**Casco Bay Aquatic Systems Survey 2023**

**Beach seine, water quality, and eDNA water sampling protocol**

**Motivation**

CBASS is a long-term data collection effort to monitor ecosystem health and community structure within Casco Bay. Data collection commenced in 2014 with more than a dozen sites in the greater Portland area. In 2023, data collection expanded to include 6 sites near Harpswell in partnership with the Quahog Bay Conservancy. Sampling methods have occasionally included acoustics and jigging, but have always included a beach seine for sampling the inshore fish and crustacean community and an eDNA component for identifying and quantifying other organisms less likely to be caught in a seine. This document will outline the standard operating procedures for completing the beach seine, water quality, and eDNA water collection components of CBASS. The motivating force for all protocols is **CREW MEMBER SAFETY.**

**Human Hazards**

Working with equipment out of a boat is an inherently dangerous situation. Make sure to communicate with all crew members, especially whoever is at the helm, when deploying anything out of the boat. Watch out for environmental hazards. Life jackets are required to be worn when working out of GMRI boats. Wear sunscreen and drink plenty of water while working outside.

**Equipment Hazards**

The crew member piloting the boat must be cautious not to tangle the seine in the prop. All crew members must watch the seine while hauling in the catch—make sure it does not catch on any boulders, logs, or other hazards. This can rip it.

**Supplies**

* Knotless seine – Delta mesh – 3/16” sq. mesh, 8 ft long – with bag
* Storage container for seine
* Fish measuring boards with 1mm increments (2-3)
* Common fish species ID booklet (Green binder)
* Pre-printed seine datasheets (1 for each site)
* Pre-printed eDNA sample bottle ID datasheet (1 per day)
* Sampling permit (paper or digital copy)
* Pens, Sharpies, pencils, and clipboard for recording data
* Watch or cellphone for recording time
* Labeled brown plastic sampling bottles for eDNA (3 per site)
* Cooler or tub for holding brown plastic sampling bottles
* 2 unopened store-bought bottles of drinking water (as an eDNA control)
* Boating safety equipment (life jackets, radio, cellphone, flares, horn)
* Gaff or long pole for adjusting seine from inside the boat
* Zip ties, duct tape for field repairs
* Plastic bags for collecting unknown or notable species

**Procedures**

*Pre-departure checklist*

1. Print out data sheets for the sampling event, place inside clipboard with an assortment of pens and pencils.
2. Make sure brown plastic sample bottles are sterilized, labeled 1-x (number of sites times 3), and placed in a clean cooler or sampling tub. Put two unopened store-bought bottles of drinking water in container as controls.
3. Collect all supplies and safely store on boat.
4. Alert a shore contact that you are leaving. Always leave a cruise plan detailing which sites you will visit and when you expect to return.

