

**Sediment Respiration and Nitrogen Cycling along a
Eutrophic Gradient in a Shallow, Coastal Estuary
SES Final Research Project, 2004**



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**Sediment Respiration and Nitrogen Cycling along a Eutrophic Gradient in a
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Abstract.

Changes in land use can have a dramatic effect on estuarine nutrient loading. New England coastal areas have experienced rapid land development over the past 100 years. One result of residential and commercial land cover is an increase in non-point source nutrient pollution. In this project we studied the effects of increasing nitrogen pollution on nitrogen cycling in West Falmouth Harbor, a shallow, coastal estuary on Cape Cod. Our project also researched the effect that eelgrass beds may have on nitrogen cycling. We analyzed the carbon and nitrogen content as well as the isotope composition of the sediments. To measure rates of respiration, ammonification, nitrification and denitrification we incubated sediments and took water samples from the cores over a time series. After these initial *in-situ* flux measurements we added allylthiourea, a nitrification block to estimate the rate of nitrification. In a second set of cores we determined nitrification and denitrification potentials. We found that the more eutrophic sites had higher respiration and ammonification. Our data shows that eelgrass affects aspects of the nitrogen cycle however the affect may change during different seasons. Although some of our data was not conclusive we speculate that the more eutrophic sites have higher rates of coupled nitrification/denitrification.

Key Words and Phrases. *Eutrophication, nitrogen loading, eutrophic gradient, eelgrass, sediment cores, sediment respiration, nitrogen cycle, nitrification, denitrification, nitrification block, shallow coastal estuary, West Falmouth Harbor, Falmouth Wastewater Treatment Plant.*

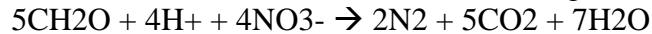
Introduction

Estuaries are highly productive systems which receive water, nutrients and potential contaminants from the surrounding watershed via groundwater and stream flow. Although the high productivity of estuaries is largely due to nutrient inputs from terrestrial watersheds, nutrient concentrations in excess leads to detrimental changes in the ecosystem. Eutrophication is a process that can occur when high amounts of nutrients such as nitrogen and phosphorous flow into a body of water. Nutrient over-enrichment stimulates primary production and excessive amounts organic carbon accumulates. Decomposition of this organic matter leads to depletion of oxygen in the water and creates anoxic or hypoxic conditions in the estuary (Diaz, 2000). Several other undesirable effects on the ecosystem may include; changes in species composition and

trophic structure, degradation of habitat quality, and increased harmful algal blooms. Commercial and recreational uses of coastal ecosystems are severely compromised by estuarine nutrient pollution.

Changes in land use can have a dramatic effect on estuarine nutrient loading. New England coastal areas have experienced rapid land development over the past 100 years. One result of residential and commercial land cover is an increase in non-point source nutrient pollution from septic systems and sewage treatment plants. Inputs of both nitrogen and phosphorous contribute to degradation of coastal areas however nitrogen is generally the limiting nutrient and thus particularly damaging to these systems. Nitrogen pollution from non-point sources represents the largest pollution problem threatening the nation's coastal waters. Approximately two-thirds of the nation's coastal areas are degraded by nitrogen pollution (Howarth et al., 2000a).

At the land-water interface, coastal ecosystems have the potential to play a crucial role in maintaining water quality by acting as sinks for polluting nutrients such as nitrate (Seitzinger, 1988). Denitrification is the mechanism of reducing nitrate (NO_3^-) to N_2 gas:



Denitrification can act effectively as a buffer to increased nutrient loads to coastal ecosystems by removing nitrogen from the system. The process of denitrification is generally carried out by heterotrophic, facultative anaerobic bacteria (Seitzinger, 1988). The bacteria use nitrate as the terminal electron acceptor during the oxidation of organic matter. Decreasing the concentration of nutrients limits phytoplankton production (N-limited). In this way, denitrification may help to regulate the degree of eutrophication in the coastal ecosystem.

Conceptual models for eutrophication effects for deep (>12 m) coastal marine ecosystems show a positive linear relationship between inorganic N-loading and increasing rates of primary production (Nixon et al., 1996). Shallow ecosystems (<12 m) are prevalent on much of the Atlantic and Gulf of Mexico coastlines. Unlike the deeper coastal areas, shallow ecosystems respond nonlinearly increased N-loading (Valiela, 1997) (Figure 1). In shallow estuaries light can penetrate to the bottom, allowing for growth of benthic vegetation. These systems are an important habitat for many species and are extremely sensitive to eutrophication (Valiela, 1997). It is more difficult to characterize the response of shallow ecosystems to eutrophication due to the diversity of benthic primary producers and coupled sediment processes (Valiela, 1997). A general scenario for eutrophication in shallow estuaries is as follows; as nitrogen inputs increase, rooted eelgrass is replaced by drift macro algae. Eelgrass, *Zostera marina*, is an estuarine plant that obtains nutrients from the sediments via roots. Macro-algae do not have roots that penetrate into the sediments and obtain nutrients directly from the water column. Due to these structural differences macro-algae species are able to take advantage of increased water column nutrients and out-compete the eelgrass. At the highest stage of eutrophication the dominant primary producer shifts from macro-algae to phytoplankton (Valiela, 1997). Understanding the sedimentary biogeochemical cycling and the effects of benthic primary producers on this cycling is necessary to evaluate how the ecosystem retains or removes nutrients.

This project studies sediment respiration and nitrogen cycling in West Falmouth Harbor in Falmouth, MA. This is a typical shallow, New England estuary surrounded by residential development (Figure 2&4). The estuary was originally dominated by eelgrass,

Zostera marina, although due to nutrient loading much has been diminished (Valiela, 1997). The estuary receives high nutrient loading from septic systems as well as from municipal wastewater. The estuary has an inner harbor and a well flushed outer harbor. Residence time in the inner harbor is approximately two to three days whereas the outer harbor residence time is only approximately half a day. This sets up a gradient where different stages of eutrophication are present at the same time. The inner harbor has more nitrogen pollution is more eutrophic than the well-flushed outer harbor. Eelgrass beds are found in both the outer and the inner harbor, although the inner harbor has significantly reduced amounts and much is covered with epiphytic algae.

