

# The Effect of Changes in Salinity and Sulfate Concentration on Potential Nitrification in Freshwater, Estuarine, and Marine Sediments

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## **Abstract:**

Coupled nitrification-denitrification is generally more efficient in freshwater environments than in marine environments. Differences in nitrification rates between these two environments are thought to contribute to this difference in efficiency. In this study I examined two potential limitations on nitrification efficiency through their effect on the potential nitrification rates of sediments collected from freshwater, estuarine, and marine systems. I used intact cores from freshwater and marine sites with altered sulfate concentrations in the water column to investigate the influence of sulfate on nitrification rates, and used sediment slurries from all three sites incubated in water of 0, 10 or 32ppt solution to investigate the influence of salinity on nitrification.

Initial potential nitrification rates were highest in freshwater sediments measured at 0ppt and marine sediments measured at 32ppt. After incubation, marine cores incubated without sulfate displayed higher potential nitrification rates than control cores, and freshwater cores experienced little change in rate when incubated with sulfate addition to overlying water. Salinity incubations show that in marine sediments salinity may be an important factor regulating growth of nitrifying bacteria. Estuarine sediment incubated at 0ppt shows higher potential nitrification when measured at 0ppt than when incubated at either 10ppt or 32ppt. Marine sediment incubated at 0ppt and 10ppt has higher potential nitrification at all measured salinities than in control slurries incubated at 32ppt. This data suggests that nitrifiers from both estuarine and marine sediments may be able to adapt to changes in ambient salinity within three weeks of salinity change, which is a much shorter adaptation time than previously suggested.

**Keywords and phrases:** *nitrification, potential nitrification, salinity, sulfide inhibition, nitrifying bacteria, nitrogen cycling, adaptation*

## **Introduction:**

Sedimentary processes are important for controlling nitrogen cycling and dynamics in estuarine systems. Nitrification by bacteria within sediments is an important process in determining the amount of nitrate available for uptake and denitrification within the system. This is particularly important in the recycling of nitrogen within water bodies, since

nitrification is an essential process contributing to loss of total nitrogen from systems through denitrification.

It has been frequently observed that rates of nitrification are slower in waters with higher salinity, for which two explanations have been suggested. The first is that salinity itself drives the depression of nitrification rates, through both its impact on ammonium availability (Boatman and Murray 1982, Rysgaard et al 1999, etc) and through its physiological impact on nitrifiers (Mondrup et al, in press, Rysgaard et al 1999, etc). The second is that higher sulfate concentrations and accompanying rates of sulfate reduction present in salt water produce sulfides that inhibit nitrification (Joye and Hollibaugh 1995).

When comparing rates of nitrification among freshwater, estuarine, and marine systems, both salinity and sulfate concentrations vary, and it is important to understand the importance of both factors in limiting nitrification within these systems. There also has been evidence from culture experiments that suggests some species of nitrifiers are able to adapt to salinity changes (Finstein and Bitsky 1972), and that the time required to adapt to changes varies among species. However, the majority of these experiments have been conducted on cultures of nitrifiers, rather than on intact sediment samples from ecosystems, with a few exceptions (ex. Rysgaard et al 1999, Mondrup et al in review). These studies also were conducted on nitrifiers from estuarine systems that may be more used to fluctuating salinity, while few studies have looked at the response of nitrifiers from ecosystems with a stable salinity. Additionally, prior studies have demonstrated the ability of nitrifiers to adapt to salinity changes over the course of 2½ months (Mondrup et al, in press), however none have investigated this over a more complete time course to see how long it takes nitrifiers to respond to a change in salinity.

The goal of this experiment was to examine the effect of differences in salinity and sulfate on rates of potential nitrification in sediment slurries and intact cores from freshwater, estuarine and marine sites. I attempted to answer three questions in this study. The first was how the presence of sulfate influences rates of potential nitrification in marine and freshwater systems. I hypothesized that the presence of sulfate would depress rates of potential nitrification. Secondly, I wanted to examine how changes in salinity impact nitrification rates, focusing on how sediment from an ecosystem responds when incubated under different salinities. I hypothesized that sediments would have the highest rates under salinities similar to their natural environment, and that after several weeks' incubation under a different salinity regime they would begin to adapt and increase rates of nitrification. For my final question I wanted to compare the effects of salinity and sulfate on nitrification rates, to see which seemed to have a larger effect in the environments studied.

## **Methods:**

### *Study Sites and Field Techniques:*

I took cores from three sites for this experiment in early November; Massachusetts Bay (Marine), Childs River (Estuarine), and Johns Pond (Freshwater). Massachusetts Bay cores were taken offshore, where there would be little influence of freshwater runoff from land, and the salinity remains relatively constant around 32ppt. Estuarine cores were taken from the Childs River in a location that would see varying salinity depending on the recent storm activity and season. When sampling occurred, the surface salinity in this area was approximately 12ppt. Johns Pond cores were taken from the bottom of the pond, which has no saltwater influence and a salinity of ~0ppt. At Johns Pond, divers collected eight cores in 3" core tubes. In the Childs River, cores were taken manually in waist-deep water. In Massachusetts Bay, cores were taken by box coring. These cores were then brought back to the lab and aerated until subsampled.

In the lab, the top two centimeters of core were separated from the 2 cores for analysis, representing the oxic layers of the sediment. This sample was homogenized and used for the subsequent salinity manipulations. Four additional cores from Massachusetts Bay and Johns Pond were left intact and set up for the sulfate experiment.

#### *Sulfate Manipulation:*

One set of cores from Massachusetts Bay and Johns Pond were used solely for this portion of the experiment. Two cores from each site were used as controls, and filled with water from the site. In the other two cores from each site, the water was siphoned out and replaced with water of the correct field salinity, but with altered sulfate concentrations. In the marine cores, artificial seawater was used that was sulfate-free, and in the freshwater cores, sulfate was added to water from the field site to a concentration of 2mM.

I took initial potential nitrification rates for the control cores using sediment samples from identical cores taken at the same time. Each of these cores was then incubated at X°C for approximately 26 days. After this time, the top 2cm were subsampled and used for a potential nitrification assay (as described below) in water of 0,10, and 32ppt, and with 300µM NH<sub>4</sub><sup>+</sup> and 60µM PO<sub>4</sub><sup>3-</sup>.

#### *Salinity Manipulation:*

I removed the top 2 cm from the cores from each of my three sites and homogenized it to make three sediment samples, labeled fresh (F), estuarine (E) and marine (M). I used a subsample of this sediment to take initial potential nitrification measurements of each of these three sediments in water of 0, 10, and 32ppt. The rest of the sediment from each of the three sites was used to make slurries in 2L flasks for the salinity incubations.

Nine flasks were set up for salinity manipulation. From each site, I put equal masses of sediment into three flasks and filled each one to 1.2L with water of a different salinity (0, 10 or 32ppt; the incubation salinity). I designated these flasks with a letter for the sediment type and number for the incubation salinity. For example, marine sediment incubated in 10ppt water is designated M10 (Table 1). The flasks were placed on an orbital shaker to prevent the slurries from becoming anoxic. I then subsampled each flask after 6, 11, and 20 days to do a potential nitrification assay. I sampled the water in the flasks each week and ran ammonium analysis to ensure that adequate ammonium was present. The water in the freshwater and estuarine flasks was changed after day 13 to prevent excessive ammonium accumulation, and 1mL of 300µM NH<sub>4</sub><sup>+</sup> was added to the marine flasks to ensure the presence of ammonium. Water designated 0ppt in this experiment was composed of a solution of 1:500 diluted seawater.

