



## Standard Operating Procedure

|   |                      |                             |                 |
|---|----------------------|-----------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br>01/01/24 | Page 1 of<br>16 |
|---|----------------------|-----------------------------|-----------------|

| Revision History |                |   |
|------------------|----------------|---|
| Version No.      | Effective Date | Description                               |
| 1.0              | 01/01/2024     | <i>Original composition by M. Kachmar</i> |
|                  |                |   |
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**Procedure Owners:**

**Date:**

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**Approved By:**

**Date:**

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Lisa Milke, EAD Chief

## Standard Operating Procedure

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 2 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

### **1 Purpose**

The purpose of this SOP is to provide concise guidance and methodology on how to sample quadrats for intertidal oyster population surveys to determine density and size class distribution.

### **2 Scope**

This SOP is pertaining to the EPA Long Island Sound Study funded Oyster Health project where quadrats will be used to quantify live oyster densities, mortality ratios, and size distributions as part of biannual oyster bed surveys included in the project efforts. For subtidal oyster beds, divers will conduct the quadrat excavations and transport them to a shore team for measurements.

### **3 Definitions/Acronyms**

spat = recently settled juvenile oyster

### **4 Safety Precautions**

All survey team members will wear appropriate clothing dependent on weather conditions including but not limited to waders, rubber boots or protective footwear, gloves, hats, sunglasses, long sleeve shirts, and pants. Thick protective gloves (e.g garden gloves) should be worn when handling oysters. Team members will wash hands thoroughly after each sampling day. A first aid kit will be present for any injury. Extra water will be provided to avoid dehydration or heat stroke. Team members will take regular breaks when needed.

Exercise weather-appropriate field safety measures by monitoring conditions before and during the trip. Do not perform fieldwork during dangerous conditions (e.g. lightning, extreme winds, extreme floods). Do not visit field sites alone (use buddy system). Inform PIs of dates and times of fieldwork. Confirm safe return to the lab to PIs or Project Leads. At intertidal sites, perform procedures during low tide. At subtidal sites, divers are to follow NOAA diving regulations according to the instructions of the lab diving coordinator (Barry Smith).

### **5 Supplies/Materials**

- Field Backpack (See Appendix A)
- Folding chair and tables
- Gray bins
- Permits and permission letters
- Spare data sheets (on waterproof paper)

## Standard Operating Procedure

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 3 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

- Waterproof paper
- Mechanical pencils
- Ruler
- Copy of survey instructions
- First aid kit
- Povidone Iodine scrub
- Hand lotion
- Hand sanitizer
- Bug spray
- Sunscreen
- Specimen collection bags
- Laminated SOPs
- Cooler with ice
- Buckets (x8)
- 2-3 Quadrats, 25 x 25 cm
- Field Gloves
- Calipers (plastic for back up)
- Tool box
- Waders
- Boots
- Scrubbing brushes
- Shucking knives
- Tally counters
- Gray trays (x6)
- Towel
- Kneeling pads
- Diving:
  - Towable dive flag
  - Diver collection bags (x2)
  - Personal Flotation Device (PFD) for row boat
  - Wire cutters on lanyard
  - Large rubber bands
  - Dive tanks
  - AED
  - Oxygen supply

## 6 Equipment

- Field tablet (charged)
- Tablet charger and spare battery

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 4 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

- Digital bluetooth calipers

## 7 Quality Control

All team members will be trained to complete all field tasks, including training on data entry requirements for each specific task. To ensure completeness, [field notebooks](#) will include a checklist of all data that needs to be recorded during each visit. All datasheets will be screenshotted as back up in the event data is lost before connecting to the network.