In this study we are interested in observing the effects of increasing nitrogen pollution on the biogeochemical nitrogen cycling in the sediments. To determine the effect that eelgrass beds may have on nitrogen cycling, we chose two sites at the same observed stage eutrophication. One site was in an eelgrass bed area while the other site was in an area without eelgrass. We compared the different sites rates of respiration, ammonification, nitrification and denitrification. We also measured nitrification and denitrification potentials. The data collected on these microbial processes enables us to evaluate the stage of eutrophication occurring at the different sites in the harbor. Additionally, the data provides important information on the response of shallow estuaries to increasing levels of nitrogen pollution over time.

Methods

West Falmouth Harbor Site Description:

West Falmouth Harbor (WFH) is a shallow coastal marine estuary located on Cape Cod in Falmouth, MA. There is an inner and an outer harbor which opens to Buzzard's Bay (Figure 2). The total area of the estuary is 79 ha. The main sources of N-loading to the estuary are home septic systems and the Falmouth Wastewater Treatment Plant (FWTP). Nutrient inputs from these sources are high and increasing (Figure 3). The nitrogen load from the FWTP takes approximately 10 years to reach the estuary. So the current load to WFH was emitted from the FWTP in 1994. The inner harbor receives high levels of nitrogen loading from the FWTP and residence time here is approximately 2 to 3 days. In the inner harbor the dominant vegetation cover is macro-algae. Of the few eelgrass beds that do remain in the inner harbor most are covered with epiphytic algae. The outer harbor is well-flushed and has a residence time of approximately half a day. Therefore, nitrogen loading to the outer harbor is much less than the inner harbor. Outer harbor eelgrass beds still are prevalent. The flushing and geography of the estuary creates a gradient where there are varying levels nitrogen pollution. Sites in the inner harbor are at higher levels of eutrophication than the outer harbor.

Sediment Core I. Experiments:

In this study I chose 4 sites along a proposed eutrophic gradient in WFH (Figure 4). Of the 4 sample sites at WFH, 2 sites are from the more eutrophic inner harbor and the other 2 are from the "cleaner" outer harbor. In each of the harbors one site has eelgrass and the other site does not have eelgrass. Eelgrass sites are expected to be less eutrophic than non-eelgrass sites. Site 1 is in the outer harbor where eelgrass is still the dominant primary producer. Site 2 is in the outer harbor but no eelgrass is present. Site 3

is in the inner harbor where eelgrass is present and Site 4 is in the inner harbor where no eelgrass is present.

SCUBA divers took duplicate sediment cores from these sites on November 15, 2004 and November 30, 2004. We used these cores to determine how the microbial processes involved in nitrogen mineralization respond to increasing nitrogen in a given ecosystem. We used the first set of cores to run in-situ nitrogen flux experiments. We used the second set of cores to determine nitrification and denitrification potentials.

The first set of cores have a 10.5cm diameter and a 50cm height. We took the sample cores back to the lab and set up to run our in-situ nitrogen flux measurements. The sediment cores were immediately placed in the dark in incubators set to ambient water temperature of 10° C. The cores consist of approximately 30cm of sediment. We siphoned out the overlaying water on the sediments and filled them with GF/F filtered sea water taken from the outer harbor. At the start of the flux measurements we put airtight lids on each of the cores.

We monitored the oxygen level in the cores using an oxygen probe. We took five water samples from each of the cores before the oxygen concentration fell below 3mg/L. The time course ranged from 30 to 50 hours depending on the specific core respiration rate. At each of the five time intervals we collected water samples for nitrate, ammonium and N₂ gas analysis. We put water samples for nitrate and ammonium analysis into plastic 20ml scintillation vials. We measured nitrate concentrations by colorimetric analysis using a Lachat Flow Injection Analyzer (FIA). Methods used for nitrate analysis are described by Wood et al., 1967. We determined ammonium concentrations on a Shimadzu 1601 Spectrophotometer following the phenol-hypochlorite method described by Solarzano, 1969 and Strickland, 1972. To measure N₂ gas concentrations we put water samples into 10ml glass tubes. We stored the N₂ samples underwater at 10°C in the dark. We measured the N₂ gas concentration from the N₂/Ar ratio determined by running the sample through a Membrane Inlet Mass Spectrometer (MIMS) (Kana et al., 1998).

After we collected all the initial flux experiment data we uncovered the cores and bubbled them with air to bring oxygen levels back up. We then conducted a nitrification block experiment to determine the approximate amount of ammonium used in nitrification. According to the Redfield ratio (Redfield, 1958) O₂ uptake to ammonium production should be 6.625. Nitrification and plant assimilation cause this ratio to increase by removing ammonium from the sediments. By adding a nitrification block one can determine the approximate amount of ammonium used in nitrification. Since nitrification and denitrification are closely coupled we also can get an estimation of denitrification. We added allylthiourea, a nitrification block to all eight sediment cores (Ginestet et.al, 1998, Roy and Knowles, 1995). The final concentration of allylthiourea was 50µM in the cores. We sampled five times over a 50 hour time period for NH₄⁺, NO₃⁻ and N₂ gas. Methods used for the analysis of these compounds are described above in the first flux experiment.

Sediment Core II. Experiment:

We collected the second set of sediment cores in the same way as the first set. We collected duplicate cores from the same four sites in WFH. We used core tubes that are 6.5cm in diameter. We used these sediment cores to test nitrification and denitrification potentials. We homogenized the top 2cm from each core.

To measure potential nitrification we put 1-1.5g of wet sediment into four 50ml plastic centrifuge tubes. We added 30ml of 300 μ M ammonium 60 μ M phosphate seawater solution to each tube. We then placed all tubes on their side in the dark on a shaker table at room temperature. At 12, 24, 48 and 72 hours we harvested one tube for each core. We centrifuged the tubes and filtered the supernatant into scintillation vials for nitrate analysis (Wood et al., 1967).

To measure denitrification potential we put approximately 5g of wet sediment from the homogenized 2cm core sample into six 30ml glass centrifuge tubes for each core. In an anoxic glove bag we bubbled a 500 μ M $^{15}\text{NO}_3^-$ seawater solution with nitrogen gas for 15 minutes. In the glove bag we filled the glass tubes with sediment samples to the top and capped them. We then placed all the tubes underwater at room temperature. At 24, 48 and 72 hours we harvested two tubes from each core. One tube we injected with 300 μ l of HgCl_2 and placed back into the water. These tubes were analyzed for $^{30}\text{N}_2$ (Kana et al., 1998). For the second tube we centrifuged and filtered the supernatant into two plastic scintillation vials for nitrate analysis (Wood et al., 1967) and ammonium analysis (Solarzano, 1969 and Strickland, 1972).