#### *Potential Nitrification Assay:*

For the assay, sediment was taken from the top of the cores (in the case of the sulfate and initial salinity samples) or subsampled from the flask after allowing the sediment to settle (for salinity samples). The sediment slurry obtained was then centrifuged to remove much of the water in the sample, and the remaining sediment in the tube was homogenized.

For the salinity manipulation experiment I subsampled each of the nine flasks and measured potential nitrification at three salinities (0, 10, 32ppt). I assigned the potential nitrification measurements symbols that begin with the flask designation and have the measurement salinity appended on the end (ex. M10-0 for marine sediment incubated at 10ppt and measured at 0ppt). This gave me a total of 27 different salinity treatments.

For each of the 27 salinity treatments (and the initial and final core samples) I set up five centrifuge tubes for potential nitrification measurement. Each tube contained the

sediment sample from the flask and 25mL of solution at the appropriate salinity. These solutions also contained 300 $\mu$ M NH<sub>4</sub><sup>+</sup> and 60 $\mu$ M PO<sub>4</sub><sup>3-</sup> to ensure that these nutrients were not limiting nitrification rates. I put the tubes on the shaker table at approximately 28°C to ensure mixing of the water, sediment and headspace so they remained oxic at a sufficient temperature to support microbial growth. After approximately 3 hours I removed one tube from each treatment, centrifuged it, and filtered the supernatant, which I then froze for future nitrate assessment. I took replicate tubes at 24 and 48 hours for the same analysis. I then ran these water samples for nitrate concentration using a Cd-Cu reduction (Wood et al. 1967), converting NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> on the Lachat QuikChem 8000 flow injector analyzer.

Potential nitrification rates were calculated using a linear regression for the nitrate increase in each treatment. The standard error for the slope of the regression was calculated in Microsoft Excel. Twice the standard error was used to indicate the 95% confidence interval for each slope when comparing the response among different treatments.

## **Results:**

### *Salinity Incubations:*

Initial rates of potential nitrification from all sediment types were low, below 1  $\mu$ molNO<sub>3</sub>/g/day (Figure 1). The highest rates were found in the freshwater sediment measured at 0ppt (F0), and in the marine sediment measured at 32ppt (M32). The nitrification rates of sediment incubated at other salinities were not statistically different, and were all substantially lower than the F0 and M32 rates. The estuarine sediment did not initially display significant rates of potential nitrification at any salinity measured.

The marine sediment showed the most consistent response to incubation, with the overall pattern of nitrification rates increasing over the duration of the experiment for most treatments. When incubated at 0ppt (M0 samples), nitrification rates increased over time for all treatments (Figure 2a). At all three measured salinities, M0 sediment did not display statistically different nitrification rates. When incubated at 10ppt (M10 samples), marine sediment nitrification rates again increased with incubation length (Figure 2b), and by day 20 the sample measured in freshwater (M10-0) had significantly lower nitrification rates than the sample measured in saltwater (M10-32). When incubated at 32ppt (M32 samples), marine sediment showed the highest nitrification rates when measured at 32ppt (Figure 3c), and rates of nitrification when measured at 0 and 10ppt were lower, but not statistically different from each other. Marine sediment incubated at 0 and 10ppt does not seem to show a strong correlation between measurement salinity and potential nitrification after 20 days of incubation (Figure 3). Also, potential nitrification rates are lower for sediment incubated at 32ppt than at either 0ppt or 10ppt (Figure 3).

For freshwater sediment incubated at 0ppt, nitrification rates initially were highest when measured in freshwater (Figure 4). After the incubation began, nitrification rates dropped rapidly in all F0 treatments, and by day 11 all rates in F0 samples had dropped to near zero, with the exception of the 32ppt measurement, and remained low for the duration of the experiment. The freshwater sediment incubated in 10 and 32ppt solution (F10 and F32 samples) and measured at all three salinities did not demonstrate that nitrification was occurring. The rate of nitrate increase in these incubations wasn't statistically different from zero.

The estuarine cores displayed very low or rates of potential nitrification for the first 11 days of the experiment (first three measurements), with rates of nitrate production at or near zero. Because of this, the change in nitrification rate over the duration of the incubation is not shown. However, by day 20 these cores displayed significantly increased rates in all treatments (Figure 5). For all incubations (E0, E10 and E32), the sediment had the highest

nitrification potential when measured at 10ppt. For cores incubated at 10 and 32ppt, the next highest rate occurred when measured at 32ppt, and the next highest rate occurred at 0ppt for the sediment incubated at 0ppt.

The nitrification rates of the control slurries (sediment incubated and measured in its natural salinity) at the end of the experiment were different from initial nitrification rates when first brought in from the field (Figure 6). For the estuarine and marine sediments, the final rates were higher than initial rates, and for freshwater sediment the final rate was lower than the initial rate. Additionally, all slurries maintained positive ammonium concentrations over the duration of the experiment (Table 2), with the freshwater and estuarine slurries maintaining higher ammonium levels than the marine slurries.

#### *Sulfate Manipulation:*

The initial potential nitrification rates for the experimental sulfate control cores are the same as the initial rates for the salinity samples F and M, since the sediment was taken from cores from the same site. Final potential nitrification rates were taken on day 20, and for the freshwater control cores the final nitrification rates were higher than the initial rates (Figure 7). In the marine control cores, final and initial nitrification rates were essentially the same (Figure 8). Also, the magnitude of potential nitrification was larger for the freshwater control cores than the marine control cores.

The cores that had manipulated sulfate concentrations displayed different behavior than the control cores for both freshwater and marine cores. In freshwater cores where sulfate was added, nitrification rates after 25 days were similar to the control cores, and slightly lower when measured at 0ppt (Figure 9). In marine cores where sulfate was removed from solution, nitrification rates were higher than in control cores after 25 days when measured at 10ppt and 32ppt (Figure 10).

#### *Incubation Response:*

The sediment slurries used for the salinity manipulation displayed different potential nitrification rates than the intact core samples used for the sulfate manipulation. For the freshwater control slurries and cores, potential nitrification rates at the end of the incubation were much higher in the cores than in the slurries at all measured salinities (Figure 7), with the highest rate in the F0-0 sample. For the marine control slurries and cores, potential nitrification rates at the end of the incubation were much higher in the slurries than in the cores at all measured salinities (Figure 8), with the highest rate in the F32-32 sample.

#### **Discussion:**

Nitrification rates are thought to be inhibited when high sulfate concentrations are present. A study by Joye and Hollinbaugh (1995) showed that the addition of hydrogen sulfide to estuarine sediments could reduce nitrification by 50 to 100%. In this experiment, sulfate was manipulated rather than sulfide with the assumption that sulfate reduction would occur in the anoxic layers of the core sediment, thereby producing sulfides. When sulfate was added to intact freshwater cores, there was little change in rates of nitrification, with the possibility of a slight decrease in potential nitrification rates that would need to be verified with additional replicates (Figure 9). There is also a possibility that the addition of NaSO<sub>4</sub> to the cores, which may have caused a subtle salinity effect on nitrifying bacteria.

