eDNA Results Update

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Background

DNA extraction and qPCR analysis have been completed for a subsample of 80 QBC CBASS water samples. These samples represent water collected approximately 50m from shore at every sampling site during the 10 weeks of data collection in 2023. Each extracted sample was run to amplify any Atlantic herring (*Clupea harengus*) environmental DNA present, as identified by a relatively short known sequence of herring DNA. Post amplification, a standard curve was used to translate amplification cycle (Cq) values to a relative index of herring eDNA concentration. The association between seine- and eDNA-based detections was quantified, and eDNA concentrations were visualized to identify any seasonal or spatial patterns of herring nearshore environment use.

Relationship to seine-based herring catch

Though eDNA is a powerful tool to detect the presence of species in complex environments, several factors cloud the relationship between eDNA concentration (derived from qPCR) and the abundance of nearby individuals for any species. Temperature, flow, and light conditions could all affect eDNA shedding rates, degradation rates, and detection probabilities. Additionally, the temporal duration of a detectable signal (sufficient eDNA in the sample to be detected after amplification) is highly reliant on the length of the DNA sequence chosen by the analyst to identify the species. In these analyses, we used a shorter sequence to identify herring. These shorter sequences will take longer to degrade in the environment and as such, we must expect that at least some of our detected signal has been “lingering” in the environment after herring have passed nearby at some point in the recent past. Further studies need to be conducted to determine the length of time these short DNA sequences will persist in a variable marine environment.

Despite these complications, we can still assess the correlation between eDNA- and seine-based herring detection. In this approach, thresholds are set for each variable above which herring are considered “detected.” Clearly, this threshold is 1 herring for the seine-based detection. The threshold for eDNA-based detection was 39 amplification cycles (Cq); beyond 39 cycles, amplification is so weak as to suggest any signal present is more likely noise than true detection. We then used Pearson’s Phi-coefficient to test the relationship between detection using the seine and detection using eDNA. This test provides a scale to see if the relationship between variables is very strong in a negative direction (values close to -1), very weak (values close to 0), or very strong in a positive direction (values close to 1). The resulting value (0.12) suggests a very weak relationship, driven by the many instances in which herring were not caught, but eDNA was detected. However, this should not be seen as a disappointing result. Because we were using the short DNA sequence as our identifier, it is more than likely that we are detecting a signal from herring that *were* in the area but left before seining commenced. It is

also possible that herring were nearby at the time of seine sampling, but were just outside the area sampled by our net or evaded capture. A bright spot of these results is that there were no instances where we caught herring in the seine but did not detect their eDNA presence using qPCR. We should also note that similar studies have found a low correlation between detection using seines and eDNA (e.g, Plough et al. 2018).

	eDNA detected	eDNA not detected
Herring caught	10	0
No catch	39	3

Table 1: Pairwise comparison of herring detection using a seine (rows) and herring eDNA detection using qPCR (columns).

Temporal patterns of herring eDNA concentration

We know from scientific literature, fishermen's ecological knowledge, and our 11 years of monitoring that herring have a fairly predictable seasonal use pattern of nearshore regions in Casco Bay. In general, we observe small numbers of herring at the beginning of our sampling season (early June), which rapidly increases to a peak by late June. Then, our catch of herring quickly declines to a consistent absence by mid-July. We plotted the temporal pattern of herring eDNA concentration across the QBC study area and found a similar temporal pattern. We saw a rapid increase in herring relative eDNA concentration from mid-June through early July, then a sudden collapse to a consistently low value that held from mid-July through early August. This result speaks to a high confidence in our ability to detect herring at longer temporal scales.

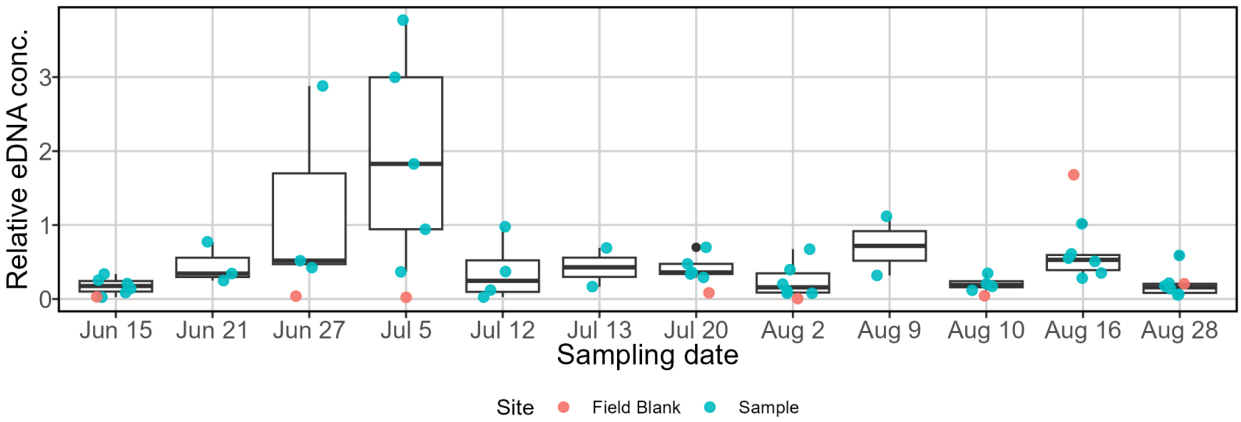

Figure 1: Relative eDNA concentration for each sampling day. Blue points represent field-collected water samples. Red points represent field-collected blanks. Blanks should contain no herring eDNA and are used to check for contamination and amplification of undesired material. Boxplots show the distribution of eDNA concentration values in water samples.

