Chapter 11

Florida Fish and Wildlife Conservation Commission
Fish and Wildlife Research Institute
Oyster Monitoring Procedures

In:
Oyster Integrated Mapping and Monitoring Program
Report for the State of Florida No. 2

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Chapter 11

Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute Oyster Monitoring Procedures

Introduction

The Florida Fish and Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute (FWRI) has routinely monitored oysters in estuaries across Florida since 2005 (Fig. 11.1). While monitoring stations and parameters have varied over time and among estuaries, FWRI monitoring represents the most widespread and comprehensive oyster monitoring in Florida. The methods described in this chapter include the monitoring procedures in most common practice by the Molluscan Fisheries Research Group at FWRI in many of the estuaries in Fig. 11.1. They are provided here as a resource for other monitoring efforts.

This document provides instructions for field monitoring, construction of monitoring equipment such as spat trees and quadrats, and related laboratory analyses. While monitoring and laboratory procedures are written following the International System of units, construction instructions and materials are given in United States Cus-

Figure 11.1. Locations and years of FWRI oyster monitoring in Florida estuaries.

tomary units, reflecting the dimensions of construction materials and equipment most commonly available in the United States.

Contents of this chapter include monitoring procedures for water quality, oyster size and density, oyster spat settlement, disease and reproduction, condition index, shell pests, growth and mortality, and shell budget. Several of these monitoring procedures involve the measurement of shell height, length, or width (Fig. 11.2). These measurements are defined as follows:

- Shell height: Maximum distance from the umbo to the opposing edge of the ventral margin
- Shell length: Maximum distance from shell ventral and dorsal margins parallel to hinge axis
- Shell width: Maximum distance between the outside surfaces of closed valves, measured at a right angle to the plane of closure of the valves

Many of the monitoring procedures described in this document include the collection of live oysters for laboratory analysis. Practitioners should investigate state and local regulations regarding shellfish collection and acquire necessary permissions prior to collection.

Water quality

Overview

Water-quality parameters (depth, temperature, salinity, pH, dissolved oxygen, and turbidity or Secchi depth) are recorded during each monitoring activity described below. They are measured using a calibrated water-quality sonde and data recorder (e.g., Fig. 11.3) near the oyster reef. Secchi depth is measured using a Secchi disk (Tyler 1968, Preisendorfer 1986, Bowers et al. 2020).

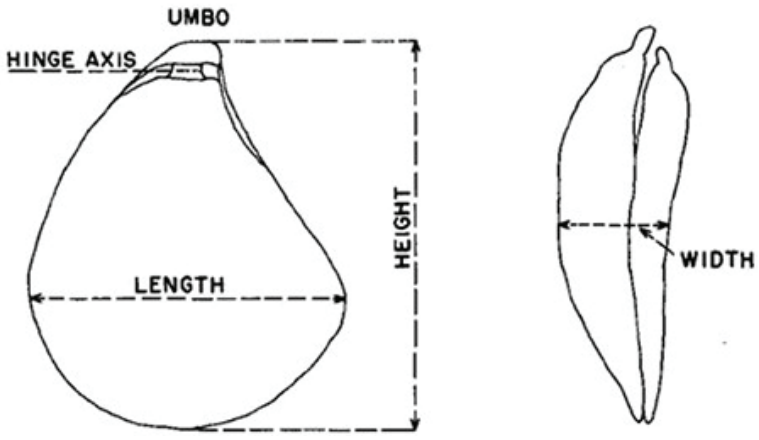

Figure 11.2. Diagram of the locations for measurement of shell height, shell length, and shell width. Figure from Galtstoff (1964).

Figure 11.3. Recording water-quality data.

Water quality field procedure

Materials needed for field monitoring

- Calibrated sonde instrument capable of measuring temperature, salinity, pH, and dissolved oxygen
- Secchi disk on a line
- Metric measuring tape
- Meter stick or sounding line
- Field book and pencils
- Optional: Water-sampling bottles or turbidity probe

Field monitoring procedure

1. At each station, measure water depth using a meter stick or sounding line and measure water-quality parameters using a sonde instrument and a Secchi disk. Record the time, depth, temperature, salinity, pH, dissolved oxygen, and Secchi depth in the field book. For shallow stations near intertidal reefs, a subsurface measurement using the sonde is sufficient. At stations deeper than 2 m, measure water quality at the top and bottom of the water column. For very deep stations, if desired, take mid-column measurements, or measurements every meter from surface to bottom, to assess whether the water column is stratified. While instantaneous water-quality measurements are commonly recorded once the sonde has stabilized, options to record an integrated signal over a period of time can also be explored.

2. Calculate water clarity as a Secchi penetration value. This is calculated as the percentage of the water column through which the Secchi disk could be seen. Be aware that

in shallow stations the sediment may be disturbed by the approach and anchoring of the boat, therefore sediment should be given time to settle before collecting Secchi measurements.

3. As an alternative to Secchi depth, and for a more quantitative measurement in shallow water, water samples can be collected for analysis of total suspended solids (TSS). TSS can be measured by filtering a known volume of water through a pre-weighed filter, washing the filter to remove salts, then weighing the filter again after drying (e.g., APHA 2007). Alternatively, TSS can be measured by absorbed or scattered light with a calibrated turbidity meter.

Oyster size and density

Overview

Oyster size and density surveys are conducted at least twice per year, once in spring and once in fall, to capture the beginning and end of the spawning season. Additional surveys can supplement this monitoring, as winter and summer monitoring adds to the understanding of temporal variability. Preseason surveys (conducted as close as possible to the opening of fishing season), midseason surveys, and postseason surveys (conducted as soon as possible after a zone is closed to harvest) can aid in monitoring of harvested stocks.

The oyster size and density monitoring methodology described here is a modification of procedures used by Lenihan and Peterson (1998) and by Grizzle et al. (2005). The number of sampling stations (i.e., reefs) may vary between estuaries and within an estuary depending on the monitoring program. In the FWRI monitoring procedure, there are commonly three or more sampling stations in each estuary of interest (Parker et al. 2013). Quadrat size and replication have varied; earlier monitoring (2005–2007) used 10 replicate 1-m^2 quadrats per reef (e.g., Parker et al. 2013), and later efforts (2008 onward) used 15 replicate 0.25-m^2 quadrats (e.g., Parker 2015). The appropriate number of quadrats and sampling stations vary with studies' monitoring objectives (see Baggett et al. 2014 for further information on sample-size determination); methods presented here call for 15 replicate 0.25-m^2 quadrats. Quadrats are designed with lead weights and holes for air venting to allow the quadrat to sink in water. They also include a float on a rope to help locate the quadrat in deep or murky water.

In brief, field monitoring includes counting the number of all live oysters and dead oysters with articulated shells (known as boxes) within each quadrat (Fig. 11.4). Oyster shell heights are measured for live oysters from each quadrat. The number of oysters measured per quadrat has varied from 10–50 in different types of FWRI oyster monitoring. The methods presented here call for the measurement of 50 live oysters per quadrat.

Oyster size and density field procedure for shallow environments

Materials needed for field monitoring

- 0.25-m^2 quadrats (see Inset 1)
- Knee pads (gardening pads)
- Metric rulers (~15 cm)

Figure 11.4. Removing oysters from a 0.25-m^2 quadrat on an intertidal reef (top) and counting and measuring oysters on a boat (bottom).

Inset 1. Construction of quadrats

Quadrat materials (makes 1 quadrat; Fig. 11.5)

- 10-ft length of ¾-inch Schedule 40 PVC pipe
- 4 ¾-inch PVC elbows
- PVC cutters
- PVC primer and PVC cement
- Drill fitted with 5/16-inch drill bit
- 5-m length of twisted 3/8-inch polypropylene rope
- 3½-inch donut float
- 8 lead line weights (5/16-inch diameter, 2 3/8-inch length cylindrical lead weights used in fishing net construction)
- Slip joint pliers

Quadrat construction procedure

1. Use the PVC cutters to cut the PVC pipe into four 50-cm sections.
2. Join the four 50-cm pieces of PVC pipe with the PVC elbows, creating a square. Measure interior sides of the quadrat to verify that each side is 50 cm long when assembled with elbows; trim if needed.
3. Disassemble the quadrat and apply the PVC primer to the interior of an elbow and the end of a 50-cm section of PVC pipe. Wait 10 seconds, then apply the PVC cement to the same surfaces and insert the pipe into the elbow. Repeat the process for all corners to construct a square and let cement dry.
4. Use the drill and the 5/16-inch bit to drill three equally spaced holes through each side of the quadrat.
5. Into the two outer holes on each side, place a lead line weight. Crimp down each side of the weight toward the exterior of the pipe using the pliers. This locks the weights in place so they will not fall out. Leave the center hole open to allow air venting and water drainage.
6. Tie one end of the 5-m rope to a 3½-inch donut float and the other end to one of the sides of the quadrat.

Figure 11.5. 0.25-m^2 quadrat for oyster monitoring.

- Digital scale with precision of ≤ 0.1 kg
- Large volumetric buckets with precision of ≤ 0.25 L
- Small volumetric buckets with precision of ≤ 0.25 L
- Oyster knives
- Field gloves
- 5-gallon buckets (in many situations it is convenient to drill the bucket with numerous holes in the bottom that are large enough to allow drainage, but small enough to retain all shell pieces)
- Data sheets and field books
- Clipboards, pencils, and permanent markers

Field monitoring procedure

1. Select one person to serve as data recorder; one or more other people will process the quadrats.
2. Haphazardly deploy (e.g., throw blindly over shoulder) a quadrat onto the oyster reef at the sampling station. If any shell substrate is encountered, the quadrat is considered to be valid; collect all the surface material within the quadrat. If no substrate is detected, it is assumed the quadrat fell outside the boundary of the reef. Deploy the quadrat to a different location at the station.
3. Using a metric ruler, measure and record the shell heights (mm) of 50 live oysters (spat included) collected from within the quadrat.
4. Count and record the total number of all remaining live oysters (spat included) and all dead oysters with articulated shells within the quadrat.
5. Repeat the process for the remaining 14 quadrats at the station.
6. Variations on field monitoring can include the following additional steps for each quadrat, which include counting oyster predators and measuring the weight and volume of oysters and substrate:

a. Remove all material within the quadrat, place in a drilled 5-gallon bucket, and record the weight of the bucket with oysters and substrate.

b. Pour 10 L of water into a volumetric bucket. Place oysters and substrate into the bucket, and record oyster and substrate volume based on volume of water that is displaced. If material is still exposed to the air, slowly add water until the material is covered. Remove the oysters and substrate, then record the decrease in water volume.

c. Weigh the empty drilled 5-gallon bucket. Subtract bucket weight from the weight of the oysters and substrate in the bucket.

d. Count the oyster drills (*Urosalpinx* spp. and *Stramonita* spp.; Fig. 11.6) in the sample.

e. Return oysters and substrate to the reef.

