J. Great Lakes Res. 27(1):84–97
Internat. Assoc. Great Lakes Res., 2001

Nitrogen Dynamics in Sandy Freshwater Sediments (Saginaw Bay, Lake Huron)

Wayne S. Gardner[1,*], Longyuan Yang[2], James B. Cotner[3,†], Thomas H. Johengen[4], and Peter J. Lavrentyev[4,‡]

[1] *National Oceanic and Atmospheric Administration*
Great Lakes Environmental Research Laboratory
2205 Commonwealth Blvd.
Ann Arbor, Michigan 48105

[2]*Nanjing Institute of Geography and Limnology*
Chinese Academy of Sciences
73 East Beijing Rd.
Nanjing, Jiangsu 210008, P. R. China

[3]*Texas A&M University*
College Station, Texas 77843

[4]*Cooperative Institute for Limnology and Ecosystem Research (CILER)*
The University of Michigan
Ann Arbor, Michigan 48109

ABSTRACT. *Sediment-water nitrogen fluxes and transformations were examined at two sites in Saginaw Bay, Lake Huron, as a model for sandy freshwater sediments. Substantial ammonium release rates (74 to 350 µmole $NH_4^+/m^2/h^1$) were observed in flow-through cores and* in situ *benthic chamber experiments. Sediment-water ammonium fluxes were similar at the inner and outer bay stations even though inner bay waters are enriched with nutrients from the Saginaw River. The high net flux of remineralized ammonium into the overlying water from these sandy sediments resembles typical data for marine systems (11 to 470 µmole $NH_4^+/m^2/h^1$) but were higher than those reported for depositional freshwater sediments (0 to 15 µmole $NH_4^+/m^2/h^1$; Seitzinger 1988). Addition of montmorillonite clay (ca. 1 kg dry weight/m^2) to the top of the sandy cores reduced ammonium flux. Mean "steady-state" ammonium flux following clay addition was 46 ± 2 (SE) % of the initial rates as compared to 81 ± 8% of the initial rates without clay addition. Zebra mussel excretion dominanted ammonium regeneration in the inner bay where the bivalve was abundant, but addition of zebra mussel feces/psuedofeces (3.0 g dw/m^2) to sediments did not increase ammonium or nitrate flux. Partial nitrification of ammonium at the sediment-water interface was suggested by removal of added $^{15}NH_4^+$ from lake water passing over dark sediment cores. Sediment-water fluxes of nitrogen obtained from flow-through sediment cores resembled those from* in situ *benthic chambers. However, extended static incubations in gas-tight denitrification chambers caused more of the regenerated nitrogen to be nitrified and denitrified than occurred with the other two measurement systems.*

INDEX WORDS: *Ammonium flux, sediment-water nitrogen dynamics, nitrification, denitrification, Saginaw Bay, zebra mussels.*

[*]Present address: The University of Texas at Austin, Marine Science Institute, 750 Channelview Drive, Port Aransas, Texas 78373. Corresponding author: E-mail: gardner@utmsi.utexas.edu

[†]Present Address: Department Ecology, Evolution and Behavior, The University of Minnesota, St. Paul, Minnesota 55108.

[‡]Present Address: Department of Biology, The University of Akron, Akron, Ohio 44325.

INTRODUCTION

The forms and supply rates of available nitrogen are important factors regulating primary and secondary productivity as well as composition of phytoplankton and other organisms in coastal ecosystems. Internal nutrient regeneration processes in the water column and at the sediment-water interface must be quantified to understand ecosystem dynamics in coastal regions (Billen 1978). Denitrification, the bacterial reduction of nitrate to dinitrogen gas via nitrite, is an important sink for biologically available nitrogen in coastal regions (Seitzinger 1988, Nixon *et al*. 1996). Much of the nitrate substrate that is denitrified at the sediment-water interface is derived from ammonium produced from organic matter mineralization in surface sediments (Seitzinger 1988). Denitrification rates are often limited by nitrate supply rates (Seitzinger 1987). Understanding factors that control organic nitrogen-mineralization and nitrification rates is therefore necessary to define the dynamics of denitrification as a nitrogen sink in coastal and tributary regions.

Physical, geochemical, and biological factors interact in complex ways to control nitrogen dynamics in coastal ecosystems. Sandy sediments, rather than depositional sediments with high clay content, dominate much of Saginaw Bay because of strong winds and currents (Wood 1964). It is possible that nitrogen dynamics in sandy sediments may differ from those in depositional sediments in low salinity environments because sand has a low cation exchange capacity and may allow some regenerated ammonium to diffuse into overlying water before it is nitrified. Sediment cation exchange sites associated with clays may increase nitrification/denitrification in freshwater sediments by binding ammonium at the sediment-water interface where it is nitrified and denitrified before being released into the overlying water. This binding is minimized in marine sediments where sea salt ions dominate exchange sites (Gardner *et al.* 1991, Seitzinger *et al*. 1991). The high sand to clay ratio in several regions of Saginaw Bay (e.g., Table 1) make it an attractive site to examine the effects of clay on nitrogen dynamics in sediments because nitrification rates at these locations are not affected by such factors as salinity (Rysgaard *et al*. 1999) or high sulfide concentrations that sometimes characterize marine estuaries (Joye and Hollibaugh 1995).

The zebra mussel (*Dreissena polymorpha*) is an important biological factor affecting nutrient dynamics in Saginaw Bay (Gardner *et al*. 1995b). It invaded the bay in 1991 and has formed dense populations in the inner bay where pebbles or other solid substrates are present (Nalepa *et al*. 1995). It affects trophic and nutrient-cycling processes by filtering large volumes of water and regenerating nutrients (Nalepa *et al*. 1995, Johengen *et al*. 1995, Gardner *et al*. 1995b). Large populations of zebra mussels excrete significant amounts of ammonium and phosphorus on a lake- or bay-wide basis (Johengen *et al*. 1995, Gardner *et al*. 1995b). Changes in water quality were noted in Lake Erie and Lake St. Clair after this species became established. Phytoplankton abundance and chlorophyll decreased dramatically with a concurrent increase in water clarity (Holland 1993, Nalepa *et al*. 1995). At the time of this study, zebra mussels were abundant (more than 4,000 individual per square meter) in the inner bay but not in the outer bay where hard substrates are less common (Nalepa *et al*. 1995).

Water-column nitrogen concentrations (Johengen *et al*. 1995) and cycling rates (Gardner *et al*. 1995b) have been reported for Saginaw Bay, but only a few studies (Ulman and Aller 1989, Lavrentyev *et al*. 2000) have examined nitrogen dynamics at the sediment-water interface in this physically-dynamic system. In this study nitrogen fluxes and transformations in Saginaw Bay sediments were quantified and compared using manipulated flow-through cores, *in situ* benthic chambers, and gas-tight denitrification chambers. The questions addressed are: (1) What are the rates of nitrogen transformations at

TABLE 1. Physical chemical properties of sediments collected from Sites 1 and 2 during summer 1995. The percent sand was measured with a 63 µm sieve and percent silt and clay were measured by the pipette method (Folk 1980). These results were obtained from duplicate measurements that gave identical results except for the ones where ranges are indicated.

