CARICAS PARTNER'S PRACTICAL FIELD AND LABORATORY GUIDE

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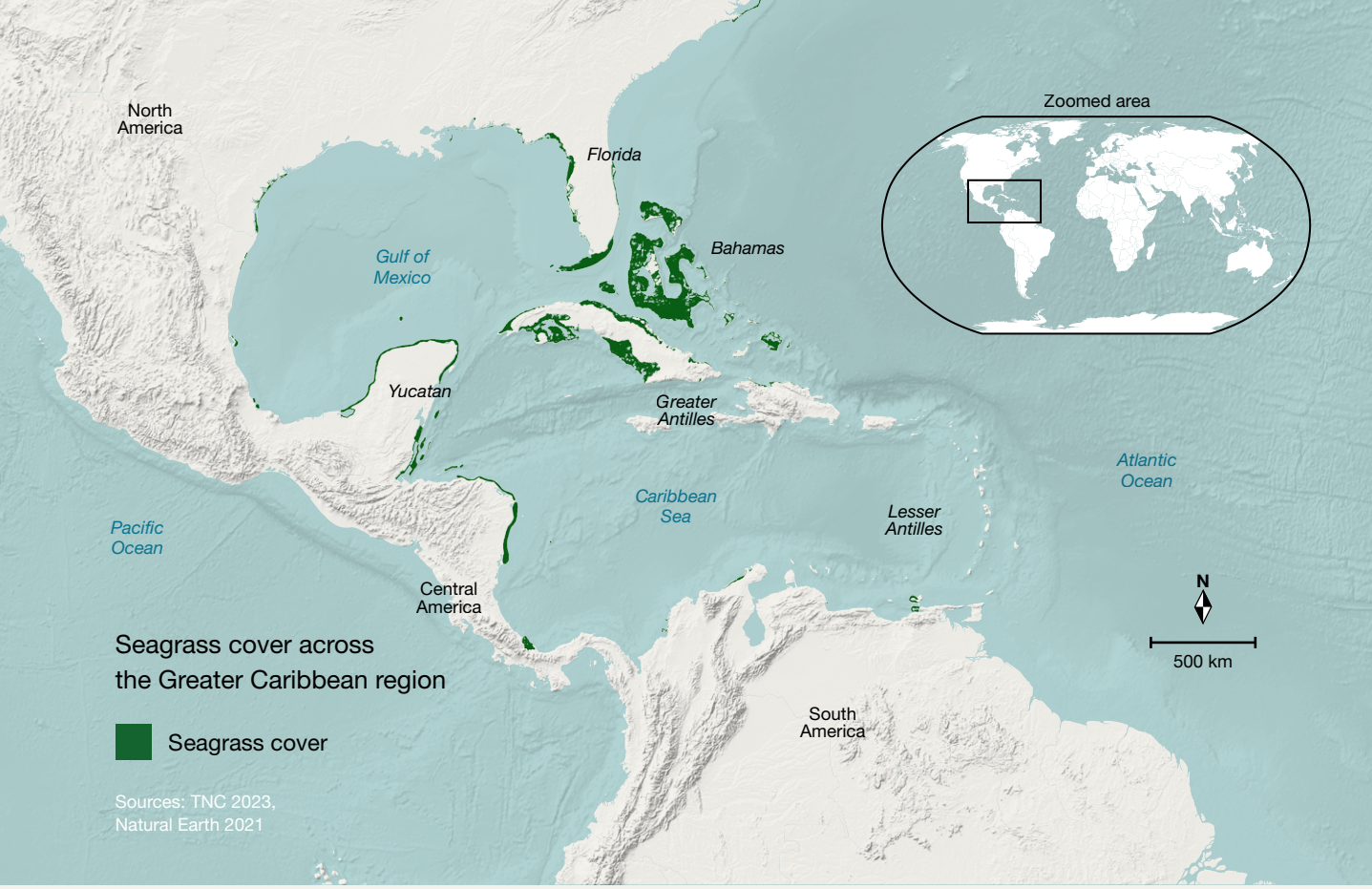
EXECUTIVE SUMMARY

The capability of some coastal vegetated ecosystems (seagrass meadows, mangrove forests, and tidal marshes) to sequester CO_2 and store large organic carbon stocks is drawing increasing attention as a potential means of conservation-based climate change mitigation. Despite the fact that the Caribbean region supports large expanses of seagrass meadows, information on the status and trends and carbon density of Caribbean seagrass meadows is surprising sparse. Further, evidence of widespread declines of seagrasses across the region suggest that Caribbean seagrass blue carbon stocks are at risk to add to global warming. To address these uncertainties, the CariCAS project aims to 1) build a collaborative network of Caribbean seagrass scientists interested in blue carbon and to 2) outfit local experts from the new network to collect the data needed to construct inventories of seagrass blue carbon to a sediment depth of 1 m from as many seagrass sites across the Caribbean as possible to understand the range, variation, and environmental correlates of seagrass C stocks. These data will be combined with seagrass mapping to generate first-order estimates of the amount of C stored in seagrasses across the region.



This field guide complements online workshops for CariCAS project partners and describes the field and laboratory methods used to characterize blue carbon in seagrass meadows. At each project site, seagrass abundance will be assessed at 16 (sixteen) 0.25 m^2 quadrats placed at random locations within the site. Eight 20 cm diameter cores will be taken to assess seagrass biomass and to provide the material for seagrass biomass carbon and nutrient content. All seagrasses within each of the eight cores will be separated by species and tissue type, washed and scraped to remove epiphytes, then dried and weighed. Following the collection of seagrass biomass cores, a piston core will be taken of uncompressed soils, making an attempt to drive the core 1 m into the sediment or until refusal. Cores will be subsampled at 5 cm depth intervals using small subcores. All subcores will be weighed wet to permit the calculation of porosity and soil dry bulk density. Seagrass tissue and sediment samples will be oven-dried at 60°C, and dry weight recorded. Finally, samples will be shipped to FIU's Blue Carbon Analysis Laboratory for determination of Loss on Ignition, total carbon content, inorganic carbon content, organic carbon content, and carbon and nitrogen stable isotope ratios. The resulting data will be used to estimate seagrass carbon stocks and their relationship with covariates, as well as being integrated with seagrass mapping efforts led by The Nature Conservancy to quantify seagrass carbon stocks of the Caribbean region.

STATEMENT OF GOALS

To assess stocks and controls of blue carbon across the Greater Caribbean region, the following parameters will be sampled:

Quadrat scores

- Seagrass abundance
- Species composition
- Canopy height

Seagrass shoot collection and biomass cores

- Tissue nutrient content (N, P)
- Tissue stable isotope content ($\delta^{13}C$)
- Seagrass biomass
- Shoot density

Rapid field assessment

- Sediment grain size

Sediment core

- Organic carbon stocks
- Inorganic carbon stocks

To generate ground-truthing data for blue carbon mapping across the Caribbean. Remotely sensed imagery will be informed by geolocated quadrat observations of:

- Seagrass cover
- Species composition
- Seagrass biomass
- Shoot density
- Substrate type

1 SITE SELECTION AND LOCATIONS OF OBSERVATIONS

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Each partner will collect information from two sites from their location. Local knowledge and the presence of historical work at a location should be considered when selecting sites. If practical, each site should be typical of a large proportion of the seagrasses from the local area, with each site representing a different identifiable type of seagrass habitat. In order to inform predictive models of carbon content across the Greater Caribbean region, we aim to get representation of sites across broad environmental gradients, such as water depth, exposed vs. sheltered, estuarine vs. marine, stable vs. disturbed sites, species composition, seagrass density, etc. However, each CariCAS project partner samples only two sites and uses their judgment to locate those sites, given the overall project goals. The final selection of sites will be determined in consultation with the project management team before beginning of sampling activities.

