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Elizabeth A. Redon for the degree of Master of Science in Marine Resource Management on March 14, 2001. Title: Application of Nitrogen Stable Isotopes to Identify Sources of Nutrient Pollution: A Management Tool?

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Nutrient pollution may perhaps be one of the oldest water quality problems and has recently been considered as one of the greatest threats to estuarine and coastal waters. Excessive nutrient loads have had a vast array of impacts on estuarine and coastal ecosystems globally. Direct negative effects include: shading out of benthic plant communities, hypoxia and anoxia due to increased biological oxygen demand, compositional change in plant and faunal community structure and increased occurrences of harmful algal blooms. This has lead to decreased biodiversity, habitat, fisheries, tourism and aesthetics. So far environmental managers have had to rely on *a posteriori* assessments of the status of nutrient pollution, using indicators such as taxonomic shifts and changes in biological abundance, which have already occurred. By the time nutrient pollution is detected, restoration of habitats is costly and sometimes no longer an option. Therefore, there is a need for management tools to assess sources of nutrient loads before damage to estuarine and coastal ecosystems

progresses beyond recovery. Early work using stable isotopes of dissolved inorganic nitrogen in groundwater samples demonstrated the potential for using stable isotopes to identify anthropogenic sources of nitrogen. These studies revealed that nitrogen sources such as fertilizers, soil organic nitrogen and wastewater nitrogen have isotopically distinct signatures that could be used to assess anthropogenic nitrogen contributions. Therefore, application of nitrogen stable isotope methods could potentially provide information on the anthropogenic nitrogen sources stimulating nutrient pollution in estuarine and coastal waters. However, when I assessed this approach for application, two sources of errors became apparent. First, laboratory isolation of dissolved inorganic nitrate from seawater delivered results depleted in ^{15}N relative to the standard used to assess the method. Second, isotopic fractionation associated with nitrogen cycle processes complicates usage of an isotopic mass balance to solve for the nitrogen source and its fractional contribution based on isotopic data alone. As a result, isotopic analysis alone of dissolved inorganic nitrogen to determine anthropogenic sources of nutrient loads to estuarine and coastal waters is not a practical approach to obtain management information concerning nutrient pollution.

Application of Nitrogen Stable Isotopes to Identify Sources of Nutrient Pollution: A Management Tool?

by
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Elizabeth A. Redon, Author

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Dedication

This thesis is dedicated to my devoted husband who provided endless support and hours proofreading for me.

Application of Nitrogen Stable Isotopes to Identify Sources of Nutrient Pollution: A Management Tool?

Chapter 1: Introduction-Nutrient Pollution in Estuaries and Coastal Waters

Nitrogen (N) serves as an essential building block for plant biomass and is commonly the nutrient limiting primary production in temperate estuarine waters (EPA 1998; Carpenter at al. 1998; NRC 2000). However, an excessive load of this nutrient can create a vast array of water quality problems, habitat alterations and biological impacts (Table 1). In recent decades, human activities have accelerated the nutrient delivery to estuarine and coastal ecosystems by approximately doubling the rate of N inputs into terrestrial regions (Vitousek et al. 1997; Bricker et al. 1999). By the mid-1990s human activities such as agriculture, urbanization and industry made new N available at a rate of 140 Tg per year globally (Vitousek et al. 1997). This rate is roughly equivalent to the natural rate of biological N fixation on all the world land surfaces (NRC 2000). In comparison, atmospheric carbon dioxide has increased 20% since 1900 while global N fixation has increased 100% since 1900 (NRC 2000). Therefore, human activities have altered global N availability on a scale that far exceeds human impacts on the global carbon cycle (NRC 2000). Furthermore, the National Research Council, GESAMP (Group of Experts on the Scientific Aspects of Marine Pollution) and the Environmental Protection Agency have listed nutrient pollution as one of the greatest threats to the

integrity of coastal waters globally (NRC 1995; Nixon 1995; Pelley 1998; EPA 1998).

Table 1. Summary of negative impacts caused by nutrient pollution (Bricker et al. 1999; NRC 2000).

Impacts	Primary Effects	Secondary Effects	Environmental Impacts	Economic and Social Implications
Increased Biomass	Shading	Loss of Benthic Plant Community	1. Habitat Loss 2. Loss of Nursery Grounds 3. Decreased Biodiversity	1. Decreased Fisheries 2. Decreased Aesthetics/ Tourism
	Increased BOD	Hypoxia/ Anoxia	1. Dead Zones 2. Habitat Loss 3. Accumulation of H_2S 4. Shift in Faunal Community 5. Decreased Biodiversity	1. Decreased Fisheries 2. Noxious Odor 3. Decreased Aesthetics/ Tourism
Change in N Concentration	Shift in Dominant Plant Community	Loss of Benthic Plant Community and Increase in Pelagic Plant Community	1. Habitat Loss 2. Change in Food Web 3. Loss of Corals 4. Decreased Biodiversity	1. Decreased Fisheries 2. Decreased Aesthetics/ Tourism
Change in Si:N	Shift in Phyto-plankton Community	Increased Occurrence of HABs	1. Toxins Released 2. Fish and Other Fauna Kills 3. Physical Damage to Fish and Larva	1. Health Effects 2. Decreased Fisheries 3. Decreased Aesthetics/ Tourism

Negative Impacts of Nutrient Pollution

Cultural Eutrophication

There have been several definitions proposed to describe the process of eutrophication. Some authors use the Greek derivation of the term which breaks it down into two words, *eu* which means "well" and *trope* meaning "nourishment" (Richardson and Jørgensen 1996). When put together the definition refers to the process by which a water body's nutritional status changes by an increase in nutrient resources, usually by addition of inorganic nutrients such as nitrogen (N) and phosphorus (P) (Richardson and Jørgensen 1996). Other authors define eutrophication as an increase in the organic matter (OM) to an ecosystem which can have negative impacts on water quality (Nixon 1995). Lastly, eutrophication has also been described as a process of increased nutrient inputs stimulating aquatic plant growth that results in high levels of OM in the ecosystem which cause negative impacts on water quality, such as an increase in biological oxygen demand leading to hypoxic and anoxic conditions (Vollenweider 1992; Bricker et al. 1999).

According to Bricker et al. (1999), it has recently become clear that nearly all the estuaries in the United States exhibit some level of eutrophic symptoms. In this National Oceanic and Atmospheric Administration study, 138 estuaries were evaluated (Bricker et al. 1999). A third (44) of those estuaries were determined to have acute eutrophic symptoms and an additional 40 estuaries showed moderate eutrophication. Therefore, 60% of U.S. estuaries have moderate to high expression

of this water quality problem (Bricker et al. 1999). Furthermore, the estuaries assessed as having moderate to high expression of eutrophication were those most studied; there could perhaps be many other less studied estuaries with serious water quality problems (Bricker et al. 1999). This particular study found 17 estuaries that have inadequate data to determine eutrophication status (10 of which are in Oregon- Table 2) (Bricker et al. 1999).

Table 2. Oregon estuaries listed as having insufficient data to assess eutrophic conditions (Bricker et al. 1999).

Oregon Estuaries
Alsea River
Coquille River
Coos Bay
Nehalem River
Netarts Bay
Rogue River
Siletz River
Siuslaw River
Tillamook Bay
Umpqua River

The immediate effect of excess nutrients in most estuarine and coastal ecosystems is stimulation of ephemeral benthic macroalgae (not typically present in estuarine benthic communities), epiphytic macro- and microalgae and phytoplankton growth, causing an increase in plant biomass (Richardson and Jørgensen 1996; Valiela et al. 1997b; Bricker et al. 1999; NRC 2000). Plant

biomass is a function of production or growth and loss processes (Sand-Jensen and Borum 1991; Richardson and Jørgensen 1996). Plant growth depends on light, nutrient availability and to a lesser degree temperature (Borum 1996; Richardson and Jørgensen 1996; NRC 2000) while losses of plant biomass can be due to grazing, sedimentation, senescence followed by decomposition and dilution (Sand-Jensen and Borum 1991; Richardson and Jørgensen 1996). Increased plant biomass has two direct impacts to coastal and estuarine ecosystems. First, the enhanced growth of phytoplankton and epiphytic algae reduce light availability to submerged aquatic vegetation, such as seagrasses, leading to their degradation (Duarte 1995; NRC 1995). Second, the increased productivity results in increased oxygen consumption when the OM is decomposed causing hypoxic and anoxic conditions (NRC 2000).

Increases in phytoplankton biomass and total suspended particles resulting from eutrophication cause shading that reduces light penetration through the water column to benthic plant communities thus leading to their degradation (Sand-Jensen and Borum 1991; Bricker et al. 1999; NRC 2000). Furthermore, epiphytic algae grow on the surfaces of macrophytic leaves thereby reducing light penetration and gas and nutrient exchange, decreasing photosynthesis (Bricker et al. 1999; NRC 2000). Ephemeral benthic macroalgae have significantly lower light requirements compared to perennial benthic macrophytes, such as seagrasses (Sand-Jensen and Borum 1991; Borum 1996; Duarte 1995; Valiela et al. 1997b). Therefore, they proliferate in response to nutrient additions, despite the decreased

light availability, and accumulate in great masses of filamentous sheets over seagrasses and sediment surfaces, thus contributing to the decline of seagrasses by decreasing sunlight availability (Valiela et al. 1997b; NRC 2000). As a result, massive and persistent ephemeral macroalgal blooms, epiphytic microalgae and phytoplankton can ultimately displace seagrasses and perennial algae through shading effects (Sand-Jensen and Borum 1991; Duarte 1995; Borum 1996; Valiela et al. 1997b).

These benthic plant communities serve many roles in coastal and estuarine ecosystems. Among these is their role in supporting a rich and diverse faunal community by providing both food and shelter (Borum 1996). They also provide a protected nursery for juveniles of many deeper water fish species (Valiela et al. 1997b; EPA 1998). Their degradation and replacement through these shading effects causes habitat losses which lead to changes in the faunal community, including a decrease in biodiversity (Norko and Bonsdorff 1996). Overgrowth of algal species over fish spawning substrate can replace benthic spawning species with pelagic species, which are often less valuable commercially (Kerr and Ryder 1992). Seagrass roots and rhizomes also serve to stabilize sediments; therefore, the loss of roots and rhizomes increases sediment resuspension, releasing nutrients stored in the sediments into the water column, further promoting algal blooms (Duarte 1995; NRC 2000).

Aquatic ecosystems can to some extent be viewed as a two-compartment system in which oxygen is produced in the surface layer by photosynthesis and

either diffuses into the atmosphere and/or into the second compartment, the benthos, where oxygen is consumed (Cruzado 1990). These compartments, respectively, cause net oxygen generation via photosynthesis and oxygen consumption by respiration to become spatially and temporally separated (Nixon 1993). Displacement of benthic macrophytes by phytoplankton and other blooming algae further intensifies this separation by shifting the majority of photosynthesis to surface waters and decreasing the role of benthic macrophytes in aerating sediments (Sand-Jensen and Borum 1991).

The augmented productivity of nutrient loaded ecosystems creates an increased biological oxygen demand (BOD) due to plant and animal respiration (NRC 2000). The increased plant biomass eventually dies off, increasing delivery of OM to the bottom compartment via sedimentation (Richardson and Jørgensen 1996). Microbial processes can respire this OM aerobically. When oxygen consumption exceeds oxygen delivery from photosynthesis and mixing, hypoxia will occur (Richardson and Jørgensen 1996; Bricker et al. 1999; NRC 2000).

Occurrences of hypoxic and anoxic events in coastal waters related to cultural eutrophication have been increasing in frequency, duration, spatial coverage, and intensity (Diaz and Rosenberg 1995; Richardson and Jørgensen 1996). For example, the dead zone along the Gulf of Mexico inner continental shelf has expanded from 9,500 km^2 in 1991 to 20,000 km^2 in 1999, this is approximately a 110% increase (Rabalais et al. 1991; NRC 2000).

Hypoxic conditions can lead to habitat loss, lower biodiversity, fish kills, altered migratory patterns of fish populations and lower recruitment of fish populations (Bricker et al. 1999; NRC 2000; Pelley 1998). The initial response of the faunal community to lowered oxygen availability is behavioral in nature. Mobile species will migrate into adjacent oxygenated waters and burrowing species will emerge from the sediments in search of oxygen (Boesch and Rabalais 1991; Diaz and Rosenberg 1995; Hagerman et al. 1996; Richardson and Jørgensen 1996). Less mobile species and species sensitive to low oxygen levels will die, leading to changes in the benthic and pelagic faunal community and contributing more OM for microbial degradation (Boesch and Rabalais 1991; Diaz and Rosenberg 1995; Richardson and Jørgensen 1996).

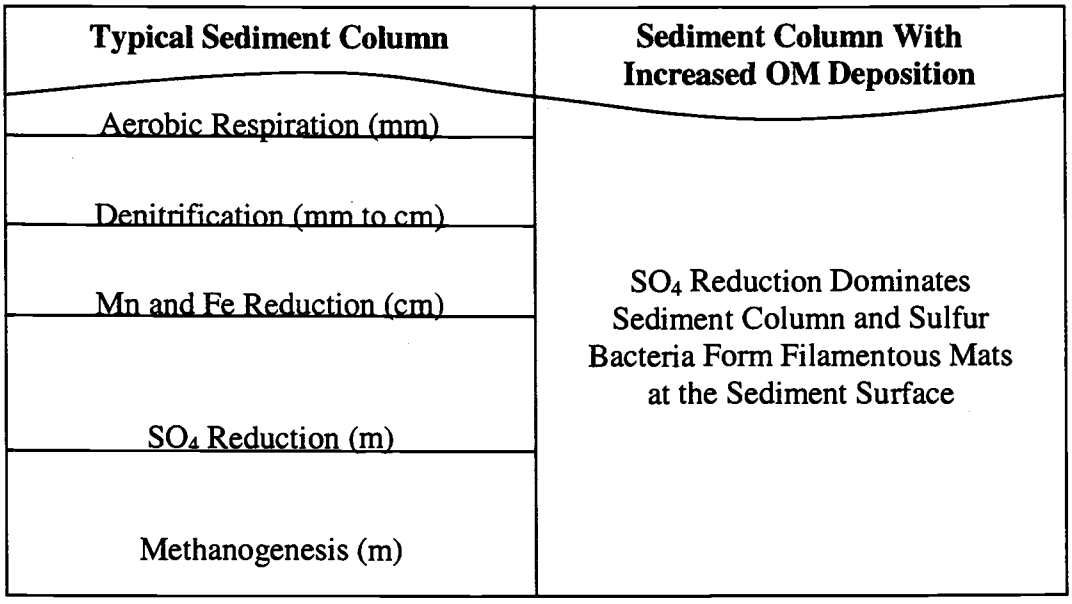
Hypoxia can also change predator-prey interactions that can lead to shifts in the energy flow within the ecosystem (NRC 2000). For example, low oxygen levels in bottom waters could prevent vertical migration of zooplankton, a mechanism used for predator avoidance during the day. As a result, these organisms could be more susceptible to fish predation if they are forced to restrict their activities to the surface oxic layer (NRC 2000). For example, anoxia in the bottom waters of Chesapeake Bay disrupted the behavior of copepods by disrupting their vertical migration into bottom waters (Roman et al. 1993). Increased fish predation on zooplankton could then reduce the grazing pressure on phytoplankton further enhancing deposition and decomposition of OM in bottom waters (NRC 2000). Roman et al. (1993) also found evidence that low oxygen levels could

decrease hatching of copepod eggs causing a decrease in copepod recruitment. This can lead to an uncoupling of the interaction between phytoplankton and copepods thereby reducing grazing pressure and allowing phytoplankton to proliferate and contribute more OM to the bottom waters intensifying hypoxia (Roman et al. 1993).