We dried the sediments from the four sites and ground them into a fine powder. We ran the sediment powder samples on a CHN analyzer and a mass spectrometer. The CHN analysis allows us to get percent carbon, percent nitrogen and the carbon to nitrogen ratios of the sediments. From these ratios and sediment respiration we can determine expected ammonium production. We used the mass spectrometer to measure the amount of the stable isotope ^{15}N present in the samples. Wastewater from the Falmouth Wastewater Treatment Plant is ^{15}N rich with $\delta^{15}\text{N}$ values ranging from 8 to 20‰ (Valiela, 1995). We used presence of this stable isotope in the sediments to evaluate the influence of the wastewater from treatment plant on the specific sites.

Results

The percent carbon values and percent nitrogen values are higher in the inner harbor than in the outer harbor (Table 1). Sites with eelgrass have higher percent carbon values, although the vegetation effect is less dramatic than the inner versus harbor effect. The outer harbor percent carbon values ranged 0.24% (no eelgrass, site 2) to 0.95% (eelgrass, site 1) whereas the inner harbor values ranged from 3.46% (no eelgrass, site 4) to 4.48% (eelgrass, site 3). The carbon to nitrogen (C: N) ratios for WFH sediments are highest in the outer harbor ranging from 9.63 to 9.75 (Sites 1 and 2 respectively). Inner harbor sites C: N ratios are lower but still higher than the Redfield ratio (Redfield, 1958). Site 3 C: N ratio is 8.59 and Site 4 C: N ratio is 8.83. Sites without eelgrass (2 and 4) show slightly higher C: N ratios than the Sites where eelgrass is still prevalent (1 and 3) however again the vegetation effect is less dramatic than the inner versus outer harbor effect (Table 1).

The inner harbor has lower $\delta^{15}\text{N}$ isotope values than the outer harbor and eelgrass sites are slightly lower than non eelgrass sites (Table 1). Inner harbor sites range from 5.63‰ (eelgrass, site 3) to 6.38‰ (no eelgrass, site 4). Outer harbor sites range from 6.88‰ (eelgrass, site 1) to 7.73‰ (no eelgrass, site 2). Difference in isotope values between the inner and outer harbor are more dramatic than the difference between eelgrass and non eelgrass sites.

In the first set of sediment cores used for *in-situ* flux measurements, oxygen was consumed linearly. The highest oxygen consumption rate observed is from the inner harbor site with eelgrass (cores 5&6). The flux of oxygen at this site is $-31.3 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$. The other inner harbor site without eelgrass (cores 7&8) also has a high respiration rate at $-28.3 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$. Both of the outer harbor sites show lower respiration rates than the inner harbor. The outer harbor eelgrass site (cores 1&2) respiration rate is $-15.6 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$. The outer harbor site without eelgrass (cores 3&4) has the lowest respiration rate compared to the other three sites. The respiration rate for this site is only $-5.9 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Figure 5).

The inner harbor site with eelgrass (Site 3, cores 5&6) has the highest flux of ammonium out of the sediments, $2084 \mu\text{mol m}^{-2} \text{ d}^{-1}$. The flux is nearly 8 times greater than the next highest flux site. Site 1 (outer harbor, eelgrass cores 1&2), has an ammonium flux of $271 \mu\text{mol m}^{-2} \text{ d}^{-1}$. Both the other two sites (Sites 2&4), show a negative ammonium flux. The outer harbor site without eelgrass (Site 2, cores 3&4) ammonium flux is $-116 \mu\text{mol m}^{-2} \text{ d}^{-1}$ and the inner harbor site without eelgrass (Site 3, cores 7&8) ammonium flux is $-48 \mu\text{mol m}^{-2} \text{ d}^{-1}$ (Figure 6).

Using these C: N ratios and respiration data, we determined the expected ammonium production for the different sites (Figure 7). These estimates are considerably higher than the actual *in-situ* ammonium fluxes measured (Figure 6).

All sites except for Site 3 (inner harbor with no eelgrass, cores 5&6), show an increase in the ammonium flux after the nitrification block (Allylthiourea) addition. Both sites with negative ammonium fluxes in the *in-situ* experiment show the largest increases in ammonium flux after the nitrification block. Site 4 (inner harbor with out eelgrass, cores 7&8), increased by $552 \mu\text{mol m}^{-2} \text{ d}^{-1}$ and Site 2 (outer harbor with out eelgrass, cores 3&4) increased by $168 \mu\text{mol m}^{-2} \text{ d}^{-1}$. Site 1 (outer harbor with eelgrass, cores 1&2) shows an ammonium flux increase of $76 \mu\text{mol m}^{-2} \text{ d}^{-1}$. The only site showing little change in ammonium flux is Site 3 (Figure 8).

We calculated the estimated amount of ammonium used in nitrification by two methods. In the first method we subtracted the *in-situ* ammonium production from the expected ammonium production determined from respiration rates and C: N ratio (Figure 9). In the second method we subtracted the *in-situ* ammonium production amount from the amount of ammonium produced after the nitrification block (Figure 10). This method estimates that only small amounts of ammonium are used in nitrification. The sites with out eelgrass have the highest ammonium used and the inner harbor site has the highest amount ($552 \mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$). The inner harbor site with eelgrass shows no ammonium used for nitrification. The first method estimates of ammonium used in nitrification are considerably higher than the estimates calculated using the nitrification block method. The respiration and C: N ratio estimates suggests that relatively high amounts of ammonium are used in nitrification. Again the highest amount of ammonium used is in the inner harbor site without eelgrass ($3251 \mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$). However, the presence of eelgrass seems to have less of an effect on these data (Figure 9).

Under ideal conditions for nitrification the sites with eelgrass show the highest potential rates (Figure 11). The inner harbor Site 3 nitrate flux is $980 \mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ and the outer harbor Site 1 flux is $691 \mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. Both sites without eelgrass show lower nitrification rates compared to the eelgrass sites. Inner harbor Site 4

nitrification potential flux is $151\mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. The lowest potential is observed in the outer harbor Site 3, where we measured a nitrate flux of $22.5\mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. Even under these ideal conditions nitrification values are much lower than the predicted based on the ammonium flux estimate from respiration and C: N ratios (Figure 9).

