Quality Assurance Project Plan for the Peconic Estuary Eelgrass Assessment Services

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Under Contract Agreement to:
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On behalf of the Peconic Estuary Program
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Prepared for:
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A2: Table of Contents:

List of Tables

List of Figures

A3. Distribution List

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A4. Project/Task Organization

Bradley Peterson, PhD, of Stony Brook University, will serve as the project leader and project QA/QC manager and be responsible for maintaining the official, approved QA Project Plan. In addition, he will validate the data generated from this project, prior to the completion of the draft final report. He will also plan all internal and external meetings and be primarily responsible for report generation.

Kaitlyn O'Toole, of Stony Brook University, will be responsible for the planning, conducting, and assembling of data collection at field sites and acquisition of supplies. She will be in charge of data collection and analysis with assistance from research assistants with proper training and monitoring performed by Kaitlyn O'Toole. She will be responsible for the QA/QC oversight of the project and write the draft and final report.

Elizabeth Hornstein, PEP State Coordinator, will be responsible for management of this project for PEP, technical review, participation in meetings and communication with the USEPA.

Sarah Schaefer, Program Coordinator**.** She will be responsible for technical review, participation in meetings and communication with the United States Environmental Protection Agency (USEPA).

Joyce Novak, PhD, PEP Director, will be responsible for overseeing this project for PEP, technical review, participation in meetings and communication with the United States Environmental Protection Agency.

Sheri Jewhurst, Clean Water Division, USEPA, Region 2, Peconic Estuary Program Project Officer. She will participate in technical reviews and internal PEP communication of results.

A5. Problem Definition/Background

Seagrasses are an integral part of coastal estuarine systems and provide a multitude of beneficial ecosystem services to the surrounding waters (Hughes et al. 2013). These services include nurseries for shellfish and fish, sediment stability, current/wave reduction, and increased water quality (Dennison et al 1993). Unfortunately, seagrasses have been in decline globally largely due to human activity (Hughes et al. 2013). *Zostera marina*, or eelgrass, is the dominant seagrass throughout waters of Long Island, NY (NYS Seagrass Taskforce 2009). Among the many species that depend on eelgrass, the survival of bay scallops, *Argopecten irradians*, has been linked to its presence (Thayer and Stuart 1974). Globally seagrass coverage has declined dramatically since the 1930's due to light limitation and temperature stress (Orth et al. 2006). In Peconic Bay, in eastern Long Island, eelgrass has declined by approximately 50% from 2000 to 2014 (Pickerell and Schott 2017). Without sufficient light, seagrasses respiration rates outpace photosynthesis, resulting in a net negative production (Dennison and Alberte 1985). *Z. marina* requires a minimum of ~20% surface light attenuating to depth (Dennison et al. 1993). Increasing ocean temperatures raises the metabolic rates of *Zostera marina*, exacerbating physiological stress on the plant. The optimal temperature range is between 10-25°C, and signs of stress can be observed above 25°C (Zimmerman et al. 1989). Prolonged exposure (30+ days) over 25°C can be lethal as well as short exposure from 27-35°C (Zimmerman et al. 1989) . Although higher temperatures and lower light levels are detrimental to the survival and productivity of seagrasses, improvements in either environmental variable can help alleviate the stress associated with the other. Higher water temperatures call for higher light requirements to compensate for higher metabolic rates (Zimmerman et al. 2015). Studying the effects of light and temperature limitations of *Zostera marina* species in Peconic Bay is essential to determine future implications of eutrophication, disease, and global warming, and ultimately improve efficacy of restoration work.

Peconic Bay, comprised of several small bays, lies between the north and south "forks" of Long Island (Figure 1). Plagued by wasting disease in the 1930's and brown tide beginning in 1985, there was a significant die-off of eelgrass (Dennison et al. 1985) and bay scallop populations.

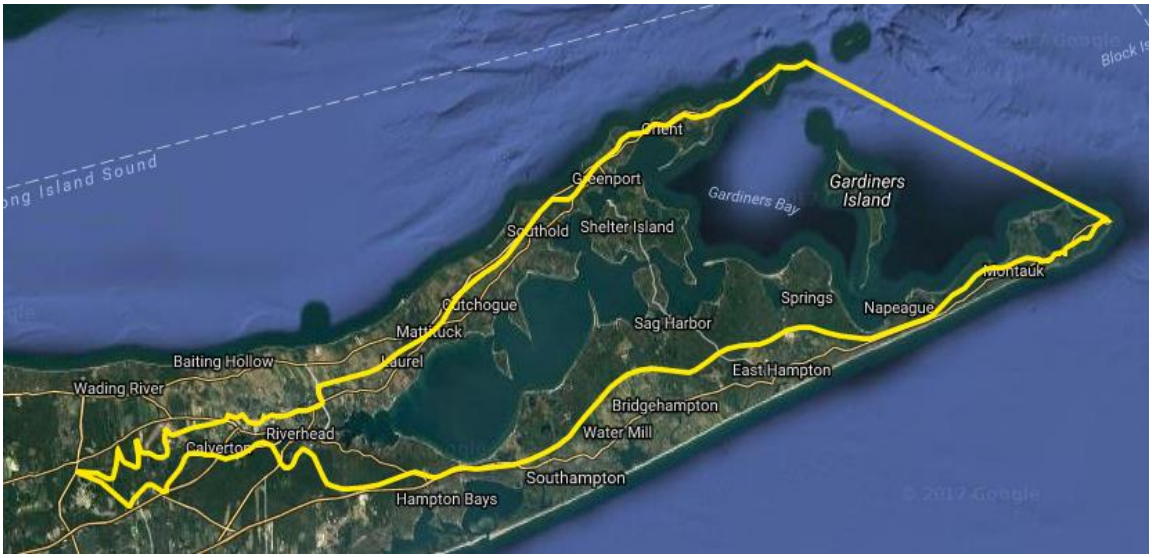

Figure 1: Peconic Estuary Boundary (Image Source: https://www.peconicestuary.org/news-and-blogs/maps-gis/maps-watershed-boundaries/)

