

**Tracing anthropogenic nutrient inputs to coastal plain ponds using stable isotopes**

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## **Abstract**

Coastal plain ponds harbor some of the world's rarest plant species. Consequently, they are vital to manage for conservation. Urbanization promotes the introduction of invasive species by providing additional nutrients, ultimately decreasing species richness. The purpose of this study is to determine if invasive species *Phragmites australis* is dependent on sewage-derived nitrogen using stable isotope analysis, which is possible due to the enriched signal of effluent. I also evaluated variations in DIN concentration in surface and ground water, soil N pools, and the  $\delta^{15}\text{N}$  of POM and deep pond sediments. An evaluation of two coastal plain ponds, one affected by nutrient pollution (Duck Pond), and one pristine (Mary Dunn), was done to demonstrate the propagation of  $^{15}\text{N}$  through biotic components. I found that Duck Pond ground water (referred to as GW) was no longer influenced by effluent from nearby wastewater treatment facilities. DIN levels at the surface water reflected long-term accumulation of N, which was verified by the  $\delta^{15}\text{N}$  values. Quantity of soil DIN beneath invasive species was also greater. All biotic tissues in Duck Pond expressed elevated  $\delta^{15}\text{N}$  values.  $\delta^{15}\text{N}$  of *Phragmites australis* suggested that it is dependent on anthropogenic nitrogen. A profile of sediments from the pond floor depicted a rapid shift in the source of N in Duck Pond. Stable isotopic values of shoreline species can be useful for identifying coastal plain ponds potentially impacted by nutrient loading from treated wastewater and septic systems.

## **Introduction**

Coastal Plain ponds are unique, delicate ecosystems that maintain specific biological conditions. These ecosystems are predominantly concentrated in the Cape Cod area. Coastal plain ponds have high biodiversity and are habitats of many extremely rare plant species for such a small unit area. The water level of a coastal plain pond is derived from the level of the GW table. These bodies of water experience seasonal shifts of flooding in the winter and drought in the summer. The periodic shift of inundation and receding waters controls the types of species along the shoreline from season to season. Coastal plain ponds have been adversely affected by urban sprawl and development. Cape Cod has experienced a 600% rise in population since 1920. Threats to these ecosystems include disturbances such as increased destruction of habitat like driving off-road vehicles & campers and construction of pond front homes. There has also been an increase in

pumping from the water table because of raised water consumption. Urban sprawl and development is an environmental concern because it increases septic inputs into coastal ponds.

Nutrient rich ground water is a problem because it causes eutrophication, which leads to alteration of nutrient pools and cycling, resulting in a loss of biodiversity. Native species are accustomed to low nutrient environments because the parent material originates from nutrient deficient glacial outwash (Kniffin 2008). High nutrients concentrations may favor invasive species because of their high demand of N. By declining the frequency of wet seasons in the pond due to increased municipal pumping, opportunities are created for invasives to migrate farther into the landscape. This combined with increasing disturbance is a catalyst for invasive colonization.

*Phragmites australis* is known in North America as the common reed, but has a genetic lineage that implies a European ancestry (Ehrenfeld 2003). Unlike the cultivar native to the U.S, this more vigorous and invasive strain of the species is deemed responsible for the domination of coastal aquatic systems in New England. Studies have shown that both physical disturbance and nutrient addition have directly increased *Phragmites* production. Competitive advantage can shift when nutrient limitation is no longer a factor, which is partly controlled by the quantity of belowground structures. *Phragmites* is a poor competitor in low nutrient environments due to minimal allocation of biomass to roots. Minchinton & Bertness (2003) showed that with competition present, fertilization had insignificant impacts on biomass production of *Phragmites*. This suggests that nutrient availability for *Phragmites* is limited by the neighboring plants. *Phragmites* is also limited by physical inhibition of aboveground shoot growth. Once released from belowground competition, *Phragmites* is a far better above ground competitor. *Phragmites* is exceptionally resilient, waiting to capitalize on ideal conditions. Due to *Phragmites* substantial allotment of resources to aboveground structures, it is able to outcompete previously dominant species when nutrients are not the limiting factor. It accomplishes this by growing more rapidly than native vegetation, shading out competitors. Production of *Phragmites* is promoted by physical disturbance which allows the clonal plant which reproduces vegetatively through propagation of the rhizome, to increase density. Minchinton and Bertness (2003) found that when matrix

