



## Scientific Standard Operating Procedure

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### Standard Operating Procedure:

*Oyster Recruitment Monitoring using Shell Stingers*

Revision History		
Version No.	Effective Date	Description
1.0	01/01/2024	<i>Original composition</i>

**Procedure Owners:**

**Date:**

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## 1 Purpose

The purpose of this SOP is to provide concise guidance and methodology for deploying and processing shell stringers at intertidal and subtidal oyster beds to monitor oyster recruitment and reproduction.

## 2 Scope

This SOP is pertaining to the EPA Long Island Sound Study funded Oyster Health Project that is incorporating recruitment and reproductive revaluations to understand oyster population health within Long Island Sound.

## 3 Definitions/Acronyms

spat = juvenile oyster (shell length < 40 mm)

## 4 Reagents/Media

REScue, 1:16 dilution in water ([REScue Concentrate](#), 4.25% accelerated hydrogen peroxide)

## 5 Supplies/Equipment

- Drill press
- Magnifier lamp
- Calipers
- Clicker counters
- Scrub brush
- Shucking gloves / protective work gloves
- Weighted galvanized wire
- Washers
- Pliers
- Wire clippers
- Cured oyster shells (6 month minimum)
- PVC
- Rebar

## 6 Safety Precautions

All collection 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 field trips end. A first aid kit will be present for any injury.

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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. 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 [barry.smith@noaa.gov](mailto:barry.smith@noaa.gov)).

Laboratory members will wear safety gear including gloves and goggles when cleaning cured shells in preparation to build stringers and while using the drill press. Members will follow standard laboratory procedures in the event of an accident and all team members will have knowledge of the location of the nearest first-aid kit. All Safety Data Sheets (SDS) for chemicals can be found in the binders on lab cabinet doors.

### 7 Laboratory Quality Control

*Cured shells are sourced from the Fairfield Shell recycling program and will have a minimum of 6 months curing duration on land to ensure there are no spread of disease or invasive species with the shells.* Multiple members of the lab will process samples at the same time to ensure everyone is using the same methods and corrections can be made when necessary. Guidelines for measuring and other steps in the process will be discussed among members prior to starting to ensure uniformity. Duplicate reads of 10% of the shells should be conducted as a quality assurance step and discrepancies discussed while observing the shells. 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. ***Any notes should be recorded in the site specific field notebook and recruitment notes file.***

### 8 Procedures

#### 1. **Prior to field deployment, prepare shell stringers.**

- Each oyster bed site will need 6 shell stringers, containing 6 cured oyster shells per stringer.
- To make shell stringers for one site, obtain 36 cured oyster shells (100–120 mm in height). The shells should be smooth with no spat scars or remnants of biofouling organisms such as barnacles or boring sponge. This will be beneficial when counting spat. If the shell is in good condition,

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- spat scars and biofouling can be removed with a shucking knife to create a smooth surface.
- c. Using a drill press (located in the workshop - Building 3 of the Milford Lab), drill a single hole in the center of the shell. Be sure to wear appropriate protective gear such as gloves and goggles when using the drill press.
    - i. Must have proper training and approval before operating the drill press (*details forthcoming*)
  - d. Once holes have been drilled in all oyster shells (36 total per site), they can be strung (6 per stringer) using weighted galvanized wire and washers (Figure 1). The shells should be oriented with the inner surface (concave) facing down.



**Figure 1:** Depiction of washers and weighted galvanized wire.

- e. Once the shells have been strung they can be attached to a PVC T-bar by looping the wire through pre-drilled holes in the PVC (Figure 2). Loop the galvanized wire through the holes on either side of the T bar and twist many times to securely fasten to the structure. A [NOAA tag](#) should be attached to the stringers with contact information for the Lead Field Scientist.

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**Figure 2:** Example of shell stringer attached to T-bar. (Photo from [Florida Fish and Wildlife Conservation Commission](#))



**Figure 3:** Underwater photograph of oyster shell stringers suspended from a PVC T-bar frame, as used by McFarland et al. (2022).