In marine cores incubated without sulfate there was a significant increase in nitrification rates (Figure 10). This suggests that when sulfate is removed from the water column, what is left in the sediment is utilized and rates of sulfide production decrease. The

removal of sulfides from surface (oxic) sediment within the core could allow larger populations of nitrifiers to grow, thereby increasing overall potential nitrification.

There were significant differences between the nitrification rates of the core incubations and the sediment slurries over the duration of the experiment. Potential nitrification began the same for both types of treatment, and by the third week the control cores of each type differed drastically (Figures 6,7). Freshwater core potential nitrification increased over the course of the experiment while marine core rates decreased, and the opposite trend occurred in slurry incubations over the same period.

The community of nitrifiers present in the sediment of Johns Pond did not appear to respond well to slurry culture within the laboratory. Initially freshwater sediment slurries displayed the predicted trend for freshwater sediment; rates of nitrification were highest when the sediment was in a freshwater solution (Figure 1), presumably because the freshwater bacteria were adapted to optimally function in water of zero salinity. When measured in water with a higher salinity, their nitrification potential decreased as expected, either because the salinity change caused mortality of some portion of the nitrifying population, or because the increased salt impaired the individual bacteria's ability to nitrify. Because excess ammonium was added to the tubes during the potential nitrification measurement, this difference is not likely due to the difference in ammonium availability that is a result of higher salinity in solution. However, by the next sampling time potential nitrifications had dropped to near zero while at the same time microbes in the freshwater cores appeared fine. The specific reason for this is not clear, and may be an effect of the particular assemblage of bacteria present in these samples. While the freshwater nitrifying community did not culture well in the slurry experiment, the marine sediment nitrifiers responded well to incubation, increasing their nitrification rates drastically over the course of the experiment (Figure 2c).

If salinity alone is the major factor inhibiting nitrification we would expect to have seen the initial freshwater sediment have higher potential nitrification than the marine sediment. However, the initial potential nitrification rates of marine and freshwater controls were the same. Part of the explanation for this similarity could be the influence of field dissolved oxygen levels on the microbial communities. Johns pond was stratified until shortly before cores were taken, with DO levels of 0.17mg/L (1.5% saturation) in September (Korth et al, 2003). The marine cores taken from Mass. Bay were taken from an area that likely is oxygen saturated much of the time (Giblin, personal communication). The presence of hypoxic conditions at the bottom of Johns Pond (freshwater site) may have limited the growth of nitrifiers so that when initial rates were measured in the laboratory they were lower than they would have been if the water had not been hypoxic prior to sampling. To test this theory I ran a potential nitrification assay on an anoxic core from Johns' Pond. The nitrification of the anoxic sediment was only 19% that of the control core. Because the actual nitrification assay is done aerobically, this shows that prolonged anoxia (3 weeks in this case) can decimate the nitrifying population so that the community's ability to produce nitrate under oxic conditions is impaired. This suggests that the low dissolved oxygen at the bottom of Johns Pond may have decreased the nitrifying population and contributed to the low nitrification values seen in the initial freshwater slurries that are similar to the initial marine rate.

The estuarine sediment did not display significant rates of nitrification initially when brought into the lab or shortly after incubation began. This may be a result of low populations of nitrifying bacteria in the sediment in the field. This suggestion is supported by the nitrification rates observed on day 20 (Figure 5). Estuarine sediment after 20 days shows significant rates of nitrification, compared to previous samplings when the rates were

at or near zero. Estuarine sediment measured in 10ppt water had the highest nitrification rates for all incubation salinities. This implies that the nitrifying bacteria were present, but not nitrifying in prior experiments. It also implies that over the course of a 20-day incubation in water of other salinities, these bacteria still function optimally in water of 10ppt salinity, their native salinity.

While estuarine nitrifiers have highest nitrification rates when measured at 10ppt, the data also indicate that these bacteria have the ability to adapt to other salinities. Estuarine sediment incubated at 0ppt for 20 days shows higher potential nitrification when measured at 0ppt than either of the other incubations (Figure 5). Likewise, sediment incubated at 32ppt for 20 days shows higher potential nitrification when measured at 32ppt than either of the other incubations. This suggests that after three weeks estuarine bacteria can begin to adapt to an environment with altered salinity, either increasing their growth rate while nitrifying with the same efficiency or nitrifying more efficiently with the same population. Previous work by Mondrup et al. (in press) indicates that this adaptation can occur within 2.5 months of a salinity change, but this suggests that the transition can occur much more rapidly.

The marine sediment salinity incubation data also suggests that marine bacteria have the potential to adapt to salinity changes in their environment. The marine slurries started with the same initial sediment and thus presumably the same community of nitrifiers. If these communities had remained the same, we would see little variability in the potential nitrification rates after 20 days of incubation (Figure 3). While there is a lot of noise in the data, one clear trend is that when incubated in 0ppt or 10ppt water, slurries have higher potential nitrification rates than when incubated at 32ppt. This is counter to the argument that microbes that are native to marine sediment should be best adapted to 32ppt conditions. One possible explanation is that salinity is impeding the growth of nitrifiers at 32ppt. If this is true, then what may be occurring is that in the original sediment sample the high salinity was limiting the growth of nitrifying bacteria. When this sediment was incubated at lower salinities this limitation was removed or relaxed, allowing the bacteria population in the flasks to increase. As a result, when I tested for potential nitrification there were more bacteria nitrifying in the sample, which is responsible for the higher rates.

Since there wasn't a significant correlation between the measured salinity and the nitrification rate in all incubated marine slurries, it could suggest that salinity has a more important impact on the growth of nitrifiers, rather than on an individual bacterium's ability to nitrify. If salinity had an impact on the physiology of the present bacteria, then we would be likely to see a decreased potential nitrification when measured at 32ppt, which was not observed. Instead we see the lower potential nitrification values when the sample was incubated at 32ppt. Even if this mechanism isn't responsible for the observed trend in nitrification rates, the data show that short-term salinity changes (on duration of a few days as in the potential nitrification assay) don't influence nitrification ability, but that over long time scales it does. So a long duration salinity change could influence the population of microbes present, but may not affect their nitrification ability on the short term

### **Summary:**

Data collected in this study suggests that there is the potential for nitrifying bacteria in estuarine sediments to begin showing adaptation to changes in ambient salinity in as short a time period as three weeks, as has also been shown by Mondrup et al. (in press) to occur after longer time periods. The data also suggest that marine nitrifiers might have similar ability to adapt to other salinities, but might take longer to conclusively exhibit this adaptation. Prior studies have not examined the adaptability populations of nitrifiers in systems with stable salinities, and this work suggests that a closer look at these organisms

