NSF Eelgrass Disease Project
2019 Handbook

Version 1.2

Questions? Contact MarineGeo@si.edu

Table of Contents

Field Sample Cheat Sheet 5
Codes 5
Field sampling (in rough temporal order) 5
Don't come back from field without the following! 6
Identifying the 3rd Rank Blade 8
Eelgrass Epifauna 10
Measured Parameters 10
Requirements 10
Materials 10
Fieldwork: 10
Post-processing (per site): 10
Preparation 11
Fieldwork 11
Post-processing 11
Macrophyte wet mass 11
Sieve Processing 12
Eelgrass microbiome 13
Summary 13
Goal 13
Sampling process 13
Supplies (provided by Stachowicz lab): 13
Instructions 14
Selecting Lesions for qPCR 16
Eelgrass Epiphytes 17
Measured Parameters 17
Requirements 17
Materials Checklist: 17
Field 17
Post-processing 17
Preparation 17
Fieldwork 18
Sample Processing: 18
Data Submission 19
Sampling for Disease Scans 20

Materials 20
Processing Eelgrass Blades 20
Roles 21
Sample processing....21
Special notes about cleaning and scanning subtidal blades 22
Scanning Eelgrass Blades....22
Materials....22
Scanning 23
General notes about processing blades 25
Disease Scan Transparency Diagram....26
Identifying and Measuring Lesions....27
Uploading Eelgrass Scans for scoring by *EELISA* 27
Eelgrass Density 28
Measured Parameters 28
Requirements 28
Materials: 28
Survey Design:....28
Fieldwork: 28
Preparation 28
Fieldwork....29
Data Submission....29
Water (eDNA) sampling protocol 30
Goal....30
Field sampling process 30
Supplies....30
Instructions....30
Lab filtering process....31
Supplies....31
Instructions....31
qPCR Laboratory Protocol 33
Materials needed....33
qPCR Sampling protocol....33
Prepare for the field....33
In the field....34
On return to the lab....34

Now you are ready to start sampling the leaves!....34
Appendix I: Seagrass Macroalgae....36
Measured Parameters....36
Requirements....36
Materials:....36
Survey Design....36
Fieldwork....36
Post-processing....37
Methods....37
Preparation....37
Fieldwork....37
Post-processing....38
Data Submission....38

Field Sample Cheat Sheet

Codes

Site codes

[2 letter region code]-[1 letter site code]
Example: WA-A (site A in Washington FHL region), BB-E (site E within Bodega Bay region)

Transect codes

3 upper shore(U): labeled by number, 1-3, left to right when looking at shore from water
3 lower shore (L): labeled by number, 4-6, left to right when looking at shore from water
Example: WA-A-U1 = leftmost upper transect at Friday Harbor (WA) site A

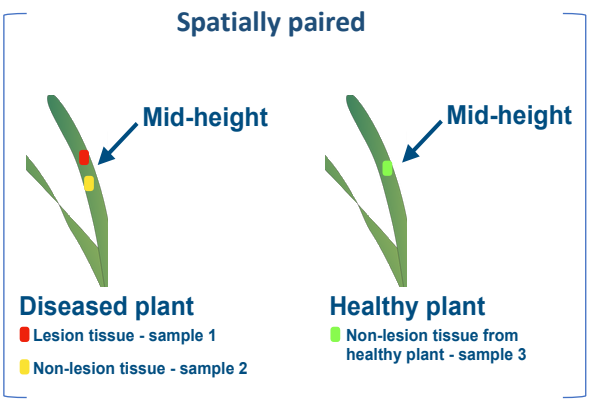
Quadrat codes

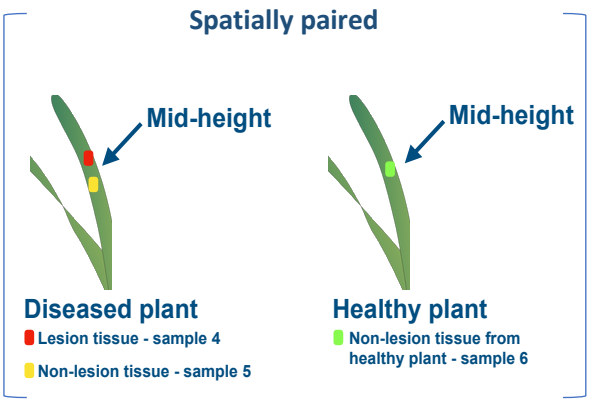
Labeled by [site code] – [transect code] – [quadrat position in m]
Example: WA-A-U1-4 = quadrat at 4 m along transect WA-A-U1

Field sampling (in rough temporal order)

1. Sample epifauna (falling tide, preferably while eelgrass is still submerged)
   Sample with mesh bag at 4 and 16 m along each transect (n=2 per transect, n=12 per site)

2. Sample microbiome and diseased blades (for qPCR)
   Sample at 2 points on each transect:
      Collect a pair of shoots (1 diseased, 1 non-lesioned)
   From each diseased shoot: Remove 2 microbiome samples (1-2cm long piece of leaf) and place in labeled vials of Zymo buffer
      1 Lesioned tissue sample
      1 Non-lesioned tissue sample
   From each diseased shoot: Retain remainder of leaf for qPCR, place in whirl-pak with filtered seawater
   From each non-lesioned shoot: Remove 1 microbiome sample (1x1 cm) and place in labeled vial of Zymo buffer





3. Sample epiphytes
   Collect shoot (or 3rd rank blade) at 5 positions (4,8,12,16, 20 m) along each transect
   Place sample in pre-labeled Ziploc bag into cooler

3a. Sample canopy height and sheath length
   Only If epiphytes were sampled from 3rd ranked blade (rather than whole shoot), then collect the following data:
   -Measure eelgrass longest blade length and width (at mid-leaf) at 5 positions along transect
   -Measure eelgrass sheath length at same 5 positions along transect

4. Sample quadrats for cover and shoot density
   Record percent cover and shoot density at 4 positions (4,8,12,16 m) along each transect (n=4 per transect, n=24 per site)
   Record data on standard data sheet

4a. Sample macroalgae in quadrats
   Collect all macroalgae within 3 of the 4 quadrats (positions 4,8,12 m)
   Place in prelabeled mesh bag or ziplock (one bag per quadrat)

5. Sample diseased blades for scanning
   Sample 15 blades, roughly evenly spaced (one per meter) per transect to be scanned for disease (for a total of 20 blades when combined with epiphyte samples)
   Blades from each transect can be combined in a single ziplock bag
   (n=15 per transect, n= 90 per site)

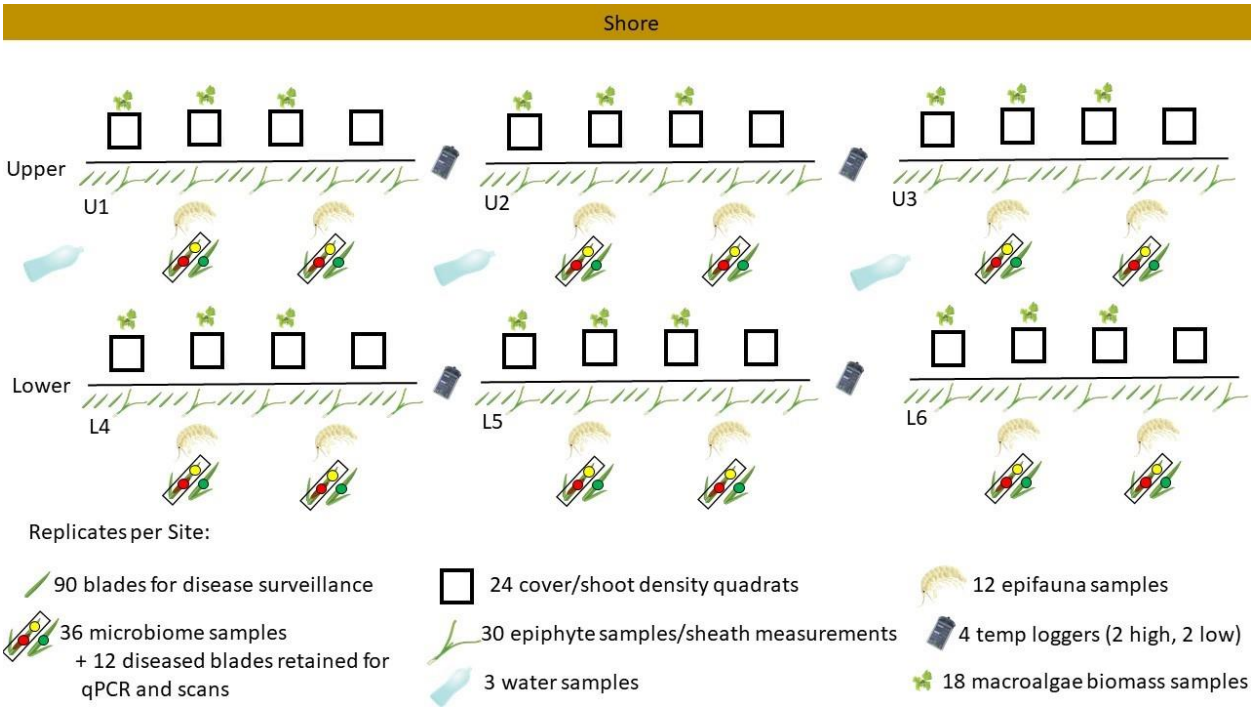
6. Sample water for microbiome (sample at end because must be filtered within 2 hours)
   Collect 3 water samples per site for eDNA, place in sterile 500 ml bottles provided

7. Get environmental data
   Check hobo temperature loggers and ideally download data
   Record salinity with refractometer and record on site metadata sheet