Sediments and associated benthic communities tend to be the components of estuarine ecosystems most sensitive to eutrophication and hypoxia because the depth of the oxic-anoxic interface within sediments easily changes in response to OM deposition rate and variations in oxygen concentrations of the water (Jørgensen 1996). The sediment oxic zone is a layer generally only a few millimeters thick at the sediment surface and surrounding macrofaunal burrows (Revsbech et al. 1980; Jørgensen 1996). Sedimenting OM in this zone is primarily respired by aerobic microorganisms and animals of the benthos (Jørgensen 1996). As OM inputs increase, this zone shrinks causing the oxic-anoxic interface to become more shallow (Diaz and Rosenberg 1995). When oxygen is depleted, only specialized microorganisms that respire OM using electron acceptors such as sulfate, nitrate, and manganese dioxide are able to survive (Nixon 1993; Diaz and Rosenberg 1995). This type of metabolism typically occurs below the oxic zone (Jørgensen 1996). Generally, there is a vertical zonation in which members of the microbial food web distribute by metabolic types, each type uniquely exploiting an alternative compound to metabolize OM (Figure 1) (Jørgensen 1996). Increased deposition of OM and subsequent hypoxia of the lower water column due to

eutrophication causes oxidized sediments to become reduced, leading to a shift towards increased anaerobic respiration with a dominance of sulfate reduction (Jørgensen 1996). Consumption of the oxidants nitrate, manganese (IV) and iron (III) becomes greater than their transport rate down into the sediments leading to a dominance of sulfate reduction to consume OM (Jørgensen 1996). Consequently, eutrophication preferentially stimulates sulfate reduction and eventually filamentous sulfur bacteria will cover the sediment surface (Jørgensen 1996; Richardson and Jørgensen 1996). It is the reduction of sulfate to hydrogen sulfide that causes the rotten egg smell sometimes associated with poor water quality or low to zero oxygen levels (Nixon 1993).

Figure 1. Sediment column under typical conditions and under the influence of increased sediment loads.



Hypoxia can play an important role in structuring the benthic community because of differing sensitivities of organisms to reduced oxygen, thereby reducing and altering habitat available to them (Diaz and Rosenburg 1995). Generally, there tends to be a shift from a dominance of larger long-lived benthic species (e.g. mussels) to a dominance of opportunistic smaller shorter-lived species (e.g. polychaetes) (Sand-Jensen and Borum 1991; Diaz and Rosenberg 1995). Furthermore, regions with persistent hypoxia remain in a pioneering state because low oxygen levels tend to halt successional development (Diaz and Rosenberg 1995). In extended cases of hypoxia or anoxia, complete die off of most macroorganisms can occur causing the flow of energy to shift from the macrobenthal community to the microbial community (Diaz and Rosenberg 1995). This reduces the energy available for recruitment of benthic fauna when oxic conditions return (Diaz and Rosenberg 1995; NRC 2000). As a result, macrofauna could be replaced by flourishing sulfur bacterial mats along the benthos (Diaz and Rosenberg 1995; Richardson and Jørgensen 1996). These bacteria reduce sulfate to hydrogen sulfide which causes an unpleasant noxious smell and is toxic to benthic fauna (Boesch and Rabalais 1991; Nixon 1993; Diaz and Rosenberg 1995). Decimation of animal populations also decreases grazing pressure on phytoplankton and epiphytes which increases their shading effect and accelerating the decline of seagrasses (Duarte 1995). Hypoxia and anoxia thus lead to reduced

diversity, abundance and biomass of benthic communities (Diaz and Rosenberg 1995).

Change in Plant Species Composition

Temperate estuarine and coastal waters are typically colonized by rooted seagrasses and benthic microalgae on sediments, and macroalgae on rocks and hard bottom sediments (Borum 1996; Valiela et al. 1997b). These benthic plant communities can significantly contribute to autotrophic production in pristine coastal areas (Borum 1996; NRC 2000). Under pristine conditions, submerged aquatic vegetation can attain dense populations that are as productive as the most productive terrestrial ecosystems (Charpy-Roubald and Sournia 1990; Borum 1996). These plant species located along the benthos are less dependent on nutrient levels within the water column than phytoplankton and ephemeral macroalgae are, therefore, nutrient enrichment rarely stimulates these macrophyte populations (Sand-Jensen and Borum 1991; Duarte 1995). Consequently, when these ecosystems are overloaded with nutrients, a qualitative shift in the plant communities often is observed (Sand-Jensen and Borum 1991; Borum 1996). Nutrient enrichment can change the composition of estuarine and coastal autotrophic communities from dominance by perennial macroalgae and seagrasses to dominance by ephemeral macroalgae and pelagic microalgae due to differences in their nutrient utilization (Borum 1996).

Internal concentration of nutrients (e.g. total N content per unit plant biomass) required for maximum growth varies between plant species (Borum 1996). Generally, smaller plant species require more nutrients to attain their maximum growth rates, which are usually higher than rates of larger plants found in the benthic communities (Sand-Jensen and Borum 1991; Duarte 1995). Smaller plants have larger surface to volume ratios allowing them to have higher specific rates of nutrient uptake to accommodate their growth needs compared to larger benthic plants that typically dominate systems under pristine conditions (Borum 1996). Despite the fact that microalgae and ephemeral algae have higher specific nutrient uptake rates, larger benthic plants are more capable of supporting new growth under low external nutrient concentrations (Borum 1996). This is partly due to the slow-growing benthic vegetation's ability to rely on internal stores of nutrients in their leaves, stems and rhizomes to satisfy nutrient requirements by retranslocating nutrients from older parts of the plant to younger actively growing regions (Sand-Jensen and Borum 1991; Valiela et al. 1997b). The storage capacity, expressed as the time nutrient reserves can sustain maximum growth, is longer for slow-growing benthic vegetation compared to the fast-growing plant species thus allowing the former plants to better withstand fluctuations in external N availability (Borum 1996). Internal nutrient storage of fast-growing plant species, such as phytoplankton, can sustain maximum growth for only about a day without external replenishment from the water column (Borum 1996). High nutrient requirements and lack of internal nutrient storage of smaller plants may mean that small algae

require higher external nutrient concentrations to satisfy growth requirements compared to the large slow-growing macrophytes (Borum 1996). As a result, high N loads caused by nutrient pollution promote phytoplankton blooms and inhibit growth of benthic plant communities (Duarte 1995).

Since benthic plant species are able to satisfy their nutrient requirements in low nutrient conditions, nutrient enrichment rarely stimulates these macrophyte populations and sunlight distribution plays a more important limiting role determining their depth penetration and areal coverage (Sand-Jensen and Borum 1991; Duarte 1991; Borum 1996; Valiela et al. 1997b). As a result, nutrient enrichment seems to inhibit benthic plant communities, rather than enhancing their growth, by stimulating planktonic communities that shade out these benthic plant communities (Sand-Jensen and Borum 1991; Borum 1996). Furthermore, nutrient enrichment may not necessarily increase total primary production per unit area (i.e. stimulate a bloom) but rather shift the majority of productivity from the benthic macrophyte community to the planktonic community (Borum 1996). This replacement of benthic macrophytes with faster growing micro- and macro-algae with rapid nutrient uptake potentials under enriched nutrient conditions can lead to decreased biodiversity, decreased habitat and can have significant trophic consequences due to the change in the type of primary production occurring (Borum 1996; Duarte 1995; Hein et al. 1995).

Nutrient pollution can change the composition of phytoplankton communities primarily by altering the silica to nitrogen ratio (Si:N) (Paerl 1997).

Anthropogenic land uses over the last century have resulted in a doubling of nitrate concentrations and a halving of silica concentrations in coastal and estuarine waters (Turner and Rabalais 1991) causing a decline in the dissolved Si:N ratio in these environments (Turner and Rabalais 1994). Reduced Si:N ratio can lead to altered phytoplankton community composition and has been implicated in the increasing occurrences of harmful algal blooms (HABs) (Smayda 1990; Rabalais et al. 1996; Humborg et al. 1997).

Phytoplankton species exhibit differences in their kinetic ability to take up and assimilate nutrients, their growth and metabolism on those nutrients and behavioral mechanisms used to ensure they obtain a continuous supply of growth limiting nutrients (Paerl 1997). Uptake capacity of phytoplankton species at different nutrient concentrations varies, allowing system productivity to be maintained across a broad range of nutrient regimes (NRC 2000). Some phytoplankton species excel in waters with chronically low nutrient levels while other species prefer high nutrient levels (Pelley 1998; NRC 2000). Furthermore, phytoplankton species require different ratios of nutrients thus a shift in the relative availability of nutrients should result in changes in the phytoplankton community (Officer and Ryther 1980; Smayda 1990).

Silica is required by diatoms and, when it is abundant, diatoms usually dominate the local community (NRC 2000). Silica can become trapped upstream of estuarine and coastal waters due to bloom events that take silica out of the water as the diatom population proliferates (NRC 2000; Rabalais et al. 1996).

Subsequent sedimentation and integration of that silica as plant material stores the silica in the sediments of freshwater portions of streams and rivers (NRC 2000; Rabalais et al. 1996). This reduces the riverine supply of silica to coastal and estuarine waters (Rabalais et al. 1996). A 30% decrease of silica delivery by the Mississippi River has been observed for the Gulf of Mexico due to this phenomenon (Turner and Rabalais 1991). Also, silica could possibly become trapped in reservoir sediments on the upstream side of dams (Humborg et al. 1997). The dissolved silica load of the Danube River has been reduced by two-thirds since the dam was constructed in the early 1970's (Humborg et al. 1997). Decreased silica along with increased dissolved inorganic N delivery to the Black Sea has resulted in large decreases in Si:N which are responsible for shifting the phytoplankton species composition from diatoms to coccolithophores and flagellates (Humborg et al. 1997). Blooms of dinoflagellate, coccolithophore and toxic species have increased by a factor of six due to this change in the nutrient regimen (Humborg et al. 1997).

In the Western Baltic, a new late-spring flagellate (*Dictyocha speculum*) bloom appeared in the early 1980's in response to altered nutrient supplies due to nutrient pollution (Jochem and Babenard 1989). The lack of silica in these coastal waters terminated the diatom spring bloom without exhausting the nitrate pool (Jochem and Babenard 1989). The remaining N was adequate to support a bloom of an unknown "naked form of the silicoflagellate *Dictyocha speculum*," which lacks a siliceous skeleton (Jochem and Babenard 1989). This phenomenon

demonstrates that a species, such as *Dictyocha speculum*, is able to exploit an ecological niche created by the environmental change set up by nutrient pollution and becomes a recurrent event in species succession (Jochem and Babenard 1989). Other flagellate blooms, including some nanoflagellate species, have occurred in response to changed nutrient regimes of the Western Baltic (Jochem and Babenard 1989). Many toxic algae are among these nanoflagellates, therefore, nutrient pollution can increase the probability of toxic HABs (Jochem and Babenard 1989). If one of these toxic algal species also became part of the natural species succession as *Dictyocha speculum* has, there could be detrimental impacts on the ecosystem (Jochem and Babenard 1989).

Phytoplankton community shifts in response to changes in the ionic ratio of essential nutrients have been observed in coastal and estuarine waters globally (Smayda 1990). Significant blooms of non-siliceous species are increasing in intensity, frequency and are even replacing diatoms as the dominant biomass group in some regions (Smayda 1990). Long-term monitoring of phytoplankton in the German Bight showed a 10-fold increase in flagellates as Si:N decreased (Radach et al. 1990 quoted in Paerl 1997). In the Mississippi River decreasing Si:N due to N loading and silica loss has led to the dominance of lightly-silicified diatoms and non-diatom species, and either decreased or completely eliminated heavily-silicified diatoms (Rabalais et al. 1996). Furthermore, several phytoplankton species posing risk to human health are now present, but previously were either absent or less dominant in these environments (Rabalais et al. 1996). HABs have

also been reported in the Baltic Sea, Japan's Seto Inland Sea, Black Sea, North Sea, Hong Kong Harbor, American east coast, and other areas (Paerl 1997; Smayda 1990).

HABs impact coastal and estuarine ecosystems through a variety of modes. Some of the algal species stimulated by changes in nutrient ratios produce toxins that are dangerous to other organisms and humans (NRC 2000; Bricker et al. 1999; Smayda 1997). These toxins can lead to mortality as an endotoxin (through direct ingestion), an exotoxin (exposure to secreted toxin in the water) and/or by vectoring through the food web (the toxin accumulates in mussels which are eaten by organisms at higher trophic levels) (Smayda 1997). The toxins produced are responsible for the human ailments: paralytic, diarrhetic, neurotoxic and amnesic shellfish poisoning (NRC 2000). These toxin producing algal species have caused mortalities in fish, shellfish, marine mammals, seabirds, other marine fauna and humans (NRC 2000). HABs can also cause physical damage to fish and larva of many faunal species (Smayda 1997). High densities of harmful algal species collide with larval stages causing damage that leads to their death (Smayda 1997). Furthermore, HAB species with setae, a hard bristle-like structure, have also pierced fish gills which can lead to respiratory failure, hemorrhaging or bacterial infection (Smayda 1997). Lastly, high densities of HAB species die and decompose, causing oxygen depletions that lead to mortalities of fish and other marine fauna (Smayda 1997).

Nutrient pollution has also been shown to cause changes in community composition of coral reefs leading to degradation of these environments. Coral reefs are among the most productive, diverse and complex ecosystems in the world and are distributed in nutrient poor waters of the tropics and subtropics (Bell 1992; Lapointe 1997; NRC 2000). Nuisance algal growth stimulated by enriched nutrient levels can lead to the replacement of corals with algae and benthic filter and detrital feeders (Bell 1992). Even slight increases in the ambient nutrient concentrations can lead to increases in the algae that replace corals due to higher temperatures and irradiance associated with these regions (NRC 2000). Macroalgae tend to dominate coral reef communities at dissolved inorganic N levels of only 1 μM (Bell 1992; Lapointe 1997). Increased phytoplankton biomass shades out the symbiotic zooxanthellae, inhibiting photosynthesis (Bell1992). Furthermore, enhanced sedimentation of OM derived from phytoplankton blooms encourages growth of filter and detrital feeders that will compete with corals for space (Bell 1992). Also, many algal species that are stimulated by enhanced nutrient loads will out-compete corals, including attached algal species, boring type species that damage corals and possibly infectious algal species associated with black band disease (Bell 1992). Stimulation of filamentous algal species may inhibit coral larval settlement and survival, which can reduce coral recruitment (Bell 1992). These shifts in the flora and fauna result in decreased biodiversity in these communities (Bell 1992).

Compositional changes in the dominant primary producers due to nutrient pollution change the character and the relative size of lower levels of the food web

and thereby probably affect higher trophic levels (Kerr and Ryder 1992). Changes in the primary production of an ecosystem will have profound effects on the heterotrophic populations that they support and the types of detrital material that are produced (Laws 1983). For example, diatoms are typically the food base for filter feeding fish and zooplankton and contribute to fishable populations in coastal waters (Officer and Ryther 1980). Their displacement could lead to alterations in higher trophic levels, which could negatively impact the fisheries industry by reducing populations of marketable fish. This is only one example of a possible impact on the food web. More information is needed to understand fully the impact of compositional changes of the primary producers on higher trophic levels.

Economic and Social Impacts

The negative ecological impacts of nutrient pollution on estuarine and coastal ecosystems can have economic consequences. Decreased water quality as a result of nutrient pollution damages the fisheries industry through fish kills caused by hypoxia or anoxia, HABs and decreased amounts of spawning and nursery habitat (Volterra and Kerr 1990). As a result, both commercial and recreational fishing industries are affected (Bricker et al. 1999; NRC 2000). Other seafood industries, such as shellfisheries, are also affected by hypoxic and anoxic waters and habitat reduction. Furthermore, tourism along coastal margins is affected by

interference of algal blooms with boating and swimming activities along with decreased recreational fishing (EPA 1998; Bricker et al. 1999; NRC 2000).