The nitrate flux out of the sediments is negative for all sites in WFH. Nitrate consumption shows little variation between sites, ranging from $197\mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ (Site 2) to $352\mu\text{mol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ (Site 4) (Figure 12). The nitrification block created the NO_3^- flux (production or consumption) to be minimal in all WFH sites.

Even with high variability, denitrification potential is substantial (Figure 13). Ranging from $1922\mu\text{mol NO}_3^- \text{ consumed m}^{-2} \text{ d}^{-1}$ (inner harbor, eelgrass) to $6149\mu\text{mol NO}_3^- \text{ consumed m}^{-2} \text{ d}^{-1}$ (inner harbor, no eelgrass). Outer harbor sites show little variation from each other ranging from $4187\mu\text{mol NO}_3^- \text{ consumed m}^{-2} \text{ d}^{-1}$ to $4323\mu\text{mol NO}_3^- \text{ consumed m}^{-2} \text{ d}^{-1}$. Unfortunately, these denitrification potentials could not be compared to MIMS $^{30}\text{N}_2$ data do to a problem with the instrument. Only data for site 2 (outer harbor, no eelgrass) was obtained. These data show a clear linear increase in $^{30}\text{N}_2$ concentrations over time (Figure 14) and a denitrification potential value similar but lower than those calculated from NO_3^- consumption, $1100\mu\text{mol N}_2 \text{ produced m}^{-2} \text{ d}^{-1}$ (Figure 15).

Discussion

Based on our observations, percent carbon, C: N ratios, isotope data and measured respiration rates, the inner harbor sites 3 and 4 are much more eutrophic than the outer harbor sites 1 and 2. The Giblin et al. model suggests that the inner harbor is at stage 2 where as the outer harbor sites are still at stage 1 of eutrophication (Figure 1).

The percent carbon values are nearly 7 times higher in the inner harbor signifying that there is a much greater amount of organic matter accumulating in the sediments. The C: N ratio is greater in the outer harbor, which indicates that nitrogen may be more limiting there. The $\delta^{15}\text{N}$ isotope data also shows that nitrogen is more limiting in the outer harbor. The inner harbor shows lower $\delta^{15}\text{N}$ values than the outer harbor. This may be due to the fact that when nitrogen is abundant, plants can discriminate against the heavy isotope and select the ^{14}N over ^{15}N . This will result in sediments with a lighter isotopic composition than sediments in areas where nitrogen is more limiting. Plants in these environments can not afford to be as selective in their nitrogen intake, resulting in heavier sediments.

Respiration rates measured are higher in the inner harbor than in the outer harbor. These data correspond to our percent carbon data. They show that increased levels of organic matter in the sediments increases decomposition and decreases oxygen levels. Eelgrass sites have higher respiration rates since eelgrass is likely to contribute substantial amounts of organic matter to the sediments.

The net ammonium flux is much lower than expected from respiration rates measured. This initially caused us to believe that the lost ammonium was used in nitrification/denitrification since the other ammonium removal mechanisms such as algal uptake and microbial immobilization were supposedly accounted for. We incubated the cores in the dark to diminish algal production and ammonium uptake and microbial immobilization in sediment cores from coastal environments is thought by many to be minimal. Unfortunately our two methods of testing whether the lost ammonium from

decomposition did in fact get nitrified gave as slightly different stories to decipher. The respiration, C: N ratio method indicated that a large amount of ammonium was being converted and used up in nitrification/denitrification processes. However, the nitrification block method data implied that little ammonium was being for nitrification/denitrification.

The different story told by these two methods is curious and may indicate that ammonium is being lost by a mechanism other than nitrification/denitrification. Even though we incubated the cores in filtered sea water, in the dark, it may be possible that algal ammonium uptake occurred in these cores either before or during sampling. Even though microbial immobilization is thought to have an insignificant role in ammonium uptake in these sediment cores, it could prove to be a potential sink for ammonium. Another important factor to consider is cold temperatures like our incubation temperature of 10° C inhibits the activity of many microbes and may change the species dynamic that normally exists during warmer conditions. In addition, since there was such a small measured response to the allylthiourea nitrification block, it may be that the block didn't get to the sediments and didn't really do its job blocking nitrification/denitrification. This would explain why the amount of ammonium estimated to be used in nitrification/denitrification is so much lower than the estimate using respiration rates and C: N ratios.

The data collected for nitrification potential is fairly robust and shows nitrification rates up to 1000 $\mu\text{mol NO}_3^- \text{ produced m}^{-2} \text{ d}^{-1}$. Even under the ideal nitrification conditions we incubated the sediment slurries in, nitrification potential rates are still lower than those estimated using respiration and C: N ratio. These data seem to suggest that active nitrifying bacteria communities are low in the sediments, perhaps due to seasonal pressures. The data shows a strong effect of eelgrass on nitrification potential. We originally hypothesized eelgrass beds to have lower nitrification/denitrification potentials since eelgrass takes up ammonium from the sediments and this would decrease the nitrifying bacterial community. However, in this study we found that eelgrass sites in both the inner and the outer harbor have much higher nitrification potentials than the non eelgrass sites. The inner harbor eelgrass site nitrification potential is nearly 7 times greater than the non eelgrass site in the inner harbor and the outer harbor eelgrass site is approximately 30 times greater than the non eelgrass site in the outer harbor. Although these data are robust the results may be due to some seasonal effect and it would be particularly important to conduct nitrification potential measurements at different times of the year. If further study proves that eelgrass areas have dramatically higher potential for nitrification, this may have important implications for the estuary since abundance of eelgrass beds are dramatically reduced with increasing eutrophication.

Nitrate consumption in the original *in-situ* flux measurements are variable and low. This indicates that little uncoupled denitrification is occurring. However, under ideal conditions and abundant nitrate, denitrification rates are high. This indicates that when nitrate is made available (mainly by nitrification), denitrification does occur. The data do not show strong differences between sites as expected. We hypothesized that inner harbor sites would have higher denitrification rates than the outer harbor and non eelgrass sites to have higher denitrification rates than the eelgrass sites. We also expected nitrification and denitrification potential data to show similar values and patterns since the processes are thought to be coupled in these sediments. Obtaining

accurate MIMS $^{28}\text{N}_2$ and $^{30}\text{N}_2$ data from the *in-situ* flux and potential flux would help in measuring robust denitrification rates.

