Collecting Specimens from Settlement Plates

Goals:

- Examine each of your assigned plates to identify the organisms on the plate
    - Which of these can you readily identify?
    - Some species might require careful examination in dissecting or compound microscopes
- Make an inventory list of each species that you can find on the plate.
- Take a picture with a scale bar of the whole plate.
- Take pictures of each individual organism selected as a voucher specimen or selected for DNA extraction. Be sure to include a scale bar.
- Label your selected organisms using your initials and a consecutive number. For example, RWTx1, RWTx2, RWTx3….

Specimens for DNA Extraction: We need individuals of each species that we can use for DNA extractions. This process will destroy the specimen.

Voucher Specimens: Ideally, we would like to have individuals of each species that we can save for use as voucher specimens in the future. These specimens will potentially be deposited in a museum collection.

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Procedures

1. Locate the plates to be processed along with their metadata and photos.

Note: Plate labels are composed of important information, including the abbreviated site name, replicate block number, plate orientation, plate number, and the date when the block was collected from the field.

- Example of label formatting:

Site - Block# - Plate# - PlateOrientation – DateCollected

Site Label	Site Abbreviation	Block #	Plate #	Plate Orientation	Date Collected
SH1-2U-10-08-2021	SH	1	2	U	10-08-2021
TB2-4D-10-08-2021	TB	2	4	D	10-08-2021

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2. For each plate, take photos of the plate using a phone camera and/or the dissecting scope camera.

- Photos should include the **Site label** and a **ruler** in the background of the photo as a size reference
- Compare your photos to the photos available on the course Google Drive.

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3. Identify the [major] organisms on the plate. These data will be used to build a presence/absence matrix, so be as specific as possible for identifications.

Use the Weiss 1995 guide to key out the organisms (*Marine Animals of Southern New England and New York: Identification Keys to Common Nearshore and Shallow Water Macrofauna*).

Be sure to check for an updated taxonomic name using the World Register of Marine Species.
https://www.marinespecies.org/index.php

Major phyla that you are likely to encounter:

- Annelida https://www.marinespecies.org/aphia.php?p=taxdetails&id=882
- Arthropoda https://www.marinespecies.org/aphia.php?p=taxdetails&id=1065
- Bryozoa https://www.marinespecies.org/aphia.php?p=taxdetails&id=146142
- Cnidaria https://www.marinespecies.org/aphia.php?p=taxdetails&id=1267
- Mollusca https://www.marinespecies.org/aphia.php?p=taxdetails&id=51
- Porifera https://www.marinespecies.org/aphia.php?p=taxdetails&id=558

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4. Select the most common organisms for individual-based DNA sequencing and voucher preservation.

- Sample at most 5 individuals of a single species from a single plate for DNA sequencing.

- Take photos of each individual organism using the dissecting scope camera. Include a ruler in the background for scale. *Note: If you are not able to include the ruler in the background, write down the microscope magnification that you are using.*

- Label your selected organisms using your initials and a consecutive number. For example, RWTx1, RWTx2, RWTx3…. would be your specimen ID numbers if your initials were RWT.

- Re-name your photographs to include important metadata, including the site, year collected, and specimen ID number. For example, “RWTx3_IMG1234”

- Place specimens for DNA extraction into 1.5 mL sterile microcentrifuge tubes containing 95% ethanol. Label the tube with the specimen ID number.

- Larger voucher specimens can be placed into 50 mL conical tubes, again with 95% ethanol. Be sure to label the tube with the specimen ID number. Also create a label using pencil on waterproof paper; place this label inside the 50 mL tube.

- Small voucher specimens should also receive a label inside their 1.5 mL tube.

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5. Quality Control

Once you have finished identifying and selecting vouchers plus samples for DNA extraction, re-check the plate to see if other organisms might have been missed.

- Enter your data into a spreadsheet for specimen inventory.
- Make sure that all your specimens are represented on your inventory list.
- Make sure that all your specimens are clearly labeled for future use. Do not use the same label codes for different specimens. For example, do not use RWTx1 for all the first specimens you found in different plates.

6. Be sure to return your settlement plates to 95% ethanol.

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