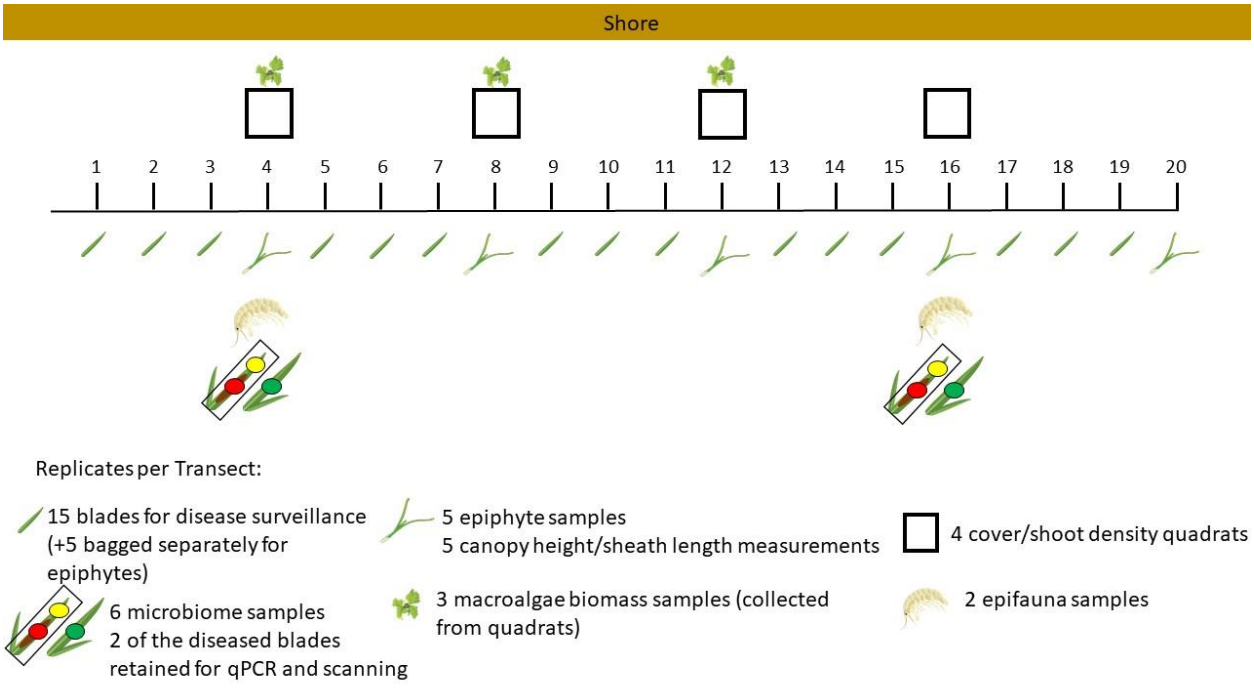
Don't come back from field without the following!

- ☐ Epifauna: 12 mesh bags containing epifauna (2 from each transect)
- ☐ Microbiome: 36 zymo tubes with tissue samples (6 from each transect)
- ☐ Blade samples for qPCR: 12 whirl-paks containing diseased blades (2 per transect)
- ☐ Epiphyte samples: 30 ziplock bags with blades or shoots (5 from each transect)
- ☐ Blade length, width, sheath length data: completed data sheet
- ☐ Eelgrass percent cover and shoot density data: completed data sheet
- ☐ Macroalgae: 18 mesh bags or ziplocks containing macroalgae hand collected from quadrats
- ☐ Blade samples for disease scans: 6 ziplock bags containing 15 blades each (1 bag per transect)
- ☐ Water samples for eDNA: 3 500-ml bottles per site
- ☐ Completed site metadata field sheet

ENTIRE SITE



SINGLE TRANSECT



Identifying the 3rd Rank Blade

For assessing seagrass wasting disease, collect the 3rd-rank eelgrass blade, as this is consistent with other methods.

1. Hold an eelgrass shoot from the base of the sheath to steady it as you work.

2. Looking at the top of the shoot, identify the 1st (innermost, generally shortest) blade growing out of the sheath.

3. Then look closely for the 2nd blade growing on the opposite side of the sheath.
    a.) It should be the next blade you come to on the sheath.

4. If you look on the opposite side of the sheath (again), the next blade along the sheath will be the 3rd rank blade.
    a.) This 3rd blade is the one to collect for disease measurements.
    b.) Gently pinch/tear off the 3rd blade at the top of the leaf sheath.

Note: Even if the 3rd blade is torn, still collect it for measurements for consistency. This method of ordering blades (determining blade rank) is consistent with Short and Duarte 2001.

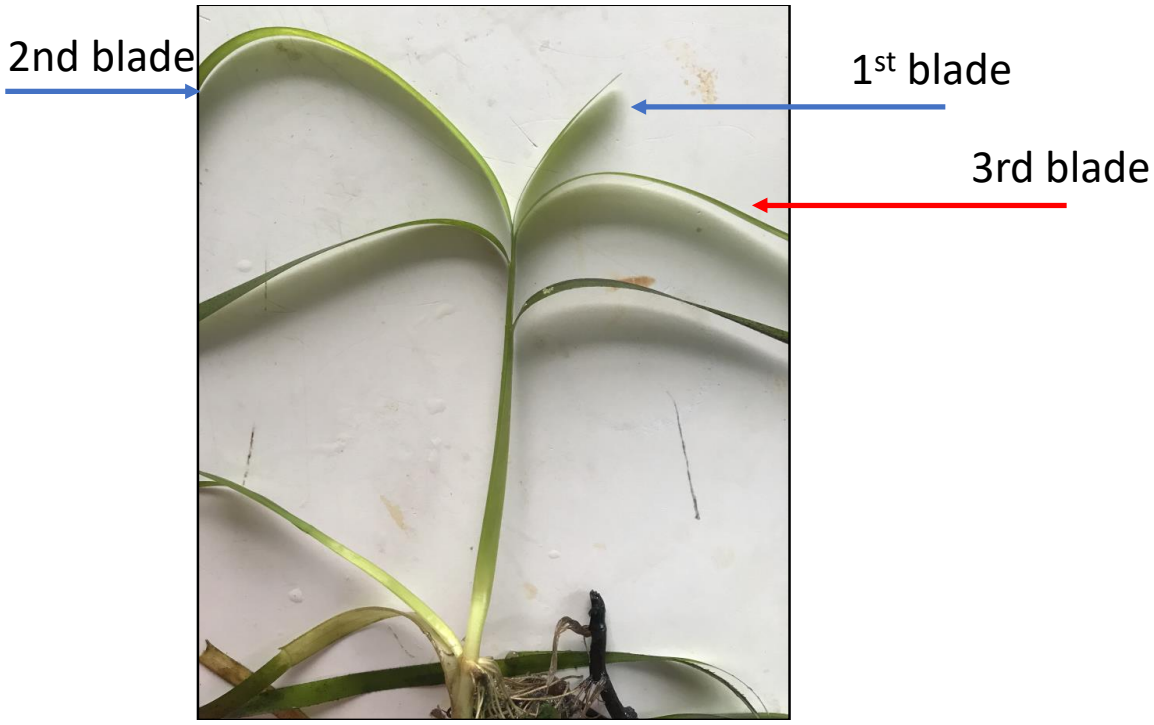


Figure 1: Blades ranked 1-3 on an eelgrass shoot.

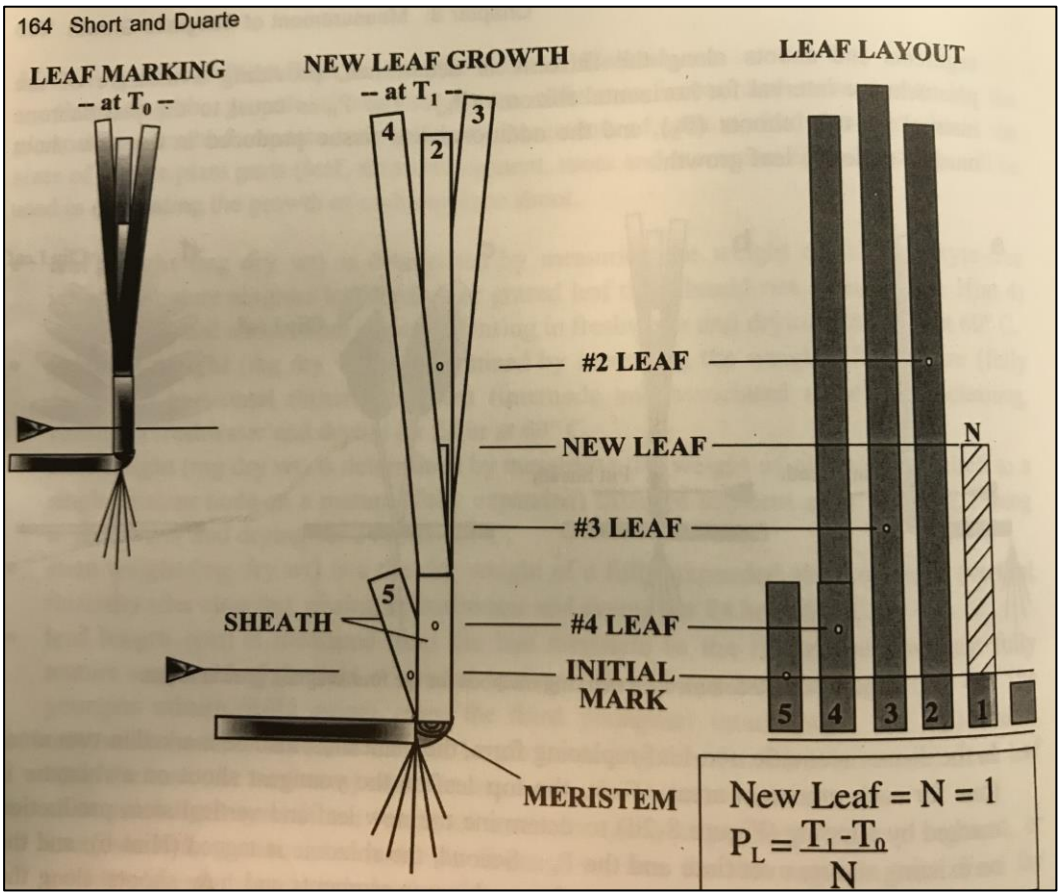


Figure 2: Leaf numbers and layouts from: Short FT and Duarte CM. 2001. Methods for the measurement of seagrass growth and production. In FT Short and RG Coles (Eds.), Global Seagrass Research Methods (pp. 164). Amsterdam, The Netherlands: Elsevier Science B.V.