This study shows that increased nitrogen loading and the presence of eelgrass increases organic carbon content in the sediments and increases decomposition and respiration. We speculate that the increased amount of ammonium produced from decomposition will be nitrified and then denitrified in coupled microbial processes. Thus increasing nitrogen loading will increase denitrification rates to a certain point where oxygen concentration in the top portion of the sediments is too low to enable nitrification. The effect of eelgrass on the coupled nitrification/ denitrification process is undetermined. We suspect that during times of high growth and productivity eelgrass beds may hinder nitrification by taking up ammonium from the sediments. However, our data taken in the late fall indicates that eelgrass beds have a considerably higher nitrification potential. Clearly further research conducted at different times during the year is necessary.

Nitrogen cycling in shallow coastal ecosystems is complex and requires thorough research. Future research should take more cores from each site and take more replicate water samples for nutrient analyses during flux experiments. This may help to reduce data variability. Microbial processes are greatly affected by temperature and season. Therefore, in future studies cores should be taken at multiple times during the year. Additionally, measuring nitrogen fixation will give a more complete study of nitrogen cycling in these estuaries.

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Figure 1- Conceptual model of changes in available nutrients (top), nitrogen cycle microbial processes (middle), and primary producers (bottom)

(Giblin et al., NSF Project Proposal)

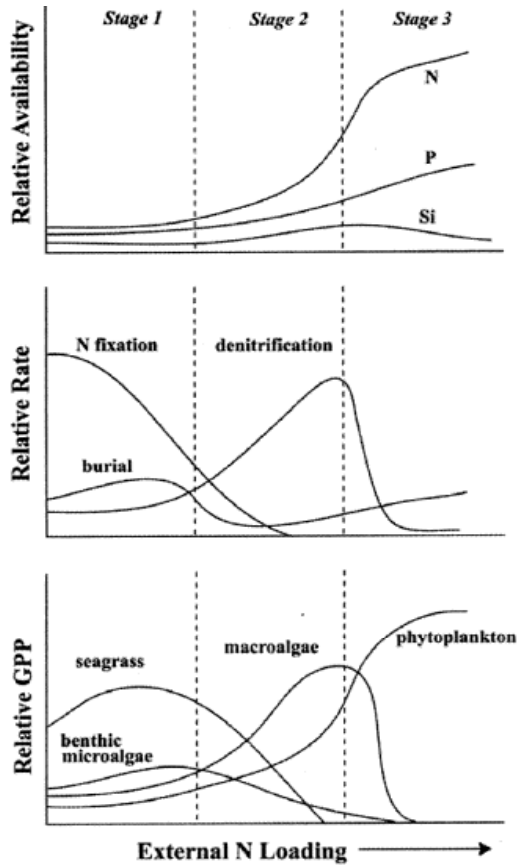


Figure 2- West Falmouth Harbor

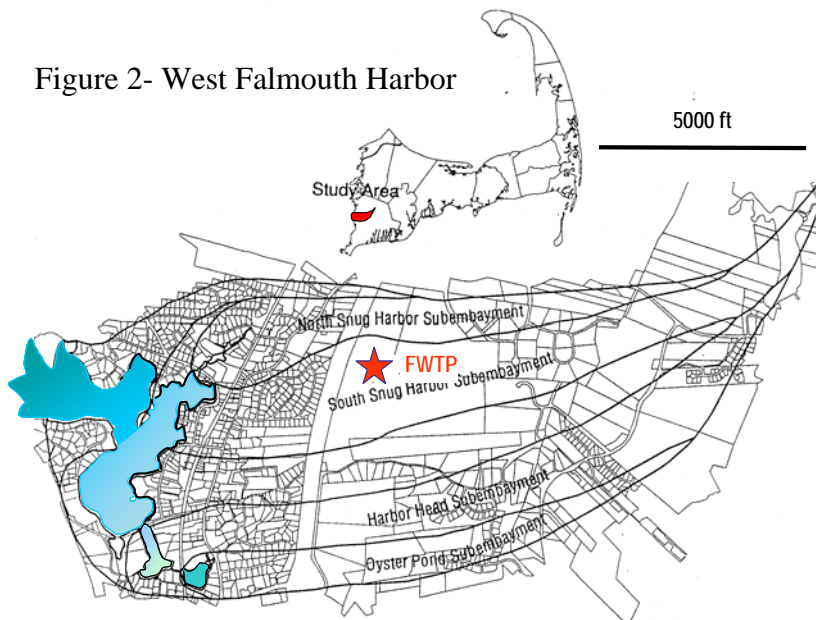


Figure 3- Nitrogen loading to West Falmouth Harbor from Falmouth Wastewater Treatment Plant

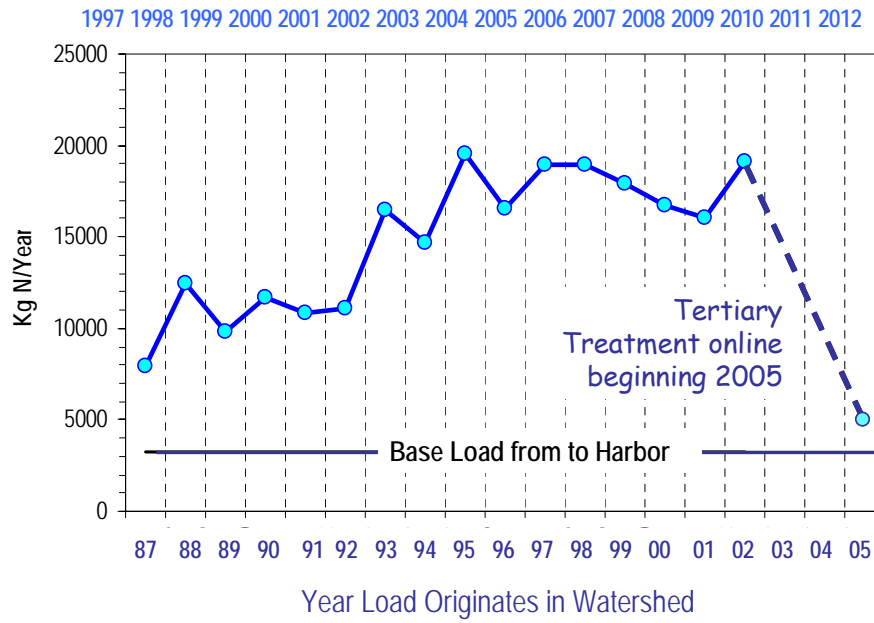


Figure 4- Project coring sites in West Falmouth Harbor



Table 1- Percent carbon and nitrogen and d15N Isotope data of West Falmouth Harbor sediments