vegetation was removed and nutrients were added to the landscape of a coastal salt marsh (experimental disturbance) that biomass and density of *Phragmites* was increased.

Previous data from studies of *Phragmites* invasions in brackish marshes suggest that decreases in  $\text{NH}_4$  pools in soils are correlated with increased uptake, which is linked to additional above ground biomass (Ehrenfeld 2003). They produce litter that decays more easily, suggesting it more nutrient rich. Ecosystem process may be altered, and possibly experience variation of spatial distribution and timing of fluxes in soil nutrient pools even when there is no net change in the amount of nutrients (Ehrenfeld 2003).

Stable isotopes have been useful in delineating anthropogenic sources of DIN into coastal estuaries because of the heavily enriched signal in sewage which can range from +8 to +22 (Cole 2004). A study by McClelland et al., showed a positive relationship between % contribution of wastewater and  $\delta^{15}\text{N}$  of primary producers in estuaries. This technique can be used to trace the source of DIN in coastal plain ponds. In this paper I will explore how point sources of enriched N can be traced in the biomass of shoreline species in coastal plain ponds using stable isotope analysis. I will assess level of dependence of invasive species on sewage derived nitrogen. I will also evaluate the effects of nutrient pollution on soil N pools. To accomplish this, I measured DIN concentrations from pond surface water and GW. I also measured extractable DIN from soils at ground water seep and a shallow, submerged area of the pond bottom. The  $\delta^{15}\text{N}$  of POM, and deep pond sediments were also determined.

This experiment will focus on many time scale factors to help further assess N utilization indicated by stable isotope analysis.  $\delta^{15}\text{N}$  of GW N will be indicative of current nutrient loading into the ponds. Surface water will indicate accumulation of sewage N over a long term period. POM will be an indicator of influence in the pond on the order of days. Plant tissues analysis will be explicative of the nutrients inputs to the system during the period of the life of the plant itself or one growing season. Lastly,  $\delta^{15}\text{N}$  profile of a sediment core will show the change in N loads in a historical context in the order of decades.

I speculate that a comparison of affected and pristine ecosystems will reflect a differences in nutrient pools. GW and surface water should be enriched. I also propose

that del  $^{15}\text{N}$  values of biotic components in Duck Pond will be elevated and reflective of sewage inputs. I hypothesize that *Phragmites* in Duck pond is released from belowground competition through the increased N supply derived from the Wastewater treatment plant.

## Methods

Sampling was performed at two coastal plain ponds in the Hyannis area. The two sites evaluated were a historically pristine pond-Mary Dun part of the Hyannis Ponds Complex in Barnstable, MA and Duck pond, which is adjacent to the Barnstable wastewater treatment plant in Hyannis, MA. This site was chosen because sewage disposal has been occurring on site since 1938. Also, expansion of infiltration beds since late 1960's have increased to potential for eutrophication. Plant sampling focused on two representative plant species in each pond. *Juncus militaris*, was collected from Mary Dunn and *Phragmites australis*, was collected from Duck Pond. Tissues of each species were ground and placed in scint vials for stable isotope analysis. Ponds sediments were collected at two locations, the GW seep and pond floor from each site in replicates of five. A KCl extraction was performed in order to determine the quantity of soil nitrogen.  $\text{NH}_4$  and  $\text{NO}_3$  concentrations of microbial extracts were determined through colorimetric analysis method presented in the *SES lab packet*.