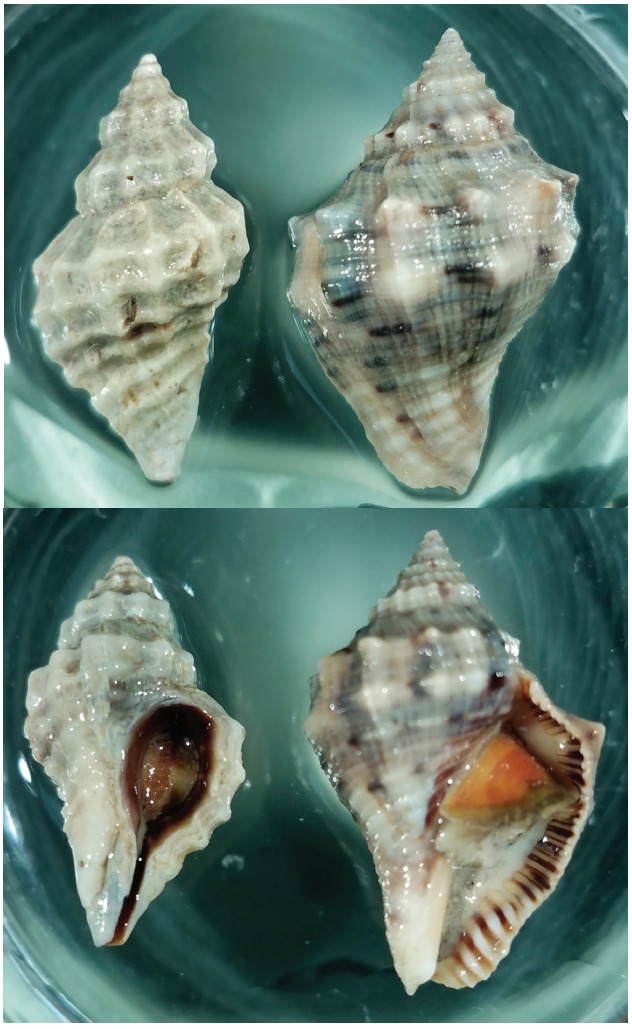

Figure 11.6. Tops and bottoms of *Urosalpinx* sp. (left in each photograph) and *Stramonita* sp. (right in each photograph).

Figure 11.7. Floating buckets are useful in collecting oysters from submerged quadrats.

7. General tips for monitoring in shallow water:

   a. Use knee pads for kneeling on the reef.

   b. Oysters can be collected and placed into 5-gallon buckets and assessed on the boat.

   c. When the reef is submerged, a floating ring can help support the bucket and sample (Fig. 11.7).

   d. Use oyster knives to break up large clumps of oysters to allow for more accurate counts.

   e. When weighing each sample, remember to subtract the weight of the empty bucket or tare the scale with the empty bucket.

8. Complete data analysis of oyster density and shell height (see Inset 2).

Oyster size and density field procedure for deep environments

Materials needed for field monitoring

- 0.25-m^2 quadrats (see Inset 1)
- Metric rulers (~15 cm)
- Digital fishing scale with precision of ≤ 0.1 kg
- Large volumetric buckets with precision of ≤ 0.25 L
- Small volumetric buckets with precision of ≤ 0.25 L
- Oyster knives
- Field gloves
- 5-gallon buckets
- Stainless steel carabiners, at least 5 inches long
- Mesh catch bags with galvanized handles
- Data sheets and field books
- Clipboards, pencils, and permanent markers
- Scuba diving or snorkeling equipment, as appropriate

Inset 2. Size and density data analysis

Calculate the mean shell height of live oysters for each station. For analysis of oyster density, multiply the live and dead oyster counts in each 0.25-m^2 quadrat by 4 to extrapolate the number of oysters found per 1 m^2. Use the extrapolated numbers from the 15 quadrats to calculate the mean live density and standard deviation per station. Divide the total number of dead oysters by the total number of live and dead oysters to calculate the dead:total oysters ratio for each sample. Calculate the mean dead:total oysters ratio per station. Alternatively, these data can be represented as:

% live = (#live / (#live + #dead)) * 100

Field monitoring procedure

1. Knot two loops in each quadrat's rope. Attach one carabiner to each loop, and attach several empty mesh catch bags to one of the carabiners.
2. Divers enter the water on opposite sides of the boat and haphazardly deploy their quadrat.
3. Remove one catch bag from its carabiner and fill it with all the surface material collected from the quadrat.
4. Place the full catch bag on the other carabiner.
5. Move at least 1.5 m (3 quadrat widths) in a haphazard direction and repeat steps 3–4 using a new bag for each new sample.
6. Return to boat, swapping full bags for empty bags until all 15 samples have been collected.
7. On the boat, select one person to serve as data recorder while the others process the samples.
8. Weigh each catch bag containing a sample using the scale, and record weight to the nearest 0.1 kg. Remember to subtract the weight of the empty catch bag.
9. Using volumetric buckets, determine the displacement volume of each sample (see step 6 in previous procedure).
10. Measure and record the shell heights (mm) of 50 of live oysters (spat included) from each sample.
11. Count all remaining live oysters and all dead oysters with articulated shells in the sample.
12. Count all oyster drills (*Stramonita* spp. and *Urosalpinx* spp.) within the sample (Fig. 11.6).
13. Return oysters and substrate to the water and repeat the steps above for all samples.
14. Complete data analysis of oyster density and shell height (see Inset 2).

Oyster spat

Overview

To monitor spat settlement, three spat trees are deployed at each monitoring location and retrieved approximately one month later. Spat monitoring arrays are adapted from Southworth and Mann (2004). T-shaped spat trees with oyster stringers (Fig. 11.8) are used for intertidal oyster reefs, and floating spat arrays with oyster stringers (Fig. 11.9) are used in deeper waters or waters with strong currents. Construction and deployment instructions are included here for oyster stringers, spat trees, and floating spat arrays.

Spat monitoring field procedure

Materials needed for field monitoring

- Clean set of oyster stringers (see Inset 3)
- Spat trees or floating floating spat array materials, as needed (see Inset 3)
- 1-gallon resealable plastic bags
- Oyster knives and paint scrapers or putty knives
- Scrub brushes and scrub pads
- Field books, pencils, and permanent markers

Field monitoring procedure

1. Take the 45 completed spat trees or floating spat arrays and the 90 oyster stringers for deployment at the monitoring stations. Place three spat trees or floating spat arrays (with two stringers each) on each reef.
2. Hang one oyster stringer through the holes on each side of the PVC spat tree or floating spat array. Wrap the loose end of the wire around the wire hanging from the PVC to secure.
3. For spat trees: Select a location where the stringers will rest at an elevation similar to that with the greatest density of oysters on the reef. Deploy by inserting the base of the T into the sediment until the lowest strung oyster shell is approximately 5 cm above the sediment surface.
4. For floating spat arrays: Lower the spat array into the water by the rope. Ensure that the top float remains visible with just enough slack to remain at the surface

Inset 3. Construction of oyster stringers, spat trees, and floating spat arrays

Oyster stringer materials (makes 90 stringers)

- 540 adult-size (5–10 cm shell height) oyster shells
- Oyster knife
- Bleach
- Bucket or tub
- Drill fitted with 1/8-inch drill bit
- Wire cutters
- 2 rolls of 16-gauge galvanized wire
- Needle-nose pliers

Oyster stringer construction procedure

1. Using an oyster knife, scrape off evidence of other organisms (oysters, barnacles, etc.) on the adult-size oyster shells.
2. Soak the oyster shells in a strong bleach solution for at least 2 days.
3. Remove shells from the bleach and rinse well with fresh water. Allow the shells to dry.
4. Fit the drill with the 1/8-inch bit and drill a hole through the center of each shell.
5. Using the wire cutters, cut 90 20-inch lengths of the galvanized wire.
6. Use the needle-nose pliers to create a small pigtail (loop) at one end of the wire to keep the shells from sliding off the end of the wire.
7. String 6 drilled oyster shells onto the wire, their inner surfaces facing down toward the pigtail. After stringing the shells, bend about 3 inches of the top of the wire down and wrap it around itself to keep the shells on the wire. Repeat for the other wires.
8. Place the appropriate number of stringers in boxes for field deployment.

Spat tree materials (makes 45 spat trees; Fig. 11.8)

- 16 10-ft lengths of ¾-inch Schedule 40 PVC pipe
- 45 ¾-inch PVC T-joints
- PVC cutters
- PVC primer and PVC cement
- Drill with a 9/32-inch drill bit
- Wire cutters
- Handheld drill or drill press

Spat tree construction procedure

1. Use the PVC cutters to cut the PVC pipe into 45 30-inch sections and 90 6-inch sections (any leftover pipe can be used for floating spat arrays).
2. Apply the PVC primer to the interior of the T-joint and the end of the 30-inch piece of PVC pipe. Wait 10 seconds, then apply the PVC cement to the same surfaces and insert the pipe into the T joint. In a similar manner, use the primer and cement to bond a 6-inch piece of PVC pipe to each side of the T-joint. You should now have a device resembling a T. Let the cement dry.
3. Fit the drill with the 9/32-inch bit and drill a hole through each of the 6-inch pieces, from which the oyster stringers will be hung. Drill each hole horizontally through the PVC (perpendicular to the 30-inch base of the T), about 1 inch from the end of each piece.
4. Repeat for the other 44 spat trees.

Figure 11.8. Deployed T-shaped spat trees.

Figure 11.9. Floating spat array used for deeper water after retrieval.

Inset 3, continued

Floating spat array materials (makes 45 spat arrays; Fig. 11.9)

- 10 10-ft lengths of ¾-inch Schedule 40 PVC pipe
- 45 ¾-inch PVC T-joints
- PVC cutters
- PVC primer and PVC cement
- 45 half cinder blocks
- 90 6-inch crab floats
- Black paint
- Small paintbrushes
- Spool of twisted 3/8-inch polypropylene rope
- Electric rope cutter
- Electrical tape
- Drill with ½-inch and 9/32-inch drill bits
- Hammer drill with ½-inch masonry drill bit

Floating spat array construction procedure

1. With the PVC cutters, cut the PVC pipe into 45 6-inch sections and 90 8-inch sections.
2. Fit the drill with the ½-inch bit and drill a hole through the top center of the T-joint (so that a rope can pass through the T-joint and into the 6-inch PVC pipe, which forms the base of the T; Fig. 11.9).
3. Use the PVC primer and PVC cement to bond the center of the PVC T-joint to the 6-inch piece of PVC. Bond an 8-inch piece of PVC to each side of the T-joint. You should now have a device resembling a short T.
4. Fit the drill with the 9/32-inch bit and drill holes through the 2 8-inch pieces. Drill a hole horizontally through each piece (perpendicular to the base of the T), about 1 inch from the end.
5. Fit the hammer drill with a ½-inch masonry bit and drill a hole completely through the center of one side of a half cinder block. The block will serve as an anchor for the floating spat array.
6. Complete steps 2–5 for the other short T devices and half cinder blocks.
7. Use an electric rope cutter to cut the black polypropylene rope into 45 5-m sections. For ease in storing the rope sections, coil each one and bind it with electrical tape. At the end of the piece of tape used, fold a small piece of the tape onto itself to create a tab that will allow for later removal of the tape.
8. String the half of the crab floats on several lengths of polypropylene rope. Use a small paintbrush to paint the agency initials onto the crab floats in black paint. Hang up the strings of floats to dry.
9. Steps 9–11 can be completed in the field to conserve deck space on the boat. Untape a length of rope, then thread it through the drilled hole in the half cinder block. Tie a figure-eight knot on the end of the rope inside the half cinder block to keep it from slipping through the hole.
10. Thread a short T device onto the rope so that the bottom of the base rests on the top side of the half cinder block (Fig. 11.9). Tie an overhand knot in the rope just above the short T device to keep it near the half cinder block.
11. Thread an unpainted 6-inch crab float onto the rope and tie an overhand knot just above it so that it will stay near the top of the short T device. This float will be underwater when the device is deployed and will help keep the short T device upright.
12. Thread a second 6-inch crab float, painted with the agency initials, onto the rope and finally secure the rope with a figure-eight knot at the end. This float will be at the surface and can be used to locate and retrieve the device.

