Variation in dissolved nitrogen and stable isotopes in groundwater and organisms as indicators of wastewater pollution in estuaries

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Semester in Environmental Science, 2019

ABSTRACT

Nitrogen pollution entering coastal waters from wastewater and fertilizers escalates with urbanization along coasts. In this study, I investigated nutrient concentrations and $\delta^{15}$N stable isotopes in groundwater as well as aquatic organisms as bioindicators of nitrogen pollution at Little Pond, West Falmouth Harbor, and Sage Lot Pond of Falmouth, MA. At these estuaries, I collected water samples from groundwater wells as well as surface water from the entering stream at Little Pond. In addition, I collected mud snails ($Nassarius obsoletus$), ribbed mussels ($Geukensia demissa$), and macroalgae ($ulva lactuca$) from each site and compared their $\delta^{15}$N stable isotopes. We observed greater NO$_3^-$ concentrations on the west shore of Little Pond and greater NH$_4^+$ concentrations on the east shore of Little Pond which we suspect is due to the gradient and hydraulic conductivity of the shores. With a higher gradient, we observed more dissolved oxygen and therefore more NO$_3^-$ on the west shore. The $\delta^{15}$N stable isotopes did not reflect the trends in dissolved inorganic nitrogen at Little Pond, but we observed a distinct difference between the $\delta^{15}$N of our pristine estuary, Sage Lot Pond ($\delta^{15}$N= -4.6 ‰), and our impaired estuaries, Little Pond ($\delta^{15}$N = 1.1-22.4 ‰) and West Falmouth Harbor ($\delta^{15}$N = 6.0-14.7 ‰). Organisms throughout Little Pond experienced a tight range of 10.5 to 12.9 ‰, showing no localization of nitrogen loading. However, we observed an obvious difference between Sage Lot Pond and both Little Pond and West Falmouth Harbor. Our findings confirm organisms as bioindicators between sites but not within sites. In addition, this study further suggests the successful removal of wastewater from Little Pond by the sewer system installation, a possible solution to eliminating nitrogen pollution in estuaries.

Keywords: Nitrogen pollution, groundwater, Little Pond, stable isotopes, wastewater, bioindicators, West Falmouth Harbor, eastern mud snail ($Nassarius obsoletus$), ribbed mussels ($Geukensia demissa$), macroalgae ($ulva lactuca$)
INTRODUCTION

Increased urbanization along coastal shores across the nation raises concern for eutrophication as a threat to aquatic ecosystems (Valiela et al. 2016). Accelerated human activity and development in coastal communities has increased pollution to rivers, lakes, estuaries, and oceans, often spiking algal blooms and harming aquatic life (Anderson et al. 2002). Fertilizers and wastewater specifically contribute to nutrient enrichment by producing excess nitrogen (N) and phosphorus (P), inducing eutrophication in water systems around the globe (Nixon 1995).

While the scientific community has endured a half-century long debate over N and P as the drivers of eutrophication, studies have revealed N as the main cause of eutrophication in estuaries and saltwater systems (Howarth & Marino 2006; Nixon 1988). As sea water becomes sulfate-rich with salinity, sulfide is produced in sediments and combines with iron (Fe), decreasing P sequestration (Hartzell & Jordan 2012). With greater P availability in estuarine systems than in freshwater, N is the limiting nutrient in estuaries and causes substantial effects when imbalanced. In estuaries and coastal waters, eutrophication is increasingly recurring (Cloern 2001), and as much as two-thirds of the United States’ coastal waters are degraded from eutrophication (Bricker et al. 1997; Howarth & Marino 2006). Therefore, finding and eliminating sources of N would help protect waters from eutrophication.

Nitrogen loading (N-loading), in particular, results from N-rich runoff from fertilizers and septic systems of coastal neighborhoods (Valiela 1997). In fact, humans have doubled the amount of biologically active nitrogen in nature since the 18th century (Galloway & Cowling 2002; Watson et al. 2017). Dissolved N most commonly enters aquatic systems through non-point sources such as groundwater, and depending on the hydrology (water table depth, hydraulic conductivity, hydraulic flow, etc.) of banks and shorelines, groundwater may play a significant role in N-loading (Pearl 1997). With non-point sources of N pollution, there is no direct method in managing eutrophication. However, identifying indicators of N enrichment can advance us toward implementing proper land management in eutrophic areas.