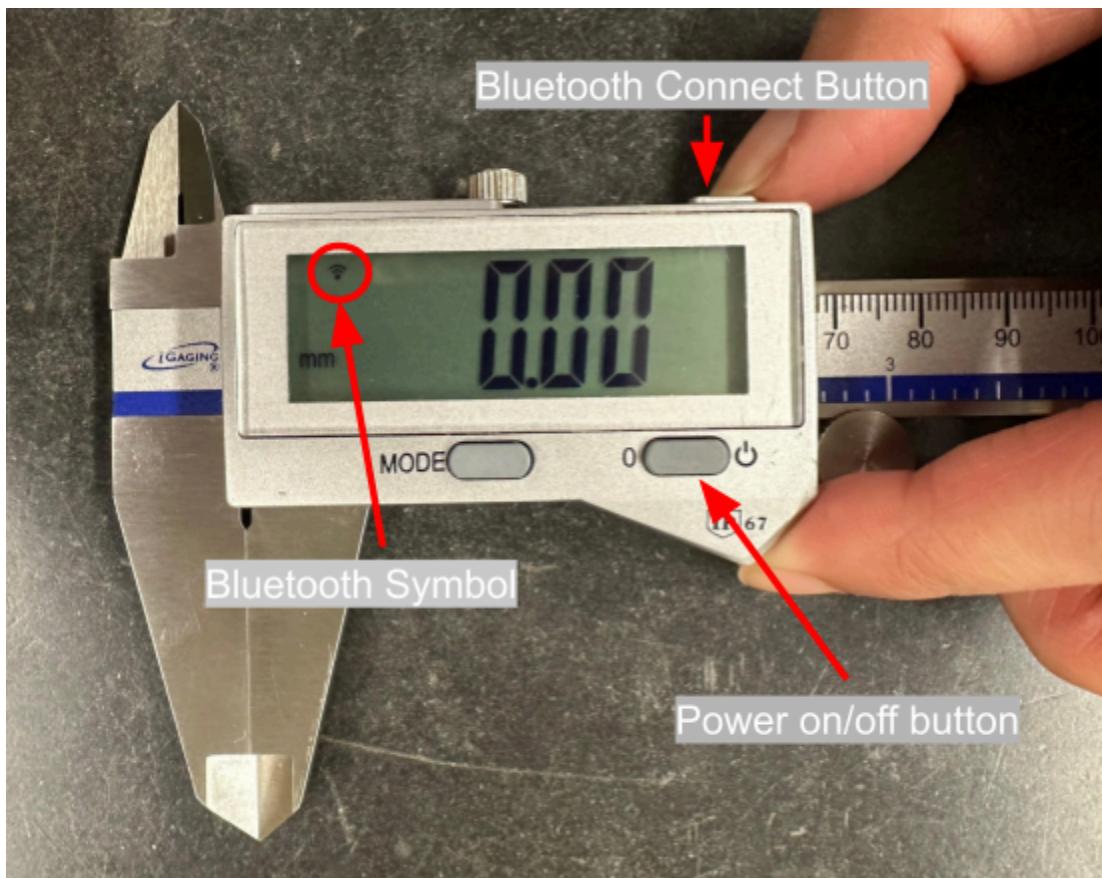
## 8 Preparation

1. At the lab:
  - a. Pre-label all collection bags and sorting bins or buckets. Collections bags should have the site name. Sorting bins and buckets should be labeled for the number quadrat (ex: 1-4).
  - b. Load the field vehicle with all equipment and supplies necessary to conduct the surveys using the checklist.
  - c. Make sure all team members have appropriate clothing and gear to proceed with the day (both diving and on land).
  - d. Assign team members tasks for the survey such as excavators and measurers.
  - e. Prepare the [data sheets](#) by ensuring the proper site labels, dates, and number of quadrats is updated.
2. On Site:
  - a. Before starting the survey, a measuring station should be set up. This includes setting up a folding table with all supplies and bins needed to sort and measure oysters. Each station should include two pre-labeled gray bins, a bluetooth digital caliper, a tablet, a bucket of seawater, and field gloves of appropriate size.
  - b. Make sure that all calipers are working and connected to the designated tablets. The calipers are labeled #1 and #2 and are matched with a like-numbered tablet.
    - i. Enter the bluetooth settings on the tablet.
    - ii. Turn the caliper on (Figure 1). Hold the top right button in until there is a blinking bluetooth symbol in the top left corner of the screen.
    - iii. The caliper name should appear in the devices list in the bluetooth connection settings.
    - iv. Click on the appropriate caliper device (Caliper 1 or Caliper 2).

