MarineGEO Oyster Reef Habitat Monitoring Protocol

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MarineGEO
THE TENNENBAUM MARINE OBSERVATORIES NETWORK

Smithsonian Institution

Background

Oysters are filter-feeding bivalves found in nearshore brackish or marine waters that coalesce with each other as they grow, forming structurally complex intertidal or subtidal reefs. Oysters are ecosystem engineers and provide critical ecosystem services including enhanced water quality via their strong filter-feeding capabilities and protection to submerged aquatic vegetation and waterfront communities. Oyster reefs also provide important biogenic habitat for a diverse suite of marine life including vital nursery grounds for many commercially valuable species in addition to many other species.

The eastern oyster, Crassostrea virginica, is found along the Atlantic and Gulf of Mexico coasts and is a critical component to the health of nearshore ecosystems. However, increasing threats including overharvesting and disease threaten oyster populations and the benefits they provide. Ongoing restoration is a continued conservation priority and this protocol was therefore developed to provide a means of monitoring both natural reef as well as restored reef to assess the long-term health of this important species as well as its critical function as refugia for associated fauna.

Introduction

In this document, we provide MarineGEO's standard survey design for sampling eastern oyster reef habitat including key measurements on reef attributes (reef area and height), reef composition, oyster density and size, rugosity, and associated biodiversity. Additionally, we provide best practices for site selection, layout, workflow, and data submission.

The overall design and replication adhere as closely as possible to other oyster reef monitoring guidelines and in particular, much of this protocol was developed using the Oyster Habitat Restoration Monitoring and Assessment Handbook (2014), complied by NOAA, The Nature Conservancy, and others. Although the handbook was designed for restoration monitoring, it adopts well to naturally occurring reefs. Our goal is to provide a standardized sampling design that can be used in different regions and for restored or natural reefs, while still being comparative in both space and time.

Methods

The following MarineGEO protocols provide a standardized set of measurements for characterizing the health of oyster reefs and their associated communities. The methodology was adopted from the Oyster Habitat Restoration Monitoring and Assessment Handbook, created by a working group including agencies from NOAA, the Natural Conservancy, as well as others and using "Universal Metrics" of study for best practices. These methods are specific for eastern oysters (Crassostrea virginica). Oyster reef monitoring has two main components: 1) collection of a series of measurements to characterize the size, structure, and health of a reef and 2) collection of associated fauna to monitor changes in biodiversity over space and time. In general, fieldwork for a reef can be easily completed in a single day. MarineGEO recommends that 3 separate reefs be included per partner site for annual monitoring.

Protocols

Core protocols below are **required** for MarineGEO partners:

- Sampling Event & Environmental Monitoring (annual)
- Oyster reef area and height (every 3 years)
- Oyster reef composition (annual)
- Oyster density and size frequency (annual)
- Oyster reef associated fauna (annual)
- Oyster reef rugosity (annual)

Brief Workflow

Preparation:

1. Identify and become familiar with the required modules listed above.
2. Download copies of protocols, field datasheets, and data entry templates.
3. Contact marinegeo-protocols@si.edu to schedule a brief conference to discuss your project and address any questions before proceeding to the next steps.
4. Acquire all necessary permits and permissions at your sites. There are no planned collections of oysters required for this protocol.
5. Review the necessary safety requirements from your institution. MarineGEO is not responsible for any loss or injury incurred during sampling.

Site Selection:

1. Identify 3 separate sites to sample on a permanent basis. Sites should be: a) typical of your region, b) reasonably accessible, and c) generally persistent. This protocol requires 3 transects (ideally 30 m in length) be used at a single site, however, transects can be distributed among separate patch reefs at a site if each reef is small.
2. Contact marinegeo-protocols@si.edu to verify your sites with our team and to receive permanent site codes to be used when submitting data.
3. There are several ongoing efforts to restore oyster reefs and this protocol can be utilized to track the success of those efforts overtime. The ultimate goal of restoration is to enhance the population and the ecosystem services they provide. It is therefore important to not only build a restorative habitat but to follow that habitat through time to monitor its success or failure as well as lessons learned. If a restored reef is to be monitored using these protocols, it is imperative to know as much of the history of that reef as possible. Though there is no associated data sheet, please provide marinegeo-protocols@si.edu with a thorough synopsis of restoration efforts including but not limited to:
    - If previous natural reef was present at the location
    - When the restoration occurred and by whom
    - What methods were used in the restoration (oyster bags, limestone, etc.)
    - What were the goals of the restoration effort
    - What monitoring was conducted after restoration was initiated

- Are there any natural reefs in the surrounding area and have data been collected on them

Brief Summary of Fieldwork:

1. Monitoring of oyster reefs should be done annually during the optimal time for the partner site.
2. Approximately 1.5 – 2 months prior to sampling, deploy preconstructed bioboxes (n = 3) at each of the reefs that are planned to be sampled. Bio-boxes are used to sample associated invertebrate biodiversity within oyster reefs (see Associated Fauna protocol for details).
3. For intertidal reefs, arrive at the reef during low tide conditions when oysters are exposed. Because low tides are short-lived events, it is expected that 1 reef be sampled per day. This is not the case for subtidal reefs though for monitoring sites on snorkel or SCUBA, sampling should be done when water clarity is maximized.
4. At each site, first record site metadata and measure environmental conditions using the Sampling Event and Environmental Monitoring Protocol.
5. Measure the reef area and height using the Oyster Reef Area and Height protocol. If multiple patch reefs are used, measure the area and height of each.
6. Lay out three 30 m transect lines with the first through the approximate average density of live oysters. Lay a second to the left and a third to the right, at least 1.5 m away from the center, and in areas that are characteristic of the reef. Transects need not be straight and can follow the shape of the reef. For smaller patch reefs, a single transect per reef can be used and laid across where the average live oyster density occurs. This is repeated for nearby patch reefs equating to 3 transects per site.
7. Along each transect conduct the following:
    a. Use the Oyster Reef Composition protocol to survey the composition of the reef at predetermined meter marks (n = 5 per transect at 5, 10, 15, 20, and 25 m) along the transect.
    b. For patch reefs that are > 30m, drop quadrats along the transect at least 1 m from the previous one (example: 1 m, 3 m, 5 m, etc.).
    c. Once along each transect, and at one of the previously sampled spots from 7a., use the Oyster Density and Size Frequency protocol to excavate a known area of oysters to count the density and measure the size of oysters as well as other sessile invertebrates encountered.
8. Collect and process each bio-box according to the Oyster Reef Associated Fauna protocol.
9. Return any collected epifaunal samples from the bio-boxes to the lab for post-processing.

Core Protocol Summary

1) Reef Area and Height

Overview:

The area of a reef is a valuable metric critical to estimating the health and persistence of the reef over time and the quality of ecosystem services the reef provides to the surrounding environment. Different methodologies can be used to get an accurate estimate of the total reef area, however, because oyster reefs are asymmetrical in shape, careful measurements are vital for spatio-temporal comparisons.

The height of a reef is the average height of the reef at its crest as compared to its surrounding, non-reef area. Reef height is a useful measure of reef growth, accretion, and persistence that might not be captured in areal measurements. Height can also be informative on how the reef provides habitat to associated species as well as information on erosion control and storm protection to neighboring areas. Both reef area and height are relatively static over short timescales. It is recommended that these measurements initially to provide a baseline and then done in 3-year intervals or after a major event.

Measured Parameters

- Area of reef where the edge extends to at least 25% live or dead shell (m2)
- Height of the reef with respect to the surrounding edge of the reef (cm, m)

2) Oyster Reef Composition

Overview:

The composition of oyster reefs can be variable in both space and time and can change depending on recruitment, survival, and environmental conditions. The abundance of live and dead oysters on a reef, taken in a non-destructive way using transects, is an easy way to characterize the entirety of a reef. The percent cover of reef substrate including oyster status (live, box, or cultch), presence of sediment, and non-oyster species is a critical way to identify the health of a reef and provides a quick and non-destructive standardized measurement to compare within and between regions.

Measured Parameters

- Percent cover of substrate (point-counts from quadrates)

3) Oyster Density and Size Frequency

Overview:
The density of live oysters on a reef is difficult to measure when doing non-destructive sampling because of the structural complexity and depth of a reef. However, the number and size of live oysters is an important metric that provides information on population structure including oyster density and size frequency, as well as recruitment and survivorship. This protocol uses a minor destructive technique to accurately count the number of live oysters in a particular area as well as classify the size frequency of those live oysters on the reef.

Measured Parameters
- Oyster density (individuals m-2)
- Oyster size frequency (shell height of live and box oysters (mm))

4) Oyster Reef Associated Fauna

Overview:
Oyster reefs provide essential habitat and refugia for a diverse suite of species including many commercially important ones as well as others that form important links to higher trophic levels. Oysters as a habitat are a typically overlooked function of reefs and the interactions of oysters and associated species on a broader scale is generally not known. Methods provided here quantify the abundance and diversity of associated small invertebrates using “bio-boxes”, a standardized tray, filled with shell, deployed within the reef for an extended amount of time to allow for associated faunal colonization. Collected trays are processed and fauna are identified and enumerated in the lab. The presence of these species plays a vital role in the trophic ecology of oyster reefs and is essential to understanding the connectively of oyster reefs and the surrounding environment.

