NEDDS CHC Manitaring Protocol						
NERRS GHG Monitoring Protocol						
Using portable sensors to measure CO ₂ greenhouse gas fluxes						
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Waquoit Bay, Narragansett Bay, Great Bay, Wells, & Connecticut National Estuarine Research Reserves						

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Introduction

Salt marshes provide a variety of ecosystem services – chief among them is their ability to sequester carbon (C). In fact, salt marshes sequester more carbon per unit area than any terrestrial ecosystem (McLeod *et al.*, 2011). This carbon sequestration is an important component of blue carbon – that is the atmospheric carbon captured and stored by marine ecosystems. Measuring fluxes of carbon dioxide (CO₂) between the atmosphere and coastal systems (Fig. 1) is a key component in quantifying coastal blue carbon and its role in climate change mitigation (Crooks *et al.*, 2018). Despite its importance, there is a severe lack of salt marsh greenhouse gas carbon dioxide flux data. Further, carbon dioxide data that we do have for salt marshes are plagued with a high degree of uncertainty.

A primary factor contributing to the uncertainty in carbon dioxide fluxes from coastal systems is their inherent spatial and temporal heterogeneity across scales both large (i.e., across multiple salt marshes) and small (i.e., within marsh variability). Therefore, if we are to better understand C dynamics in salt marshes, we also need higher resolution flux data. The paucity of data is a major barrier to developing complete C sequestration values for salt marshes. It also limits our knowledge of how climate change impacts (e.g., rising sea levels) and habitat restoration efforts (e.g., remediating impaired hydrology or sediment placement to help keep pace with sea

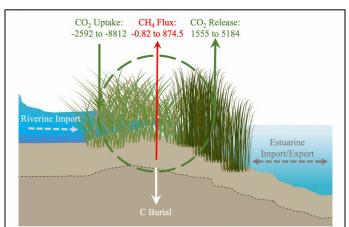


Figure 1. Overview of carbon uptake, storage, and release in salt marsh ecosystems. Green arrows show CO₂ flux (data from Geoghegan et al., 2018), red arrow shows CH₄ flux, white arrow show carbon burial rates (data from Rosentreter et al., 2021), and dashed grey arrows indicate lateral carbon fluxes. Fluxes are in mg C m⁻² d⁻¹.

level rise) alter blue C. The lack of data quantifying salt marsh carbon dioxide (CO₂) emissions and C storage is due in part because traditional methods are time consuming and labor intensive, while more recent technologies are cost prohibitive and destructive to the salt marsh. Monitoring salt marsh carbon dioxide emissions is not yet a routine measurement. We seek to change this.

We have developed a low-cost sensor package that can measure CO_2 concentrations along with temperature and humidity. When paired with a closed chamber, it can reliably, efficiently, and quickly measure changes in CO_2 concentrations across the saltmarsh-air interface. This light-weight, portable system can be deployed during other routine marsh monitoring or on-going research efforts.

Methods

1. GHG Sensor Calibration and Prior Validation

Prior to deployment for monitoring or research, calibration of the greenhouse gas (GHG) sensor is performed using a 2-point calibration with 0 ppm (helium) and 400 ppm CO₂ gas standards. The CO₂ sensor is directly calibrated by attaching the sensor to a computer running the software Gaslab. The input of the CO₂ sensor is connected with tubing to the standard tank and the "Configure sensor" button used to calibrate the sensor to each standard. The calibration is checked for drift by measuring three CO₂ standards of 0 ppm, 200 ppm, and 400 ppm between every three field sessions or before and after every two-week loan. In addition to biweekly checking for drift from the calibration, sensors are re-calibrated using Gaslab every 2 months, or when sensors have been out of use for several months. Prior to field deployment, the battery pack inside the sensor is charged using the external port and wall plug before each field day.

The CO₂ concentration readings by the sensors were validated in the field through comparison to state-of-the-art instrumentation, a backpack Picarro (GasScouter G4301) (Fig. 2). Readings by the GHG sensors and Picarro instrument were compared at salt marsh sites during year-round sampling. The Picarro and one sensor were attached to the same chamber for directly comparable results. Comparisons to each individual sensor were conducted at all salt marsh locations included in this protocol. Regression of the readings by the sensors and Picarro yields an R² of 0.97, supporting the high accuracy of GHG sensor readings to interpret CO₂ flux values.