## Standard Operating Procedure

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 5 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

- v. The caliper should now be connected to the tablet and ready for use. To check connection, go into the appropriate datasheet and do a few test measurements before beginning the survey.
- vi. **Note that both calipers will have trouble connecting to their designated tablet if within close proximity. Try connecting a few feet away from each other**



**Figure 1:** Image of digital bluetooth enabled calipers. Arrows point to both the power on/off and bluetooth connect buttons as well as the bluetooth symbol. The bluetooth symbol flashes when searching for an enabled device and will appear static when connected.

## Standard Operating Procedure

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 6 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

### 9 Procedures

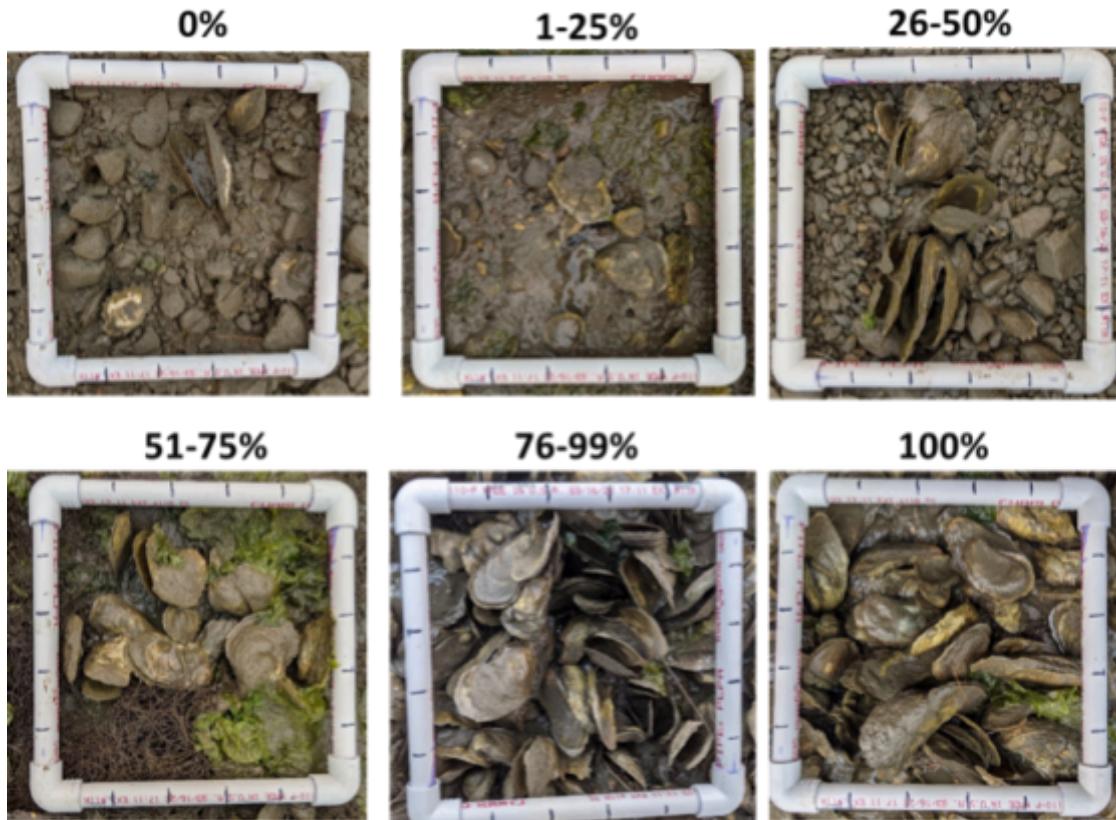
*Note: techniques for quadrat collection may vary slightly between subtidal and intertidal sites. Variations will be specified in instructions.*

1. Randomly lay the 25cm x 25cm quadrat within the target zone on the bed (See Appendix B for maps of reef locations). At subtidal locations, 3-4 quadrats will be taken from the oyster bed (Janiak, 2021). To account for the greater variation in reef composition and structure at intertidal locations, 5-6 quadrats will be collected.
  - a. All quadrats should be either weighted or have holes drilled to allow water to fill and sink the quadrat. All quadrats for subtidal sites will be weighted.
2. Percent coverage:
  - a. Estimate percent coverage within the quadrat's inner perimeter (inside of the quadrat square). This will be done for each quadrat.
    - i. Coverage includes all live, gapers, and box oysters, but not random loose half shells that may have washed up (Table 1).
    - ii. Coverage options are: 26-50%, 51-75%, 76-99%, and 100%. At these sites, if a quadrat has 0-25% coverage, then it is too far into the mud flat or bottom substrate and off of the oyster bed. Examples are shown in Figure 2.
  - b. Record percent cover in the appropriate quadrat datasheet.