However, a brown tide event has not occurred since 1995. Water quality declines from east to west as the estuary gets shallower and less mixed, into Flanders Bay and meets the Peconic River, a freshwater source (Hardy 1976). Nutrient loading is highly influential in this area, as the embayment is surrounded by residential, commercial, and agricultural land (Pickerell and Schott 2017). A historical discharge influence of nitrogen and phosphorus due to sewage treatment plants in Riverhead has since lessened, yet the influence of leaky septic systems and cesspools has increased, contributing to 43% of total nitrogen loading to the Peconic Bay Estuary (Hardy 1976, Lloyd 2014). Fertilizers and agriculture also play a major part in nutrient runoff, especially on the north fork of the island. Nutrients runoff directly into the estuary, but also can leach into the groundwater and persist for over 100 years (Lloyd 2014). Nutrient levels are below established criteria in eastern Peconic Bay for a majority of the year, according to the Peconic Estuary Program; however, seagrass beds have continued to decrease and are considered outside of optimal environmental parameter range west of Shelter Island. Meadows west of Shelter Island (Bullhead Bay) are found in areas of significant submarine groundwater discharge, which cools the rhizomes and results in lower respiration and thus, lower light requirements (Pickerell and Schott 2017). More research into groundwater discharge in Peconic Bay is currently being conducted. However, typically, extended temperatures (~30 days) above 25°C and lower light availability west of Shelter Island have resulted in unsuccessful restoration attempts by Cornell Cooperative Extension (Pickerell and Schott 2017).

A bio-optical model allows for the addition of multiple water absorbing, scattering, and refracting parameters to model light attenuation to a specified depth. Bio-optical models have previously been used to determine water column irradiance by representing the effect of biological material on optical properties and using this data to create a model to predict natural waters (Baker and Smith 1982). These models are typically used to find euphotic zone depths in

open ocean, as well as used in coastal areas to model light limiting depths for seagrass restoration (Gallegos and Kentworthy 1996, Koch 2001, Gallegos 2001, Biber et al. 2008, Zimmerman et al. 2015). The availability of previous bio-optical models for *Zostera marina* introduces the parameters and methods to be used for future models and different systems. These models are variations on one another, but all have the same underlying factors to be sampled for: temperature, depth, light attenuation (chlorophyll-a, Total Suspended Solids, and Colored Dissolved Organic Matter), and dissolved total phosphorus and nitrogen (Zimmerman et al. 2015, Staver and Staver 1993, Kemp et al. 2004, Dennison et al. 1993, Biber et al. 2008, Gallegos and Biber 2004).

