

Florida Bivalve-Seagrass Restoration Consortium Coordinated Network Experiment Manual

Version 1
September 19, 2025



This initial version of the coordinated network experiment manual provides a high-level overview of the proposed experiment and is designed to give prospective site leads information necessary to understand general activities associated with the experiment to evaluate capabilities, time commitments, and costs. Specific guidelines and protocols are subject to modifications through consensus among coordinated network experiment site leads.

The development of this manual and the larger project it will support was made possible through funding from the Florida Department of Environmental Protection. This manual was conceptualized and developed by attendees of the Florida Bivalve-Seagrass Restoration Consortium Coordinated Network Experiment Workshop in August 2025.

For questions about this manual, please contact Katie Lohr (kelohr@ufl.edu).

Introduction

The purpose of this manual is to provide a set of standardized guidelines for a coordinated network experiment to quantify ecological interactions between hard clams (*Mercenaria* spp.) and seagrasses (primarily *Halodule wrightii*) at a series of sites across Florida. The experimental protocols in this document must be followed by all participating teams to ensure the data generated are comparable. The coordinated network experiment will address the following questions:

- (1) What factors influence interactions between seagrasses and clams?
- (2) How do clams and seagrass influence one another under different environmental conditions?
- (3) What conditions influence the success of clam-seagrass co-restoration (i.e., benefit one or both taxa)?

All teams interested in participating in the coordinated network experiment must, at minimum, execute the experiment laid out in this plan. However, if teams are capable and interested, they may also propose supplementary studies (see the requests for proposals for more information).

Experimental Design

A factorial design will be used to evaluate the effects of adding native aquacultured clams in areas with and without seagrass (Figure 1). The two levels of the “seagrass” factor (seagrass, unvegetated substrate) will be established by locating plots in naturally vegetated and unvegetated areas, not by planting or removing seagrass. However, the two levels of the “clams” factor (clams added, no clams added) will be achieved by adding clams to some plots.