## 2. Deployment

- a. At each site and each deployment, 6 shell stringers, each containing 6 cured oyster shells, will be deployed for spat collection and quantification.
- b. Stringers will be deployed at three time points within a sample year, in June and August (totaling 18 stringers per site per season).

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- c. The stringers will be deployed along the growing edge of the oyster bed.
- d. Stringers should be suspended at least 5 cm above the seabed on their T-bar frames. This will aid in avoiding predation on settled spat (Figure 3).

**e. Site specific instructions:**

- i. **Intertidal sites (Ash Creek and Fence Creek):** PVC T-bars holding the shell stringers are securely deployed using rebar on the oyster bed. Rebar should be driven deep enough to ensure it will remain secure in the seabed. The PVC will have holes drilled through it to add zip-ties for additional security.
  - 1. At Ash Creek the stringers are deployed on the fringe of the reef on the far side of the left reef bed (Figure 4).
  - 2. At Fence Creek the stringers are deployed at the base of the bridge on the ‘lease’ side (Figure 5).



**Figure 4:** Shell stringer deployment location at Ash Creek, Fairfield, CT.

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**Figure 5:** Shell stringer deployment location at Fence Creek, Madison, CT.

- ii. **Subtidal- Gold Star Beach:** Shell stringers will be deployed on T-bars secured to cement blocks deployed alongside the oyster mounds (Figure 6). Shell stringers will be deployed and retrieved by divers.

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**Figure 6:** Shell stringer structure deployment for Goldstar Beach, Huntington Bay, NY.  
 (Original photo from Pensacola & Perdido Bay Estuary Program)

3. Bimonthly at each site, all shell stringers will be retrieved and new shell stringers deployed for spat collection and quantification.
  - a. ***Therefore, June deployment will be assessed in August and August deployment will be assessed in October.***
    - a. Retrieved shell stringers will be removed from the T-bar by cutting the galvanized wire with wire clippers and strings will be held in a bucket of seawater or in a cooler on ice with their labeled tags for the site until ready to return to the lab. ***Note: Shells should remain in order and on their individual strings with tags until counted in the lab.***
      - i. At the end of the field season, the PVC T-bar from the final deployment should be returned to the lab. The rebar can be left in place for the following season.
    - b. Stringers should be transported to the lab for analysis on ice in the cooler.

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- c. Once the stringers have been returned to the lab, they can be disassembled (being careful to keep the shells in order and replicates separated). Lightly rinse each individual shell with fresh water, and gently scrub to remove any fouling organisms such as algae or mud to increase visibility of settled spat (Figure 7).



**Figure 7:** Depiction of fouled oyster shell stringer ready to be disassembled and cleaned.

- d. Cleaned shells should be placed on a tray with an assigned shell number (#1-6) for each stringer (Figure 8).

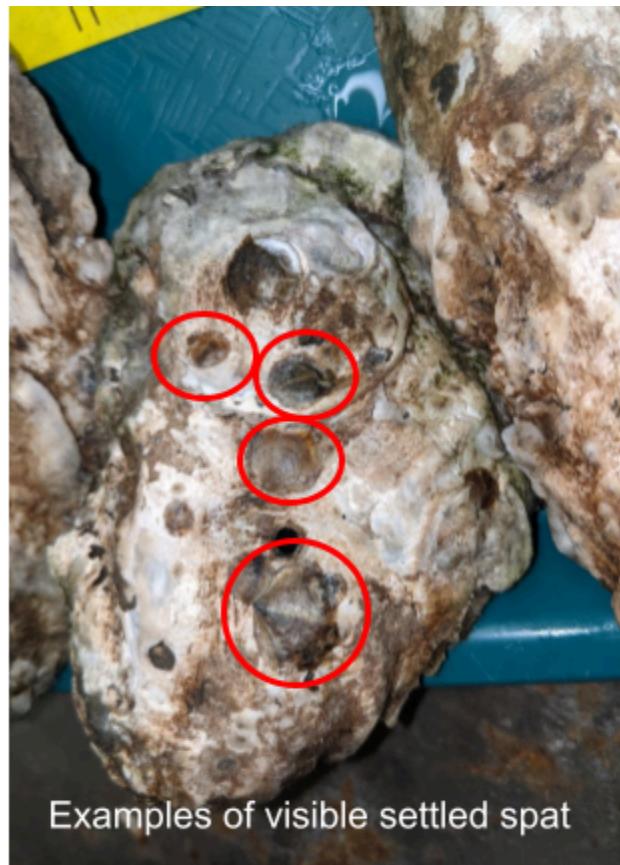
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**Figure 8:** Depiction of shell stringers disassembled, cleaned and placed on a tray with an assigned number.