Eelgrass Epifauna

Measured Parameters

This assay quantifies characteristics of seagrass epiphyte and mobile epifaunal communities, measured as:

- Macrophyte biomass (mg)
- Epifaunal abundance and taxonomic composition (individuals)
- Epifaunal biomass (mg dry or ash-free dry mass)

Requirements

Replication: Two (2) epifaunal samples taken using mesh bags along six (6) transects (total n = 12 per location).

Materials

Survey Design:

☐ At least 2 20-m metric transect tapes
☐ Hand-held GPS unit
☐ 2 PVC marker poles (diameter and length as needed)

Fieldwork:

☐ 12 draw-string mesh bags (500 µm mesh size, approximate dimensions: 75 cm x 20 cm, with 20 cm opening)
☐ Waterproof paper for internal labels
☐ 1 cooler with ice (optional)

Post-processing (per site):

☐ 20+ pre-weighed foil tins
☐ 1000 µm mesh sieve
☐ Sorting tray
☐ 20+ scintillation vials (20-mL) with lids
☐ 70% ethanol (0.5-1.0 L)
☐ Petri dishes
☐ Forceps (fine-tip)
☐ Pen/pencil
☐ Drying oven
☐ 1mm sieve

Preparation

1. This protocol assumes n = 2 bags taken at meters 4 and 16 along a 20-m transect, replicated along 6 separate transects.
2. Use 12 of the labels provided for your site (printed on waterproof paper), and place inside 12 mesh bags (500 µm mesh size).
    a. Recommended: Add an additional label to the drawstring using duct or electrical tape, just in case the paper label falls out.
3. Fill a cooler with ice immediately before departing for the field.

Fieldwork

1. Record site metadata on field sheet: start time, date, tidal height. Take several photos to document conditions at the site.
    a. For intertidal meadows, make an effort to collect epifauna samples immediately after deploying transects, on a falling tide.
2. At each replicate along the transect, randomly select a patch to the right hand side of the transect when standing at the 0m mark facing the 20m mark. Be sure NOT to sample within the quadrat used for quantifying percent cover, as this may affect surveys in subsequent years.
3. Position the mesh bag over the canopy and gently lower it over the eelgrass, being careful to avoid disturbing or dislodging any organisms or macroalgae. It may be necessary to move the bag from side to side to gently guide the eelgrass blades through the opening.
    a. Once the opening of the bag is just above the surface of the sediment, close the bag by pulling the drawstring and either cut or tear the exposed shoots at the sediment surface to release them into the bag. (Avoid uprooting belowground material)
    b. Invert the closed bag and bring it up to the surface, flushing the contents fully into the bottom of the bag, then close the drawstring and tie a knot at the top of the bag to prevent the bag from accidentally opening.
4. Place the bag with contents on ice in the cooler.
5. Repeat steps 1-4 at the next location along the first transect until all 3 replicates are taken.
6. Repeat steps 1-5 for the remaining five transects for a total of 12 samples.
7. Transport cooler with samples back to the lab for processing.

Post-processing

Samples are best processed immediately (within 24 hours) upon returning from the field. Samples can be stored for longer in the freezer but risks damaging the organisms and making them difficult to identify.

Macrophyte wet mass

1. Print lab data sheets.
2. Weigh foil tins and record the weight of the tin directly on the foil using a pen. Tins can be either pre-made, or constructed by folding an aluminum foil square over on itself and sealing the sides.
3. Open a mesh bag and record the metadata from the internal label on the lab data sheet.

4. Gently rinse the contents of the bag into a shallow sorting tray with water. Rinse both the bag and all plant material, separating epifauna from eelgrass and algae.
5. For eelgrass blades, select a pre-weighed tin and label with the sample metadata (replicate number, date, location).
    a. Separate the eelgrass blades and macroalgae and place into separate corresponding labeled tins. ***Be careful that no animals are transferred with the epiphytes.*** This may require picking animals one-by-one out of more complex substrates.
    b. Record the sample data, species name, and the empty tin weight on the lab data sheet.
6. Once no plant or algal material remains in the sorting tray, pass the contents through a 1.0 mm mesh sieve. Gently rinse any loose material through the sieve, and then transfer the remaining contents to an internally and externally labeled 20-mL vial filled with 70% ethanol. Multiple vials may be required for larger samples or organisms. Set the vial aside for epifaunal processing at a later date.
7. Repeat steps 3-6 for each sample.
8. Weigh macrophytes (wet) to nearest mg. Record this weight (including tin weight) on lab data sheet.

Sieve Processing

(To be completed at SERC, mailing instructions to follow)
This step can be done at your leisure as organisms are now preserved in 70% ethanol.

9. Print lab data sheets.
10. Stack the sieves from the smallest mesh size on the bottom to the largest mesh on top.
11. Select a 20-mL vial with epifauna (Step 1, #7), open the top, and gently invert it over the top sieve, allowing the contents to pass onto the sieve tower.
12. Use a hose or squirt bottle to gently rinse out the vial with tap or distilled water and empty its contents into the sieve tower until the vial is empty.

Eelgrass microbiome

Summary

From each transect, there will be a total of six samples collected and preserved in a total of six 2-mL Zymo buffer tubes. However, these samples will be spatially paired in two groups as described below. Sample collection and preservation will occur in the field. Between every sample please sterilize your utensils (scissors and tweezers) with provided alcohol wipes. If you find the tweezers too difficult to work with, and need to use your gloved-hands to assist the collection process, make sure to sterilize your gloved-hands with alcohol wipes between each sample. We have provided packs of Ultra-Thin Polyurethane Coated Nylon Safety work gloves in assorted sizes. Please wear these gloves or something similar (nitrile or latex gloves are OK too) throughout the entire sampling process. The palm sides of the gloves provided in this kit are coated with polyurethane and should be sterilized with alcohol wipes at the start of sample collection. The purpose of sterilizing utensils (tweezers and scissors) between every sample is to avoid cross-contamination of bacterial DNA between samples. This is critical to successful characterization of bacterial microbiomes of eelgrass between healthy and diseased blades! Thank you for your attention to detail here.

Goal

Two diseased plants and two healthy plants along each transect will be sampled. Select diseased plants that have an obvious lesion on them (do not try to pick a representative plant for that site, but rather something that looks convincingly like a lesion— *see lesion guide*). From the 1st diseased plant, you will collect a small section (1 cm piece of leaf) of lesioned tissue (SAMPLE 1) from the 3rd rank leaf mid-height and the nearest part of the same blade that is lesion-free (SAMPLE 2). Each of these samples will be preserved in separate 2mL tubes with Zymo buffer (tubes are pre-filled and labeled). Make sure that the buffer completely covers the sample. If there are no lesioned plants at a site, then skip this step and leave the tubes for sample 1 and 2 empty. Next, find the nearest plant that does not have an obvious lesion, and take a small section of lesion free tissue (1 cm piece of leaf) of healthy blade from the 3rd rank leaf mid-height (SAMPLE 3). Repeat this process again for samples 4-6 (SAMPLE 4,5,6) with a new set of plants along transect separated by at least 3m from the first pair of plants. The color of 2mL Zymo buffer tube stickers correspond to tissue type and are depicted in the diagrams at the end of this section.

Sampling process

We strongly encourage you to designate one pair of samplers as the “microbial team” and have them collect samples from each transect as soon as transects are laid out.

Supplies (provided by Stachowicz lab):

- Metal tweezers
- Metal scissors

- 2 mL Zymo-filled buffer tubes – 36 tubes per site
- Sterile alcohol pads (2 per sample plus 10% extra)
- Polyurethane-coated gloves

Instructions

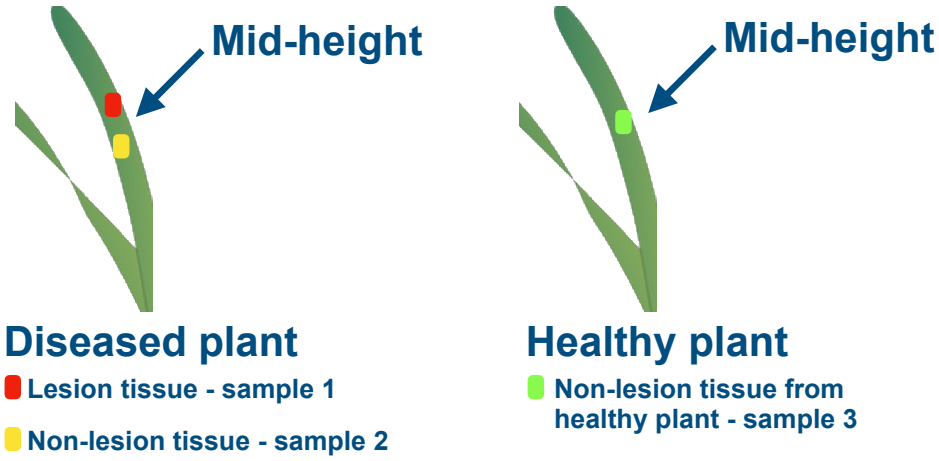
1. sterilize your gloves, scissors, tweezers using an alcohol wipe
2. find 3rd leaf from diseased plant at transect #1
3. use sterile tweezers to hold tissue sample that will be preserved
4. use sterile scissors to cut a 1 cm long section of leaf around the tissue section you are holding with your tweezers.
5. place tissue sample in appropriate 2 mL Zymo buffer tube (see color scheme below). Ensure that leaf sample is completely covered by buffer.
6. For the diseased plant only, place the sampled leaf into a whirl pack pre-filled with ~25mL 0.22 filtered seawater (or artificial seawater). Place on ice and bring back to the lab for DNA tissue processing. See qPCR protocol for further details.