**Table 1.** Description of oyster categories that will be assessed for population demographics.  
Source: Tarnowski, 2021.

| Oyster category | Description  |
|-----------------|--|
| Live            | Live oyster  |
| Gaper           | Dead or moribund oyster with gaping valves and tissue still present. |
| Box             | Pairs of empty shells joined together by their hinge ligaments.      |

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 7 of<br>16 |
|---|----------------------|------------------------------------|-----------------|



**Figure 2:** Examples of estimated oyster coverage.

3. Excavating the quadrat:
  - a. Wearing protective gloves, excavate all oysters (Live, Gapers, and Box; see below) from inside the quadrat. (Figure 3 - Left Image) ***Oyster clumps should be preserved as well as possible to avoid killing animals and disrupting the physical structure.***
  - b. If  $\geq 50\%$  of the oyster is within the inner perimeter of the quadrat frame, it is counted as “in the quadrat.” If  $< 50\%$  of the oyster is outside of the inner quadrat perimeter (shaded green in Figure 4) it is counted as “out of the quadrat.”
    - i. There will be difficult calls from time to time (eg. the question mark in the bottom right quadrat of Figure 4). The quadrats do not lay perfectly and oysters are not flat on the bottom. In these cases it is

## Standard Operating Procedure

|   |                      |                                    |                 |
|---|----------------------|------------------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 8 of<br>16 |
|---|----------------------|------------------------------------|-----------------|

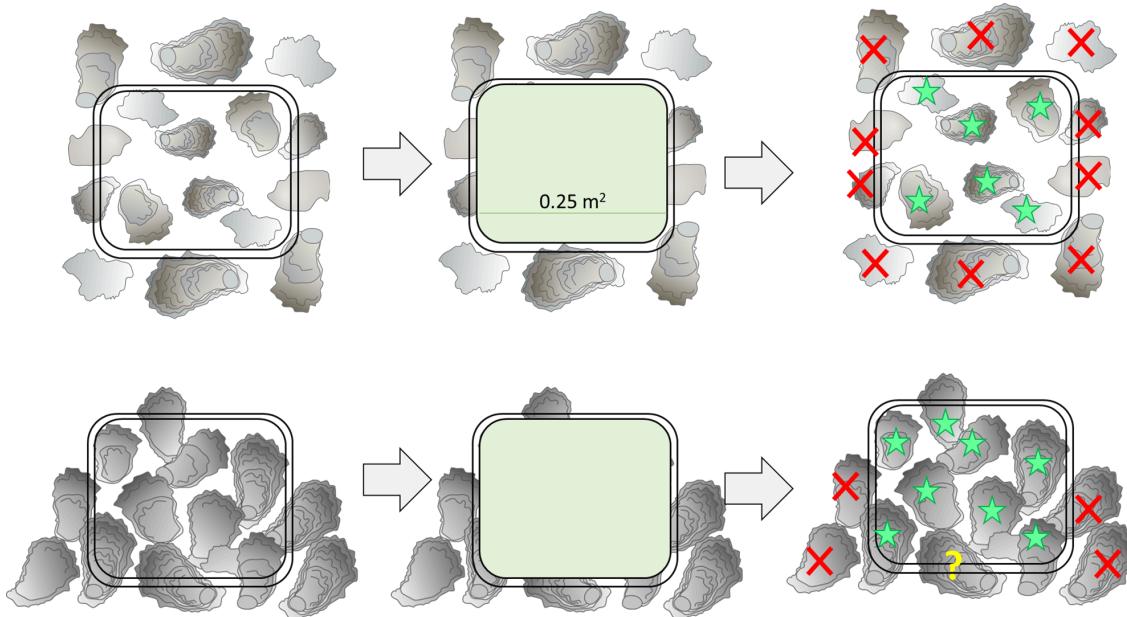
important to have confidence in your best judgment and remain consistent in your calls among quadrats.