In aquatic systems, organisms, such as macroinvertebrates, are useful as bioindicators of pollution and environmental stress (Pearson & Rosenberg 1978 Wildsmith et al. 2011; Hale t al. 2016). Studying the diversity and composition of organisms can show the impact of pollutants on aquatic communities (Pearson & Rosenberg 1978). In addition, macroinvertebrates provide a measure of how conditions vary over time (Watson et al. 2017). Therefore, investigating the N-loading in macroinvertebrates may indicate N-loading at an ecosystem level across timescales.

In this study, we explore eastern mud snails (Nassarius obsoletus), ribbed mussels (Geukensia demissa), and macroalgae (Ulva lactuca) as bioindicators of eutrophication in Cape Cod, MA estuaries. Mud snails, ribbed mussels, and macroalgae are relatively abundant in intertidal zones throughout Cape Cod. As opportunistic grazers of diatoms, mud snails live and grow on the sediment in estuaries and other saltwater systems (salinity > 15 %), serving as primary consumers in their ecosystem (Watson et al. 2017). Ribbed mussels, in comparison, are filter-feeding bivalves that favor peaty edges of shorelines and are coined as primary consumers of phytoplankton in marine systems (McKinney et al. 2001). Macroalgae float within estuaries and coastlines in large mats, directly taking up nutrients in the water by photosynthesis as primary producers (Teichberg et al. 2010). Investigating a combination of mud snails, ribbed
mussels, and macroalgae may provide a more comprehensive picture of the N-loading in estuaries.

Stable isotopes are often used in ecosystem studies to analyze the composition of elements in living organisms (McClelland and Valiela 1997; Peterson & Fry 1987). Isotopic ratios of nitrogen ($\delta^{15}N$) are used to trace the retention of nutrients in organisms, showing how they are affected by nutrient enrichment. Previous studies on primary producers show that heavier N isotopic ratios occur in areas of high N-loads (Fox et al. 2012; Valiela et al. 2016). Specifically, N isotopes are used by comparing the isotopic composition of atmospheric nitrogen ($\delta^{15}N = 0 \%o$), which is the standard, to that of anthropogenic sources of nitrogen. Wastewater contains human excretions which have heavier isotopes ($\delta^{15}N_{wastewater} = 10$ to $20 \%o$) (Valiela et al. 2000). Therefore, we can trace anthropogenic sources of N by the weight of isotopes.

A predominantly residential area, Falmouth, MA of Cape Cod exhibits 19 small bays along its coast to the Vineyard Sound, and 15 of these bays are designated by the Commonwealth of Massachusetts as having “impaired” water quality (Cole 2018). Little Pond is an estuary located in East Falmouth, MA with suburban houses lining its shorelines, receiving approximately 13 kg N per day. In comparison, West Falmouth Harbor is an estuary located in West Falmouth, MA with similar residential life around its shores and receives approximately 12 kg N per day. However, West Falmouth Harbor also receives effluent from the Falmouth Wastewater Treatment Plant which provides a gradient of N-loading from the plume. With these anthropogenic influences, investigating the organisms in these developed coastal zones could indicate the effects of N-loading on these estuaries.

This experiment investigates nitrogen pollution in groundwater and organisms at a landscape level at Little Pond, West Falmouth Harbor, and Sage Lot Pond. Our objectives were to investigate (1) whether N inputs from humans are reflected in the nutrient concentrations and stable isotopes ($\delta^{15}N$) of groundwater in estuaries, (2) possible relationships between the amount of N and $\delta^{15}N$ in groundwater, and (3) whether the $\delta^{15}N$ in organisms reflect the $\delta^{15}N$ in groundwater in estuaries. I hypothesize that the $\delta^{15}N$ will match the N-loading of groundwater at and within each site, showing a relationship between the amount of N and the $\delta^{15}N$. In addition, I hypothesize that organisms exposed to high N-loading will show heavy $\delta^{15}N$ ratios and low N-loading will show light $\delta^{15}N$ ratios, allowing us to use organisms as bioindicators of N pollution. I expect that sampling organisms from sites of varying N-loading and investigating their $\delta^{15}N$ will show how estuary life retains N and the index of eutrophication in aquatic systems. In addition, comparing the N-loading in organisms to that of the entering groundwater will capture a greater understanding of how much N is entering the ecosystem from human inputs.