Measured Parameters
- Individuals m-2

5) Oyster Reef Rugosity

Overview:
Rugosity is defined here as a measure of habitat or substrate complexity. The complexity of a substrate is an important ecological characteristic and habitats with high rugosity are likely to provide more cover or refuge for both sessile and mobile associated species as well as for the oysters themselves. Rugosity is a simple measure to take and is useful for comparisons of habitat heterogeneity for restored and natural reefs and between different types of habitat (e.g. coral reefs or rocky intertidal). The most common protocol used is a chain method, whereby an index is calculated using the ratio of a fixed line transect to a flexible line hung over the substrate. This protocol provides a useful, standardized metric that can be directly related to other parameters measured within the reef as well as across different habitats.

Measured Parameters:

- Ratio of fixed distance to actual distance

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet: https://marinegeo.github.io/data-submission
4. Contact us if you have any questions: marinegeo-protocols@si.edu

Oyster Reef Area and Height

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Introduction

This protocol provides methods on standardized data collection for the areal dimensions and height of an oyster reef. The area is defined as the reef itself with a continuous edge extending to approximately 25% cover of living or dead shell. The height of an oyster reef is defined as the mean height of the reef above the surrounding adjacent substrate, excluding the terrestrial shoreline. At a particular site, several reefs or patch reefs could be present and height and area should be measured for all reefs from which any other data are collected. For expansive reefs where such measurements are not practical, it is still important to give some estimation of the size of the reef (example: > $100m^2$). It is also possible to use recent satellite imagery (Google Earth, Landsat, etc.) to acquire areal extent. In general, the area and height of a reef will change little from year to year. Because of this, MarineGEO requests that this be done at least initially to serve as a baseline and every 3 years thereafter or following a major event. This protocol provides 3 alternative methods to obtaining reef area. All methods have some inherent error associated with them. A priority here is to establish the relative size of each reef from which other forms of data come from.

<u>Measured Parameters</u>
- Oyster reef areal dimensions (m^2)
- Mean reef height (cm, m)

Requirements

Personnel: 2 people

Estimated Total Time Per Location (n = 3 sites)
  Preparation: 1 person x <1 day
  Fieldwork: 2 people x <1 day per location
  Post processing: None
  Data processing: 1 person x <1 day

Replication: At least 3 oyster reefs per region

Materials:
<u>Fieldwork:</u>
- ☐ Hand-held GPS or better
- ☐ String with line level
- ☐ Meter stick
- ☐ Oyster reef area and height data sheets
- ☐ Transect tape

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:
1. Review the MarineGEO Oyster Reef Habitat Survey Design for selection of permanent sites.
2. Become familiar with GPS equipment or other methods that will be used in the field. Test the device and make sure that it is collecting data and that this data can be moved to mapping software.
3. For intertidal sites, sampling is done at a low tide when the oyster reef is exposed. For subtidal reefs, sampling should be done when water clarity is optimal.

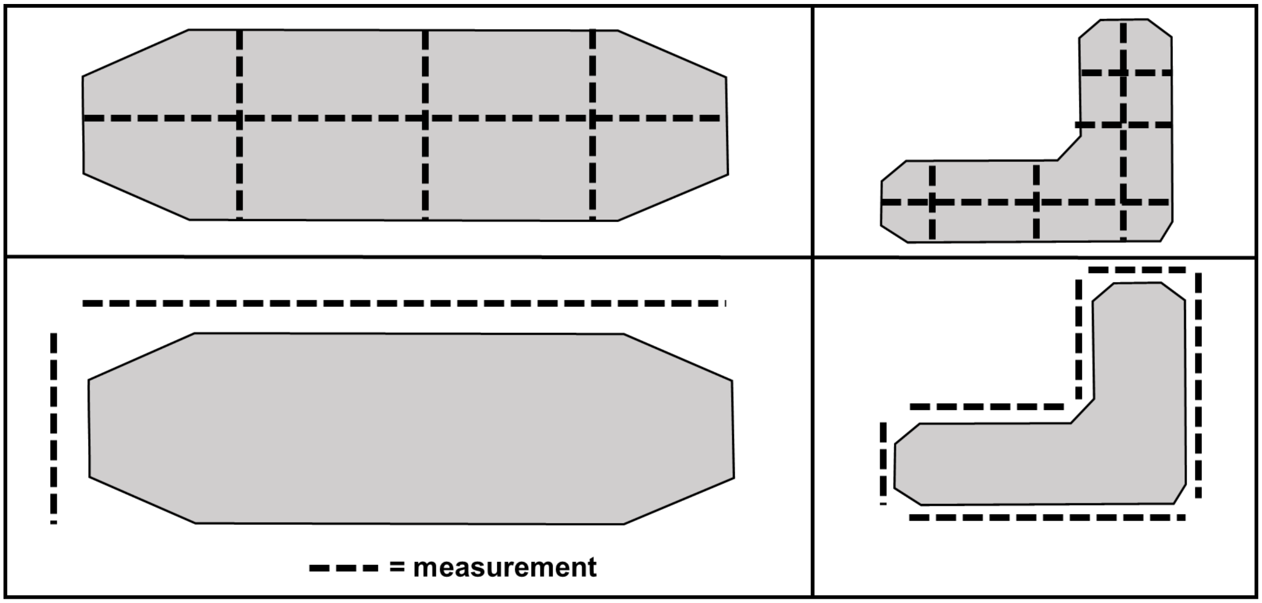
Fieldwork: Reef Area
1. It is helpful to sketch the general shape of the reef or patch reefs and obtain GPS coordinates. The perimeter of reef is the continuous edge where live or dead shell make up about 25% of the substrate.
2. Choose a method from below to measure reef area.