WFH Site	%C	%N	C:N	d15N o/oo vs. air
OH, Eel	0.95	0.1	9.63	6.88
OH, No Eel	0.24	0.025	9.75	7.73
IH, Eel	4.48	0.525	8.59	5.63
IH, No Eel	3.46	0.4	8.83	6.38

Figure 5- West Falmouth Harbor Sediment Core Respiration: *in-situ* O₂ flux

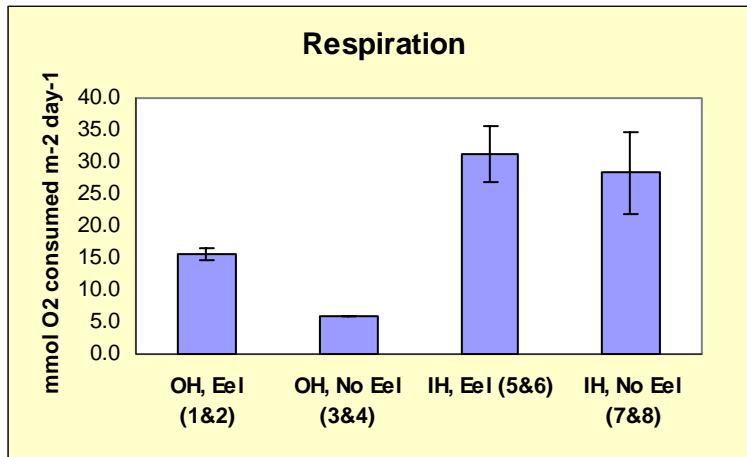


Figure 6- West Falmouth Harbor sediment core net ammonium *in-situ* flux

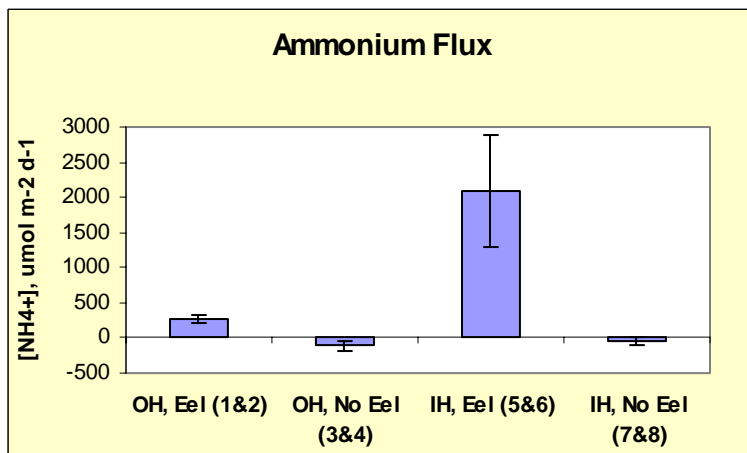


Figure 7- Total expected NH_4^+ production from ammonification based on respiration and C: N ratios of West Falmouth Harbor sediments

Note: These flux numbers represent the total amount of NH_4^+ produced in ammonification before NH_4^+ loss in nitrification/ denitrification, immobilization or algal uptake

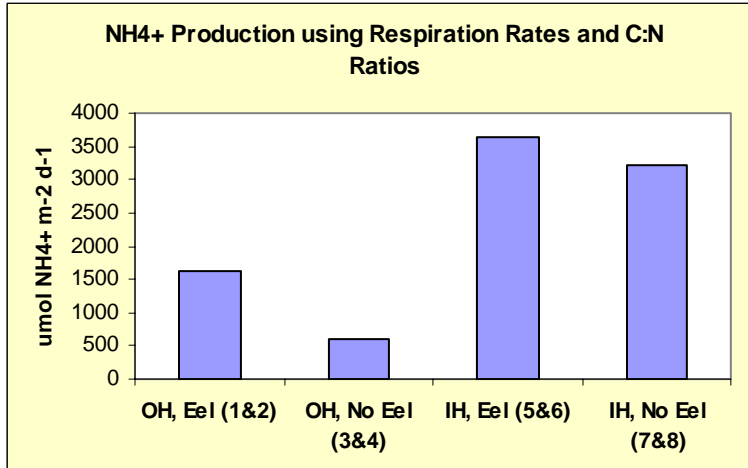


Figure 8- Total NH_4^+ production from ammonification measured by nitrification block experiment

Note: These flux numbers represent the total NH_4^+ produced from ammonification by blocking nitrification/ denitrification using Allylthiourea

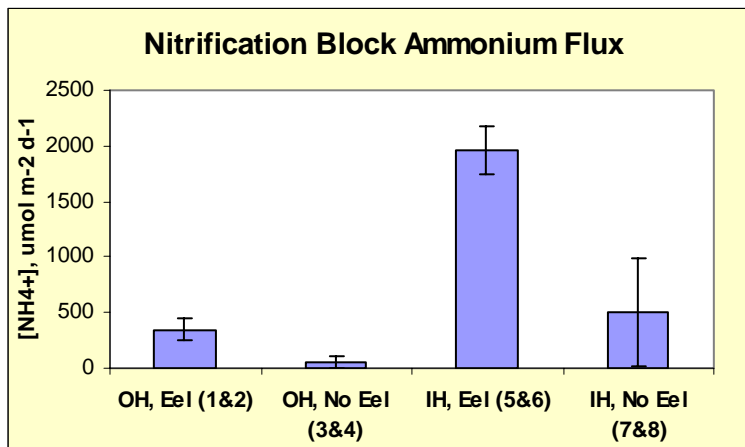


Figure 9- Estimated NH_4^+ used in nitrification/denitrification based on respiration and C: N data

Note: These numbers are calculated from the estimated total NH_4^+ production (total ammonification) minus the net *in-situ* NH_4^+ flux.