Figure 11.10. Recently collected spat stringers (left) and shells set out for processing (right) in the laboratory.

of the water during high tide. Avoid loops or extra floating line at the surface. The rope length may need to be cut for some stations.

5. Collect the deployed oyster stringers every month (Fig. 11.10). Wrap the wire from the oyster stringers from the same spat tree/floating spat array around each other, then place the three pairs of stringers from the replicate spat trees from each station into a labeled plastic bag and take them back to the lab for spat counts (FWRI does not differentiate between the three replicate spat trees, provided all three sets of stringers from a station are kept together).
6. Clean the spat tree, scraping/scrubbing away any epibionts or algae on the PVC.
7. Attach new stringers to the spat trees and redeploy.
8. In the field book, note how many stringers were retrieved and how many were deployed at each station. Store stringers in the refrigerator or freezer upon return from the field until processing.

Spat monitoring laboratory procedure

Materials needed for spat counting and cleaning

- Magnification lamps or dissecting microscope
- Large plastic trays
- Forceps and probes
- Masking tape
- Oyster knives and scrub brushes
- Bleach
- Buckets or tubs
- Wire cutters
- 2 rolls of 16-gauge galvanized wire
- Needle-nose pliers
- Data sheets, pencils, permanent markers

Spat counting and cleaning laboratory procedure

1. Determine oyster spat settlement by counting the number of spat on the underside and topside of strung shells with the aid of a magnification lamp or dissecting microscope (Figs. 11.10 and 11.11). Examine each shell systematically with the aid of a probe or forceps. Keep track of the location of the strung shells on each of the two stringers per spat tree (Stringer 1: top shell = 1, next shell = 2, … bottom shell = 6; Stringer 2: top shell = 7, next shell = 8, … bottom shell = 12).
2. Record the numbers of spat on each shell on a data sheet. When all the shells on a stringer have been examined, prepare stringers for independent confirmation of spat count by another person by replacing the shells on the stringer in their original order, bending the end of the wire, and labeling each stringer with a numbered piece of tape.
3. After two people have independently counted and confirmed the number of spat on each shell, complete spat data analysis (see Inset 4).
4. Place all the processed shells in a tub of bleach water for 48 hours.
5. Rinse, then scrub the shells and remove all spat and other fouling organisms. Rinse and set out to dry for 24–48 hrs.
6. Cut more galvanized wire and restring shells for the next month's deployment.
7. Shells, spat trees, and floating spat arrays can be reused each month as long as they remain in good condition. Spat trees should be replaced at least once per year.

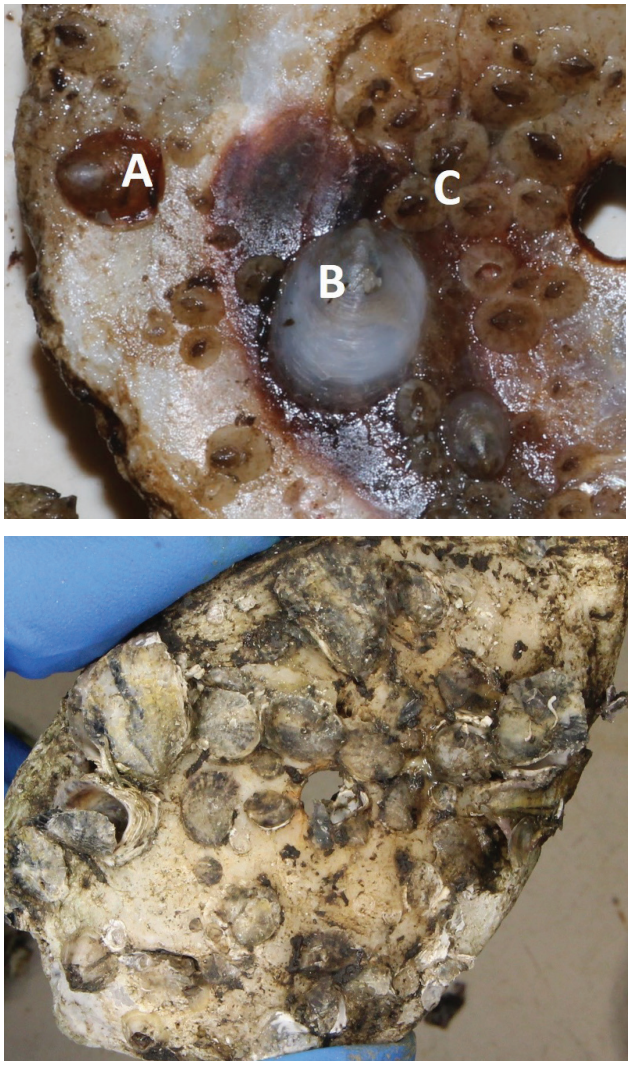

Figure 11.11. Oyster spat arrays may accumulate organisms other than oysters (top photograph) such as jingle shells (A), slipper shells (B), and barnacles (C). Organisms should not be confused with oyster spat (bottom).

Inset 4: Spat data analysis

Juvenile oyster recruitment is determined using the number of settled spat on the underside of the middle 4 shells on each stringer (Southworth and Mann 2004). Calculate recruitment rates by dividing the raw number of spat per shell by the number of days the shell had been deployed, and then standardize to a 28-day month. Use these standardized values to calculate the mean number of spat per shell per month for each successfully retrieved spat monitoring array, resulting in a maximum of 3 data points per station each month.

Disease and reproduction

Overview

To evaluate the reproductive state of oysters and the prevalence of the disease dermo (caused by *Perkinsus marinus*), five oysters at each station are collected and transported live to the laboratory for analysis. The oysters are dissected, and tissues are removed for microscopic analyses of reproduction and disease. In January 2017, FWRI simplified the qualitative 0–10 sex and reproductive stage classification it had been using (modified from Fisher et al. 1996) and implemented a simplified classification (described below) comprising just four stages.

Ray's fluid thioglycollate media (RFTM) method is used to diagnose prevalence and intensity of dermo (Ray 1966). In this method, sections of mantle and gill tissue are incubated in tubes with RFTM, antibiotics, and antifungals for seven days. Tissues are then placed on glass slides, macerated, stained, and examined under a microscope for the presence of *P. marinus* hypnospores. Procedures for the preparation of chemicals needed for reproduction and disease analysis are included in the laboratory procedure below.

Disease and reproduction field procedure

Materials needed for disease and reproduction field work

- Oyster knives
- Field gloves
- 1-gallon resealable plastic bags
- Cooler with ice
- Field books, pencils, and permanent markers
- Scuba diving or snorkeling equipment, as appropriate

Disease and reproduction field procedure

1. At each station, collect 5 oysters (plus 1 or 2 extra because some oysters that initially appear alive may be dead and filled with mud) and separate them into single oysters. Clean off all fouling organisms, place the oysters in a labeled resealable plastic bag, and put them in a cooler. If there are not enough live oysters at a station (or if there are none), collect more at another station to meet the required number for the site quota.
2. In the field book, note how many oysters are collected at each station.
3. Transport oysters live and chilled to the laboratory for processing.

Disease and reproduction chemical preparation

General materials for chemical preparation

- Stirring hot plate
- Stir bar
- Weighing boats
- Spoon
- Scale with precision of ≤ 0.001 g
- Funnel
- Laboratory gloves and goggles
- Graduated cylinders of assorted sizes
- Beakers and flasks of assorted sizes
- Deionized (DI) water

Materials for RFTM preparation

- Thioglycollate
- NaCl (crystalline/biological, certified)
- Thermal gloves
- Thermal-resistant surface
- Volumetric pipette
- 15-mL polypropylene centrifuge tubes (see Table 11.1 for number)
- 6 autoclave-safe tube racks
- Autoclave

Materials for preparing antibiotics and Nystatin

- Penicillin (broad-spectrum gram-positive antibiotic)
- Streptomycin (broad-spectrum gram-negative antibiotic)
- Nystatin (antifungal)
- 50-mL conical centrifuge tubes
- 15-mL polypropylene conical centrifuge tubes

Materials for preparing Dietrich's solution

- Very fine sieve
- 20-L polypropylene carboy with spigot
- Ethanol (95%)
- Formalin (37%)
- Glacial acetic acid
- Aquarium salt
- Fume hood

Important: Use appropriate personal protective equipment (gloves, goggles, etc.) for all chemical preparations and perform them under a fume hood.

RFTM preparation procedure

1. Into a flask, add the amounts of thioglycollate medium, NaCl, and DI water sufficient for the required number of 15-mL centrifuge tubes (see Table 11.1).
2. Add the stir bar to the flask and place on the stirring hot plate. Set the stir plate temperature to 550 °C and increase the rotation of the stir bar to the point at which it maintains a vortex in the solution.
3. Bring the contents to a boil, constantly stirring with the stir bar, and ensure that the thioglycolate and NaCl are fully dissolved. As the contents warm, the liquid will sequentially change color from purple to red to yellow.
4. Once the contents have turned yellow, the RFTM should be noticeably boiling. Boil for approximately 1 more minute. Then, wearing thermal gloves, remove the flask from the stir plate. Place the flask on a thermal-resistant surface to cool.
5. Once the liquid has cooled to room temperature, the color will change back to reddish purple. Transfer the liquid to a beaker and use a volumetric pipette to dispense 9.5 mL into 15-mL centrifuge tubes labeled with *RFTM* and the date prepared.
6. Loosely cap the tubes, place them in a test tube rack, and autoclave them at 121 °C for 15 minutes. A piece of autoclave tape can be placed on each test tube rack to ensure that the tubes reached the appropriate temperature for sterilization.
7. Once the tubes cool to room temperature, tighten the caps. Store in a cool, dark place. Sterilized RFTM will last approximately one month. If the media becomes cloudy, do not use for dermo dissections, as this is a sign of contamination.

Table 11.1. Supplies needed for preparing RFTM for analysis of various numbers of samples.

