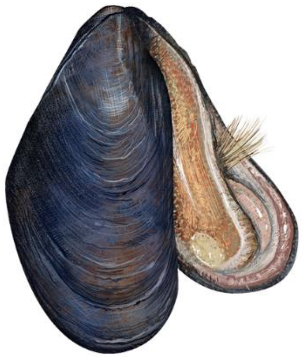

Blue mussel (*Mytilus edulis*) stable nitrogen isotope signatures ($\delta^{15}N$) to monitor human-derived nitrogen inputs to Casco Bay

Graham Sherwood*, Zachary Whitener, Adam Baukus, Andrew Clement

Gulf of Maine Research Institute
350 Commercial Street, Portland, ME 04101
*Corresponding author: gsherwood@gmri.org

Abstract

Nitrogen loading to nearshore marine ecosystems can have deleterious effects including promotion of algal blooms which can lead to shellfish toxicity or eutrophication, which in turn can cause oxygen depletion and coastal acidification. Nitrogen monitoring can be costly and sporadic and the full picture of the ultimate fate of nitrogen throughout coastal waters is usually incomplete. Here we present data from a pilot stable nitrogen isotope monitoring study which can help fill the gaps spatially and direct future monitoring efforts. Specifically, we measured stable nitrogen isotope signatures ($\delta^{15}N$) in blue mussels which integrate temporal variations in human nitrogen inputs to specific locations. We sampled 100 sites around Casco Bay to construct a heatmap of mussel $\delta^{15}N$ which can then be used to visualize locations where human nitrogen sources (e.g., sewage/septic but also domestic animals) are potentially problematic. Not surprisingly, the east end of Portland showed signs of higher human nitrogen loading, but the spatial extent of this influence was relatively limited. On the other hand, a relatively large plume of higher $\delta^{15}N$ was detected in the eastern half of the bay suggesting either high inputs or poor flushing of terrestrial sources. Stable carbon isotope signatures ($\delta^{13}C$) were also measured and provided further insights into freshwater vs. marine water masses in Casco Bay. Taken together, mussel stable isotope monitoring is a promising technique for inferring sources and fate of freshwater and nutrient inputs to nearshore marine ecosystems.

Introduction

Casco Bay is the second largest embayment in Maine and is adjacent to the largest population density in the state. Portland is the largest city but a number of smaller towns and cities also lie within its catchment including South Portland, Cape Elizabeth, Westbrook, Falmouth, Yarmouth, Cumberland, and Freeport. Higher population densities are often associated with higher nutrient loading (notably nitrogen) and this may also involve environmental issues including eutrophication (algal blooms, which may involve harmful toxic species), oxygen depletion and coastal acidification. Nitrogen loading to Casco Bay has been monitored periodically in the last decades by a number of agencies and groups including the Friends of Casco Bay and the Maine Department of Environmental Protection. However, these monitoring efforts may be limited in terms of spatial and temporal coverage (e.g., Figure 1), thus hampering our ability to observe trends and pinpoint sources.

Dolenec et al. (2005) used stable nitrogen isotope signatures ($\delta^{15}N$) of sea anenomes (*Anemonia sulcata*) in an embayment of the central Adriatic Sea to monitor the extent of human sewage impacts on this nearshore marine ecosystem. The approach they took is quite simple. Nitrogen exists in two isotopic forms, the common ^{14}N and the rarer and heavier ^{15}N (extra proton). The relative abundance of each isotope can be measured in samples via mass spectrometry and the ratio of the two in relation to a standard (N_2 in air) gives us the nitrogen signature ($\delta^{15}N$) of a sample. In food webs, ^{15}N biomagnifies so that higher trophic level organisms tend to have more ^{15}N and higher $\delta^{15}N$ signatures (Vander Zanden & Rasmussen 2001). Human-derived nitrogen that enters the food web via uptake at the base (i.e., primary production) can also increase the $\delta^{15}N$ of consumers in an area. Thus, $\delta^{15}N$ of aquatic organisms is a valuable tracer of food-web processes, as well as variations in nitrogen loading to a system, particularly from human sources.