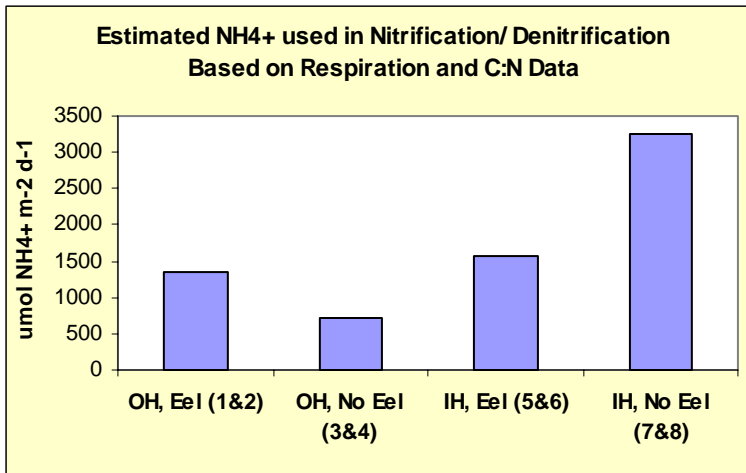


Figure 10- Estimated NH_4^+ used in nitrification/ denitrification using nitrification block

Note: These numbers are calculated from the estimated total NH_4^+ production (total ammonification) minus the net *in-situ* NH_4^+ flux.

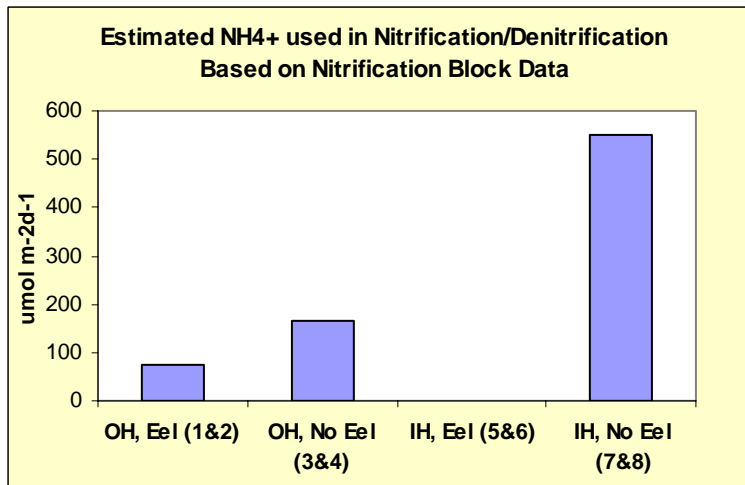


Figure 11- Nitrification potential

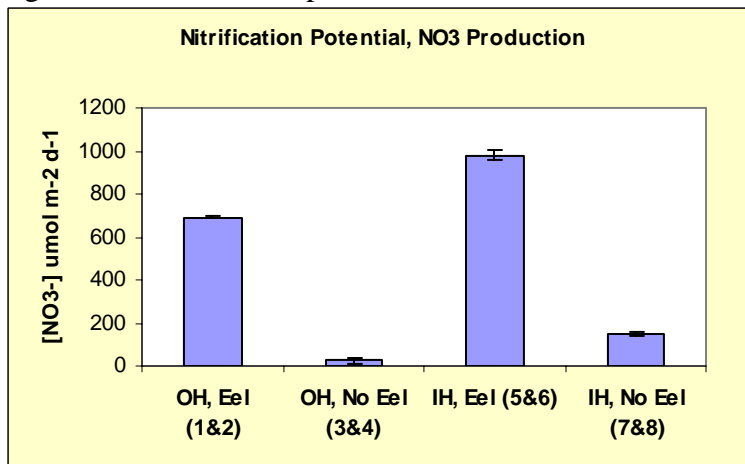


Figure 12- West Falmouth Harbor sediment core *in-situ* nitrate consumption

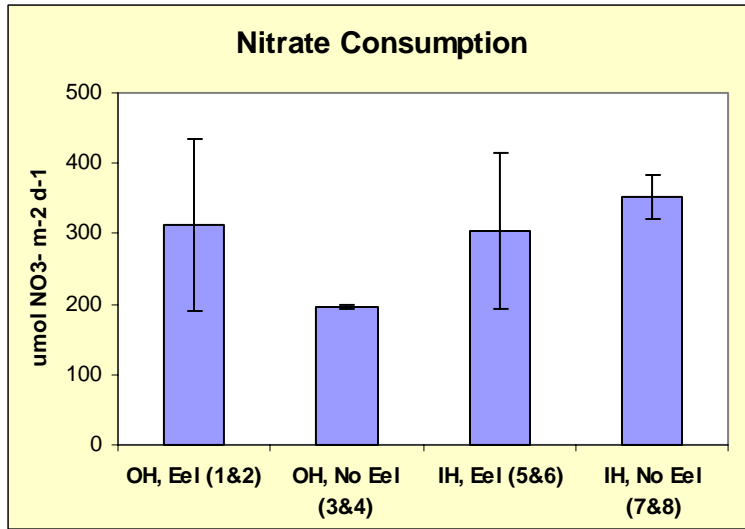


Figure 13- Denitrification potential

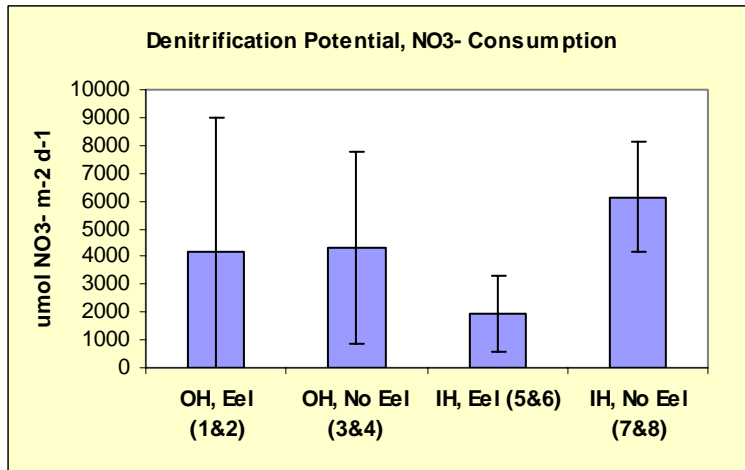


Figure 14- Potential denitrification rate for outer harbor with no eelgrass: $N_{2(g)}$ measured using MIMS

