Nueces and Guadalupe Estuaries (kg-atom N/d).

Area	Nueces Estuary 492 km2	Guadalupe Estuary 410 km2	Time interval	Reference
Riverine input				
DIN (nitrate + nitrite + ammonium)	16	550	A	Dr. T. Whitledge' s report
DON (dissolved organic nitrogen)	na	370	A	Dr. T. Whitledge' s report
Total	na	920	A	Dr. T. Whitledge' s report
Anthropogenic Input	181	na	A	Oppenheimer et al. (1975)
Denitrification	620	400	A	This study
Outflow of DIN from estuary	na	4.3	A	Dr. T. Whitledge' s report
Benthic ammonium flux	1650	3060	S	Drs. Benner and Yoon's report (III)
Total ammonium regeneration (benthic + water column)	4030	5210	S	Drs. Benner and Yoon' s report (III)
Phytoplankton N-demand	6917	5998	A	Dr. D. Stockwell's report

* A; Annual average
* S; Summer time data
* na = not available

Figure 1. Flux of N2 gas in the killed control chambers.

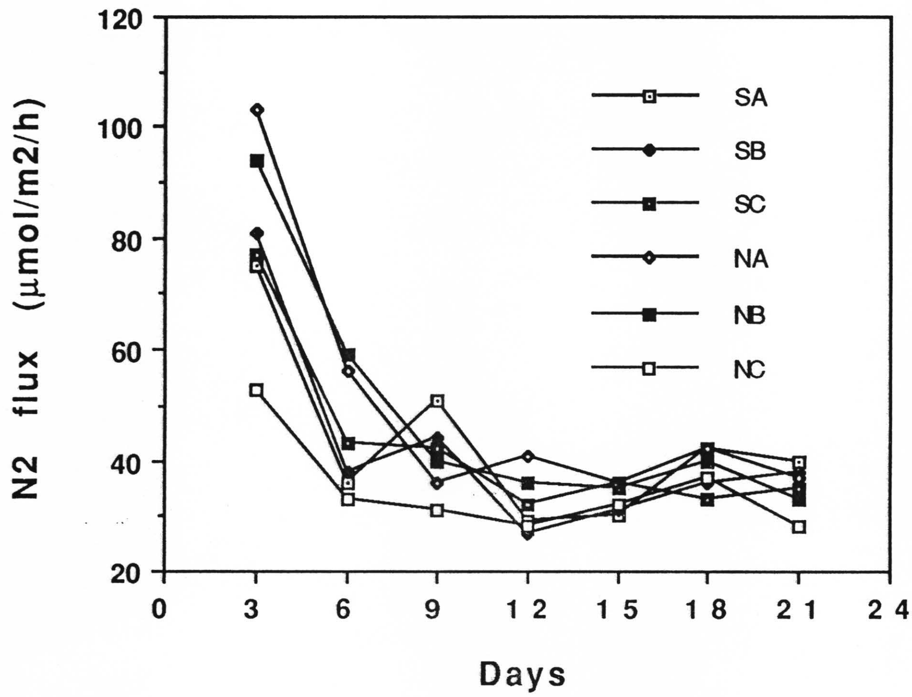


Figure 2. Fluxes of N2 gas after 10 d of incubation.