Water was collected in 20L carboy at the surface of the pond. 1- 2L of pond water was filtered once with 47mm GF/F filter to collect POM (assumed to predominantly phytoplankton) at each site. Filters were then dried and prepared for stable isotope analysis. GW was extracted in triplicate from each test site. Approximately 3.79L of GW was filtered twice (initially with 124mm & next with 47mm GF/F filter) to remove all POM.  $\text{NO}_3$  concentrations of pond and GW were verified using reagents from the *LaMonte nitrate test kit* and colorimetric analysis which was performed on a spectrophotometer in order to determine the volume necessary for diffusions.  $\text{NH}_4$  concentrations of were also determined by colorimetric analysis. The del  $^{15}\text{N}$  of  $\text{NO}_3$  in pond and GW samples were determined by performing a diffusion using the method from the *Linx Project*. Filters were then dried and prepared for stable isotope analysis. Sediment cores approximately 30cm in length were taken from the bottom of each pond. Sediment were sectioned in 5cm increments, ground using a mortar and pestle, and prepared for stable isotope analysis on the mass spectrometer.

## Results

GW concentrations of DIN were greater in Mary Dunn than in Duck Pond, but do not vary significantly. I measured no  $\text{NH}_4$  in Mary Dunn where concentrations of Duck Pond surface water were 80.9  $\mu\text{M}$  (**Fig 1**). Total DIN in Duck Pond is approximately 10x greater than Mary Dunn. Soil extracts from Duck Pond had a greater average quantity of both  $\text{NH}_4$  and  $\text{NO}_3$  than Mary Dunn in the GW seeps and as well as pond bottom. Soils from the bottom of Duck pond constituted 26.68  $\mu\text{g N-NH}_4/\text{g}$  soil compared to 2.93  $\mu\text{g N-NH}_4/\text{g}$  in Mary Dunn (**Fig 2**). Due to an inadequate % of N recovery during the diffusion procedure (<50%), I could not accurately determine the stable isotope values of the surface and GW. However, surface water samples from each site were recovered at a consistent ratio, and therefore interpretable. The  $\delta^{15}\text{N}$  value surface water at Duck Pond was much higher than Mary Dunn (**Fig 3**). All biotic components of Duck Pond were heavily enriched in comparison to Mary Dunn (**Fig 4**). The  $\delta^{15}\text{N}$  profile of the sediment core in Duck Pond show that top 10cm of sediments are highly enriched (**Fig5**).

## Discussion

GW concentrations of  $\text{NH}_4$  and  $\text{NO}_3$  were significantly lower than expected in Duck Pond. The Barstable wastewater treatment plant in Hyannis, MA is releasing effluent with N concentration of 5mg/L, much higher than what was collected at Duck Pond. This suggests that effluent is no longer being incorporated with the GW. Another hypothesis is that due to the heterogeneous nature of GW flow, I was unable to locate a plume influence by anthropogenic waste. However, concentration of DIN in the surface water is high, due to the legacy of nutrient loading from the WTP. Duck pond has no outlet, and nitrogen can only collect and recycle unless denitrified.

Exotic species generally have greater biomass, NPP, and growth rates than the species that they displace (Ehrenfeld 2003). The substantial amount of primary production of *Phragmites* in Duck pond has created thick layer organic matter on the shoreline and in the shallow depths of the pond. With an adequate carbon source, mineralization creates nutrient rich soils. Active nutrient cycling is depicted by the difference in levels of  $\text{NH}_4$  and  $\text{NO}_3$  at the GW seeps of both sites. Nitrification is occurring due aerobic activity in the soils along the shoreline where GW was sampled.

NH<sub>4</sub> may be less at the seep due to selectivity of plant nutrient uptake. The quantity of labile nitrogen further verifies Duck Pond as choice for eutrophic coastal pond.