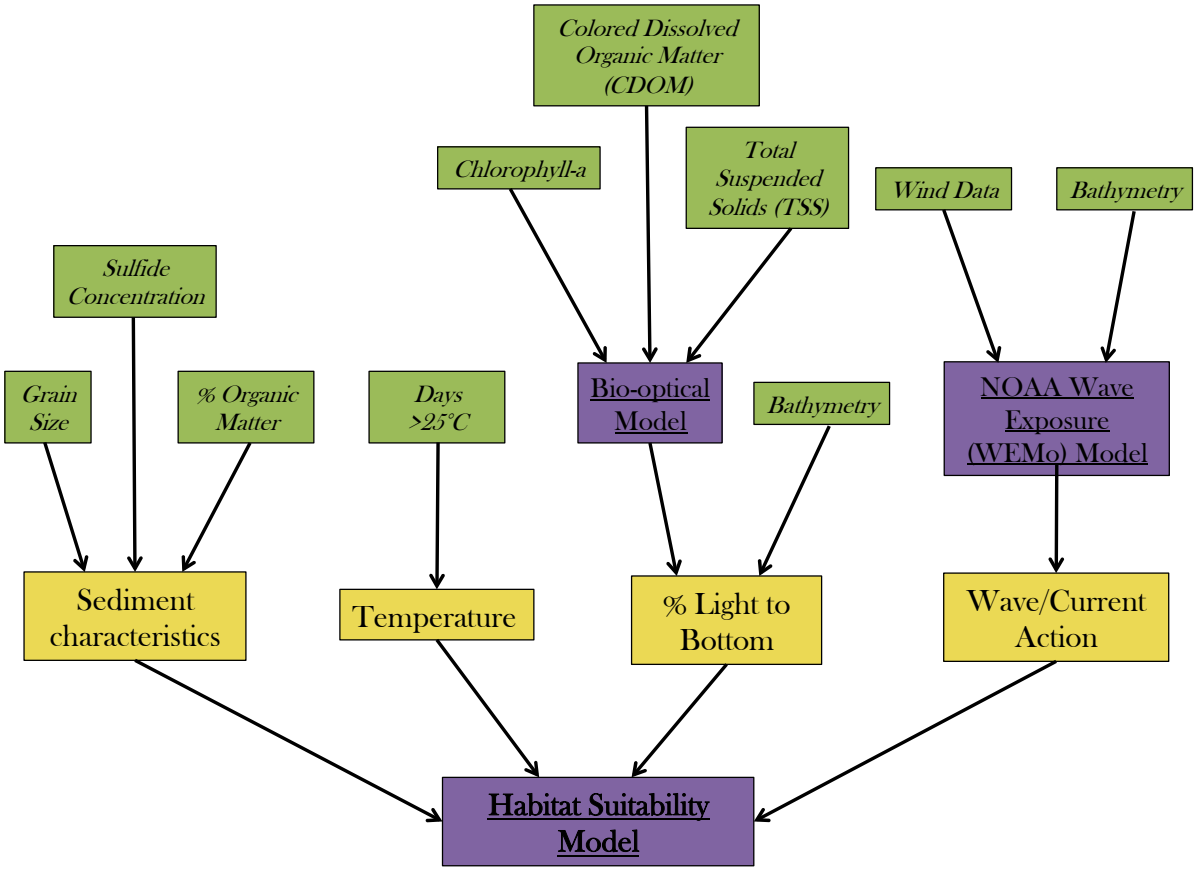

Figure 2: Green italicized boxes indicate model inputs, purple underlined boxes indicate models used, yellow boxes indicate parameters used for the habitat suitability model.

The bio-optical model can be combined with sediment characteristics (sulfide concentrations, organic content, and grain size), water column properties, depth in relation to tide, wind, and wave exposure data, to create a habitat suitability model (Figure 2). This habitat suitability model can be used to predict areas of ideal eelgrass restoration potential. This model can also be manipulated with projected future parameters to demonstrate how climate change or eutrophication will affect certain areas and the restoration potential of these areas in the face of global warming. Improvement in water quality has allowed for successful restoration efforts of seagrass, *Thalassia testudinum* (Florida) and *Zostera marina* (mid-Atlantic region), in the Indian

River Lagoon (FL) and Tampa Bay, Chesapeake Bay areas, and coastal Virginia (Reynolds et al. 2016, Greening et al. 2014), through development of habitat suitability models.

A6. Project/Task Description

Project Schedule:

- Month 1-4: Develop QAPP and submit for comments.
- Month 4-6: Edit QAPP for comments.
- Month 7-8: Purchase supplies and calibrate instruments, prepare instruments and datasheets for field season.
- Month 9-12: Collection and analysis of samples, monitoring of seagrass meadows and temperatures, data review/validation.
- Month 10-12: Modeling and data analysis as samples are processed and verified.
- Month 10-12: Report and presentations to PEP and Stony Brook University.

Water quality, whole water samples, and light attenuation measurements will be obtained bi-monthly at 15 locations, all of which have a continuous HOBO data logger, and will be deployed prior to field water quality data collection. These 15 sites consist of 5 extant seagrass meadows and 10 previously inhabited seagrass locations (prior to 2015) stretching from Flanders to Gardiner's Bay (Figure 2, Table 1).

Table 1: Latitude and longitude of HOBO and water sampling locations.

Location	Latitude	Longitude
WQ 1	40.9127525	-72.5658267
WQ 2	40.9529667	-72.5507833
WQ 3	41.0299667	-72.4099
WQ 4	41.0760247	-72.3964622
WQ 5	41.115532	-72.336705
WQ 6	41.1268208	-72.2873789
WQ 7	41.0899389	-72.3183861
WQ 8	41.0568828	-72.276302
WQ 9	41.0359681	-72.274549
WQ 10	40.922783	-72.456506
SG 1	41.088629	-72.355629
SG 2	41.104182	-72.326866
SG 3	41.073786	-72.276237
SG 4	41.012005	-72.275087
SG5	41.048973	-72.242523