	Site 1	Site 2
Porosity (mL/cm^3)	0.47	0.50
Organic matter (% dw)	0.67–0.73	0.70
Chlorophyll a (µgC/g dw)	3.3–6.2	2.7–6.6
Grain size (% dw)		
Gravel	11.2	1.9
Sand	87.8	93.4
Silt	1.0	4.6
Clay	0	0

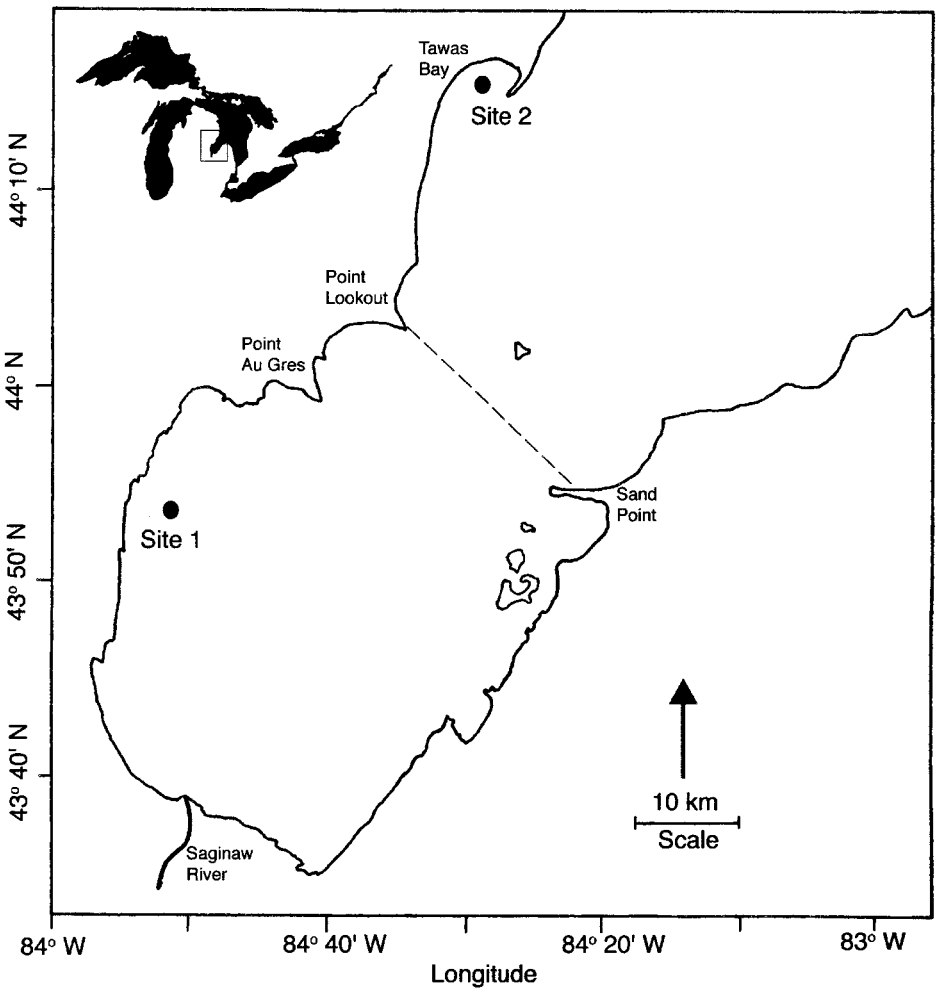

FIG. 1. Map of Saginaw Bay with sampling sites. Site 1 is in the Inner Bay and Site 2 is in the Outer Bay.

the sediment-water interface in Saginaw Bay? (2) Would the addition of montmorillonite clay to sandy freshwater sediments decrease ammonium flux? (3) What is the importance of zebra mussels in benthic nitrogen regeneration?

EXPERIMENTAL PROCEDURES

Sampling Sites

Saginaw Bay (Fig. 1) is an extension of Lake Huron, approximately 49 km wide and 80 km long. It receives drainage from an area of 21,000 km^2 via the Saginaw River and other smaller tributaries (Smith *et al*. 1977). The inner region of the bay (Fig. 1) has a surface area of about 1,550 km^2, with a mean water depth of 5.1 m and a water volume of 7.9 km^3. The outer region of the bay has a surface area of about 1,200 km^2, with a mean depth of 13.7 m and a volume of 16.6 km^3 (Smith *et al*. 1977). Water flows into the bay from Lake Huron along the north-western shore, mixes with Saginaw River water, and flows out along the south-eastern shore (Danek and Saylor 1977). Most of the nutrient load to the bay is derived from the Saginaw River, which accounts for about 70% of the total tributary flow into the bay and drains nearly 80% of the total drainage basin (Canale and Squire 1976). The inner bay has high levels of nutrients, suspended solids, and phytoplankton biomass and is more productive than the outer bay that resembles the nutrient-poor waters of Lake Huron (Dolan *et al*. 1978).

Experiments were conducted on sediments from

an inner bay site (Site 1) located at Great Lakes Environmental Research Laboratory (GLERL) Station 5 (43°56′N, 83°52′W) and an outer bay site (Site 2) in Tawas Bay near GLERL Station 21 (44°15′N, 83°30′W; Fig. 1). The water depths at both sites were about 4 m. Comparison of the sediment characteristics at the two sites in 1995 did not indicate major differences (Table 1). Clays were not detected in surface sediments from either site. Sediments at Site 1 contained some pebbles, which are effective substrates for zebra mussels.

Field Experiments

Schematic diagrams of the enclosure devices used to measure nitrogen fluxes and transformations are shown in Figure 2. The sampling times, sites, and types of the respective experiments are outlined in Table 2.

Sediment Core Flow-through Experiments

In 1994, Site 1 sediment cores were reconstituted from sediments collected by divers on 22 June and 18 July. Surface sediments at this site consisted of a mixed homogenous multi-centimeter layer of "green sand" with some silt. The sandy material was collected in bulk, sieved through a nylon window screen to remove large particles, and sub-cored for experiments.

Flow-through core chambers (Fig. 2) were constructed from 60-mL syringes following the design of Gardner *et al.* (1991). Cores were taken with a truncated syringe and carefully transferred via a rubber sleeve connector into a second unmodified incubation-syringe barrel. The bottom of each incubation-syringe barrel was sealed with a septum. The syringe plunger, containing inserted Teflon inlet and outlet tubes, was gently lowered to about 1 cm above the sediment surface to give an overlying volume of about 5 mL. Saginaw Bay water was passed over the surface of each sediment core at a rate of 0.1 mL/min with a peristaltic pump (Technicon II). This flow rate was reasonable because it allowed measurement of chemical changes without depleting the oxygen supply in the flow water. The cores were maintained in a dark box at near *in situ* temperature (ca. 21°). Unfiltered feed water from the lake was held under fluorescent lights (daytime) during incubations.