Number of 15-mL centrifuge tubes	Thioglycollate (g)	NaCl (g)	DI water (mL)
15	4.9	3.3	161.7
30	9.7	6.7	323.3
45	14.6	10	485.0
60	19.5	13.3	646.7
75	24.3	16.7	808.3
90	29.2	20	970.0
105	34.1	23.3	1,131.7
120	38.9	26.7	1,293.3
135	43.8	30	1,455.0
150	48.7	33.3	1,616.7

Antibiotics and Nystatin preparation procedure

1. Weigh out 0.315 g of penicillin and 6.65 g of streptomycin.
2. Add these dry ingredients to 500 mL of DI water in a 500-mL beaker.
3. Place a stir bar in the beaker and place on a stir plate. Mix thoroughly.
4. Label 50-mL conical centrifuge tubes with *Antibiotics* and 15 ml tubes with *Nystatin*. Label all tubes with the date prepared.
5. Dispense 50 mL of antibiotics into the conical centrifuge tubes and store in a freezer.
6. Thaw tubes of antibiotics as needed for dermo dissection. Thawed antibiotics will last for approximately one month.
7. Nystatin suspension comes in a 100-mL bottle. Place 5 mL into labeled 15-mL centrifuge tubes. Freeze tubes and thaw as needed for dermo dissection. Leftover Nystatin can be refrozen and used within one month.
8. The volume of the antibiotic subsample preparation can be adjusted to monthly sample needs.

Dietrich's solution preparation procedure

1. Make sure the 20-L container for Dietrich's solution storage is clean before starting preparation. Do not add new Dietrich's to leftover solution, as precipitate builds up in the container over time. Dispose of any leftover Dietrich's solution as chemical waste.
2. In another container, add 192 g of aquarium salt to 3.6 L of DI water. Mix well to dissolve the salt.
3. Add 6.0 L of DI water to the Dietrich's storage container. Note: Add the following components in order.
4. Add 4.8 L of 95% EtOH to the Dietrich's container. Mix well.
5. Add 320 mL of glacial acetic acid to the Dietrich's container. Mix well.
6. Add 1.6 L of 37% formalin to the Dietrich's container. Mix well.
7. Pour the saltwater mix (3.6 L) through a very fine sieve into the Dietrich's container and discard any particles collected in the sieve. Mix the solution in the Dietrich's container well. Note: If saltwater is added to 95% EtOH a precipitate will form. If the solution was not well mixed and precipitate forms, discard the mixture as chemical waste.
8. Label the container with the date the solution was prepared and store in a chemical cabinet. Dietrich's solution will last approximately two months. If excess precipitate forms at the bottom of the storage container, discard the remaining solution and make a fresh preparation.

Disease and reproduction laboratory procedures

Materials needed for disease and reproduction laboratory procedures

- Bunsen burners
- Oyster knives
- Vernier calipers
- Digital scale with precision of ≤ 0.01 g
- Plastic trays
- Dissection scissors and forceps
- Labeled (station, oyster #, and collection date) 15-mL tubes with RFTM
- 4-ounce glass jars filled approximately half full of Dietrich's solution
- Labels on waterproof paper with station, oyster #, and collection date
- Oysters collected from monitoring stations
- Antibiotic solution, thawed
- Nystatin, thawed
- Volumetric pipettes (50–200 µL and 100–1,000 µL)
- Pipette tips (50–200 µL and 100–1000 µL)
- Tissue cassettes for histology (Fig. 11.12)
- Rinse containers (Fig. 11.12; polypropylene containers with 1/8-inch diameter holes drilled in sides and lids)
- Benchtop orbital shaker
- Paraffin-embedment and slide-mounting equipment (see step 5 of reproduction laboratory procedure below)

Dissection laboratory procedure

1. Clean all fouling organisms from the oysters with a scrub brush and an oyster knife.
2. Measure and record the shell height, length, and width (mm; Fig. 11.2) of each oyster with Vernier calipers.
3. Measure and record the weight (g) of each whole oyster.
4. Place oysters in a labeled pan after they have been measured to keep track of the sample numbers.
5. Open each oyster with a freshly sterilized oyster knife (flamed in the Bunsen burner).
6. Use freshly sterilized dissection scissors and forceps (flamed in the Bunsen burner) to remove a 1-cm^2 piece of gill tissue and a 1-cm^2 piece of mantle tissue. If

possible, these tissues should be sampled from widely separate areas of the oyster. Flame the dissection scissors and forceps between oysters to prevent cross contamination.

7. Place each tissue piece in the appropriately labeled RFTM tube.
8. Add 500 µL of antibiotic solution and 50 µL of Nystatin to each tube. Make sure the Nystatin is well mixed before adding to the tube. The antibiotics and Nystatin should be thawed for a maximum of one month.
9. Place the rest of the tissue into a jar of Dietrich's solution with the appropriate waterproof label. This tissue will be used for reproduction analysis (see below). The tissue should fill no more than ~25% of the volume of the jar, with the rest of the jar filled with preservative to ensure proper fixation.
10. Remove any remaining tissue, then measure and record the wet weight (g) of each shell.
11. Save shell (5–10 cm shell height) as needed for stringer construction. Discard excess shell.
12. Cap the RFTM tubes, put them in a rack and place them in a dark space (RFTM drawer) at 25 °C for 7 days. This tissue will be used for dermo analysis (see below).
13. Label the group of tubes with a piece of tape indicating the date the tissues were placed in incubation.
14. Place the jars with Dietrich's solution and tissues on the benchtop shaker for a minimum of 2 days.

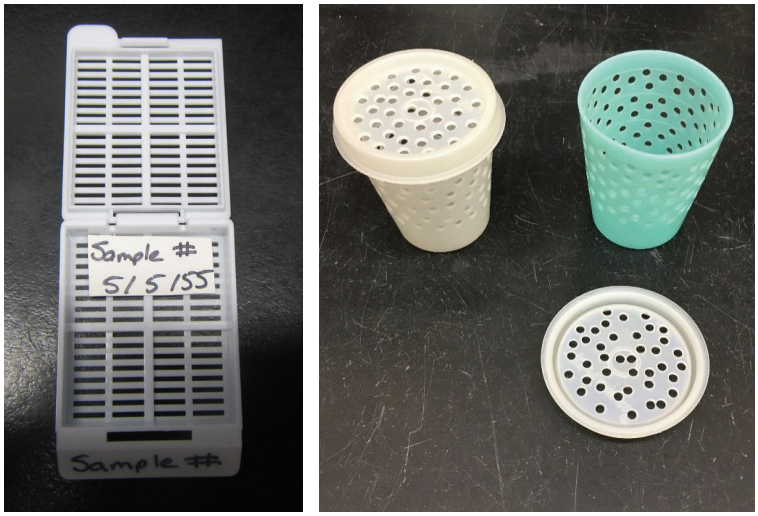

Figure 11.12. Example of cassette used for rinsing and preserving oyster tissue for reproductive analysis (left) and rinse containers for holding remaining tissue (right).

Reproduction laboratory procedure

1. Once fixed for 2 days in Dietrich's solution, rinse each preserved sample in tap water to remove sediment that could cause nicks or tears during sectioning. Take a cross section with a fresh razor blade approximately halfway between the adductor muscle and the anterior margin, including the gonad (see Figure 3-49 in Howard et al. 2004 for illustration of cross-section location). Place cross-sectioned tissue into an appropriately labeled tissue cassette (Fig. 11.12) along with the waterproof label. If the oyster is very small, place the entire cross section in the cassette. If the oyster is very large, remove gill tissue only to allow it to fit.
2. Place the remaining tissue in rinse containers (Fig. 11.12).
3. Rinse the tissues in the cassettes and rinse containers, the jars, and the lids in tap water for a minimum of 2 hours. Leave tap water running at a low rate during the rinse process to remove formalin. Briefly scrub the bottom and sides of jars with a bottle brush to remove any protein buildup.
4. After rinsing, place the cassettes into a labeled plastic jar (station, sample number, date) with 70% ethanol. The leftover tissue may be disposed of after rinsing.
5. Deliver tissues and histology data sheets to a histology laboratory for paraffin embedment and slide mounting (Day 2014). The procedure followed for FWRI samples is summarized here for reference, but procedures and equipment will vary among laboratories. Infiltrate tissue samples into paraffin in a tissue processor and then embed tissues into a paraffin–polyisobutylene tissue-embedding medium. Cut paraffin and tissue sections with a thickness of 4.0 µm on a microtome, and transfer sections to a 40 °C DI-water bath. Mount sections on acid-cleaned glass slides, and dry slides on a slide warmer at 40 °C. To ensure attachment before staining, heat slides in a 70 °C oven for 15 minutes. Stain slides following Weigert's hematoxylin and eosin (H&E) method (Feldman and Wolfe 2014) in a slide autostainer.
6. When the completed slides return from the histology laboratory, let them air dry in a ventilated space for 24 hours.

7. Examine slides at a minimum of 40× on a compound microscope. Examine all the reproductive tissue in the cross section. Three technicians should read and stage each slide independently.
8. Determine sex (male, female, or male/female) and assign each sample to a reproductive stage (from 1 to 4) following the classification scheme shown in Table 11.2 and Figures 11.13–11.17. Record the presence of the flatworm *Bucephalus polymorphus* and any other parasites. Record *Z* for sex if a) the oyster is in reproductive stage 4 (see Table 11.2 and Fig. 11.16) or if b) slide quality is poor or no reproductive tissues are present in the sample. Record *Z* for stage if a) the oyster is infected with *B. polymorphus* (Fig. 11.17), b) the oyster has both male and female gametes (i.e., it is hermaphroditic, Fig. 11.17), or c) slide quality is poor or no reproductive tissues are present in the sample. See published literature for further information on identifying reproductive structures and stages in oysters (Coe 1932, Galtsoff 1964, Eble and Scro 1996, Lango-Reynoso et al. 2000, Rodríguez-Jaramillo et al. 2008).
9. The results from all three technicians should be compared to determine the final sex and stage for each slide. If all three readings agree, the sex and stage are final. If any readings are different, re-examine the slide as a group and record the group consensus on the data sheet.

Materials needed for reading disease slides

- Bunsen burner or ethanol for sterilization
- Forceps
- Razor blades
- Glass slides
- Lugol's iodine working solution
- Cover slips
- Compound microscope capable of 40–50× and 100× magnification

Disease laboratory procedure

1. After the 7-day incubation period in RFTM, use freshly sterilized forceps to remove the tissues from each tube and place on clean and labeled glass slides. Place mantle to the left and gill to the right.
2. Use a freshly sterilized razor blade to macerate the tissue.
3. Add 1 or 2 drops of Lugol's iodine working solution to the macerated tissue.
4. Add a cover slip and gently press to smash the tissue. Place prepared slide on a plastic slide tray.
5. Rinse the forceps and razor blades with 70% ethanol after each oyster to prevent cross contamination.
6. Examine each slide at a minimum of 40× for the presence of blue-black spheres (Fig. 11.18). Spheres will be perfectly round and large (about 50 μm in diameter).
7. Count and categorize the spheres (cells); count mantle and gill tissue from the same oyster separately. Categorize and record infection intensity according to the Mackin scale (Table 11.3; Mackin 1962).
8. Calculate mean infection intensity for each oyster as the average infection intensity of mantle and gill tissues. Calculate infection prevalence as the percentage of oysters infected per station, regardless of infection level.