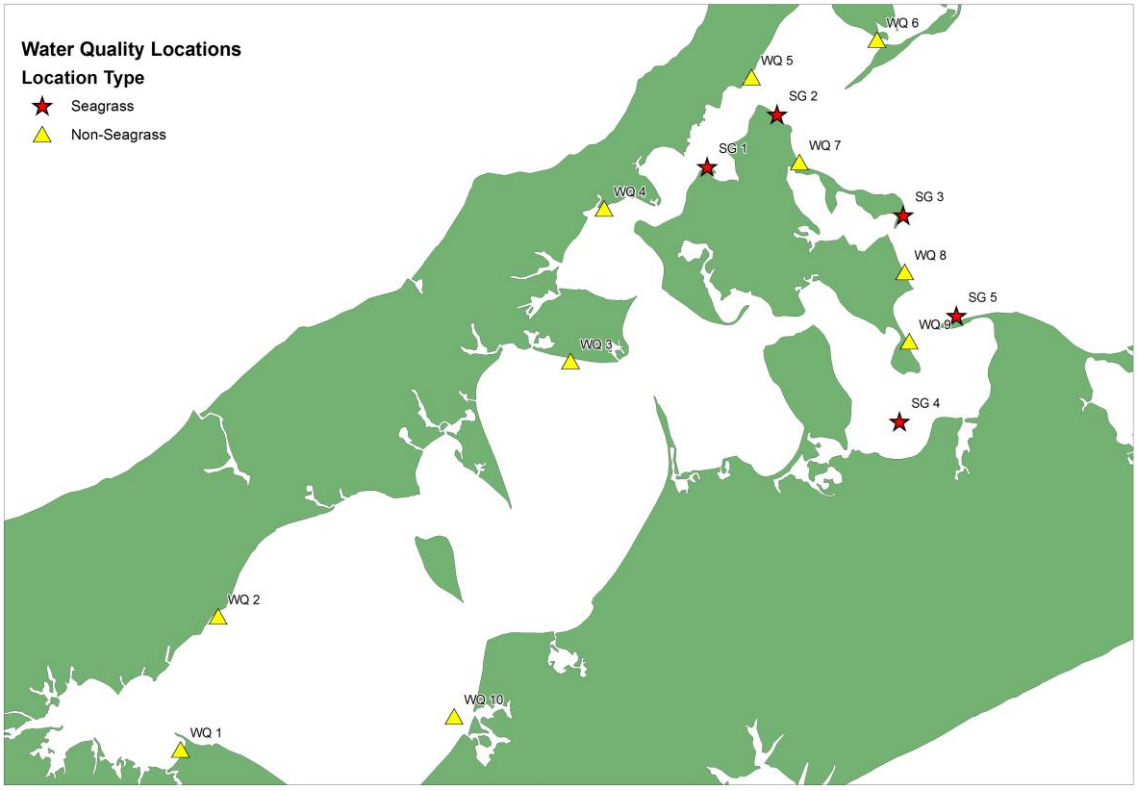

Figure 3: Map of selected bi-monthly water quality locations for Peconic Bay Estuary System.

Water quality measurements will be taken using a YSI 6600 sonde from a boat. At each station we will measure depth, dissolved oxygen, temperature, salinity, and turbidity, at surface and <1m from the bottom, which will be used in the bio-optical and habitat suitability models. The Li-Cor 1400 datalogger displays and converts the underwater and deck PAR sensor irradiation values to the attenuation coefficient of downwelling PAR (K_d) and will be taken at every station. The constituents of the water column that affect the attenuation of light, and therefore are included in the bio-optical model, include chlorophyll-a (an indicator of shading by phytoplankton present in the water column), CDOM (colored dissolved organic matter), DIN (dissolved inorganic nitrogen: nitrate, nitrite, ammonia), DIP (dissolved inorganic phosphate: orthophosphate), and TSS (total suspended solids). Whole water samples will be taken in replicate at each station for TSS and chlorophyll-a. CDOM, DIN, and DIP samples taken in replicate will be filtered on site at each station monthly. In addition, HOBO Pendant temperature and light data loggers will be deployed at all 15 stations, taking measurements every 15 minutes.

Seagrass deep edge measurements will also be performed to establish the parameters for eelgrass success. The deep edge of a seagrass bed represents the limitation of multiple stressors on the eelgrass, particularly in regards to light availability. Physical limitations to the eelgrass are determined by sampling at several eelgrass sites along the deep edge. Seagrass deep edge measurements will be performed one time during the field season to determine depth of deep edge, % cover of macroalgae and/or seagrass, and shoot density. Sediment cores will be taken once at each station for total organic matter and grain size. Eelgrass productivity and nutrient

analysis measurements will be performed and collected every 3-4 weeks. A relative exposure index (REI) will be calculated using wind data from the past year to generate a wave model (WEMo), which will determine the amount of stress presented by tides, waves, and currents to the seagrass. Too much physical stress to the eelgrass results in unsuccessful restoration efforts for the plants, as the plants cannot establish themselves (Koch 2001).

Based on the field sampling and analysis, a bio-optical model will be created that shall predict light attenuation from water quality constituents based on field measurement of light attenuation coefficients co-located with water quality constituents. The bio-optical model will be created using the Zimmerman and Gallegos *Grasslight* model (EPA Opportunity Number: EPA-R3-CBP-14-01, Zimmerman et al. 2015) with inputs (TSS, chlorophyll-a, CDOM, turbidity) collected during water quality sampling. This model will determine what is decreasing light availability to eelgrass, determine whether nitrogen loading, suspended sediment, and/or other factors should be managed, and quantify the nitrogen load that a Peconic Estuary eelgrass community can withstand, according to the Contract Agreement (Task 2: Field Sampling and Analysis of Water Quality and Bio-Optical Model):

- Field Sampling:
    - Water quality, water clarity, and inherent optical properties
    - CDOM, chlorophyll-a, TSS
    - DO, turbidity, salinity, temperature
    - PAR
- Bio-Optical Model: Based on the field sampling and analysis, a bio-optical model produced will predict light attenuation from water column constituents based on field measurements of light attenuation coefficients co-located with water column constituents, revealing:
    - Determination of what is decreasing light available to seagrass
    - Determination of whether nitrogen loading, suspended sediment, and/or other factors
    - Quantify the nitrogen load that Peconic eelgrass can withstand

A habitat suitability model will then be created from the bio-optical model, REI, tidal amplitudes, temperature, and water column and sediment nutrient concentrations. This habitat suitability model will be used to predict areas of ideal eelgrass restoration potential according to the Contact Agreement (Task 3: Light, Temperature, and Combined Thresholds for Eelgrass Survival; and Task 4: Site Suitability Index):

- Transects at five eelgrass beds within the Peconic Estuary and visually estimate seagrass cover, macroalgal cover and shoot density in quadrats. Terminal shoots, canopy height, and cover will be used to estimate the biomass of the bed.
- Analyze shoots to assess epiphytism, grazing, wasting disease and nutrient content (C, N, P).
- Sediment samples for grain size, type, sulfide content, and organic content
- Water depth at deep edge relative to local tidal stage.
- Temperature and light loggers deployed at each deep edge.
- Monthly productivity measurements of seagrass.
- Habitat Suitability Model: identify a set of environmental conditions that should support seagrass recruitment.