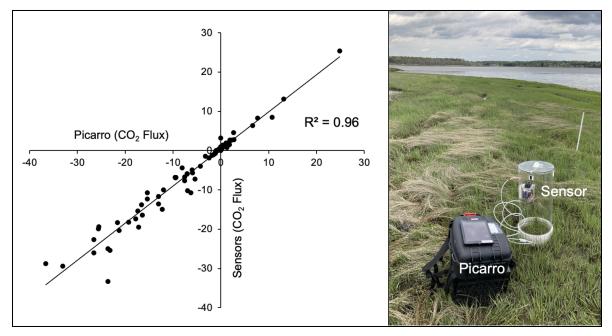


Figure 2. Field validation of GHG sensors using direct comparison to a backpack Picarro. Left graph shows the comparison of sensor readings relative to Picarro readings and the right image shows the field setup to simultaneously measure with a GHG sensor and the Picarro.

2. Deployment Timing and Locations

This protocol is written for GHG sensor deployment in five New England Reserves (NERRs) including Connecticut NERR in CT, Narragansett Bay NERR in RI, Waquoit Bay NERR in MA, Great Bay NERR in NH, and Wells NERR in ME.

Sensors are deployed along established salt marsh vegetation transects including vegetation dominated by *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*, and other tidal wetland graminoid and forb species.

2.1. Alignment with vegetation monitoring

GHG sensors are deployed simultaneously with vegetation monitoring, which is conducted at peak plant growth during late August to early October for New England marshes. Deployment occurs when the marsh is not flooded. For the low marsh sites, deployment occurs during tidal timing when these sites have maximal draining of standing water. During monitoring, two paired sensors are deployed at 20 vegetation monitoring plots (including both high marsh and low marsh sites). The same configuration of plots should be repeatedly monitored (e.g., annually, between multiple seasons, etc).

GHG flux measurements are taken in proximity to 1m x 1m vegetation monitoring quadrats. Vegetation monitoring plots are located along established transects which span from the upland to lowland marsh at the edge of a tidal channel or bay water, the primary inundation source. Two replicate GHG measurements are collected at each vegetation monitoring plot along the transect, excluding upland plots dominated by tall vegetation (e.g., *phragmites, typha, iva, baccharis*, etc.) that are too tall to fit in the chamber. The two GHG sensors within the chambers are placed within 1.5 m away from the vegetation plot edge in order to avoid a zone of disturbance from walking around the plot.

GHG sensors are placed in a visually similar vegetation community to the plot. To capture a representative plant community, the GHG measurements can either be placed parallel or perpendicular to the transect (Fig. 3). The orientation of GHG measurements relative to the vegetation plot will vary by reserve depending on nearby features including the locations of pools, changes of vegetation zones, and worn walking pathways along the transect line which should be avoided when placing GHG chambers. The location of the GHG measurements relative to the vegetation plot will be recorded using these classes: Landward (towards the upland), Seaward (towards the channel or bay), Left (Left of the plot when facing the water), or Right (Right of the plot when facing the water).

When choosing locations for the GHG sensor placement, follow these steps:

- 1. What side of the plot is the transect marker post? Avoid this side because it is typically a walking path for monitoring.
- 2. Of the other three sides of the plot, are there any pools, or a drop off in elevation, or bare patches? *Avoid these features when choosing a location.*
- 3. Of the other three sides, which two are most similar to the plant community within the vegetation plot? *Choose sites with a visually similar plant community to the plot.*

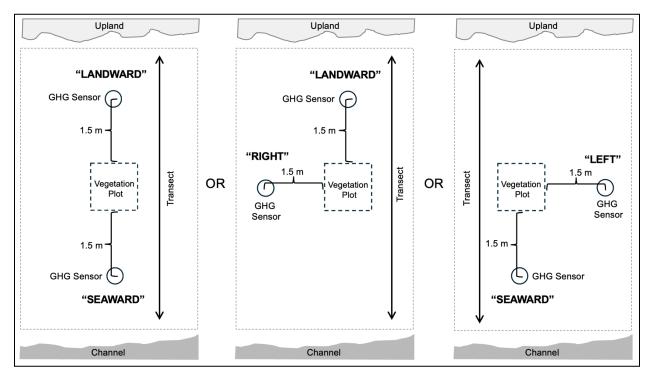


Figure 3. Diagram of several placement options of the two GHG chambers in proximity to the vegetation monitoring plots along the transect. Any combination of two sides, apart from the side with the transect post, can be chosen based on visual similarity to the plant community within the plot.