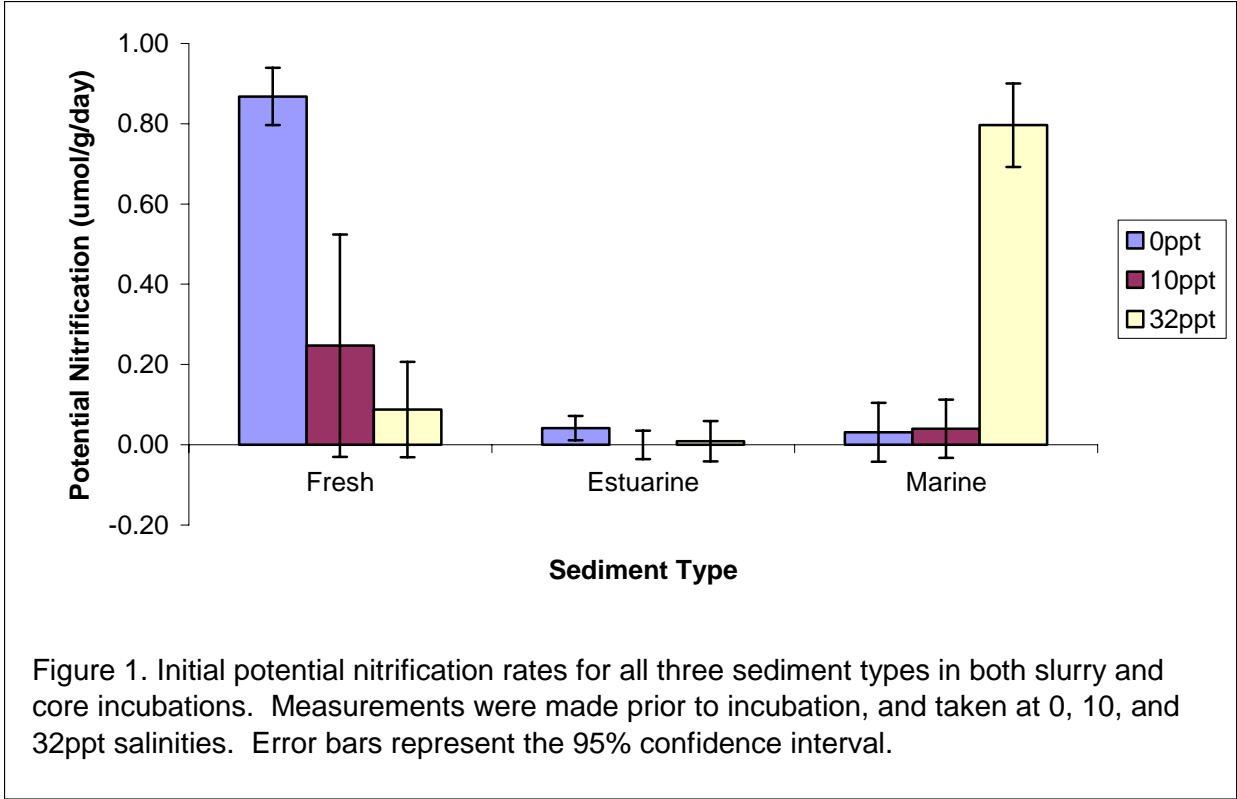
may be in order, since marine nitrifiers appear to respond and adapt to salinity changes over three weeks. Devising a way to culture an assemblage of freshwater nitrifiers might provide interesting data nitrifiers from the other extreme of stable salinity environments. Sediments in this experiment appeared to show both salinity limitation of nitrification and sulfate limitation of nitrification in some of the incubations. A larger data set would allow these trends to be explored more fully, and verify the observation that sulfate enrichment of the water column can reduce the population of nitrifiers and/or their nitrification efficiency. The current data suggest that given ample ammonium substrate, nitrifiers are co-limited by salinity and sulfate in marine waters, and further studies will be necessary to discover the relative importance of these two limitations.