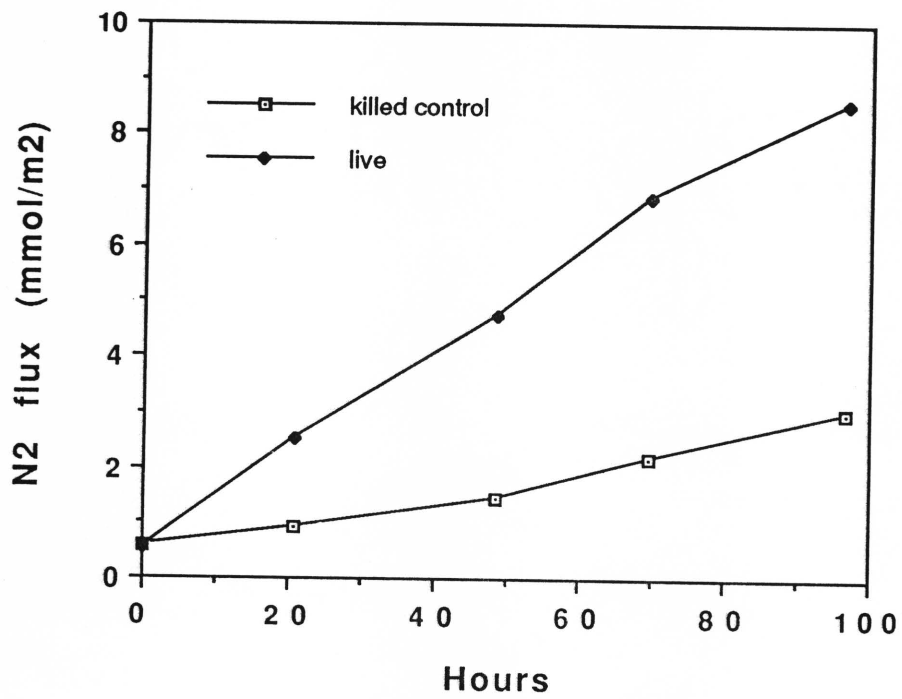


Figure 3. Oxygen consumption by formalin-killed sediments.

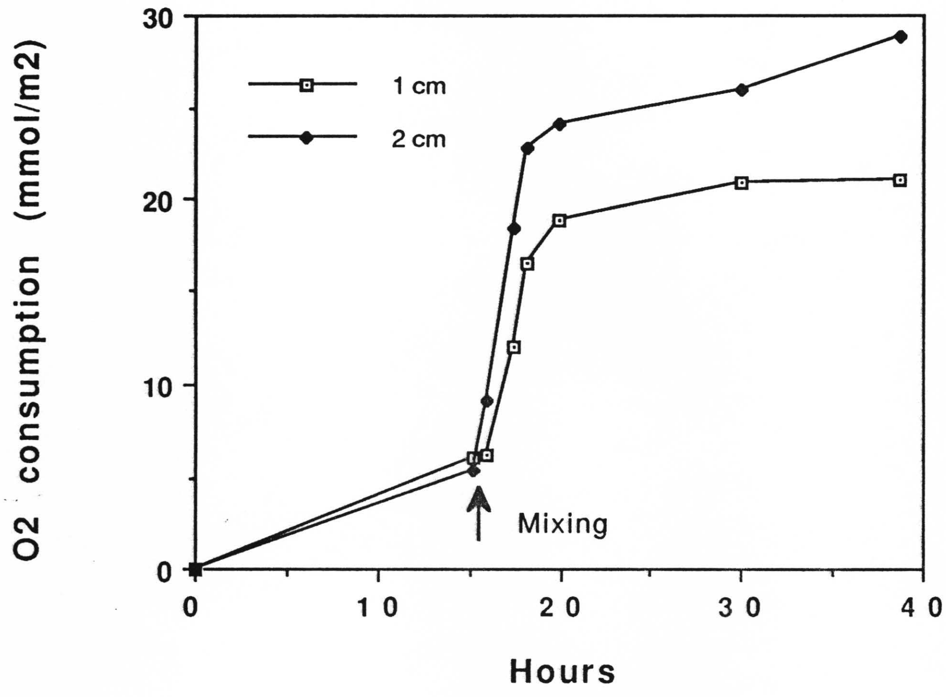
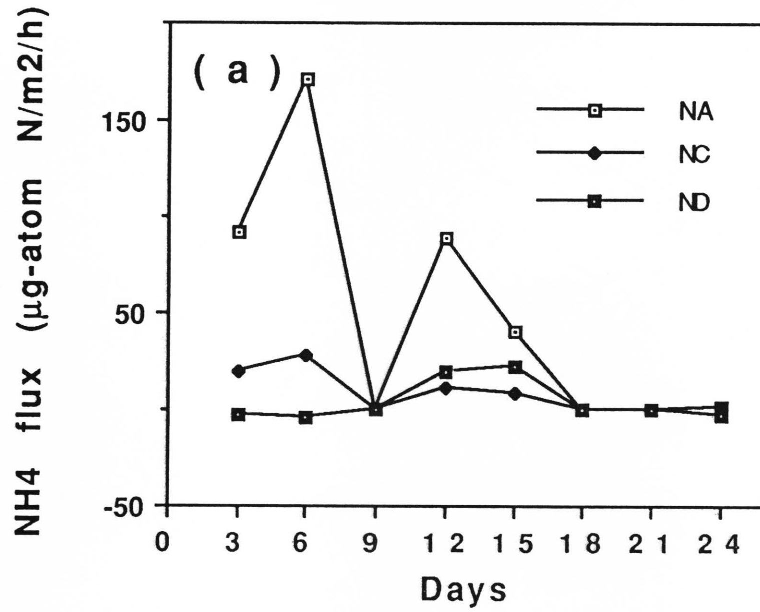


Figure 4. Ammonium fluxes from sediments of Nueces (a) and Guadalupe (b) Estuaries.