Table 11.2. Qualitative reproductive-staging criteria for oysters, *Crassostrea virginica*, collected from Florida waters. See Figures 11.13–11.17 for reference. Record the letter *Z* for reproductive stage if the oyster is infected with *Bucephalus polymorphus*, the oyster is hermaphroditic, the slide is of poor quality, or the slide lacks reproductive tissue.

Stage	Observations
1	**Developing:** Gametogenesis has begun, with immature gametes located on the follicle walls. May have mature gametes.
2	**Ripe/Spawning:** Follicles distended and full of ripe gametes. Ova compact/sperm with visible tails (light pink). No immature gametes on follicle walls. Active spawning has begun. Fewer than 2/3 of gametes are depleted.
3	**Spent/Recycling:** Spawning has progressed to the point that most gametes have evacuated from the follicles. More than 2/3 of gametes are depleted.
4	**Indifferent**: Immature or postspawning. Gonads devoid of gametes. Cytolysis ongoing. Sex cannot be determined (record *Z* for sex).

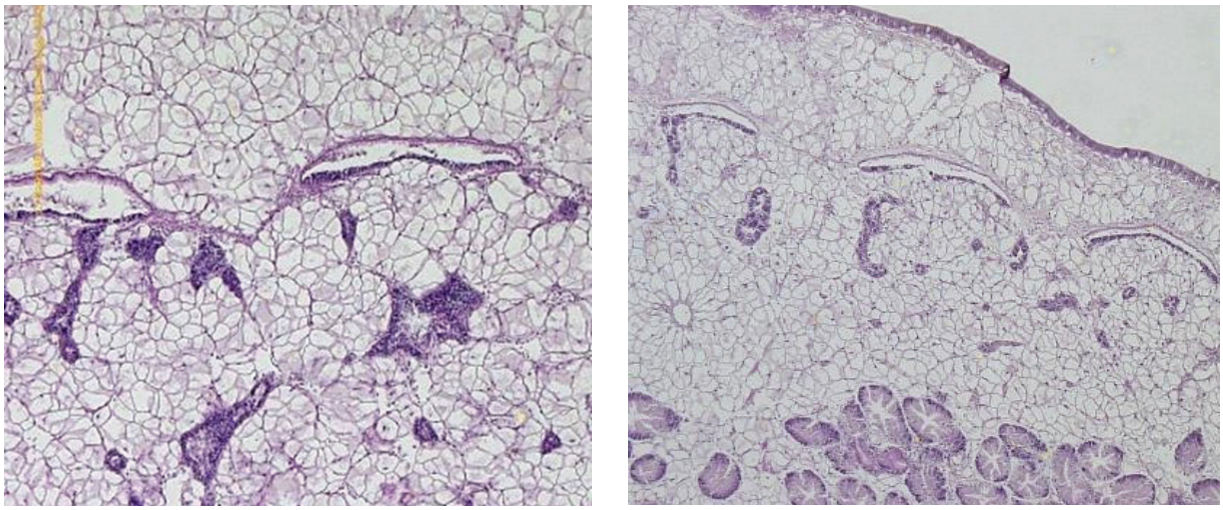

Figure 11.13. Stage 1 male (left) and female (right) oyster tissues at 50× magnification. Maturing gametes line the follicle walls.

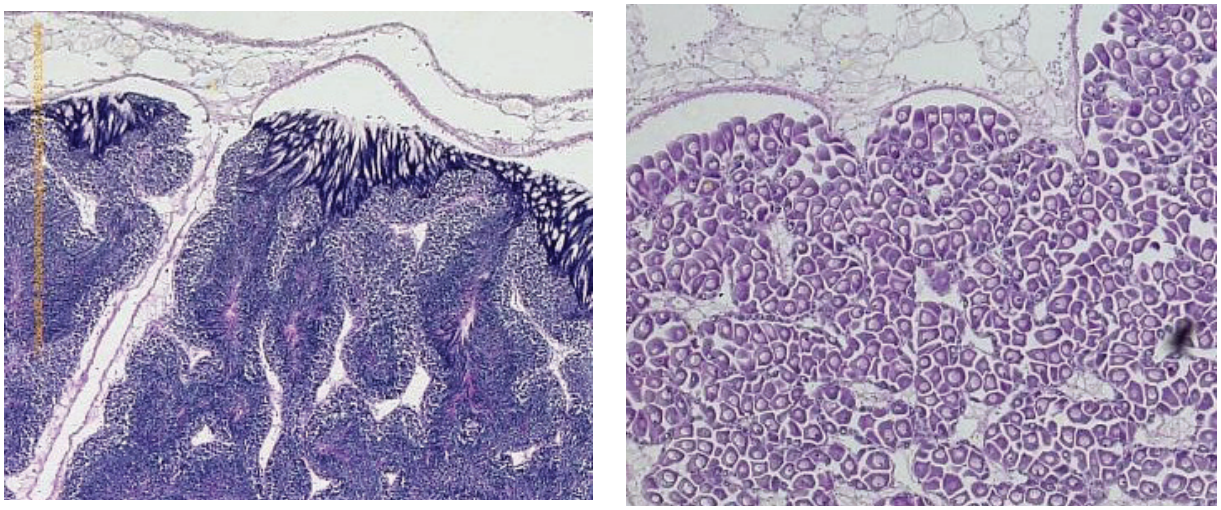

Figure 11.14. Stage 2 male (left) and female (right) oyster tissues at 50× magnification. Follicles are filled with mature gametes, and mature gametes in the gonoducts indicate that active spawning has begun.

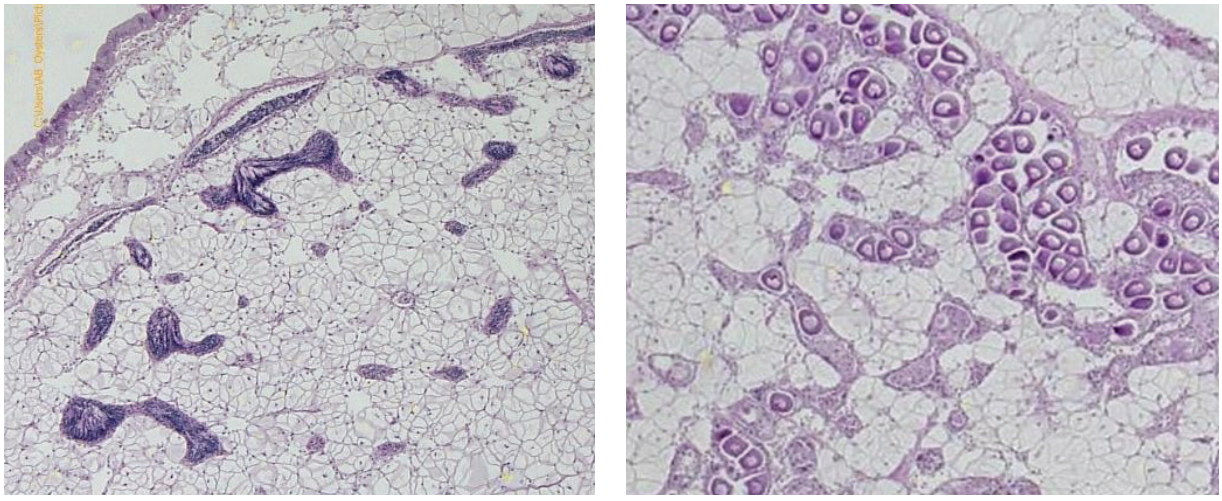

Figure 11.15. Stage 3 male (left) and female (right) oyster tissue at 50× magnification. More than 2/3 of mature gametes have been evacuated from the follicles.

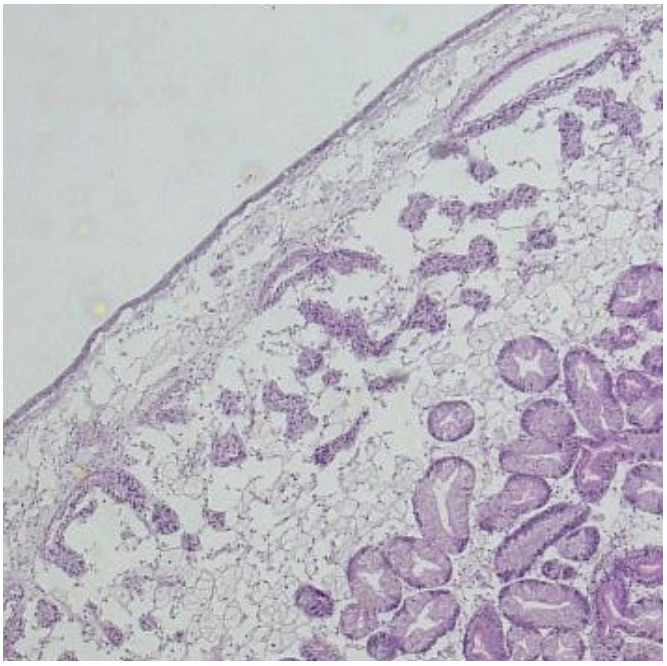

Figure 11.16. Stage 4 oyster tissue at 50× magnification. No gametes are present, so sex cannot be determined. Postspawning, ongoing cytolysis is indicated by spaces in the reproductive tissue and hemocytes in and around the follicles.