Six cores were prepared for the June 1994 flow-through experiment. Ammonium and nitrate concentrations were measured at intervals in both

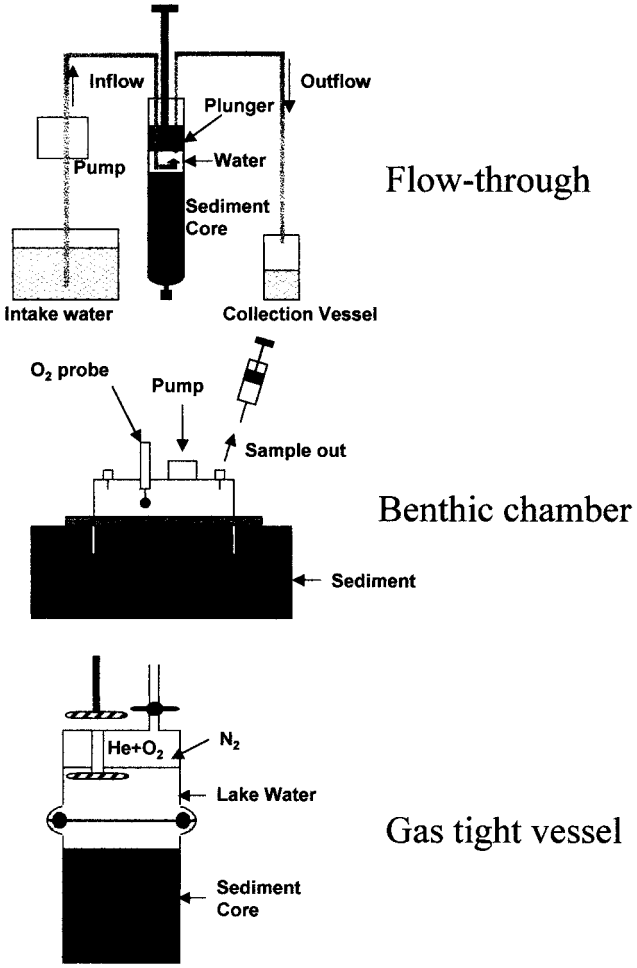

FIG. 2. Schematic diagram of three experimental systems for measuring sediment-water nitrogen fluxes.

inflow and the outflow waters from each core. On Day 3 of the experiment, thawed zebra mussel feces/pseudofeces (materials that are ingested but not assimilated [feces] and filtered but not ingested [pseudofeces]), which had been collected from zebra mussels held in flowing lake water over a screen, were added as a slurry to three of the cores at a concentration of 3.0 g dry weight/m^2 surface area. This concentration of deposits was higher than typical amounts of non-zebra mussel benthic biomass in sandy sediments of Saginaw Bay (0.08 to 0.94 g dry weight/m^2) but in the range of that found in depositional zones (1.2 to 8.3 g dry weight/m^2; personal communication, T. Nalepa, NOAA Great Lakes Environmental Research Laboratory). On Day 7 of the experiment, 4 μM $^{15}NH_4^+$ was added

TABLE 2. Summary of nitrogen flux experiments conducted at two sites in Saginaw Bay during spring/summer in 1994 and 1995. Flow-through core results for June 1995 at Site 1 are reported elsewhere (Lavrentyev* et al. *2000) but results from control cores without zebra mussel additions are included here for methodological comparisons (Table 8). FPF = feces/pseudofeces.

	Flow-through cores	In situ Benthic Chambers	Gas-tight Chambers
Site 1			
Natural	June 1994, July 1994	June 1995, Aug 1995	June 1995, Aug 1995
	June 1995	—	—
+ FPF	June 1994	—	—
+ Clay	July 1994	—	—
Site 2			
Natural	—	June 1995, Aug 1995	June 1995, Aug 1995

to the inflow water of three cores as a tracer to examine the proportion of ammonium lost by nitrification or other uptake processes under steady state conditions. Ammonium concentration and atom % ^{15}N composition in inflow and outflow waters were measured by high performance liquid chromatography (Gardner and St. John 1991, Gardner *et al*. 1995a) on Days 8 and 9. Nitrate (including nitrite) concentrations were measured by standard colorimetric procedures (APHA 1990).

In a second flow-through experiment conducted in July 1994, the effects of adding montmorillonite clay on sediment-water fluxes of ammonium and nitrate were examined. The premise for adding clay was that it would increase cation exchange capacity of the sediments and increase ammonium retention and nitrification at the sediment-water interface under steady-state conditions. Six cores were taken from sieved sediments and set up for flow-through experiments. Saginaw Bay water was passed over each core at a flow rate of 0.1 mL/min. Ammonium concentrations in the inflow and outflow waters were monitored daily for 8 days. After samples were collected on Day 2, four of the cores were covered with a montmorillonite clay slurry (ca. 1 kg dw/m^2) so steady-state ammonium fluxes from sediments with clay additions could be compared to both initial rates and to rates in controls without added clay. The added montmorillonite was either untreated (2 cores) or pre-soaked with 0.2 mM NH_4^+ (2 cores) to establish whether some clay-associated differences may have been caused by non steady-state absorption of ammonium by the clay particles.

Benthic Chamber Measurements of Nutrient Fluxes and Respiration

Fluxes of dissolved oxygen and dissolved nutrients (NH_4^+ and $NO_3^- + NO_2^-$) were measured in benthic chambers at both sites in 1995. Duplicate opaque (dark) and clear (light) benthic chambers (design similar to Rowe *et al*. 1994; Figure 2**)** were positioned over zebra mussels in the sediments to a depth of ca. 4 cm by divers using SCUBA. The light chambers were placed in locations with a moderate zebra mussel biomass (0 to 1,063 mg dw/m^2) so any signals associated with primary production would not be obscured by the presence of zebra mussels. The dark chambers were placed in locations with abundant zebra mussels (384 to 19,110 mg dw/m^2). Chambers were purged with water for 10 min before closing all ports and beginning the incubation outside to eliminate disturbances caused during placement. Dissolved oxygen concentrations were measured with a YSI oxygen probe contained within the chambers and logged every 5 min. An electric pump circulated water within the chamber. Sediment plus zebra mussel benthic oxygen demand was corrected for water-column biological oxygen demand. Water column oxygen demand was determined in dark bottles at ambient temperature with a two-point determination of dissolved oxygen concentrations (Winkler method). Nutrient concentrations were measured on

samples that were removed from the chambers at the initiation and completion (ca. 2 to 5 hours) of incubations.

Fluxes in Gas-tight Denitrification Chambers

Nitrogen fluxes (N_2, NH_4^+, NO_3^- + NO_2^-) and oxygen consumption rates were measured on intact cores in June and August 1995 using the gas-tight N_2 gas production method developed by Seitzinger *et al.* (1980) and modified by Tomaszek *et al.* (1997) for measuring denitrification rates (Fig. 2). Atmospheric N_2 was sparged from the water overlying the sediments with He:O_2 (80:20) at multiple intervals over a period of 4 days before measurements were begun. Nitrogen and oxygen gases were analyzed with a Shimadzu gas chromatograph (model GA-8A) equipped with dual Molecular Sieve (5A) columns, a thermal conductivity detector and a gas-tight sampling syringe. At the end of the sequential series of experiments, the water-sediment cores were flushed with He gas to deplete O_2 and thereby stop coupled nitrification/denitrification. Denitrification rates were estimated by subtracting these mean background N_2 fluxes (due to N_2 degassing from pore waters; Nowicki 1994) from the mean N_2 fluxes observed in the presence of oxygen for each chamber.