The ecological impacts of nutrient pollution can also have social consequences. The increasing occurrences of HABs can affect human health. Toxins produced by some of these bloom species become incorporated into seafoods, such as shellfish, which results in paralytic, diarrhetic, neurotoxic, domoic acid and amnesic shellfish poisoning (Bricker et al. 1999; NRC 2000). Also, degradation of coastal, estuarine and coral reef habitats result in aesthetic losses of these environments (NRC 2000).

Anthropogenic Sources of N

Table 3. Summary of N sources (Schlesinger and Hartley 1992; Vitousek and Matson 1993; Galloway et al. 1995; Kumar 1995; Prospero et al. 1996 Schlesinger 1997; Vitousek et al. 1997).

Natural Sources of N	**Tg N/yr**	
Lightning	3	
Terrestrial Biological N Fixation	90-130	
Marine Biological N Fixation	40-200	
Anthropogenic Sources of N		
Fertilizers	80	
N_2-Fixing Crops	40	Total of 140
Fossil Fuel Burning	20	
Mobilization		
Draining Wetlands	10	
Clearing Land for Crops	20	Total of 70
Burning Forests and Grasslands	40	

Under pristine conditions in which the landscape remains largely undisturbed by human activities (such as during pre-industrial times), most of the N within the N cycle is contained within the massive, well-mixed pool of N gas (N_2) that resides in the atmosphere (Vitousek et al. 1997), which must be fixed to be biologically available (Schlesinger 1997). As a result many ecosystems are N limited (Schlesinger 1997). Lightning and N_2-fixing microorganisms convert N_2 into biologically available forms (Table 3) (Galloway 1995; Vitousek et al. 1997; Schlesinger 1997; Pelley 1998). Lightning globally fixes about 3 Tg N annually (Galloway et al. 1995; Kumar 1995; Schlesinger 1997). Biological N_2-fixation in marine ecosystems is estimated to range from 40 Tg N yr^{-1} to 200 Tg N yr^{-1} while terrestrial biological N_2-fixation prior to extensive human activity is estimated to have ranged between 90 and 130 Tg N yr^{-1} (Galloway et al. 1995). The export of N into estuaries and coastal waters under these "pristine" conditions was very small, particularly in temperate zones (NRC 2000). About one third of the N fixed on land was transported to coastal waters via riverine inputs (Galloway et al. 1995). The net effect of human activities has been to increase the amount of biologically available N and disrupt this carefully balanced system in which the majority of N remains unavailable in the atmosphere until it is fixed biologically or by lightning. Human activities such as the application of synthetic N fertilizers, fossil fuel combustion and cultivation of N_2-fixing crops now convert N_2 into biologically available forms at a rate comparable to pre-industrial biotic N_2-fixation, contributing an estimated 140 Tg N yr^{-1} (Table 3) (Galloway et al. 1995; Vitousek

et al. 1997; Pelley 1998). Furthermore, human perturbations such as land clearing and conversion, wetlands drainage and biomass burning mobilize an additional 70 Tg N annually (Table 3) (Vitousek et al. 1997). This anthropogenically generated fixed N reaches estuaries via groundwater, surface runoff, riverine input and atmospheric deposition where it contributes 25 to >50% of the new production (excluding regions with upwelling) (Paerl 1997).

Agricultural

N sources derived from agricultural practices include synthetic fertilizer application, high-density animal feeding operations and cultivation of N_2-fixing crops. Industrial fixation of atmospheric N_2 into synthetic fertilizers after World War II accounts for more than half of the total human alteration of the N cycle because it is the largest process whereby humans can fix N into biologically available sources (Vitousek and Matson 1993; Vitousek et al. 1997; NRC 2000). Currently, about 80 Tg N yr^{-1} are fixed industrially through the Haber process into fertilizer (Schlesinger and Hartley 1992; Prospero et al. 1996; Vitousek et al. 1997; Schlesinger 1997). Overall, the utilization of synthetic N fertilizers by crops is very inefficient (NRC 2000). In the United States an estimated 45 to 75% of the N contained in the synthetic fertilizers applied are removed with crop harvest, the remaining N is stored as soil organic N, volatilized to the atmosphere or leached into surface and groundwater (Bock 1984; Nelson 1985; NRC 2000). The soil

type, climate, fertilizer type, rate of fertilizer application and farming practices influence the allocation of the N among these endpoints (Howarth et al. 1996; Bouwman et al. 1997; NRC 2000). For example, between 10 and 40% of synthetic fertilizers applied to loam and clay arable soils leaches into surface and groundwaters and between 25 to 80% leaches into surface and ground waters when applied to sandy arable soils resulting in an estimated 20% loss of the total amount of synthetic fertilizers applied in the United States (Howarth et al. 1996; NRC 2000).

High-density animal feeding operations have also increased during the post World War II period. The general trend has been a decrease in the number of livestock farms accompanied with an increase in livestock populations. For example, United States census information has revealed an 18% increase in the number of hogs along with a 72% decrease in the number of hog farms from 1990 to present (NRC 2000). Over the same period the number of dairy farms has decreased by 40% while the herd size increased by 50% (NRC 2000). This concentration and intensification of livestock operations reduces production costs and raises the industry productivity (Mallin 2000). These facilities produce highly concentrated sources of nutrients and are a major challenge to waste management (Smetacek et al. 1991; Mallin 2000; NRC 2000). Generally, livestock are raised in small areas and their feces and urine are collected into holding ponds from which the waste is pumped onto sprayfields of cover crops such as Bermuda grass (Mallin 2000). These holding ponds can leak into nearby soils and groundwater as

demonstrated by a study of eleven unlined swine waste lagoons in North Carolina in which 55% showed moderate to severe seepage losses (Huffman 1995 quoted in Mallin 2000). Furthermore, these storage ponds can run over during flood events causing high concentrations of nutrients to enter surface runoff, adjacent streams, and associated estuaries threatening the water quality (Mallin 2000). In 1995, a waste-holding lagoon spilled approximately 25 million gallons of waste into New River and Estuary, North Carolina, stimulating toxic and persistent algal blooms (Mallin 2000). In comparison, the Exxon-Valdez disaster in 1989 spilled 11 million gallons of oil into Alaska's Prince William Sound (NRC 2001). Ammonium from the animal waste stored in lagoons and pumped onto sprayfields also volatilizes into the atmosphere and is then deposited over the landscape as atmospheric deposition (Mallin 2000). A proportionately greater amount of ammonia is lost by these operations compared to when cattle are diffusely spread across pasture lands (Smetacek et al. 1991). A study in Sampson County, North Carolina demonstrated a concurrent rise in atmospheric ammonia and swine population from 1990 to present (Mallin 2000). Linear regression analysis indicated that 72 % of the variability in the atmospheric ammonia could be explained by increased swine populations (Mallin 2000).

Cultivation of N_2-fixing crops, such as legumes (e.g. soybeans, peas, alfalfa), increases the conversion of N_2 to fixed N by increasing the rate of biological N-fixation (Galloway et al. 1995). Agricultural production of N_2-fixing crops replaces natural communities causing intensively managed regions to become

a source of biologically available N in excess of that typically produced by native vegetation (Vitousek et al. 1997). The most recent estimate of N fixation by such cropping systems is estimated from 32 to 53 Tg N yr^{-1}, with an average of 40 Tg N yr^{-1} (Galloway et al. 1995; Vitousek et al. 1997).

Atmospheric

Anthropogenic consumption of fossil fuels along with industrial and agricultural discharges of N-containing gases, aerosols, and airborne particles into the atmosphere are having a great impact on the N loading of estuaries through wet and dryfall deposition (Paerl 1993). Current estimates for the proportion of new N derived from direct atmospheric deposition and indirect deposition (i.e. wet and dryfall over the landscape that collects in runoff and is channeled into estuaries) range from 10 to over 50% depending on location, hydrological regimens and human activities (i.e. agriculture, industrial discharge, urban waste water discharge) (Paerl et al. 1993). Waters adjacent to less populated, non-industrialized regions tend to lie towards the lower end of the range while waters near urbanized, industrialized and extensive agricultural activities tend to lie near the 50% plus end of the range (Paerl et al. 1993). Affected regions have annual N atmospheric deposition rates ranging from 20 to 100 mmol $m^{-2}yr^{-1}$ which is a function of the human population growth and urbanization, industrialization and agricultural expansion worldwide (Paerl 1993).

A large proportion of N in atmospheric deposition has a chemical composition that is readily utilizable by aquatic plants such as phytoplankton (e.g. nitrous oxides, nitrates, nitrites, ammonia and ammonium) (Paerl 1993). The combustion of fossil fuels releases fixed N via oxidation of organic N stored in fossil fuels and by fixing N_2 under high temperatures and high pressures of combustion (Carpenter et al. 1998). Fossil fuel combustion generates large amounts of nitrous oxides (NO_x) which form nitrite or nitrate anions when dissolved in water, both of which serve as plant nutrients (Vitousek and Matson 1993; Paerl 1993). Approximately 20 to 25 Tg N yr^{-1} of fixed N is released from combustion of coal, petroleum products and natural gas globally (Vitousek and Matson 1993; Prospero et al. 1996; Galloway et al. 1995; Vitousek et al. 1997).

Volatilization of N compounds ammonia, ammonium, and nitrous oxide into the atmosphere from agricultural practices also contributes a form of biologically available N to atmospheric deposition (Paerl 1993). Manures from swine, cattle, and poultry applied to land can lose 70 to 80% of their ammonia to the atmosphere through volatilization (Sommer et al. 1993 quoted in Paerl 1997). Volatilization of fertilizers is influenced by temperature and pH of soils along with the type of fertilizers used (Schlesinger and Hartley 1992; Bouwman et al. 1997). N losses via volatilization tend to be greater for ammonia, urea and ammonium-sulfate (Schlesinger and Hartley 1992). At a pH below 5.5 there is less than 2% N loss due to fertilizer volatilization while at high pH, above 7.4, fertilizer volatilization can cause up to 50% N loss (Whitehead and Raistrick 1990).

Distance between the source of N atmospheric deposition and the receiving waters can be substantial ranging from 10 to > 1000 kilometers depending on the sources and chemical forms of the N containing compounds (Propero et al. 1996; Paerl and Whitall 1999). As a result, the contribution of atmospheric deposition to the water quality issue of nutrient pollution could be regional and possibly even global in nature (Galloway et al. 1995; Paerl and Whitall 1999).

Urban

Sources of N associated with urbanization include, but are not limited to, municipal waste treatment effluent, construction sites, runoff of fertilizers applied to lawns, septic tank leachates, waste disposal site leachate and overflows of combined storm and sanitary sewers (Carpenter et al. 1998). The contribution of these sources is largely dependant on the population size versus the surface area of the estuary (NRC 2000). For example, wastewater effluents into Long Island Sound account for 60% of the N loading (NRC 2000) while the same source only accounts for 10% of the total N flux into the mouth of the Mississippi River (Howarth et al. 1996) and 25% into Chesapeake Bay (Nixon et al. 1996; NRC 2000).

Mobilization By Human Activities

Human activities also liberate N from long-term biological storage pools, such as plants and soils, in addition to enhancing N_2-fixation of new N (Vitousek et al. 1997). Biomass burning mobilizes between 15 and 46 Tg N from terrestrial vegetation to the atmosphere (Crutzen and Andreae 1990). Land clearing and conversion to agricultural lands mobilizes 10 Tg N yr^{-1} (Vitousek et al. 1997). Mature forests tend to hold tightly to N, therefore, their removal can mobilize N (Nixon 1993). Draining wetlands and the subsequent oxidation of their organic soil mobilizes 1.5 to 18 Tg N yr^{-1} (Vitousek and Matson 1993; Vitousek et al. 1997). Furthermore, wetlands play a significant role in removing N via denitrification and incorporating it into OM, therefore, loss of wetlands further increases N mobility to estuaries (Howarth et al. 1996; Vitousek et al. 1997).

Susceptibility of Estuarine and Coastal Systems to Nutrient Pollution

The extent to which excessive N loads affect a particular estuary or coastal environment varies greatly (Pennock et al. 1994; Bricker et al. 1999). Varying levels of N loading can evoke very different responses because different estuaries and coastal environments have varying degrees of sensitivity to nutrient enrichment (NRC 2000). Sensitivity is determined by assimilation capacity, or the ability to absorb nutrients (Bricker et al. 1999). Factors that determine an estuarine and

coastal ecosystem response to high nutrient loads are hydrologic, physical and biological in nature.

Hydrologic Factors

The primary hydrologic factor is freshwater discharge, which subsequently affects flushing and water residence time in an estuary. Freshwater discharge affects nutrient concentrations in an estuary through dilution of nutrient concentrations (Pennock et al. 1994). Estuaries receiving high nutrient loads and high freshwater discharges tend to have low nutrient concentrations which limit primary production (Pennock et al. 1994). However, estuaries receiving high nutrient loads and low freshwater discharge tend to have high nutrient concentrations that support high rates of primary production (Pennock et al. 1994). Therefore, larger volumes of freshwater discharge dilute the nutrient load which decreases the maximum biomass attainable (Pennock et al. 1994). Freshwater discharge can also affect sunlight penetration through the water column, which can limit photosynthesis. At low discharges, sunlight tends to penetrate more deeply, extending the photic zone to lower depths, probably because there are fewer particles resuspended and smaller discharges of sediment from the watershed (Howarth et al. 2000).

Flushing time is the time required to replace the existing freshwater in an estuary at a rate equivalent to river discharge (Officer 1976; Dyer 1997) and is

related to freshwater discharge and tidal mixing (Church 1986; Miller and McPherson 1991; Pennock et al. 1994; Slinger et al. 1994; Bricker et al. 1999). Estuaries with faster flushing times will remove nutrients more quickly and therefore tend to be less susceptible to nutrient pollution because nutrients are diluted and removed (Bricker et al. 1999; Smetacek et al. 1991).

Water residence time, also referred to as the retention time, is the average time water remains in an environment (Mitsch and Gosselink 1993) and is related to the flushing time (Church 1986). Water residence times in coastal water bodies range from a few days to months (Nixon et al. 1996), while phytoplankton divide about 0.5 to 3 times a day, which is partly a function of the supply of limiting nutrients such as N (Valiela et al. 1997b). In estuaries and coastal waters where water residence time is short due to high discharge and short flushing times, nutrients will more likely be flushed out of the system decreasing N available for phytoplankton growth (Valiela et al. 1997b; Howarth et al. 2000; Bricker et al 1999). However, in estuaries where water residence time is long due to low discharge and long flushing times, nutrients tend to spend more time in those areas allowing N to accumulate and support phytoplankton growth (Valiela et al. 1997b; Bricker et al. 1999; Howarth et al. 2000). For example, if both water residence time of an estuary and phytoplankton turnover time, the times it takes for the phytoplankton population to double, were hypothetically one day, then a bloom cannot occur because the phytoplankton population and nutrient load will be advected out of the system at the same rate they are producing new generations thus

limiting any potential bloom through dilution (Howarth et al. 2000; NRC 2000). As water residence time increases above one day, phytoplankton blooms are more likely to occur because more generations are produced before being flushed out of the system, assuming the appropriate factors such as nutrients and sunlight are available above limiting levels (Howarth et al. 2000; NRC 2000). Anthropogenic engineered water flows, such as dams, can reduce freshwater discharge into an estuary which decreases the flushing time and increases the residence time therefore making the estuary more susceptible to nutrient pollution (Bricker et al. 1999).

Physical Factors

Physical factors affecting the response of an estuarine ecosystem to nutrient enrichment include: mixing, stratification, dilution and tides. Dilution results from a variety of mixing processes that occur when nutrient loads from a watershed enter an estuary (NRC 2000). As the nutrient load is entrained into the water, it disperses and will have a different effect whether it is distributed into a water volume of 10^6 m^3 or 10^{10} m^3 because of the difference in resulting nutrient concentrations (NRC 2000). However, the degree to which a nutrient is diluted in an estuary depends on whether the estuary is well-mixed or stratified. In vertically homogenized or well-mixed water columns of estuaries, nutrient loads are more likely to be diluted; but

in stratified water columns, nutrients are assumed to remain in the upper freshwater portion (Bricker et al. 1999).

