Herring qPCR subsample analysis

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0.1 Background information

A subset of 12 QBC CBASS water samples from 2023 and 2024 were run for qualitiative analysis in summer 2024. The objective was to determine if herring DNA was present in water samples taken immediately preceeding large herring catches, and to further confirm that no DNA was identified in "blanks" (sterile water samples exposed to air in the field, but not otherwise coming into contact with materials likely to host any marine DNA). Results indicated that the sterility check was passed, and that some herring DNA was identified.

Further qPCR analysis was conducted to estimate herring DNA concentrations, using quantification cycle (C~q) values as a proxy for concentration. In short, C~q is the number of amplification cycles necessary to reach a threshold fluorescent signal which signifies detection of DNA matching a chosen primer. Low C~q values indicate a high concentration of the chosen species' DNA, and therefore few amplification cycles were needed to detect it. High C~q values indicate a low concentration, and therefore more amplification cycles for detection.

0.2 Available data

Data run in these analyses came from July 5, 2023 and June 6, 2024. It is important to note that a water collection protocol change took place between the two years. In 2023, water samples were collected at three distinct distances from shore (at shore, 50m from shore, 100m from shore) prior to each beach seine. Samples were labeled as Inner, Middle, and Outer, respectively. In 2024, all samples were taken at the same time and at the same distance from shore– approximately 10m from shore, which is close to the distance between the back of the bag and the shoreline when the beach seine is fully deployed. All samples were taken at the surface.

For our analyses, the 2023 samples were all from the "Middle" sampling location. The 2024 samples were randomly selected from the triplicate samples of each site. The thought process was that these should be the most similar in environmental conditions and habitat characteristics, and therefore likely the best way to control for patterns in species habitat use.

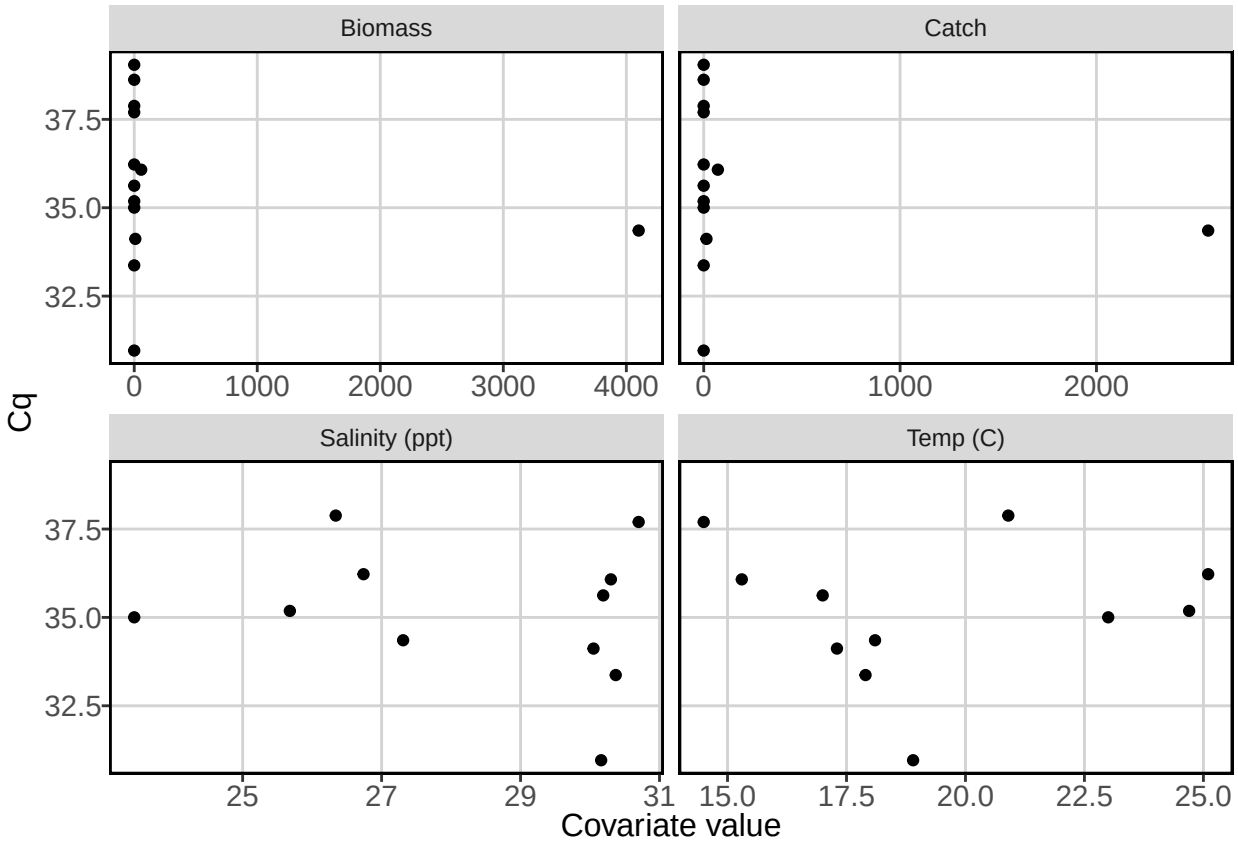
Data for each site pertinent to both organism presence and eDNA degradation include surface water temperature and salinity, time of day, weather conditions, catch of herring, and an estimated biomass of herring caught. Weight was not collected in the field, so individual weights had to be estimated by use of a Length-Weight relationship based on growth parameters from the literature. Biomass was then estimated as the mean weight per individual multiplied by the number of individuals caught.