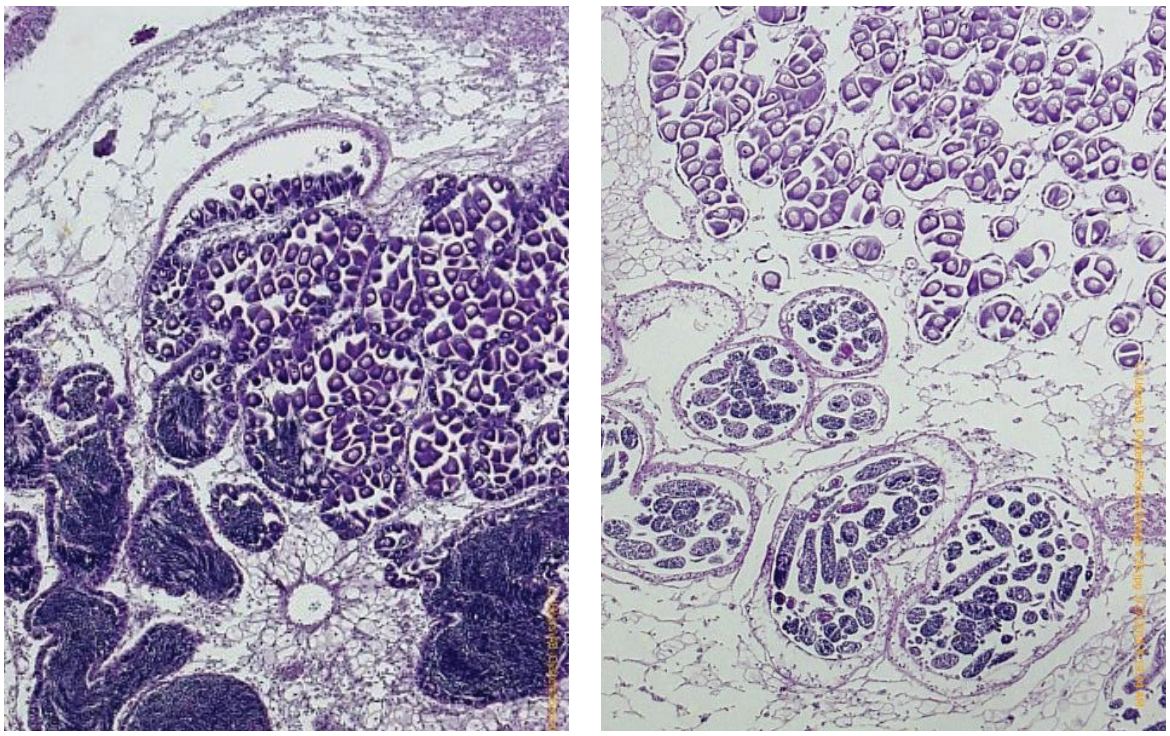

Figure 11.17. Reproductive stage cannot be assigned when both male and female gametes are present (left) or when tissue is infected with *Bucephalus polymorphus* (right). When both male and female gametes are present (may be in separate follicles or intermixed within a follicle), sex is assigned *M*/*F* and stage is assigned *Z*. Sex can be assigned when tissue is infected with *B. polymorphus* only if gametes are present.

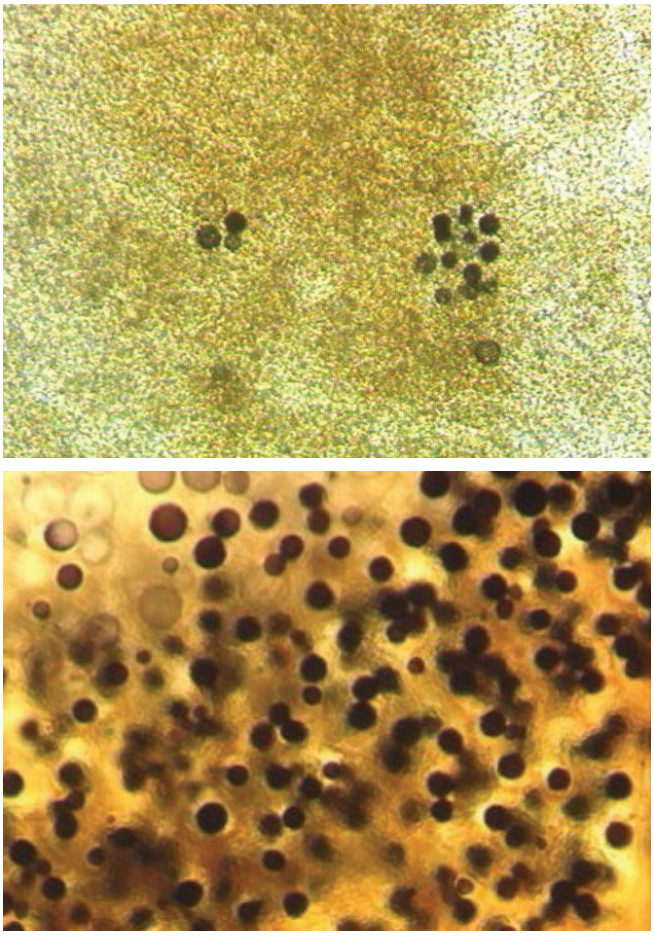

Figure 11.18. Microscopic view of oyster sections prepared for analysis of density of *Perkinsus marinus* hypnospores (dermo). (Light infection, Stage 1, top; heavy infection, Stage 5, bottom).

Table 11.3. Mackin scale of *Perkinsus marinus* infection intensity stages (Mackin 1962).

Stage	Category	Number of cells
0	Uninfected	None detected
0.5	Very light	≤ 10 cells
1	Light	11–100 cells
2	Light to moderate	≥ 101 or more cells, local concentrations of 24–50 cells (3 or more cells may be visible in some fields at 100× magnification)
3	Moderate	3 or more cells in all fields at 100× magnification
4	Moderate heavy	Large numbers of cells throughout tissue; less than half of tissue appears blue-black macroscopically
5	Heavy	Enormous number of cells; more than half of tissue appears blue-black macroscopically

Condition index

Overview

The oyster condition index (CI) provides a method of comparing the physiological health of oysters across multiple locations (Lawrence and Scott 1982, Crosby and Gale 1990, Baggett et al. 2014). Condition index is determined by weight (Fig. 11.19) and is calculated as follows:

$$CI = \frac{(\text{soft-tissue dry weight} \times 100)}{(\text{whole oyster wet weight} - \text{shell wet weight})}$$

This dry-to-wet-weight ratio can provide a metric of the proportion of water in the tissue of an oyster. A large amount of water in the tissue is a sign of depleted energy reserves (as occurs after spawning) or food limitation (Lucas and Beninger 1985, Rheault and Rice 1996).

Figure 11.19. Oyster shell (top) and tissue (bottom) separated for determination of oyster condition.

Condition index field procedure

Materials needed for CI field work

- Oyster knives
- Field gloves
- 1-gallon resealable plastic bags
- Cooler with ice
- Field books, pencils, and permanent markers
- Scuba diving or snorkeling equipment, as appropriate

CI field procedure

1. If conducting condition analyses at each station, collect 5 additional oysters (plus 1 or 2 extra) during monitoring and separate them into single oysters. Different oysters should be collected for disease monitoring and condition index because of the subsampling necessary for disease analysis, which would bias subsequent condition index measurements. Clean off all fouling organisms, place the oysters in a labeled resealable plastic bag, and put them in a cooler. If there are not enough live oysters at a station (or if there are none), collect more at another station to meet the required number for the site quota.
2. In the field book, note how many oysters were collected at each station.
3. Transport oysters live and chilled to laboratory.

Condition index laboratory procedure

Materials needed for CI laboratory procedure

- Oysters from monitoring station
- Scrub brushes
- Vernier calipers
- Benchtop scale
- Oyster knives (or scalpels)
- Aluminum weighing dishes, labeled with station and sample number
- Metal pans labeled for staging dissections and drying oyster shells
- Drying oven
- Data sheets, pencils, and permanent markers
- 1-gallon resealable plastic bags

CI laboratory procedure

1. Clean any remaining fouling organisms from the oysters with a scrub brush and an oyster knife.
2. Measure and record the shell height, length, and width (mm; Fig. 11.2) of each oyster with Vernier calipers.

3. Measure and record the total weight (g) of each whole oyster.
4. Place oysters in a metal pan after they have been measured (Fig. 11.19). Label the pan and place oysters in the pan in the order of measurement to keep track of the station and sample numbers.
5. Label aluminum weighing dishes with station and sample number.
6. Measure and record the total weight (g) of each empty dish.
7. Open each oyster with an oyster knife.
8. Fully remove the oyster body tissue with the oyster knife and transfer it into the appropriately labeled aluminum weighing dish (Fig. 11.19).
9. Measure and record the total wet weight (g) of the oyster soft tissue in the aluminum weighing dish.
10. Measure and record the wet weight (g) of each shell.
11. Place the shell back in the appropriate place in the labeled metal pan. After all dissections are completed, place the pan with shells and the pan with tissue in the aluminum weighing dishes in the drying oven at 65 °C for a minimum of 48 hours.
12. After drying, remove and record shell dry weights (g) and tissue dry weights (g).
13. If needed for shell pest analysis (see below), write the sample number inside each shell valve with a permanent marker. Place shells into a resealable plastic bag labeled with station, date, and sample number.
14. Dried oyster tissue and aluminum pans may be disposed of in the trash.

Shell pests

Overview

Clean, dry shells from CI or disease and reproduction samples can be labeled and saved for quantitative analysis of shell pests. Marine organisms including boring sponge (*Cliona* spp.), boring polychaetes (*Polydora* spp.), and boring clams (*Pholadidae*, especially *Diplothyra curta*) inhabit living oysters and are considered shell pests due to the damage caused to the oyster shell and the presumed energetic costs this inflicts on the oyster (De Baets and Huntley 2021). This procedure describes how to measure the area of shell impacted by pests using photographs and image-processing software.

Shell pest procedure

Materials needed for shell pest analysis

- Digital camera
- Digital camera stand that can mount the camera at various heights (Fig. 11.20)
- White dive slate
- Large tray
- Pencil and data sheets
- Ruler
- Computer with image-processing software capable of making calibrated measurements

Shell pest laboratory procedure

1. Organize the clean, dry, labeled oyster shells on the tray by sample number, pairing both valves.
2. Place a white dive slate on a horizontal surface to serve as the background of each photo. Use the camera stand (Fig. 11.20) to position the camera directly above the dive slate with the dive slate in the field of view.
3. Proper image calibration is required to obtain true-to-life measurements from each photograph using the imaging-processing software (Fig. 11.21). Consult the software manual for specific instructions on

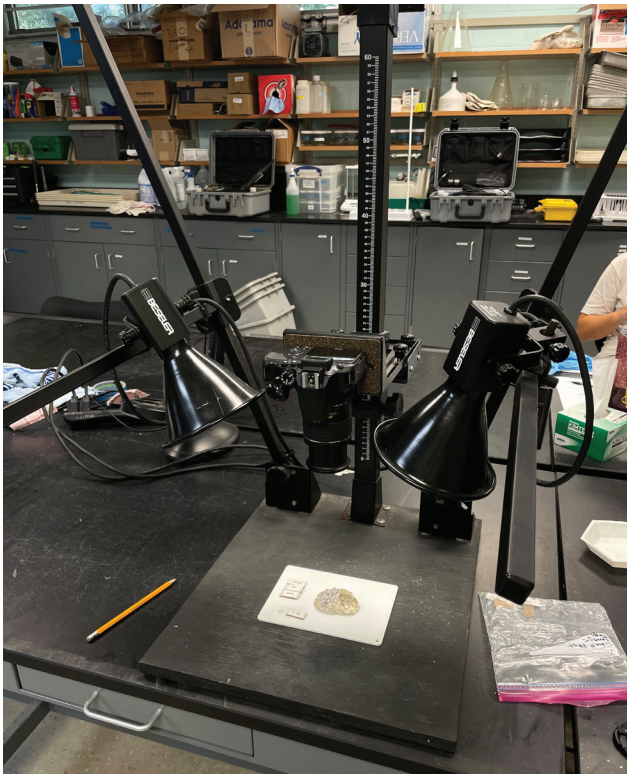

Figure 11.20. Camera stand used for shell pest analysis.