Repeat, starting with:

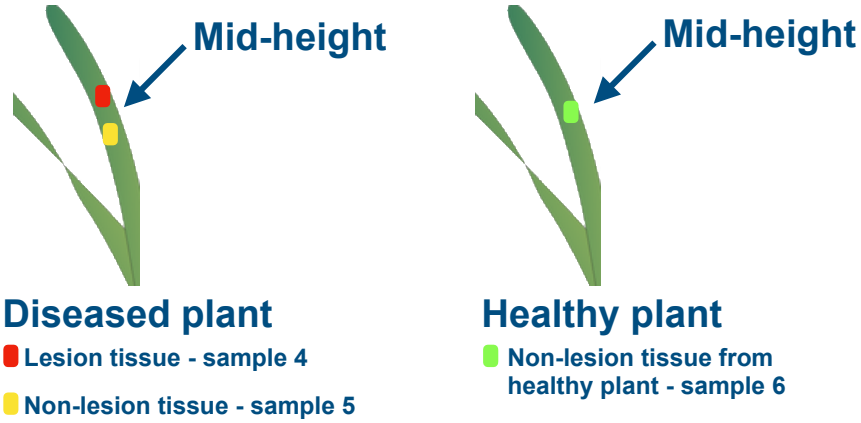
Step 1: sterilize your gloves, scissors, tweezers …

Samples 1-3 and 4-6 are spatially paired. Colors in the diagram below correspond to tissue type. Each 2 mL Zymo buffer tube will have the appropriate color sticker (red – lesioned tissue from diseased plant, yellow – healthy tissue from diseased plant, or green – healthy tissue from healthy plant). To select a lesion refer to the diagram on lesion selection provided by the Harvell lab.

SAMPLES 1 – 3:
These samples are from 2 spatially paired plants:



SAMPLES 4 – 6:
These samples are from 2 new spatially paired plants



The samples are arranged in your boxes by transect, replicate and sample for easy location, but are also individually labeled in case they fall out or get disrupted.

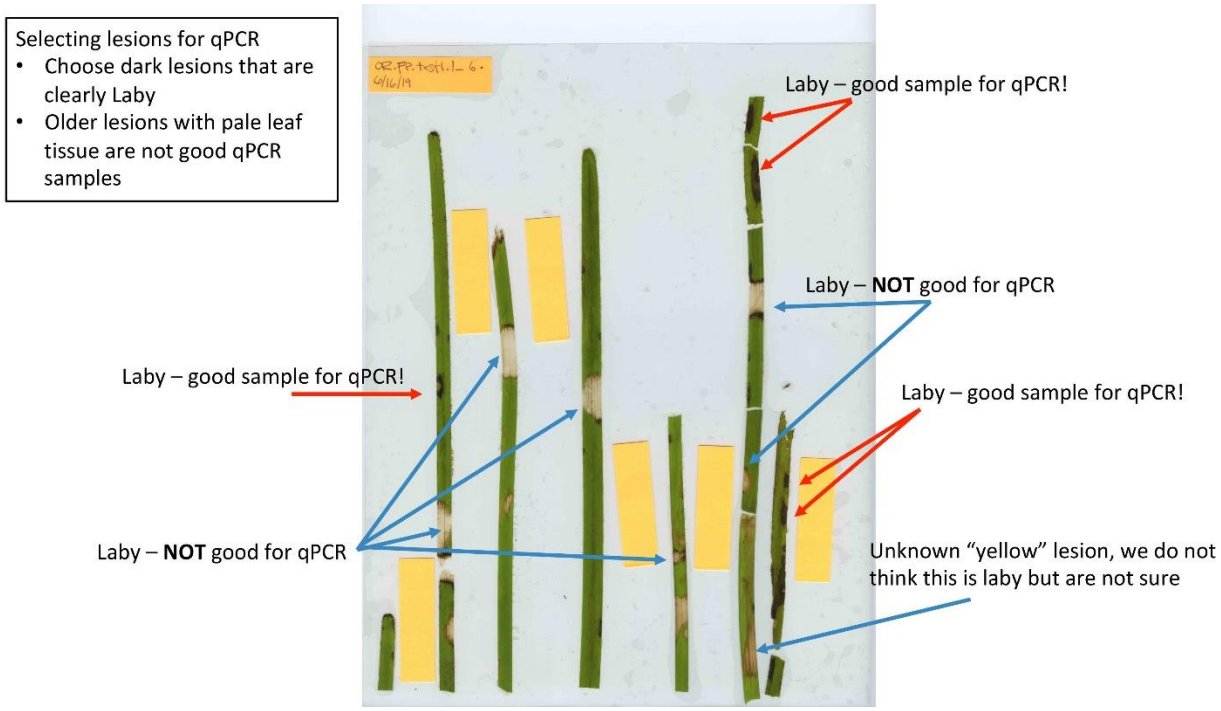
When you return to the lab, microbiome samples should be maintained at room temperature and then shipped to Jay Stachowicz at UC Davis by FEDEX as soon as possible (you can wait to send all the samples in one package, assuming they are all collected within a few weeks of each other). Make sure that caps are tightly snapped onto each tube so that buffer does not leak during shipment. See other notes about how to process qPCR samples and where to send them (reference page number).

Shipping Info for microbiome samples only (qPCR samples go to a different address):

Jay Stachowicz
Department of Evolution and Ecology
2320 Storer Hall
University of California
Davis CA 95616 USA
Phone 530-752-1559
FEDEX account number: 152860439

Please email jjstachowicz@ucdavis.edu to let us know that you are shipping the sample. Please ship early in the week since we cannot receive deliveries on the weekend.

Selecting Lesions for qPCR



Eelgrass Epiphytes

Measured Parameters

This assay determines physical characteristics of seagrasses and fouling community, measured as:

- Blade length (cm)
- Blade width (cm)
- Sheath length (cm)
- Disease lesions (number and length in cm)
- Grazing scars (number)
- Fouling biomass (mg)

Requirements

Personnel:	2 persons
Time:	Preparation: 2 persons x 1 hr.
	Field work: 2 persons x 0.5 days.
	Post processing: 1 persons x 3 days.
	Data processing: 1 persons x 1 hr.
Replication:	1 blade x 5 locations x 6 transects = 30 samples

Materials Checklist:

Field

- ☐ 30 plastic bags with external and internal labels
- ☐ 1 cooler (with ice)
- ☐ Measuring stick (optional)

Post-processing

- ☐ Sorting tray
- ☐ Pre-weighed foil tins (≥ 36)
- ☐ Pencil/pen
- ☐ Microscope slide
- ☐ Ruler (mm)
- ☐ Drying oven

Preparation

1. Label 30 disposable plastic bags using NSF Eelgrass labeling scheme:
    a. Region(2 letters)-Site(1 letter)-Depth(UorL)Transect(1-6)-Position(meters)

   b. Example: WA-A-U1-4 = epiphyte sample from 4 m position along transect WA-A-U1
2. Place 30 internal labels with the same metadata written on waterproof paper inside the corresponding plastic bag.
3. Fill a cooler with ice immediately before departing for the field.

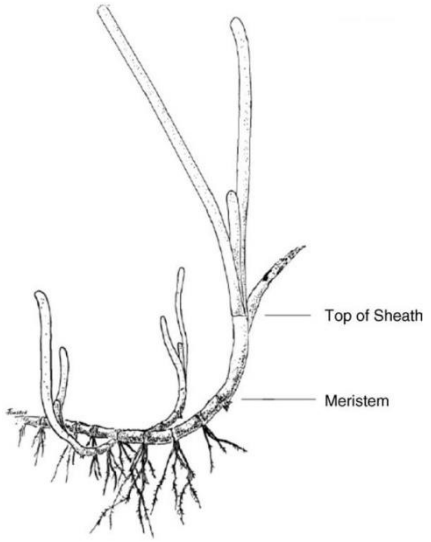


Figure 1. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. Adapted from Gaeckle et al. (2006, Aquatic botany 843:226-232).

Fieldwork

1. Sampling every 4 meters along the 20 meter transect (meter markers 4, 8, 12, 16, and 20).
2. At each location, use a measuring stick to measure the sheath of a haphazardly selected eelgrass shoot. Measure the length and width of the longest leaf. Record the measurements on datasheet. Then, select the third rank blade and gently break the blade from the shoot, being careful not to disturb attached material.

OR

3. At each location, use your fingers to gently break off 1 eelgrass shoot at the sediment surface. Be careful not to disturb attached material.
4. Gently place the blade or shoot and attached material into the corresponding labeled plastic bag.
5. Place the bag and contents on ice in the cooler.
6. Repeat steps 2-4 at the at the remaining 29 sampling locations.
7. Transport cooler with samples back to the lab for immediate processing.

Sample Processing:

Samples are best processed immediately upon returning from the field. Samples can be stored for up to 24 hours in the refrigerator; any longer risks decay.

13. Print lab data sheets
14. Making and weighing foil tins:
    - Use a balance to pre-weigh foil tins (either manufactured, or made by folding an aluminum foil square over on itself and sealing the sides)
    - Record the weight of the tin directly on the foil using a pen.
15. Select a labeled bag and record the metadata on the lab data sheet
16. Gently transfer the blade or shoot from the bag into a shallow sorting tray without any water. If you collected the whole shoot, discard any below-ground material
17. Use a microscope slide to lightly scrape the fouling material from the surface of the blade into one of the pre-weighed tins
18. As long as you are confident that the water you used to transport the sample contained only epiphytes from the 3rd rank blade, sieve the water to collect any epiphytes that fell from the blade.
19. Record the tin's empty/dry weight on the datasheet and label the tin with the sample number
20. If you collected a whole shoot, measure and record:

- The length and width of the longest leaf. Measure width at the approximate midpoint of the blade.
- The sheath length from the top of the sheath surrounding the leaf bundle to the meristem (the visible constriction at the shoot base) to (Figure 1)

21. Whether you collected the whole shoot or just the 3rd rank blade, record:
    - Presence or absence of disease lesions on the 3rd rank blade (1 for present, 0 for absent)
    - Whether the tip of the 3rd rank blade was broken (Y or N)
22. Examine the blades for any evidence of grazing scars on the 3rd oldest blade and record the number on the lab data sheet.
23. After removal of epiphytes, blades will be processed according to the Processing and Scanning Protocol
24. Place all the tins containing fouling material in a drying oven at 60°C. Dry samples until they register a constant weight (usually 1-3 days, depending on the volume of material).
25. Remove tins from the oven and weigh each to the nearest mg. Record the epibiont/fouling dry mass of (including foil) on the lab data sheet.
26. Fouling load will be calculated as mass of epiphytes per unit area of eelgrass blades. Eelgrass blade area will be calculated in post-processing.