- Incorporate all data on sediment grain size, type, and organic content. Subsurface irradiance and light attenuation characteristics, water temperature, salinity, dissolved oxygen, depth, tidal amplitude, chlorophyll-a, total suspended solids, colored dissolved organic matter, and sediment sulfide concentrations.
- Interpolate all point and bathymetric data by inverse density weighted (IDW) or Kriging techniques and all remaining vector data rasterized and clipped to the PEP extent. Resulting grids shall be reclassified by the Contractor by using a binary (In/Out of Range) schema based on the literature threshold values. Depending on the coarseness of input layers, the Contractor shall strive to restrict cell or pixel sizes to one-hundred (100) squared meters.
- Combine model data using simple arithmetic models designed to give all layers equal weight in the model output. The resulting grid shall be clipped to the Peconic Estuary boundaries and crosschecked against the most recent distribution map. Model fit shall be quantitatively assessed using a variety of metrics, including percent of cells correct, rates of omission and commission errors, as well as Kappa statistics. Unsatisfactory model runs shall be adjusted using additional threshold values obtained during the literature search and re-run until the Kappa statistic and overall accuracy percentage reach a maximum.

- Restoration Potential Model: in order to assess the relative suitability of potential restoration sites, a Restoration Potential Model will be developed:
  - Reclassify the habitat suitability model layers using a 1-10 ranking scheme (10 being most suitable) using published threshold values and combined with derived distance datasets for known seagrass stressors as well as for the proximity to historical seagrass distributions. The layers shall then be recombined using simple arithmetic models to partition each parameter's influence on the output cells final ranking.
  - Calculate the relative exposure index and wind data for at least one year prior to the most recent mapping effort to further assess the hydrodynamic environment present at each cell location. The relative frequency of the values found within the current distribution shall be used to generate the ranked order of the exposure values that should be suitable for potential restoration sites.

A7. Quality Objectives and Criteria

The goal of this project is to obtain the data necessary to build a seagrass habitat suitability model for the Peconic Bay Estuary. Collecting accurate, precise, representative, complete, and comparable data will be of the utmost priority. Table 2 and 3 addresses accuracy, detection limits, and sensitivity for each parameter sampled for, where applicable. All data gathered from the HOBO Datalogger, YSI 6600, Li-Cor 1400, and Turner Trilogy Laboratory Fluorometer that registered above the minimum detection limit as listed in Table 2 & 3 will be used in the Restoration Potential model. This follows on from Zimmerman et al 2015 with the addition of TSS, chlorophyll-a, CDOM, turbidity of which values above the minimum detection limit are used. Table 4 addresses nutrient analysis specifications. Duplicates will be taken to ensure precision of whole water and nutrient samples (Table 6), samples that do not meet the duplicate requirements are not included in analysis. Sampling locations were chosen from west (no seagrass) to east (seagrass) based off of historical and current seagrass data to ensure a

representative sampling of field conditions within the bay. Holding times for each sample will be followed and notated if otherwise violated. Field and laboratory SOP's will be followed. YSI 6600, Li-Cor 1400, Chlorophyll-a, and sediment core collection procedures were obtained from A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region (Kopp and Neckles 2009), as the team is familiar with the protocol having done water quality monitoring for the National Park Service since 2005. The TSS protocol will follow EPA method 160.2 Residue, Non-Filterable (Gravimetric, Dried at 103-105°C). The habitat suitability model will be compared with known Peconic eelgrass distribution (Pickerell and Schott 2017) and calibrated in order to enhance the modeled and observed pixel values for eelgrass and where eelgrass restoration is deemed feasible. The goal of this model is to create 85% agreement between observed and modeled seagrass areas (Table 5).

Table 2: Specifications for the YSI 6600 EDS V2-2 Sonde Sensors, HOBO dataloggers, Li-Cor 1400, and GPSMAP 78sc (Field Measurements)