- e. At the time when recruitment is assessed, oyster spat can be verified under a magnifier lamp. Recruitment will be quantified as the mean number of newly settled oysters per oyster shell (top and bottom), deployment date, and site. Spat counts will be done in duplicate for quality assurance purposes (Figure 9). *Note: There are other species that may look like spat such as slipper snails. See Figure 10 to help identify differences.*
- f. Counts will be tallied using a clicker counter and measurements of spat will be taken using calipers. Data will be recorded in [data sheets](#).
- g. **Any notes should be recorded in the site specific [field notebook](#) and [recruitment notes](#) file.**

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**Figure 9:** Depiction of counting under a magnifier and examples of visibly settled spat on shell.

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**Figure 10:** Depiction of slipper snail juveniles attached to shell stringers and oysters.

## 9 Calculations

$$\text{Top shell count} + \text{Bottom shell count} = \text{Total spat count per shell}$$

$$\frac{\text{Sum of total spat count from 6 individual shells}}{6 \text{ individual shells}} = \text{mean spat count per shell stringer}$$

## 10 Clean up

1. All oyster shells should be gently scrubbed clean of any biofouling, including the spat, and set to dry outside (~24 hrs). These shells will be used in the following season after 6 months of dry storage.
2. All tissue and growth scraped from the shells should be thrown in the trash and removed from the laboratory at the end of the day.
3. All tools and trays should be washed with soap, dried, and put away.

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4. All lab surfaces should be sprayed with disinfectant (REScue, 1:16 dilution in water), allowed to sit for 5 minutes contact time, and wiped down.
5. Inventory of supplies should be taken to prepare for the next sampling period.
6. All laboratory notebooks and data sheets should be updated and checked for quality assurance and control.
7. ***Any notes should be recorded in the site specific field notebook and recruitment notes file.***

## 11 Data Quality Control

Laboratory data will be recorded on data sheets and transcribed to digital data records on the secure NOAA server within 3 business days of the date of collection and processing. This will ensure all notes are correctly updated while the events are fresh in the team's memory. Digital data files will be QC'd by a second individual alongside the data sheets to ensure all data was correctly entered within one week of data entry. Discrepancies in the data will be resolved by comparing data sheets and digital logs alongside lab notebooks that contain site and date specific information that could explain outliers in the data. A record of the lab recorder, person entering the data, and QC completion will be logged to ensure all tasks are completed prior to final data collation. Each month after QC, data will be collated with the previous data and exploratory plots updated. This will serve as an added check for potential error in the data collection and archiving process.

All laboratory data sheets will be scanned and filed on the NOAA server in the project folder. Physical copies will also be filed for archiving at the NOAA Milford Laboratory. Purely digital files (eg. sonde data, photos, etc.) will be uploaded directly to the NOAA server which is backed up regularly.

## 12 References

McFarland, K., Rumbold, D., Loh, A.N., Haynes, L., Tolley, S.G., Gorman, P., Welch, B., Goodman, P., Barnes, T.K., Doering, P.H., Soudant, P., Volety, A.K., 2022. Effects of freshwater release on oyster reef density, reproduction, and disease in a highly modified estuary. Environ Monit Assess 194, 96.  
<https://doi.org/10.1007/s10661-021-09489-x>

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