Method 1: For large intertidal reefs, walk the continuous edge (<25% dead/live shell) of the reef(s) using a standard hand-held GPS. Collect several points that are 3 – 5 m apart along the edge of the reef. The more points collected will increase the accuracy of the area calculated during post-processing, however, GPS units have an estimated accuracy of roughly 3 m and an excessive amount of points will not help accuracy. Coordinates are later entered into mapping software (e.g. ArcGIS) in order to calculate reef area. GPS points can also be loaded into Google Earth and a polygon can be created to establish the areal extent of the reef. Using a GPS device is not recommended for small reefs and it is more accurate to pick a method below.

Method 2: For intertidal reefs or subtidal reefs where water clarity is good, a drone can be used to collect areal dimensions. Fly the drone at a consistent height that captures the entire reef. Include a meter stick, a PVC pole, or something similar with a known length on the reef and in view of the drone. This is needed and used in post-processing to estimate the area. Image J (https://imagej.net) is a well-known free program that can easily estimate area from photos. Other, more sophisticated programs can be used at the user's discretion.

Method 3: For all reefs including those that are large or patch, subtidal, or asymmetrical, a transect tape can be used to take several measurements of the reef and used to calculate the area. There are simple ways to calculate area for known shapes (circle, square, triangle, etc.) and if the reef takes on these shapes, the appropriate measurements should be taken. However, most reefs are asymmetrical and therefore several length x width measurements should be taken to estimate the shape of the reef (Figure 1). First measure the longest axis and then take several other measurements perpendicular to the longest axis. In Google Earth, a polygon can be constructed, and area calculated using these measurements.

Figure 1: Different reef shapes and how to estimate areal coverage.



Fieldwork: Reef Height

1. If available, more sophisticated GPS equipment can be utilized to capture the mean height of an intertidal reef, however, in most cases practitioners will not have access to this and therefore they can proceed as follows below.
2. First, one person should find the highest point on the reef. At the selected point, hold a string (with line level attached, Figure 2) against the oysters. The other end of the string is held by another along with a meter stick. When the line level is straight, measure the height on the meter stick. Starting at the oyster reef edge (<25% living/dead shell cover), walk the perimeter of the reef and take at least 4 measurements in each direction (N, S, E, and W) from the highest point. The reef height is calculated as the mean height from these measurements. Do this for each reef that data are collected from.

Figure 2: Line level used for reef height

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet: https://marinegeo.github.io/data-submission
4. Contact us if you have any questions: marinegeo-protocols@si.edu

Oyster Reef Composition

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Introduction

This protocol provides non-destructive standardized data collection on the composition of an oyster reef including both living (oysters, algae, other bivalves, etc.) and non-living substrate (dead shell, rock, sediment, etc.). Oysters are classified as live, gaper (dead or dying with visible tissue), box (dead and gaping with both bivalved shells still attached at the umbo), and cultch (single shell or shell fragments). The protocol is designed to accommodate both large and patch intertidal and subtidal reefs. Permanent transect locations are not necessary for this protocol but can be used if a partner site chooses to do so.