METHODS

Study area and sampling sites

Little Pond is a small, tide-influenced estuary (20 ha) located in East Falmouth, MA that receives seawater from the Vineyard Sound and freshwater from the surrounding watershed through groundwater seeps and a stream (MEP 2006; Figure 1). A residentially dominated area, Little Pond historically receives high N inputs from septic tanks of the surrounding houses. However, in 2015-16, a sewer system was constructed in the Little Pond watershed, and over
1,000 homes of the total 1,400 homes are connected as of June 2018 (Figure 2). At this site, I sampled groundwater and organisms at 12 stations along the shorelines as well as one station at the stream entrance to the estuary (Figure 1). As shown in previous studies, these stations vary in N-loading, from 59-234 μM Dissolved Inorganic Nitrogen (DIN) (Duran 2018), with the highest at station LP-5 and the lowest at station LP-11. I sampled at these 13 stations to record the full story of N-loading at Little Pond as well as to contribute to previous data collections for analyzing N-loading trends over time.

West Falmouth Harbor is a larger estuary (76 ha) located in West Falmouth, MA, receiving seawater from Buzzards Bay, freshwater from a stream, and groundwater from the surrounding watershed (Mosemann-Valtierra et al. 2015) (Figure 3). Similar to Little Pond, West Falmouth Harbor is primarily residential and is influenced by high nitrogen loads leaching from wastewater of septic tanks. In addition, West Falmouth Harbor receives effluent from the Falmouth wastewater treatment plant, contributing about 67% of the total N-load (Hayn et al. 2014). At this site, I sampled groundwater and organisms at three stations: SC1, SC3, and SC7 (Figure 4). These stations range in N-loading, with the highest at SC3, where the effluent from the wastewater treatment plant enters. I sampled at SC1, an upstream freshwater entrance to the harbor, and SC7, a boat dock location closer to Buzzards Bay, to observe the N-loading surrounding the wastewater treatment plant plume.

Sage Lot Pond is a sub-estuary of Waquoit Bay in East Falmouth, MA with pristine conditions of N-loading, with wastewater comprising only 16% of its total N-load (McClelland et al. 1997). For this study, I used Sage Lot Pond as a control site, to better evaluate the anthropogenic impact of Little Pond and West Falmouth Harbor.

Groundwater Sampling & Processing

At each station in Little Pond and West Falmouth Harbor, I sampled from 2-5 previously established, multi-depth groundwater wells varying from 1-10 meters in depth. Before sampling from each well depth, I used a Geo-pump and HydroLab Quanta to collect the temperature, conductivity, salinity, pH, DO (mg/L), and % DO saturation. Using GF/F filters, filter heads, and silicone tubing, I filled a 60 mL water sample bottle with groundwater from each available well depth. In addition, I filled a 1 L water sample bottle with equal parts from each well depth (e.g. 250 mL from each depth at a well with four depths). At the stream of Little Pond, I inserted a 60 mL syringe into the flowing stream water multiple times and filtered the collected water into a 1 L sample bottle. Upon return to the lab, I temporarily stored the groundwater samples at 4 °C in a cooler or refrigerator. These samples were later used for nutrient analyses and isotopic analyses.

Sage Lot Pond is a site that does not have permanent groundwater wells. Therefore, I used well-points to collect groundwater. This involved inserting a hollow, metal rod into the ground at a depth within the groundwater table (2-4 meters). From the rod, I attached tubing and a vacuum pump to pull groundwater up through the rod and into a connected Erlenmeyer flask, using a filter syringe to filter the water into a sample bottle. Before collecting groundwater as a sample, I used a refractometer to check for low salinity and confirm the water was majorly freshwater. Since the N-loading at this site is pristine by comparison, I collected 4 L of sample water to ensure I had enough for isotopic analysis. Upon returning to the lab, I temporarily stored
the groundwater samples at 4 °C in a cooler or freezer. These samples were later used for nutrient analyses and isotopic analyses.

All water samples were analyzed for phosphate (PO$_4^{3-}$), nitrate (NO$_3^-$), and ammonium (NH$_4^+$) within 72 hours of collection, and total dissolved nitrogen (TDN) concentrations two weeks after collection. PO$_4^{3-}$ and NH$_4^+$ were detected by adding reagents and measuring absorbances on a spectrophotometer, and NO$_3^-$ was analyzed using the Lachat. TDN concentrations were detected by fixing samples with an oxidizing agent, sterilizing by autoclave, and analyzing TDN by Lachat. All instruments and materials were provided by the Marine Biological Laboratory’s Semester in Environmental Science laboratory.