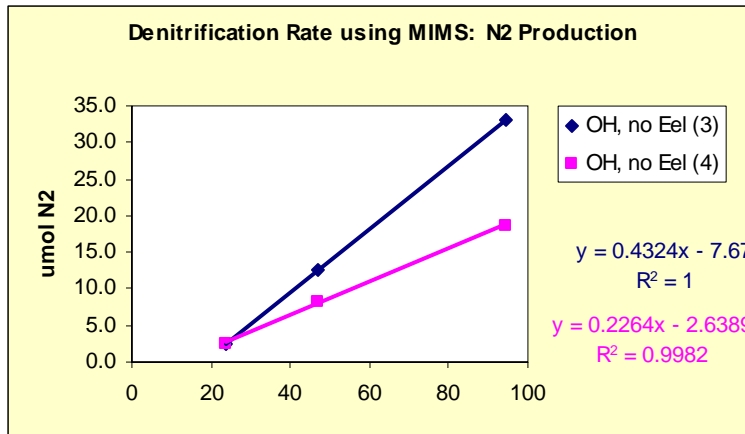
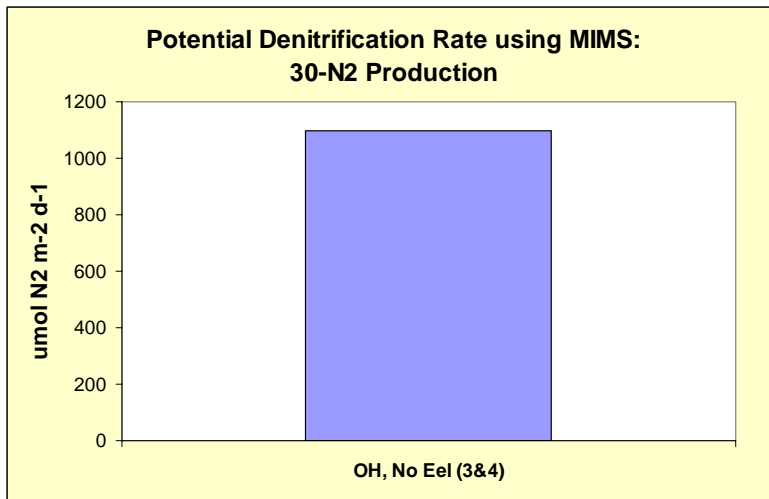


Figure 15- Denitrification potential $N_{2(g)}$ flux measured using MIMS



Appendix A: CHN, Isotope and West Falmouth Harbor Sediment Core *In-situ* Flux Data

CHN and Isotope Data

	%C	%N	C:N	d15N o/oo vs. air	
OH, Eel		0.95	0.1	9.63	6.9
OH, No Eel		0.24	0.025	9.75	7.7
IH, Eel		4.48	0.525	8.59	5.6
IH, No Eel		3.46	0.57	8.83	6.4

Sediment Respiration

	O2 (mmol m-2 d-1)	AVG O2	error
OH, Eel (1&2)	-14.61	-15.63	
	-16.65		1.02
OH, No Eel (3&4)	-5.92	-5.88	
	-5.83		0.04
IH, Eel (5&6)	-35.70	-31.34	
	-26.98		4.36
IH, No Eel (7&8)	-21.76	-28.29	
	-34.82		6.53

In- Situ Fluxes

	NH4+ (umol m-2 d-1)	AVG NH4+	error	NO3- (umol m-2 d-1)	AVG NO3-	error
OH, Eel (1&2)	322.16			-190.55		
	220.37	271.27	50.90	-435.54	-313.04	122.49
OH, No Eel (3&4)	-44.58			-193.77		
	-186.61	-115.60	71.01	-199.87	-196.82	3.05
IH, Eel (5&6)	2884.66			-414.02		
	1283.72	2084.19	800.47	-192.02	-303.02	111.00
IH, No Eel (7&8)	-106.70			-322.28		
	11.27	-47.71	58.98	-382.49	-352.39	30.10

Nitrification Block Fluxes

	B NH4+ (umol m-2 d-1)	AVG B NH4+	error	B NO3- (umol m-2 d-1)	AVG B NO3-	error
OH, Eel (1&2)	250.46			58.93		
	444.21	347.34	96.88	4.34	31.64	27.30
OH, No Eel (3&4)	1.71			-17.15		
	101.71	51.71	50.00	-33.61	-25.38	8.23
IH, Eel (5&6)	2174.44			-42.41		
	1736.91	1955.67	218.76	2.69	-19.86	22.55
IH, No Eel (7&8)	13.89			-2.92		
	994.61	504.25	490.36	33.81	15.44	18.37

Redfield expected N

	R NH4+ (umol m-2 d-1)	C:N Ratios	Expected N using C:N (umol m-2 d-1)
OH, Eel (1&2)	2359.04	9.63	1623.76
OH, No Eel (3&4)	887.01	9.75	602.71
IH, Eel (5&6)	4730.76	8.59	3648.82
IH, No Eel (7&8)	4270.79	8.83	3203.50

Appendix B: Nitrification and Denitrification Potential Data

Denitrification Potential Fluxes

	NH4+ (umol m-2 d-1)	avg. NH4+ error		NO3- (umol m-2, d-1)	avg. NO3- error	
OH, Eel (1&2)	39.47			634.65		
	21.18	30.33	9.14	-9007.95	-4186.65	4821.30
OH, No Eel (3&4)	38.69			-867.50		
	3.72	21.20	17.49	-7777.79	-4322.65	3455.15
IH, Eel (5&6)	21.65			-553.22		
	24.60	23.12	1.48	-3290.69	-1921.95	1368.73
IH, No Eel (7&8)	771.81			-4152.01		
	11.19	391.50	380.31	-8146.36	-6149.18	1997.18

Nitrification Potential Flux

	NO3- (umol m-2 d-1)	Avg. NO3- error	
OH, Eel (1&2)	684.27		
	698.04	691.16	6.89
OH, No Eel (3&4)	32.94		
	13.76	23.35	9.59
IH, Eel (5&6)	957.99		
	1002.98	980.49	22.50
IH, No Eel (7&8)	142.02		
	159.97	150.99	8.98