Not all aquatic organisms are suitable for the baseline approach described by Dolenec et al. (2005). Small planktonic organisms turn over nitrogen very fast such that their $\delta^{15}N$ values are indicative of only a few days of source nitrogen $\delta^{15}N$. Larger organisms may integrate $\delta^{15}N$ over much longer time periods. However, since many are mobile, it is difficult to assign their $\delta^{15}N$ values to a given location (i.e., they may integrate nitrogen sources from multiple areas). Larger, mobile organisms also tend to feed across a broad range of trophic levels and therefore variation in their $\delta^{15}N$ may be driven primarily by feeding differences. The best organisms for this purpose are medium- to large-sized, *sessile* primary consumers (e.g., Cabana & Rasmussen 1996). Primary consumers (e.g., filter feeders of phytoplankton) have known and invariable diets, thus eliminating the influence of trophic level. The fact that they are sessile means that the nitrogen they incorporate is from sources very close. Finally, their larger size means that turnover of nitrogen is slower and their $\delta^{15}N$ values are therefore integrating weeks to months of source variations. In essence they are like continuous monitoring instruments.

The purpose of this pilot study was to apply stable isotope analysis of a sessile primary consumer, blue mussels (*Mytilus edulis*) to examine fine-scale spatial variations in human nitrogen loading to Casco Bay. Blue mussels have undergone marked declines in the abundance and distribution of large beds. However, they are still found in small quantities at most intertidal and subtidal sites throughout Casco Bay making them useful for our purposes. We hypothesized that $\delta^{15}N$ signatures of mussels would be highest adjacent to the largest population center (i.e., Portland) and would reflect more pristine natural values further away from towns, cities and rivers.

Methods

A total of 100 sites (Figure 2) were sampled for blue mussels in the summer of 2018 from July to August over 5 dates. At each site, 1-3 individual mussels of varying size (20 – 94 mm) were collected from the intertidal zone, or just into the subtidal, and brought back to the lab where they were dissected to remove the foot tissue. Foot tissue was subsequently dried to constant weight in a drying oven (70^0C for 48 hrs), crushed using a mortar and pestle, and a small (~ 1 mg) subsample was packed into a tin capsule for stable isotope analysis at the Colorado Plateau Stable Isotope Laboratory. Stable isotope results are expressed in delta notation. For example, for nitrogen, the parts per thousand (‰) deviation of the ratio of $^{15}N/^{14}N$ in the sample to the same ratio in the standard (N_2 in air; standard for carbon was peedee belemnite limestone) was calculated. Only one mussel sample per site was analyzed to increase spatial resolution. There was a range in mussel sizes analyzed. However, there was no significant relationship between size (length, mm) and $\delta^{15}N$ or $\delta^{13}C$ over the entire sample (Figure 3 and 4). Resulting nitrogen and carbon stable isotope values were visualized by spatial interpolation (kriging) performed using the ‘gstat’ package in R (Pebesma 2019).

Results

Stable isotope results are shown in Figures 5 and 6. Figure 5 shows the variability in stable nitrogen isotope signatures ($\delta^{15}N$) around the bay. As expected, higher $\delta^{15}N$ values were found in the Fore River, adjacent to the East End of Portland, and as far to the east from there as Mackworth Island (see Figure 2 for place names). Moving east from Portland towards Peaks, Great Diamond and Long Islands, the values drop off considerably and appear to be closer to pristine values which were observed on the seaward side of the outer islands and out to Halfway Rock. Unexpectedly, the highest $\delta^{15}N$ values were observed in the eastern end of the bay near Bustins and Upper and Lower Goose Islands and into Maquoit and Middle Bays. Similarly, higher values were seen adjacent to the Harraseeket and Royal Rivers which drain from Freeport and Yarmouth. This plume of high $\delta^{15}N$ values extended relatively far out towards the outer eastern portion of Casco Bay (i.e., through Broad Sound).

Carbon isotope results are shown in Figure 6. Carbon isotope signatures ($\delta^{13}C$) are measured simultaneous to $\delta^{15}N$ and can inform patterns of freshwater versus marine influences in a given area. This is because $\delta^{13}C$ values of freshwater are much more negative than those of open marine systems (by about 10 delta units, ‰). There is very little influence of trophic level on $\delta^{13}C$ (Vander Zanden & Rasmussen 2001), and therefore $\delta^{13}C$ of a primary consumer is related to carbon source (i.e., freshwater versus marine). In Figure 2, green areas represent areas more influenced by freshwater sources (more negative $\delta^{13}C$ values), whereas blue areas represent mostly marine influences (more positive $\delta^{13}C$). In this case, the very western side of the bay near Portland appears to be mostly marine influenced (blue), even well into the Fore River. There appears to be a plume of mild freshwater influence in the middle part of the bay and along the shore from Mackworth Island to Maquoit Bay (greener). The lowest (most negative or most fresh) $\delta^{13}C$ values appeared in the eastern side of Casco Bay in Maquoit and Middle Bays (very green). It should be mentioned that the absolute difference in what is interpreted as freshwater versus marine is relative. The greenest values are around -20‰ which is still largely marine. These values happen to be slightly less marine, in terms of carbon values, than sites where mussel $\delta^{13}C$ values are around -19‰.