With consideration to the difficulty with execution of the NO<sub>3</sub> diffusion, stable isotope analysis suggest that Duck pond is receiving more septic nutrient loading than Mary Dunn. According to the treatment facility, current NO<sub>3</sub> composition of effluent is 2mg/L or 40% of the total nitrogen expelled. Consequently this particular assessment may be an underestimate of heavy N in the pond waters.

The  $\delta^{15}\text{N}$  values of all the biotic tissue illustrate active N assimilation. POM, assumed to be *mostly* phytoplankton, has a turnover rate of approximately 10 days (SES) and is absorbing N directly from the pond. Previous work by Hannson et al. found that POM in the Baltic Sea that were in close proximity to outfall from a wastewater treatment plant with sewage that had  $\delta^{15}\text{NH}_4$  of +23.6 and  $\delta^{15}\text{NO}_3$  of +29.3, had POM with a  $\delta^{15}\text{N}$  between 8 and 10. POM furthest away from outfall had a  $\delta^{15}\text{N}$  of 2.9 and 4. The macroalgae azolla, is has been either suspended or floating on the surface of Duck pond for nearly 30-45 days and has included enriched N within its biomass. Nutrient uptake by *Phragmites australis* has been occurring for the entire growing season, and has structural biomass that will last until the following growing season. When the  $\delta^{15}\text{N}$  of biomass of *Juncus Militaris* and *Phragmites* are compared, it is clear that *Phragmites* has a greater nitrogen demand, resulting in the accumulation of  $^{15}\text{N}$ . Therefore *Phragmites* establishment is dependent on wastewater derived nitrogen.

Sediment Core profile from Duck Pond is long term record of nitrogen inputs for over 100 years. Although the core sections are not dated, there is a sharp transition to septic nitrogen deposition in the more recently developed sediments. These max  $\delta^{15}\text{N}$  values roughly match that of the primary producers of Duck Pond which exhibit fractionation. The profile also verifies that Mary Dunn has no contributions from septic wastewater.

Stable isotopic values of shoreline species can be useful for identifying coastal plain ponds potentially impacted by nutrient loading from treated wastewater and septic systems. Because *Phragmites* is known to require high levels of nutrients, its emergence can be an indication increased N supply. Stable isotopes may be employed by determining septic nutrient loading before ecological processes and plant community

composition is significantly altered. Efforts of determine correlation between  $\delta^{15}\text{N}$  of groundwater and % domination of landscape by *Phragmites* would be ideal. This is also a mechanism to survey of multiple ponds, made possible by unproblematic sampling procedure. A more thorough evaluation of GW is needed across the pond perimeter.