0.3 Caveats

This is not going to be a statistically robust exercise. We only have 11 field samples, 2 field blanks, and 2 laboratory controls to build from. This is not enough, especially when only three of the beach seines associated with these water samples had observations of herring. We need to build a larger dataset of herring catch vs. C~q values to fully address any research objective. That being said, I think we can take a quick look at herring catch vs. C~q. This might help us formulate stronger research objectives.

0.4 Visualization

We would expect eDNA concentration to vary with herring density in the area immediately around the collection site. We can use numeric abundance or estimated biomaass to check this assumption. We also expect eDNA concentration to be affected by environmental conditions that are known to degrade DNA: we can use temperature and salinity collected at the field sites. We'll plot these relationships to get a sense of what analyses are possible.



These plots confirm what we stated before: data volume is a massive issue. We'll need to run many more samples to make any robust quantitative analyses.

As is, there appears to be no strong relationship between salinity and C~q or temperature and C~q. It is hard to assess the nature of the relationship between measures of herring density (biomass or catch) and C~q, but there may be a slight negative relationship; increased herring presence decreases C~q.

One bright spot is the similarity of our field negative controls to the laboratory negative control.

```
##         Sample       Cq
## 7       ABlank 39.04328
## 13      DBlank 38.61919
## 14 Neg Control 39.59454
```

The rest of our C~q value are between 30 and 38. Our two lowest C~q values come from sites that had no recorded catch of herring. This is not necessarily an issue– it's possible the herring just weren't using the nearshore location capable of being sampled by the seine. It's also possible that they saw and evaded the net. But we would obviously have no way of confirming true absence of herring with the seine-eDNA combo, only true presence.

```
##    Sample       Cq catch
## 1     A21 30.95903     0
## 2     A22 33.36944     0
## 3     A23 34.11858    14
```

```
## 4     D25 34.35341  2569
## 5     D23 35.00264     0
## 6     D21 35.18390     0
## 7     A26 35.62264     0
## 8     A25 36.07593    72
## 9     D22 36.22267     0
## 10    A28 37.69926     0
## 11    D26 37.88022     0
```

0.5 Models

We obviously meet none of the accepted standards for normal statistical analyses. With more data, I'd love to run some linear mixed-effects models to test for relationships between C~q and abundance (or biomass), temperature, salinity, and a random effect for site. It's not worth running these models now. They won't tell us anything with only 11 samples.

Still, I'll use a simple Spearman rank correlation to assess the relationship between C~q and our covariates for samples taken in the field (this excludes laboratory positive and negative controls).

```
## [1] -0.2823816
```

```
## [1] -0.2823816
```

This is a weak negative correlation. That's a good sign– the number of cycles needed to amplify herring DNA to surpass detection thresholds decreases slightly with increasing catch or biomass.

0.6 Takeaways

- Let's run more data before we share any quantitative analyses. Starting with a full year of either the middle site (2023) or a single replicate (2024) would be useful.

- We can currently share some good signs– we get herring data where we expect to see it, and low quantities of total DNA in our field blanks. This indicates our sterility protocols are working to clean the bottles but NOT destroy DNA with bleach.

- I'd love to run some spatial analyses on 2023. We have some contrast with the Inner-Middle-Outer scheme. I'll have to think about the best methods to do that.