Using Stable Isotope Signatures to Identify Possible Anthropogenic Nutrient Sources

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Introduction

Geukensia demissa, ribbed mussels, are marine bivalves in the phylum Mollusca. *G. demissa* are filter feeders, a subgroup of suspension feeders. The species intakes water, which is then filtered by ctenidia and the excess is released through the excurrent siphon. The mussels feed on what is left over after the filtering process – particulate matter (PM). Furthermore, mussels are sessile animals, meaning they are fixed in one location, thus constantly accumulating nitrogen and carbon in their tissues that can be indicative of the isotopic levels regarding their environment. Increases in anthropogenic sources of nitrogen have been linked to an increase in occurrences of eutrophication, which can have negative impacts on the environment, such as promotion of growth of harmful bacteria (Paerl 1997). The average δ^{15}N levels for unimpacted groundwater range from -1.5 to 4.5% (McClelland et al. 1998). When affected by human waste these levels can reach 10-20% (Bannon 2008). Increased isotopic levels can be indications of source material that have the potential to cause harmful effects, making it important to monitor these parameters and be aware of the anthropogenic sources in an area.

Methodology

Ribbed mussel and water samples were collected from five locations along the Connecticut coast – Sandy Point Bird Sanctuary (SP), Morris Creek at Lighthouse Point Park (MC), Quinnipiac Meadows Nature Preserve (QM), Clinton Town Beach (C) and a salt marsh located on Grand Avenue (GA). One group of samples was collected in June, and a second in July.

Mussel Preparation

The mussels were placed into a container holding artificial seawater and an aerator to undergo a 24-hour depuration period. The samples were then frozen overnight before extraction. The mussels' tissues were then dried in an oven at 60°C and were checked intermittently to endure there was no moisture left in the samples. The dried samples were wrapped in aluminum and transported to the Yale Analytical and Stable Isotope Center for further processing.

Water Sample Preparation

300mL of each water samples was filtered via vacuum filtration and transferred to a glass beaker. The beakers were dried in an oven until only the PM remained. The dried samples were wrapped in aluminum and transported to the Yale Analytical and Stable Isotope Center for further processing.

Sample Analysis

Each sample was frozen with liquid nitrogen and ground into a powder using a mortar and pestle. Between 0.4 and 0.6mg of powder for each sample was weighed and placed into a tin capsule, which was then folded into a small square. The samples and standards were loaded into a Costech 4010 Elemental Analyzer paired to a Thermo Delta Plus Advantage Isotope Ratio Mass Spectrometer and run overnight. The results were transferred to a flash drive for the completion of analysis.

Figure 1 Map of sampling locations

Figure 2 Sampling *Geukensia demissa* at Sandy Point

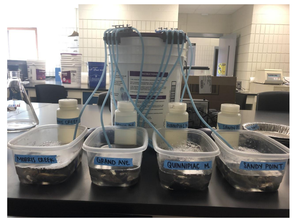

Figure 3 *Geukensia demissa* undergoing a 24-hour depuration period

Results

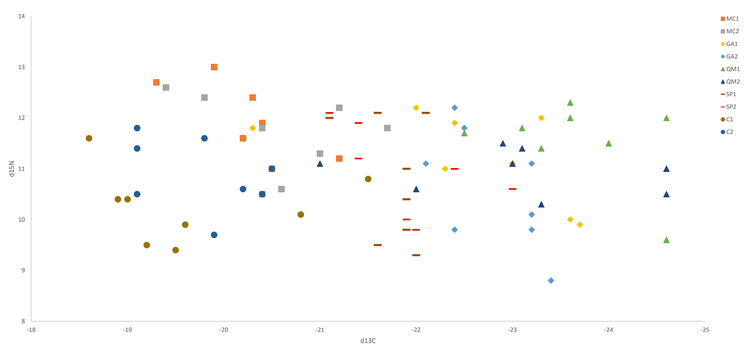

Figure 4 δ^{15}N and δ^{13}C levels of mussel tissue

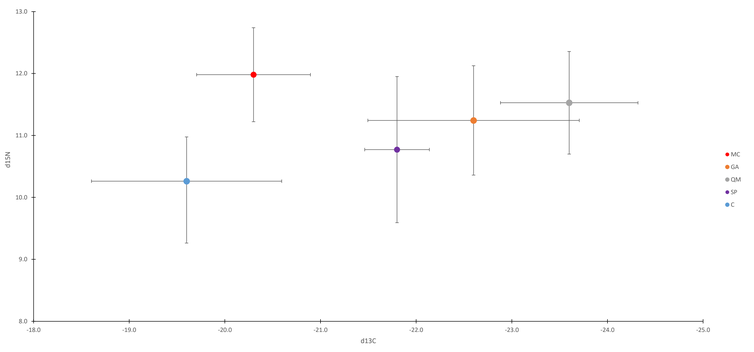

Figure 5 Average δ^{15}N and δ^{13}C levels of each location with standard deviation

Discussion

Five sites were used for this study. While the sites showed a small range of δ^{15}N levels, there was a broader range of δ^{13}C levels. All samples produced δ^{15}N levels between 10.4% and 12% indicating that there are anthropogenic sources affecting these areas. The average levels at Sandy Point were less than the expected values regarding waters in the vicinity of wastewater treatment plants. This could be due to the fact that the output pipe for the facility released the waste further into the body of water than the area from which the samples were gathered. Morris Creek produced the greatest δ^{15}N levels of any location. The creek was adjacent to a popular beach for the summer season, a boat launch as well as a residential area, whereas other sampling locations were located in close proximity to fewer possible sources. Being that the creek was exposed to these factors, it was put at a higher risk of being exposed to an excess of anthropogenic wastes which could explain these increased levels. Despite the small range in both δ^{15}N and δ^{13}C levels, individual samples from each site displayed scattered results with small areas of clustering, similar to the results obtained from same sites during the winter months. The results of the δ^{15}N and δ^{13}C levels in the particulate matter from all sites except Quinnipiac Meadows were inconclusive. This was most likely due to the high percentage of salts that made up the final PM sample.

Conclusions

The goal of this study was to use stable isotope analysis of tissue samples from *G. demissa* and compare the to both literature values and the values determined in a prior study (Strohm 2019) to look for variability in results due to seasonality. It was hypothesized that likewise to the prior research conducted, Sandy Point would have the highest average isotopic levels due to its proximity to a wastewater treatment plant, and that Clinton Town Beach would have the lowest average levels. Though there was clustering visible in the results, the clustering was more distinguishable in the results from the winter months. Though Sandy Point showed elevated levels of both δ^{15}N and δ^{13}C levels, two other sites, Grand Avenue and Quinnipiac Meadows, displayed greater levels of both isotopes. As expected, Clinton Town Beach produced results with the lowest isotopic levels. In future studies more samples should be collected per site, and other species of filter feeders can be tested to further ensure the results' integrity.

References

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