- c. All oysters counted as “in the quadrat” should be moved to a labeled bucket or gray bin for height measurements (Figure 3 - Right Image). Divers will have varying colored collection bags that are pre-labeled for specific quadrats to fill with excavated oysters. These bags will be brought to shore for the measuring team to sort.
- d. Take the bucket or bin to the measuring station for designated team members to begin processing. Divers will bring collection bags to shore.



**Figure 3:** Left image - Excavation of a quadrat. Right image - Excavated oysters in gray bins for sorting while measuring. All oysters begin in the first bin and are moved to the 2nd bin as they are counted and measured.

|   |                      |                             |                 |
|---|----------------------|-----------------------------|-----------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br>01/01/24 | Page 9 of<br>16 |
|---|----------------------|-----------------------------|-----------------|



**Figure 4:** Examples of oysters to be considered inside (green stars) and outside (red X's) of the quadrat. Only the inner perimeter of the frame counts as inside the quadrat (green shaded box).

#### 4. Determining Survival:

- The survival status of all excavated oysters should be recorded alongside the shell measurements.
- Mud oysters can sometimes appear as though they are live oysters. In particularly muddy regions, shaking the oyster clumps in a bucket of seawater can help to open mud oysters and dislodge the mud suctioning them closed
- Oysters can be classified as Live, Gaper, or Box (Table 1; Figure 5).
  - Live oysters will remain fully closed when removed from the water and during handling.
  - Boxes are fully open with no tissue remaining inside the shell.
  - Gapers are open with partial or all tissue remaining (in some cases only the adductor muscle will remain) indicating a recent mortality.

|   |                      |                             |                  |
|---|----------------------|-----------------------------|------------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br>01/01/24 | Page 10 of<br>16 |
|---|----------------------|-----------------------------|------------------|

- d. Only oysters where both shells are articulated at the hinge should be counted. Disarticulated single shells are not included in the survival counts and shell measurements.



**Figure 5:** Examples of box and live oysters. Live oysters will remain fully closed when handled, boxes are fully open with no tissue remaining inside the shell, and gapers are open with partial or all tissue remaining.

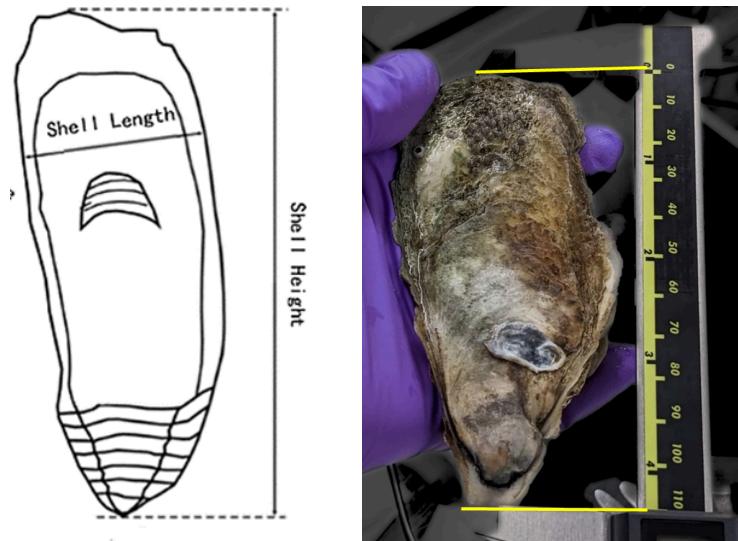
## 5. Shell Measurements

- Using a bucket of seawater, rinse oyster clumps to remove all mud from oysters prior to measuring to ensure that no animal is being missed.
- As shown in Figure 6, using the digital bluetooth calipers, measure the shell height of up to 100 oysters in the quadrat. Record shell height in the column reflecting the survival status (Live, Box, Gaper [Example data sheet](#)). Remaining oysters in each quadrat (>100) will be counted, but not measured for each category (Live, Box, Gaper) for proper mortality estimates. *Ensure that each measurement is in the proper column or row in the spreadsheet (Live, Box, Gaper) and the caliper is recording the numbers properly.*
  - Shell height is measured from the hinge tip to the bill in millimeters and to the nearest decimal (Figure 6).