Once the two sites are selected, locate as accurately as possible the center point of each site with GPS. It is best to take multiple readings of the location and average them for the location. Mark this location with a stake driven into the soil.

Generate 16 Braun-Blanquet cover-abundance quadrat locations at each site thusly (Fig. 1):

1. Generate 4 random compass directions (from the stake, referenced to magnetic north) in each of 4 quadrats, NE, SE, SW and NW. Order these directions from smallest to largest
   Example: NE, between 1-90 degrees: 6°, 25°, 50°, 56°, 85°; SE, between 91-180 degrees: 105°, 122°, 142°, 155°, 156°; etc.
2. For each random compass direction, generate a random distance between 1 and 30.
   Example: NE: 7 m, 30 m, 16 m, 9 m, 3 m; SE: 4 m, 12 m, 1 m, 14 m, 22 m, etc.

3. Generate radial coordinates by matching directions with distances.
   Example: 6°, 7 m; 25°, 30 m; 50°, 16 m; etc.
4. Number each of the 16 locations generated by the above randomization from 1-16. These locations will then be recorded for all data collected at each of those sites (Table 1). Note radial coordinates on the field datasheet 01 (Appendix B).
5. Attach the measuring tape to the center stake. Using an underwater compass, swim in the direction of the first quadrat location until the distance is reached.
6. Without biasing the placement of the quadrat by what is on the bottom, place a PVC 0.5 m by 0.5 m square quadrat at the indicated distance. When facing the stake, the quadrats' lower right corner should touch the indicated number on the measuring tape and the quadrat should be positioned to the left side of the tape. In each quadrat, do a rapid field assessment of benthic species cover and abundance and sediment texture (see methods below).
7. Place a surveyor's flag marked with the quadrat number in the middle of every odd-numbered quadrat location. These will designate the locations for collection of core samples for biomass from 8 of the 16 quadrats at each site. Alternatively, you may collect the biomass core immediately after completing the rapid field assessment of benthic species. In this case, no survey flags are needed.
8. Return to the center stake and navigate to the next point until all 16 quadrats have been assessed.

Table 1. Example of 16 randomly generated radial coordinates for quadrat locations

Quadrat	Quadrat #	Angle	Distance	Biomass core?
NE 	1	26°	20 m	Yes
	2	46°	27 m	No
	3	60°	25 m	Yes
	4	71°	12 m	No
SE 	5	96°	12 m	Yes
	6	109°	7 m	No
	7	115°	21 m	Yes
	8	142°	29 m	No
SW 	9	187°	20 m	Yes
	10	201°	9 m	No
	11	219°	25 m	Yes
	12	262°	10 m	No
NW 	13	303°	21 m	Yes
	14	318°	24 m	No
	15	333°	23 m	Yes
	16	346°	11 m	No

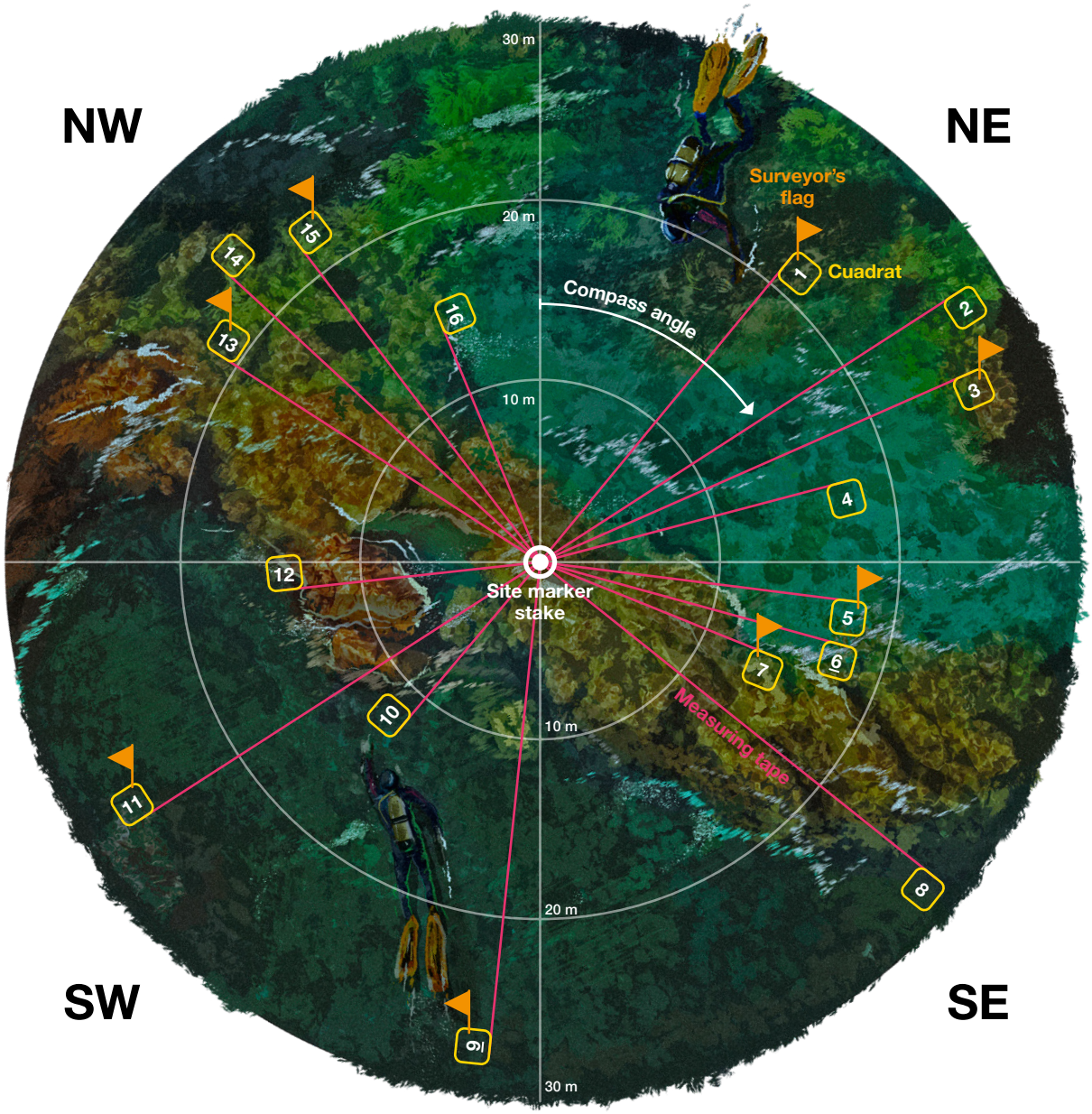

Figure 1. Illustration of sampling positions at each site. Quadrat positions are located from the site marker stake using a compass and tape measure. Every odd-numbered quadrat is marked with surveyor's flags for biomass core collection.