Discussion

The purpose of this pilot study was to use mussel stable isotope values to infer spatial patterns in human nitrogen loading to Casco Bay. A major caveat to this approach is that $\delta^{15}N$ is useful for tracing the relative influence of human sources (including sewage/septic, livestock and animals which all have higher $\delta^{15}N$ than natural sources; Townsend et al. 2002) but does not indicate total nitrogen loading. Another source of anthropogenic nitrogen is fertilizer which has a lower $\delta^{15}N$ than natural sources (Townsend et al. 2002) so the balance of human sources may actually drive lower $\delta^{15}N$ in receiving waters. Nonetheless, based on Dolenec et al. (2005) who studied $\delta^{15}N$ enrichment in a very similar system, we expected to find more human (sewage) influence near Portland in the western half of Casco Bay and more pristine values in the outer bay. As expected mussels near Portland showed signs of some human influence in that their $\delta^{15}N$ signatures were higher. However, this influence did not appear to extend very far into the rest of the bay, particularly eastward (Figure 5). The area between Falmouth and Long and Chebeague Islands had fairly low $\delta^{15}N$ values suggesting that human nitrogen sources are low here. The reason that the $\delta^{15}N$ plume from Portland may not penetrate very far to the east is that the area between Falmouth and Great Diamond to Chebeague Islands is relatively shallow and may cut off any flow from west to east. Indeed, the bulk of the flow in this area is directly in and out and associated with flood and ebb tides (True & Manning 2005). The approach shown here is the first to provide nitrogen data at a scale that can resolve this type of nitrogen transport in Casco Bay.

Unexpectedly, the highest mussel $\delta^{15}N$ values were observed in the eastern half of the bay (Figure 5). There may be multiple explanations for this. First, this side of the bay is influenced by the Royal and Harraseeket Rivers which drain from Yarmouth and Freeport. Thus, these rivers may carry a large load of human nitrogen into the system. This is consistent with total nitrogen monitoring results carried out by the Friends of Casco Bay near the outflow of these rivers (Figure 1). The extent of the eastern "plume" may also be related to multiple small-scale point sources throughout the islands in the eastern half of Casco Bay. Individual failed septic systems or raw sewage from single houses may add up to a large contribution of human nitrogen to the system in this area. Finally, currents may concentrate nitrogen in this area through recirculation of water masses within Maquoit and Middle Bays (True & Manning 2005). Support for this comes from mussel $\delta^{13}C$ results (Figure 6). In this case, Maquoit and Middle Bays reflect the highest influence of freshwater carbon suggesting less of a marine influence and perhaps more stagnant water masses (less tidal flushing). On the other hand, there appears to be less freshwater influence in the very western side of Casco Bay (off Portland) suggesting a high degree of tidal flushing. This may act to dilute some of the human nitrogen entering here from Portland.

Conclusions

This pilot study demonstrates the usefulness of measuring stable isotope signatures of blue mussels for monitoring the relative influences of human nitrogen on Casco Bay at a fine scale. Nutrient monitoring through direct sampling is time consuming and costly and cannot always achieve high spatial and temporal coverage. As such, our understanding of nutrient fate in nearshore marine ecosystems is not always complete. The method described here provides a relatively cost-effective and robust means for identifying potential problem areas over a broad scale that can be followed up with more direct measurements. We intend to repeat this study and increase sample size so that nutrient loading in discrete bays (e.g., Harpswell Sound not sampled here) can be added. There is also the possibility of coordinating sampling with other monitoring groups (e.g., Friends of Casco Bay and Maine DEP) so that direct comparisons may be made between mussel $\delta^{15}N$ and total nitrogen concentrations.