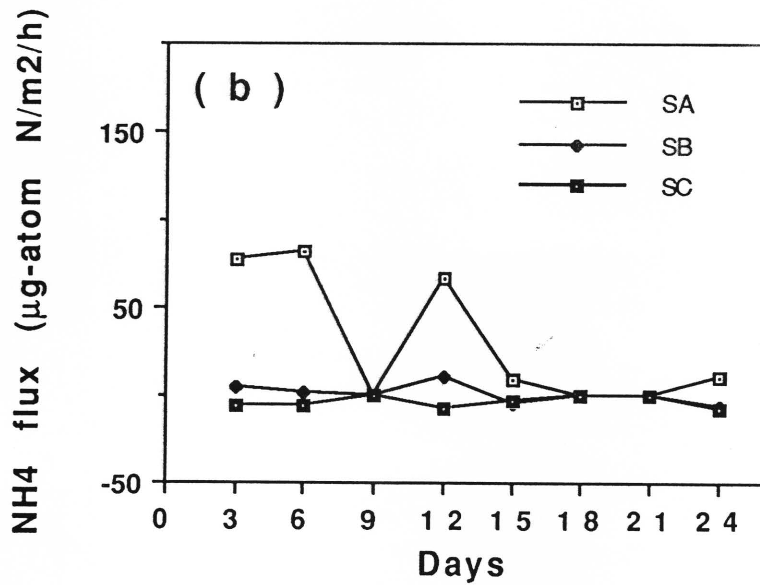
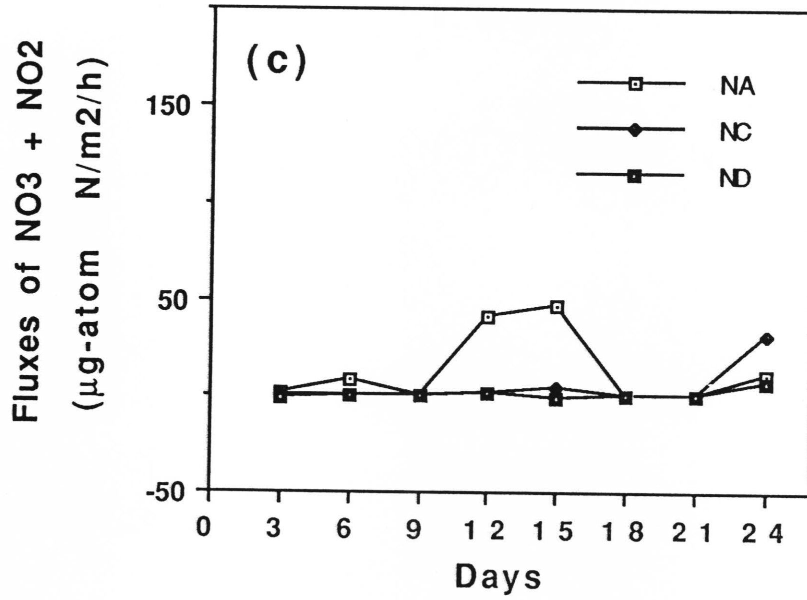
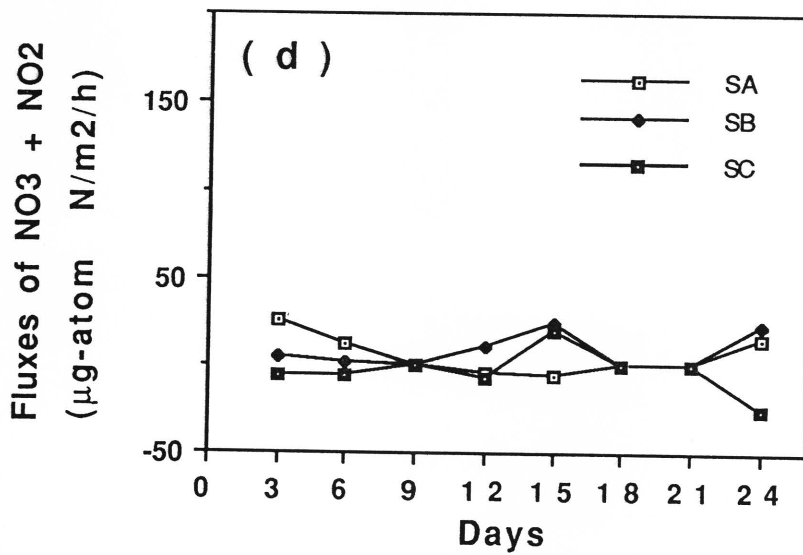


Figure 4. Nitrate plus nitrite fluxes from sediments of Nueces (c) and Guadalupe (d) Estuaries.