2.2. Sampling during other seasons

The sensors have been validated with a backpack Picarro (GasScouter G4301) year-round during the summer (July), spring (May), and winter (February). Therefore deployment can occur during any season in addition to the peak vegetation monitoring. Sampling during dormant seasons could occur at the same locations along the monitoring transects, or at other salt marsh sites.

Considerations for sampling during the winter include that recording the dormant vegetation species may be challenging, but if sites are in proximity to the monitoring transects then the vegetation community can be inferred from the summer monitoring. When the ground is frozen, ice should be cleared away from the chamber location before placement. Even when covered

with snow and ice, the collar can usually still be pushed into the sediment of the marsh to form a seal of the chamber.

3. Deployment Instructions

3.1 Materials needed for deployment

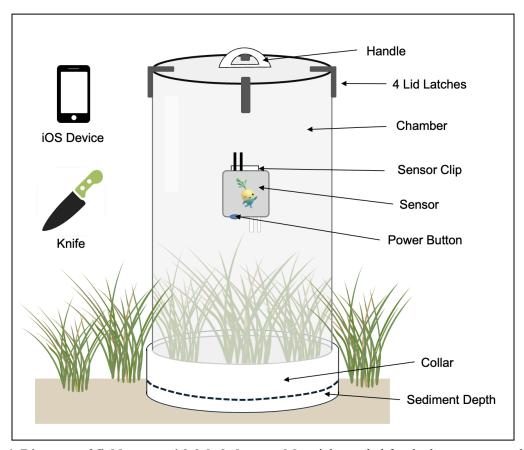


Figure 4. Diagram of field setup with labeled parts. Materials needed for deployment are a chamber with detachable lid, collar, sensor, iOS device, and a knife. Icons from Integration and Application Network (ian.umces.edu/media-library).

3.2 Step-by-step field deployment instructions

- 1. Place the PVC collar with bevel side down. Arrange grass so stems growing within the collar are inside.
- 2. Cut ~3 cm deep in sediment around the collar ring using a knife (provided) and push the collar down to the dashed line on the collar.
- 3. Place the chamber on top of the collar and push it down to the gasket ring.
- 4. Turn the sensor on (you will see the power button light up, hear the pumps and the fan turn on).

- 5. Latch the sensor onto the side of the chamber by sliding it down. Reduce shading by turning the chamber so the sensor is on the side of the chamber facing away from the sun.
- 6. Close the top lid and latch all four clips on the sides of the lid.
- 7. Ready to start measurement.
- 8. Measure for at least 10 minutes between Start and End time. During the measurement do not shade the chamber and avoid walking or stepping close to the chamber. The measurement should run for a least 10 minutes before stopping the sensor, but it is okay if the sensor runs longer.

Appendix A shows a handout of the field setup instructions with photos, which can be printed for reference during fieldwork.

3.3 Recording metadata during deployment

When turned on, the sensor will automatically record the date and time, CO₂ concentration, temperature, and humidity. This data is stored on an SD card in the sensor which will be readout after fieldwork.

During the fieldwork, we need to record information about which sensor, start and end times, location, and vegetation so the GHG sensor data can be linked to the specific deployment. The metadata we need to record are:

- 1. Location: Reserve name, Marsh name or section number, Transect and plot number, Location relative to vegetation plot, and Latitude and Longitude coordinates
- 2. Time: Date, Start and End time of deployment
- 3. Vegetation: Species and percent cover, other cover types including bare, dead vegetation, and standing water, and a photo of the plants in the collar (see below for details)
- 4. Name of the person who collected the data

In order to standardize the data collected and share it to a central location, this information will be entered into an app. The App platform is called "Epicollect5" and there is a specialized form for this Project called "GHG fluxes at NERRs". For data entry, someone will need an iOS device (phone or ipad) with the Epicollect5 app downloaded. Step-by-step instructions for using the app:

- 1. Download the Epicollect5 app.
- 2. Add Project, then Search for "GHG Fluxes at NERRs..."
- 3. Click on the project name, then ADD ENTRY in the top right corner.
- 4. Follow all instructions and answer all questions in the form.
- 5. To save the entry, click on SAVE ENTRY. Review the data for accuracy. You can return to edit any entry in the list. They are labeled as "End time, Sensor name", so you can use the End time as reference to know when that sensor is done measuring.