Mixing can also affect the distribution of phytoplankton in the water. In deeper, turbid estuaries mixing can push phytoplankton into deeper areas of the photic zone where there is insufficient light to support photosynthesis in excess of phytoplankton respiration (Howarth et al. 2000). In stratified water columns with little or no mixing, phytoplankton remain buoyed at the water surface where light no longer limits photosynthesis (Howarth et al. 2000; NRC 2000).

Stratification plays a role in determining the extent to which oxygen depletion affects marine ecosystems. Oxygen depletion is not always a product of eutrophication; it requires hydrographic conditions such as stratification in order to isolate a water mass from reaeration (Møller 1996). A water column is said to be stratified when the density varies with depth and density is a function of temperature and salinity (Møller 1996). Generally, there are two ways stratification can occur in an estuary. First, under slow flushing times, solar heating of the surface waters can generate a warmer and lighter surface layer thus stabilizing the water column and hampering downward movement of oxygen (Nixon 1993; Møller 1996). Second, lighter freshwater entering an estuary from a riverine input tends to flow on top of the heavier salt water thus creating a stratification due to salinity differences (Nixon 1993; Møller 1996). As a result of stratification, vertical exchange is limited, thus spatially separating primary productivity at the surface and mineralization in the bottom waters (Boicourt 1992; Møller 1996). Bottom

waters will become more depleted in oxygen as OM from the surface waters decomposes (Boicourt 1992; Møller 1996; NRC 2000). Because the density gradient inhibits mixing of layers, stratification prevents reaeration of bottom waters (Møller 1996). The degree of stratification depends on the balance between factors increasing the buoyancy of surface waters (i.e. salinity and temperature) and the energy available for mixing water layers of different densities (e.g. tidal energy) (Nixon 1993).

Tidal mixing changes stratification and flushing characteristics inhibiting the effects of nutrient pollution (Geyer et al. 2000). Furthermore, tidal currents can stir up and suspend bottom sediments reducing water clarity and sunlight penetration, which can inhibit photosynthesis (Geyer et al. 2000). Tidal influence is most pronounced when freshwater discharge is low (Howarth et al. 2000). When the tidal amplitude is low during low discharge, higher rates of gross primary production, longer water residence times, greater stratification and deeper light penetration are observed (Howarth et al. 2000).

Biological Factors

Biological factors influencing estuarine ecosystem responses include trophic interactions and nutrient processing in the estuarine and coastal environment. Benthic filter feeders clear particles from the water column and limit accumulation of algal biomass (Cloern 1982). Depending on the water depth and

density of the benthic population, benthic filter feeders can recycle water more rapidly than can the hydrodynamic residence time and at rates comparable to phytoplankton growth (Officer et al. 1982). In the Northern San Francisco Bay area, bivalve suspension feeders may be responsible for persistent and widespread reduction of phytoplankton biomass (Alpine and Cloern 1992). Furthermore, increased phytoplankton biomass was observed during periods when benthic suspension feeders were absent or scarce (Alpine and Cloern 1992). Filter feeders, therefore, are capable of raising an estuary's assimilation capacity (Bricker et al. 1999). Also, grazers of phytoplankton and macroalgae may control blooms and help maintain dominance of macrophytes like seagrasses (Valiela et al. 1997b).

Processing of nutrient loads by estuarine and coastal wetlands, marshlands and mudflats contributes to the assimilative capacity of these environments. First, the primary production base determines how much nutrient is removed from the water for assimilation into plant biomass. Primary production base refers to the various primary producers, each of which has unique temperature, substrate, light and nutrient requirements and, therefore, responds differently to enhanced nutrient loads (NRC 2000). Different estuaries will promote different communities of primary producers such as emergent marshes and swamps, attached intertidal algae, seagrasses and phytoplankton (NRC 2000). Each of these communities cope with enhanced nutrient loads differently thus creating a variation in estuarine susceptibility or assimilation capacity. For example, seagrasses are less dependent

on water column nutrient levels than are phytoplankton and ephemeral macroalgae with their rapid nutrient uptake potentials (Sand-Jensen and Borum 1991).

Wetlands, marshlands and mudflats adjacent to estuaries and coastal waters, can remove N before they reach the latter environments via denitrification and biological uptake (Correll et al. 1992; Howarth et al. 1996; Vitousek et al. 1997). Denitrification is the process whereby nitrate and/or nitrite is converted to N_2 during the oxidation of OM by anaerobic bacteria (Seitzinger 1988). This process may help control the degree of eutrophication by decreasing the amount of anthropogenically derived N transported from the landscape to coastal water bodies (Seitzinger 1988). Reported rates of denitrification in estuarine and coastal areas have ranged from 0 to 1.067 mmol N $m^{-2}h^{-1}$, the highest rates have occurred in eutrophic conditions (Seitzinger 1988). Denitrification is greater in shallow systems with longer water residence times because these environments allow for greater contact of N in waters with the required anoxic environment for this process to occur (NRC 2000). Human alterations to these habitats, including dredging and draining, change the nutrient assimilatory capacity of these systems (Bricker et al. 1999).

Status of Nutrient Pollution Management

Nutrient pollution may perhaps be the oldest water quality problem caused by humans (Vollenweider 1992; Richardson and Jørgenson 1996). Because

nutrient pollution is predominately a nonpoint source problem, with the exception of sewage outfalls, it is more difficult to control compared to point sources (Pelley 1998). Furthermore, mandatory control efforts are rejected by N contributors, such as agricultural producers and industry, because of the lack of information concerning sources of N loads to coastal water bodies (Pelley 1998). Also, implementation of voluntary efforts has mostly failed (Pelley 1998). For example, voluntary goals for reducing nutrients into the Tar-Pamlico estuary in North Carolina were unmet two years after the program began and best management practices were instituted on only 13% of the agricultural lands (Pelley 1998). Implementing efficient and cost-effective control measures requires information concerning the sources of N inputs.

Currently, there are no reliable methods for identifying sources of nutrient pollution available to managers working with this water quality problem (NRC 2000). Assessment of nutrient pollution has relied on taxonomic shifts and changes in the relative abundances of producers and consumers (McClelland et al. 1997). While these indicators may prove to be useful, they rely on changes that have already occurred thus providing only an *a posteriori* assessment (McClelland et al. 1997). Restoration of these habitats is difficult once these changes have occurred (McClelland et al. 1997). Identification of N sources when rising N levels are still relatively low would allow management responses to become more effective in protecting these critical environments (McClelland et al. 1997). So far nutrient sources to estuarine and coastal waters have been poorly or even incorrectly

characterized (NRC 2000). As a result, there is a demand for improved approaches or methodologies for elucidating the sources and magnitude of nutrient loads (NRC 2000). The development of techniques to measure N stable isotopes may provide the first step to creating such a method. N stable isotopes have the potential for providing information on nutrient sources and magnitudes of anthropogenic nutrient loads through unique isotopic signatures characterized by the N source.

Chapter 2: Using N Stable Isotopes to Identify Sources of Nutrient Pollution

The stable isotopes of N on earth, ^{14}N and ^{15}N, occur in a fixed global proportion of approximately 273 ^{14}N to every one ^{15}N (Peterson and Fry 1987; McClelland et al. 1997). However, isotopic ratios of ^{15}N and ^{14}N in each N pool of the environment differ (Peterson and Fry 1987; McClelland et al. 1997) because slight differences in the atomic masses of ^{14}N and ^{15}N causes biological, physical and chemical processes to preferentially discriminate between one or the other isotope (Hoefs 1987; Fogg et al. 1998). As a result, products become enriched in the preferred isotope and reactants are left enriched in the other isotope (Fogg et al. 1998). This isotopic fractionation yields N products with different isotopic ratios (Figure 2) (Fogg et al. 1998). Isotopic compositions of inorganic N are therefore a function of its source and any isotopic segregation or fractionation that may have occurred during its generation and/or transport to receiving waters (Macko & Ostrom 1994). Thus, N stable isotopes have the potential to provide information on sources of N pollution and their pathways into estuarine and coastal ecosystems (Figure 3). However, in order for N stable isotopes to be utilized as a tracer of nutrient pollution, primary sources of interest must be isotopically distinct from each other and isotopic signatures of nutrient sources must not change as materials are transported and transformed in the environment, or they must change in a predictable manner (Owens 1987; Macko and Ostrom 1994).

Figure 2. Summary of $\delta^{15}N$ values for N sources (Heaton 1986; Freyer and Aly 1974; Kreitler and Jones 1975; Gormly and Spalding 1979; Kreitler 1979; Heaton et al. 1983; Kreitler and Browning 1983; Aravena et al. 1993; Paerl 1993; Macko and Ostrom 1994).

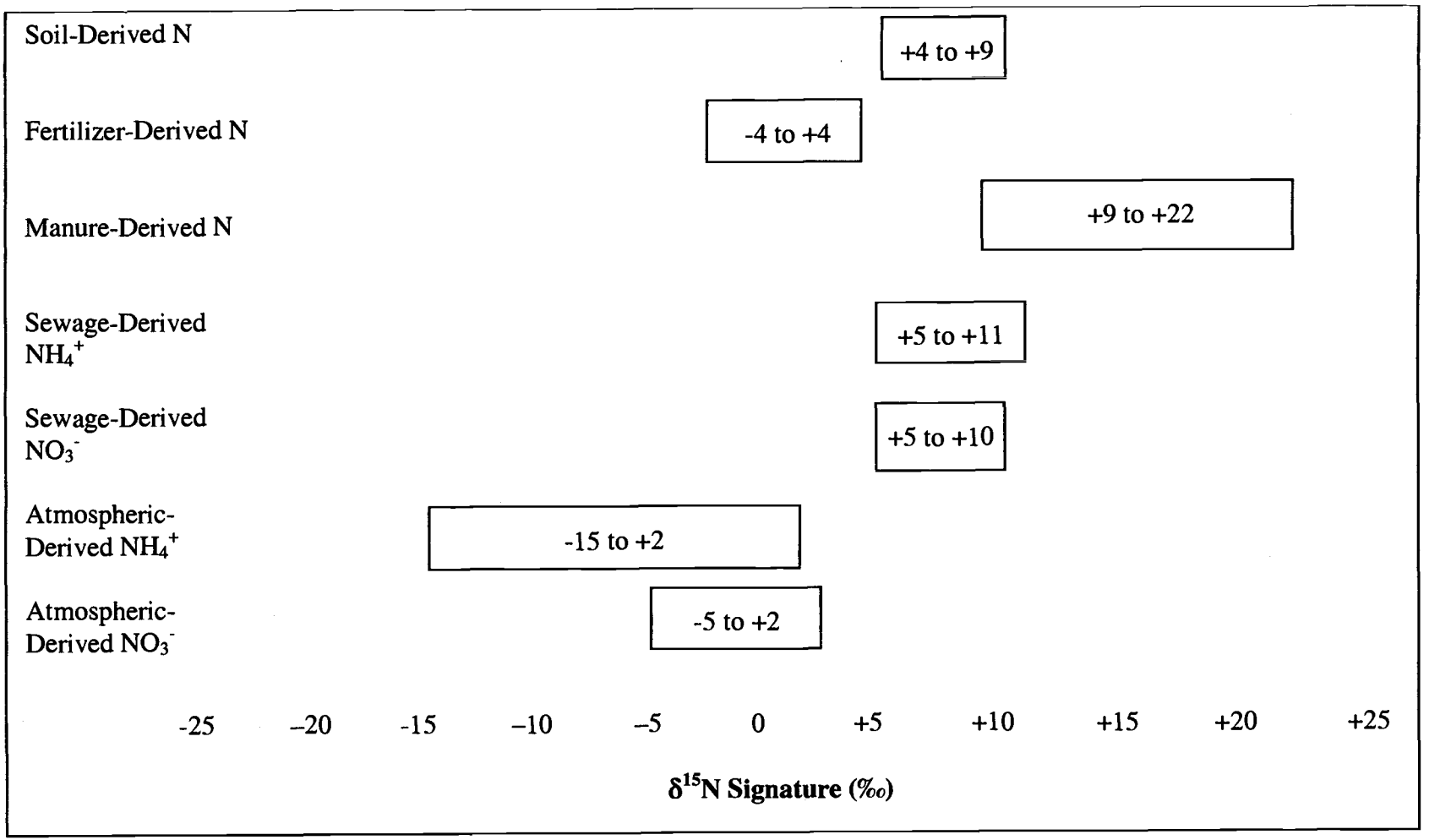
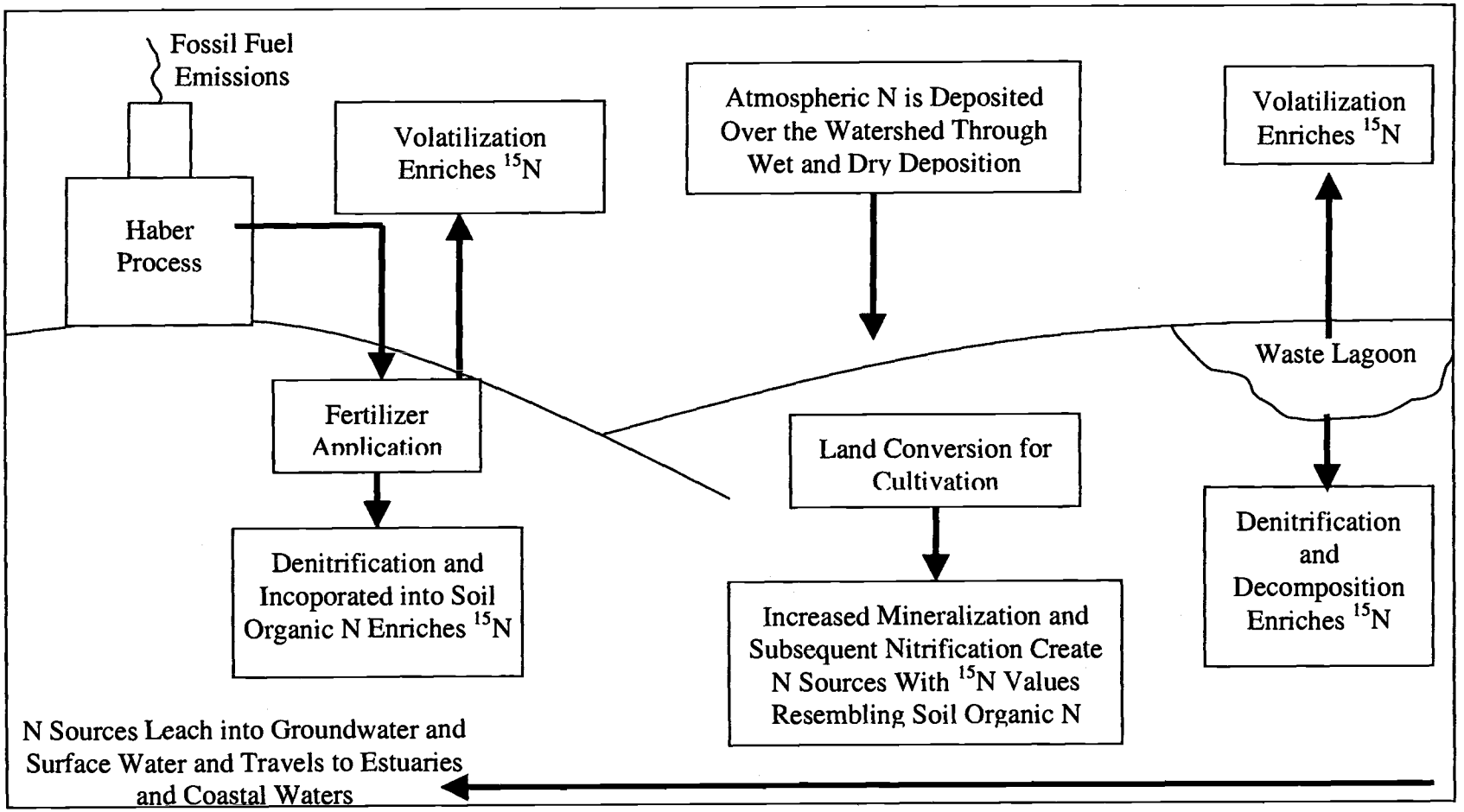


Figure 3. Summary of N sources and the processes affecting their $\delta^{15}N$ values (Freyer and Aly 1975; Kreitler and Jones 1975; Meints 1975; Gormly and Spalding 1979; Kreitler 1979; Heaton et al. 1983; Kreitler and Browning 1983; Heaton 1986; Aravena et al. 1993; Macko and Ostrom 1994).