Spatial patterns of herring eDNA concentration

We are also interested in describing the spatial pattern of eDNA concentrations among the sampling sites. To do this, we calculated the mean relative eDNA concentration at each sampling site and then mapped those values to our site locations. We completed the same procedure for seine-caught abundance of herring. The spatial patterns do not seem to match; sites with higher mean eDNA concentrations have among the lowest mean catch. However, we need to consider the physical features of these sampling sites. Orrs Cove, Snow Island Cove, Lowell Cove, Garrison Cove, and Long Point Cove are all highly protected sites. Though tidally influenced, the retention time of water in these coves may be much higher than for Cedar Beach (the southernmost site depicted on the map, on the northeast corner of Bailey Island), which is more directly exposed to the rest of Casco Bay. Thus, we could be observing herring presence over a longer time scale in the shallow cove sites, and more immediate herring presence at the less-protected site.

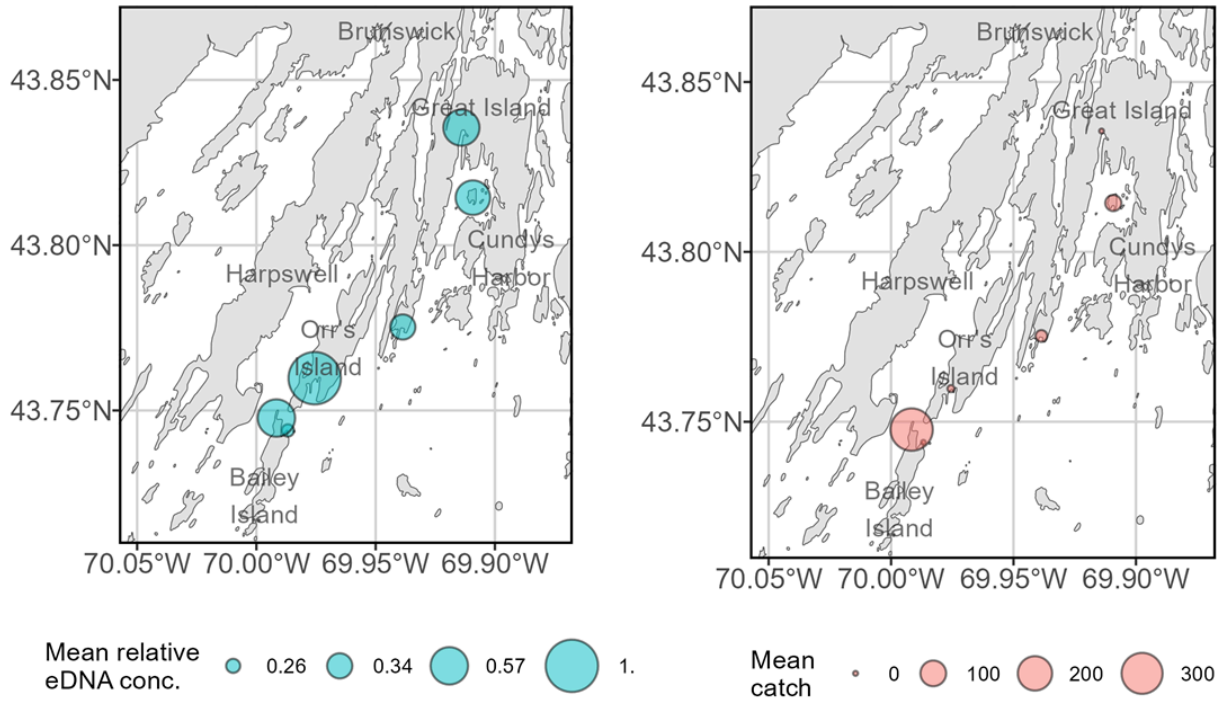

Figure 2: Mean 2023 relative concentration of herring eDNA (left, blue points) and mean 2023 seine catch of herring (right, red points) at each sampling site. Points are scaled to represent higher values as larger points. Cedar Beach and Garrison Cove points overlap, as they are very close to each other on the north end of Baily Island.

The finest-detail way to view our results is to look at temporal patterns of eDNA concentration at each sampling site. We plotted these results and identified Garrison Cove and Lowell Cove as having strong pulses of herring eDNA in early July. Sampling in Garrison Cove on July 5th, 2023 produced both the highest overall concentration of herring eDNA and the largest catch of herring in the seine (more than 2500 individuals) for the season.