- 6. "UPLOAD" in yellow box to upload all data entries to the shared project.
- 7. Click on BOTH "UPLOAD DATA", and then "UPLOAD PHOTOS". If you make edits to an entry, make sure to re-upload the data to save your edits.

Appendix B shows instructions with photos for entering data using the app, which can be printed for reference in the field. Note: If you have downloaded Epicollect in the past, check that you have the newest version of the app updated for the current operating system of your phone. Appendix C shows a backup datasheet that can be printed in case of any challenges with using the app form (for example, low charge of your device, additional information that needs to be added, etc.)

3.4 Approach for vegetation cover

CO₂ fluxes have been shown to vary across different salt marsh vegetation communities (Barry *et al.*, 2023; Moseman-Valtierra *et al.*, 2016; Mueller *et al.*, 2020). In order to verify that the plant community in the GHG measurement location is similar to the vegetation monitoring plot, the plant species are recorded within the circular collar of the GHG chamber. Ocular cover, or recording visual estimates of cover categories that sum to 100%, will be used over other methods (i.e. point intercept, cover classes) (Burdick *et al.*, 2020, Moore 2017). Plant species, bare cover, dead vegetation, and wrack are recorded as a percentage of the cover within the collar.

Because standing dead vegetation can be important for GHG fluxes from the sediment, this is an important variable to include (Carmichael and Smith 2016; Kuehn and Suberkropp 1998). We treat "dead vegetation" as a part of the overall percentage and it should be counted when summing the cover classes to 100% overall within the circular collar. Dead vegetation is split into standing (vertical) and lying (or horizontal) categories. Wrack (unattached vegetation) is distinguished from standing dead vegetation and also counted in the total sum. Bare cover is also counted as a part of the total sum to 100%. Percent cover should be recorded in increments of 5% (for percent cover values of 5-100%) and increments of 1% (for trace percent cover of <5%) (Fitzgerald *et al.*, 2021, Moore 2017, James-Pirri and Roman 2004). Percent cover should be forced to sum to 100% (e.g. if there is a plant with a percent cover of 2%, the percent cover of another species should be adjusted to accommodate for it). Two observers should separately determine the percent cover and then reach a consensus. Additionally, every veg plot will be photo documented from ~2 feet above to aid with quality control.

4. Processing the Data

After returning from the field, the data will be read off from the SD card within the sensor (using a microSD card reader to transfer the data to a computer). All readings collected on a sensor will be listed in one sheet (.csv), sequentially, which is labeled as the sensor's name. Columns of the

datasheet are: "Date time", "CO2 (ppm)", "CH4 reference", "CH4 (mv)", "Temperature (°C)", and "Humidity (%)". The CO₂ values are concentrations in parts per million, and the sensor is pre-calibrated, so no post calculations are required. Values of "-295" indicate a missing reading by the sensor and will be removed in processing.

Download the metadata from the Epicollect site for this project. The project can be found by searching "GHG fluxes at NERRs" on the Epicollect5 website (https://five.epicollect.net/project/ghg-fluxes-at-nerrs). Under the project, navigate to "View Data" and then "Download". You can select the date range of interest when downloading the .csv file since all project data will be listed.

A script written in R coding language is used to calculate the GHG fluxes using the concentration readings and the metadata collected in the field. Multiple measurements collected on one sensor can be processed with the script, but each different sensor is calculated separately by changing the sensor name in the code where indicated.

Input files to the code are:

- 1. The data output from one sensor. This file will be named "SENSORNAME.csv" when copied from the GHG sensor SD card.
- 2. The metadata collected using the Epicollect App form. Important columns include site and plot names, date, start and end times. Additional metadata includes the standard size of this chamber as described in the code. This file will be named "form-1 ghg-flux-data.csv" when downloaded from the Epicollect site.

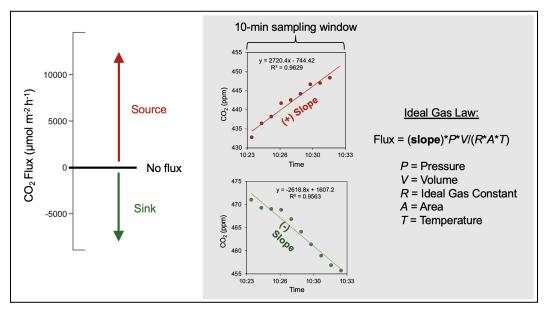


Figure 5. Diagram of the approach for calculating and interpreting GHG flux values. The slope of CO₂ concentration within the measurement window and the chamber size and conditions are used to calculate the flux as a source or sink from the marsh.