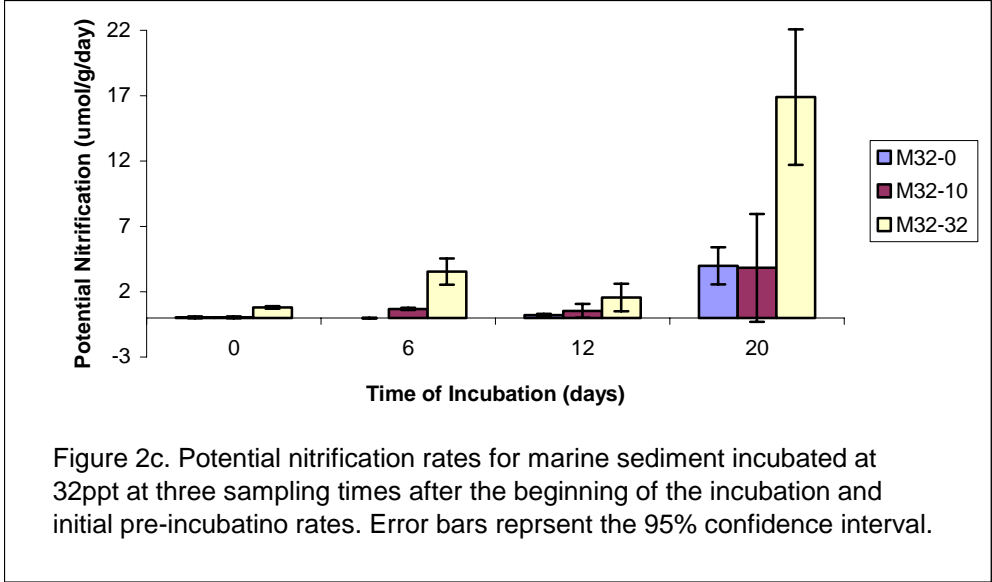
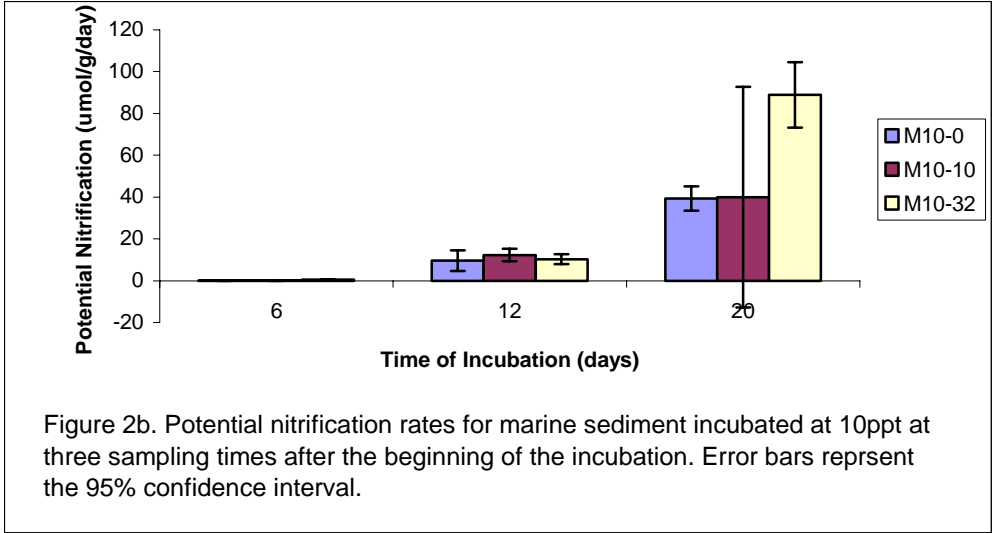
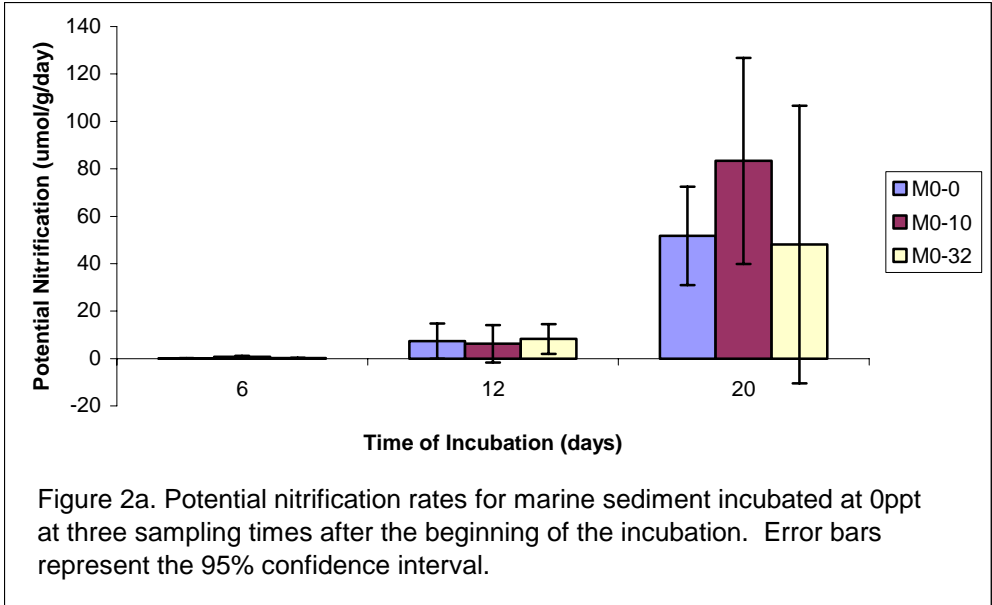
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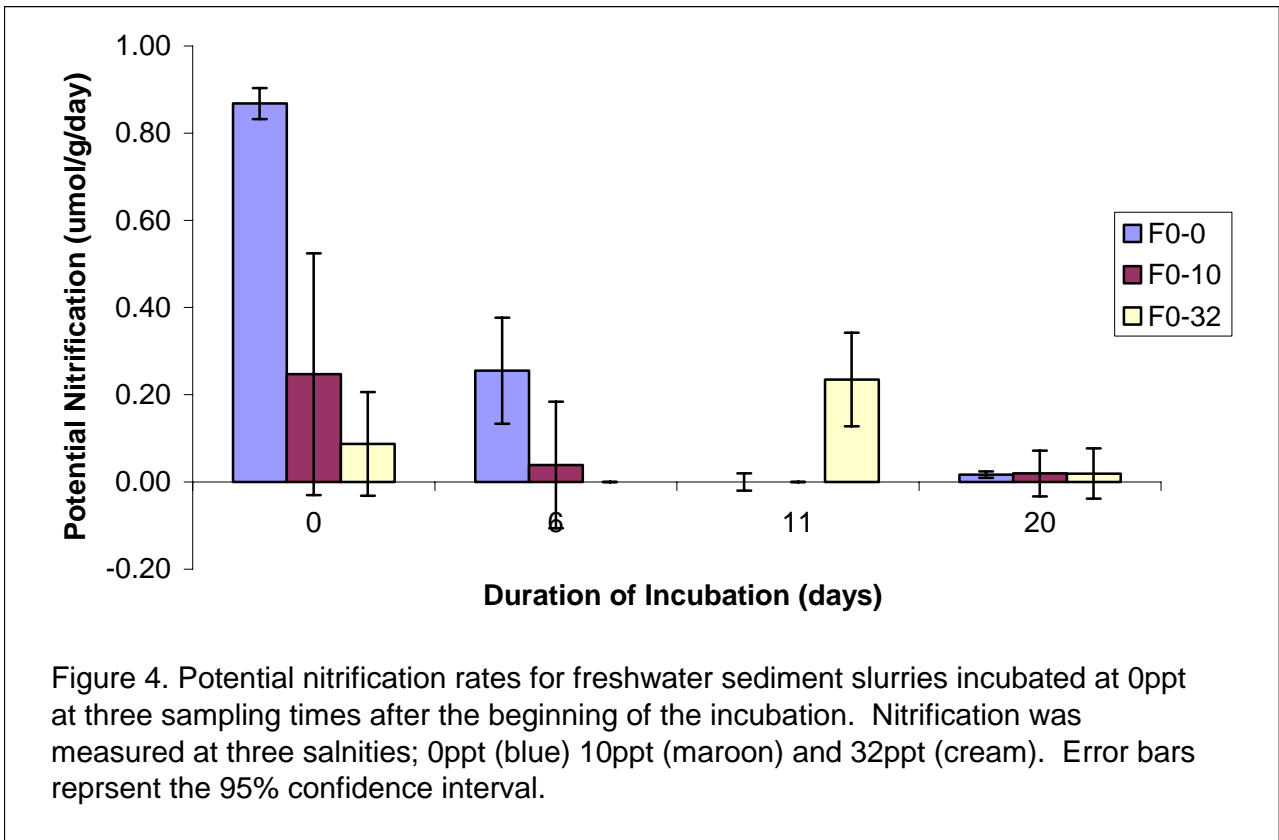
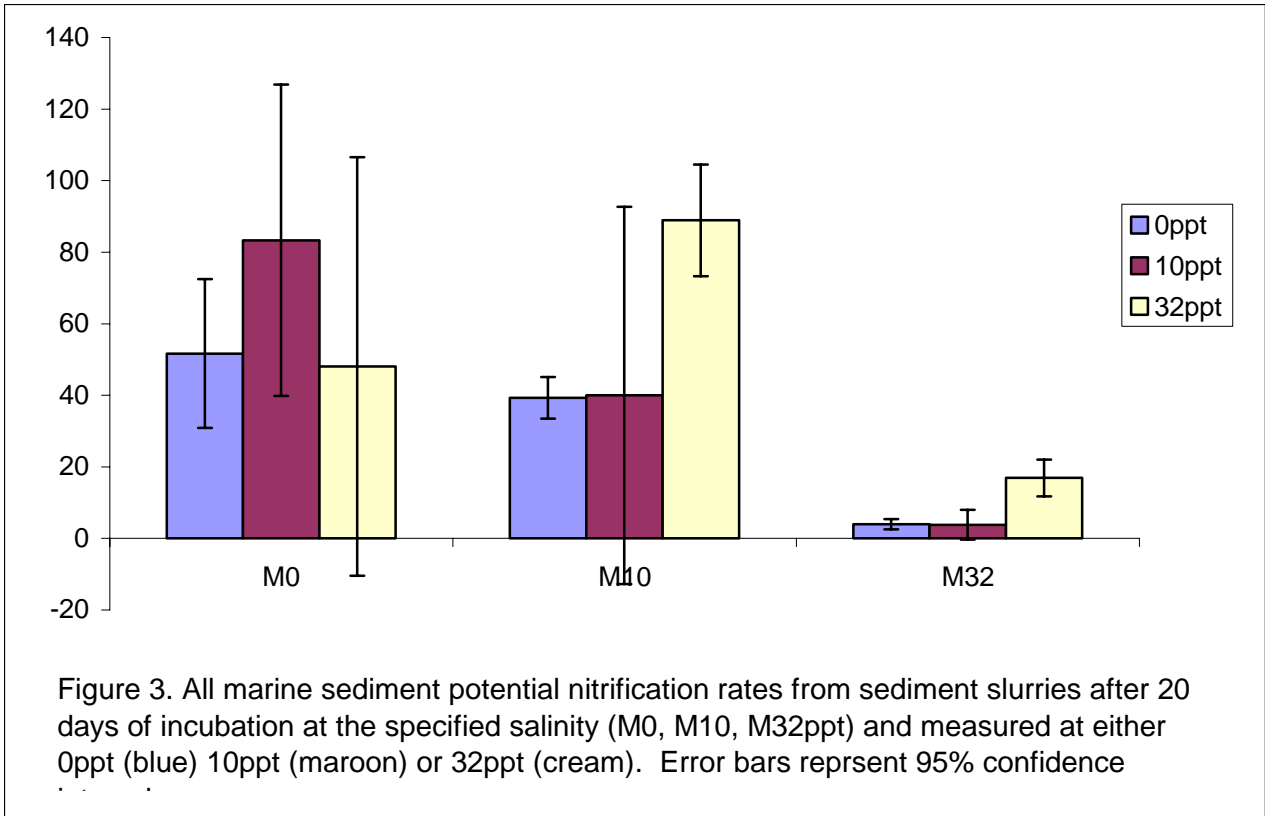
Thanks to the Semester in Environmental Science program for allowing me the opportunity to conduct this research, and Anne Giblin for her advice, support and enthusiasm helping me with my project. I would also like to thank Linda Deegan for her statistical advice, Ian Washbourne for his endless help running nitrate samples, Samuel Kelsey for his diving skills obtaining the cores, and Pat Micks, Leslie Graham, and Becky Karasack for their help in the laboratory.