Acknowledgements

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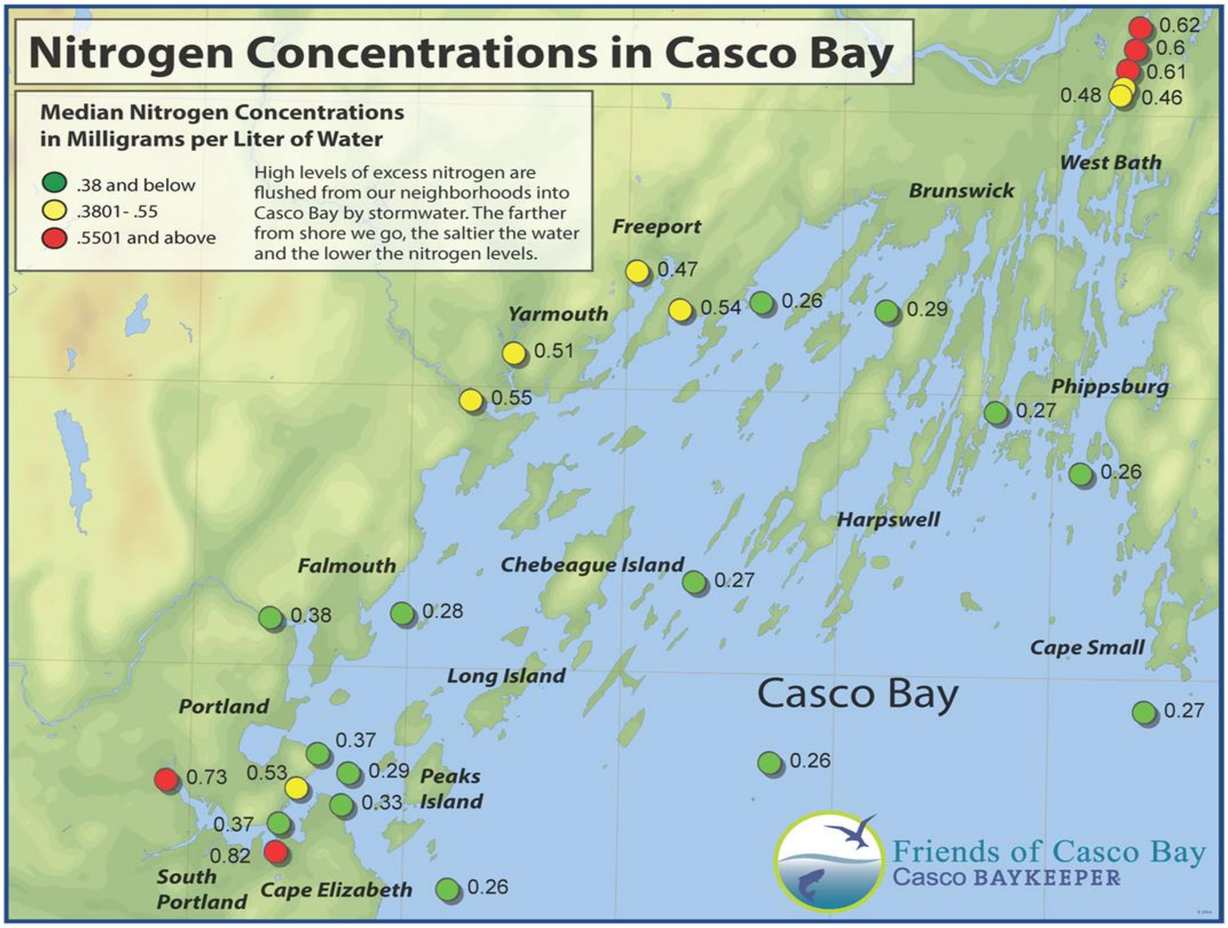

Figure 1. Total nitrogen concentrations measured at various locations around Casco Bay. From Friends of Casco Bay (Mike Doan, pers. comm.).