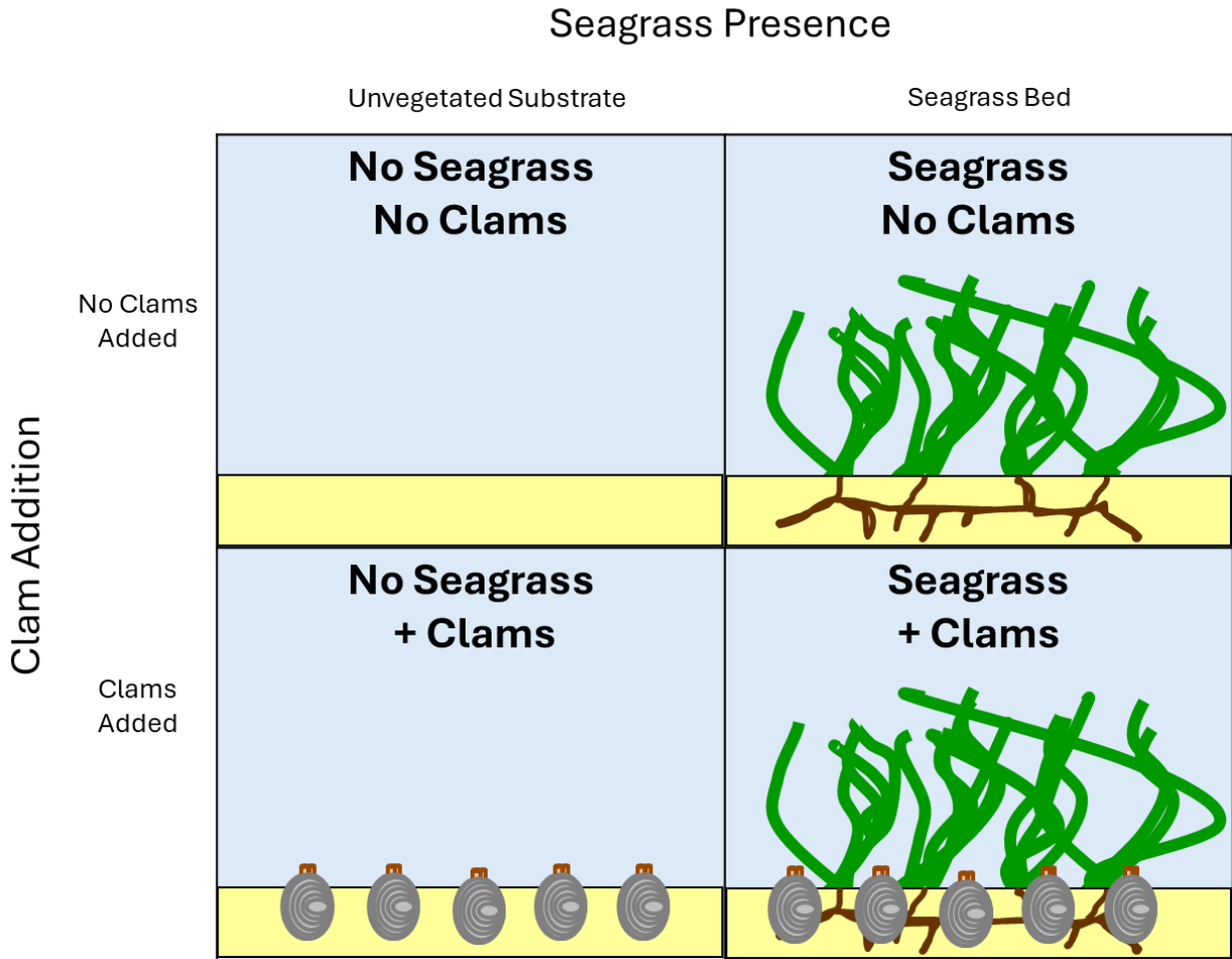


Figure 1. Experimental treatments and controls.

At each site, six replicate 0.25 m² (0.5 x 0.5 m) plots will be established for each treatment or control type for a total of 24 plots per site:

- **Unvegetated Substrate (No Seagrass)** consists of a naturally bare area, i.e., one with no seagrass cover. Ideally it should be close to the seagrass bed and have similar depth and other environmental characteristics.
- **Seagrass Bed (Seagrass)** consists of a naturally vegetated area with moderate to high cover of *Halodule wrightii*.
- **No Clams Added (No Clams)** means a plot that is a control treatment with respect to clams; it may have some naturally occurring clams, but none have been added.
- **Clams Added (+ Clams)** denotes a plot to which aquacultured clams (*Mercenaria* spp.) are added.

Plots will be established in April 2025 and field experiments will run through October 2025 (approximately 7 months, or one growing season).

Site Selection

Sites proposed for the coordinated network experiment should conform to the following characteristics (modified from Campbell et al., 2024; Campbell et al., n.d.). A patch of continuous seagrass bed of 5 m x 7.5 m is needed to accommodate the seagrass plots in 3 rows of 4 plots with 2 m separation between plots. An equally sized bare area is needed to accommodate the array of unvegetated plots. If the seagrass is too patchy to accommodate the 3 x 4 grid of plots, a different spatial arrangement of plots can be used.

Biological Characteristics	Preferred
Depth	<3 m
Seagrass cover	25–100%
Seagrass area	5 m x 7.5 m (minimum)
Seagrass species	>70% <i>Halodule wrightii</i>
Epiphyte load	None, or light/patchy epiphyte coating
Seagrass bed characteristics	Uniformity of depth, canopy height, density
Canopy height	No requirement

Physical Characteristics	Preferred
Exposure	Protected from prevailing storm winds
Wave energy	Low
Flow	Low to moderate
Distance from boat launches/docks	≥200 m
Distance from public access point	≥100 m