2 RAPID FIELD ASSESSMENT OF BENTHIC SPECIES COVER

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Figure 2. Example of Braun-Blanquet 0.25 m^2 quadrats.

At each sampling location, a 0.25 m^2 (0.5 m by 0.5 m) quadrat is placed on the bottom. Measure the effective canopy height, defined as the estimated average height of the tops of seagrass leaves above the soil surface, with a ruler. Take a photograph of the quadrat from directly above, so that the entire quadrat is in the frame. Either note picture ID on datasheet 01 (Appendix B) or take a photo of the datasheet after photographing the quadrat, so that each quadrat photo position can be reliably identified later on. Perform the rapid field assessment of sediment texture (see below) and note the sediment class on datasheet 01. Identify all conspicuous benthic plants and sessile animals to a meaningful and reliable taxonomic level. It is important that all sites across the network use the same categories for benthic plants and animals, so only the scores for agreed-upon categories listed on datasheet 01 are recorded. Other benthic taxa not on the list should be scored as “other” with a notation on the datasheet with the taxonomic ID. If the observer can reliably identify organisms to a finer taxonomic level than the agreed-upon categories, this finer-scale taxonomic information can be recorded in comments for local use. Seagrasses will be identified to species level and voucher specimens for each species will be collected and pressed in an herbarium press (see methods below).

Table 2. Braun- Blanquet abundance scores (S). Each benthic taxon will be scored in each quadrat using this scale.

S	Interpretation	
5	Taxon covers 75% - 100% of the quadrat area	
4	Taxon covers 50% - 75% of the quadrat area	
3	Taxon covers 25% - 50% of the quadrat area	
2	Taxon covers 5% - 25% of the quadrat area	
1	Taxon represented by > 5 shoots or individuals, <5% cover	
0.5	Taxon represented by 2-5 shoots or individuals, <5% cover	
0.1	Taxon represented by a solitary shoot or individual, <5% cover	
0	Taxon absent from quadrat	

Macroalgae (exclusive of seagrass epiphytes) will be identified in broad categories (Green algae, brown algae, red algae, calcareous green algae). In addition to seagrasses and macroalgae, the Phylum Porifera (sponges), Subclass Octocorallia (octocorals or soft corals) and hard corals (comprised of Order Scleractinia [stony corals] plus Order Anthoathecata, Family Milleporidae [fire corals]), will each be assigned a cover ranking according to a modified Braun-Blanquet scale (Table 2; Fourqurean et al., 2001). Cover is defined as the fraction of the total quadrat area obscured by the species, genus or group when observed by a diver from directly above. All information is recorded on datasheet 01 (Appendix B).

Tools needed
GPS
Stake as site marker
Hammer
30 m underwater tape measure
Underwater compass
0.25 m^2 square quadrat, negatively buoyant
8 survey flags (if needed)
Clipboard, underwater data sheets, pencils.
Ruler
Underwater camera
Datasheet 01

Requirements
Personnel: 2 divers / snorkelers, 1 topside assistant
Estimated total time: 1 hour

3 RAPID FIELD ASSESSMENT OF SEDIMENT TEXTURE

Assess the predominant sediment texture in each quadrat, using the 9-category classification scheme (Table 3; Howard et al 2021). The diver can use this qualitative test to help classification:

1. Pick up a small (ca. 5 mL) volume of surficial sediment without compacting it.
2. Rub some of the sediment between bare fingertips. If it does not reel grainy, it is scored as Mud (1)
3. Lift it above the sediment-water surface and drop the sample.
4. Observe the sample fall through the water.

    i. If the sample forms a cloud in the water, and very few particles are big enough to sink, score it as Mud (value = 1)

    ii. If the sample forms a cloud but *less* than half of the released sample falls rapidly as particles, it is scored as Sandy Mud (2).

    iii. If the sample forms a cloud but *more* than half of the released sample falls rapidly as particles, it is scored as Muddy Sand (3).

    iv. Small grains fall quickly in water column, very little cloud forms, it is scored as Sand (4).

    v. The remaining categories are determined by estimating size of predominant grains.

In cases where quadrats contain multiple sediment textures (like a living coral amongst sandy soils, for example), base the score on the sediment texture that covers the largest section of the quadrat. Note the sediment type on datasheet 01 (Appendix B).

Table 3. Sediment texture categories for rapid field observations of seagrass soils.

Grain size category	Numeric value	Description
Mud	1	Individual grains indistinguishable, easily compress in hand, sediment remains clumped after compression.
Sandy mud	2	Majority of grains indistinguishable but textured upon touch, easily compress in hand, sediment remains clumped after compression
Muddy Sand	3	Sandy texture upon touch but compresses in hand, sediment dissociates upon release with most grain falling in water column
Sand	4	Clearly distinguishable grains, difficult to compress in hand, grains fall quickly in water column
Coarse Shell	5	Shell and shell remains dominate sediments (approx. 5-10 mm in size)
Halimeda-hash	6	Remains of carbonate segments from *Halimeda* detritus (approx. 5-10 mm in size)
Rubble	7	Medium size rock or coral skeletal fragments (approx. 10-25 mm in size)
Live Coral	8	Living hard coral
Rock	9	Bedrock or solid biogenic carbonate formations

4 FIELD COLLECTION OF SEAGRASS PLANTS FOR BIOMASS, MORPHOLOGY, $\delta^{13}C$ AND C, N, P CONTENT

First, collect seagrass shoots from within 5 m of the central stake at each site. Collect whole shoots selected haphazardly by digging down into the soil with your fingers enough to pluck the shoot off the underground stem without dislodging any of the leaves on that shoot. Because of the differences in sizes of different seagrass species, collect 7 shoots of *Thalassia testudinum*, 30 shoots of *Syringodium filiforme*, 40 shoots of *Halodule* species and 50 shoots of *Halophila* species. All seagrasses for each species present in the site are then collected in a 1-gallon ziplock bag that has been labelled with the site name. Keep these samples cool and out of the sun until they are processed.

For sampling seagrass above- and belowground biomass, 20 cm diameter cores are collected at 8 quadrat positions, either directly after recording benthic species cover or, when survey flags were used, at their locations. Carefully place the PVC core on the sediment surface. It is extremely important to check both the inside and outside edges of the PVC ring to ensure that seagrass leaves are not trapped underneath. If there are leaves inside the ring that originate from shoots outside the PVC ring, carefully pull these leaves out. Conversely, if there are leaves outside the PVC ring that originate from shoots inside the ring, carefully pull these leaves inside. Only after this has been completed, insert the ring into the

sediment while simultaneously twisting the core to sever belowground rhizomes. Carefully remove the core and place all captured aboveground and belowground vegetative biomass into a mesh bag, and insert an identifying label with core ID written on a small piece of waterproof paper in each bag. Gently shake the bag underwater to remove loosely attached sediment. Note on datasheet 01 whether a biomass core was taken at each quadrat location.