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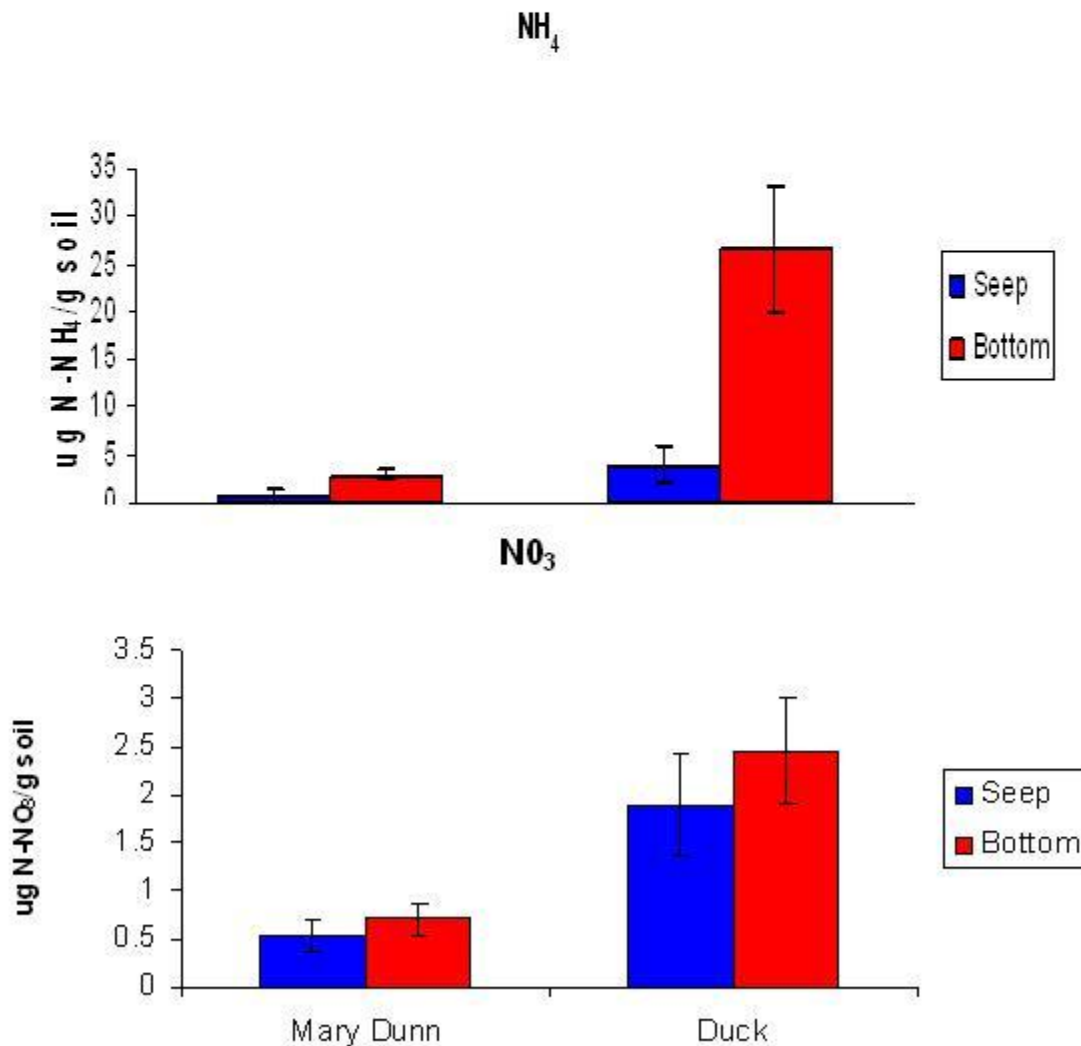
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**Figure 1.** Ground Water & Pond Water Concentrations at Mary Dunn and Duck Pond

Site		NO <sub>3</sub> (uM)	NH <sub>4</sub> (uM)	Total DIN
<b>Mary Dunn</b>	Surface Water	10.7	0	10.70
	Ground Water	4.79	0.53	5.33
<b>Duck</b>	Surface Water	14.8	<b>80.9</b>	<b>95.70</b>
	Ground Water	2.53	0.47	3.00

**Figure 2.** NH<sub>4</sub> & NO<sub>3</sub> of soil in Mary Dunn and Duck Pond



**Figure 3.** Delta <sup>15</sup>N of Pond & Ground Water

	<b>Mary Dunn</b>	<b>Duck Pond</b>
Pond water	-4.4	<b>10.0</b>
GW1	12.6	3.5
GW2	8.6	8.3
GW3	9.2	-0.2

**Figure 4.** Delta <sup>15</sup>N values of Biotic Tissues

	<b>Mary Dunn</b>	<b>Duck Pond</b>
POM	<b>2.9</b>	<b>12.4</b>
<i>Azolla</i> (macroalgae)	n/a	<b>12.2</b>
<i>Phragmites australis</i>	n/a	<b>10.1</b>
<i>Juncus militaris</i>	<b>5.1</b>	n/a
<i>Eleocharis acicularis</i>	<b>3.2</b>	n/a
<i>Coreopsis rosea</i>	<b>2.8</b>	n/a

**Figure 5.** Delta  $^{15}\text{N}$  Sediment Core Profile of Mary Dunn and Duck Pond