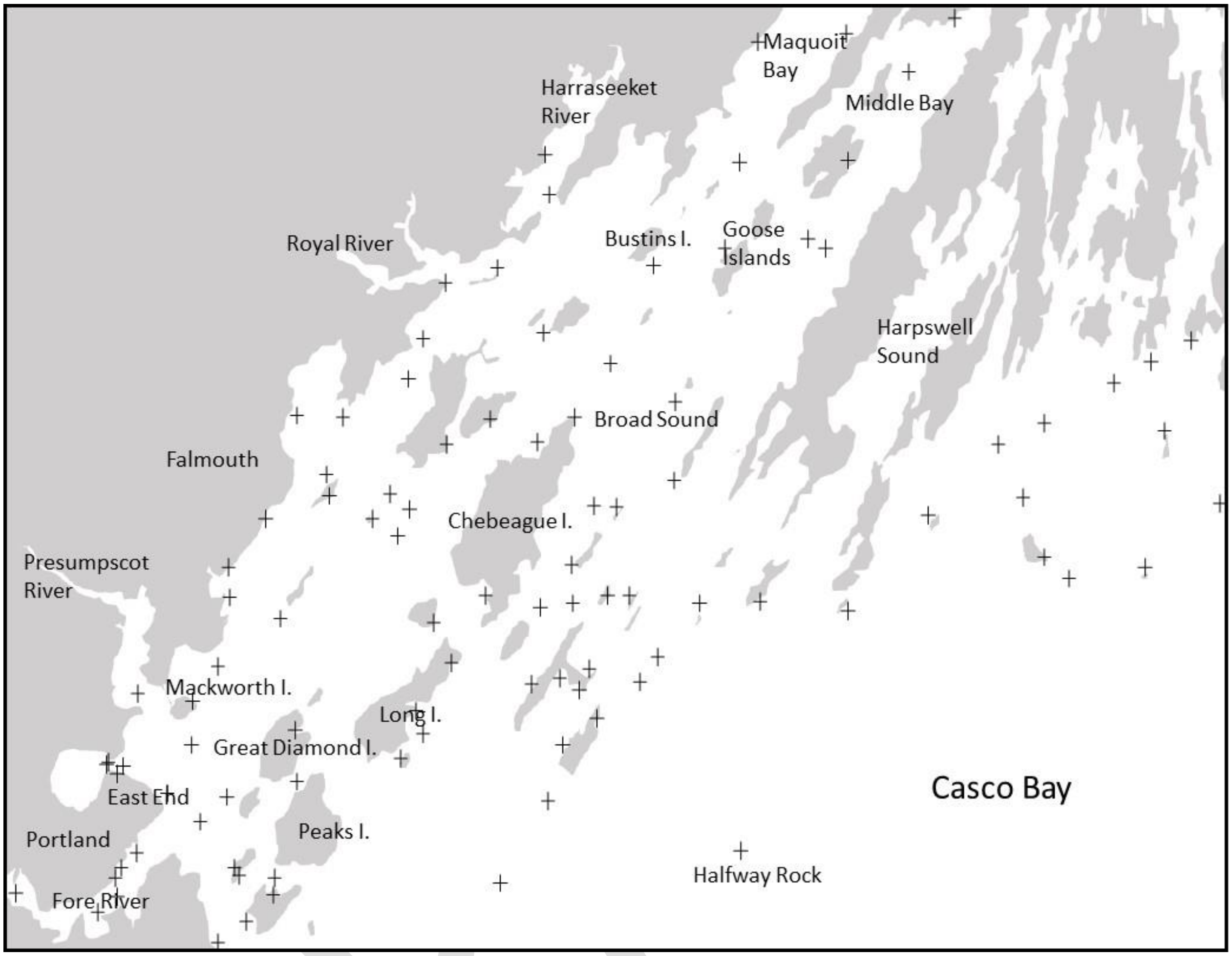

Figure 2. Map showing location names throughout Casco Bay. Treated sewage water from Portland enters the bay off East End. Sampling sites are shown by crosses.