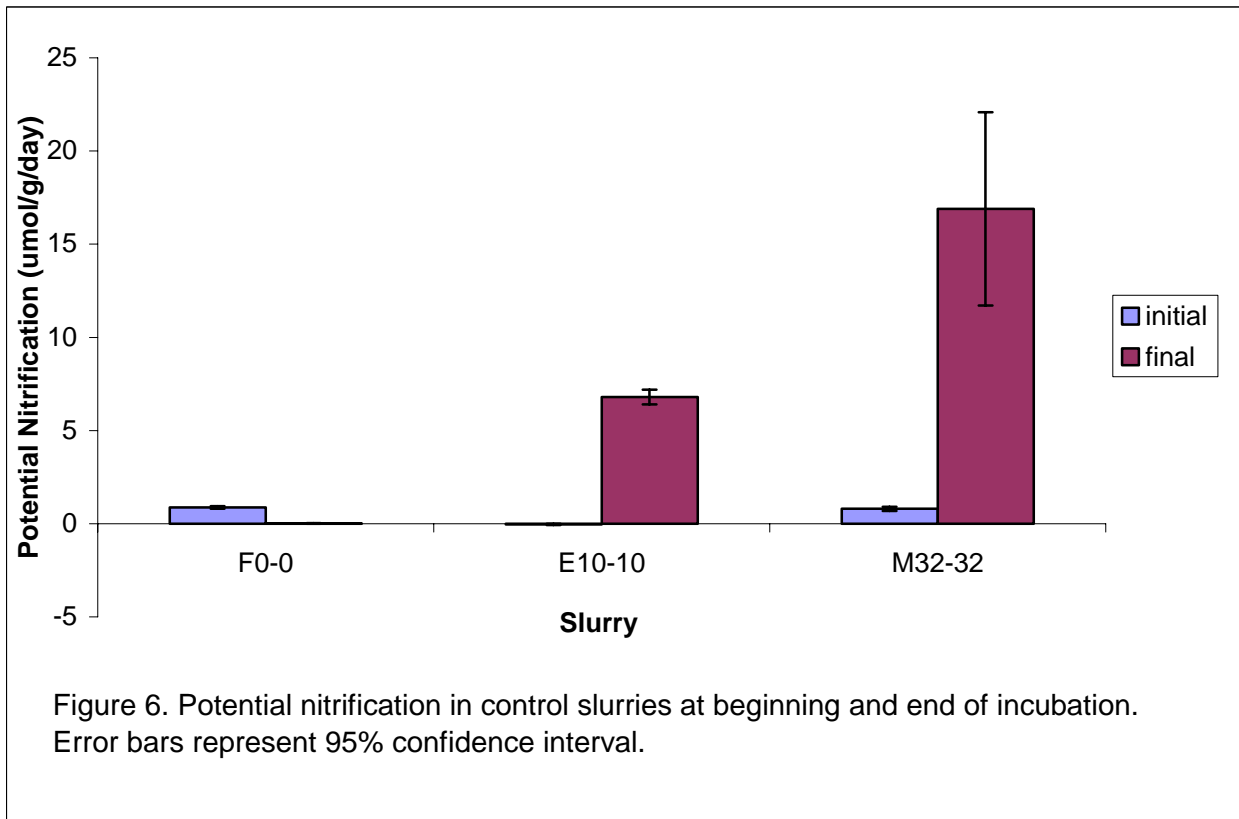
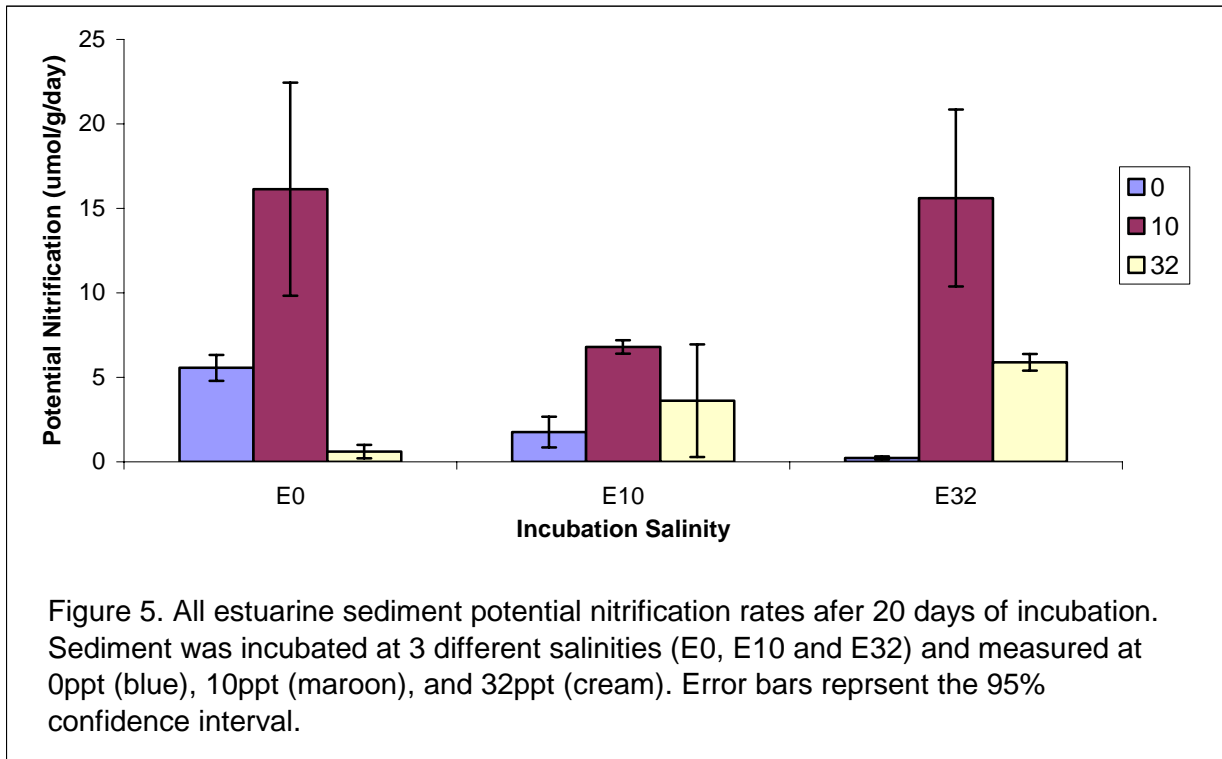
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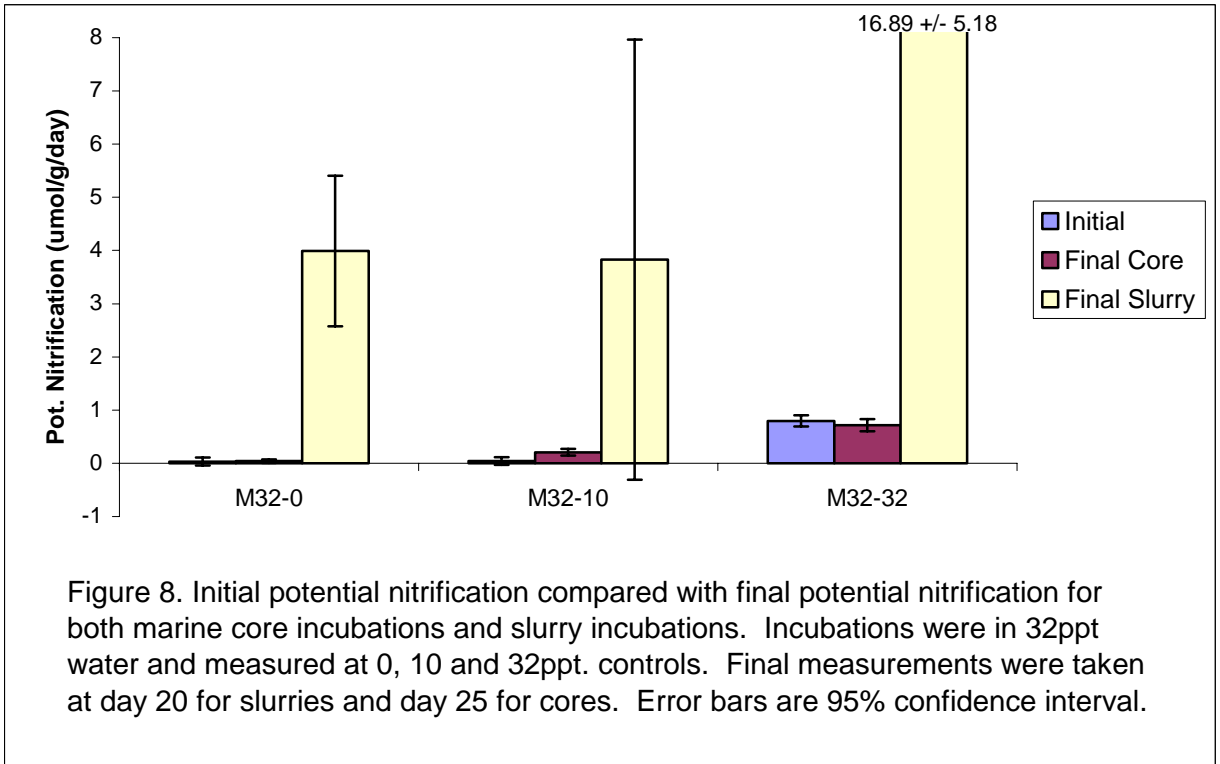
