

Background Information on Oyster Larvae Settlement

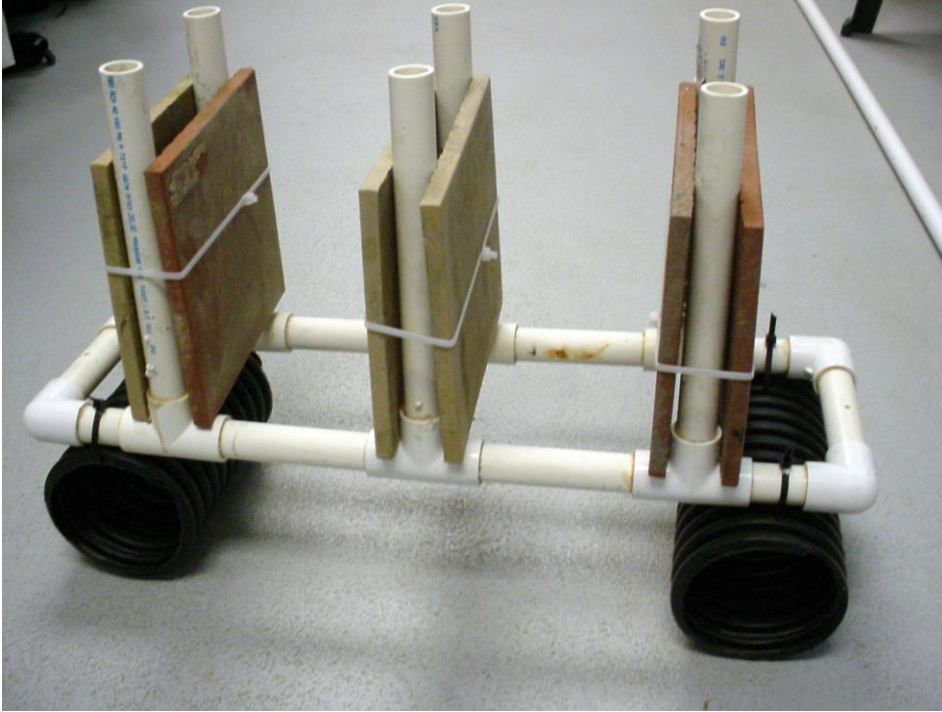
Oysters within an area tend to spawn all at one time, although they may spawn more than once in a given season depending on timing and conditions. Since oysters demonstrate a degree of synchronized spawning and the larvae are only able to settle for a short time it is important to understand how the timing of larval settlement may change from location to location through time. The project that you are participating in is one of the first efforts to coordinate sampling for oyster larvae up and down the NC coast. The key to this project is to get our artificial settlement substrate into the water and keep them in the water year round.

Oyster larvae begin searching for suitable settlement substrate when they reach the “eyed” stage. This occurs a few weeks after the larvae have been spawned. This pigment patch is not a true eye but is sensitive to light so the larvae will remain near the substrate. When the “eyed” larvae encounter a good settlement substrate they secrete a form of biological glue to attach themselves and begin life as an oyster. Larvae are “competent”, or able to settle, for about two weeks. If they have not found a suitable settlement location by this time they will die.

Although many studies have tried to determine exactly what cues the larvae use to settle, it is still not clear. We do know, however, and without any doubt, that they prefer the shells of other oysters. For our study, we are using a ceramic tile for our settlement substrate. The tiles will serve as a good surrogate for actual oyster shell and, because of their uniform surface area, will simplify our sampling and data parameters.

Oyster Spat Monitoring Protocol

I. Spat Rack Assembly (Two spat racks, with six tiles each, deployed per site)



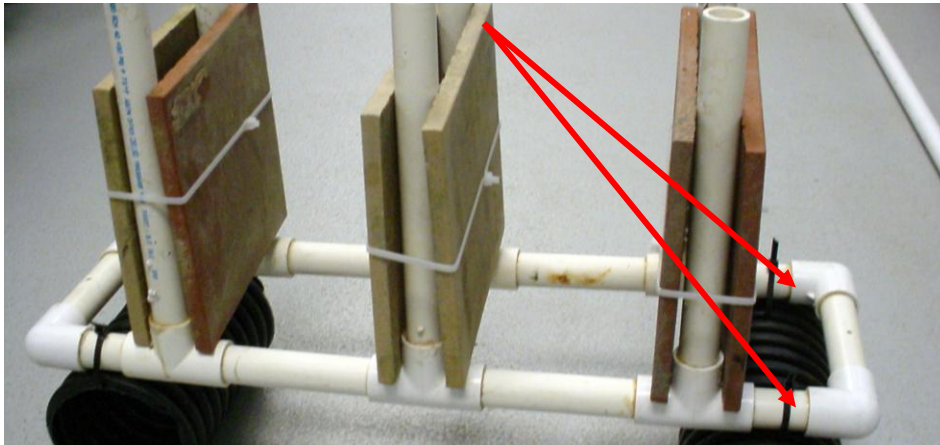
-Assemble the racks as follows:

- 1) Attach two tiles to each pair of posts, smooth side (SIDE A) facing out (i.e. away from posts), rough side (SIDE B) facing in (i.e. facing each other, touching posts).
- 2) Attach two 14" plastic cable/zip ties together to make a loop.
- 3) While holding two tiles in place against the posts, pull the looped cable ties over the posts and around the tiles (to the approximate middle of the tiles).
- 4) Pull the ends of cable ties until tight.
- 5) Cut excess cable tie ends with scissors or wire cutters.
- 6) To secure tiles firmly, pull cut ends of cable ties with pliers (tiles can slide off if rack accidentally flips over).