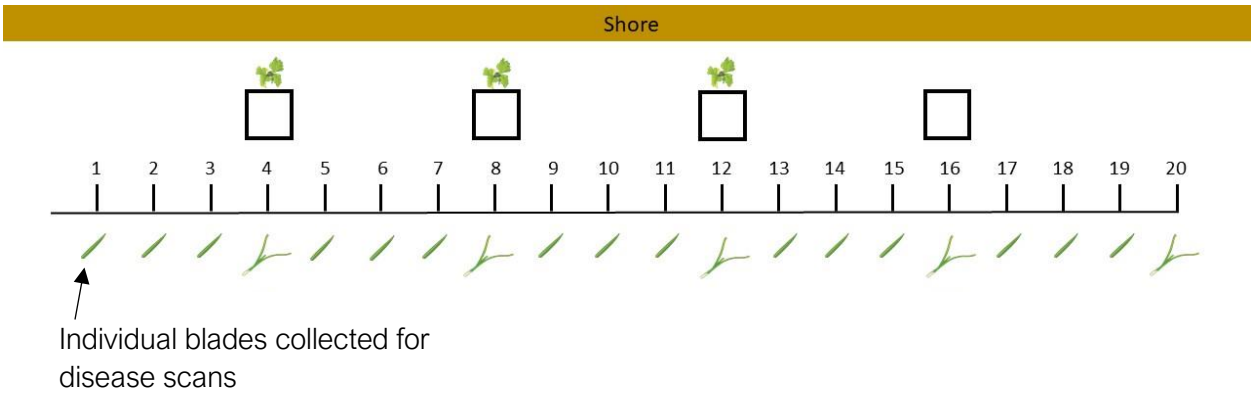
NOTE: Weighing blades may be necessary for a subset of samples.

Data Submission

1. Enter data into provided excel data entry template.
2. Scan the completed lab data sheets as PDFs and save both paper and electronic versions
3. Place all documents (excel datasheet, scanned lab datasheets, and any photos in a new file and rename
4. Upload data entry file, scanned lab data sheets, and any photos to team Google Drive.

Sampling for Disease Scans

In addition to the 5 blades (or shoots) collected to analyze epiphyte biomass (these will be scanned as well!), collect 15 3rd rank eelgrass blades along each of the 6 transects. (N=90 per site) These blades should be placed into one prelabeled ziplock bag per transect. You should leave the site with six bags of 3rd rank blades collected specifically for disease scans.



Lesion Identification & Measurement Protocols: The methods listed here are those used by the Harvell Lab for processing eelgrass blades collected in field surveys. Once we clean, cut, and scan them, we measure the individual lesions on each blade for disease prevalence and severity measurements.

Materials

- Clean transparencies
- Paper towels
- Thin plastic rulers (15 cm) cleaning blades
- Meter sticks
- Wet lab/area that can get dirty while cleaning blades
- Small container with seawater (for sprinkling water onto blades)
- Printed and cut data labels for blades

Processing Eelgrass Blades

Depending on the number of people processing blades, you may be able to set up a "factory line"/processing stations. For example, one person can clean eelgrass blades, one person can cut them and place them on transparencies, one person can collect the transparencies and scan them (in a clean, dry area), and a fourth person can clean transparencies. If the eelgrass is covered in epiphytes, cleaning them can take a considerable amount of time.
The purpose of scanning eelgrass blades, rather than measuring lesions by hand, is to create optimal images of eelgrass for consistent digital image analysis of Labyrinthula zosterae lesions. This way, we can also create an electronic archive of collected blades for future reference.

Roles

- Eelgrass "scraper": cleans blades using ruler. This requires the most work, and is the most-needed job!
- Data recorder: records measurements. Note: We only record the full blade length and width for subtidal blades—not intertidal—since they're too long to fit onto a transparency sheet. See notes below for additional tips.
- Transparency preparer: places measured blades on transparencies in order they were measured, places appropriate label on each
- Scanner: scans transparencies into computer, saves and files images appropriately
- Transparency cleaner: cleans transparencies once scanned (throws away seagrass, rinses
- transparencies in freshwater, layers between paper towels)

Sample processing

1. When you return to lab, store all blades collected from the field in a refrigerator or cooler with ice.
   a.) Note: Keep the blades separated in their transect bags; don't dump them all into a sea table or mix them.

2. Empty your eelgrass samples into a plastic tub, working on one bag of samples at a time. (optional)
   a.) You can also keep the samples in bags on ice while you work.

3. Select a blade of eelgrass. Placing it on your clean works surface, gently remove debris using the edge of a plastic ruler. If you have stubborn debris, you may need to use a little more force. Be careful not to rip the eelgrass.
   a.) Clean the blade thoroughly! This is key, since epiphytes can look like lesions on scanned images. The cleaner the blade, the better the image.

4. Get a nice, clean transparency for your blades. Transparencies should be squeaky clean and free of dried water spots, debris, etc. for optimal images.

5. Leaving a 1.5 inch margin at the top of the transparency for your paper label(s), place you cleaned blade on a clean transparency sheet. Once you reach the bottom of the transparency, gently tear off the rest of the eelgrass with your fingers.
   a.) Align the remaining pieces of the blade next to one another, but not overlapping.
   b.) Align the bottom of the blade fragments so that they're even. This way, it's easy to distinguish which blade fragments.
   c.) Once you place all the pieces from a given blade onto the transparency sheet, leave a noticeable space before placing the other blades on the sheet.

6. Repeat with remaining blades of eelgrass, leaving room for paper metadata labels at the top of each sheet (make your own customized labels for each site HERE [OG: will include link]).
   a.) Labels should contain: processing date, blade numbers on transparency, site name, transect, depth penciled in.

7. As you're filling your transparency with blades, occasionally spritz them with seawater by dipping your hands into a container with seawater then flicking the water onto the blades.
   a.) This prevents the blades from drying out and shriveling.
   b.) Note: The transparency shouldn't be saturated with water. You just want to keep the blades moist.

8. When your transparency sheet is full, carefully place another clean transparency on top of the blades to sandwich them.
   a.) Gently press down to prevent the top transparency from slipping off, and wipe off any excess water with a paper towel.
   b.) Ensure there aren't any air bubbles trapped between the blades and transparencies, as this will make it harder to view lesions!
      -To avoid this, gently rub a paper towel along each blade sandwiched between the transparencies. It should look nice and clear!
   c.) To help EELISA distinguish between blades, place a neon sticky note (on the top transparency) in between each blade.

Special notes about cleaning and scanning subtidal blades

1. Because subtidal blades are so long and wide, we measure the full blade length and width (at the widest section) in lab (using a meter stick, record in mm on paper data sheet), measuring from tip to top of sheath.
   a.) This is in contrast to smaller intertidal blades for which we use EELISA for all blade measurements.

2. Then, we gently tear/cut out sections with lesions or questionable spots, place them on the transparency in grouped vertically together, then repeat for other blades.

3. If your blades are small enough to scan the entire blade, go for it! For us, though, usually 1 subtidal blade will easily fill 1-2 transparencies.

Scanning Eelgrass Blades

Once you cleaned all blades and placed them on transparencies, you can begin scanning!
Note: The layout of the scanning software will likely change, as we're switching from an older, Canon scanner to faster, Epson scanners. The general procedures below will remain the same, but Olivia will update protocols with screenshots of the new scanner software in the coming weeks.

Materials

- Canon Scanner
- External hard drive (for backing up image scans)
- Laptop/computer
- Paper towels for cleaning scanner
- Prepared eelgrass transparencies

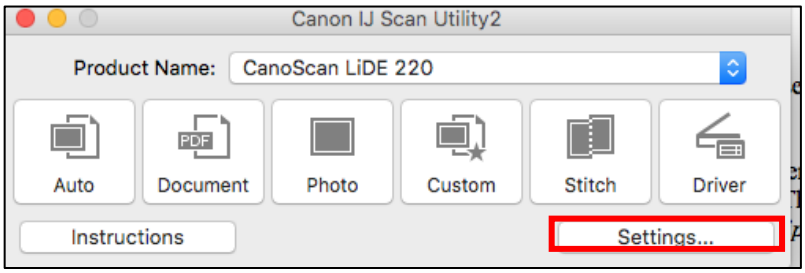
Scanning

1. Before you begin, make a series of folders on your computer for all scanned images, using the following naming structure:

   Geographic Region→Scans→Field Site Name→Depth

   a.) Note: Having a consistent filing structure across all sites and regions helps organize scans before running them through EELISA.