*On-site data collection*

1. Steam to site coordinates.
2. At approximately 100m from shore, the “outer” eDNA water sample will be collected. Select a brown plastic sample bottle. Record its number on the appropriate row of the eDNA water sample data sheet under the proper site name and outer/middle/in location. Remove the lid, making sure to not touch the inside of the bottle or the underside of the lid. Fill the bottle with surface water and pour out, letting the water wash over the underside of the lid. This is called a field rinse. Fill the bottle once again with surface water, filling as much as possible, and tightly close the lid. Replace the bottle in the tub with all the other sample bottles.
3. At approximately 50m from shore, the “middle” eDNA water sample will be collected. Repeat the procedure for collecting the “outer” sample.
4. At approximately the shoreline, the “inner” eDNA water sample will be collected. Repeat the procedure for collecting the “outer” sample.
5. Record the weather and tidal conditions at the sample site.
6. Once all eDNA water samples have been collected and weather/tidal information have been recorded, the seine will be deployed. The crew member piloting the boat will gently nose into the shoreline. Two crew members will lift the storage container with the seine onto the bow and exit the boat. Do not attempt to lift the seine without a partner, especially if the net is wet—it will be very heavy. One of the shore-based crew members will hold the rope at one end of the seine (this is called the bridle) in place as the boat backs off the shoreline. Once the crew member has a hold of the seine, the deployment start time will be noted.
7. As the boat motors backwards, a crew member will guide the seine out over the bow and gunwales. It is important that the lead line (sinking side) and cork line (floating side) do not cross each other as the net is deployed. This will cause tangles and reduce capture efficiency. In the center of the seine is what is called the “bag,” which is a large pouch where all caught organisms will be concentrated. The bag has two lead lines and two float lines. These should be deployed simultaneously as the boat maneuvers to be parallel to the shoreline. Tangles in the bag can be fixed by boat-based crewmembers after the full length of the net has been deployed. After the bag is deployed, the boat will return to shore. The final path of the boat will be similar to a semi-circle. The second shore-based crew member will take the bridle on the end of the seine and hold it in place. Crew members remaining on the boat will fix any tangles and note any potential hazards to the net.
8. Once the seine is free of tangles, the shore-based crew members will be instructed to haul the net in. Pull from the cork line to avoid lifting the lead line off the bottom, which would allow fish to escape. As the net is pulled, crew members on the boat will assist in lifting the seine over any potential hazards (tree branches, logs, rock ledges, moorings, etc). Do not continue pulling if the net is snagged—this will damage the net.
9. The crew member piloting the boat will anchor and bring equipment (ID booklet, fish measuring boards, data sheets, plastic bags) ashore.
10. Crew members hauling the seine will slowly move towards each other as they haul. Once the edges of the bag are reached, they will lift the entire bag from the lead line and bring it onshore. It is recommended to leave a small section of the bag partly in the water to prevent killing the fish inside.
11. Net contents will be sorted. Assign a scribe to record catch and site information. The remainder of crew members will sift through the net. For each species caught, 25 individuals will be measured to the nearest millimeter. Fish will be measured with total length (nose to caudal fin tips) and crabs will be measured with carapace length (widest part of shell). If more than 25 individuals of a species are present in the bag, the remainder only need to be counted, not measured. Make sure to shake through any debris and seaweed brought up in the net to look for organisms.
12. Unknown organisms can be either extensively photographed and released OR collected in a labeled plastic bag for further ID at the lab. If photography is the preferred method make sure to note identifying features that may not show up in photographs, such as number of dorsal spines.
13. Grass shrimp and hermit crabs do not need to be measured, but their presence should be noted. Hermit crabs are noted as present or absent. Grass shrimp are noted as absent or present with high, medium, or low abundance. Low abundance is 1-10 individuals. Medium abundance is 11-100 individuals. High abundance is more than 100 individuals. You can estimate grass shrimp abundance.
14. Once all organisms have been noted, collect all materials and return to the boat. The scribe will record the substrate conditions at the site and any other notable events pertaining to the seine.
15. Two crew members will retrieve the seine as the boat backs out. One crew member will haul in the cork line and one will haul in the lead line. Fold the net into the storage container so that the lines do not cross, as this will make the next deployment much harder. Try to shake off as much debris and seaweed as possible as you retrieve the seine. Once the seine is fully in the storage container, the two crew members will lift the container and place it back onto the deck of the boat. The scribe will record the time that the seine is stored on the boat as the end of the haul.
16. As the seine is being retrieved, a crew member will use a handheld YSI sonde to collect and record data on water quality (DO, salinity, and temperature) of the surface water.
17. Steam to the next sampling site and repeat the process.

*Cleanup and data storage upon return*

1. Alert your shore contact that you have returned.
2. The seine will be laid out on the lawn to dry. This process takes approximately a day. Do not keep the seine out in the sun much longer than a day, as UV can damage the material.
3. All other equipment will be rinsed with fresh water and stored.
4. Identify any species that were unable to be identified in the field. Make any necessary amendments to the paper data sheets.
5. Scan paper data sheets in as PDFs. Upload these files and any photographs taken to the relevant cloud-based storage platform. Data that is not backed up might as well not exist.
6. Update the yearly digital datasheet with the information collected.
7. Process eDNA water samples within 1 day of collection (use separate eDNA water filtration protocol sheet as a guide).

*Troubleshooting*

* Thousands of fish of the same species
  + Occasionally, a school of thousands of the same species of fish will be caught in the net. Usually these will be small baitfish like herring, silversides, or menhaden. To prevent all these fish from dying, grab handfuls at a time and estimate how many you are holding when counting fish. Speed is key.