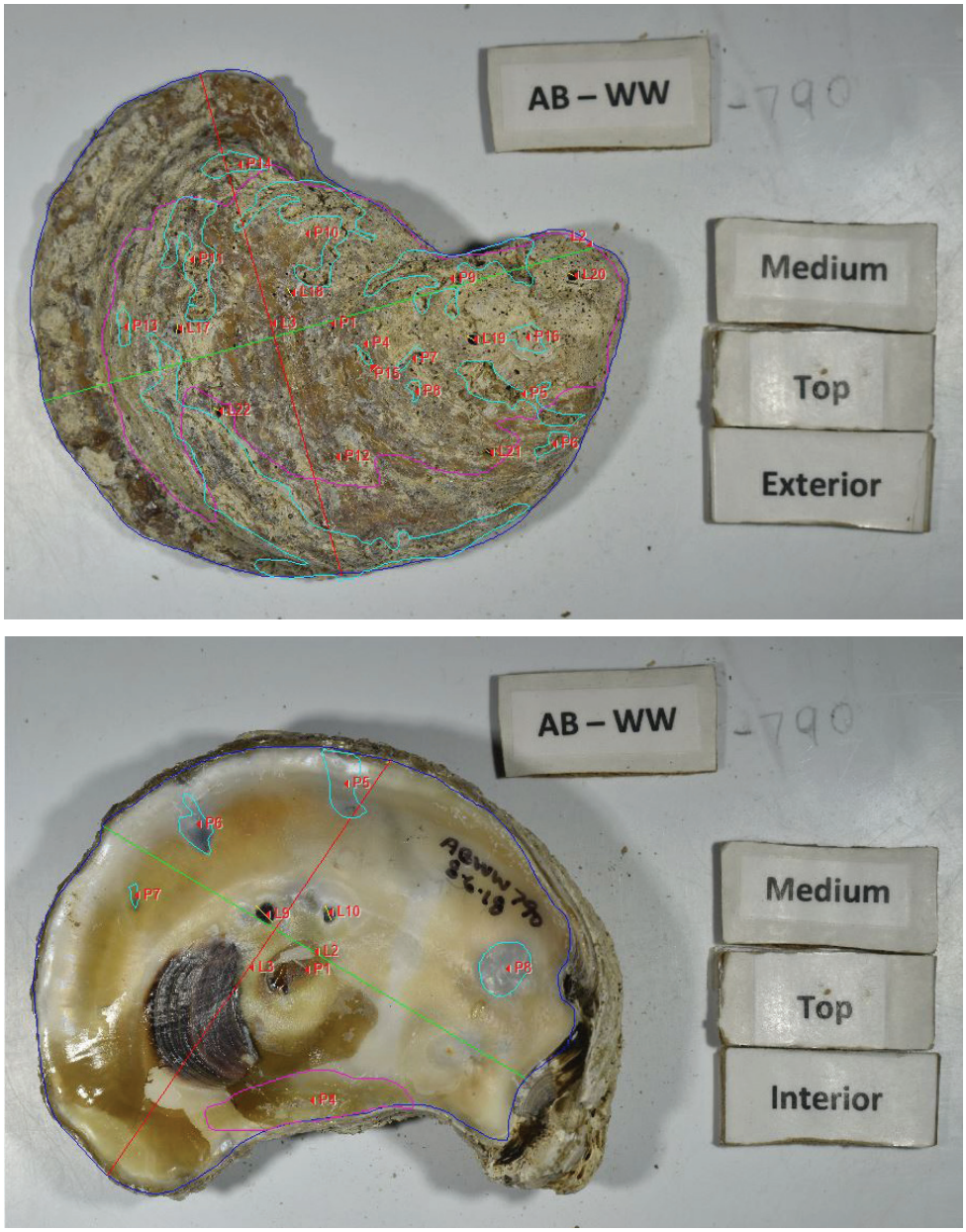

Figure 11.21. Example measurements of shell dimensions and areas impacted by shell pests on the exterior (top) and interior (bottom) of an oyster shell.

calibrating images, measuring and counting objects, and exporting data. Some image-processing software requires the calibration to be set manually for each photo by measuring an object of known dimensions in the photo, such as a ruler. Other types of software allow the user to save calibrations for a known height and apply them to subsequent images. In the instructions provided here, reference photos of the ruler were taken from a set of fixed camera heights; these calibrations are saved and applied to subsequent photos using the same height and camera settings.

4. Photograph the external and internal surfaces of both valves (top and bottom) of each shell in the same order for each oyster following the steps below:

   a. Adjust the camera to a height setting that maximizes the area of shell in the frame without exceeding the field of view.

   b. In the frame of view on the white dive slate, write in pencil the sample number and shell surface (top/bottom valve and internal/external side). Also note the camera height setting (or include a ruler in the frame for image calibration).

c. Focus the image and take the photograph.

5. Once all the photos have been taken, save the images in an appropriate location on the computer, and rename each image with the sample number and shell surface (T = top valve, B = bottom valve, I = internal surface, E = external surface).
6. Open the first image in the image-processing software. Set the image calibration according to the camera height setting or using the ruler included in the image.
7. All measurements below (Fig. 11.21) should be made using the image-processing software. Measure the total area of the shell. For images of the external surface, the area includes everything within the margins of the shell. For images of the internal surface, exclude the hinge so that the area measured only includes the white nacre that surrounds the mantle. Note: To ensure measurement accuracy, also reference the original physical shell while analyzing an image.
8. Measure the shell height and length (Figs. 11.2 and 11.21). For images of the internal surface, measure shell height and length only within the white nacre.
9. Measure the area of the shell with *Cliona* damage (Fig. 11.22) by outlining the affected area using the image-processing software. On the external surface, *Cliona* damage looks like a series of very small, closely spaced holes (1–1.5 mm diameter). On the internal surface, *Cliona* damage will cause the normally white nacre to appear yellow, with closely spaced very small black holes.
10. Measure the area of the shell with *Polydora* damage (Fig. 11.23) by outlining the affected area using the image-processing software. *Polydora* creates U-shaped tunnels in the oyster shell. On the external surface, a pair of closely spaced entrance and exit holes (~1 mm diameter) can be seen. The tunnels may be seen if part of the shell has been chipped away. On the internal surface, *Polydora* damage causes mud blisters—dark-colored chambers covered by a thin layer of nacre.
11. Count the *Diplothyra* boring clams (Fig. 11.24) present by measuring the diameter of each cavity containing a clam. On the external surface, boring clam holes are singular and larger than *Polydora* holes, and the remains of the clam can be found with a probe. On the internal surface, boring clam holes are indicated by a singular dark-colored spot surrounded by a ring of yellowed nacre.
12. Once all the measurements have been collected, save all measurements to an appropriately labeled data file.

Repeat steps 4–9 for each image until all shells have been processed.

Growth and mortality

Overview

In this monitoring procedure, oysters are left in cages in order to track growth and mortality through monthly monitoring of shell height and survivorship. Open cages can be used to track overall rates of oyster mortality, while a comparison of mortality rates in open and closed cages provides a way to determine rates of mortality resulting from predation.

Growth and mortality field procedure

Materials needed for cages (per station)

- Three constructed open-top, Vexar-lined growth cages (construct cages with 25.4-mm plastic-coated wire mesh with dimensions of approximately 0.6 m L × 0.6 m W × 0.2 m H. Line the bottom and sides of the cages with 6.35-mm plastic mesh to prevent loss of smaller oysters)
- 6 2.5-m sections of twisted 3/8-inch polypropylene rope
- 3 half cinder blocks with a hole drilled through the center of one side
- 6 donut floats for initial deployment (2 red, 2 yellow, 2 brown)
- 6 spare donut floats (2 red, 2 yellow, 2 brown)
- Metric rulers (~15 cm in length)
- Data sheets and field books
- Clipboards, pencils, and permanent markers

Field monitoring procedure

1. At the beginning of the monitoring, place 30 adult oysters per cage into 3 open cages per station. Oysters should have shell heights of 10 mm or more. These oysters do not need to be thoroughly cleaned of fouling organisms such as barnacles or algae but should be separated from any other oysters. The source of oysters for these cages has varied in FWRI monitoring activities; sources have included broodstock from breeding programs and oysters collected from shell left at monitoring sites.
2. Each cage should have a 2.5-m polypropylene line attached to it with a donut float at the end. Each of the 3 cages should be identifiable by the color of its float (one each of brown, red, and yellow). Secure the cages at each station by placing a half cinder block in them with a 2.5-m black polypropylene line passed through

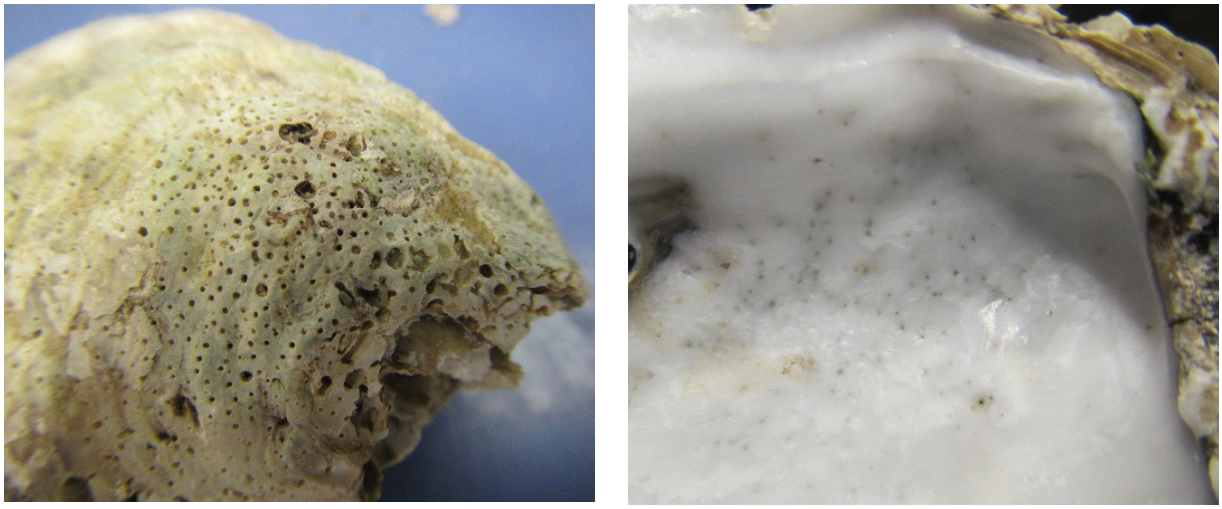

Figure 11.22. External (left) and internal (right) examples of the small, closely spaced holes caused by *Cliona* boring sponges.