After all other sampling at a site is completed, collect voucher specimens of all seagrass species present to mount on herbarium sheets in a plant press. Try to collect at least 2 fragments of *Thalassia testudinum*, 3 of *Syringodium filiforme*, 5 of *Halodule* species and ca. 10 of *Halophila* species. The best samples are excavated plant fragments that have 3-5 connected and intact shoots with roots and a rhizome apical meristem attached (see example photos in the Figure 6).

Tools needed
20-cm diameter PVC core
Clipboard, pencil
Datasheet 01 on underwater paper
Mesh bags
Zip-lock bags
Waterproof paper for labels
Underwater tape measure

Requirements
Personnel: 2 divers / snorkelers, 1 topside assistant
Estimated total time: 1 hour

5 COLLECTING SOIL CORES FOR QUANTIFYING SEAGRASS SOIL CARBON STOCKS

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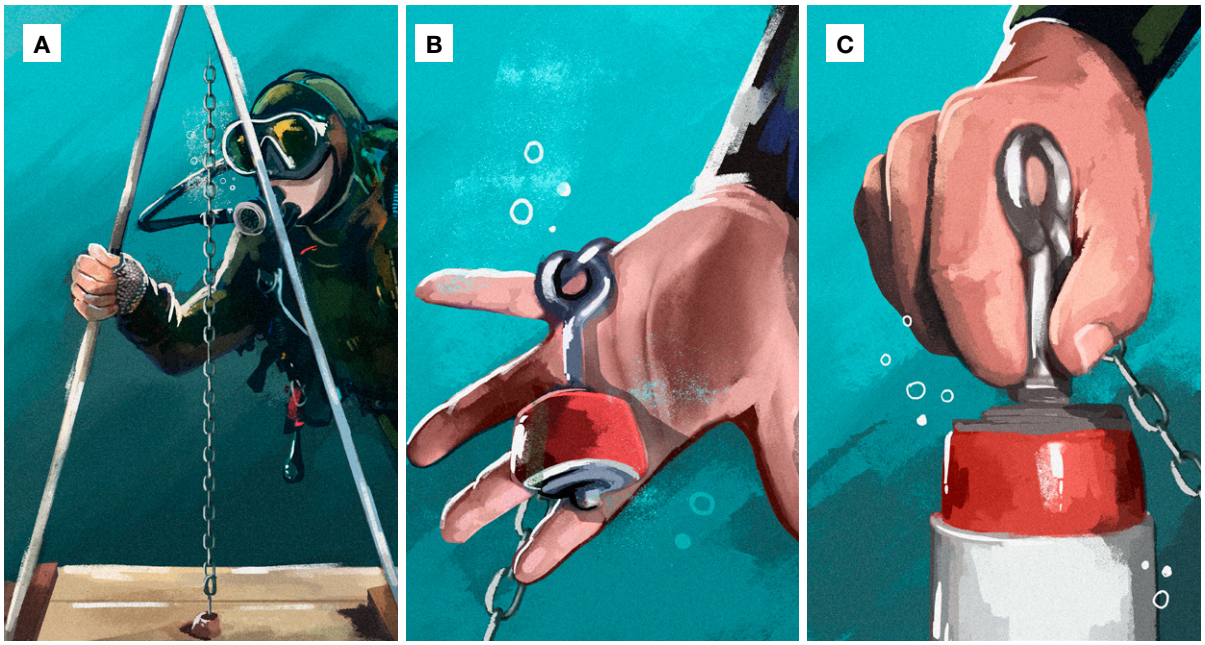
We will collect one core per seagrass meadow (two per partner site) for quantifying seagrass soil carbon stocks. Cores will be driven to refusal, or to 1 m deep in the soil if the soils are deeper than 1 m. A tripod and a piston corer (diver-operated in water depths >1.5 m) are used following the methods of Sansone et al. (1994). Cores will be driven through the soil until they reach underlying rock, up to 1 m deep. These cores will be sub-sampled every ~5 cm for determination of porosity, dry bulk density, soil inorganic carbon, organic carbon, nitrogen, and phosphorus. Stable carbon isotope ratios ($\delta^{13}C$) of soil organic matter will also be determined.

Steps for taking soil samples in seagrass systems are unique because the soils are saturated with water, do not hold their shape well, and are more susceptible to compaction. Further they are typically underwater, requiring the operator to use SCUBA. We will use thin-walled stainless steel tubing and a piston constructed from a rubber bung, an eye bolt, washers and nuts (Fig. 3). A portable tripod constructed from iron pipe (or a ladder) is used to hold the piston in place (vertical position) and a winch can be attached to aid in removing the core. For improved stability, a wooden board with a hole in the center can be used as a coring platform (Fig. 3). A chain-block is used to keep the core straight as it is being removed (Fig. 4). Another option is to excavate the core barrel out of the surrounding soil.

These methods are reproduced from Chapter 3 "Field Sampling of soil carbon pools in Coastal Ecosystems" from the Blue Carbon Initiative's "Blue Carbon Manual", available free of charge in Spanish and English from https://www.thebluecarboninitiative.org/manual

Please see this manual for all things Blue Carbon!

Figure 3. Piston constructed from a rubber bung, eye bolt, washers, and nuts (B). A chain can be attached to the piston (A), which should fit snug on the coring pipe (C)



CORING TUBE PREPARATION

The coring tube needs to be prepared before entering the water. First, rinse the coring tube with freshwater, then dry it thoroughly with a towel. Next, cover the holes on the side of the tube with duct tape. To do this, run one stripe of duct tape along the core directly above the holes. It is important that there are no wrinkles in the tape, because they could cause the tape to detach when the core is driven into the sediment. Reinforce the duct tape by attaching two additional stripes of tape on top of the first one, each 50 % offset to either side of the first stripe, again ensuring there are no wrinkles in the tape. Below the bottom hole and just above the top hole, wrap several layers of duct tape around the core, to reinforce the tape stripes covering the holes. The upper tape will serve as a visual guide for the maximum depth to which the core should be driven into the sediment, so using a colored duct tape can be useful. It is important that the coring tube is not driven deeper into the sediment, because the top hole is the first subsample that can be retrieved from the core and it should correspond to the sediment surface. Lastly, insert the piston and chain into the coring tube, making sure it fits snugly. Modulate the piston size by turning the wingnut to ensure a tight fit. Once the tube is taped and the piston is inserted, it can be used to collect the sediment core.

Click, tap or scan to see more

Figure 4. The core is inserted into the sediment using a sledgehammer. A tripod and metal chain ensure that the core is kept in a vertical position and can assist in removing the core from the soil.

TAKING THE SEDIMENT CORE

Before taking the sediment core, measure the sediment depth at the center stake. Insert a 2 m sediment depth rod until it reaches 2 m or a solid horizon. Measure this depth and record on datasheet 02 (Appendix C) under 'Total Sediment Depth'. If the rod does not reach a solid horizon, record the depth as >200 cm. If the rod reaches a solid horizon before 1 m, make sure you don't hammer the core tube below that depth.