Groundwater samples were prepared for stable isotope analyses by extracting NO$_3^-$ and NH$_4^+$ isotopes into GF/F filters through gas diffusion (Sigman et al. 1997; Holmes et al. 1998). This protocol was modified in order to catch both NO$_3^-$ and NH$_4^+$ in the filters rather than NO$_3^-$ or NH$_4^+$ independently. Modifications included adding magnesium oxide (MgO) and DeVarde’s alloy simultaneously to capture NO$_3^-$ and NH$_4^+$ in gaseous forms in the filters. Quarter-filled HDPE bottles of treated groundwater sample were incubated at 40 °C and shaken for eight days with the filters in floating filter packs. Filters were removed and dried in a desiccator for 72 hours until used for $\delta^{15}$N analysis.

**Organism Sampling & Processing**

Snails, mussels, and macroalgae were collected by hand along the shores of each sampling site. Due to varying salinities, snails and mussels were not observed at all sites, but collected from all sites where they were observed. Snails were observed on the sandy bottom in the shallow water near shore and mussels were found amongst the peat, grassy shorelines. I observed macroalgae floating on the surface near shore. These organisms were collected as close to the shore as possible within the vicinity of the groundwater wells. With late-fall weather conditions, small hand nets were often used to retrieve snails from the frigid water. Sampled organisms were kept in plastic Ziplock bags labeled with site, organism type, and date of collection and temporarily frozen at 0 °C in a freezer. These samples were later used for isotopic analyses.

While the groundwater samples shook, I prepared the collected organisms for isotopic analyses. Organisms were thawed, briefly rinsed, and dipped in ethanol to remove any extra sediment or debris. Removing snails from their shells with dissection tools, I extracted the foot muscle from snails at each station, grouping their extracted tissue into a scintillation vial labeled with site, station number, type of organism (snail, mussel, or macroalgae), and date collected. Adductor muscles were extracted from mussels at each station and grouped into a scintillation vial labeled with site, station number, type of organism, and date collected. Scintillation vials with organism tissue were placed in a drying oven at 65 °C for 36 hours to dry the tissue. Using a mortar and pestle, the dried tissue was grinded up into powder for stable isotopic analyses.

$\delta^{15}$N Analysis

I used the groundwater samples and organism powders for stable isotope analysis. The Stable Isotope Lab at the Marine Biological Laboratory processed the samples that I prepared for
stable isotopic analyses. The ratios of stable nitrogen (N) isotopes were expressed as and \( \delta^{15}N \). The following equation was used to calculate the ratios:

\[
\delta^{15}N = \left( \frac{R_{\text{sample}} - R_{\text{atmosphere}}}{R_{\text{atmosphere}}} \right) \times 1000
\]

where R is \(^{15}N/^{14}N\) for \( \delta^{15}N \) (Lloret & Marin 2009).

**RESULTS**

*Groundwater profile measurements*

At Little Pond, salinity increased and dissolved oxygen decreased with proximity to the Vineyard Sound (Figure 5). Specifically, salinity also increased with well depth, observing surface water more fresh and deeper water more saline. While conductivity mirrored this trend in salinity, pH and temperature relatively decreased with well depth (Table 1).

*Nutrient concentrations*

At Little Pond, the west shore experiences more N loading in comparison to the east shore (Figure 6). Stations 1, 2, and 3 in the northwest show less total N than that of stations 5, 6, and 7 in the south, but we see an overall large influx of NO\(_3^-\) on the west shore. The east shore, however, shows a similar longitudinal pattern, but with more NH\(_4^+\), especially in stations 10, 11, and 12. Groundwater DIN showed various trends across all stations and depths (Figure 7). We observed peak DIN concentrations in shallower depths (0-4 meters) at stations 3, 4, 5, 8, and 9, while we observed peak DIN concentrations in deeper depths (6-8 meters) at stations 1, 2, 6, and 10. Relatively uniform patterns of DIN concentration were observed at stations 7 across all depths. In context with DIN concentrations from 2016, 2017, and 2018, we see a clear decrease in DIN at stations 3, 7, 8, and 11 (Figure 8). While other stations do not explicitly show a decrease in DIN, they do show a vertical trend of peak DIN at similar depths across time. Station 9, for example, shows high DIN at 2 meters and low DIN at 4-6 meters across all years of data, but not an obvious decrease in DIN concentration over time.