Sensor	Sensor Type	Range	Accuracy	Resolution	Temperature Range	Calibration Requirements	Calibration Frequency
HOBO Datalogger (UA-002-64)							
Temperature		-20 to 50°C	+/- 0.53°C	0.14°C at 25°C (drift <0.1°C/ year)	-20°C to 50°C	Replace desiccant and battery, check o-ring	Yearly
Light Intensity		0 to 320,000 lux	Measures relative light levels, see plot A				
YSI 6600 EDS V2-2 Multi-parameter sonde							
Depth (Non-Vented Level)	Stainless steel strain gauge	0-200 ft (61m)	+/- 0.4 ft (0.12m)	0.001 ft (0.001m)	-5 to 45°C	Calibrated by YSI	Every 2 years
Temperature	YSI 6560 Conductivity and Temperature Probe, thermistor	-5 to 50°C	+/- 0.15°C	0.01°C	N/A	Compared to certified thermometer	Bi-monthly
ROX Optical Dissolved Oxygen Probe, % saturation	YSI 6150 ROX Optical Dissolved Oxygen Probe, Optical Luminescence Lifetime	0 to 500% air saturation	0 to 200% air saturation (+/- 1% of the reading or 1% air saturation whichever is greater) 200-500% air saturation (+/- 15% of reading); relative to calibration gases	0.1% air saturation	-5 to 50°C	Air saturated water calibration, 1-point	Bi-monthly
ROX Optical Dissolved Oxygen, mg/L	YSI 6150 ROX Optical Dissolved Oxygen Probe, Optical, Luminescence Lifetime	0 to 50 mg/L	0 to 20 mg/L (+/- 1% of the reading or 0.1mg/L whichever is greater), 20-50mg/L (+/- 15% of the reading), relative to calibration gases	0.01mg/L	-5 to 50°C	Air saturated water calibration, from % saturation	Bi-monthly
Turbidity	YSI 6136 Turbidity Probe, optical, 90° scatter, with mechanical cleaning	0 to 1000 NTU	+/- 2% of the reading or 0.3 NTU (whichever is greater)	0.1NTU	-5 to 50°C	2-point calibration with 0 (D.I. water) and 126 NTU YSI standard	Bi-monthly
Conductivity	YSI 6560 Conductivity and Temperature Probe, 4 electrode cell with autoranging	0 to 100mS/cm	+/- 0.5% of reading +0.001mS/cm	0.001mS/cm to 0.1mS/cm (range dependent)	-5 to 60°C	1-point, specific conductivity calibration using 10mS/cm standard	Bi-monthly
Salinity	Calculated from conductivity and temperature	0 to 70ppt	+/-1.0% of reading or 0.1ppt (whichever is greater)	0.01 ppt	-5 to 50°C	Done by conductivity calibration	Bi-monthly

Li-Cor 1400							
Irradiance (PAR)	High stability silicon photovoltaic detector (blue enhanced) Underwater Sensor (LI-192) Terrestrial Sensor (LI-190)	0 to 10,000 µmol s^{-1} m^{-2}	+/- 1%	0.01 µmol s^{-1} m^{-2}	-40 to 65°C	Return sensors to manufacturer	Every 2 years, calibration check twice in season
Garmin GPSMAP 78sc							
Location	GPS / DGPS	NA	<10m / 3-5m	NA	-15-70°C	Software Update	Whenever available

Table 4: Specifications for the Turner Trilogy Laboratory Fluorometer modules.

Sensor	Minimum Detection Limit	Linear Range	Operating Temperature	LED	Excitation	Emission
CDOM/$NH4^+$ module	0.1 ppb	0-1000 ppb	15-40 °C	365 nm	350/80 nm	410-450 nm
Chlorophyll-a Extracted (Non-Acidification)	0.025µg/L	0-300µg/L	15-40 °C	460 nm	436/10 nm	685/10 nm

Table 3: QuikChem Nutrient Analysis Specifications

Nutrient	Method	Minimum Detection Limit	Linear Range	General QC Procedures
Ammonia	Lachat QuikChem 31-107-06-1-Q	0.0022 mg NH_3-N/L	0.005 to 2.0 mg NH_3-N/L	Calibration curves are established for all nutrient methods; MDLs calculated; LRBs, QCSs at low, mid and high analyte concentrations, matrix spikes, and duplicate samples analyzed.
Nitrate and Nitrate	Lachat QuikChem 31-107-04-1-G	0.05 mgNO^-_2/L 0.002 mg NO^-_3/L	0.25-10.0 mg NO^-_2/L 0.01-1.0 mg NO^-_3/L	
Orthophosphate	Lachat QuikChem 31-115-01-1-H	1.0µg P/L	5-400µg P/L	

Table 5: Proposed pixel agreement between modeled and observed eelgrass habitat suitability model.

	Measured	
Modeled	Present	Absent
Present	>85%	<40%
Absent	<15%	>60%

A8. Special Training/Certification

All individuals involved in field sampling are SCUBA certified through nationally recognized agencies (i.e. PADI or NAUI). Copies of dive certification cards will be kept on file with the QA/QC project leader. Familiarity with equipment used will be performed over several weeks of on-site field and laboratory training by the team leader. Bradley Peterson has been performing water quality under the National Park Service since 2007 and has trained the team following A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region using the equipment found in this document. Kaitlyn O'Toole trains the team on equipment by first exposing a new team member to the equipment a the whole water collector, the next time in the field, as data recorder (to understand the data being collected and logical values), then trained on the instruments with Kaitlyn O'Toole supervising at all times. The data is validated by National Park Service staff for quality assurance and reports back to Bradley Peterson. The Gobler Laboratory which performs the QuikChem analysis is in the process of becoming a NYSDOH ELAP accredited laboratory with a certified technician run by Jennifer Goleski and has been following procedure since 2014. All training and certification records are kept on the lab premises and can be verified.

A9. Documents and Records

Data collected in the field will be recorded on prepared waterproof datasheets and later transcribed into digital files. These datasheets will include date, time of sampling, weather conditions, parameters sampled for, and crew names. Labels on TSS and chlorophyll bottles will include only the site ID, as samples are processed before subsequent sampling. Nutrient samples are labeled with the site ID, replicate number, and date, they are placed in a zip lock bag labeled with the date collected in the freezer. Laboratory data will be recorded on prepared data sheets. Calibration of instruments will be recorded and stored in the laboratory file cabinet before being deposited electronically. Electronic and paper copies of the data will be kept by the project leader, and deposited in the Stony Brook Electronic Repository.