Next, set up the coring station directly at the center stake. Make sure the exact coring location was not disturbed by the stake, depth rod, or field personnel during previous tasks. Set up the tripod on top of the board and attach the chain that is attached to the piston. Make sure the coring tube is upright and that the chain holding the piston does not have any slack. The core tube can then be driven into the sediment using a sledgehammer or a post pounder (Fig. 4). After the core barrel is driven to the desired depth, fix the chain holding the piston to the coring tube using a vice grip. This is holding the piston in place when the core is removed. Remove the core carefully and cap the bottom immediately to prevent sediment loss. The core barrel may be very difficult to remove and the use of a chain (or other non-stretching line) along with a hand-held winch is recommended.

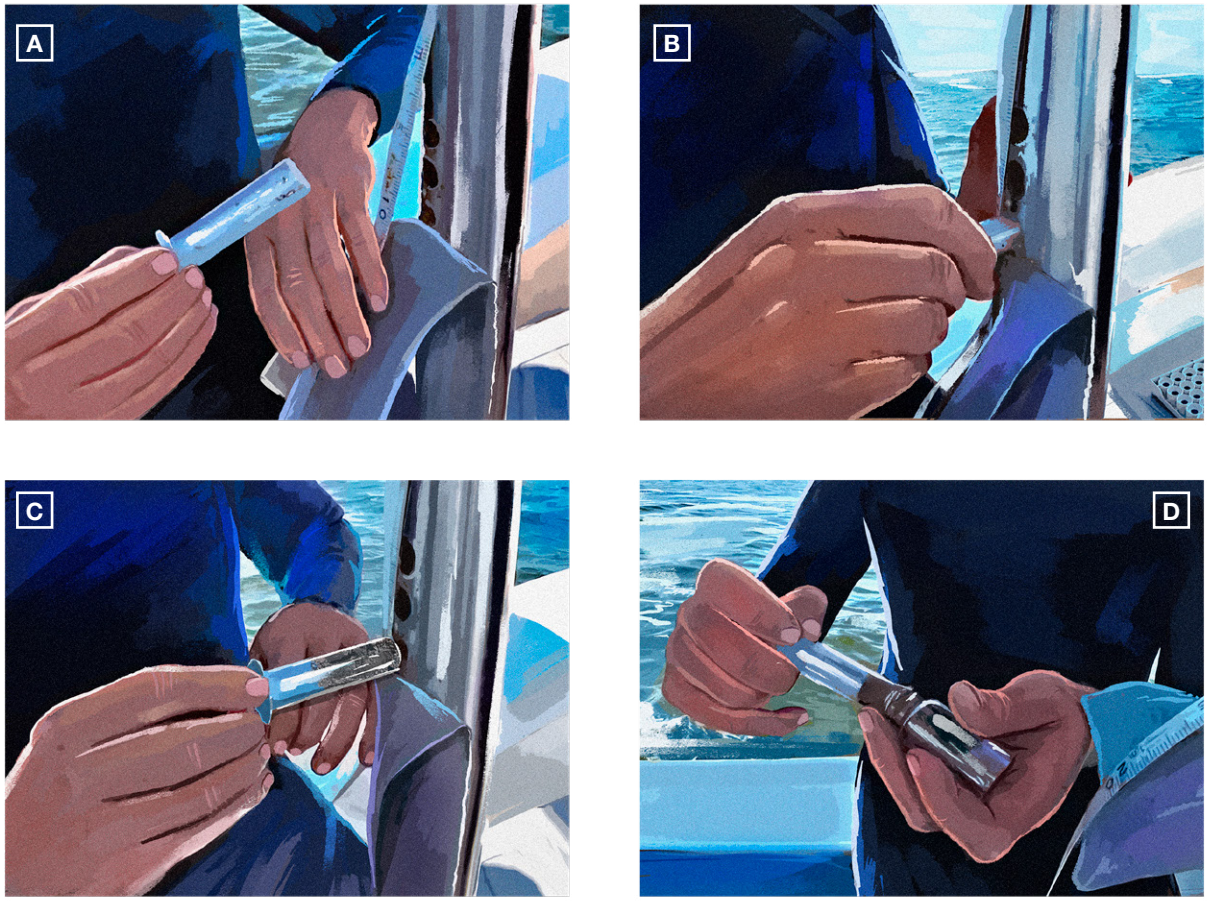
Once the coring tube is removed and capped, keep it upright while it is being transferred to the boat or shore for subsampling. If subsampling at the lab, seal the plastic cap with duct tape and keep the core upright during transport. Note that it is very important to keep cores upright and to avoid water loss during transportation so that the core layers do not mix within the tube. If it is logistically difficult to transport the entire core vertically to the lab, subsamples should be taken at the site.

Requirements
Personnel: 2 divers / snorkelers, 1 topside assistant
Estimated total time: 1 hour core tube preparation, 1 hour taking the core

6 COLLECTING SUBSAMPLES OF THE CORE WITH DEPTH IN THE SOIL

There is a risk of mixing the soil layers if the core is laid on its side for transport or subsampling, so perform the next steps holding the coring tube upright. Before subsampling, identify the location of the sediment surface inside the coring tube. If the core did not reach 1 meter depth, slowly peel back the duct tape starting at the top hole until the sediment surface is reached. Attach the measuring tape to the coring tube where the sediment surface is located, so that the depth of each subsample can be determined. A 25 mL, cut-off polyethylene syringe (2 cm diameter) can be used as a mini-corer to accurately subsample loose and saturated soils inside the coring tube (Fig. 5). The core tube is predrilled with 2.5 cm diameter sampling ports at 2.5 cm intervals. The duct tape covering the holes for subsampling is slowly peeled downward (Fig. 5A), starting from the top, to uncover one hole at a time. At each hole, the sub-corer is inserted (Fig. 5B), starting at the top, to extract a soil sample of known volume. When inserting the syringe core, hold the plunger so that it stays in contact with the surface of the subcore, then push the syringe barrel with a twisting motion into the core tube to collect the subsample. It is important to note the volume sampled each time (Fig. 5C, D). To estimate the volume, use a spatula or knife to remove any sediment material protruding the sub-corer. Record the volume and the depth of each subcore on datasheet 02 (Appendix C). Transfer the entire subsample from the syringe into pre-weighed glass vials, making sure not to lose any fine sediment or water.