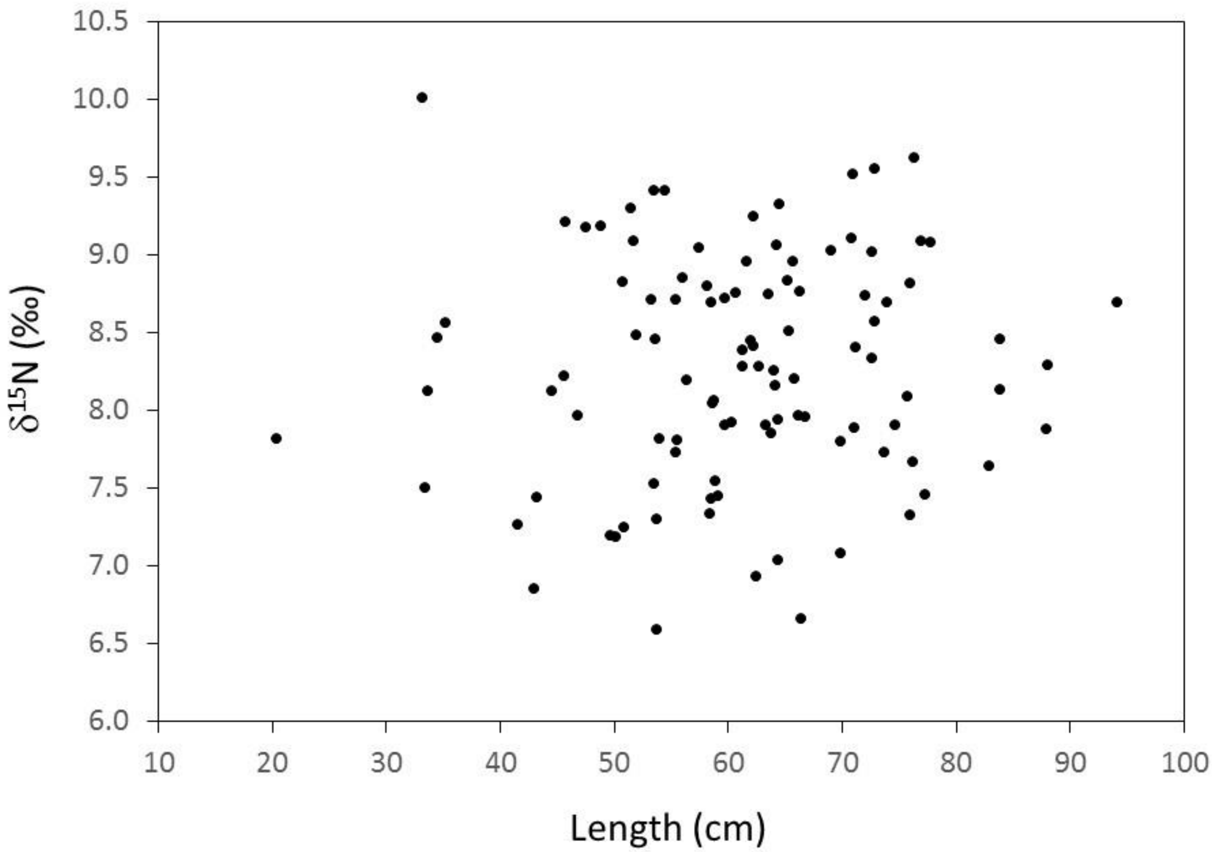

Figure 3. Blue mussel $\delta^{15}N$ versus blue mussel length. There was no significant relationship.