B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design (Experimental Design)

The sampling locations (Figure 2) will range from eastern Flanders Bay (Red Cedar Point, Southampton) to western Gardiner's Bay (Cedar Point County Park, East Hampton). 5 current seagrass locations and 10 previously inhabited seagrass areas (no seagrass currently) were chosen based off of relation to hardened shorelines and stream runoff areas. Locations for

sampling will be chosen based off of aerial and satellite imagery, hardened shoreline, and hydrography data (Google Maps, CUGIR). The non-seagrass areas were chosen based off of historical aerial imagery displaying eelgrass presence confirmed by datasets obtained from Cornell Cooperative Extension (Steve Schott, unpublished data). These areas were then narrowed down based on distance to dredging, bathing, runoff, and hardened shoreline areas, choosing the most natural area (Peconic Estuary Program Long-Term Eelgrass Monitoring Program 2013, GoogleImagery). The seagrass locations were chosen based off of location to one another, and shoot density and meadow size, both indications of seagrass health, also obtained from CCE (Steve Schott, unpublished data). The deep edge is considered to be the area of highest environmental stress for *Zostera marina* (Zimmerman 2015), increased depths result in lower amounts of light reaching the eelgrass but provides cooler temperatures typically. Therefore, minimum light, sediment, and water column requirements are measured here and used as the minimum in the range of parameters for potential eelgrass restoration areas. These seagrass sites will be used to create a more accurate range of the environmental parameters for the seagrass in Peconic Bay together with ranges for *Zostera marina* published previously from other water bodies.

Stations will be identified by water quality (WQ) or non-seagrass sites, numbered 1-10, and seagrass (SG) sites, numbered 1-5. Garmin GPS units are used to locate sites within 30m of the GPS location (coordinates found in Table 1). HOBO data loggers will log temperature and light attenuation at all 15 sites. These loggers will record light and temperature every 15 minutes. They will be cleaned off of fouling monthly and data will be offloaded. The sampling locations, 5 seagrass, 10 non-seagrass locations (15 total), will be sampled bi-monthly for physical and biological parameters at the surface and bottom of the water column, denoted by 0 (surface) and 2 (bottom) by boat. Light measurements will occur alongside water quality measurements. Water quality and light measurements will take place on the same day within the timeframe of 9-5 pm, unless due to unforeseen circumstances. Data that cannot be collected within the day due to unforeseen circumstances will be continued within 24 hours of the first sample collected. Sediment cores will be taken annually at each location. Deep edge analysis will take place once, as long as the edge does not deviate from previous measurements. Productivity measurements will occur at the edge every 3-4 weeks.

B2. Sampling Methods

Field Procedures

Continuous monitoring

Continuous HOBO data loggers (UA-002-64) will be deployed prior to water quality sampling at all 15 sites and are cleaned monthly. Sites with seagrass will have the HOBO loggers placed at the deep edge of the seagrass patch. The depth of non-seagrass HOBO loggers will be approximately 1.5m at deployment and no less than 1.0m at low tide. These loggers will be deployed in April before waters have significantly warmed and log temperature and light data every 15 minutes. They will be deployed at the bend in the dog stake approximately 15-20cm from the sediment surface, facing south to minimize shadows from the stake or buoy. There will also be a small surface cricket buoy tied securely to the top for future cleaning and retrieval, so that no interference with the light logging occurs (Figure 4). The loggers will be offloaded of

data monthly using a handheld Onset HOBO waterproof shuttle (U-DTW-1) and uploaded to the laboratory computer using HOBOware Pro.

Water Quality Location:_____

Station Code:	YSI used:
Date:	Time:
Field Crew Names:	Recorder:
Wind Direction (degrees):	Cloud Cover (+/-25%):
Beaufort Scale:	Water Depth:

LiCor Light Profile

Depth of Measurement (m)	Channel I1 Underwater Irradiance PPFD ($\mu Em^{-2}s^{-1}$)	Channel M2 Underwater Irradiance % of I_{Air}
0.1		
0.2		
0.4		
0.8		
1.4		
2.0		
3.0		

YSI Hydrocast

	Depth (m)	Temp (C)	Sp. Cond (mS/cm)	Salinity (ppt)	DO (% sat)	DO (mg/L)	Turbidity (NTU)
B							

****LOGGED: 9-QA/QC_____ 0-Suface_____ 1-Mid-depth_____ 2-Bottom_____**

HOBO found/offloaded: _____

Notes:

Figure 4: (A) Field datasheet for recording water quality data. (B) HOBO datalogger set-up.

Bi-Monthly Monitoring

Water quality samples will be taken every other week at 15 sampling sites throughout Peconic Bay within 200 feet of the HOBO location. Approximate wind speed, direction, cloudiness, time, and depth will be taken at each site, as well as any unusual characteristics, such as nearby construction or noticeable algal blooms. Site details are recorded on waterproof printed field sheets (Figure 4).