2. Open the "Canon IJ Scan Utility2" application and click on "Settings."

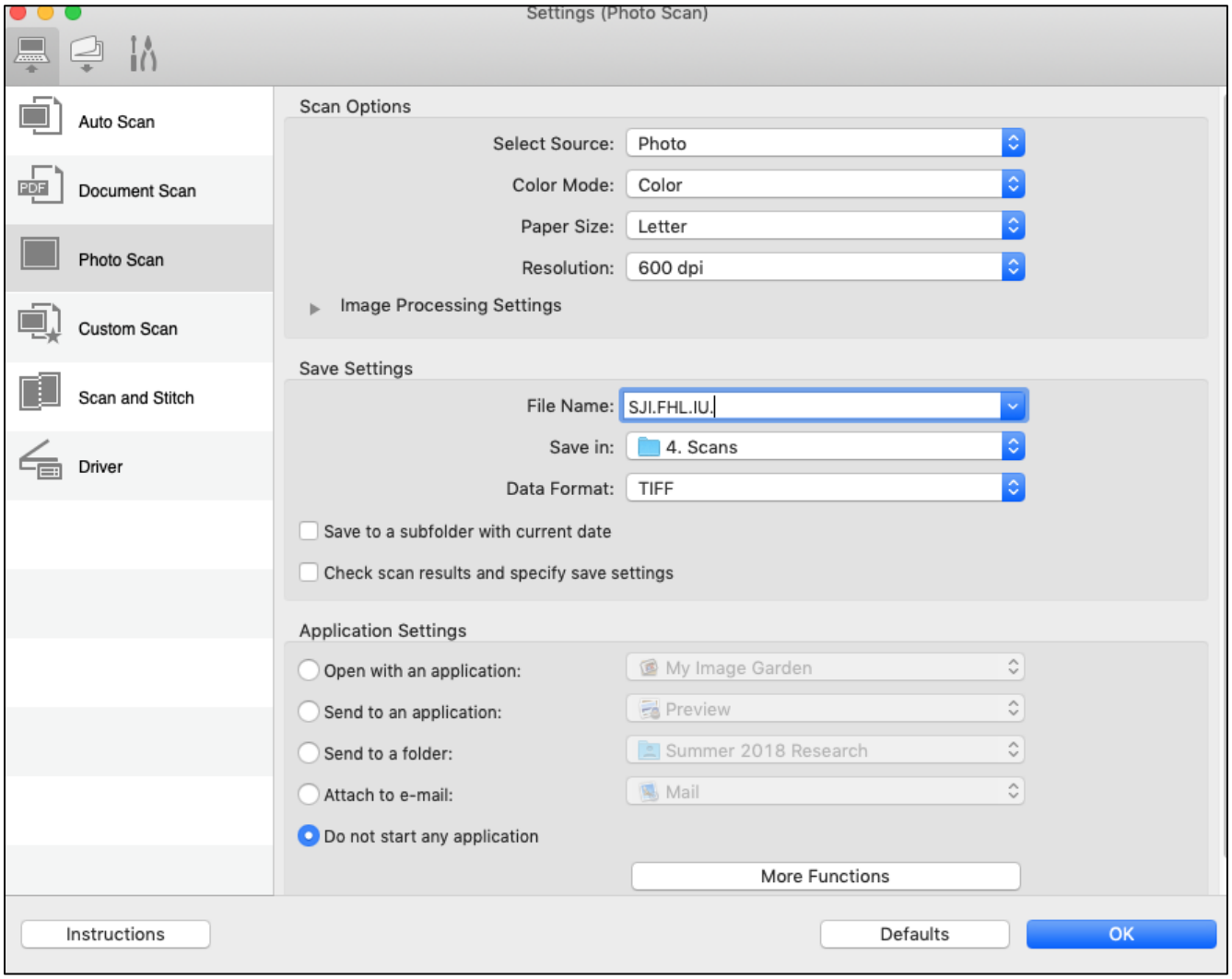


3. Under "Settings", select "Photo Scan" on the left side.
a.) Under "Scan Options", select 600 dpi as the resolution.
b.) Under "Save Settings," click the "Save in" drop down menu. Click "add" and find the folder you just created for the scans. When done, click ok.
   -You'll need to change the folder each time you scan blades from different depths, sites, etc.
   c.) Under Data Format, select "TIFF" –this is important!! Do not save as JPEG or another file format.
   d.) Your screen should look something like the figure below.

4. Open the scanner lid and place the transparencies face-down on the scanner, orienting the top of the transparency (with the metadata label) towards the bottom of the scanner. (See provided examples for reference.)
   a.) Double check that none of the eelgrass blades are overlapping or covered by the neon sticky notes.
   b.) Scanner glass should be clean and dry before scanning.
   c.) Avoid getting sediment and salt water on the scanner. Sand scratches the scanner glass, and salt water forms crusty spots that influence image quality.

5. Close scanner lid gently, and click "Photo" to begin scanning.

6. When complete, remove transparency, gently dry scanner glass and lid, then scan the next transparency.



7. Every so often, check that the scans to ensure they're high-quality: no debris, no labels/sticky notes covering blades, no blades overlapping, no air bubbles between blades and transparencies, etc.

8. When done scanning all transparencies, name each one using the standardized naming system:

Region.SiteName.Depth.Blade#s
Ex: SJI.FB.IU.1_10 would be:
San Juan Islands→False Bay→Intertidal Upper→Blades 1-10

Conversely, if you are working in subtidal meadows, you'd use an S to indicate subtidal. For depth, U = first set of transects in upper intertidal (i.e. higher on shore). L = lower set of transects (i.e. deeper in the water)

a.) Note: Since naming each transparency takes a while, it saves time to just name them all when you're done scanning (all blades from a given depth), referring to the metadata label on each scan for details.

9. When done scanning and naming all transparencies, backup files 2x:
   a.) Upload files to shared project Box folder.
   a.) Backup scans to an external hard drive.
   b.) Lots of high-resolution scans take up significant—and precious!—space on computers, so you'll want to back them up in multiple places.

10. When completely done scanning, you can clean the transparencies.
   a.) Note: Do not leave transparencies for more than a few hours with eelgrass stuck to them! They will dry, stink, and the transparencies will be very hard to clean.
   b.) Throw away blades, rinse each transparency in fresh water, shake to get big droplets off, sandwich in between paper towels and let air dry.
   c.) You can also soak transparencies in a basin with freshwater once you've removed the seagrass and before drying them if you need to resume cleaning later.
   d.) Note: Scanning requires lots of transparencies, especially when scanning blades daily. To ensure you have enough, stay on top of cleaning/drying them!

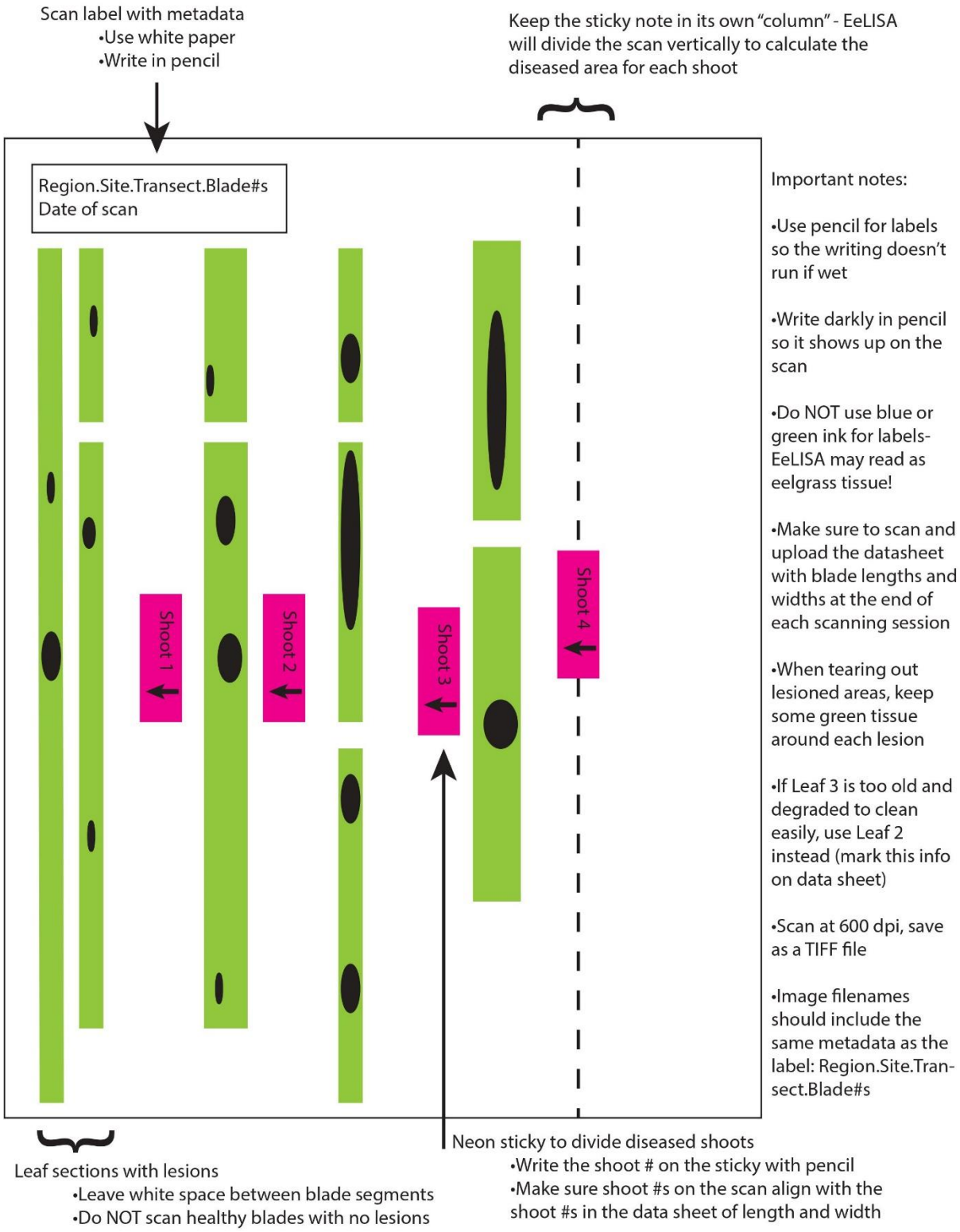
General notes about processing blades

1. Do not leave blades for more than 24 hrs in fridge before scanning, as lesions continue to develop and may bias disease measurements.

2. Unless you have lots of field/scanning support, try to collect and scan blades from just one site per day.

3. Time commitment:
- Fieldwork: 3-4 people x 2.5 hrs (*just for seagrass collections—not epiphyte, epifauna, etc.)
- Cleaning and scanning: 3-4 people x 1.5-3.5 hrs (*varies widely based on epiphyte load, size of blades)

Disease Scan Transparency Diagram



Identifying and Measuring Lesions

Lucky us, we have a snazzy, smart application to measure lesions! Once you've scanned all eelgrass blades from your site and upload them to the Box folder, the Cornell team will upload them to *EELISA* for image analyses. Thanks for your help!