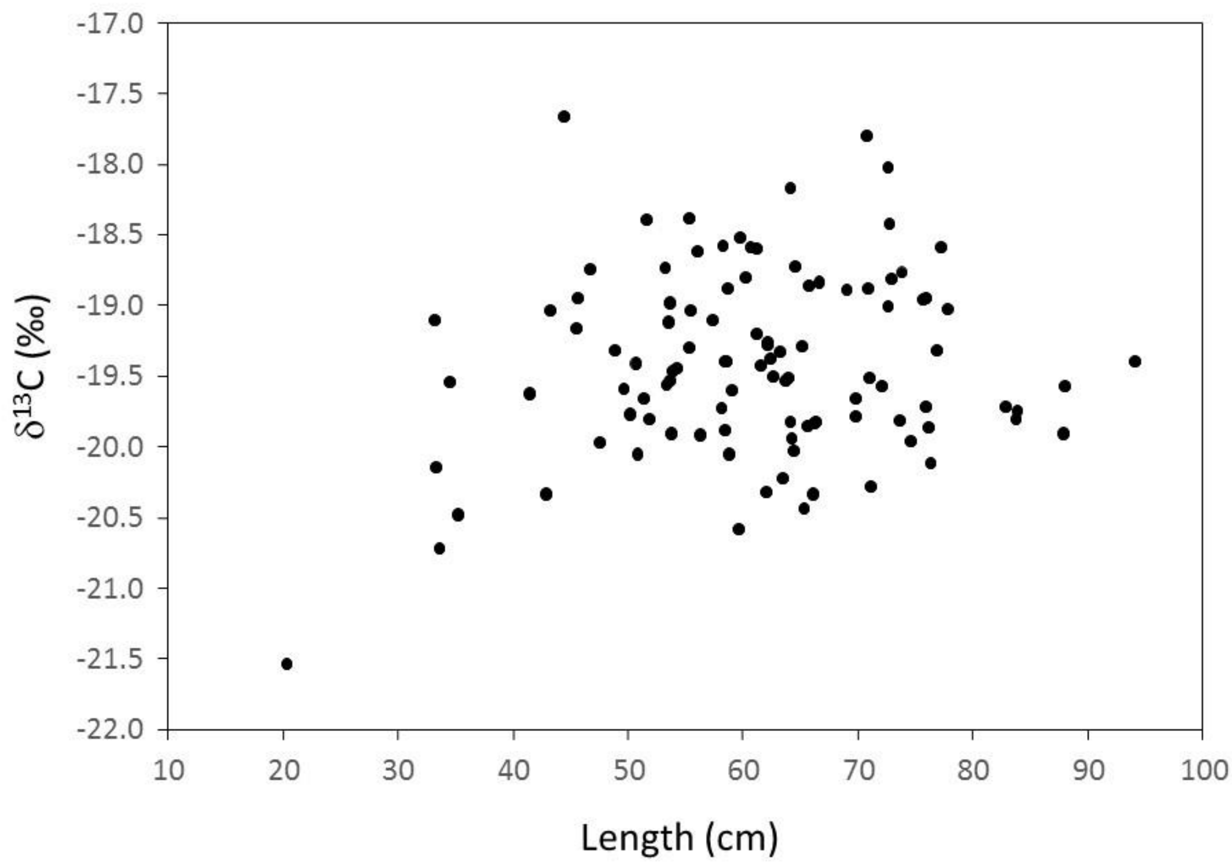

Figure 4. Blue mussel $\delta^{13}C$ versus blue mussel length. There was no significant relationship.

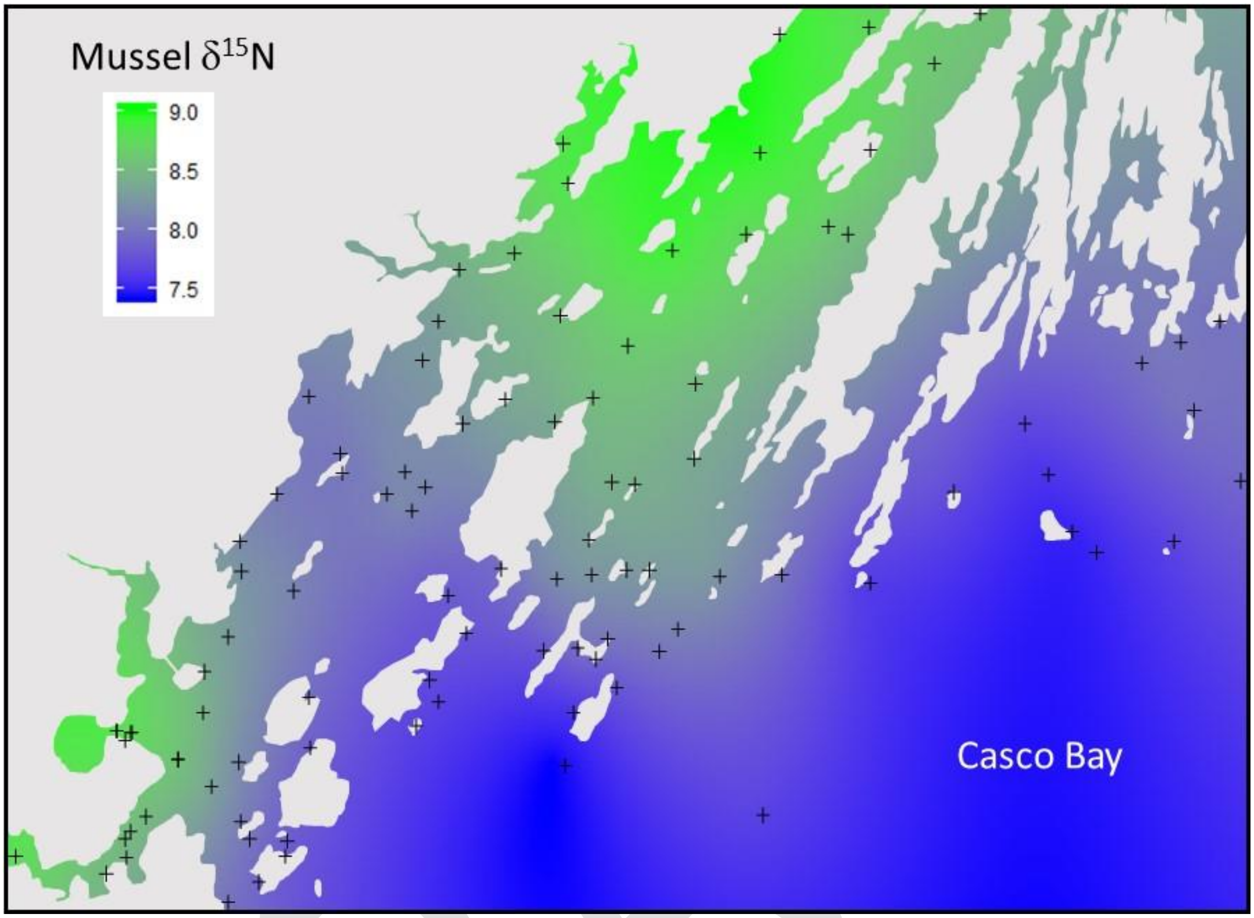

Figure 5. Spatial distribution pattern of blue mussel $\delta^{15}N$ values in Casco Bay. Note that $\delta^{15}N$ values were kriged without barriers. Thus, shading in bays and reaches without sampling sites is spurious (e.g., Harpswell Sound; see Figure 2).