<u>Measured Parameters</u>

- Percent cover of living and non-living substrate

Requirements

Personnel: 2 people

Estimated Total Time Per Location (n = 3 transects per site)
- Preparation: 1 person x <1 day
- Fieldwork: 2 people x <1 day per location
- Post processing: None
- Data processing: 1 person x <1 day

Replication: 5 replicate quadrates per transect, 3 replicate transects per reef, 3 reefs per region

Materials:

<u>Fieldwork:</u>

- ☐ 81-point PVC quadrat (Figure 1)
- ☐ 30 m transect tape (3)
- ☐ Camera
- ☐ Oyster reef composition data sheets

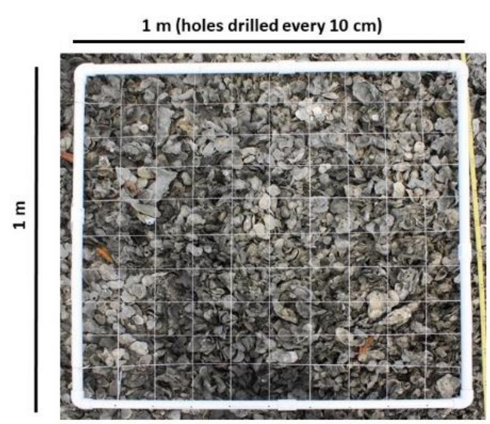


Figure 1. 1x1 m quadrat with 81 intersecting points, 9 holes drilled every 10 cm on each side with string pulled through, PVC can be ¾'' – 1'' in diameter.

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

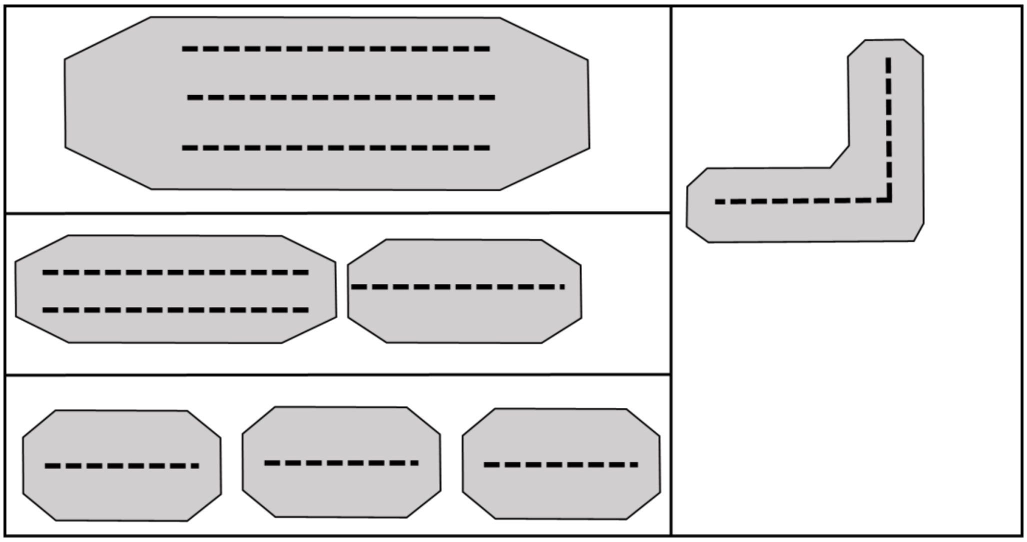
Preparation:

1. Review the MarineGEO Oyster Reef Habitat Survey Design for selection of permanent sites.
2. Become familiar with the methodology prior to going out into the field to conduct sampling.
3. Print datasheets on waterproof paper.
4. This protocol assumes that n = 5 replicate quadrats for percent cover are taken per each transect. Three (n = 3) transects are done per each site.
5. For intertidal reefs, sampling is done at a low tide when oysters are exposed. For subtidal reefs, timing of sampling is site dependent but when water clarity is maximized.

Fieldwork:

1. Lay out three 30 m transects across a single reef. The first should go along the area where the continuous average density of oysters are and should not start at the reef edge. The subsequent 2 transects should go to the left and to the right of the initial transect and at least 1.5 m from the center transect. In many cases, the crest of the reef is not an area with an extensive amount of live oysters and so it is important to not default to this location for the central transect.
2. Ideally 3 transects should be used per reef and sampling from 5 replicate 1x1 m quadrats are done per transect. However, if a single reef is small and there is more than one reef per individual site, several reefs can be used. If this is the case, it is important to note which transects belong to each reef so that the same reef can be sampled in the future. Permanent transects are not necessary for this protocol, however, repeated sampling along the same reef over time is required. For patch reefs and those less than 30 m, lay out a transect to each edge of the reef where the oyster density is average to that throughout the transect. If multiple transects fit parallel on a patch reef, they need to be at least 1.5 m from each other. The overall goal is to obtain 15 replicate quadrat samples per site though because oyster reefs are not uniform in size and shape, some sites might have to adapt a more complex design or reduce the amount of sampling. For example, some reefs might only be large enough to fit 2 quadrats along a transect, though if multiple small reefs are within the vicinity, enough replicates can be obtained to get a robust average of oyster composition. See Figure 2 for potential placement of transects. In such scenarios, please contact MarineGEO to verify altered methods.
3. For a transect reaching 30 m, 5 replicate quadrats are taken along predetermined points. At 5, 12, 15, 20, and 25 meter marks, lay the quadrat to the right of the transect tape with the meter value touching the lower left corner of the quadrat. For transects that do not reach the desired 30 m, quadrats can be placed at points determined by the practitioner but should be at least 1 m from each other (e.g. 1 m, 3 m, 5 m, etc.) and this should be noted on the data sheet.