The ratio of heavy and light isotopes in a sample is measured with a mass spectrometer and is expressed in del (δ) notation with per mil (‰) units:

$$\delta^{15}N\ (‰) = [(R_{sample}-R_{standard})/R_{standard}] \times 1000 \text{ or } (R_{sample}/R_{standard}-1) \times 1000$$

where R represents the ratio ^{15}N to ^{14}N and the standard is atmospheric N (Lajtha and Michener 1994). A sample enriched in ^{15}N relative to atmospheric N has a positive $\delta^{15}N$ value while samples depleted in ^{15}N relative to atmospheric N have negative values (Kreitler and Jones 1975).

Ground and Surface Water Application

During the early 1970's, Kohl et al. (1971) produced the first paper to use N stable isotopes to distinguish dissolved N sources in the Sangamon River watershed of Illinois. This research was initiated by concern over rising nitrate levels in surface waters in the United States corresponding with increased application of fertilizers. Kohl et al. (1971) measured natural enrichment of ^{15}N in soil-derived nitrate, fertilizer-derived nitrate and surface water nitrate along with concentrations of nitrate in surface waters to determine fractional responsibility of fertilizer application to rising levels of nitrate in surface waters. They found that a minimum of 55 to 60% of nitrate-N concentrations in surface waters in the Sangamon River watershed during spring 1970 originated from fertilizer (Kohl et al. 1971). These results were then used in a testimony during a hearing on legislation to regulate

agricultural use of fertilizers (Bremner and Tabataboi 1973). However, this research also faced a barrage of peer criticism. Most of it was based on concerns over the absence of information regarding natural ^{15}N abundance in the environment and isotopic fractionation in N cycling processes (Hauck et al. 1972; Meints et al. 1975).

High nitrate levels in excess of the World Health Organization and the United States Environmental Protection Agency recommended limit and national drinking water standards (10mg/L) in both surface and groundwaters (Kendall 1998) prompted continued development of N stable isotopic research despite criticism given to Kohl et al. High nitrate levels were a concern because of its toxicity to both humans and animals through conditions such as methaemoglobinaemia (Heaton et al. 1986; Macko and Ostrom 1994; Kendall 1998).

Enhanced N levels in ground and surface waters have been attributed to urbanization, cultivation of soils, livestock operations and waste disposal such as septic tanks (Kreitler 1979). Amounts of excess N in ground and surface waters from primary sources in any given region largely depend on dominant land uses in that region (Kreitler 1979). Three main sources of excess N in surface and groundwater have been recognized: (1) enhanced mineralization of soil organic N resulting from conversions of "virgin" lands for cultivation, (2) addition of nitrogenous fertilizers, and (3) disposal of concentrated animal or human sewage

(Gormly and Spalding 1979; Kreitler 1979; Kreitler and Browning 1983; Heaton 1986; Macko and Ostrom 1994).

Soil-Derived N

Clearing for cultivation of agricultural lands can increase rates of mineralization, the process whereby organic material (OM) is converted into ammonium (Figure 3) (Heaton 1986; Macko and Ostrom 1994). Ammonium under aerobic conditions could then undergo nitrification thus producing nitrate, a more mobile compound that is easily released into groundwater (Kreitler and Jones 1975). Therefore, enhanced mineralization following cultivation of soils could lead to a source of increased nitrate levels in groundwaters (Macko and Ostrom 1994). Nitrate produced from mineralization of OM tends to have a $\delta^{15}N$ value similar to soil organic N, from which it is derived, ranging from +4 to +9‰ (Heaton 1986).

In a groundwater study in Runnels County, Texas, Kreitler and Jones (1975) found that high nitrate levels in groundwater were due predominately to nitrate derived from soil organic nitrogen. Isotopic signatures of nitrate in groundwater samples correlated with those values measured in nitrate derived from mineralization of organic N, ranging from +2 to +8‰ (Kreitler and Jones 1975; Gormly and Spalding 1979; Kreitler and Browning 1983). Nearly every acre of this region had been converted to arable lands since the early 1900's, therefore, high nitrate levels in the groundwater have been a result of oxidation of humic

material that developed in the grasslands that once occupied this region (Kreitler and Jones 1975). Furthermore, the age of the groundwater corresponded with a period of extensive terracing which allowed the groundwater to rise to the surface and increase nitrate leaching (Kreitler and Jones 1975). This study was one of the first to show that soil organic N can serve as a significant source of nitrate to groundwaters.

Fertilizer-Derived N

Most fertilizer N retains $\delta^{15}N$ signatures of atmospheric nitrogen gas (0‰), from which it is derived through the Haber process, which has little fractionation associated with it (Heaton 1986; Macko and Ostrom 1994). However, further processing of N into nitrite, nitrate or urea-based compounds can cause a wider spread of $\delta^{15}N$ values between their compounds and atmospheric N (Macko and Ostrom 1994). For example, ammonium and ammonia fertilizer N tend to have lower $\delta^{15}N$ values compared to the $\delta^{15}N$ value of atmospheric N while nitrate fertilizer N tends to have a higher $\delta^{15}N$ compared to atmospheric N (Freyer and Aly 1974; Freyer and Aly 1975; Kreitler 1979). Reported $\delta^{15}N$ values for fertilizers range from –4 to +4‰ (Freyer and Aly 1974; Gormly and Spalding 1979; Kreitler 1979; Heaton 1986).

The $\delta^{15}N$ signatures of fertilizers can change after application due to volatilization, denitrification, interactions with soil organic N and leaching into

groundwater (Figure 3) (Gormly and Spalding 1979; Macko and Ostrom 1994). Ammonium fertilizers tend to volatilize after application, releasing ammonia into the atmosphere (Kreitler 1979; Macko and Ostrom 1994). Ammonia escaping into the atmosphere is enriched in ^{14}N (depleted in ^{15}N) causing residual ammonium pools to have more enriched $\delta^{15}N$ values compared to values of the fertilizers originally applied (Kreitler 1979; Macko and Ostrom 1994). Kreitler (1979) found that groundwater nitrate was isotopically more enriched with ^{15}N compared to applied fertilizers and attributed this difference to volatilization of ^{15}N-depleted ammonia upon fertilizer application. Altered ammonium fertilizer N that does not volatilize can then be converted to nitrate via nitrification, a process also associated with some fractionation creating nitrate depleted in ^{15}N (Freyer and Aly 1975).

Denitrification of fertilizer-derived nitrate into N_2 results in ^{15}N enrichment of the residual nitrate pools because the N_2 escaping into the atmosphere is enriched in ^{14}N (Freyer and Aly 1975; Kreitler 1979; Gormly and Spalding 1979). Denitrification is not a ubiquitous process and requires presence of denitrifying bacteria, reducing or anoxic conditions, and available labile organic carbon (Macko and Ostrom 1994). Results of an analysis of Nebraskan groundwater samples with high levels of nitrate showed that 75% of the samples had $\delta^{15}N$ values between 3.5 and 9.5‰, an isotopic value that does not allow for source identification according to the authors because the $\delta^{15}N$ values have too broad of a range (Gormly and Spalding 1979). However, environmental conditions such as those found in water-logged soils, where anoxic conditions existed and high amounts of carbon were

present, provided evidence that denitrification was probably responsible for variations observed in groundwater $\delta^{15}N$ values (Gormly and Spalding 1979). A 20% nitrate loss can result in a ^{15}N enrichment of up to 8‰ (Gormly and Spalding 1979).

Fertilizer-derived N can also become integrated into the soil-plant system via biological transformations causing it to lose its distinct isotopic signature (Meints et al. 1975; Heaton 1986). Meints et al. (1975) found that increasing additions of fertilizer N, with $\delta^{15}N$ values around 0‰, to soils had no impact on $\delta^{15}N$ values of soil N. They concluded that since soil N is a larger pool compared to the amount of N in the applied fertilizers, the $\delta^{15}N$ signatures of fertilizers simply do not contribute greatly to the processes that enrich soils with ^{15}N (Meints et al. 1975). Furthermore, as fertilizer N cycles through soil organic N pools it becomes enriched causing it to lose its distinct isotopic signature (Meints et al. 1975). However, over long-term fertilizer application, soil $\delta^{15}N$ values will inevitably change because of continuous fertilizer inputs and the consequent decrease in natural soil organic N that was present before the fertilizer applications occurred (Kreitler 1979).

Animal- and Sewage Waste-Derived N

Animal- and sewage-derived N tend to have more enriched $\delta^{15}N$ values, ranging from +9 to +22‰, due to fractionation during volatilization of ammonia,

organic N decomposition and denitrification (Figure 3) (Kreitler 1975 quoted in Kreitler 1979; Kreitler and Jones 1975; Gormly and Spalding 1979; Kreitler 1979; Heaton et al. 1983; Kreitler and Browning 1983; Heaton 1986; Aravena et al. 1993; Macko and Ostrom 1994). The extent of these reactions controls the degree of fractionation that occurs and, therefore, the range of $\delta^{15}N$ values for waste-derived N (Kreitler 1979). The $\delta^{15}N$ values of fresh animal excretion (between +1.7 and +4.8‰) typically are not distinguishable from values of soil-organic-derived (between +4 and +9‰) and fertilizer-derived (between –4 and +4‰) N (Freyer and Aly 1974; Gormly and Spalding 1979; Kreitler 1979; Heaton 1986; Macko and Ostrom 1994). Soon after the waste is deposited on the ground, ^{14}N enriched ammonia quickly begins to volatilize into the atmosphere causing the remaining ammonium to become enriched in ^{15}N (Kreitler 1979; Gormly and Spalding 1979; Heaton 1986; Macko and Ostrom 1994). Residual ammonium pools enriched in ^{15}N undergo nitrification, producing a nitrate product generally greater than 10‰ (Kreitler and Jones 1975; Kreitler 1979; Macko and Ostrom 1994). This nitrate can be further enriched due to preferential use of the light isotope during denitrification (Kreitler 1975 quoted in Kreitler 1979; Aravena et al. 1993).

The $\delta^{15}N$ values of nitrate collected from septic tank plumes were enriched in ^{15}N compared to values of nitrate in surrounding groundwater samples (Aravena et al. 1993). The $\delta^{15}N$ values for groundwater nitrate collected from a region with domestic septic tanks in Ontario, Canada were consistent with that of N from human waste that has been enriched due to volatilization of ammonia and

conversion of waste into nitrate via nitrification (Aravena et al. 1993). However, $\delta^{15}N$ values ranging from +8 to +14‰ for nitrate from septic systems generally tend to be at the lower end of the range for waste-derived nitrate because volatilization can often be inhibited in septic tanks and/or drainage tiles (Aravena et al. 1983; Komor and Anderson 1993). As rural areas become more urbanized, onsite treatment systems such as septic tanks have been used where municipal sewers are absent (Aravena et al. 1993). If this trend continues, this may become a more important source of nutrient pollution.

Estuarine and Coastal Water Application

Application of amounts of N stable isotopes to identify sources of nutrient pollution in estuarine and coastal waters has been mostly limited to food web analyses where the isotopes were used to detect anthropogenic sources of N entering the food web. In a study in Waquoit Bay, Massachusetts (McClelland et al. 1997), primary producers and consumers from five different estuaries associated with subwatersheds experiencing different degrees of wastewater loading were compared. Wastewater is considered the largest source of N into many coastal regions, due to increasing urbanization (McClelland et al. 1997). Earlier work indicated that $\delta^{15}N$ signatures of primary producers reflect the $\delta^{15}N$ of their inorganic sources along with some amount of fractionation during N uptake (Fogel and Cifuentes 1993). In general, primary producer $\delta^{15}N$ values increased with

increasing wastewater loading (McClelland et al. 1997). The $\delta^{15}N$ values of eelgrass and macroalgal species showed considerable variability, which could be attributed to mixing of different N sources (fertilizers and atmospheric deposition are other possible sources of N to these estuaries) (McClelland et al. 1997). Furthermore, the amount of ^{15}N enrichment in different producers, including eelgrass, phytoplankton, macroalgae and cordgrass, varied in response to increased wastewater loading (McClelland et al. 1997). This may have been a result of different fractionation processes or because the different producers rely predominately on either N in the water column (e.g. phytoplankton) or in the sediments (e.g. eelgrass) (McClelland et al. 1997). As a result, wastewater N may not be as influential an N source for some producers (McClelland et al. 1997). The $\delta^{15}N$ signatures of consumers reflected values of producers along with enrichment of ^{15}N due to fractionation caused by preferential metabolism of ^{14}N (McClelland et al. 1997). Differences in $\delta^{15}N$ values of consumers between the estuaries were similar to respective differences found between producers, indicating that increased wastewater loading was identified in both primary producers and consumers (McClelland et al. 1997).

In subsequent studies, $\delta^{15}N$ of groundwater nitrate and ammonium entering Waquoit Bay was analyzed to demonstrate the increasing importance of wastewater as a source of N to the bay (McClelland and Valiela 1998). The $\delta^{15}N$-nitrate values for groundwater originating from the unaltered forested portion of the watershed ranged from –1.5 to +4.5‰, typical of rainwater inputs (McClelland and Valiela

1998). The $\delta^{15}N$-nitrate values for groundwater originating from portions of the watershed with higher densities of septic tanks were more enriched in ^{15}N (McClelland and Valiela 1998). The $\delta^{15}N$ for nitrate delivered to the estuary associated with the areas containing a higher density of septic tanks was 11‰ higher compared to the estuary receiving groundwater from the unaltered forested watershed (McCleland and Valiela 1998). Furthermore, $\delta^{15}N$ values of producers corresponded with $\delta^{15}N$ values of groundwater nitrate (McClelland and Valiela 1998).

In Saldanha Bay, South Africa, $\delta^{15}N$ signatures of primary producers were used to determine sources of excess N that may have caused an *Ulva* algal bloom that replaced the economically important red alga, *Gracilaria varrucosa* (Monteiro et al. 1997). The potential anthropogenic sources of N included sewage treatment effluent, a desmersal fish (hake) processing plant and a pelagic fish (anchovy and pilchard) processing plant (Monteiro et al. 1997). The signature of the *Ulva* bloom species was enriched in ^{15}N (10.8‰) compared to *Ulva* in other bays (Monteiro et al. 1997). Furthermore, the isotopic signature resembles that measured for muscle tissue found in the anchovy factory's waste products released from the processing plant (Monteiro et al. 1997). Leftover fish pieces are released into the bay where they are remineralized into ammonium, a N form that the blooming *Ulva* species is more efficient in utilizing relative to uptake rates of the displaced red algal species (Monteiro et al. 1997). Thus, $\delta^{15}N$ signatures of primary producers were used to elucidate N sources causing an algal bloom.

These early groundwater and estuarine food web studies hint at the potential of applying stable N isotopes to determine sources of dissolved inorganic N in estuarine and coastal waters experiencing nutrient pollution. Therefore, the purpose of my research was to develop a reproducible method for isolating inorganic nitrate and ammonium from estuarine and coastal waters, which can also be applied to other sample types such as groundwater, river water, rainwater and soil solutions. This method could then be used to elucidate sources and pathways of excess N nutrients to assist resource managers faced with the problem of nutrient pollution. Despite the potential for this application, difficulties in developing this method confound the reliability of using N stable isotopes as a management tool. Furthermore, natural variability of isotopic ratios, as a result of fractionation at each step of the N cycle, can alter anthropogenically derived N pools, further shadowing prospects of confidently using this type of data as a basis for enforceable management measures or even legislation without some concurring data to support the isotopic evidence.