RESULTS

Continuous-flow Results for Sediment Cores (Site 1)

In the June 1994 flow-through experiment, mean net ammonium regeneration rates (based on flow rate, cross-sectional area, and concentration differences between inflow and outflow waters) ranged from about 150 to 350 µg atom N/m^2/h (Fig. 3). Addition of 3 g dw/m^2 of thawed zebra mussel feces/pseudofeces did not increase rates of ammonium (Fig. 3) or nitrate (data not shown) release over controls. Only about 40% of the $^{15}NH_4^+$ in the inflow water on average was recovered in the outflow water on Days 8 and 9 (data not shown) in the $^{15}NH_4^+$-addition experiment conducted on Days 7 to 9.

In the July 1994 flow-through experiment, ammonium release rates among replicate cores varied from about 50 to almost 200 µg atom N/m^2/h before the addition of montmorillonite clay on Day 2. Successive measurements on individual cores were more precise than the among-core variations for some treatments (control treatments, Fig. 4). Large

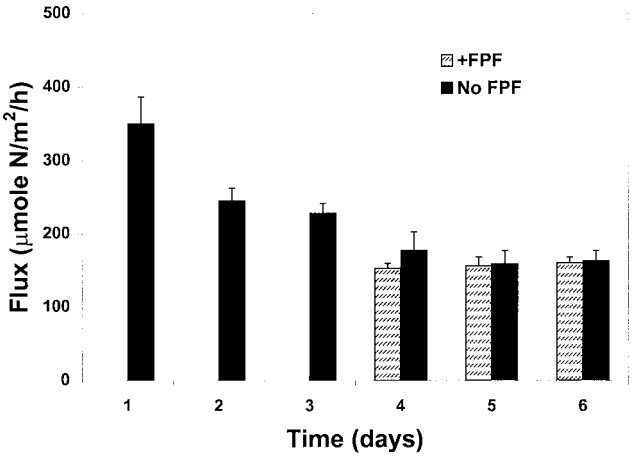

FIG. 3. Sediment-water ammonium flux from reconstituted Site 1 cores in Saginaw Bay as measured with a lake water flow through system in June 1994. Zebra mussel feces/pseudofeces (FPF) were added to one half of the cores at a density of 3 g/m^2 after samples were collected on Day 3 of the incubations.

decreases in net fluxes over initial rates were observed for clay-treated cores on the first day after clay additions. The cores with untreated montmorillonite showed the greatest initial decreases, but ammonium release rates did not differ between ammonium-treated and untreated montmorillonite cores after steady-state conditions were approached (about 2 days after clay additions; Fig. 4). Average net ammonium regeneration rates from clay treated cores were 50 to 58 % lower on Days 5 to 8 than on Days 1 and 2 before the clay had been added (Table 3). By comparison, mean rates in the cores without clay additions decreased by about 20 % over the same interval. The mean net flux of ammonium during Days 5 to 8 of the experiment was 49 ± 7 µg atom N/m^2/h for the four montmorillonite-treated cores and 98 ± 26 µg atom N/m^2/h for the two control cores (calculated from data in Table 3).

Benthic Chamber Results

Net fluxes of ammonium and nitrate, and uptake of oxygen (oxygen demand), in the light and dark benthic chambers at the two sampling sites in June and August 1995 are presented in Tables 4 and 5. Oxygen depletion was observed in all dark chambers, but net production of oxygen occurred in

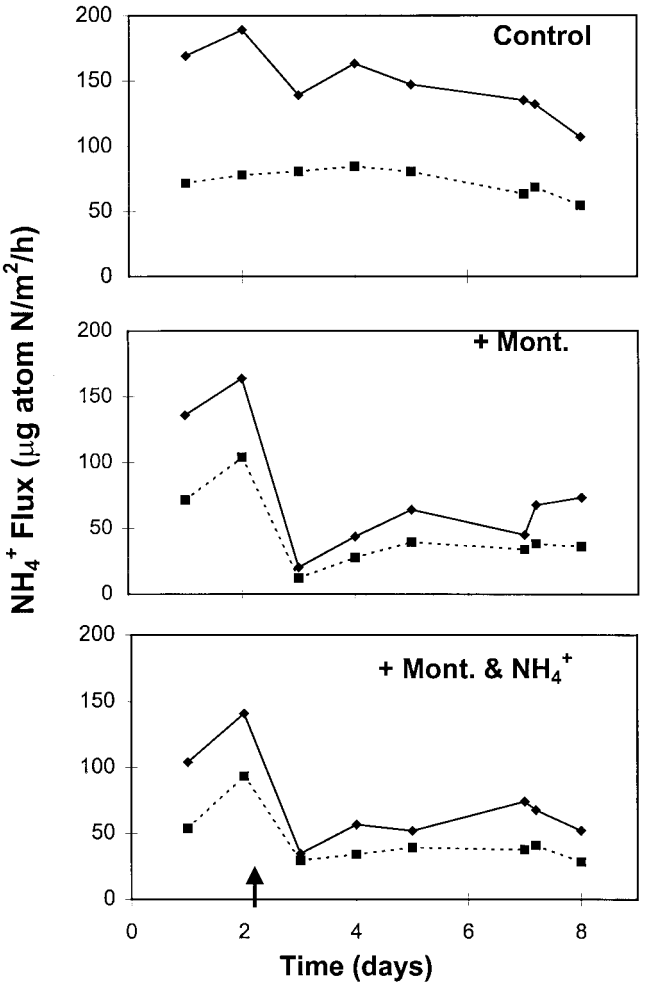

FIG. 4. Time sequence of net ammonium regeneration rates (μg atom N/m²/h) from Saginaw Bay cores without and with additions of a layer of montmorillonite clay. After an initial incubation of 2 days (see arrow), duplicate sediment cores were treated with (a) lake water only (control), (b) untreated montmorillonite, or (c) montmorillonite pre-soaked with ammonium chloride. Samples for analysis were collected from inflow and outflow waters. Net regeneration rates were calculated as the increase in ammonium concentration in outflow water over inflow water times the flow rate (0.1 mL/min) divided by the cross sectional area of the core (5.23×10^{-4}/m²). The lines between points connect results from successive measurements on individual flow-through cores.

some light chambers due to primary production in the water and/or at the sediment surface. The oxygen demand was proportional to zebra mussel biomass ($r^2 > 0.88$) at both the inner and outer bay sites under dark conditions and at the inner bay site in the lighted chamber (Table 6). This relationship was not significant in the light at the outer bay site where zebra mussel biomass was lower than at the inner bay site.

TABLE 3. Mean "steady-state" core-specific ammonium fluxes (μg atom N/m²/h) from reconstituted Saginaw Bay cores in July 1994 before (Days 1–2) and after (Days 5–8) treatment with montmorillonite clay and percent decrease in ammonium flux for each core. Standard errors were calculated from successive measurements for the same core within the time intervals stated above.