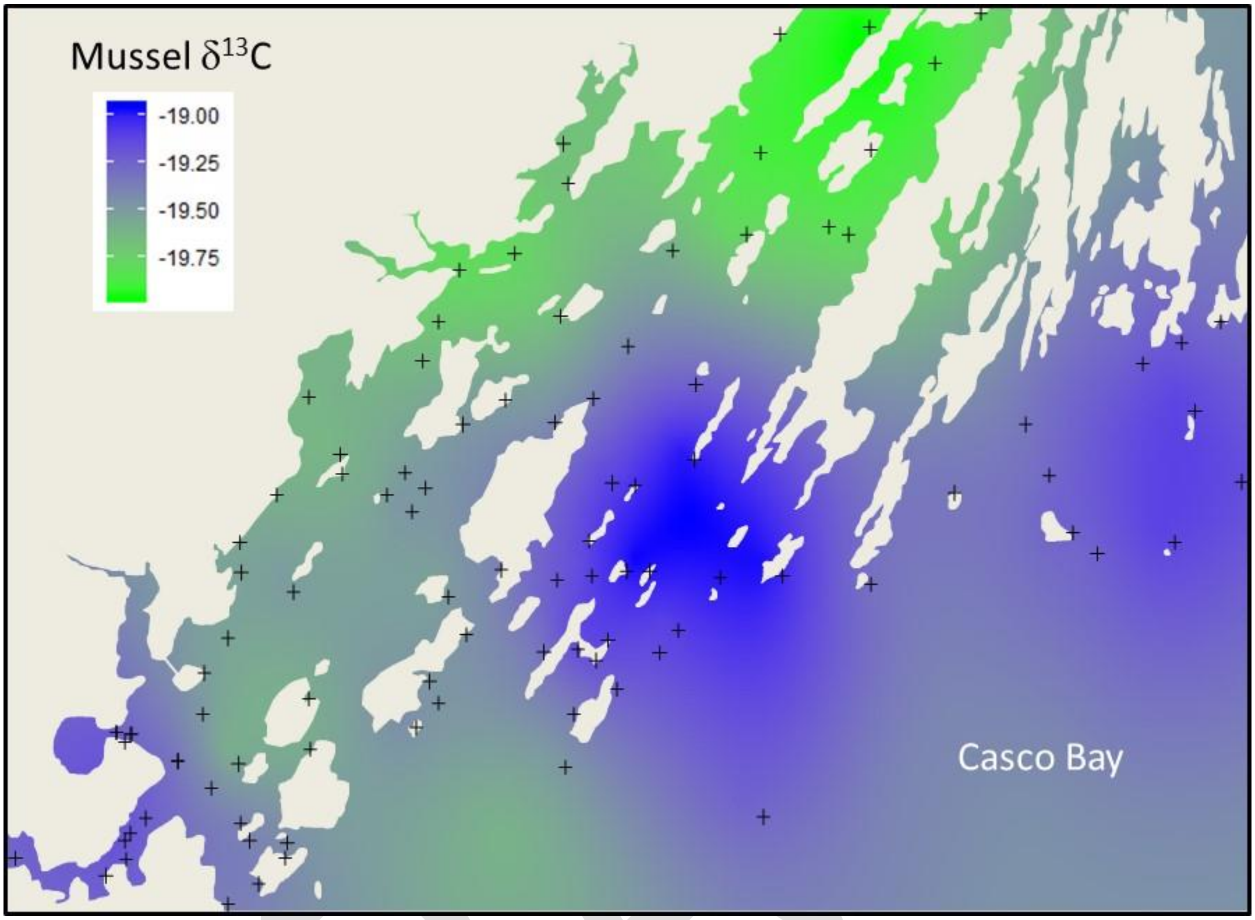

Figure 6. Spatial distribution pattern of blue mussel $\delta^{13}C$ values in Casco Bay. Note that $\delta^{13}C$ values were kriged without barriers. Thus, shading in bays and reaches without sampling sites is spurious (e.g., Harpswell Sound; see Figure 2).