II. Spat Rack Deployment (Racks will be deployed at mid-intertidal position, between high tide line and low tide line, but *always* below the marsh grass; if your area is subtidal, or only intermittently exposed, use your best judgment to determine mid-tide.)

A. Method One- Hang racks from dock

- 1) You will need three pieces of rope for each rack- two approximately three feet long and one long enough to secure rack to dock (length varies by site).
- 2) For EACH piece of short rope: tie to the two corners of the end of the rack (as shown in by arrows).



- 3) Pull two pieces of short rope together (forms two triangles that meet in center of rack). Tie together with long piece.
- 4) Lower rack until sitting on mud/sand. Secure long line to dock with about one foot of slack line to keep rack upright if wave action, boat wakes, etc.

B. Method Two- Place racks on sandflat

- 1) Wait until low tide when sandflat is exposed.
- 2) Set spat racks on mud/sand.
- 3) Secure racks by pushing “J” shaped rebar anchors into the sand, hooking curved end of rebar over the pvc at the end of the rack (one piece of rebar per side of rack).

Note: CHECK RACKS ON MUD/SAND FLAT EVERY WEEK TO ENSURE THAT SPAT RACKS REMAIN UPRIGHT.

III. Spat Rack Rotation (for each rack, six weeks in, two weeks out, six weeks in, two weeks out and so on for the duration of the monitoring project, with a two week lag between rack #1 and rack #2)

- 1) Put spat rack #1 in the water (will remain in water for SIX weeks).
- 2) **After spat rack #1 has been in water FOUR weeks, put spat rack #2 in water.**
- 3) Remove spat rack #1 from water at SIX weeks from deployment date.
- 4) Record data for tiles from rack #1 (SEE *DATA COLLECTION*).
- 5) Clean tiles (SEE SECTION V on procedure) from spat rack #1 and wait two weeks until redeployment (rack will now be named spat rack #3).
- 6) Leave spat rack #2 in water for SIX weeks.
- 7) Remove spat rack #2 at SIX weeks from deployment date.
- 8) Record data for tiles from rack #2 (SEE *DATA COLLECTION*).
- 9) Clean tiles (SECTION V) from spat rack #2 and wait two weeks until redeployment (rack will now be named spat rack #4).
- 10) Repeat.

IV. Data Collection

1) Record air temperature, water temperature and salinity measurements at least two times per week; RECORD DATE AND TIME OF DAY whenever temperatures and salinity are recorded (we can determine tides with this information). PLEASE USE PENCIL ONLY.

Note: Thermometer must remain submerged for at least 3 minutes to get an accurate water temperature and should be read immediately after pulling out of water (even a light breeze will drive temperature reading down).

Note: Hydrometer must be rinsed with FRESH water BEFORE AND AFTER use.

2) Remove tiles from rack (cut cable ties) on designated date and record DATE and TIME then record organisms PER TILE NUMBER (1, 2, 3, 4, 5, 6) AND SIDE (A=smooth side, B=rough side), for example:

1A: live oysters 4, oyster scars 1, live barnacles 50

1B: mussels 20, sea squirts present

2A:and so on

3) Record *number of each* found of the following:

- Live oyster spat
- Oyster spat scars
- Live barnacles
- Dead barnacles
- Barnacle scars
- Slipper shells
- Mussels (do not have to identify which species)
- Atlantic oyster drills
- Boonea (gastropod/snail)

4) Record *whether present* (no count necessary) of the following:

- Worm tubes
- Hydrozoans
- Bryozoans
- Sea squirts
- Sponges

Note: It is likely you may find some things that are difficult to identify or not included on this list. If this happens, call us with a description or send a digital photo and describe in your field notebook.

5) Record any general comments/observations on conditions around the site (high boat traffic, storms, etc.).

6) Record rainfall amounts if you have a rain gauge

V. Cleaning Tiles for re-use

1) After counting/recording organisms on tiles, use a putty knife (or something similar) to scrape off any animals/algae (best for smooth side). Can also use a hard fingernail brush or any stiff brush (best for rough side).

2) If you are not able to remove barnacle scars, barnacles, oyster spat, oyster spat scars or worm tubes with scraping/brushing, pour 4 cups distilled white vinegar into a 3 gallon bucket (does not have to be this exact size- any standard bucket should be fine), fill with water, put tiles in and let sit for one week (or until you are ready to put them back in the water for the next six week rotation- remember: six in, two out, and so on).

3- Take out and rinse off and scrape/brush again- tiles should be clean and ready to put back on rack for the next rotation!

IMPORTANT!!! Anytime you make any observation or write any data in your field notebooks PLEASE record the **DATE AND TIME** and name of the person(s) who took the observation.

Note: Your observations are very important to the project, so while we want you to be concise in your comments it is very important that you make any observations that you consider significant. These comments will be used to help evaluate the data.