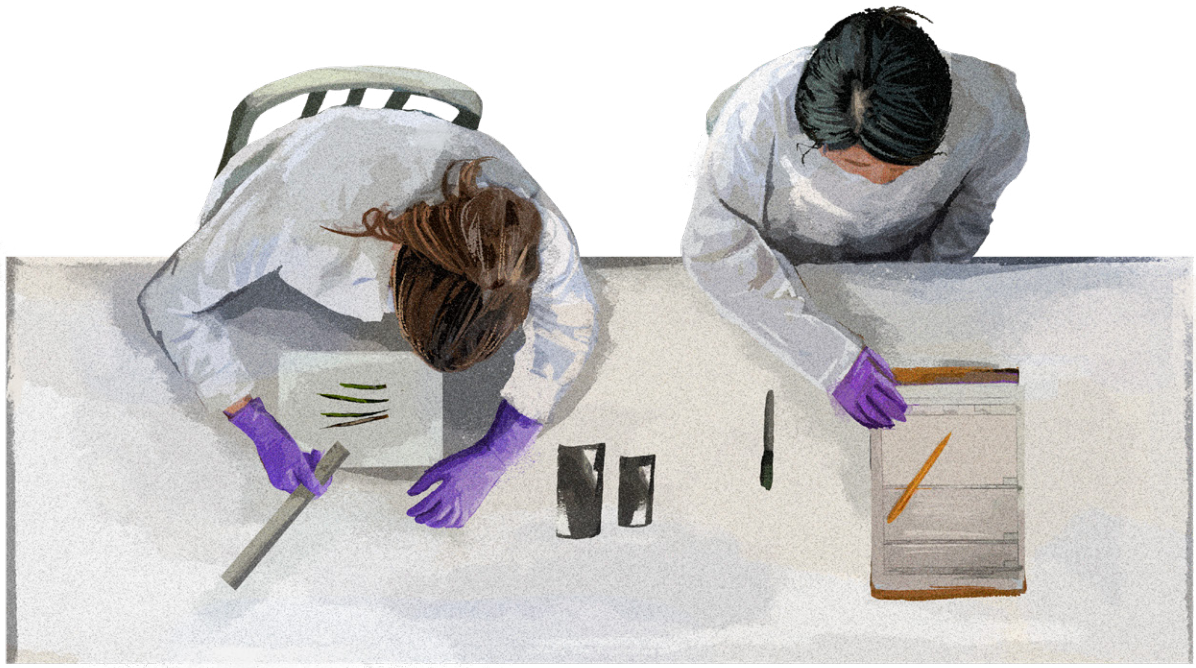
Figure 5. Subsampling of the core using a 25 mL cut-off polyethylene syringe. The duct tape is peeled back starting at the top of the core (A); the syringe is used to remove a sediment sample form the core (B,C); the exact volume of sediment within the syringe is noted on a datasheet (D) before transferring the subsample into a pre-weighed glass vial.



Tools and supplies needed	
2 m rod for sediment depth	Towel to dry coring tube
Coring tube (supplied by project management)	Freshwater and wipes to clean coring tube
Piston to fit in core barrel	Duct tape (contrasting color, if possible)
Metal tripod and chain	Knife or spatula
Sledgehammer or post pounder	Rope
25 mL cut-off polyethylene syringes (subcores)	Come-along / winch
20 mL pre-weighted glass scintillation vials	Board as coring platform
Datasheet 02	Vice-grip

Requirements
Personnel: 2 persons
Estimated total time: 1 hour

7 LABORATORY ANALYSIS OF SEAGRASS BIOMASS AND MORPHOLOGY



In the laboratory, take each mesh bag with biomass core material and carefully transfer its contents into a separate tub filled with freshwater. Gently agitate the seagrass material in the freshwater to further remove loosely attached sediment. On datasheet 03 (Appendix D), record the total number of shoots for each seagrass species.

Separate the aboveground green leaf material with a razor blade from the belowground sheath. For the genera *Thalassia*, *Syringodium* and *Halodule*, arrange the green leaf material on a glass plate, and gently slide a razor blade down the length of both sides of each leaf of the shoot to remove attached epiphytes. Make sure you have removed all epiphytic material (including calcareous algae). Set aside the epiphytic material. Place the scraped leaves of each species into a single pre-weighed aluminum tare and label the aluminum foil with site name, species name, date, and 'AGB' for aboveground biomass. Now turn your attention to the belowground biomass. Rinse all root and rhizome material thoroughly to remove intermixed sediment particles. Carefully separate live root and rhizome biomass from dead material and pool roots and rhizomes into a pre-weighed aluminum foil tare for each species. This material will be dried and used for estimating seagrass biomass. Any additional vegetative biomass (macroalgae, including calcareous taxa, and other seagrass species), as well as the epiphytic algae scraped off the seagrass can be placed in separate aluminum tares for drying in the oven at 60 °C. After samples are dry (48 hours) record weights on datasheet 03 (Appendix D).

To process seagrass samples collected for morphology and elemental content, empty the ziplock bag containing seagrass shoots into a tub filled with fresh water. Then, process one seagrass species after another by taking out one shoot at a time and separating the blades from the rhizome, cutting with a razor blade at the intersection of the photosynthetic, green material and the rhizome. Separate the leaves of the shoot and remove epiphytes by gently scraping along each leaf with a razor blade. Collect the scraped-off material and discard. Arrange the scraped leaves from youngest to oldest on an even surface (e.g., glass plate) and measure their length in mm. For *Thalassia* leaves only, also measure the width in mm. Record each leaf length and width (for *Thalassia*) on datasheet 04 (Appendix E). Collect all measured leaves on pre-weighed aluminum tares, one tare per species. Dry all tares in the oven at 60°C for 48 hours. Finally, weigh the tares with dried seagrass leaves and record the weight on datasheet 04. Store the dried content in each tare in a cool and dark place until shipment to Florida International University.

By the end of lab processing, for each of your two sites you should have:

- 8 aluminum tares (1 per biomass core) for each seagrass species in the core, with pooled aboveground green leaf material from all shoots of a species (dried for 48 hours in a 60°C oven)
- 8 aluminum tares (1 per biomass core) for each seagrass species in the core, with pooled belowground biomass of a species (dried for 48 hours in a 60°C oven)
- 8 aluminum tares (1 pre biomass core) with any additional vegetative biomass / epiphytes (dried for 48 hours in a 60°C oven)
- Datasheet 03 with biomass core data, above- and belowground biomass, and shoot counts
- Datasheet 04 with shoot morphometric measurements and dry weights of below and above-ground biomass
- One aluminum tare per seagrass species with dried leaf material (dried for 48 hours in a 60°C oven), to be sent to FIU for CNP content determination

Tools needed
Tub / container
Ruler
Razor blades
Datasheets 03 and 04
Pencil
Smooth surface (glass plate)
35 Pre-weighed aluminum tares
Drying oven (at 60°C)
Mortar and pestle
Lab analytical scale
Herbarium sheets and press

Requirements
Personnel: 2 lab technicians
Estimated total time: 2 days lab processing

8 PRESERVING SEAGRASS VOUCHER SPECIMENS

Carefully clean the voucher specimens off to remove adhering sediment but without breaking the leaves or roots. Carefully lay these specimens out on herbarium paper (see Fig. 6 for examples). It sometimes helps to float the specimen over the herbarium sheet in a shallow pan of water and slowly draining off the water. Then use forceps and a paintbrush to lay the specimens out on the herbarium sheets. The multiple fragments per species can be arranged on the same sheet of herbarium paper if they will fit. Top the specimens with a sheet of wax paper, and then press in an herbarium press between layers of absorbent paper and leave to dry in a cool, dry place. When dry, label the herbarium sheets with location name, latitude and longitude, water depth, collector's name, and date in the lower right-hand corner using a pencil.