V. Ammonium Regeneration and Utilization in Guadalupe Estuary, Texas

Bottom water (collected at approximately 0.5 m above the sediment-water interface) was used for determination of ammonium regeneration and utilization rates in Guadalupe Estuary. Ammonium regeneration rates of in Guadalupe Estuary ranged from 0.13 to 0.23 µg-atom N $l^{-1}h^{-1}$ during the study period (Fig.1). This range is similar to that of surface water in Nueces Estuary (0.07 - 0.39 µg-atom N $l^{-1}h^{-1}$) (Fig. 1a, Section II). The highest rate was observed at Station A, and the lowest rate was observed in Station C in April. The range of ammonium utilization rates was from 0.15 to 0.39 µg-atom N/l/h. This range is also similar to that of surface water in Nueces Estuary (0.07 - 0.66 µg-atom N $l^{-1}h^{-1}$) (Fig.1b, Section II). The highest rate was observed at Station A in November, and the lowest rate was observed at Station C in January. Both ammonium regeneration and utilization rates were usually higher at the upper estuary station (Station A) than at the lower estuary station (Station C).

Ammonium utilization rates were usually higher than ammonium regeneration rates (U/R > 1) in Guadalupe Estuary. This suggests that ammonium utilized in the water column was supplied from either riverine input or benthic fluxes. The U/R ratioes at Station A were generally higher than that of Station C except for April, 1987 when the U/R ratio at Station C was 4.92. Higher ratioes at Station A may be related to a greater influence of freshwater inflow in Station A than in Station C. Ammonium utilization rates were nearly balanced with regeneration rates in both stations (U/R is close to 1) in July, 1987 when the overall salinity was the lowest during the study period.

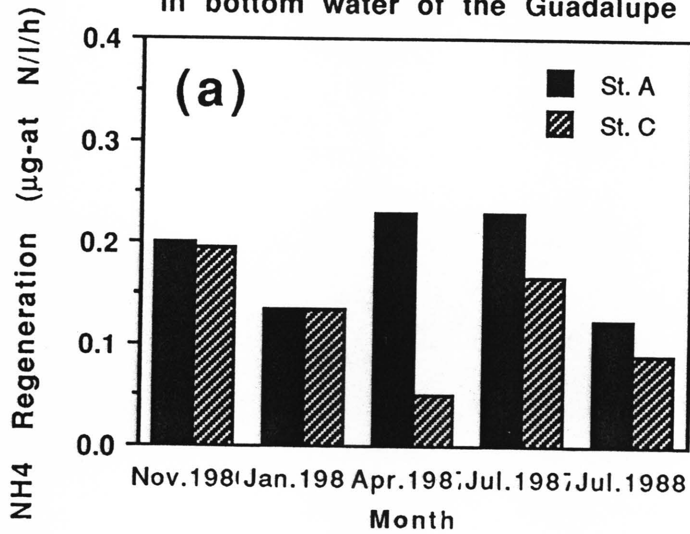
Pool size of nitrogenous compounds in the upper 10 cm in the Guadalupe Estuary are shown in the Table 1. Ammonium pool size

ranged from 2.4 to 38.2 μg-atom $N^{-1}m^{-2}$ during the study period. Of the four stations, the largest pool size was found at the lower estuary (Stastion D) in November, March and July(1987), but was found at the upper estuary (Station A) in January. The overall pool size (the mean of four stations) was largest in November and was smallest in April. The pool size of ammonium did not show a significant relationship either to salinity ($p > 0.05$) or to temperature ($p > 0.05$).

The range of pool size of nitrate plus nitrite was from 0.2 to 1.5 μg-atom N m^{-2} which is approximately one order magnitute less than that of ammonium. The spatial variations of this pool was much smaller than that of ammonium. The overall pool size was largest in March the smallest in July (1987). There was no significant relationship either to salinity ($p < 0.05$) or to temperature ($p > 0.05$)

Pool sizes of primary amines varied from 2.9 to 10.5 μg-atom N/m2 in the study period. The extent of seasonal and spatial variations were smaller than those of ammonium and nitrate plus nitrite. In general, the overall pool size was much larger than that of nitrate plus nitrite, but was smaller than that of ammonium. Pool sizes of primary amines were not significantly related either to salinity ($p > 0.05$) or to temperature ($p > 0.05$) in the study area.

Figure 1. Ammonium regeneration (a) and utilization (b) in bottom water of the Guadalupe Estuary.