The R script calculates the fluxes of CO_2 at the saltmarsh-air interface. The flux values are calculated using the slope of the gas concentration measured over time and the Ideal Gas Law (PV=nRT). The flux is equal to the change of gas concentration in the chamber during sampling times "P" pressure (standard air pressure of 101223.7 Pa) times the volume "V" of this chamber (Volume = 0.0463 m³) divided by the "R" Gas constant (8.3144621 J/kmol*K) and "A" the area of the marsh in the collar (Area= 0.0688 m²) and "T" temperature (converted from °C to K) (Fig. 5).

A positive flux is the result of an increasing slope of CO₂ (ppm) during the 10-minute sampling period in the chamber. This indicates that the concentration of the gas is increasing in the atmosphere, therefore the gas is being released from the plants and/or sediment in the chamber as a "source". A negative flux indicates that the concentration is decreasing during the sampling window, so that gas is being taken up by the marsh as a "sink". The code will generate an output file called "sensordata.csv". The column called "Report CO2 Flux" is the calculated fluxes in units of umol/m^2/h that can be used for comparison to other variables and conditions.

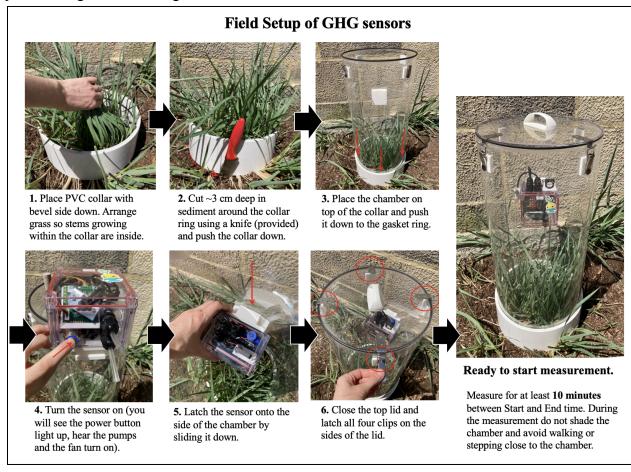
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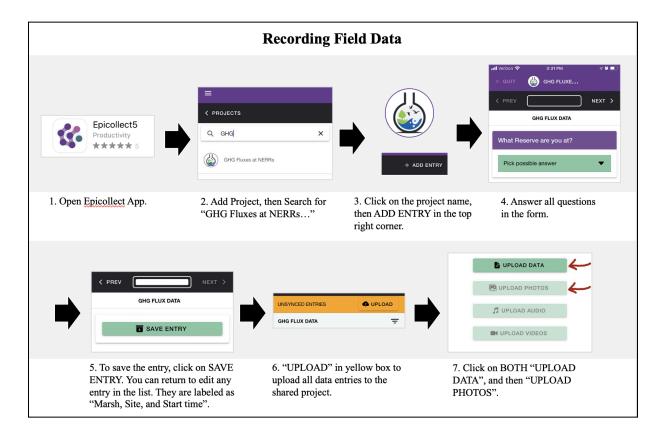
Appendix A

Example of setup instructions for field deployment with descriptive photos. This sheet can be printed for guidance during fieldwork.



Appendix B

Example of setup instructions for recording metadata using the project app with descriptive photos. This sheet can be printed for guidance during fieldwork.



Appendix C

Example field datasheet for manual data entry, which can be printed as a backup to be used to enter data by hand if any challenges occur with the Epicollect app.

CO2 Fluxes	- Field Datasheet	
Reserve & Marsh Location/Section:		
Date:		
Field Team:		
Weather conditions:		
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Plant species (% cover)	Water (% cover; depth cm)	Start Time (hh:mm:ss)	End Time (hh:mm:ss)	Which Sensor	Transect and Plot #	Location to Veg plot	Coordinates
S. pat 10%, S. alt 30%, Bare 60%	ex. 40%; 2 cm	ex. 11:34:02	ex. 11:44:25	ex. Dashi	ex. T3_P2	ex. Left (facing the water), or SW (seaward)	ex. 42.38934N, -70.99099W