Figure 2: Potential transect designations.



4. Tally what occurs under each intersecting point (totaling 81 points). Because oysters are the primary target, the tally should quantify the presence and state of oysters. In some cases, seasonal or ephemeral macroalgal blooms can occur on reefs, forming a canopy attached to and covering oysters. In other instances, occasional or rare species (algae, barnacles, etc.) can be found growing on oysters though are not numerically dominant. In either case, all species growing on oysters or forming a canopy get counted separately as secondary species (see data sheet for separate columns of primary vs. secondary species). Major categories include:
    - Live oyster
    - Gaper oyster (gaping dying oyster with visible tissue, uncommon)
    - Box oyster (gaping dead with both shells still attached, no visible tissue)
    - Cultch (dead with only single shell remaining)
    - Shell hash
    - Sediment
    - Algae (be specific if possible)
    - Rock
    - Any other groups (bivalves, ascidians, sponges, etc.) using lowest taxonomic classification as possible
5. Once finished scoring the quadrat, double count the tallied values to make sure all points were recorded.
6. Repeat for each quadrat along each transect.

7. It is recommended that for intertidal reefs, a photo of the quadrat prior to scoring be taken and labeled for historical records. For subtidal reefs this can be a bit more challenging but is still recommended if conditions are suitable.

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
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Oyster Density and Size Frequency

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Introduction

This protocol provides standardized data collection on live oyster density (>15mm) and the size frequency of oysters on a reef. Because a reef is structurally complex, the accurate number of live oysters can't be adequately measured through percent cover alone. Along with the density of oysters, the size frequency of those oysters is taken to provide information on how the oysters are distributed across different size classes. Other encountered species including bivalves and gastropods are counted and measured as well. Data on these species are important and provide detail on oyster predators and space competitors. Decapods are enumerated in the Oyster Reef Associated Fauna protocol and are NOT sampled here because of the difficulties in field identification and collection. The methods here are semi-destructive, due to excavating a portion of the reef, however, once measured, all oysters are returned to the excavation site.

<u>Measured Parameters</u>

- Oyster density (individuals per m^2)
- Associated invertebrate density (individuals per m^2)
- Oyster size frequency (length (mm) per live and box oysters)
- Associated invertebrate size (length (mm) per individual)

Requirements

Personnel: 2 people

Estimated Total Time Per Location (*n* = 3)

Preparation: 1 person x < 1 day
Fieldwork: 2 people x < 1 day per location
Post processing: None
Data processing: 1 person x <1 day

Replication: 3 0.25 x 0.25m excavated quadrates per reef and 3 oyster reefs per region

Materials:

<u>Fieldwork:</u>

- ☐ 0.25 m x 0.25 m PVC quadrat
- ☐ Calipers
- ☐ Buckets
- ☐ Work gloves
- ☐ Oyster reef density and size frequency data sheets

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:

1. Review the MarineGEO Oyster Reef Habitat Survey Design for selection of permanent sites.
2. Become familiar with the methodology prior to going out into the field to conduct sampling.
3. Print datasheets on waterproof paper.
4. This protocol assumes that *n* = 3 replicate quadrats for oyster density and size are taken per site, 1 per each transect.
5. Sampling is typically done at a low tide when the oyster reef is exposed. For subtidal sites, sampling is done when water clarity is maximized.

Fieldwork:

1. Along each transect, haphazardly choose one representative quadrat while conducting the Oyster Reef Composition protocol to do an excavation. Once the percent cover is scored from the previous protocol, place a 0.25 x 0.25m quadrat inside the area of the larger quadrat used for percent cover. Within the smaller quadrat, excavate all oysters to the sediment level or where it is assumed no living oysters still remain. All material is placed into buckets and be rinsed to remove sediment. Figure 1
2. From the bucket, randomly remove all material and measure (mm) with calipers (Figure 1) the first 50 live oysters that are **above 15 mm in length** and the first 25 box oysters encountered. For clumps, rotate the clump and measure any live oysters found. Take care to **not** break apart oyster clumps. Young oysters (< 15 mm) are not be measured because this can lead to underestimates in the average size of adult oysters and many young oysters will not survive as well. Measurements are taken on the height of the oyster (umbo to distal edge of the shell). Once the limit for measurements is reached, count all the remaining live and box oysters above 15 mm to obtain an accurate density.