In comparison to West Falmouth Harbor and Sage Lot Pond, the DIN at Little Pond (19-250 µM DIN) is comparable to West Falmouth Harbor (64-253 µM DIN) (Figure 9). We observed a low DIN concentration at Sage Lot Pond (8.8 µM DIN) as expected.

*Stable Isotopes \( \delta^{15}N \)*

We observed little variance in water sample \( \delta^{15}N \) values across stations at Little Pond (\( \delta^{15}N = 1.1-22.4\%o \)), with the exception of a station 1 (\( \delta^{15}N = 22.4\%o \)) which must be confirmed with replication (Figure 10). We saw comparable \( \delta^{15}N \) values at West Falmouth Harbor (\( \delta^{15}N = 6.0-14.7\%o \)) and a light \( \delta^{15}N \) at Sage Lot Pond (\( \delta^{15}N = -4.6\%o \)).

Among the organisms at Little Pond, we saw the lightest \( \delta^{15}N \) in *ulva lactuca* (\( \delta^{15}N_{\text{macroalgae}} = 10.5-12.5\%o \)) and the heaviest \( \delta^{15}N \) in *nassarius obsoletus* (\( \delta^{15}N_{\text{snails}} = 11.5-12.9\%o \)) (Figure 11). Nevertheless, all organisms at Little Pond fell within a tight range of 10.5 to
12.9 ‰. We observed similar $\delta^{15}$N values in the organisms collected in West Falmouth Harbor ($\delta^{15}$N = 9.7-11.4‰) and lighter $\delta^{15}$N values at Sage Lot Pond ($\delta^{15}$N = 5.8-7.4‰), as expected.

**DISCUSSION**

*Nutrients*

The nutrient concentrations found in this study suggest that the sewer system connection to Little Pond is successfully removing wastewater from Little Pond. With most houses connected to the sewer line and decreasing DIN since 2016, our results indicate that wastewater removal is occurring with less houses on septic (Figure 2 & Figure 8). However, the shores of Little Pond differ in their compositions of DIN which may be explained by elevation and hydraulic conductivity, septic tank placement, or organic matter composition.

The DIN compositions on the east and west shores of Little Pond may reflect the elevation and hydraulic conductivity of those shores. Previous studies show that elevation increases dissolved oxygen and nitrification in groundwater (Ueda *et al.* 1991) and high hydraulic conductivity may further induce nitrification. As we observed a high gradient and more dissolved oxygen on the west shore (Figure 5), this may contribute to more nitrification and therefore more NO$_3^-$ (Figure 6). The low-lying east shore had little dissolved oxygen which indicates less nitrification and more denitrification, generating more NH$_4^+$ as observed.

The high and low gradients of each shore provide contrasting layers of freshwater and saltwater within the groundwater tables which may affect the fate of wastewater leaching from septic tanks. With a higher gradient on the west shore, the septic tanks may lie above the saltwater table but within freshwater, allowing nitrification and therefore a higher composition of NO$_3^-$ in the groundwater. In contrast, the septic tanks on the low-lying east shore may lie just above or within the saltwater table where nitrification cannot occur (Santoro 2009). Instead, leaching wastewater would immediately denitrify into NH$_4^+$, causing a higher composition of NH$_4^+$ in the groundwater. Thus, our results suggest that the placement of septic tanks in the groundwater table may affect the DIN in groundwater flowing into estuaries.

With high NH$_4^+$ concentrations on the east shore, gradient and septic tank placement may not be the only factors. Instead, organic matter may drive these this trend as organic matter in sediments degrade and release NH$_4^+$ into the groundwater table (Huang *et al.* 2015). Therefore, the more organic matter in sediments, the more NH$_4^+$ in groundwater. Thus, the east shore of Little Pond may have more sedimental organic matter and, in turn, more NH$_4^+$ than the west side.