	Control		Mont. Only		Mont. + NH_4^+	
Core #	1	2	1	2	1	2
Days 1–2	179	74.8	150	87.8	123	73.6
SE	10	3.0	14	16.3	18	19.8
Days 5–8	130	66.7	62.6	37.1	61.4	36.5
SE	8.4	5.4	6.1	1.2	5.6	2.8
% Decrease	27	11	58	58	50	50

A net production of ammonium was observed in all but one light chamber. At the inner bay site, net ammonium regeneration rates ranged from 3.7 to 29 μg atom N/m²/h in the light and 82 to 182 μg atom N/m²/h in the dark (Table 4). Mean net ammonium regeneration rates in the outer bay site ranged from –7.8 to 61 μg atom N/m²/h in the light and 89 to 350 μg atom N/m²/h in the dark (Table 5). Net ammonium regeneration rates at the respective stations were related ($r^2 > 0.72$, N = 3–5) to the biomass of zebra mussels enclosed in the chambers under light or dark conditions, when rates for both sampling dates were combined at each site (Table 6).

Net uptake of nitrate was observed in most chamber experiments. On average, higher uptake rates were observed in the light than in the dark. Net nitrate uptake rates at the inner bay site ranged from 9.4 to 30.2 μg atom N/m²/h in the light and from a production (negative uptake) rate of 19 μg atom N/m²/h to an uptake rate of 19 μg atom N/m²/h in the dark (Table 4). Net uptake of nitrate was observed in all chambers from the outer bay station and rates were higher in the light (40 to 73 μg atom N/m²/h) than in the dark (17 to 52 μg atom N/m²/h; Table 5).

The net benthic oxygen demand in the light benthic chambers was either near-zero (Site 1) or

TABLE 4. Benthic oxygen demand (µg atom O/m²/h), ammonium and nitrate fluxes (µg atom N/m²/h), and zebra mussel biomass (mg dw of soft tissue/m²) in the light and dark for* in situ *benthic chamber experiments at Site 1 (inner bay) in June and August 1995. Note that negative oxygen demand represents oxygen production.

			Oxygen demand	NO_3^-	NH_4^+	ZM biomass
Light						
	June	(a)	8,240	–19.4	28.7	1,063
		(b)	–2,100	–30.2	3.73	172
	August		–5,080	–9.37	12.54	304
	Mean		353	–19.7	15.0	513
	SE		4,040	6.0	7.3	278
Dark						
	June	(a)	7,850	–19.5	138	3,071
		(b)	6,640	0	122	384
	August	(a)	19,300	18.9	182	19,110
		(b)	2,820	0	82	1,288
		(c)	2,830	0	96	966
	Mean		7,890	–0.13	124	4,964
	SE		3,910	6.05	17.5	3,565

TABLE 5. Benthic oxygen demand (µg atom O/m²/h), ammonium and nitrate fluxes (µg atom N/m²/h), and zebra mussel biomass (mg dw of soft tissue/m²) in the light and dark for* in situ *benthic chamber experiments at Site 2 (outer bay) in June and August 1995. Note that negative oxygen demand represents oxygen production.

			Oxygen demand	NO_3^-	NH_4^+	ZM biomass
Light						
	June	(a)	–6,800	–72.6	14.9	131
		(b)	–6,360	–52.7	–7.78	80
	August	(a)	1,890	–58.7	60.9	412
		(b)	–124	–40.5	11.3	0
	Mean		–2,850	–56.1	19.8	156
	SE		2,540	6.7	14.6	90
Dark						
	June	(a)	11,560	–52.0	123	3,130
		(b)	14,100	–27.1	350	6,776
	August	(a)	3,900	–33.6	91.6	504
		(b)	5,200	–17.4	89.0	646
	Mean		8,690	–32.5	163	2,764
	SE		2,840	7.3	62.6	1,467

showed net oxygen production (Site 2). The mean dark oxygen demand in the benthic chambers was 7,890 and 8,690 µg atom O/m²/h at Sites 1 and 2. If extrapolated to zero zebra mussel biomass, the mean dark oxygen demand rates in the *in situ* benthic chambers from the respective sites were 3,860 and 4,300 µg atom O/m²/h for Site 1 and Site 2, respectively.

TABLE 6. Least-squares relationship between oxygen demand (µg atom O/m²/h) and ammonium flux (µg atom N/m²/h) to zebra mussel biomass for all measurements taken in June and August 1995 (see Tables 4 and 5). Negative oxygen demand represents oxygen production.

	N	Slope	Intecept	r^2
Oxygen demand				
Site 1, light	3	13.6	–665	0.88
Site 1, Dark	5	0.811	3,860	0.91
Site 2 light	4	12.0	–4,715	0.24
Site 2, Dark	4	1.59	4,305	0.90
Ammonium flux				
Site 1, light	3	0.0257	1.80	0.95
Site 1, Dark	5	0.0042	103	0.74
Site 2 light	4	0.146	–2.93	0.81
Site 2, Dark	4	0.041	50.6	0.91

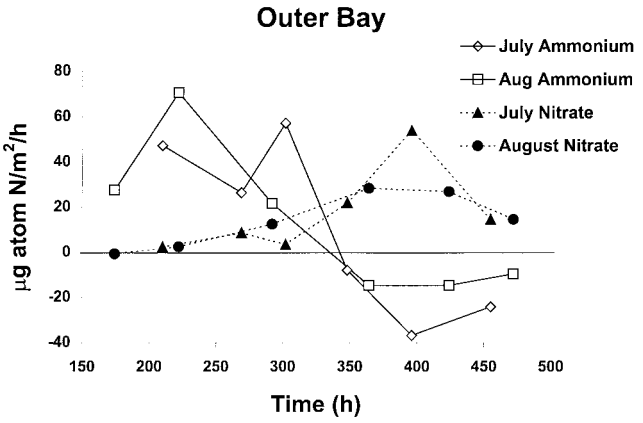

FIG. 5. Fluxes of NH_4^+ and NO_3^- during denitrification incubations conducted at Site 2 in July (sampled 27 June) and August of 1995.

Gas-tight Chamber Results

The denitrification chambers had mean oxygen demands of 1,580 and 1,870 µg atom O/m²/h at Sites 1 and 2, respectively, after being maintained in the laboratory for several days. The mean denitrification rates for intact cores measured in gas-tight chambers varied between 16 and 81 µg atom N/m²/h (Table 7). The net fluxes of NH_4^+ from the Site 1 denitrification cores were negative, and showed losses from the overlying water that averaged –8 and –4 µg atom N/m²/h over the whole incubations in July and August 1995, respectively (Table 7). In contrast, the NH_4^+ fluxes in sediments from the outer bay (Site 2) averaged 10 and 18 µg atom N/m²/h in July and August 1995 (Table 7). The patterns of ammonium and nitrate release showed that the composition of regenerated nitrogen changed during the course of the incubations at Site 2 (Fig. 5). In both Site 2 experiments, ammonium was released at relatively high rates in the early stages of the experiment, but the nitrate flux was negligible. As the experiments progressed, the ammonium flux peaked and then decreased to negative values as nitrate flux peaked. However, peak nitrate flux did not balance the decrease in ammonium flux over the same interval.