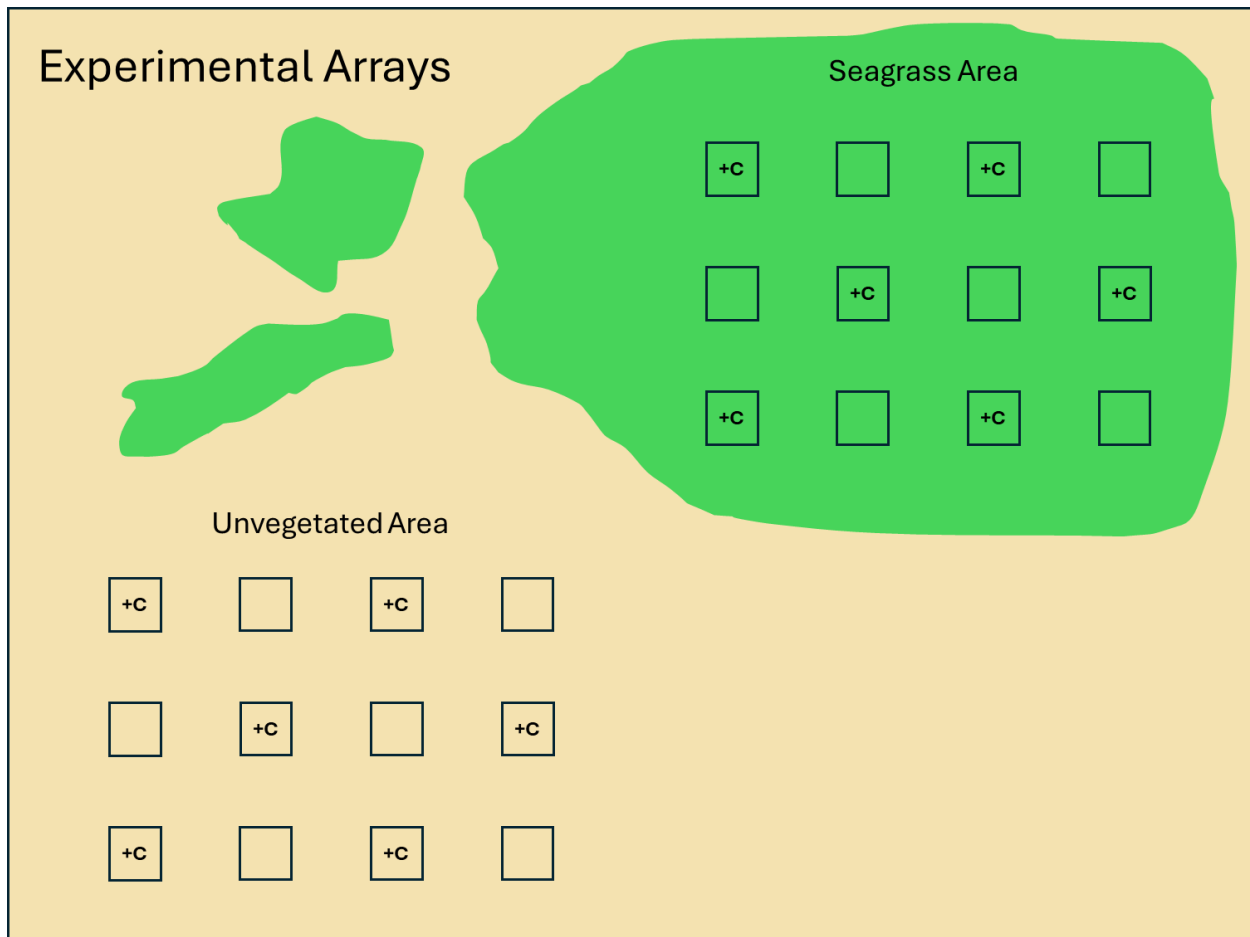


Figure 2. Ideal experimental layout. Squares marked “+c” indicate plots with added clams. Plot squares are not to scale; there should be 2 m separation between adjacent plots.

Major Materials

To Be Provided by Site Lead

In addition to access to a site that meets the criteria above, each site team must have access to the following equipment:

- Muffle furnace
- Analytical balance (to 0.000 g)
- Spectrophotometer
- Drying oven
- Vacuum pump
- YSI (with salinity, temperature, and dissolved oxygen sensors, calibrated monthly)

Additionally, consumable materials will need to be provided by each site lead (e.g., plot markers, hand rakes, scintillation vials, syringe filters). The amounts and types of these

materials (including specific brands, where necessary) will be determined collectively and agreed upon by the site leads prior to the start of the experiment.

To Be Provided by Sea Grant

The following equipment will be provided to each funded team:

- Rhizon samplers
- Salt Water Conductivity/Salinity Data Loggers
- Submersible Photosynthetic Active Radiation (PAR) Logger
- Aquacultured clams (*Mercenaria campechiensis* for Gulf sites and *M. mercenaria* for Atlantic sites)

Additionally, Sea Grant will cover the cost of the following laboratory analyses:

- Sediment porewater nutrients
- Water column chlorophyll and nutrients
- Seagrass foliar nutrients

Site Leads will be responsible for collecting, preparing, and shipping samples to a central laboratory (to be determined). Please review the sections below for additional information on sampling and collection frequency.

Experimental Setup

Site Establishment

Data loggers will be cable-tied to a separate PVC post near the experimental array. The loggers should be placed at the top of the seagrass canopy, with the sensors parallel to the sediment surface. The PAR logger must be oriented upward according to the manufacturer's specifications.

Within each site, three sentinel commercial aquaculture growout bags will be deployed to track clam survival monthly. Sentinel bags will be placed within the site in areas of bare substrate. A total of 25 clams, 20–25 mm in size, will be added to each bag.

Plot Establishment

At each site, 24 0.25-m² experimental plots will be established, ideally in two 4 x 3 arrays of 12 plots, one array in a seagrass bed and one array outside the bed (Figure 2). Each plot will be separated from adjacent plots by 2 m in each direction. Each experimental plot will be demarcated at all four corners with stakes (PVC, wood, bamboo, or rebar) and will be labeled for identification.

Twenty-five clams (*M. mercenaria* or *M. campechiensis*, depending on site region) approximately 25 mm in size will be added to each “bare substrate + clams” plot (n = 6) and “seagrass-dominated + clams” plot (n = 6).