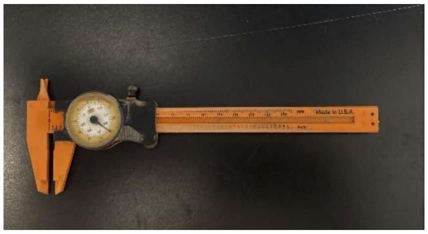

Figure 1: calipers (mm)

3. For bivalves and gastropods, count all and measure the first 25 individuals for each genus/species encountered.
4. For all other sessile invertebrates encountered including sponges, ascidians, barnacles, polychaetes, etc., mark each as present within each replicate quadrat on the data sheet. Counting these species can be impractical and misleading.
5. As material gets processed, it can be placed into an empty bucket. Once all the material has been processed, the bucket can be carefully placed back into the excavation pit.
6. Repeat this once for each transect *(n* = 3 per reef)

Alternative Methodology:

1. Because oysters coalesce with one another, it could be difficult or too destructive to excavate a portion of the reef. It might be possible to at least count and measure oysters without removing them from the substrate to get measurements of the number and size or oysters in a given area.

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
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Oyster Reef Associated Fauna

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MarineGEO
THE TENNENBAUM
MARINE OBSERVATORIES NETWORK

Smithsonian Institution

Introduction

This protocol provides standardized data collection on the associated biodiversity found living within an oyster reef. Here, the use of "bio-boxes" of a known size are deployed on the reef 1.5 – 2 months prior to sampling and allowed to be colonized by resident mobile macrofauna (crabs, shrimp, etc.). Details are given on how to construct cost-effective bio-boxes, however, these can be constructed of the practitioner's choice though must have a known area such that data can be reported as individuals per meter^2. Post-processing requirements include identification and enumeration of associated fauna done in the lab.

<u>Measured Parameters</u>

- Individuals (m^{-2})

Requirements

Personnel: 2 people

Estimated Total Time Per Location (n = 3 bio-boxes per site)

Preparation: 1 person x <1 day
Fieldwork: 2 people x <1 day per location
Post processing: 1 – 2 people x 3 days
Data processing: 1 person x <1 day

Replication: 3 bio-boxes (0.5 x 0.5m) deployed at each reef, 3 oyster reefs per region

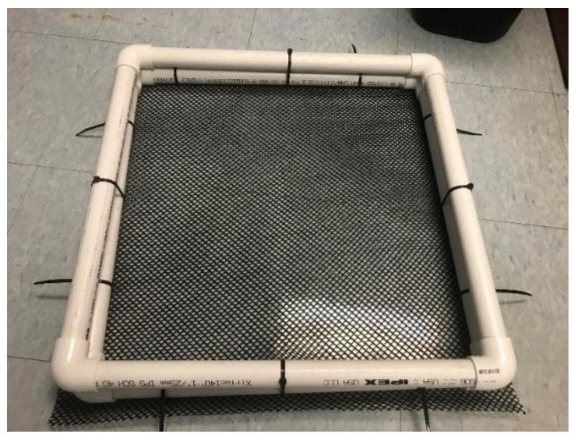

Figure 1: A fully constructed example of a biobox (0.5 x 0.5m).

Materials:
<u>Fieldwork:</u>

- ☐ Bio-boxes (3 per reef)
  - 0.5 m length PVC (1" diameter) with several holes drilled to reduce buoyancy
  - 1" PVC elbows
  - 6.25 mm vexar mesh (or smaller)
  - Cable ties
- ☐ Forceps
- ☐ Collecting jars (0.5 liter per bio-box)
- ☐ Large enough tray to place bio-box in for sorting in field

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:
1. Review the MarineGEO Oyster Reef Habitat Survey Design for selection of permanent sites.
2. Deploy bio-boxes in triplicate at each site 1.5 – 2 months prior to sampling.
3. Become familiar with the methodology prior to going out into the field to conduct sampling.
4. Print datasheets on waterproof paper.
5. Sampling is typically done at a low tide when the oyster reef is exposed. For subtidal reefs, collection of bio-boxes can be done at practitioner's choice though should be collected in the summer months.