Uploading Eelgrass Scans for scoring by *EELISA*

This protocol describes how to upload scanned images of eelgrass blades to the project folder on Box.

1. Images of the scanned blades should be saved locally on the computer during the scanning process. Images should be named according to our project meta-data style, "Year.Region.Site.TransectID.Blade#s.tiff" **Note that the style has yet to be finalized

2. Project collaborators all have access to the Box folder, titled "NSF Eelgrass Scans". **If you do not have access, email Lillian (lra53@cornell.edu)

3. To upload the images, navigate to the project Box folder using a web browser. Do NOT upload by dragging and dropping the image files into the desktop Box app.
    a. Click through to the correct folder, e.g. for the early summer practice scans in Washington, click through NSF Eelgrass Scans → 2019 → Practice Scans → Washington.
    b. Click "Upload File" in the upper right.
    c. Navigate to the image files stored locally on your computer and hit OK.
    d. After files have uploaded, open them in Box to double check.

4. If possible, also copy the image files to an external hard drive so that we have multiple backup locations.

Uploading images of field notebooks and datasheets
(same process as for the eelgrass scans)

1. In order to preserve all the field data, please photograph or scan individual pages of field notebooks and datasheets. Save the files locally on your computer using clear filenames that follow the project metadata – e.g. "Year.Region.Site.ShootDensityDatasheet.jpg"

2. Navigate to the Box folder through a web browser as follows: NSF Eelgrass Scans → 2019 → Field notebooks and datasheet scans → [Region]

3. Upload images and double-check that they open in Box.

Eelgrass Density

Measured Parameters

This assay quantifies seagrass community structure, measured as:

- Percent cover of seagrass canopy, bare substrate, and other cover types
- Shoot density (number of shoots per m^2)

Requirements

Personnel: 2 people
Replication: 4 quadrats taken along 6 transects (total n = 24 per site)

Materials:

Survey Design:

- ☐ 1 20-m metric transect tape
- ☐ Hand-held GPS unit
- ☐ 2 PVC marker poles (diameter and length as needed)

Fieldwork:

- ☐ At least one quadrat. A 0.5m x 0.5m (0.25 m^2) is recommended but other sizes may be used. For example, if eelgrass shoots are very sparse, you may use a 1m-x-1m quadrat instead (specify size on datasheet. Quadrats may be strung with a monofilament grid (see heading photo!) if using point counts for cover measurements
- ☐ Pencil
- ☐ Waterproof paper
- ☐ Clipboard
- ☐ RECOMMENDED: Waterproof camera

Preparation

1. This protocol assumes n = 4 quadrats for percent cover and n = 4 shoot density counts taken along a 20-m transect, replicated along 6 separate transects.
2. Print field data sheets on waterproof paper. You will need at least 4 sheets, but having more available is useful.

Fieldwork

1. Record site metadata on field sheet: start time, date, tidal height. Take several photos to document conditions at the site.
2. At each replicate along the transect (4m, 8m, 12m, and 16m), lay down the quadrat immediately adjacent to the transect line on the left hand side of the line when standing at the 0m meter mark facing the 20m mark.
3. Evaluate the quadrat for percent cover using either point count (with string grid) or visual estimate. In either case, also record area (or points) occupied by bare substrate, and by other sessile organisms if present (e.g., macroalgae, sponges, etc.). Be as specific as you can in identifying these organisms but do not guess if you are unsure (e.g., record 'red sponge' and add 'Acarnus erithacus?' in notes column); record a photo if possible.
    a. *Point count option*: Record the macrophyte species (e.g. *Zostera marina*) under each point (e.g., 9 x 9 grid for 81 points).
    b. *Visual estimate option*: Estimate percent cover visually using methods quality-controlled by your lab. A separate estimate should be recorded for each seagrass species, bare substrate, and any other sessile organisms.
    c. *Either way*: Measure percent cover of the eelgrass canopy (rather than eelgrass stems).
4. In every replicate, obtain a measurement of shoot density by counting and recording the number of seagrass shoots within the lower right 25 cm-x-25 cm quadrant of the quadrat. (Alternatively, count every shoot within the entire quadrat.) If visibility is poor, shoot density can be obtained by touch.
    a. Count and record the number of reproductive (i.e., flowering/fruiting) shoots within the same quadrat evaluated for total density.
    b. Record the area for which shoots, flowers, and fruits were counted!
5. Take a photo of the entire quadrat.
6. Repeat steps 1-5 for each replicate on the first transect.
7. Repeat steps 1-6 for the remaining two transects.

Data Submission

8. Enter data into provided data entry templates.
9. Scan the completed field data sheets and save both paper and electronic versions.
10. Upload data entry file, photos, and scanned field data sheets to the shared Box/Google Drive folder.

Water (eDNA) sampling protocol

Goal

Filter 200 – 500 mLs of water onto a cellulose nitrate filter. The filter will be preserved, frozen, and used for determining water microbiome community and possibly for eDNA.

3 water samples will be collected from each site. These should be taken at the same time (roughly) as microbiome samples and should be taken at a time and in a place where there has not been tons of sediment resuspension from other sampling activities. These could be taken at the end of the sampling or the beginning.

Water samples will be collected in single-use sterile 500 mL bottles (1 per sample) in the field and filtered with single-use filtration units (1 per sample) back at the lab. DO NOT RE-USE BOTTLES, TWEEZERS, OR FILTRATION UNITS. The bottles, tweezers, and filtration units are SINGLE USE to prevent cross contamination of bacterial/environmental DNA between samples.

Please sterilize your gloves with the provided alcohol wipes before collecting samples in the field and also before filtering samples at the lab.

Field sampling process

Supplies

- 3 sterile 500 mL bottle (VWR Cat. # 76299-562) (1 per sample to be collected -- 3 samples per site in total)
- Cooler with ice packs (provided by the site team)

Instructions

1. label each 500 mL sterile bottle with the date, site, and water sample number
2. open bottle, do not touch inside of cap or rim of bottle.
3. fill sterile 500 mL bottle with water, be careful to do this in a place where you have not stirred up sediment, dislodged epiphytes, etc.
4. put sample on ice to return to the lab (within 2 hours of collection)
5. place bottle of water in a 4°C refrigerator until you complete the filtration process, see below

Lab filtering process

Supplies

- 1 sterile Nalgene filtration unit (Nalgene cat. # 130-4020, 0.22um cellulose nitrate filter) per water sample
- 2 sterile individually wrapped disposable tweezers (Andwin Scientific Cat. # 2221121B11) per water sample
- 1 five mL Zymo buffer tube (Zymo Cat. # R1100250; ThermoFisher Sci. Cat. # 21-402-905) per water sample
- Gloves
- Sterile alcohol pads

Filtration will be carried out in 5 sequential steps of filtering 100 mL volumes at a time, for a total volume of 500 mLs per sample. Make sure your vacuum pump is off when you add water to the top of the chamber, so that you can properly track the total volume filtered using the demarcations for volume on the filtration unit. Note: Alaska is provided with a hand pump; other sites said they had their own pumps.

Make sure to save the filter. This is what we want to preserve!

Instructions

1. unpack your sterile individually wrapped filtration unit and attach the unit to the vacuum pump tubing
2. pour 100 mL of water into the upper chamber (use demarcation on the chamber to determine volume)
3. turn on vacuum
4. when the 100 mL volume of water has completely filtered through to the bottom chamber, turn off vacuum
5. break vacuum (pull rubber tubing off of white attachment arm on filtration unit)
6. dump water out from the bottom of the chamber down the drain (waste)
7. attach the bottom of the chamber back to the unit (as it appeared when you first opened sterile package)
8. start again with step 1, until a total volume of 500mLs has been successfully filtered through the unit OR a lesser volume if water is turbid – see note below with asterisks **
9. Record total volume of water filtered
10. Detach the bottom of the unit, dump out water down the drain
11. Sterilize your gloves and open two single-use sterile tweezers.
12. Using the tweezers, remove the filter from the unit, fold filter in half twice and place in a sterile 5 mL Zymo buffer tube labelled with the sample number. Do not touch the filter with your gloves as you fold it.

Start again with step 1 for your next water sample. DO NOT re-use filtration units or tweezers. These go in the trash after each water sample.

** If you have very turbid water you may only be able to filter a total of 100 – 200 mLs through the filter. If the flow rate is slowing substantially, your filter is likely starting to clog. In this case, the

current volume of water you are filtering will be your last 100 mL aliquot to filter, but please finish filtering that 100 mL. Please remember to record the total volume filtered; this is critical for proper downstream analyses. **

qPCR Laboratory Protocol

Materials needed

Each kit will include:

- 120 2ml screw top epi tubes w/ 0-rings, filled with ~1.5 ml 70% ethanol
- 60 2ml screw top epi tubes w/ 0-rings, filled with ~1.5ml 10% formalin
- 3 labeled cryo boxes (2 for DNA samples and 1 for histology)
- Parafilm to wrap the epi tubes
- 120 whirl packs (we need to decide on size. I used 18oz, but 4oz could be fine)
- 2 tweezers
- 2 scissors
- 2 lab markers
- 2 rulers
- Gloves

Partners need to provide:

- Artificial seawater (please let the Harvell Lab know if you need us to send some!)
- 5 Gallons Distilled Water – since some contaminants can interfere with the PCR we would prefer if you buy water for rinsing the sample
- bleach and 4 containers to sterilize equipment (glass or Tupperware is fine)
- spray bottle of 10% bleach for cleaning the counter
- Extra gloves

qPCR Sampling protocol

Before leaving for the field, label 12 whirl packs and fill them with ~25 ml artificial seawater. You just need enough water to keep your sample wet until processing! You will need two whirl packs per transect. If you would rather add a little water from the site where you get the sample, that is also fine. If you decide to do this, dump this water on return to the lab and replace with artificial seawater. For the processing back at the lab you MUST use artificial seawater! 0.22ul filtered seawater is also acceptable, but we would like everyone to make artificial seawater to use.