Tools and supplies needed
Herbarium press
20 sheets of 12"x18" herbarium paper
Roll of wax paper
Absorbent paper – newspaper works great
Forceps
Artist's paintbrush
Pencil
Shallow pan large enough to contain flat herbarium sheet

Requirements
Personnel: 1 lab technician
Estimated total time: 1 day lab processing, 3-7 days drying

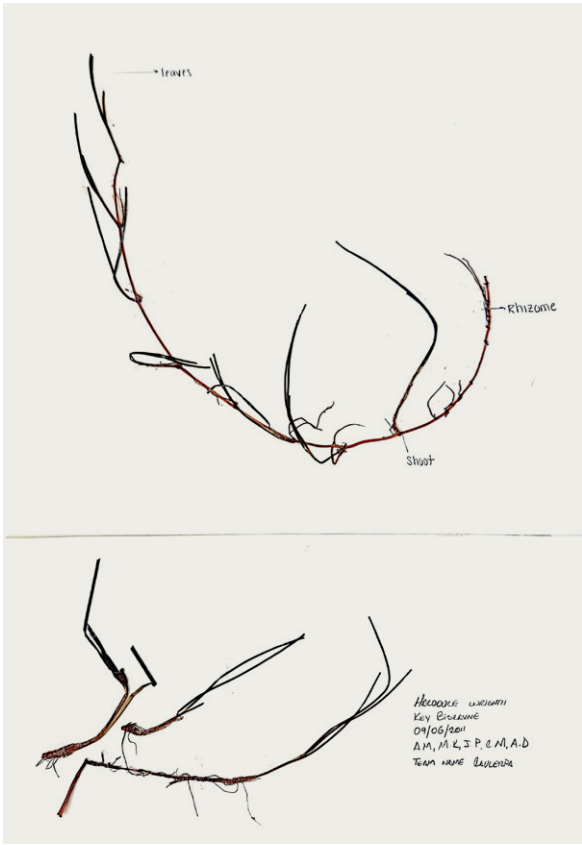
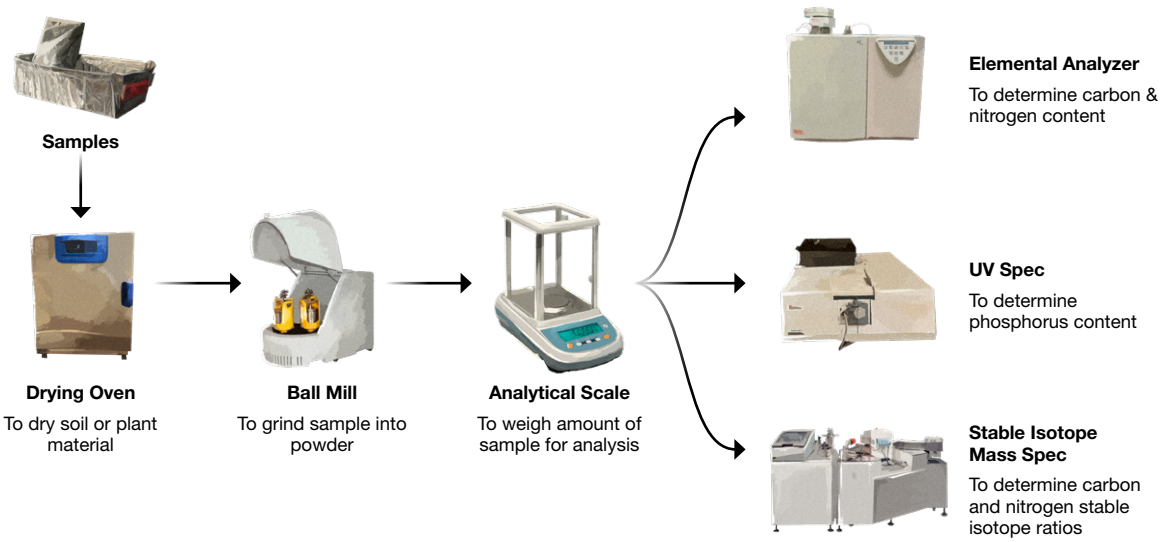


Figure 6. Example herbarium slides with seagrass specimen.

9 ANALYZING SUBCORE SAMPLES FROM DEEP CORES AND SAMPLE ANALYSIS AT FLORIDA INTERNATIONAL UNIVERSITY'S BLUE CARBON ANALYSIS LABORATORY



In the lab, remove the vial caps and record the wet weight of the vials containing the subcore samples on datasheet 05 (Appendix F). Transfer these vials into a drying oven at 60 °C, and dry until they reach a constant weight (generally overnight). Weigh the dry vials with the subcores in them and record the weights. Recap each vial with its original cap, close tightly, and ship the samples in the glass vials to Florida International University for chemical analyses.

At Florida International University, samples from each depth layer are analyzed for sediment porosity, dry bulk density, organic content and elemental content (C, N, P). Dry bulk density (DBD) is calculated as the dry weight of the sediment divided by the volume of the original soil sample, DBD will be calculated for each sample using the volumes and dry weights reported by collaborators from each site on datasheet 02. A subsample of each sample is ashed at 500 °C for five hours and organic content is calculated as loss on ignition (LOI). Dry soil samples are ground using a ceramic mortar and pestle or ball mill and total nutrients (C, N and P) are determined. Powdered samples are analyzed in duplicate for total carbon (TC) and nitrogen content using a CHN analyzer (Fisons NA1500). Ashed soils are also analyzed for total

inorganic carbon (IC) and the percentage of organic carbon (Corg) is calculated as the difference between IC and total carbon (TC). Estimation of the calcium carbonate content of the samples will be performed using the IC values and assuming a molecular formula of $CaCO_3$.

Phosphorus content is determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992). Similarly, dried plant material is analyzed in duplicate for C and N content using a CHN analyzer (Fisons NA1500). P content is determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract using a UV spectrophotometer (Fourqurean et al. 1992).

For all samples, elemental content is calculated on a dry weight basis, and elemental ratios are calculated on a mole:mole basis. Stable isotopes of C are determined using standard elemental analyzer/isotope ratio mass spectrometer (EAIRMS) procedures. The C isotopic ratios are reported in the standard delta notation (δ, ‰), presented with respect to the international standard of Vienna Pee Dee belemnite (V-PDB).