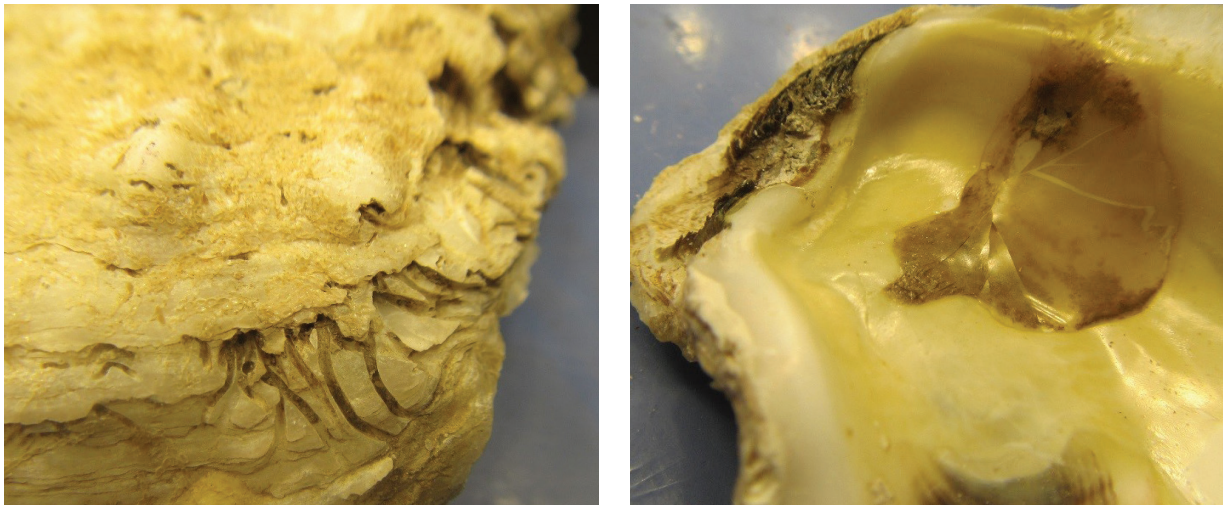

Figure 11.23. External tunnels and holes (left) and an internal mud blister (right) caused by *Polydora* boring polychaetes.

Figure 11.24. *Diplothyra* boring clam shells on external side of an oyster shell (left) and internal (right) examples of the holes and yellow nacre caused by boring clams.

the hole in the block. Tie another donut float that matches the color of the cage on the end of the line so it can float on top of the water.

3. Each month, using a metric ruler, measure and record the shell height of all surviving oysters. Record the presence of any dead oysters by writing in *DEAD* instead of their shell height. A dead oyster is classified as a complete box with top and bottom valve as long as it is mostly intact or both valves are present and can be matched up. Record any missing oysters as *Z*.
4. Each month, clean and maintain the cages as necessary. Remove excessive fouling organisms from the cage, block, and floats. Check the floats; if damaged or so much paint has worn off to hinder identification, replace them. Due to general wear, it is recommended that all cages and blocks be replaced annually.
5. This general growth and mortality procedure has been modified for some monitoring studies in order to isolate mortality due to predation vs. other causes, or to track the growth of individual oysters over time. Modifications to the procedure include the following:

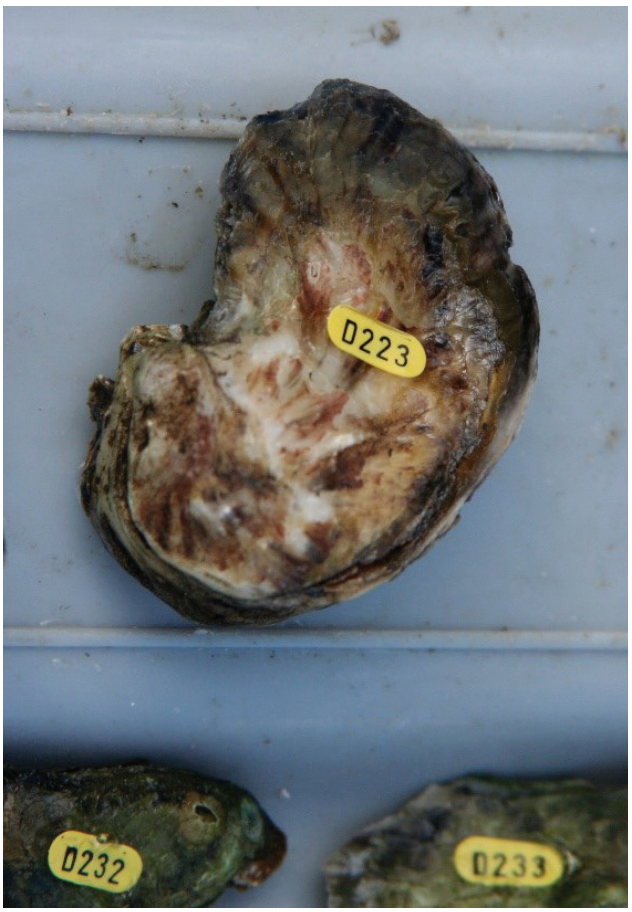

Figure 11.25. Individually tagged oyster shells.

   a. Use shellfish tags glued to the shell of the oyster to identify and track individual oysters (Fig. 11.25).
   b. Use one open and one closed cage to isolate mortality attributable to macrofaunal predation.
   c. If a sufficient oyster population is present in the estuary, replace the caged oysters monthly to ensure that each month begins with 30 live oysters in order to maintain a consistent monthly sample size. In estuaries with severely depleted populations this may not be feasible. When replacing all or a portion of the oysters, the shell height (or other measured metric) should be recorded at the beginning of the measurement period.

Data analysis

Calculate mortality rates for each cage by dividing the number of remaining live oysters by the number of live oysters initially planted in the cage.

Shell budget

Overview

Reef accretion requires that the rate of shell deposition is greater than that of shell loss; the balance between the two rates can be quantified as a shell budget. Details on the calculation and modeling of shell budgets are beyond the scope of this document, but further information is available in published literature (e.g., Powell et al. 2006, 2012, Powell and Klinck 2007, Soniat et al. 2012, 2019). At each station, 15 replicate 0.25-m^2 quadrats are haphazardly deployed, and all oysters and cultch (shell and reef substrate) in each quadrat are collected and placed into bags by divers (Fig. 11.26). Laboratory analyses include determination of the total number of live oysters and of dead oysters with articulated shells, shell height measurements for 50 live and dead oysters, and cultch volume and weight. Samples are sorted into categories (live oysters, oyster shell, planted shell, shell hash, and other substrate), and the weight and volume are measured for each. The number of oyster drills is also determined for each quadrat.

FWRI carries out shell-budget monitoring quarterly at subtidal 2-acre stations in Apalachicola Bay. Shell budget monitoring will be also included in Suwannee Sound monitoring starting in late 2021. Weather and staffing considerations necessitate laboratory processing for Apalachicola Bay monitoring, but the procedures could be adapted to field processing if needed. The procedure described here is specific to subtidal oyster reefs, but these procedures could also be adapted for intertidal locations.

Shell-budget field procedure

Materials for subtidal shell-budget field work

- 0.25-m^2 quadrats (see Inset 1)
- Marker buoys
- Stainless-steel carabiners, at least 5 inches long
- Vinyl duct tape for labeling
- Mesh catch bags with galvanized handle (15 for each station)
- 1-gallon resealable plastic bags
- Waterproof paper sample labels
- Field books, pencils, and permanent markers
- Field gloves
- Shrimp baskets, buckets, or storage containers, as appropriate
- Scuba diving or snorkeling equipment, as appropriate

Subtidal shell-budget monitoring procedure

1. Boat to the corners of the 2-acre station and mark each corner (within 30 ft) with a marker buoy. Anchor within the station boundary.
2. Knot two loops into each quadrat rope, attach carabiners to each loop, and attach several empty mesh catch bags to one carabiner.
3. Divers enter water on opposite sides of the boat and haphazardly deploy their quadrat.
4. Remove one catch bag from the carabiner with empty bags and fill with all the surface material within the quadrat.
5. Place full catch bag on the other carabiner.
6. Move at least 1.5 m (3 quadrat widths) in a haphazard direction and repeat steps 2–5 using a new bag for each new sample.
7. Return to the boat, swapping full bags for empty bags until all samples have been collected.
8. Transfer the contents of the catch bag into a 1-gallon resealable plastic bag if the sample is small enough to fit; keep large samples in catch bags.
9. Place a waterproof paper label in each in each bag (catch bag or plastic bag), seal or close, then seal with tape label with station and sample number that matches paper tag.
10. Retrieve the marker buoys and proceed to the next station.
11. Return to the laboratory and store all samples collected in a freezer.

Shell-budget laboratory procedure

Materials for shell-budget procedure

- Digital scale with high weight capacity (e.g., up to 15 kg) and precision of ≤ 0.01 kg
- Digital scale with low weight capacity (e.g., up to 200 g) and precision of ≤ 0.01 g
- Large volumetric buckets with precision of ≤ 0.25 L
- Small volumetric buckets with precision of ≤ 0.25 L
- Metric rulers (~15 cm)
- Multipurpose trays
- Polystyrene weighing dishes
- Oyster knives
- Assorted probes
- Data sheets and pencils
- 5-gallon buckets

Shell-budget laboratory procedure

1. Record identifying information on data sheet for samples to be processed (site, station, quadrat, date collected, date processed).

Figure 11.26. Supplies for divers to use in collecting oysters during subtidal oyster monitoring (top); collected material is later sorted at the laboratory (bottom).

2. Using scale, weigh each sample. Record weight in kilograms to the nearest 0.01 kg.
3. Using volumetric buckets, determine the displacement volume of each sample.
4. Separate sample into its component parts as listed below:
   a. Live oysters: Live oysters that can be separated from the substrate.
   b. Oyster drills: *Stramonita* spp. and *Urosalpinx* spp.
   c. Other organisms: Crabs, snails, fish, mussels, etc. that constitute more than 5% of the total sample weight.
   d. Oyster shell: Substrate created by past oysters. May be whole or partial shells. Tip: These pieces should be easily recognizable as oyster shells. They are often lighter, flatter, or more two-dimensional than planted shell.
   e. Planted shell: Substrate that has been placed by humans. May be rocks or fossil shell. Tip: These pieces are often heavier, thicker, or more three-dimensional than oyster shell (Fig. 11.27).
   f. Shell hash: Pieces of substrate smaller than 10 cm^2 (Fig. 11.27). Tip: Use grid paper or a shape outlining an area of 10 cm^2 to measure area when unsure. Pieces smaller than 10 cm^2 can be classified as oyster shell or planted shell if they are readily identifiable and constitute significant oyster habitat.
   g. Black and other substrate: Black substrate is subsurface material not available for oyster recruitment. Usually no encrusting organisms are present and it may have regions stained black by anoxic bacteria. Other substrate refers to materials that do not fall into any of the above categories (e.g., glass, trash, wood, unidentifiable objects).
5. Measure and record the shell height of as many as 50 random live oysters to the nearest millimeter. Count the remaining live oysters. Record the number of total live oysters.
6. Measure and record the shell height of as many as 50 random dead oysters to the nearest millimeter. Count the remaining dead oysters. Record the number of total dead oysters.
7. Record weight and volume for all components listed in Step 4.
8. Ensure that sum of components' weights and volumes are within 10% of total weight and total volume. Reweigh or remeasure volumes if errors present.
9. Once processing and checks are complete, place sample in a 5-gallon bucket to be returned back to an appropriate reef.

Figure 11.27. Examples of planted shell (top) and shell hash (bottom) that were sorted for shell budget analysis.

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