TABLE 7. Mean net sediment-water fluxes (µg atom N/m²/h ± SE) of N_2, ammonium, and nitrates (µg atom N/m²/h ± SE) and oxygen demand (µg atom O/m²/h ± SE) for sediment cores in gas-tight denitrification chambers in 1995.

	N_2	NH_4^+	NO_3^-	Oxygen demand
Site 1				
30 June 1995	16 ± 15	–8 ±7	14 ± 7	1,480 ± 110
1 August 1995	81 ± 10	–4 ± 2	21 ± 8	1,670 ± 90
Site 2				
30 June 1995	40 ± 14	10 ± 16	17 ± 8	2,190 ± 260
3 August 1995	50 ± 8	18 ± 12	14 ± 5	1,550 ± 110

Methodology Comparison

Mean dark ammonium fluxes across the sediment water interface, without zebra mussels, determined by different methods in 1995, and total dissolved inorganic nitrogen flux measured in the denitrification chambers during the same season, are presented in Table 8. The flow-through chamber data were obtained from a companion study using large (76 mm i.d.) cores (Lavrentyev *et al*. 2000). Benthic chamber fluxes were determined by extrapolating the relationship between zebra mussel biomass and net ammonium regeneration rate to zero mussel biomass at each station. At Site 1, the extrapolation gave an ammonium flux of 95 µg atom N/m²/h, compared to a mean flux of 60 µg atom N/m²/h for the flow-through cores. In contrast, the ammonium flux measured at Site 1 in the denitrification chambers was negative but near zero. The mean total dissolved inorganic nitrogen flux in the denitrification chambers (59 µg atom N/m²/h) was similar to the ammonium fluxes measured by the other methods. The benthic chamber extrapolated ammonium flux

TABLE 8. Comparison of mean net ammonium regeneration rates (mg atom N/m²/h) from sediments without zebra mussels in Saginaw Bay by different measurement procedures and of mean total dissolved inorganic nitrogen flux (mg atom N/m²/h) in the denitrification chambers. All data were collected June through August 1995. Data from the flow through cores were taken from Lavrentyev* et al. *2000. ND = not determined.

	Site 1	Site 2
Benthic chambers (extrapolated to zero zebra mussels; r > 0.85)	95	40
Flow-through cores	60	ND
Denitrification chambers		
a. Initial rate after sparging	–1	37
b. Mean rate over incubations	–6	14
Mean total DIN ($N_2 + NH_4^+ + NO_3^-$) flux in denitrification chambers	59	75

of 40 µg atom N/m²/h at Site 2 was comparable to the initial denitrification chamber readings that averaged 37 µg atom N/m²/h (Fig. 5) after sparging. However, the mean ammonium flux in the denitrification chambers changed over the course of the experiments (Fig. 5) and was only about 14 µg atom N/m²/h on average (Table 5). This value compares to a mean flux in total dissolved inorganic nitrogen of 75 µg atom N/m²/h (Table 8).

DISCUSSION

Rates of Nitrogen Transformations at the Sediment-water Interface in Saginaw Bay

The data indicate that nitrogen fluxes and transformations were dynamic at the sediment-water interface at both sites examined in Saginaw Bay. Active ammonium regeneration was observed and a portion of the regenerated nitrogen was nitrified and denitrified. Ammonium fluxes (Table 8) were similar to (or higher than) the flux of 42 µg atom N/m²/h, predicted from primary production demand as derived from stoichiometric considerations (Ullman and Aller 1989). They were much higher than the 18 µg atom N/m²/h predicted from a diffusion model in the same study. The difference between the production and diffusion estimates was attributed to consumption of ammonium from nitrification or benthic diatom uptake (Ullman and Aller 1989). Ammonium fluxes were within the range reported for hypereutrophic Old Woman Creek wetland adjacent to Lake Erie (60 to 1,033 µg atom N/m²/h; Tomaszek *et al*. 1997). They were higher than the near-zero rates observed for Lake Michigan and other freshwater sediments but resemble rates reported for coastal marine sediments (Seitzinger 1988). Denitrification rates in the gas-tight chambers were typical of rates observed for several freshwater and coastal marine systems with similar techniques but lower than rates for several other nutrient-enriched coastal ecosystems or mesocosms (Seitzinger 1988, Nowicki 1994, Tomaszek *et al*. 1997).

Comparison of Ammonium Regeneration at Inner and Outer Bay Sites

Total sediment-water regeneration rates of ammonium in the absence of zebra mussels were comparable at the two stations despite differences in trophic status. The inner bay had spring chlorophyll levels of up to 16 µg/L compared to less than 4 µg/L in the outer bay during 1991 to 1993 (Fahnenstiel *et al*. 1995). In contrast to these benthic ammonium flux results, water column regeneration rates were higher in the inner bay than in the outer bay, even without zebra mussel contributions (Gardner *et al*. 1995b). The mineral and organic properties of sediments are similar at the two sites. It can be postulated that high physical energy caused by winds and currents in these shallow waters may prevent organic materials as well as clays from accumulating in proportion to primary productivity in the two respective regions. Therefore a lower percentage of fixed organic material may be deposited at Site 1 than Site 2. Likewise, benthic biomass is an order of magnitude lower in sandy regions than in depositional regions of Saginaw Bay (T. Nalepa, pers. com., NOAA Great Lakes Environmental Research Laboratory).

Nitrate was removed from solution in the light benthic chambers at both sites in 1995, but removal rates were much higher at Site 2 (56 ± 7 µg atom N/m²/h) than at Site 1 (20 ± 6 µg atom N/m²/h), perhaps because of differences in light conditions or phytoplankton abundance or composition.

Effects of the Mineral (Sand vs. Clay) Composition of the Sediments on Nitrogen Transformations and Fate in Saginaw Bay

Sandy freshwater sediments offer a suitable matrix to investigate cation exchange effects on nitro-

gen dynamics in coastal systems because they contain fewer cation exchange sites than clay per volume. Furthermore, sulfide inhibition of nitrification is not as important in freshwater sediments as in marine systems because the former sites have low sulfate reduction rates and sulfide concentrations (Joye and Hollibaugh 1995). Likewise, decreased nitrification of regenerated ammonium cannot be attributed to salinity affecting microorganism physiology as observed in estuarine sediments (Rysgaard *et al*. 1999).

The experimental results agreed with the hypothesis that net "steady-state" ammonium flux from the sediments would decrease after the cation-exchange capacity was increased by the addition of clay particles. Adding montmorillonite decreased ammonium flux from the cores, apparently both by non-steady state uptake and by steady-state biological removal via nitrification. The low ammonium flux on the first sampling point after clay additions provided evidence for the first process. Also, the lower initial net flux in cores with untreated montmorillonite than in those with ammonium-soaked montmorillonite suggests that some regenerated ammonium was captured by the clay particles. After 2 days, ammonium release rates were still lower in clay treatments than in control cores, but rates were similar for both of the clay treatments. Mean net ammonium fluxes from the clay-treated sediment cores were about one half of the rates observed in the control cores during Days 5 to 8 of the experiment.