## Standard Operating Procedure

|   |                      |                                    |                  |
|---|----------------------|------------------------------------|------------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 11 of<br>16 |
|---|----------------------|------------------------------------|------------------|

- ii. Oysters of all ages and sizes will be measured including recently settled oyster spat (Figure 8).
    - 1. Recently settled spat are likely to be prevalent during the fall surveys and can be difficult to spot. Take care to check for small oysters (<10mm) and clean the oyster in the bucket of seawater as needed to improve visuals.
  - iii. Other juvenile shellfish can be mistaken for oysters (Figures 7 & 8), so be sure that detailed checks are made during the assessment.
  - iv. Oysters are often heavily clumped together. Try to identify the hinge and bill of each oyster to measure. Calipers will have to be fit into crevices.
6. After measurements are complete, collect 3-4 adult oysters (80mm -120mm) from each quadrat in the pre-labeled collection bag for lab processing. The remaining individuals (to reach a total of 33 oysters) should be randomly selected from across the reef. Sampled oysters should be placed on ice to be brought back to the lab. See [Monthly Sample Collection](#) for more details. Return the remaining animals to the excavated region to reduce disturbance to the reef.



**Figure 6:** Collecting shell measurements with calipers. Schematic showing height and length measurement landmarks (left). Shell height measurement on live oyster (right).

|   |                      |                             |                  |
|---|----------------------|-----------------------------|------------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br>01/01/24 | Page 12 of<br>16 |
|---|----------------------|-----------------------------|------------------|



**Figure 7:**  
Limpets can sometimes be mistaken for juvenile oysters. Yellow circles in the photos indicate limpets on oyster shells.



**Figure 8:** There are often many overlapping juvenile oysters on a single loose shell or live adult oyster. In some cases, they are completely growing on top of each other (eg. pink circle) making it difficult to get good measurements without killing the overlaying oysters to break up the clump.

## Standard Operating Procedure

|   |                      |                                    |                  |
|---|----------------------|------------------------------------|------------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 13 of<br>16 |
|---|----------------------|------------------------------------|------------------|

### 7. Take down & clean up

- a. Ensure that all datasheets are complete. Screenshot the datasheets as backup in the event data is lost before connecting to the network.
- b. Return oysters from excavated quadrats to the oyster bed where they were collected (minus those selected for lab processing). For subtidal sites, divers will return to the general area or the shore team will dump oysters back into the water.
- c. Rinse equipment off with water to remove excess mud to transport back to the lab. Ensure that no animals are residing in the buckets or bins.
- d. Using the checklist, repack equipment into the field vehicle to ensure everything is accounted for and not left behind.
- e. Wash hands or use hand sanitizer after oyster collections. Ensure that no one has any cuts or injuries.
  - i. *At Fence Creek, due to high bacterial load, use the Povidone Iodine scrub to cover your hands/arms and allow it to sit for 1 minute on intact skin or a minimum of 5 minutes (maximum of 10 minutes) on any cuts or wounds. If you are prone to skin irritation with povidone iodine scrub, discontinue/avoid use. Be sure to remove any clothing or jewelry that you do not want to become stained. Rinse thoroughly with water.*
  - ii. *Immediately follow the above procedure for povidone iodine application if a cut is acquired while at any site.*

### 10 Lab return and clean up

1. All equipment should be hosed down with fresh water including bins, buckets, tools, tables, and field clothing.
2. Metal tools (eg. clickers, calipers etc.) should be sprayed with WD-40, with focus on the hinges to prevent rusting.
3. Waders and gloves should be hung upside down to dry.
4. All equipment should be put away in their designated area in the Oyster Health Field gear area.
5. Oyster samples should be immediately taken to Rm 29 to be processed or placed in the fridge.
6. Divers are responsible for their own gear.
7. Make sure all notes have been updated in the appropriate [field notebooks](#).