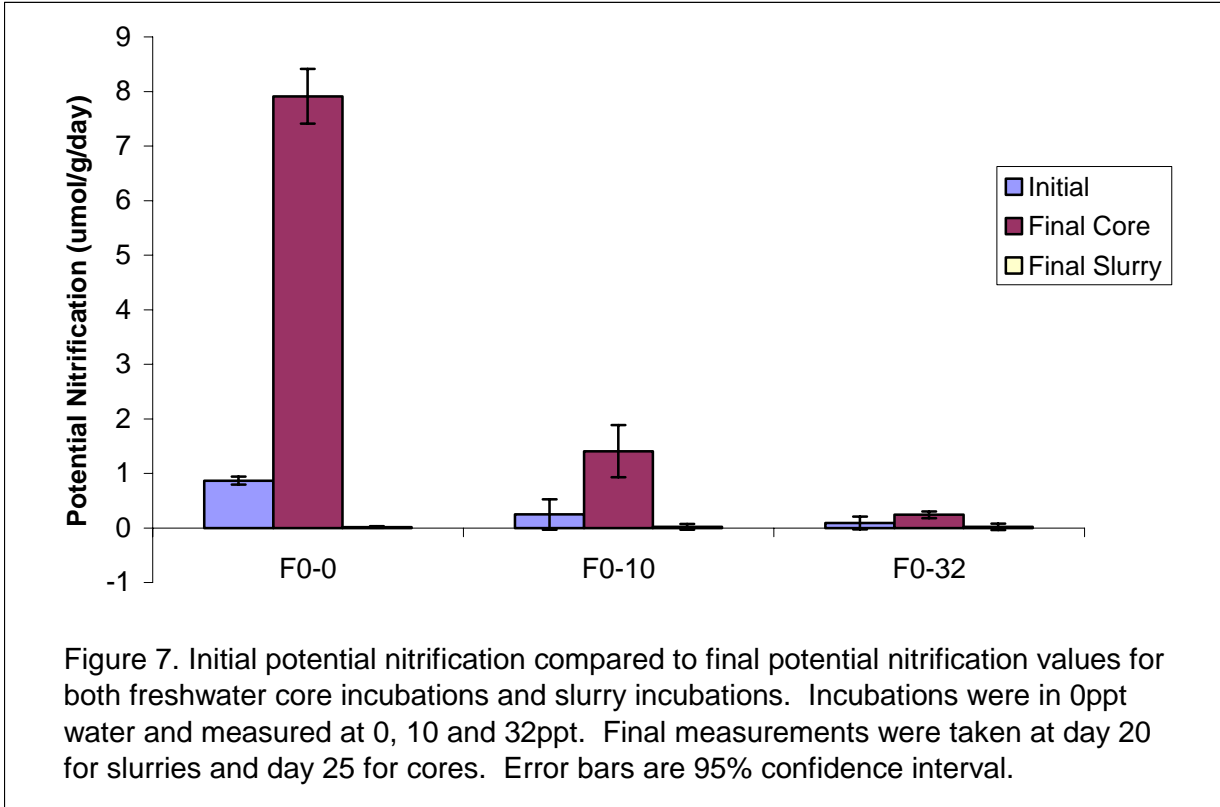
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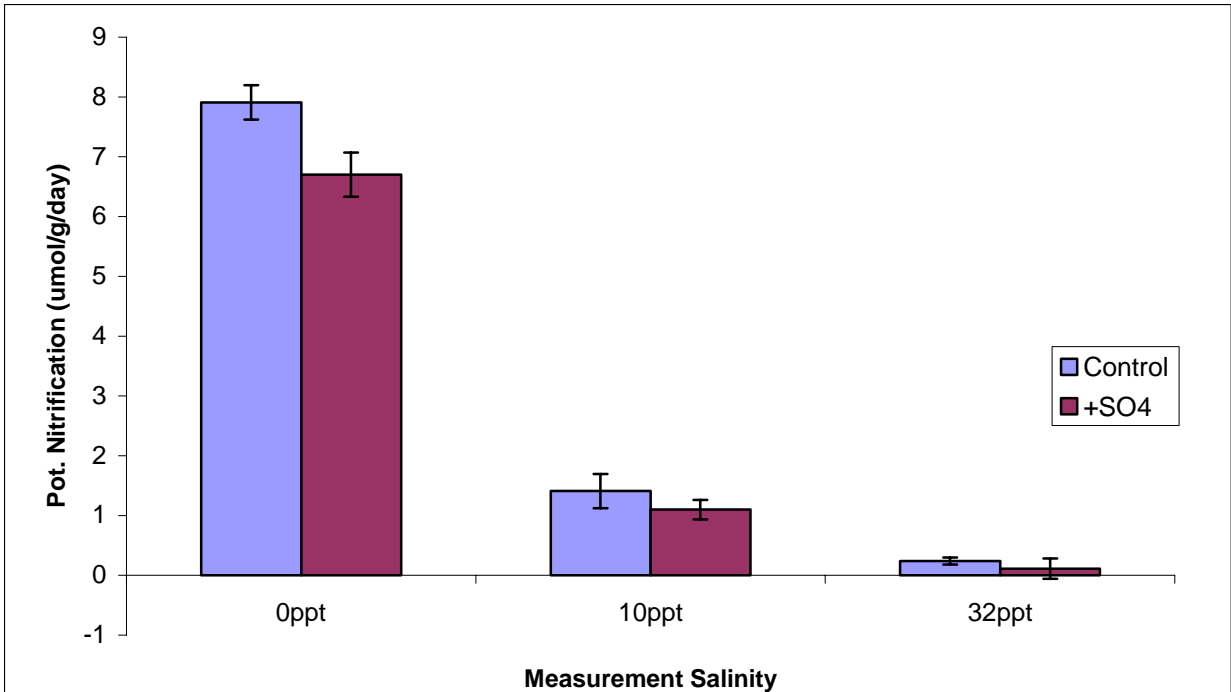


