**Project Planning**

**Sampling Strategy**

Full latitudinal sampling will be pushed off till Summer 2024. This summer, we’ll focus on obtaining three sets of samples (early season, peak temperatures, and end of season) for five sites (Key Largo, Chesapeake Bay, Long Island Sound, Maine, and Shediac Bay). These three samples will help us decide when to sample next year by helping characterize:

* Allele frequencies at the beginning of the season (for populations that are not present year round: CT, ME, and SB). This may help address where the animals that re-start the population each year come from (large, admixed shelf population, egg bank, etc.).
* How allele frequencies change after the warmest time of year.
* How allele frequencies change after water temperatures drop and (assumed) selection by high temperature is relaxed.

Proposed sampling details are included below. Early samples are based on either past work (the Key Largo sample), how quickly I can arrange travel (for the Chesapeake and LIS sites), and the emergence of Acartia tonsa (the Maine and Shediac sites). Peak sample dates are based on when each site reaches maximum temperatures (occurs slightly earlier at lower latitudes), and aim to catch the population just before water temperatures begin to decrease. Late samples aim to catch the population just before it drops out of the community (LIS, Maine, and Shediac), or after water temperatures drop substantially (Key Largo and Chesapeake).

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| --- | --- | --- | --- | --- |
| **Site** | **Location** | **Early Sample** | **Peak Sample** | **Late Sample** |
| Key Largo | Steamboat Creek | Late Feb. | Early August | Late November |
| Chesapeake Bay | **‘S lineage’ sampling sites from Plough et al. 2018** | ASAP (early July) | Late August | Early November |
| Long Island Sound | Esker Point Beach | ASAP (early July) | Very Early September | Late October |
| Maine | Machias? Sandy Point Bridge? **Darling Marine Center?** | **Late July/Early August?** | Early September | **Early October?** |
| Shediac Bay | Shediac Bridge | **Mid-July?** | Early September | Late September |

Key uncertainties are represented in bold text. Foremost among them is figuring out the beginning and end of the season of occurrence in Maine and Shediac Bay. It would also be helpful to check on the best sampling sites within the Chesapeake.

* ~~I will reach out to Jeff Runge at Darling Marine Center about the phenology of~~ *~~Acartia tonsa~~* ~~there.~~
* ~~I will reach out to Louis Plough and Jamie Pierson at Horn Point about whether they have recommendations for timing and location of samples.~~

Other notes:

* This approach would result in large differences in the number of generations between samples for the different sites. We will account for this when examining allele frequency changes (ex - % change per generation).
* If we collect a second Early Season sample in 2024, we would be able to compare the allele frequencies at the beginning of the season with the allele frequencies towards the end of the season this year.

**Phenotyping**

CTmax and body sizes will be measured for field collected animals. Temperature and salinity will be measured at the time of collection, with experimental conditions adjusted to match. Each individual will be preserved in 95% ethanol after the assay is complete. At least 20 individuals will be phenotyped per site during each seasonal sampling event. Based on this number of individuals, we would have a total of 300 copepods phenotyped and preserved by the end of the year (20 individuals, 3 seasons, 5 sites)

**Sequencing**

Low coverage whole genome sequencing using Twist Bio 96-plex library prep and new Illumina NovaSeq X Plus with Novogene. Outlined below is what we’d estimate for the full latitudinal sampling.

DNA extraction

* Would need ~20 ml Ampure beads (overlap with Alison’s project)
* Alternatively, could test out the Charm BioTech approach
  + ~~I will reach out to Charm about test kits, downstream applications, and whether it would work for epigenomics~~
    - Seems like Charm works best for 50 ng inputs at a minimum
    - Tube 50 X 1 preps (EG-920-M) - $78

96-plex library prep

* $10 per sample ($10,000 for the 960 sample kit, but there are 480 sample kits available as well)

NovaSeq X Plus

* $1999 per lane if we use between 8-15 lanes ($20,000 for 10 lanes)
* ~1x coverage for 96 individuals per lane; $30K

For this initial round of sampling, we’d need:

* The 480 sample kit from Twist (~$5000)
* 3 lanes of sequencing (~$6000)