|   |                      |                                    |               |
|---|----------------------|------------------------------------|---------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 14 of 16 |
|---|----------------------|------------------------------------|---------------|

## 11 Calculating mortality indices (adapted from Ford et al. 2006):

[Example data sheet](#) has the following calculations built in for a quick assessment of the data collected.

$$\text{Total mortality (\%)} = \frac{\text{number of gapers + box / quadrat}}{\text{number of gapers + box + live oysters / quadrat}} \times 100$$

$$\text{Gaper mortality (\%)} = \frac{\text{number of gapers / quadrat}}{\text{number of gapers + live oysters / quadrat}} \times 100$$

$$\# \text{Live oysters per } m^2 = \text{number of live oysters counted/quadrat} \times 16$$

## 12 References

Ford, S.E., Cummings, M.J., Powell, E.N., 2006. Estimating mortality in natural assemblages of oysters. *Estuaries Coast.* 29, 361–374.

<https://doi.org/10.1007/BF02784986>

Janiak, D., 2021. MarineGEO Oyster Reef Habitat Monitoring Protocol. Tennenbaum Marine Observatories Network, MarineGEO, Smithsonian Institution.

Tarnowski, M., 2021. Maryland Oyster Population Status Report: 2021 Fall Survey. Maryland Department of Natural Resources, Annapolis MD.

Whitlock, M.C., Schlüter, D., 2019. *The analysis of biological data*, Third edition. ed. Macmillan Learning, New York.

## 13 Appendices

- A. What goes into the field backpack?
  - a. Tablets
  - b. Digital Calipers
  - c. Extra gloves
  - d. Extra zip ties
  - e. Field notebook
  - f. Permits and permission letters
  - g. Spare data sheets (on waterproof paper)
  - h. Waterproof paper
  - i. Mechanical pencils
  - j. Ruler

|   |                      |                                    |                  |
|---|----------------------|------------------------------------|------------------|
| Title:<br><b>Oyster bed density and size distribution measurements using quadrats</b> | Version Number:<br>1 | Effective Date:<br><b>01/01/24</b> | Page 15 of<br>16 |
|---|----------------------|------------------------------------|------------------|

- k. Copy of survey instructions
- l. First aid kit
- m. Povidone Iodine scrub
- n. Hand lotion
- o. Hand sanitizer
- p. Bug spray
- q. Sunscreen