Among all three study sites, our average DIN concentrations show that Little Pond and West Falmouth Harbor are indeed receiving wastewater from humans (Figure 9). With slightly more DIN, West Falmouth Harbor reflects that of its two sources of N-loading: septic systems and effluent from the Falmouth Wastewater Treatment Plant. On the other hand, our evidence shows that Sage Lot Pond is in fact a pristine site and highlights the additional human impacts at Little Pond and West Falmouth Harbor.
$\delta^{15}N$ Groundwater

The $\delta^{15}N$ stable isotope values further illustrate the presence and absence of wastewater at these sites. With similar ranges in groundwater $\delta^{15}N$, Little Pond and West Falmouth Harbor show similar sources of N (Figure 10). The average groundwater $\delta^{15}N$ at West Falmouth Harbor is 8.61‰ which falls near the expected $\delta^{15}N$ range for wastewater contamination in groundwater (10 to 20‰) and confirms that wastewater is the main source of N in this estuary (McClelland & Valiela 1997). In contrast, Sage Lot Pond shows an absence of wastewater with a negative $\delta^{15}N$, suggesting a possible influence from atmospheric deposition or fertilizers. In fact, Sage Lot Pond is only 1.11 kilometers from a golf course which may account for an added source from fertilizers. Previous studies confirm a negative $\delta^{15}N$ at Sage Lot Pond and also specify fertilizers as an influence (Valiela et al. 2000). Nevertheless, in regard to wastewater, Sage Lot Pond is still pristine, with only one suspected human source, in comparison to Little Pond and West Falmouth Harbor, with multiple human sources.

Within Little Pond, the water sample $\delta^{15}N$ values of the stations do not show a pronounced trend, especially when in context with the observed DIN concentrations (Figure 12). Where DIN is high, we expected heavy $\delta^{15}N$ values. However, our results reveal no distinct trend of $\delta^{15}N$ with DIN. For example, we observed a high DIN concentration at station LP5 with a light $\delta^{15}N$, contrary to our prediction. In addition, the $\delta^{15}N$ values do not correlate with the DIN composition of each station, as we expected the isotopes to detect $\text{NH}_4^+$ (Figure 13). Thus, our results show that $\delta^{15}N$ was independent of the trend in DIN at Little Pond, with no difference in $\delta^{15}N$ between NO$_3^-$ rich and $\text{NH}_4^+$ rich water.

From these findings, we suspect that the sources of N and organic matter are similar enough to show no explicit differences in $\delta^{15}N$ values of Little Pond stations. While the DIN varies throughout Little Pond groundwater, the sources of N may be the same but processed differently based on the hydrology of the shore, as previously discussed. Moreover, N on the east shore is denitrified and N on the west shore is nitrified, showing opposite DIN compositions but similar $\delta^{15}N$ values from same sources.

$\delta^{15}N$ Organisms

The $\delta^{15}N$ values found in organisms resemble that of the water samples, with evident differences between sites but not within sites. At Little Pond and West Falmouth Harbor, we observed $\delta^{15}N$ values between 9.7 and 12.9‰ which aligns within the expected $\delta^{15}N$ range for wastewater, especially considering the biomagnification of nutrients in organisms (Figure 9; McClelland & Valiela 1997). In fact, mud snail $\delta^{15}N$ values from this study are consistent with those observed by Watson et al. in a Long Island study in 2017, reporting a mud snail $\delta^{15}N$ range of 6.6 to 14.1‰, dependent upon the magnification of wastewater loading. In contrast, we observed a $\delta^{15}N$ range from 5.7 to 7.4‰ in the organisms from Sage Lot Pond which further distinguishes this site as pristine. With clear indications of wastewater limitation in Sage Lot Pond and wastewater presence in Little Pond and West Falmouth Harbor, our study encourages the use of organisms as bioindicators between aquatic systems.

Within each of our sites, organisms were not strong bioindicators. For example, the $\delta^{15}N$ values for all the organisms at Little Pond remained within 10 to 15‰ $\delta^{15}N$, whereas we
expected these values to maximize or minimize with N-loading (Figure 11). This uniformity in $\delta^{15}$N may derive from the tidal flow entering and exiting Little Pond. While these organisms are predominantly sessile, the water around them moves with the tide, meaning the organisms are not constantly exposed to groundwater of varying N-loading but rather to estuarine water with the total N-load of Little Pond. Therefore, the $\delta^{15}$N of the organisms reflect a range similar to the overall N-load of Little Pond instead of the individual loads at each groundwater station.