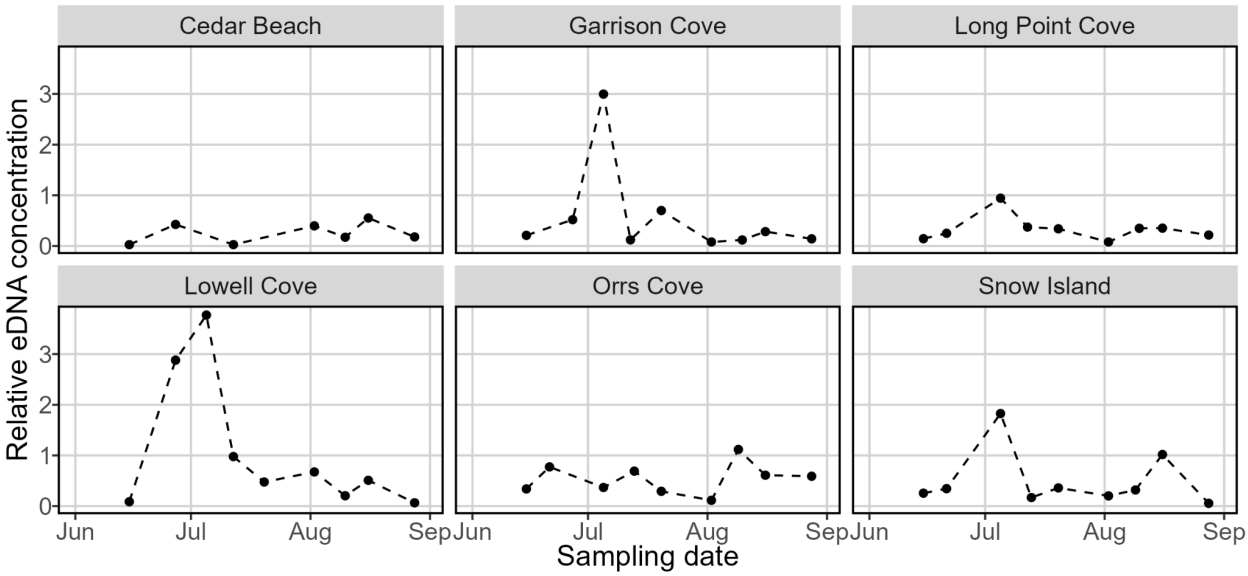

Figure 3: Relative eDNA concentration across the 2023 season for each sampling site.

Conclusions

Results suggest that our current field sampling and laboratory processing procedures are well-designed to detect finfish eDNA in Quahog Bay and surrounding areas. Our seasonal pattern of herring eDNA presence (via amplification of short herring DNA sequences using quantitative PCR) closely matched known seasonal trends of herring nearshore habitat use. Maximum estimated eDNA concentration at any site occurred simultaneously with maximum seine catch. Poor correlation between short-sequence-based eDNA detection and seine-based detection may be due to the longevity of short-sequence eDNA fragments in the nearshore environment or herring evading capture, and should not be interpreted as evidence against eDNA's usefulness.

Next steps

We did notice some unexpected or unexplainable results. Chiefly, field blanks procured in the last two sampling events produced a herring eDNA concentration suggesting herring presence. This should not be possible under the correct application of field and laboratory protocols– the field blanks are drawn from sealed bottles of purified drinking water that are opened very briefly in the field to be exposed to the same airborne eDNA as water samples. We suspect this may be a labeling error, though unintended cross-contamination of field blank and water samples may have occurred. We intend to run two more rounds of the mid-site samples through the amplification process, in the hopes that triplicate samples will both help resolve these issues and produce confidence intervals around our estimated eDNA concentrations. Moving forward, samples will be run in triplicate for all filtered water samples.

Extraction and qPCR analysis for all other samples (2023 Inner and Outer samples for each sampling site, all 2024 samples) must still be completed. This is a lengthy process but will provide the material needed to test hypotheses for multiple species and/ or multiple DNA

sequence lengths. Our next priority will be to run qPCR analysis on the extracted mid-site samples using a longer herring DNA sequence. Longer sequences have faster degradation rates, and would theoretically be a closer approximation of nearshore herring presence during the immediate time around seine sampling. We can then compare spatial and temporal DNA patterns emergent from those different sequence lengths, which will provide insight into the relative abundance and variation of longevity between those sequences.

We will also procure primers to run qPCR analysis for other species. We have identified Atlantic tomcod as a good candidate; tomcod are commonly caught in our seine samples and are known to have interesting seasonal usage patterns similar to herring. Running qPCR for tomcod would also allow us to compare the relationship between eDNA detection and seine detection for a bottom-oriented species.

References

LV Plough, MB Ogburn, CL Fitzgerald, R Geranio, GA Marafino, KD Richie. (2018). Environmental DNA analysis of river herring in Chesapeake Bay: A powerful tool for monitoring threatened keystone species. *PLoS ONE* 13(11): e0205578.