Sampling

Table 1 provides an overview of the timeline over which the following sampling activities will be carried out. Specific protocols for sample collection will be determined collectively and agreed upon by site leads prior to the start of the experiment.

Table 1. Clam, seagrass, and site characteristic sampling schedule (2026).

	APR	MAY	JUN	JUL	AUG	SEP	OCT
Site: Bivalve community	X						X
Clam: Size (plots)	X						X
Clam: Survival (plots)							X
Clam: Survival (sentinel bags; marked shells visible on plot surface)		X	X	X	X	X	X
Clam: Condition index, biomass							X
Seagrass: Above/below ground biomass	X						X
Seagrass: Percent cover, percent occurrence, shoot density, species composition, canopy height, epiphyte cover	X	X	X	X	X	X	X
Seagrass: Foliar nutrients	X			X			X
Sediment: Texture, bulk density	X						X
Sediment: Organic matter, porewater nutrients, porewater sulfide	X			X			X
Water: Chlorophyll, total suspended solids, nutrients, depth, flow (clod cards), YSI measurements	X	X	X	X	X	X	X

Bivalve Community

To evaluate native infaunal bivalve communities at each site, a combination of hand raking and sediment sampling will be completed at the beginning (April 2026) and end (October 2026) of the experiment. Sediment samples will also be used to evaluate seagrass above- and below-ground biomass (see seagrass section).

Quadrats

At the start of the experiment, 12 0.25-m² areas will be selected, six in naturally bare areas and six in seagrass-dominated areas, outside of experimental plots. A quadrat will be placed to demarcate the correct area. Within the quadrat, the entirety of the 0.25-m² area will be hand-raked to 5-cm depth and all hard clam and other macrobivalves obtained from hand-raking will be enumerated and measured.

Sediment Sampling

Adjacent to each quadrat, a sample will be collected using a 160-mm diameter sediment sampler. These samples will be passed through a 2 mm size mesh (e.g., box sieve) and the remaining contents collected and stored on ice. Later, samples will be weighed and sorted to identify and enumerate smaller bivalves. At the end of the experiment, the same process will be repeated in all experimental plots (n = 24). The same samples will be used to evaluate above- and below-ground seagrass biomass.

Clams

Before clams are added to each plot, their shells will be marked with fluorescent nail polish for identification. Each clam will be measured (shell length) prior to its addition to each plot (April 2026). A subsample (n = 30) will be collected to determine weight-to-length ratio prior to deployment, as described below. Clams will be placed by hand within each plot, as well as in three sentinel bags. Clams in sentinel bags will be visually inspected monthly to determine survival, and the surface of each plot will also be inspected to identify empty, marked shells at the surface. At the end of the experiment (October 2026), each plot will be hand-raked to recover marked clams. Each recaptured clam will be measured (shell length) and condition index will be assessed by measuring tissue ash-free dry weight-to-length ratio (weight divided by length), dry shell weight, and ash-free dry weight of tissue (which will be used to compute biomass).

Seagrass

Above- and Below-Ground Biomass

The same sediment samples collected in April and October to evaluate the infaunal community in seagrass areas will also be used to evaluate above- and below-ground seagrass biomass at the beginning and end of the experiment.

Visual Evaluation

Each month, percent cover, percent occurrence, shoot density, species composition, canopy height, and epiphyte cover will be measured.

Foliar Nutrients

At the beginning (April), middle (July), and end (October) of the experiment, foliar nutrients will be measured.

Sediment

Texture and Bulk Density

Sediment texture and bulk density will be measured at the beginning and end of the experiment. 100 g of sediment will be collected from each plot using two 7–8 cm deep samples from a 60 cc syringe.

Organic Matter, Porewater Nutrients, and Porewater Sulfide

Organic matter and porewater nutrients/sulfides will be measured at the beginning, middle, and end of the experiment (i.e., April, July, and October).

Water Quality

In addition to data collected continuously using conductivity/salinity and PAR loggers, the following metrics will be measured monthly: water column nutrients (dissolved nitrate and ammonium and phosphate), water column chlorophyll, total suspended solids, depth, and flow. Additional water quality characteristics will be measured using a YSI.

Literature Cited

- Campbell, J. E., Rhoades, O. K., Munson, C. J., Altieri, A. H., Douglass, J. G.,...& Wied, W. L. (2024). Herbivore effects increase with latitude across the extent of a foundational seagrass. *Nature Ecology and Evolution*, 8, 663–675. <https://doi.org/10.1038/s41559-024-02336-5>
- Campbell, J., Rhoades, O., Altieri, A., Douglass, J., Heck, K., & Paul, V. (n.d.). *Handbook for seagrass tropicalization network experiment* [Unpublished document]. Smithsonian Marine Station at Fort Pierce.