Although methodologies were not identical, comparing these data with previous results from depostional sediments (Gardner *et al*. 1987, Seitzinger 1988) supports the idea that sandy sediments release more regenerated ammonium than clay-dominated sediments. Ammonium dynamics in sandy Saginaw Bay sediments seem to resemble those of marine sediments (sand or clay) where dissolved sea salt ions occupy cation exchange sites and form ion pairs with ammonium (Seitzinger *et al*. 1991, Gardner *et al*. 1991). In contrast, freshwater sediments with high clay content retain ammonium and provide a more suitable environment for nitrification/denitrification. However, the addition of the clay also may have affected nitrogen dynamics by other mechanisms such as changing physical diffusion or modifying the composition or function of microorganisms at the sediment water interface. Regardless of the exact mechanism, these results suggest that sediment composition may play an important role in the regulation of nitrogen dynamics in freshwater sediments. The efficient regeneration of ammonium from sandy freshwater sediments in coastal regions is an important concept because it is a mechanism returning available nitrogen that may enhance primary production in shallow water columns. On the other hand, nitrification/denitrification (and or burial) would prevent a fraction of the remineralized nitrogen from returning, as an available form, to waters over depositional sediments that contain abundant clay particles.

Effects of Zebra Mussel on Nitrogen Dynamics at the Sediment-water Interface in Saginaw Bay

The removal of phytoplankton and other small organisms from the water by zebra mussels (Cotner *et al*. 1995, Lavrentyev *et al*. 1995) and the corresponding production of feces/pseudofeces (Griffiths 1993) affect nutrient cycling patterns within pelagic and benthic food webs (Gardner *et al*. 1995b). The potential effects of these materials on the ammonium regeneration process were examined by adding feces/pseudofeces to the flow-through sediment cores at an arbitrary level of 3 g dw/m^2. No measurable differences in ammonium or nitrate regeneration were observed for these treatments relative to controls. It can be concluded that these materials may not be a major source of regenerated nutrients. Information about the actual quantity of feces/pseudofeces deposited by zebra mussels is incomplete, but the level of addition was quite large relative to the biomass of non-zebra mussel benthic biomass expected in sandy sediments. If the feces/pseudofeces material has a high C:N ratio, it could be a net sink, rather than source, for regenerated nitrogen in the sediments. Although the C:N ratio of zebra mussel feces/pseudofeces has not been determined and would vary with food supply, it may tend to be high because the zebra mussel assimilates food efficiently (Stoeckmann and Garton 1997) and has high rates of ammonium excretion (Gardner *et al*. 1995b).

The flow-through and benthic chamber experiments indicated that zebra mussel metabolism dominated the nitrogen regeneration and oxygen consumption processes in regions where it was present. Rates were higher in the presence of added zebra mussels in flow-through cores (Lavrentyev *et al*. 2000) and proportional to zebra mussel biomass in benthic chambers under similar light conditions. These data agree with previous studies that indicate that excretion by this bivalve is the major factor

regulating community nutrient regeneration rates in regions where it is abundant (Gardner *et al*. 1995b, Johengen *et al*. 1995). Oysters have a similar effect in coastal marine systems (e.g. Dame *et al*. 1984, 1989).

Methodological Considerations

The results from the flow-through chambers resembled fluxes observed in short-term *in situ* benthic chambers. For example, ammonium was released at comparable rates from the sediments in benthic chamber experiments and flow-through core experiments. Net ammonium release rates measured by the flow-through core or *in situ* benthic chamber systems were higher than the near-zero values obtained in the gas-tight chambers after extended incubations. However, the total dissolved inorganic nitrogen flux, including N_2, in the gas-tight chambers was comparable to the ion flux observed in the benthic chambers and flow-through cores. The time-course patterns of ammonium and nitrate fluxes in the Site 2 denitrification chambers (Fig. 5) provide some insight about how incubation method and time can affect the forms of nitrogen released in the static enclosed chambers. Ammonium release rates in the denitrification chambers right after sparging were between 30 and 60 μg atom N/m^2/h in both July and August experiments and resembled extrapolated results from the benthic chambers (40 μg atom N/m^2/h). However, the ammonium flux decreased to negative values as the experiment progressed. Net nitrate flux increased as ammonium flux decreased but did not account for all of the ammonium removed. After the nitrate flux peaked, it decreased to near zero, suggesting a close coupling between nitrification and denitrification (Jenkins and Kemp 1984). The extended incubations in enclosed chambers affected the form and fate of regenerated nitrogen compounds more than the actual rates of total nitrogen regeneration. In agreement with other recent findings (Risgaard-Petersen *et al*. 1998, Cornwell *et al*. 1999), these data suggest that extended static incubations in enclosed chambers can misrepresent natural rates by increasing the fraction of regenerated nitrogen that is nitrified and denitrified. On the other hand, the *in situ* benthic chambers and flow-through cores yielded comparable and realistic fluxes for ammonium and nitrate. Both of these systems can be adapted to state-of-the-art denitrification rate measurements that quantify N_2:Ar ratio changes over short time intervals by membrane inlet mass spectrometry (Kana *et al*. 1994) or gas chromatography (An and Joye 1997). The new N_2:Ar ratio approach of measuring denitrification over short time periods in benthic chambers or cores provides a preferable method to obtain accurate estimates of denitrification rates. The data suggest that the long-term incubations provide reasonable estimates of total regenerated nitrogen if all major forms of nitrogen are quantified, but they may over-estimate the fraction of the total regenerated nitrogen lost by denitrification. Despite this potential limitation, the long-term estimates of denitrification are more valid than those determined by the acetylene-reduction technique because the latter method inhibits nitrification, a major source of nitrate substrate needed for denitrification (Seitzinger 1988).

ACKNOWLEDGMENTS

We thank D. Fanslow for help in collecting cores and in doing field experiments, H. Bootsma for analyzing nitrate in the core experiments, S. Seitzinger for providing the montmorillonite clay, J. R. Johnson, J. F. Cavaletto, and M. McCarthy for technical assistance and S. An for comments on the manuscript. This research was supported in part by NOAA Grant # NA16RGO457 to J. Cotner, Texas A&M University. The Chinese Ecosystem Research Network, Chinese Academy of Sciences, provided financial support for L. Yang to participate in this cooperative project. The Nancy Lee and Perry R. Bass Regents Chair in Marine Science, at The University of Texas Marine Science Insitute, supported manuscript preparation. This paper is GLERL Contribution #1184 and UTMSI Contribution #1174.

REFERENCES

An, S., and Joye, S.B. 1997. An improved chromatographic method to measure nitrogen, oxygen, argon and methane in gas or liquid samples. *Mar. Chem*. 59:63–70.

APHA. 1990. *Standard Methods for the Examination of Water and Wastewater.* 17th edition. American Public Health Association. Washington DC.

Billen, G. 1978. A budget on nitrogen recycling in North Sea sediments off the Belgian Coast. *Estuar. Coast. Mar. Sci*. 7:127–146.

Canale, R.P., and Squire, J. 1976. A model for total phosphorus in Saginaw Bay. *J. Great Lakes Res*. 2:364–373.

Cornwell, J.C., Kemp, W.M., and Kana, T.M. 1999. Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. *Aquatic Ecology* 33:41–54.