Fieldwork:
1. Deploy 3 bio-boxes per reef approximately 1.5 – 2 months before field sampling during a low tide. Bio-boxes can be placed either at the edge of a reef to reduce disturbance or within the reef itself. Within the reef, oysters should be excavated, and bio-boxes placed into the substrate so that the top of the box is mostly level with the substrate. Fill the bio-box with the excavated oysters such that it resembles the density of the reef. For reefs with low oyster cove, placing an excessive amount of material in the bio-box could lead to inflated counts. In high wave areas, bio-boxes can be secured with rebar or plastic dowels though in general, the weight of the oysters inside the box is sufficient to hold them in place. The PVC itself can also be filled with sand or rebar to assist is securing the bio-box in place. If placing bio-boxes at the edge of a reef, loose oyster shell and clumps can be collected in put into the bio-box with an amount the resembles the reef itself. Keep replicates several meters apart from each other.
2. After the allotted time for colonization, return to the reef to collect bio-boxes. This is typically done when other sampling is being conducted. To do this, lift the bio-box and immediately place it in a large tray. For subtidal sites, remove the bio-box from the substrate and return to the surface to place within the sorting tray.
3. Carefully pick through the material and collect all associated macrofauna either using fingers or forceps and place into a labeled sampling container. Spend a good amount of time with oyster clusters as crabs can easily hide and be difficult to locate. However, do **not** break apart oyster clumps. Larger crabs, gastropods, and fish can be noted as found and released alive. Within the reef, abundant smaller mobile fauna (polychaetes, amphipods, etc.) can be found. If possible, these can be collected and noted as present/absence, however, the focus here is on larger invertebrates (> 5 mm) and fish. Field collections of smaller species are time-consuming and often lead to underestimates.
4. Once all shells have been picked through, the rest of the sediment and smaller shell hash can be picked through in the tray or sieved. If sieving the material, a sieve size < 6.25 mm is recommended as that is the size of the mesh on the bottom of each bio-box.

5. Sampling containers should have labels, filled with 70% ethanol in the field, and brought back to the lab to be processed at a later date.
6. Material from bio-boxes should be returned to the where the bio-boxes were collected from.

Post-Processing:
1. All associated fauna is identified to the lowest taxonomic level and counted.

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet: https://marinegeo.github.io/data-submission
4. Contact us if you have any questions: marinegeo-protocols@si.edu

Oyster Reef Rugosity

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MarineGEO

THE TENNENBAUM MARINE OBSERVATORIES NETWORK

Smithsonian Institution

Introduction

This protocol provides methods on standardized data collection to measure rugosity of an oyster reef. Rugosity is defined as the three-dimensional arrangement of structural features and can be used as a proxy for habitat complexity. Rugosity (**Rq**) is measured by a chain method in which a chain of known length is hung over the substrate in a straight line. A rugosity index is calculated as **Rq = d / l** where **d** = distance (m) covered by the chain along the substrate and **l** = length of chain (m) fully extended. A value approaching 1 indicates a nearly flat surface and decreases as the substrate becomes more structurally complex.

<u>Measured Parameters:</u>

- Ratio of chain-laid distance to fixed distance (**Rq**)

Requirements

Personnel: 2 people

Estimated Total Time Per Location (*n* = 15 measurements per site)

Preparation: 1 person x <1 day
Fieldwork: 2 people x <1 day per location
Post processing: None
Data processing: 1 person x <1 day

Replication: At least 15 measurements per oyster reef or collectively for patch reefs

Materials:
<u>Fieldwork:</u>

- ☐ Stainless steel ball chain (2 mm ball width, 1 m length, amazon.com has several acceptable types)
- ☐ 30 m transect tape(s)
- ☐ Oyster reef rugosity data sheets

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:

1. Review the MarineGEO Oyster Reef Habitat Survey Design for selection of permanent sites.
2. For intertidal reefs, sampling should be done at a low tide when the oyster reef is exposed. For subtidal reefs, the timing of tides is not an issue though this should be done when water clarity is maximized.

Fieldwork:

1. In conjunction with the Oyster Reef Composition protocol, rugosity is measured at predetermined meter marks (5, 10, 15, 20, and 25) along each of the 3 transects. For reefs less than 30 m and patch reefs, rugosity measurements are taken at locations where quadrats are done.

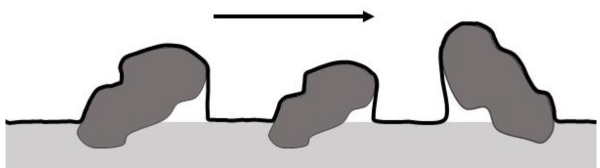

Figure 1. Chain conforming to substrate.

2. At each point, lay the chain along the substrate, parallel with the transect and conforming with the substrate (Figure 1). When laying the chain, allow it to conform with the upper surface of the substrate and once over, drop the chain straight down and continue. Do not attempt to push the chain against the overhanging portions of the substrate.
3. Measure the distance that the chain reaches using the transect tape and record this on the data sheet.
4. Rugosity: **Rq = d** / l where **d** = length of measured distance (m) and **l** = total length of chain (1 m)

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet: https://marinegeo.github.io/data-submission
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