Chapter 3: Methods: Isolating Dissolved Inorganic Nitrogen from Estuarine Waters for Isotopic Analysis

Isotopic measurements of dissolved inorganic nitrogen (DIN) pools are more complex to measure compared to particulate N pools because dissolved ammonium and nitrate must be removed from solution and concentrated and converted into a form that can be analyzed by a mass spectrometer (Kristiansen and Paasche 1989; Holmes et al. 1998). Furthermore, quantitative conversion is required at every step of the procedure to avoid the effect of isotopic fractionation (Shearer and Kohl 1993). The two main methods used to isolate these nitrogen species from environmental samples (i.e. soil extracts, freshwater and seawater) are: 1) steam distillation and 2) ammonia diffusion.

Steam Distillation

For the distillation method, NaOH or MgO are added to water samples to raise the pH above 9 facilitating the conversion of ammonium to ammonia (Cline and Kaplan 1975; Glibert et al. 1982; Hauck 1982; Velinsky et al. 1989). Ammonia, the volatile species, is then steam-distilled into a receiving flask where the condensate is captured by an acid trap (Cline and Kaplan 1975; Harrison 1978; Glibert et al. 1982; Liu et al. 1996) or an acid-zeolite molecular sieve slurry (Velinsky et al. 1989; Horrigan et al. 1990). When using an acid trap, the distillate volume is usually reduced by evaporation, then the ammonium is converted to N_2

gas for isotopic analysis by mass spectrometry (Cline and Kaplan 1975; Harrison 1978; Glibert et al. 1982; Liu et al. 1996). When using the zeolite molecular sieve, H^+ is exchanged for ammonium, capturing it onto the molecular sieve (Velinsky et al. 1989). The zeolite is collected on a glass fiber filter and combusted to N_2 gas for isotopic analysis (Velinsky et al. 1989; Horrigan et al. 1990). This method requires quantitative distillation of the ammonia and quantitative ion exchange of ammonium onto zeolite for accurate isotopic measurement (Velinsky et al. 1989). In order to remove nitrate from a sample for isotopic analysis, Devarda's alloy, an alloy of 45% Al, 50% Cu and 5% Zn (Svehla 1987), is added to the sample solution before distillation to reduce the nitrate to ammonium (Cline and Kaplan 1975; Hauck 1982; Svehla 1987; Liu and Kaplan 1989; Horrigan et al. 1990; Liu et al. 1996):

$$NO_3^- + 4Zn + 7OH^- + 6H_2O \rightarrow NH_3\uparrow + 4[Zn(OH)_4]^{2-}$$

$$3\ NO_3^- + 8Al + 5OH^- + 18H_2O \rightarrow 3NH_3\uparrow + 8[Al(OH)_4]^-$$

Ammonia Diffusion

With the diffusion method, NaOH or MgO is again used to raise the pH of the sample solution above 9 for the conversion of ammonium into ammonia which is then collected via a gas-phase diffusion onto an acidified glass filter disk that is either suspended over the sample solution or encased in a teflon packet floating on the solution surface (Brooks et al. 1989; Kristiansen and Paasche 1989; Kelley et

al. 1991; Sørensen and Steen-Jensen 1991; Lory and Ruselle 1994; Slawyk and Raimbault 1995; Sigman et al. 1997; Holmes et al. 1998; Kahn et al. 1998; Downs et al. 1999). Devarda's alloy is also added to the sample solution to reduce nitrate to ammonium (Brooks et al. 1989; Sørensen and Steen-Jensen 1991; Lory and Ruselle 1994; Slawyk and Raimbault 1995; Sigman et al. 1997; Kahn et al. 1998; Downs et al. 1999). The ammonium is then converted to ammonia due to the basic conditions and the ammonia is captured on the acidified disk (Brooks et al. 1989; Sørensen and Steen-Jensen 1991; Lory and Ruselle 1994; Slawyk and Raimbault 1995; Sigman et al. 1997; Kahn et al. 1998; Downs et al. 1999). This entire process occurs within a sealed container at either room temperature (Brooks et al. 1989) or in an oven of about 65 to 70°C (Sigman et al. 1997).

Method Errors

Both the steam distillation and the diffusion methods have drawbacks. First, both methods have considerable N blanks associated with the process of extracting DIN compounds from sample solutions. The reagents used have been known to contribute an N blank that can alter the results of the isotopic analysis. The Devarda's alloy can contribute the most significant blank, which can vary between manufacturer and lot number (Liu et al. 1996; Sigman et al. 1997). MgO can also contribute N, but this reagent can be baked in order to reduce its

contribution to the blank (Slawyk and Raimbault 1995; Sigman et al. 1997; Holmes et al. 1998).

Dissolved organic nitrogen (DON) has been suspected to hydrolyze to ammonia due to the basic conditions and higher temperatures thus contributing another blank (Kristiansen and Paasche 1989; Velinsky et al. 1989; Sigman et al. 1997; Holmes et al. 1998). Using MgO instead of NaOH to raise the pH of the sample solution can reduce the size of the DON blank because the MgO buffers the seawater at a lower pH (~9.7) compared to NaOH (can be up to 13) (Velinsky et al. 1989; Slawyk and Raimbault 1995; communications with M.A. Altabet cited in Liu et al. 1996; Sigman et al. 1997). Also, it has been demonstrated in soil and water samples that more labile organic N is hydrolyzed by distillation than by diffusion due to the higher temperatures required for steam-distillation (Mulvaney and Khan 1998).

Lastly, cross contamination between samples via the glassware can contribute an N blank, however, this can be reduced by properly washing glassware with an acid solution between sample processing (Hauck 1982; Brooks et al. 1989;Velinsky et al. 1989; Kelley et al. 1991; Saghir et al. 1993; Khan et al.1998). The steam-distillation equipment can have an N blank that originates in the steam generator which is subject to contamination from previous samples and is not easily accessible (Hauck 1982; Brooks et al. 1989; Velinsky et al. 1989). Additionally, steam-distillation methods using long drying periods to reduce the volume prior to isotopic analysis and after collecting the distillate in an acid trap are subject to an N

blank caused by contamination from the air (Hauck 1982; Brooks et al. 1989; Kelley et al. 1994).

With each method, there is potential for fractionation that causes the isotopic results to favor one of the N isotopes, usually the light (^{14}N) (Hauck 1982; Velinsky et al. 1989; Holmes et al. 1998). When processing nitrate for isotopic analysis, incomplete conversion of nitrate into ammonium can cause fractionation to occur while using either the steam-distillation method or the ammonia diffusion method (Hauck 1982; Sigman et al. 1997). Fractionation can occur using the steam-distillation method due to incomplete distillation, loss of ammonia during the distillation and/or incomplete ion exchange with the zeolite (Hauck 1982; Velinsky et al. 1989). With the diffusion method, fractionation can occur due to nonquantitative N recovery caused by incomplete diffusion of ammonia onto the acidified disks (Kristiansen and Paashe 1989; Lory and Russelle 1994; Sigman et al. 1997; Holmes et al. 1998).

Methods-Diffusion Protocol

The goal of my research was to determine a reproducible method based on published materials to isolate DIN compounds from estuarine waters for isotopic analysis. Data obtained from isolated samples would provide information about the N source that could assist managers dealing with nutrient pollution problems. Therefore, I analyzed each method to determine which method offered the most

direct approach in order to avoid introduction of error. The ammonia diffusion method appeared to have the most potential in satisfying this requirement. This method allows for extraction and concentration of nitrate and ammonium for isotopic analysis with the fewest steps within one sealed container thus reducing outside influences. Furthermore, this method requires less sample manipulation that could alter the isotopic composition of a sample, thus reducing its potential in effectively identifying sources of nutrient pollution.

The efficiency and rate of diffusion depends on pH, temperature, depth of the sample solution, and surface area of the solution (Kelly et al. 1991; Lory and Russelle 1994). Therefore, the design of the diffusion method should consider these factors to optimize the success of N recovery and accuracy of isotopic analysis. The diffusion design used was a modification of that presented by Kahn et al. (1998) which utilizes a Mason jar with a large diameter to increase the surface to volume ratio of sample solutions by decreasing sample depth and increasing surface area (Kahn et al. 1998). Also, Mason jars seal tightly without a fixative agent, and this prevents leakage of ammonia (Kahn et al. 1998).

The diffusion unit (Figure 4) was a modified version of the Mason jar unit described by Khan et al. (1998). A 1.6 mm diameter (1/16 inch) stainless steel welding rod was shaped to hold two glass fiber filter disks and suspended from a stainless steel machine screw that came down from the lid of a pint-size, wide-mouth Mason jar. The machine screw was oriented to one side of the Mason jar lid, so that when jars were placed onto an oven rack that was oriented at a 1° angle

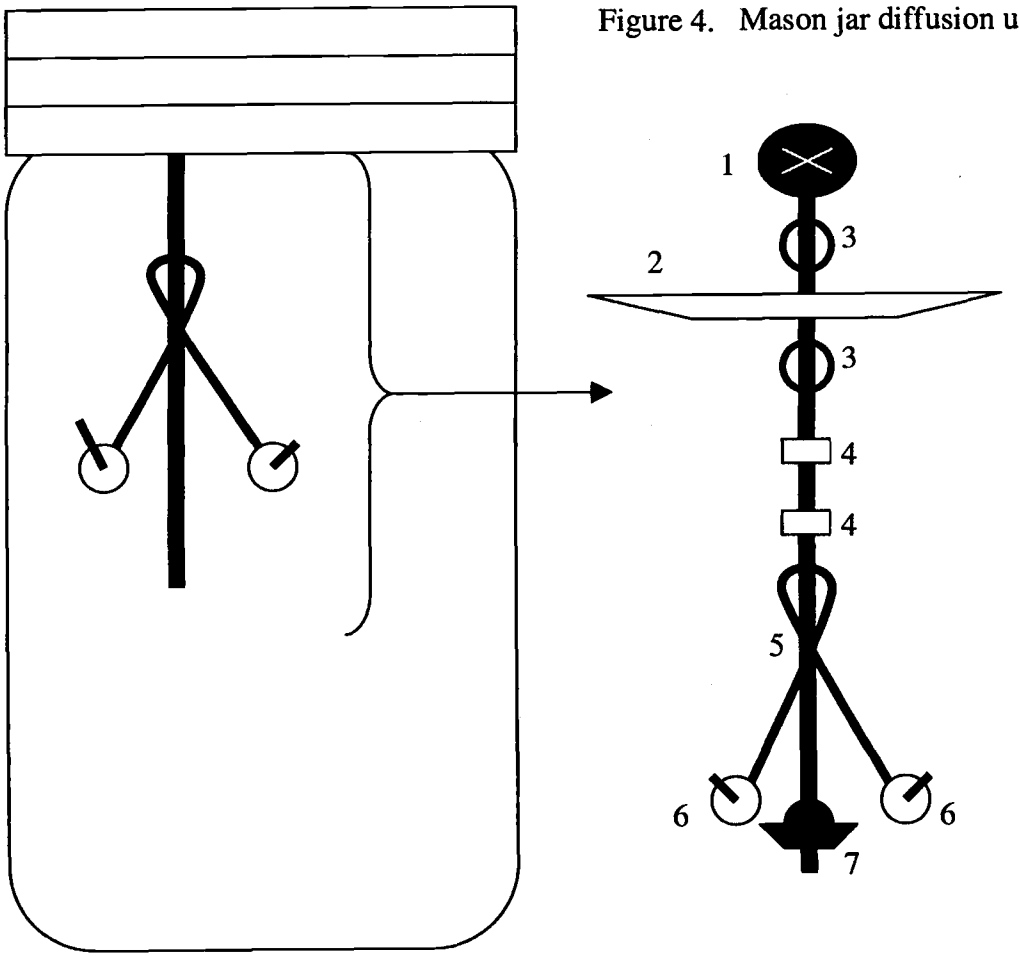


Figure 4. Mason jar diffusion unit.

1. Stainless Steel Machine Screw
2. Mason jar Lid
3. O-Ring
4. Stainless Steel Nut
5. Stainless Steel Welding Rod
6. Glass Fiber Filter Disks
7. Stainless Steel Wing Nut

Note: Mason jars are placed on an oven rack tilted 1° and oriented so that the machine screw is on the upslope side (which would be the left side of figure).

of inclination, the machine screw could be oriented upslope (Khan et al. 1998). This orientation encourages condensation to roll from the lid to the jar sides instead of onto the suspended disks (Khan et al. 1998). O-rings were used to seal the hole in the lid through which the machine screw hung down from. One stainless steel nut was used to tighten the O-ring to the lid and the other was used to hold the welding rod in place. Lastly, a stainless steel wing nut was used to hold the welding rod above the sample solution.

An aliquot of 100 ml of water amended with 125 μg of N (this is a concentration of approximately 90 μM of N) was placed in the Mason jar. This amount of N was chosen to provide a minimum of a one-volt signal during mass spectrometry analysis to ensure resulting isotopic data had minimal error. When analyzing environmental samples, the volume of water collected must be large enough to contain equivalent or greater amounts of N to ensure minimal error from the mass spectrometer. In order to maximize the efficiency of the diffusion process, samples with large volumes can be evaporated or boiled in order to reduce sample volume to about 100 ml (Sigman et al.1997). However, estuarine and coastal waters impacted by nutrient pollution can have N concentrations sufficient to collect 100 ml samples for diffusion. For example, the Delaware estuary had nitrate-N concentrations up to 140 μM in 1985 (Cifuentes et al 1988), Neuse River, North Carolina, had nitrate-N concentrations up to 120 μM in 1987 (Showers et al. 1990) and the lower Mississippi River and the northern Gulf of Mexico had nitrate-N concentrations up 114 μM in 1981 to 1987 (Rabalais et al. 1996).

The Mason jar lid was assembled as described above. Glass fiber filter disks were punched out using a paper hole punch (provides a disks of approximately 7 mm in diameter) and baked in an oven at 450°C for 4 hours (Saghir et al. 1993; Sigman et al. 1997; Holmes et al. 1998). For each diffusion unit, two disks were acidified with approximately 10-20 μL of 1 M H_2SO_4 and then placed onto the two hooks shaped into the welding rod (Khan et al. 1998). When isolating nitrate, approximately 75 mg of Devarda's alloy was added to convert nitrate to ammonium and 300 mg of MgO (baked at 450°C for 4 hours) was added to raise the pH to ~9.7 to facilitate conversion of ammonium to ammonia (Slawyk and Raimbault 1995; Sigman et al. 1997). When isolating only ammonium for isotopic analysis only 300 mg MgO was added to each sample (Holmes et al. 1998). A magnetic stir bar was placed in each jar so that sample solutions could be stirred each day in order to mix the MgO and Devarda's alloy that settled to the bottom. The lids were tightly screwed onto the jars to ensure a seal was formed and the jars were placed in an oven set at 65 to 70°C for 6 days when isolating nitrate (Anderson et al. 1997) and 3 days when isolating ammonium (Sørensen and Steen-Jensen 1991). Each day, jars were removed from the oven and placed on a magnetic plate and sample solutions were stirred twice a day for approximately 15 minutes. At the end of 3 or 6 days, the welding rods were carefully removed from the lids and inserted into a Styrofoam block, which was placed in a desiccator under vacuum to dry and store disks until they were analyzed by mass spectrometry.

Chapter 4: Results and Discussion

Results and Discussion For Method Development

To minimize the number of variables, the first experiments to isolate nitrate and ammonium out of a sample solution were conducted in de-ionized water. A 100 ml aliquot of de-ionized water was amended with 125μg of N from a potassium nitrate standard with an average $\delta^{15}N$ of –0.96 ± 0.035‰ or an ammonium sulfate standard with an average $\delta^{15}N$ of +0.74 ± 0.09‰ (Table 4).