Overall, the $\delta^{15}$N in organisms tend to increase with higher loads from wastewater. Previous studies have reported the following percentages of N-load from wastewater: 16% at Sage Lot Pond (McClelland et al. 1997), 67% at West Falmouth Harbor (Hayn et al. 2014), and 76% at Little Pond (MEP 2006). Plotting the $\delta^{15}$N values of our organisms against the percent N-load from wastewater in each site illustrates how heavier $\delta^{15}$N values are seen in sites with more wastewater (Figure 14). This shows the increase in wastewater load not only from site to site but also from organism to organism. These findings indicate that removing wastewater inputs from Little Pond and West Falmouth Harbor could potentially decrease the load to match Sage Lot Pond. With time and additions to the sewer system, we hope to see continued signs of wastewater removal specifically in Little Pond, and sequential studies should confirm these expectations.

CONCLUSION

From this study, we conclude that the nutrient concentrations over time suggest successful removal of wastewater from Little Pond by the sewer system installation. The $\delta^{15}$N values in groundwater samples, however, do not indicate a clear relationship with the amount of dissolved N in the water, and this trend may stem from the processing (i.e. nitrification and denitrification) or sources of N in groundwater at Little Pond. While organisms are weak bioindicators of wastewater within sites, they are strong bioindicators between sites, showing light $\delta^{15}$N values at Sage Lot Pond and heavy $\delta^{15}$N values at Little Pond and West Falmouth Harbor. Considering the percent N-load from wastewater at each site, we see the increase in $\delta^{15}$N with greater percentages of wastewater input. Moreover, the findings in this study confirm that removing wastewater from estuaries positively impacts the ecosystem by replenishing natural nutrient conditions.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Ken Foreman for his invaluable mentorship and unwavering guidance in all aspects of my project. I would also like to extend sincere gratitude to Rich McHorney for helping me develop and execute the methods of my project, as well as Rachel Clifford, Emily DeFelippis, and Nicholas Patel who graciously and readily assisted me in the field and laboratory. Special thanks to Marshall Otter and Anne Giblin for their help in running and analyzing my samples for stable isotopes. I am also grateful to Javier Lloret for his investment in the beginning stages of my project and to my fellow Semester in Environmental Science students who supported me in every phase of my project, through in through.
LITERATURE CITED


Figure 1. Map of Little Pond and the stations where I sampled groundwater and organisms.
Figure 2. Graph depicting the number of homes connected to the Little Pond Sewer System installed in 2016.
Figure 3. Map of all three sampling sites: West Falmouth Harbor, Little Pond, and Sage Lot Pond.
Figure 4. Stations at West Falmouth Harbor and their nitrate concentration (μM) as reported in previous studies. Star shapes signify the stations investigated in this study: SC1, SC3, and SC7.
Figure 5. Salinity and percent dissolved oxygen across all depths at all groundwater wells at Little Pond for this study.
Figure 6. Dissolved nitrogen concentrations at all groundwater stations in Little Pond, including that of the surface water from the entering stream. DON was reported from TDN results, and DIN is represented by NO$_3^-$ and NH$_4^+$. 
Figure 7. Little Pond 2019 DIN concentrations observed across all depths at all groundwater wells in this study.
Figure 8. Little Pond DIN concentrations in 2016, 2017, Summer 2018, November 2018, and November 2019 observed across all depths at all groundwater wells in this study.
Figure 9. Average DIN (μM) in 2019 at Little Pond, West Falmouth Harbor, and Sage Lot Pond.
Figure 10. δ\textsuperscript{15}N of water samples from all stations at Little Pond, West Falmouth Harbor, and Sage Lot Pond. Note that the stream sample is not groundwater, but surface water from the stream.
Figure 11. $\delta^{15}\text{N}$ of all water samples and organisms at stations in Little Pond.
Figure 12. δ^{15}N and DIN (μM) of all water samples at Little Pond, West Falmouth Harbor, and Sage Lot Pond.
Figure 13. $\delta^{15}$N and DIN (µM) compositions of all water samples at Little Pond. Note that the stream sample is not groundwater but surface water from the entering stream.
Figure 14. $\delta^{15}\text{N}$ of organisms with respect to percent N-load from wastewater. Dots depict individual $\delta^{15}\text{N}$ values for each station at each site while lines depict the trend of $\delta^{15}\text{N}$ with respect to increasing percent N-load from wastewater.
Table 1. Recorded depth-profile data of all groundwater stations at Little Pond, including conductivity, pH, and temperature.

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