Label the samples to match with the microbiome samples:
Please label each transect with a and b. Example: WA-A-U1-a for region: Washington, Site: A, Transect: Upper 1, sample: a

Prepare for the field

- Prepare a cooler with ice or cold packs to transport the samples back to the lab
- Prepare a gallon of artificial seawater (add ½ cup to a gallon of distilled water and shake to mix)
- 12 pre-labeled whirl packs

In the field

qPCR sample collection will be done in the field using the Eelgrass microbiome sampling protocol. The microbiome sample and qPCR sample will be taken from the same leaf. After collecting the microbiome sample, carefully remove surface epiphytes from the leaf (the one you just cut samples from!) with your gloved fingers and place the diseased plant leaf into a whirl pack. You can collect the whole leaf or a 10-15 cm section that contains both areas that the microbiome was samples from. Whatever is easier! You will not need to do this for the healthy microbiome plant. qPCR sample preservation will be done in the lab. Put the whirl pack in the cooler for transport back to the lab. The qPCR samples must be processed within 24 hours of collection.

On return to the lab

Keep the samples in the cooler or a refrigerator at ~4C until you are ready to process the samples. Before starting your sample processing prepare a sterile bench area. Spray down the full counter area you will be working at with 10% bleach. This is to destroy any DNA that is present. Our qPCR assay for laby quantification is very sensitive so it is important to make sure there is no tissue or DNA contamination between samples. Make sure to fully dry the counter so all the bleach is removed! If you get bleach in your samples, even a drop, it will destroy the DNA. Make sure you have a new or bleached pair of transparences for the DNA samples at the start of each new site. You only need two total for this.

Get ~1 L artificial seawater. You will need this for cleaning the leaves.

Set up a tool sterilizing station. You will need 4 containers (plastic or glass are fine, just keep the order the same each time so you always us the same one for the bleach, rinse 1, etc.). Fill container 1 with ~2 inches % bleach and fill containers 2-4 with ~3 inches RO or DI water. The fresh water rinses need to be higher than the % 10 bleach.

Label and order as follows:
1. 10% bleach
2. Rinse 1 (RO or DI water)
3. Rinse 2 (RO or DI water)
4: Rinse 3 (RO or DI water)

Sterilize the ruler between each leaf by wiping with 10% bleach and rinsing with RO water. Tools need to be sterilized between each sample. Dip in 10% bleach for 10-20 seconds, followed by dipping in each of the 3 rinses in order. Sterilize tools between every tissue sample.

Now you are ready to start sampling the leaves!

1. Remove the leaf from the bag and place on a kim wipe (provided in your kit). Dump out the water in the whirl pack. Rinse the whirl pack with RO or DI water. Keep the whirl pack for step 3. Gently remove epiphytes and diatoms from the surface of the leaf using a plastic ruler or a sterile glass slide.

2. Once the leaf is scraped, use a new kim wipe to clean the whole surface of the leaf on both sides until the kim wipe remains clean when run over the surface of the leaf. You can dampen the kim wipe with a small amount of RO water to help remove diatoms. If the leaf is very dirty you may need to do this several times.

3. Place the leaf back in the whirl pack it was collected in. Pour ~50-100 mL artificial seawater over the leaf. Close the whirl pack and gently agitate for 30 seconds. At this point, you can clean the rest of the leaves and procced to step 4 after all are ready. The ruler/glass slide needs to be sterilized between each leaf. If the leaves were all kept on kim wipes during cleaning you do not need to sterilize the counter between each leaf, but if you lay the leaf on the counter you do need to sterilize it.

4. Using sterile tweezers, remove the leaf from the whirl pack. The surface of the leaf should remain wet. Place in a new whirl pack (without water), laying it flat so the whole surface of the leaf can be seen. Your sample is now sterile and can be safely scanned. We put the leaf in the second whirl pack to provide a sterile surface to label and scan the leaf.

5. Indicate where the paired DNA samples and 2 (or 4 and 5) will be taken (further instructions on sample selection will be provided). Using a ruler as a guide, write on the surface of the whirl pack with a sharpie showing the exact area that will be cut for each sample. Number each sample. The DNA samples should be ~2X1cm. If your leaf is relatively narrow, you can cut the length and leave the width of your sample to width of the leaf. We will measure sample weight when the DNA extractions are done, so it is not important that the samples are exactly this size. It is important that we can see exactly what tissue was sampled for DNA and histology. Now select a small area that contains as much of the lesion as possible for histology. The histology sample must be 0.5 X 0.5 cm. Mark where this sample will be taken with a sharpie on the surface of the whirlpak as well.

6. Once the DNA samples are marked, put the whirl pack on the scanner with a label, placed between to transparencies to keep the leaves in place. Scan. Check the scan after to make sure all labels are visible. Since the whirlpac is sterile you do not need to use a new transparency for each sample.

7. Place the whirl pack on the counter, being careful to keep the leaf in the exact position that it was scanned and all marking in the correct place. Using sterile scissors, cut the DNA and histology samples exactly as marked, keeping the samples inside the whirl pack (you can cut the sides of the whirl pack to make this easier. Sterilize the tools between sample a and sample b. Using sterile tweezers place samples a and b in their labeled tubes (pre-filled with 70% ethanol). Make sure to completely immerse the samples in the liquid. Place the histology sample in the tube pre-filled with 10% formalin. Make sure to completely immerse the sample in the liquid.

Once you have all tubes collected place them back in the boxes. They can all be stored at room temperature or 4C. Please keep out of high temperature and out of direct sunlight. At the end of the sampling, the samples will be shipped to Friday Harbor Labs for further processing. We will send further instructions on how to pack and ship the samples.

Appendix I: Seagrass Macroalgae

NOTE: This protocol is an optional component of the field sampling effort that would make your site eligible for inclusion in the MarineGEO seagrass food webs dataset. If you choose to include this protocol in your sampling, it should only be included at one out of the five sites in your region.

Measured Parameters

This assay quantifies the biomass of macroalgae, measured as:

- Macroalgal wet weight (mg)
- Macroalgal dry weight (mg)

Requirements

Personnel: 2 people

Estimated Total Time Per Location (n = 3 transects):
Preparation: 1 person x 1 day
Field work: 2 people x 1 day
Post processing: 1 person x 3-5 days
Data processing: 1 person x 1 day

*Estimated times will vary by site and conditions

Replication: Six (6) macroalgae samples taken along three (3) transects (total n = 18).

Materials:

Survey Design

- ☐ 1 50-m metric transect tape
- ☐ Hand-held GPS unit
- ☐ 2 PVC marker poles (diameter and length as needed)
- ☐ 0.5m x 0.5m (0.25m^2) quadrat

Fieldwork

- ☐ 18 draw-string mesh bags (~1 mm mesh size, approximately 25x35 cm or sized as needed) (example)
- ☐ 1 cooler with ice (optional)

Post-processing

- ☐ 20+ pre-weighed foil tins (example)
- ☐ Sorting tray
- ☐ Pencil/pen
- ☐ Permanent marker
- ☐ Ruler (mm)
- ☐ Drying oven

Methods

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

Preparation

4. Review the MarineGEO Seagrass Habitats Survey Design for site selection and setup. Samples are collected concurrently with the MarineGEO Seagrass Density protocol. This protocol assumes n = 6 macroalgae samples taken every 8 m along a 50-m transect, replicated along 3 separate transects.
5. Label 18 disposable plastic bags with the sampling location, transect, and replicate number using a permanent marker.
6. Place 18 internal labels with the same metadata written on waterproof paper inside the corresponding plastic bag.
7. Fill a cooler with ice immediately before departing for the field.

Fieldwork

8. Set up transects and position the first quadrat. Refer to the MarineGEO Seagrass Density protocol for further instructions.
9. With the 0.5x0.5 m quadrat in place, hand collect all macroalgae within the quadrat and place in the corresponding labeled mesh bag. Limit collections to large intact macroalgae that can be picked from the bottom by hand (filamentous attached/epiphytic algae are evaluated in a separate protocol). Macroalgae with holdfasts can be broken from the substrate at the sediment surface.
10. If the macroalgal unit overlaps the bounds of the quadrat, collect the entire frond (unless it has a holdfast that is outside of the quadrat).
11. Place the mesh bag and contents on ice in the cooler.
12. Repeat steps 1-4 at the at the remaining 5 sampling locations along the transect.
13. Repeat steps 1-5 for the remaining two transects.
14. Transport cooler with samples back to the lab for immediate processing.

Post-processing

Samples are best processed immediately (within 24 hours) upon returning from the field. Samples can be stored for longer in the freezer but risk decay.

1. Print lab data sheets.
2. Weigh foil tins and record the weight of the tin directly on the foil using a pen. Tins can be either pre-made, or constructed by folding an aluminum foil square over on itself and sealing the sides.
3. Select a labeled bag and record the metadata on the lab data sheet.
4. Transfer the macroalgae from the bag into a sorting tray or bowl and rinse with freshwater. Add enough freshwater to cover the macroalgae.
5. Allow algae to soak in freshwater for approximately one minute to release any epifauna. Remove algae from the freshwater soak and check fronds for any remaining animals. Remove animals if present and discard.
6. Select a pre-weighed tin and label with the sample metadata (replicate number, date, location) and contents (macroalgae). Record tin weight (mg) on lab data sheet.
7. Place macroalgae in tin, weigh to the nearest mg and record wet weight (including tin) on lab data sheet.
8. Repeat steps 3-7 for each remaining replicate.
9. Place all the tins in a drying oven at 60°C. Dry samples until they register a constant weight (usually 1-3 days, depending on the volume of material).
10. Remove tins from the oven and weigh each to the nearest mg. Record this dry mass (including foil) on the lab data sheet.

Data Submission

5. Scan the completed lab data sheets and save both paper and electronic versions locally.
6. Enter data into provided data entry template.
7. Use our online submission portal to upload the Excel Spreadsheet (coming Fall 2019).
8. Contact us if you have any questions: marinegeo@si.edu.