Table 4. $\delta^{15}N$ signatures for the standards potassium nitrate and ammonium sulfate.

Standard	$\delta^{15}N$ (‰)	Average $\delta^{15}N$ (‰)
Potassium Nitrate	-0.99	-0.96± 0.035
	-0.91	
	-0.97	
	-0.99	
	-0.94	
Ammonium Sulfate	0.81	0.74± 0.09‰
	0.71	
	0.84	
	0.75	
	0.61	

This N quantity was chosen to provide a minimum of a one-volt signal during mass spectrometry analysis to ensure resulting isotopic data had minimal error. The

average percent recovery for nitrate was 78.8 ± 10.2% with an average $\delta^{15}N$ signature of –2.43 ± 0.66‰ (Table 6 and Table 7). For ammonium, percent recovery was 109 ± 3.3% with an average $\delta^{15}N$ signature of –0.53 ± 0.63‰ (Table 5 and Table 7). Generally the recovered $\delta^{15}N$ signatures were similar to that of the standards differing by 1.47 ‰ for nitrate and 1.27 ‰ for ammonium (Table 7). The variability in recovery is probably largely due to condensation dripping from the lid of the jar, onto the disks and back into the solution, returning recovered N to the water. When analyzing freshwater samples, salt (typically KCl or NaCl) is usually added to obtain a 2 M concentration, which tends to reduce this condensation effect (Sørensen and Steen-Jensen 1991; Khan et al. 1998).

Table 5. Ammonium diffusion results.

Ammonium in:	**N Recovered (μg)**	**Percent Recovery (%)**	**$\delta^{15}N$ (‰)**
Freshwater	135.7	108.6	-0.03
	129.9	103.9	-0.96
Sargasso Seawater	138.4	110.8	1.01
	125.3	100.2	0.9

Table 6. Nitrate diffusion results.

Nitrate in:	**N Recovered (μg)**	**Percent Recovery (%)**	**$\delta^{15}N$ (‰)**
De-ionized Water	119.0	95.2	-2.01
	110.0	80.0	-2.37
	90.1	77.6	-1.62
	86.0	72.0	-3.27
	97.0	68.8	-2.89
Sargasso Seawater	83.8	67.0	-9.69
	77.7	62.2	-7.59
	70.6	56.5	-7.77
	65.6	52.5	-6.49
Sargasso Seawater Boiled	86.1	68.9	-7.08
	83.9	67.1	-7.13
	71.0	56.8	-7.81
NaCl Solution	103.7	83.0	-1.65
	100.9	80.7	-2.44
	96.3	77.0	-1.85
	88.1	70.5	-1.98

Sargasso water was used to test the method under seawater conditions.

Average percent recovery for nitrate was 59.5 ± 6.4% with an average $\delta^{15}N$ value

of –7.88 ± 1.3‰ (Table 6 and Table 7) and average percent recovery for ammonium was 111 ± 7.5% with an average $\delta^{15}N$ value of +1.0 ± 0.08‰ (Table 5 and Table 7). While recovery for ammonium was sufficient to produce an isotopic signature similar to the standard, differing by 0.26 ‰, data from the nitrate recovery reveals that nitrate diffusions were incomplete because there is a 6.9‰ difference between the $\delta^{15}N$ values of N recovered by diffusion and the N of the standard analyzed directly by the mass spectrometer (Table 7). Furthermore, the incomplete conversion from nitrate to ammonium is causing a fractionation that favors the light isotope causing $\delta^{15}N$ values to be depleted in ^{15}N compared to the standard.

The nitrate diffusion was performed in a solution of de-ionized water and sodium chloride at the same ionic strength as seawater in order to determine if there is something about Sargasso seawater that was hindering nitrate conversion. Average percent recovery of nitrate in the sodium chloride solution was 77.8 ± 5.46% and the average $\delta^{15}N$ value was –1.98 ± 0.34‰ (Table 6 and Table 7). Both percent recovery and fractionation improved compared to Sargasso seawater diffusions. Furthermore, $\delta^{15}N$ values of recovered N were more similar to the standard (–0.96 ± 0.035‰) with only a 0.82‰ difference (Table 7).

The percent recoveries for the Sargasso seawater diffusions were lower compared to the sodium chloride solution and de-ionized water diffusions. Furthermore, the $\delta^{15}N$ values were more depleted (i.e. more negative) for the Sargasso seawater diffusions compared to the sodium chloride solution and de-

ionized water diffusions (Table 5, 6 and 7). These observations demonstrate that there is some relationship between percent recovery and the $\delta^{15}N$ values in which, generally, diffusions with larger percent recoveries tend to have less deviation from the $\delta^{15}N$ values of the standards (i.e. there is less fractionation when more complete N recovery occurs) (Table 7). Because the $\delta^{15}N$ values of the Sargasso seawater are significantly more negative than the standard, this indicates fractionation is most likely occurring in which ^{14}N proceeds through the diffusion first, followed by ^{15}N. Therefore, when the diffusion is incomplete not all of the ^{15}N will be recovered resulting in measured $\delta^{15}N$ values that are more negative than expected from the standard. As a result, incomplete N recovery in Sargasso seawater diffusions resulted in fractionation that produced more negative or depleted $\delta^{15}N$ values compared to the values of the standards.

The incomplete recovery of N by the diffusion is most likely due to incomplete conversion of nitrate to ammonium and not due to incomplete conversion of ammonium to ammonia or diffusion of the ammonia onto the acidified disk. This is evident from the diffusions of the ammonium standard in Sargasso seawater (Table 5 and 7). The percent recoveries were over 100% (due to some N contribution by the blank) and the $\delta^{15}N$ values were very similar to the standard. This demonstrates that ammonium is converting to ammonia and diffusion of ammonia onto the acidified disk is occurring to the fullest extent possible. Therefore, incomplete N recovery must be due to incomplete conversion of nitrate to ammonium.

Since incomplete recovery and more negative $\delta^{15}N$ values were a greater problem with Sargasso seawater diffusions than with sodium chloride solutions and de-ionized water, incomplete recovery could be due to either something in seawater complexing with nitrate, or something poisoning the Devarda's alloy which inhibits conversion of nitrate to ammonium.

Table 7. Summary of experiments.

List of Experiments to Isolate DIN for Isotopic Analysis	**Average N Recovered (μg)**	**Average Percent Recovery (%)**	**Average $\delta^{15}N$ (‰)**	**Difference from Standard (‰)**
Extract Nitrate from De-ionized Water	98.4 ± 12.8	78.8 ± 10.2	-2.43 ± 0.66	-1.47
Extract Ammonium from De-ionized Water	132.8 ± 4.1	109 ± 3.3	-0.53 ± 0.63	-1.27
Extract Nitrate from Sargasso Seawater	74.4 ± 8.0	59.5 ± 6.4	-7.88 ± 1.3	-6.92
Extract Ammonium from Sargasso Seawater	131.9 ± 9.3	111 ± 7.5	+1.0 ± 0.08	+0.26
Boil Sargasso Seawater Before Extracting Nitrate	80.3 ± 8.1	64.3 ± 0.41	-7.34 ± 0.41	-6.38
Extract Nitrate from a NaCl Solution	97.3 ± 6.8	77.8 ± 5.46	-1.98 ± 0.34	-1.02

The method produced a significant N blank that could also contribute to the difference between the signatures of the standard and that measured for the recovered N (Table 8). Since this blank is a source of N, the isotopic signature

associated with the blank could be mixing with and affecting the isotopic signature of the recovered standard. An isotopic mass balance was used to correct for the effect of the blank on the $\delta^{15}N$ signature of nitrate N recovered from de-ionized water, Sargasso Seawater and NaCl solutions using the following equation:

$$\delta^{15}N_{corrected}X_{corrected} + \delta^{15}N_{blank}X_{blank} = \delta^{15}N_{measured}\ X_{measured}$$

However, when the $\delta^{15}N$ signatures were corrected for the blank by solving for $\delta^{15}N_{corrected}$, the $\delta^{15}N$ signatures showed larger differences from the $\delta^{15}N$ signature of the standard (Table 7 and 9). Since correcting for the blank using an isotopic mass balance did not account for the difference between the measured $\delta^{15}N$ signatures of the diffusion samples and that of the standard, this implies that the blank is not likely a major contributor for causing the discrepancy. Therefore, the depleted (more negative) $\delta^{15}N$ values relative to the standards are more likely a result of fractionation due to incomplete recoveries and/or incomplete conversion of nitrate to ammonium. The mixing of the standard N with the blank nitrogen did not significantly contribute to the depleted $\delta^{15}N$ values. However, even though the $\delta^{15}N$ values for the blank were generally consistent, the N in these samples produced a signal lower than one-volt on the mass spectrometer. Therefore, these results are less reliable because the errors associated with these values are several times larger than for normal signal strengths (i.e. above one volt). As a result, any analysis with these values is only conjecture and the blank could be contributing more than the values calculated with the isotopic mass balance indicate.

Table 8. Blank analysis results.

Blank Results for N Contributed by Devarda's Alloy + MgO (combination used to isolate nitrate)				
$\delta^{15}N$ Signature of Blank			**Size of Blank**	
Sample Solution	***$\delta^{15}N$ (‰)***	***Average $\delta^{15}N$ (‰)***	***Sample Solution***	***µg N/100 ml***
De-ionized Water	-3.09	-3.27 ± 0.25	De-ionized Water	29.0
	-3.45			37.6
Sargasso Seawater	-4.66	-4.43 ± 2.98	Sargasso Seawater	26.9
	-3.45			23.3
	-5.35			37.6
	-8.46		NaCl Solution	32.7
	-0.25			36.0
NaCl Solution	-3.43	-3.90 ± 0.62		39.1
	-3.73			36.4
	-4.59			
	-3.48			
	-4.78			
	-3.40			
Blank Results for N contributed by MgO (combination used to isolate ammonium)				
$\delta^{15}N$ Signature of Blank			**Size of Blank**	
Sample Solution	***$\delta^{15}N$ (‰)***	***Average $\delta^{15}N$ (‰)***	***Sample Solution***	***µg N/100 ml***
De-ionized Water	-5.3	-5.17 ± 0.18	De-ionized Water	15.5
	-5.04			11.8
Sargasso Seawater	-0.03		Sargasso Seawater	18.4

Table 9. $\delta^{15}N$ signatures for nitrate diffusions calculated from isotopic mass balance calculations.

Experiment	Average N Recovered (μg)	Average Blank (μg)	Corrected N Recovered (μg)	Corrected $\delta^{15}N$ (‰)	Difference from Standard (‰)
De-ionized Water	98.4 ± 12.8	33.3 ± 6.1	65.1	-2.00	-1.04
Sargasso Seawater	74.4 ± 8.0	29.3 ± 7.7	45.1	-10.12	-9.16
NaCl Solution	97.3 ± 6.8	36.0 ± 2.7	61.3	-0.85	+0.11

Sargasso seawater amended with the nitrate standard was also boiled prior to diffusion to reduce the sample size to enhance the diffusion rate. Additionally, this was done to determine if boiling is a necessary step to convert nitrate, since this step is used in both the distillation and Sigman et al. (1997) versions of seawater diffusion. Average percent recovery was 64.3 ± 0.41% and the average $\delta^{15}N$ value was –7.34 ± 0.41‰ (Table 6 and Table 7). Since this percent recovery and average $\delta^{15}N$ value are similar to those for Sargasso seawater diffusions, boiling is not critical for nitrate conversion and can be used to reduce sample volumes to aid diffusion of larger water samples without altering the isotopic signature significantly.

In conclusion, the discrepancies between the measured $\delta^{15}N$ signatures of the diffusion samples and that of the standards is more likely due to fractionation

effects caused by incomplete recovery of N and/or incomplete conversion of nitrate into ammonium. Contribution of blank N is minimal according to the isotopic mass balance calculations. However, all of these factors could have a cumulative effect in altering the $\delta^{15}N$ signature of diffusion samples.

A more ideal approach for analyzing DIN isotopic signature would involve direct removal of nitrate and/or ammonium from sample solutions such as ion chromatography or some sort of membrane such as those used for dialysis that could remove the DIN species without converting or changing them. If one of these techniques could be developed to remove DIN from seawater for isotopic analysis then the problems associated with converting nitrate and use of reagents that contribute a N blank could be removed. However, these ideas require a lot more research and are also plagued with several complicating factors. The nature of seawater makes it difficult to use ion exchange columns because the ions can swamp the exchange sites. Also, both ideas could be prone to fractionation if incomplete N recovery occurs. There is another method that was recently published that might provide a more accurate method for analyzing $\delta^{15}N$ signature for DIN. This alternative method by Johnston et al. (1999) converts nitrate into 1-phenylazo-2-napthol (sudan-1). This method involves reducing nitrate to nitrite using cadmium and then converting the nitrite into a sudan-1 dye, which is recovered by reverse phase chromatography for isotopic analysis (Johnston et al. 1999). This method could become a useful alternative method for isolating DIN from seawater.

Environmental Challenges

Even with improvements in the laboratory method for isolating DIN species, use of N stable isotopes to identify sources of nutrient pollution can be compromised by biogeochemical processes occurring in the environment. For example, a simplified model of a hypothetical estuary receives three sources of N (Figure 5), including natural inputs of terrestrial-derived N with $\delta^{15}N$ values of DIN and particulate organic nitrogen (PON) averaging around 3.9‰ and marine-derived N with $\delta^{15}N$ values of DIN and PON averaging around 7.2‰ (Owens 1985; Owens 1987, Tucker et al. 1999) along with N contributions from anthropogenic sources such as fertilizers or sewage. An isotopic mass balance could be used to solve for the $\delta^{15}N$ of the anthropogenic source of DIN and its relative proportion using the following equation:

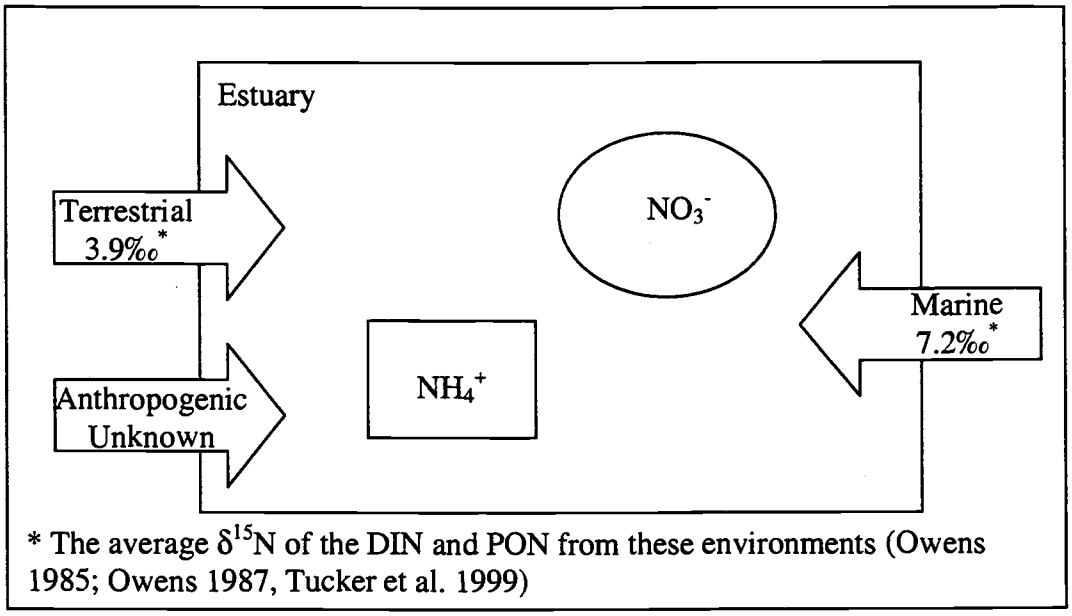
$$\delta^{15}N_{obs} = \delta^{15}N_{mar}X_{mar} + \delta^{15}N_{terr}X_{terr} + \delta^{15}N_{anthrop}\,(1-[X_{mar}+X_{terr}])$$

$$\text{if } X_{mar} + X_{terr} + X_{anthrop} = 1$$

where $\delta^{15}N_{mar}$ represents the signature for marine-derived N, $\delta^{15}N_{terr}$ represents the signature for terrestrially-derived N, $\delta^{15}N_{anthrop}$ represents the anthropogenic N source signature and X is the relative portion of each source which would have to be measured or could be estimated using a model such as that developed by Valiela et al. (1997a). Because $\delta^{15}N_{obs}$ of a sample represents the total of the N sources and cumulative effects of N cycle processes, isotopic compositions of DIN compounds are subject to variation caused by isotopic fractionation from transformations of the N cycle (Hauck 1973; Mariotti et al. 1984; Cifuentes et al. 1988; Cifuentes et al.