Figure 9. Johns Pond core potential nitrification rates after 25 day incubation either as a control (blue) or with sulfate addition (maroon), measured at either 0, 10 or 32ppt. Error bars represent 95% confidence interval.

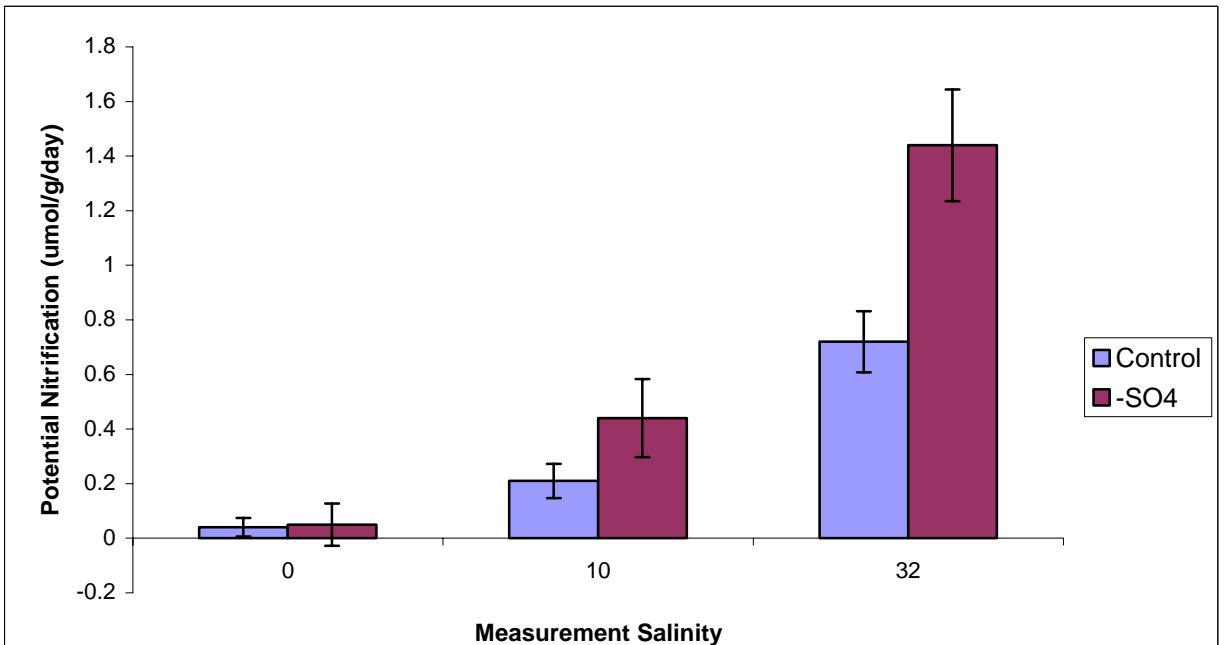


Figure 10. Mass. Bay core potential nitrification rates after 25 day incubation either as a control (blue) or with no sulfate (maroon), measured at 0, 10 or 32ppt. Error bars represent 95% confidence interval.

Sediment	Incubation	
	Salinity	Symbol
Fresh	0ppt	F0
Fresh	10ppt	F10
Fresh	32ppt	F32
Estuarine	0ppt	E0
Estuarine	10ppt	E10
Estuarine	32ppt	E32
Marine	0ppt	M0
Marine	10ppt	M10
Marine	32ppt	M32

Table 1. Salinity incubation slurry setup and symbols for each of the nine incubations.

	t=6 days	t=11 days	t=20 days
F0	15	>150	81
F10	105	>150	166
F32	119	>150	204
E0	26	>150	416
E10	22	>150	406
E32	17	>150	354
M0	-	41	8
M10	26	125	10
M32	13	104	30

Table 2. Ammonium concentration in slurry flasks at time of sub-sampling. ~500mL of water was replaced with new water of the appropriate salinity following t=11 days. Numbers “>150” were above the detection limit of the ammonium test and were not able to be diluted.