Cotner, J.B., Gardner, W.S., Johnson, J.R., Sada, R.H., Cavaletto, J.F., and Heath, R.T. 1995. Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton: Evidence for both size-selective consumption and growth stimulation. *J. Great Lakes Res*. 21:517–528.

Dame, R.F., Zingmark, R.G., and Haskin, E. 1984. Oyster reefs as processors of estuarine materials. *J. Exp. Mar. Biol. Ecol*. 83:239–247.

_____, Spurrier, J.D., and Wolaver, T.G. 1989. Carbon, nitrogen and phosphorus processing by an oyster reef. *Mar. Ecol. Prog. Ser*. 54:249–256.

Danek, L.J., and Saylor, J.H., 1977. Measurement of the summer currents in Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 3:65–71.

Dolan, D.M., Bierman, Jr., V.J., Dipert, M.H., and Geist, R.D. 1978. Statistical analysis of the spatial and temporal variability of the ratio chlorophyll a to phytoplankton cell volume in Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 4:75–83.

Fahnenstiel, G.L., Lang, G.A., Nalepa, T.F., and Johengen, T.H. 1995. Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 21: 435–448.

Folk, R.L. 1980. *Petrology of Sedimentary Rocks*. Hemphill Publishing.

Gardner, W.S., and St. John, P.A. 1991. High-performance liquid chromatographic method to determine ammonium ion and primary amines in seawater. *Anal. Chem*. 63:537–540.

_____, Nalepa, T.F., and Malczyk, J.M. 1987. Nitrogen mineralization and denitrification in Lake Michigan sediments. *Limnol. Oceanogr*. 32:1226–1238.

_____, Seitzinger, S.P., and Malczyk, J.M., 1991. The effects of sea salts on the forms of nitrogen released from estuarine and freshwater sediments: Does ion pairing affect ammonium flux? *Estuaries* 14:157–166.

_____, Bootsma, H.A., Evans, C., and St. John, P.A. 1995a. Improved chromatographic analysis of ^{15}N:^{14}N ratios in ammonium or nitrate for isotope dilution experiments. *Marine Chemistry* 48:271–282.

_____, Cavaletto, J.F., Johengen, T.H., Johnson, J.R., Heath, R.T., and Cotner, Jr., J.B. 1995b. Effects of the zebra mussel, *Dreissena polymorpha*, on community nitrogen dynamics in Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 21:529–544.

Griffiths, R.W. 1993. Effects of zebra mussels (*Dreissena polymorpha*) on the benthic fauna of Lake St. Clair. In *Zebra Mussels Biology, Impacts, and Control*, eds. T.F. Nalepa and D.W. Schloesser, pp. 415–437. Boca Raton, Florida: Lewis Publishers/CRC Press.

Holland, R.E. 1993. Changes in plankton diatoms and water transparency in Hatchery Bay, Bass Island area, Western Lake Erie, since the establishment of the zebra mussel. *J. Great Lakes Res*. 19:617–624.

Jenkins, M.C., and Kemp, W.M. 1984. The coupling of nitrification in two estuarine sediments. *Limnol. Oceanogr*. 29:609–619.

Johengen, T.H., Nalepa, T.F., Fahnenstiel, G.L., and Goudy, G. 1995. Nutrient changes in Saginaw Bay, Lake Huron, after the establishment of the zebra mussel (*Dreissena polymorpha*). *J. Great Lakes Res*. 19:449–464.

Joye, S.B., and Hollibaugh, J.T. 1995. Influence of sulfide inhibition on nitrification on nitrogen regeneration in sediments. *Science* 270:623–625.

Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E., and Cornwell, J.C. 1994. Membrane Inlet Mass Spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. *Anal. Chem*. 66:4166–4170.

Lavrentyev, P.J., Gardner, W.S., Cavaletto, J.F., and Beaver, J.R. 1995. Effects of the zebra mussel (*Dreissena polymorpha* Pallas) on protozoa and phytoplankton from Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 21:545–557.

_____, Gardner, W.S., and Yang, L. 2000. Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. *Aquat. Microb. Ecol*. 21:187–194.

Nalepa, T.F., Wojcik, J.A., Fanslow, D.L., and Lang, G.A. 1995. Initial colonization of the zebra mussel (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron: population recruitment, density, and size structure. *J. Great Lakes Res*. 21:417–434.

Nixon, S.W., Ammerman, J.W., and Atkinson, L.P., 1996. The fate of nitrogen and phosphorus at the land-sea margin of North Atlantic Ocean. *Biogeochemistry* 35:141–180.

Nowicki, B.L. 1994. The effect of temperature, oxygen, salinity, and nutrient enrichment on estuarine denitrification rates measured with a modified nitrogen gas flux technique. *Estuar. Coast. Shelf Sci*. 38:137–156.

Rowe, G.T., Boland, G.S., Phoel, W.C., Anderson, R.F., and Biscaye, P.E. 1994. Deep-sea floor respiration as an indicator of lateral input of biogenic detritus from continental margins. *Deep-Sea Research* 41:657–668.

Risgaard-Petersen, N., Nielsen, L.P., and Blackburn, T.H. 1998. Simultaneous measurement of benthic denitrification, with the isotope pairing technique and the N_2 flux method in a continuous flow-through system. *Water Research* 32:3371–3377.

Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P.B., and Sloth, N.P. 1999. Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* 22:21–30.

Seitzinger, S.P. 1987. Nitrogen biogeochemistry in an unpolluted estuary: the importance of benthic denitrification. *Mar. Ecol. Prog. Ser*. 41:177–186.

_____. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr*. 33:702–724.

_____, Nixon, S.W., Pilson, M.E.Q., and Burke, S. 1980. Denitrification and N_2O production in near-shore marine sediments. *Geochim. Cosmochim. Acta* 44: 1853–1860.

_____, Gardner, W.S., Spratt, A.K. 1991. The effect of salinity on ammonium sorption in aquatic sediments: implications for benthic nutrient cycling. *Estuaries* 14:167–174.

Smith V.E., Lee, K.W., Filkins, J.C., Hartwell, K.W., Rygwelski, K.R., and Townsend, J.M. 1977. *Survey of chemical factors in Saginaw Bay, Lake Huron.* U.S. Environmental Protection Agency Ecological Research Series. EPA-600/3-7-125. Duluth, MN.

Stoeckmann, A.M., and Garton, D.W. 1997. A seasonal energy budget for zebra mussels (*Dreissena polymorpha*) in western Lake Erie. *Can. J. Fish. Aquat. Sci.* 54:2743–2751.

Tomaszek J.A., Gardner, W.S., and Johengen, T.H. 1997. Denitrification in sediments of a Lake Erie coastal wetland (Old Woman Creek, Huron, Ohio, USA). *J. Great Lakes Res.* 23:403–415.

Ullman, W.J., and Aller, R.C. 1989. Nutrient release rates from the sediments of Saginaw Bay, Lake Huron. *Hydrobiologia* 171:127–140.

Wood L.E. 1964. Bottom sediments of Saginaw Bay, Michigan. *J. Sed. Petrol*. 34:173–184.

Submitted: 21 April 2000
Accepted: 30 November 2000
Editorial handling: Fred Soster