B. Maps of sites

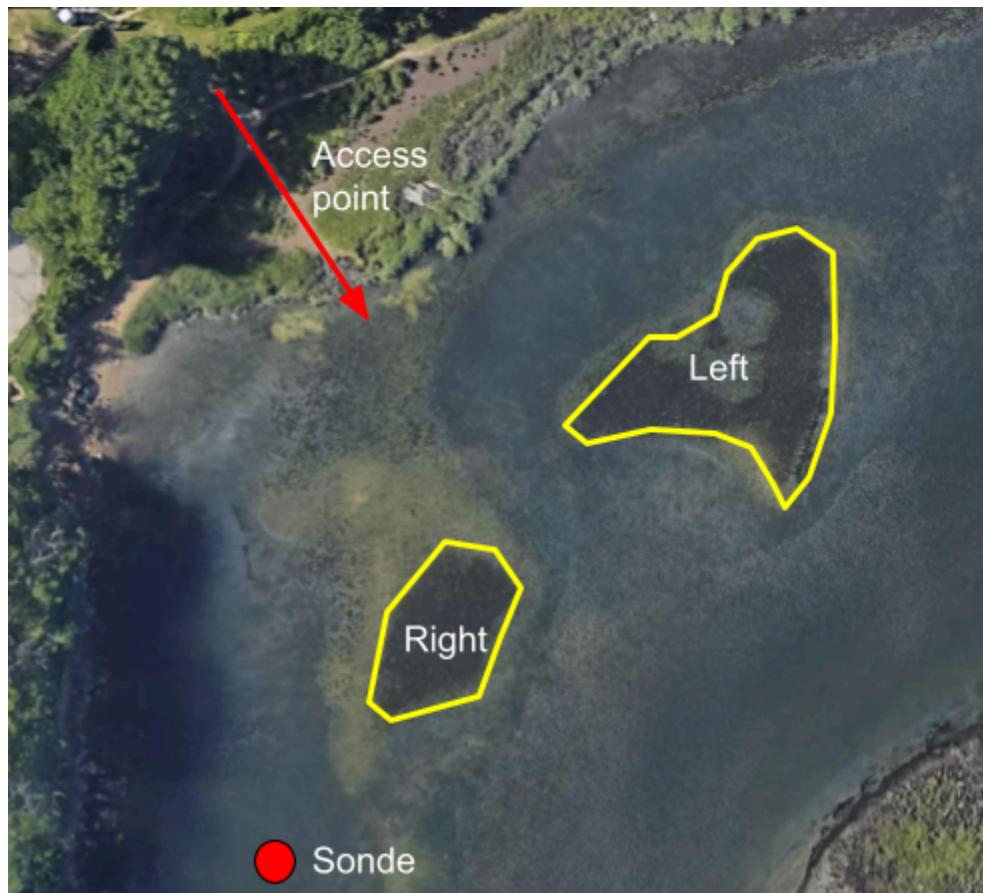


Figure 1: Aerial map of Ash Creek. Yellow perimeter indicates where the oyster bed resides.

## Standard Operating Procedure

|  |                                    |   |                      |
|--|------------------------------------|---|----------------------|
| <b>Title:</b><br><b>Oyster bed density and size distribution measurements using quadrats</b> | <b>Version Number:</b><br><b>1</b> | <b>Effective Date:</b><br><b>01/01/24</b> | <b>Page 16 of 16</b> |
|--|------------------------------------|---|----------------------|



Figure 2: Aerial map of Fence Creek. Yellow perimeter indicating where the oyster bed resides.

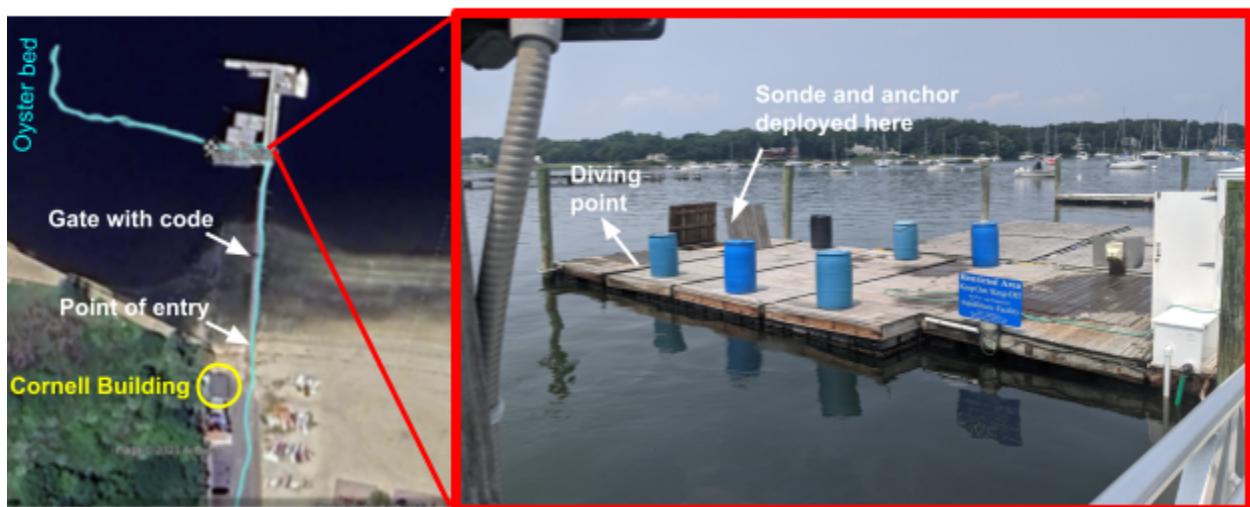


Figure 3: Aerial map of Gold Star Beach oyster bed general location off of the dock.