1989; Horrigan et al. 1990). As a result of these processes, application of this simple model to estuarine and coastal ecosystems is confounded.

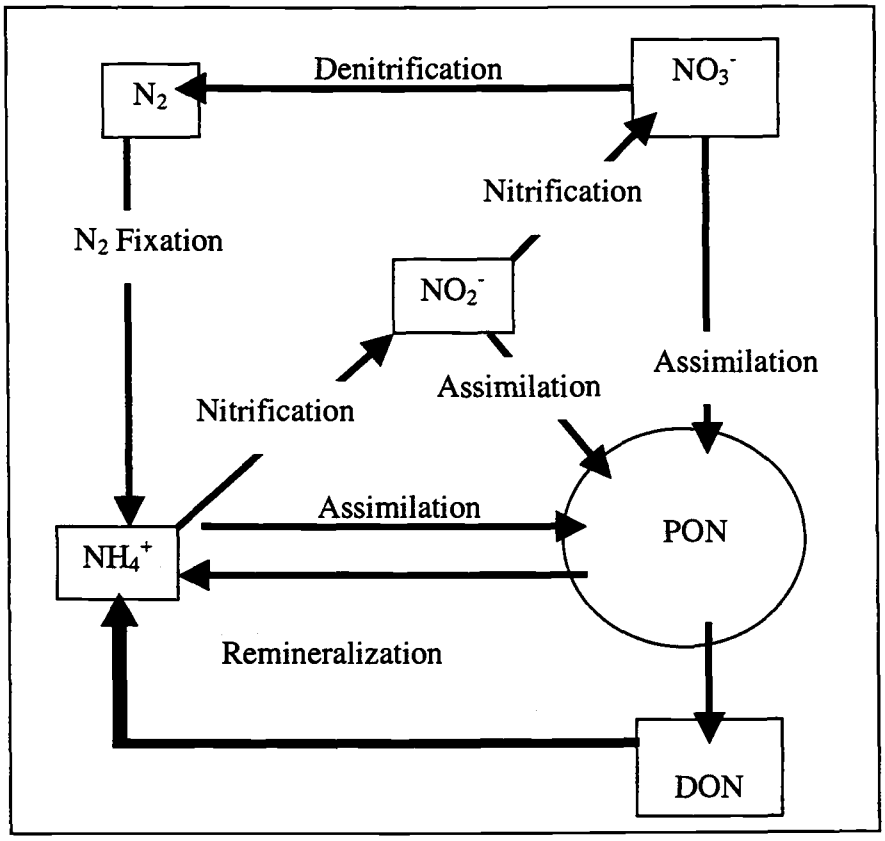
Figure 5. DIN inputs to a hypothetical estuary.



Isotopic ratios can be modified by the N cycle processes (Figure 6) N assimilation, N fixation, nitrification, denitrification and remineralization (Cline and Kaplan 1975; Mariotti et al. 1984; Horrigan et al. 1990; Kendall 1998). Generally, rate constants for ^{15}N are smaller than for ^{14}N, causing these reactions to favor the lighter isotope (Altabet and McCarthy 1985; Owens 1987; Kendall 1998). As a result, isotopic compositions of residual substrates are enriched in ^{15}N and products are depleted in ^{15}N relative to substrate pools when these reactions do not

go to completion (Altabet and McCarthy 1985; Owens 1987; Horrigan et al. 1990; Kendall 1998).

Figure 6. Estuarine N cycle (Cline and Kaplan 1975; Mariotti et al. 1984; Horrigan et al. 1990; Kendall 1998).



The magnitude of isotopic fractionation for a reaction can be determined if the isotopic composition of the substrate and product pools and the extent of the reaction is known (Montoya et al. 1991; Kendall 1998). The difference of the ^{15}N content between substrate and product pools can provide an estimate of the

magnitude of isotopic fractionation, known as the discrimination factor (D), caused by the process connecting the two pools (Heaton 1986; Peterson and Fry 1987; Montoya et al. 1991; Lajtha and Michener 1994; Kendall 1998).

$$D = \delta^{15}N_{product} - \delta^{15}N_{substrate}$$

This equation is commonly used for biological systems instead of α because natural, biologically mediated reactions rarely differ from unity by more than 5% and are dominated by kinetic, unidirectional reactions (Montoya et al. 1991; Fogel and Cifuentes 1993; Lajtha and Michener 1994; Kendall 1998). D values are negative when products become enriched in ^{14}N as a reaction proceeds and are positive when products become depleted in ^{14}N as the reaction proceeds (Fogel and Cifuentes 1993). Furthermore, large variations in D values can occur as a function of the size of the substrate pool (Kendall 1998). For example, isotopic fractionation during assimilatory uptake of DIN by phytoplankton and bacteria only occurs when DIN is present in excess, when DIN is limiting it is assumed that the entire pool is converted to OM (Mariotti et al. 1984; Altabet and McCarthy 1985; Saino and Hattori 1985; Sigleo and Macko 1985; Fogel and Cifuentes 1993; Ostrom et al. 1997). Generally, the N cycle processes tend to discriminate against ^{15}N (Altabet and MacCarthy 1985), resulting in negative D values. Table 10 provides a summary of fractionation effects resulting from N cycle processes.

As a result of all this, the isotopic mass balance requires knowledge of isotopic compositions of DIN sources along with the magnitude of fractionation caused by

N cycle processes and the degree of completeness of these reactions which determines the effects of Rayleigh distillation (Owens 1987).

Table 10. Summary of discrimination factors caused by fractionation during N cycle processes and the isotopic effect on substrate and product N pools.

N Cycle Process	D ‰	Substrate	Product	Ref
Nitrogen Assimilation	-27 to 0	NO_3^-, NO_2^- & NH_4 Enriched With ^{15}N	NO_3^-, NO_2^- & NH_4 Depleted In ^{15}N	Altabet & McCarthy 1985 Owens 1987 Fogel & Cifuentes 1993 Kendall 1998
Nitrogen Fixation	-3 to +1	N_2 Enriched With ^{15}N	NO_3^- Depleted In ^{15}N	Owens 1987 Fogel & Cifuentes 1993 Liu et al. 1996 Kendall 1998
Nitrification	-12 to -29	NH_4^+ Enriched With ^{15}N	NO_3^- Depleted In ^{15}N	Horrigan et al. 1990 Owens 1987 Kendall 1998
Denitrification	-40 to -5	NO_3^- Enriched With ^{15}N	N_2 Depleted In ^{15}N	Cline & Kaplan 1975 Owens 1987 Kendall 1998
Mineralization	-35 to 0	PON Enriched With ^{15}N	NH_4^+ Depleted In ^{15}N	Owens 1987 Kendall 1998

Variations in DIN $\delta^{15}N$ also reflect seasonal, spatial and short term changes in the estuarine N cycle (Cifuentes et al. 1989; Horrigan et al. 1990; Montoya et al. 1991). For example, seasonal DIN assimilation can create residual DIN pools depleted in ^{15}N during spring blooms (Sigleo and Macko 1985; Cifuentes et al. 1989). Seasonal and spatial dominance of nitrification, denitrification and

regeneration can cause variations in the $\delta^{15}N$ of residual DIN pools (Cifuentes et al. 1988; Cifuentes et al. 1989; Horrigan et al. 1990; Montoya et al. 1991). Horrigan et al. (1990) found oxidation of ammonium created elevated $\delta^{15}N$ values in ammonium pools during the spring at the turbidity maximum and other localized regions of Chesapeake Bay. Cifuentes et al. (1989) found biological rates of N cycling to be low during January due to low water temperatures and varied between the river and bay portions of Delaware estuary. A storm event in Chesapeake Bay induced short-term variations, on the order of several days, in $\delta^{15}N$ data of dissolved and planktonic pools of N because storm-induced mixing created a perturbation in the estuarine N cycle which changed phytoplankton nutrient regimes (Montoya et al. 1991). The storm induced mixing event resulted in an injection of ammonium into surface waters that changed the magnitude of isotopic fractionation during ammonium assimilation (Montoya et al. 1991). The isotopic fractionation was more pronounced in regions of Chesapeake Bay where there was an excess of ammonium compared to regions where the rate of supply matched the demand more closely (i.e. pre-storm event) (Montoya et al. 1991). Lastly, seasonal flood cycles can change dominant sources of DIN to an estuary. During high flows, an estuary will likely receive more terrestrial and anthropogenic derived N than marine derived N because of the increased export of materials from land (Fogel and Cifuentes 1993). These examples of seasonal, spatial and short time scale patterns of $\delta^{15}N$ values further demonstrate the importance of determining the sources of $\delta^{15}N$ variations when interpreting N isotopic data.

Isotopic fractionation associated with the reactions that move N between different pools and the spatial and temporal variations of N cycle processes that produce variation in $\delta^{15}N$ values complicate usage of an isotopic mass balance to identify anthropogenic DIN sources or the fraction of the total N of that source using $\delta^{15}N$ values alone (Owens 1987; Montoya et al. 1991). Depending on the resources (i.e. money and equipment) available to a manager, it could be very difficult to collect all the necessary data required to solve the isotopic mass balance equation, N stable isotopic analysis of estuarine DIN to identify sources of nutrient pollution, therefore, might not be practical to use as a management tool for this water quality problem (Owens 1987; Montoya et al. 1991).

N isotopic analysis of food webs and multiple isotope approaches using carbon, oxygen and sulfur isotopic compositions of organic and inorganic components can assist with analysis of $\delta^{15}N$ data to identify sources of N causing nutrient pollution (Owens 1987; Kendall 1998). These analyses of particulate N pools could serve to ground truth or collaborate with the $\delta^{15}N$ data on DIN to demonstrate an anthropogenic N source is in fact entering and impacting the system. For example, N isotopic analysis of primary producers, and primary and secondary consumers can provide evidence for an anthropogenic N source entering estuarine food webs. McClelland et al. (1997; 1998), whose research is described in more detail in Chapter 2 herein, found enrichment of ^{15}N in food webs exposed to a higher wastewater load. Research off the coast of New Jersey used $\delta^{15}N$ and $\delta^{34}S$ to detect sewage-derived OM entering benthic food webs (Van Dover et al.

1992). $\delta^{15}N$ and $\delta^{34}S$ data from Boston Harbor and Massachusetts Bay revealed presence of sewage-derived particulate and dissolved materials and identified a corresponding sewage signal in the food web as well (Tucker et al. 1999). Additional information provided by $\delta^{13}C$ data of OM from Delaware estuary revealed that $\delta^{15}N$ values measured during a Spring bloom could have been misidentified as a terrestrial or sewage source but were really a product of fractionation during assimilation of riverine DIN (Cifuentes et al. 1988; Cifuentes et al. 1989). Lastly, there is potential to use $\delta^{18}O$ to separate fertilizer and soil organic nitrate $\delta^{15}N$ signatures since fertilizer nitrate has an $\delta^{18}O$ signature of +18 to +22 ‰ and soil organic nitrate ranges from –5 to +10‰ (Aravena et al. 1993; Kendall 1998). Furthermore, there is potential to use $\delta^{18}O$ to determine the "natural" versus anthropogenic (e.g. industrial emissions, car emissions and coal burning emissions) sources of nitrate in wet deposition from the atmosphere (Kendall 1998).

Chapter 5: Conclusions: Are Nitrogen Stable Isotopes a Useful Management Tool?

Nonpoint source pollution, which includes anthropogenic sources of N, is difficult to manage because of the lack of evidence connecting the source and effect of these pollutants. For example, it is difficult to place legal restrictions or enforce Best Management Practices on a farmer applying fertilizers upstream of an estuary experiencing algal blooms when there is no concrete evidence linking that fertilizer N to that bloom. This lack of information prevents managers from effectively planning, restoring and protecting estuarine habitats from further degradation caused by nutrient pollution. Because of this, managers are reduced to relying on *a posteriori* methods for identifying occurrences of nutrient pollution, using indicators of environmental degradation that have already occurred. As a result, remediation is more difficult and costly, and preventive approaches are impossible. Therefore, there is a need for a tool to assist managers in assessing nutrient pollution problems to determine anthropogenic sources of N so that management approaches and plans can be created to protect and restore estuaries before future damage occurs.

Studies of N stable isotopes of groundwater DIN revealed that N sources could be isotopically distinct and this method could potentially be used to resolve N sources stimulating nutrient pollution in estuarine and coastal waters so that management actions could begin before estuarine habitat degradation occurred. However, there are complications associated with using N stable isotopes as a

management tool for nutrient pollution. First, methods for laboratory isolation of DIN for isotopic analysis introduce significant error mostly associated with fractionation caused by incomplete recovery of nitrate, resulting in variations of $\delta^{15}N$ values that are difficult to account for. Second, variations of $\delta^{15}N$ values caused by fractionation associated with N cycle processes, including denitrification, assimilation, nitrification, nitrogen fixation and remineralization, complicate identification of N sources using an isotopic mass balance. However, depending on the intent of the end user or natural resource manager, N stable isotopic data can still be useful to better understand N cycling in estuaries and to provide a general picture by which to base volunteer management practices to reduce nutrient inputs into these ecosystems. If the intent is to use N stable isotopic data as a basis for legislative regulations limiting nutrient uses and inputs into estuarine ecosystems, the complications in both the laboratory performing either diffusion or distillation methods and the environment could cause the validity of this tool to be easily challenged both scientifically and legally. This has already occurred when N isotopic data was used to justify regulations limiting nitrate inputs into groundwater caused by fertilizer inputs. Therefore, it would be critical to know the purpose (i.e. be clear about the goal of the project) for collecting N isotopic data to best utilize this information before saturating resources such as time and money.

This leaves the question of how managers can deal with the problem of nutrient pollution in the future. N stable isotopes could still be used to understand the N cycling that transforms N causing it to be more or less available.

Furthermore, spatial and temporal N quantity or concentration measurements along with landscape assessments of watershed N sources could be used in collaboration with N stable isotopes in order to construct N budgets and elucidate primary sources of nutrient pollution. N stable isotopes could also be used to look for nutrient loading signals into the food web. Additionally, alternative isotopic analysis of particulate pools by $\delta^{34}S$, $\delta^{13}C$ and possibly $\delta^{18}O$ could be applied in collaboration with $\delta^{15}N$ to help identify nutrient sources by providing confirming evidence through distinct signatures that a specific nutrient source is entering an estuarine ecosystem. Also, N stable isotopes could be used to confirm that an anthropogenic source of N is entering a water body. For example, if a hog farm is suspected to contribute N to a river or estuary, the $\delta^{15}N$ signature of N in water samples from areas upstream and downstream of the hog farm could be measured. This $\delta^{15}N$ data then could demonstrate the lack of hog waste-derived N upstream of the farm and confirm the presence of hog waste-derived N downstream of the farm. Lastly, managers will need to continue educating citizens about nutrient pollution, its sources and effects, and their role in its prevention.

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