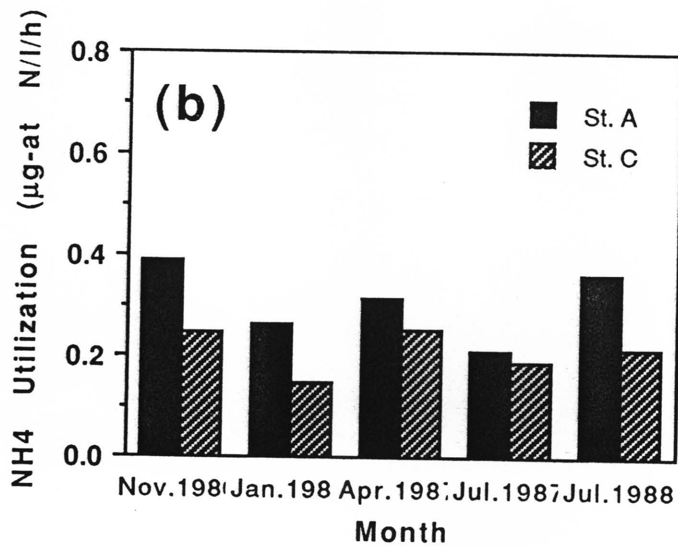


Figure 2. The ratio of ammonium utilization to regeneration rate in bottom water of the Guadalupe Estuary.

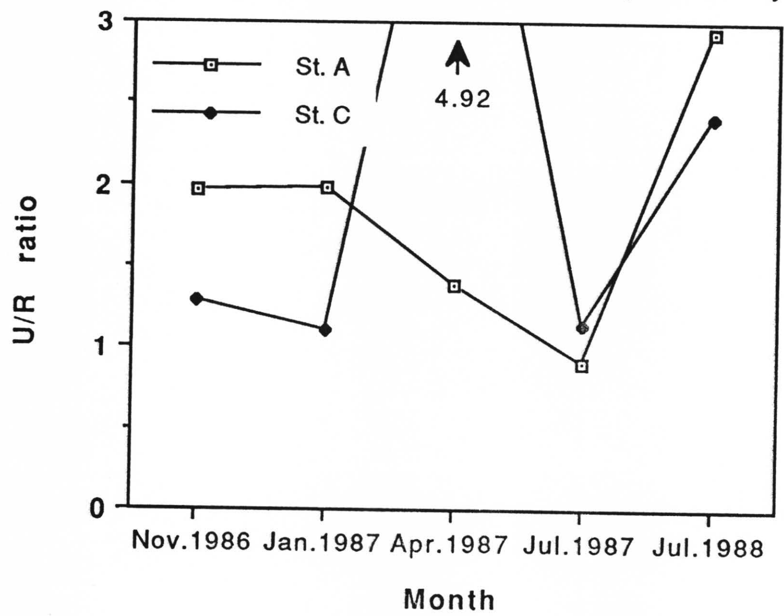


Table 1. Pool sizes of nitrogenous compounds in sediments of the Guadalupe Estuary. (μg-atom N/m2) in the upper 10 cm surface sediments.

Month	Station	NH4	NO3 +NO2	Primary Amines
Nov. 1986	A	7.0	0.7	6.7
	B	16.5	0.6	6.8
	C	7.0	0.4	7.7
	D	38.2	0.7	8.1
Jan. 1987	A	19.2	0.8	3.6
	B	9.1	1.0	4.3
	C	11.6	0.5	8.8
	D	9.1	0.5	8.1
Mar. 1987	A	6.7	0.9	5.7
	B	9.1	1.5	7.7
	C	5.5	0.8	6.6
	D	13.4	0.8	9.2
Apr. 1987	A	2.4	0.2	4.8
	B	7.7	0.3	6.4
	C	4.5	0.2	4.8
	D	7.2	0.2	10.5
Jun. 1987	A	3.6	0.3	4.7
	B	9.6	0.3	5.6
	C	3.8	0.5	4.9
	D	5.6	na	5.9
Jul. 1987	A	2.7	0.2	3.9
	B	6.7	0.2	2.9
	C	3.4	0.2	3.9
	D	11.6	0.2	4.4
Jul. 1988	A	20.9	na	7.1
	B	23.4	na	7.5
	C	9.6	na	7.0
	D	11.3	na	8.4

* Pool size of nitrogenous compounds in the upper 10 cm of sediments were calculated as follows.

Pool size (μg-atom N/m2) = the concentration of a nitrogenous compound (μg-atom N/l) * water content of sediments(l/m2)

* Water content of sediments was obtained from porosity data.

* na; Data are not available