Tools and supplies needed
Drying oven
Analytical scale

Requirements
Personnel: 1 lab technician
Estimated total time: 2 hours processing, 1 day drying

REFERENCES

Fourqurean, J.W., Zieman, J.C., Powell, G.V.N., 1992. Phosphorus limitation of primary production in Florida Bay: Evidence from C:N:P ratios of the dominant seagrass Thalassia testudinum. Limnol. Oceanogr. 37, 162–171. https://doi.org/10.4319/lo.1992.37.1.0162

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APPENDIX A: LIST OF TOOLS AND EQUIPMENT

Task	Tool	Amount	✓
Field assessment of benthic cover	GPS	1	
	Stake as site marker	1	
	50 m underwater tape measure	1	
	Underwater compass	1 per diver	
	0.5 m x 0.5 m square quadrat, negatively buoyant	1 per diver	
	Survey flags marked with quadrat numbers	8	
	Clipboard, pencil	1 per diver	
	Datasheet 01 on underwater paper	3 per diver	
	Ruler	1 per diver	
	Underwater camera	1 per diver	
Field sampling of sediment depth, biomass, morphology, and CNP content	20-cm diameter PVC core	1	
	Clipboard, pencil	1 per diver	
	Datasheet 01 on underwater paper	1 Per diver	
	Mesh bags	9	
	Zip-lock bags	5	
	Waterproof paper for labels	1 sheet	
Laboratory processing of biomass, morphology, and CNP content	Tub / container	1 per analyst	
	Ruler	1 per analyst	
	Razor blades	1 per analyst	
	Datasheet 03	Multiple	
	Datasheet 04	1	
	Pencil	1 per analyst	
	Smooth surface (glass plate)	1 per analyst	
	Pre-weighed aluminum tares	35	
	Drying oven (at 60°C)	1	
	Mortar and pestle	1	
	Lab analytical scale	1	
	Herbarium sheets	1 per species	
	Herbarium press (or similar DIY press)	1	
Herbarium specimen	Herbarium press	1	
	Sheets of 12”x18” herbarium paper	20	
	Roll of wax paper	1	
	Absorbent paper – newspaper works great	1	
	Forceps	1	
	Artist’s paintbrush	1	
	Pencil	1	
	Shallow pan large enough to contain flat herbarium sheet	1	
Soil core collection and processing for blue carbon	2-m rod for sediment depth	1	
	Coring tube (supplied by project management)	1	
	Piston to fit in core barrel	1	
	Metal tripod and chain	1	
	Sledgehammer or post pounder	1	
	25 mL cut-off polyethylene syringes (subcores)	1	
	20 mL glass scintillation vials	10	
	Datasheet 02	1	
	Towel to dry coring tube	1	
	Freshwater and wipes to clean coring tube		
	Duct tape (contrasting color, if possible)	5 m	
	Knife	1	
	Spatula	1	
	Rope	1 m	
	Come-along / winch	1	
	Board as coring platform	1	
	Vice-grip	1	

APPENDIX B: DATASHEET 01 FOR BB COVER FIELD OBSERVATIONS

Depth (m): **Lat:** **Lon:**	**Site #:**	**Time:**	**Diver:**
	Curr: None Light Mod Strong Surge		**Viz:** <10 10 20 30 40 50 >50

MARINE PLANT TAXA	Thalassia Syringodium Halodule Halophila stipulacea Halophila decipiens Ruppia
	Green fleshy algae Red algae Brown algae Calcareous Green Calcareous Red Sponges Stony Corals Octocorals Other
ABUNDANCE	**0.1** = solitary (<5% cover) **0.5** = 1-5 indv. (<5% cover) **1** = >5 indiv. (<5% cover) **2** = 5≤25% **3** = >25≤50% **4** = >50≤75% **5** = >75%
SUBSTRATE TYPE	Mud Sandy-Mud Muddy-Sand Sand Coarse Shell Halimeda-Hash Rubble Live Coral Rock

Bearing (°) / Distance from stake (m)		**Q1:** ° m	**Q2:** ° m	**Q3:** ° m	**Q4:** ° m
Q5: ° m	**Q6:** ° m	**Q7:** ° m	**Q8:** ° m	**Q9:** ° m	**Q10:** ° m
Q11: ° m	**Q12:** ° m	**Q13:** ° m	**Q14:** ° m	**Q15:** ° m	**Q16:** ° m

Quad #	Sub/ Canopy	Taxa	Abun	Biomass	Picture ID	Quad #	Sub/ Canopy	Taxa	Abun	Biomass	Picture ID
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APPENDIX C: DATASHEET 02 FOR SEDIMENT CORE FIELD NOTES AND SUBSAMPLING

Sediment Core Field Datasheet		Site:
Core ID:	Lat:	Lon:
Date (MM-DD-YY):		Seagrass species at coring location:
Total Sediment Depth (cm):		Notes:

Subcore extracted with syringe			**Subcore extracted with syringe**		
Subcore depth (cm)	**Subcore volume (cm^3)**	**Vial ID**	**Subcore depth (cm)**	**Subcore volume (cm^3)**	**Vial ID**

APPENDIX D: DATASHEET 03 FOR BIOMASS CORE LABORATORY USE

Biomass Collection		
Site:	Processing Date:	Technician:

			Aboveground Biomass			Belowground Biomass				
Quadrat Number	**Species**	**Total # Shoots**	**Tare Number**	**Tare Weight**	**Tare + Dried Plant Weight**	**Tare Number**	**Tare Weight**	**Tare + Dried Plant Weight**	**Above-ground Biomass (g)**	**Below-ground Biomass (g)**

APPENDIX E: DATASHEET 04 FOR SEAGRASS MORPHOLOGY LABORATORY USE

C:N:P Leaf Morphometrics Data Sheet		
Sampling:	Site Serial No.:	Site:

		Please record Length & Width from Youngest Leaf to Oldest Leaf for Tt						
Sp./Tare #	**SS#**	**Leaf # 1**	**Leaf #2**	**Leaf #3**	**Leaf #4**	**Leaf #5**	**Leaf #6**	**Leaf #7**
Tt	1							
#	2							
	3							
	4							
	5							
	6							
	7							

Sp./Tare #	**SS#**	**Leaf # 1**	**Leaf #2**	**Leaf #3**	**SS#**	**Leaf # 1**	**Leaf #2**	**Leaf #3**
Sf	1				16			
#	2				17			
	3				18			
	4				19			
	5				20			
	6				21			
	7				22			
	8				23			
	9				24			
	10				25			
	11				26			
	12				27			
	13				28			
	14				29			
	15				30			

Sp./Tare #	**SS#**	**Leaf # 1**	**Leaf #2**	**Leaf #3**	**SS#**	**Leaf # 1**	**Leaf #2**	**Leaf #3**
Hw	1				21			
#	2				22			
	3				23			
	4				24			
	5				25			
	6				26			
	7				27			
	8				28			
	9				29			
	10				30			
	11				31			
	12				32			
	13				33			
	14				34			
	15				35			
	16				36			
	17				37			
	18				38			
	19				39			
	20				40			

Sp./Tare #	**Sp./Tare #**	**Sp./Tare #**	**Sp./Tare #**
Hd	He		
#	#	#	#

Date Processed/Initials:

APPENDIX F: DATASHEET 05 FOR SEDIMENT SUBCORE LABORATORY PROCESSING

Sediment Core Lab Datasheet	Site:	Core ID
Date: Recorder 1:		Recorder 2:

To complete before field sampling		**To complete after field sampling**				
Vial ID	**Vial initial weight (g)**	**Vial + sample wet weight (g)**	**Vial + sample dry weight (g)**	**Dry weight of sediment (g)**	**Sample volume (cm^3)**	**Dry bulk density (g/cm^3)**