1. Spatial Water-Quality Monitoring with the YSI Sonde: Temperature, dissolved oxygen, turbidity, salinity, and depth of each measurement will be taken using the YSI 6600 multi-parameter sonde, calibrated prior to use, bi-monthly while in use, and post-calibrated (each probe described in Table 2). Measurements are allowed to stabilize before being recorded. QA/QC measurements are taken at each location before submersion; local DO % is always ±2% of 100%. If outside of range, rinse again with DI water and reassess local DO%, if outside of range again, calibration must be performed before proceeding. Once in-situ, the probes will be wiped to ensure air bubbles do not

interfere with measurements. Surface and bottom measurements within 0.5m of the sediment surface will also be taken. Bottom measurements will be recorded on a datasheet and QA/QC, surface, and bottom measurements will be recorded on the YSI 650 MDS handheld. The YSI will be turned on 5 minutes before arrival to the first site to warm up. A 0.5m line will be attached to the bottom of the sonde sensor guard so the user can feel the bottom, to lessen the risk of the Clark-type probe, for dissolved oxygen, touching the sediment and interacting with hydrogen sulfide. The YSI 6600 sonde will be cleaned using DI water after sampling at each site and wrapped in a towel moistened with DI water to keep membranes moist and the sonde accessible between sites. This procedure was taken from the A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region, SOP 4, Version 1.01 (Kopp and Neckles 2009).

2. Spatial Water-Quality Monitoring with Li-Cor PAR Instruments*:* Downwelling photosynthetically available radiation (k_d PAR, 400-700 ηm) at depth will also be taken at each site with a Li-COR LI-1400 handheld console equipped with an underwater quantum sensor (LI-192SA) and one deck sensor in air (LI-190SA). The underwater sensor measures photosynthetic photon flux density (PPFD) with depth and the deck sensor measures irradiance in air to minimize error due to possible changing solar irradiance. Multipliers for each sensor are calculated during factory calibration, which occurs every other year. The sensors are re-checked to assure data quality twice during the field season, once in May prior to sampling and once in July. Percent light reaching the underwater sensor in reference to the deck sensor is also calculated by the handheld. The sensors will be deployed in the field using a lowering frame with a weight on the bottom in case of a heavy current. The Li-COR will be deployed for at least 15 seconds at each depth to optimize the 15-second averaging function. Measurements will be taken within 3 hours of apparent noon (13:00 Eastern Time) at depths of 0.1, 0.2, 0.4, 0.8, 1.4, 2.0, and 3.0m, or to the bottom. The deck sensor must remain out of shade in full sunlight, on an even surface. The underwater sensor must also remain out of the shade from the boat and items that may block the light must be removed, especially seaweed or debris. This procedure was taken from the A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region, SOP 5, Version 1.0 (Kopp and Neckles 2009).

3. Whole Water Sampling: 2 1-L whole water samples for TSS and chlorophyll-a are taken at each site using pre-rinsed amber polyethylene wide mouth bottles. Chlorophyll *a* is utilized as the proxy for phytoplankton biomass. Each bottle is pre-labeled for each site and lowered to 20-30 cm below the surface, and rinsed three times with ambient water. The bottle is then capped underwater to ensure no air infiltration, which may alter chlorophyll concentrations, and placed on ice in a cooler. Chlorophyll *a* samples are kept on ice until filtered, immediately upon return to the laboratory and in the order collected. TSS samples are kept on ice upon return to the laboratory and either filtered after chlorophyll or kept in the refrigerator (-4°C) for less than 7 days, though typically no longer than 2 days after collection (Greenburg et al. 2005). Chlorophyll-*a* procedure was taken from the A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region, SOP 6 (Kopp and Neckles 2009). TSS

procedure follows EPA 160.2 Residue, Non-Filterable (Gravimetric, Dried at 103-105°C).

Monthly monitoring

1. Nutrient (DIN and DIP) and Colored Dissolved Organic Matter (CDOM) Sampling: At all 15 sites, duplicate samples for DIN, DIP, and CDOM are taken at 20-30 cm and are filtered on site into a 20mL amber borosilicate vial, pre-combusted at 450°C for 4 hours. Samples are obtained with a 1L plastic cup, rinsed three times with DI water prior to rinsing three times with ambient seawater. A 60mL BD luer-lock syringe (BD 309653) is then rinsed three times with this seawater. A polypropylene luer-lock syringe tip (SX0002500), fitted with a pre-combusted 25mm 0.7 GF/F filter for 2 hours at 450 °C using forceps, is then attached and used to filter the seawater from the syringe, through the filter, into the vials. These vials are then labeled with site, date, and replicate number, and put directly on ice. CDOM samples are immediately placed in the freezer (-20°C) and analyzed in less than 6 months (Branco and Kremer 2005). DIN/DIP sample are held in the freezer (-20°C) and processed within 30 days.
2. Productivity Measurements: Site productivity is measured by "punching" the shoot at 1cm above the sheath bundle with a hypodermic needle on SCUBA (Tomasko and Dawes 1989, Figure 5A). This process allows for an accurate, non-lethal marking for productivity measurements as growth of individual leaves occurs from this bundle. At least 10 terminal shoots from each site along the deep edge, near HOBO data logger, will be punched at the sheath bundle for productivity measurements. These shoots will be placed within a small metal grid with flagging tape and a small Styrofoam float, attached for easy detection when retrieved 3-4 weeks later (Figure 5B). These shoots will then be brought to the lab and analyzed for total length, canopy height, epiphyte biomass, grazing, wasting disease (Kopp and Neckles 2009), and nutrient availability (Fourqurean et al. 1997).

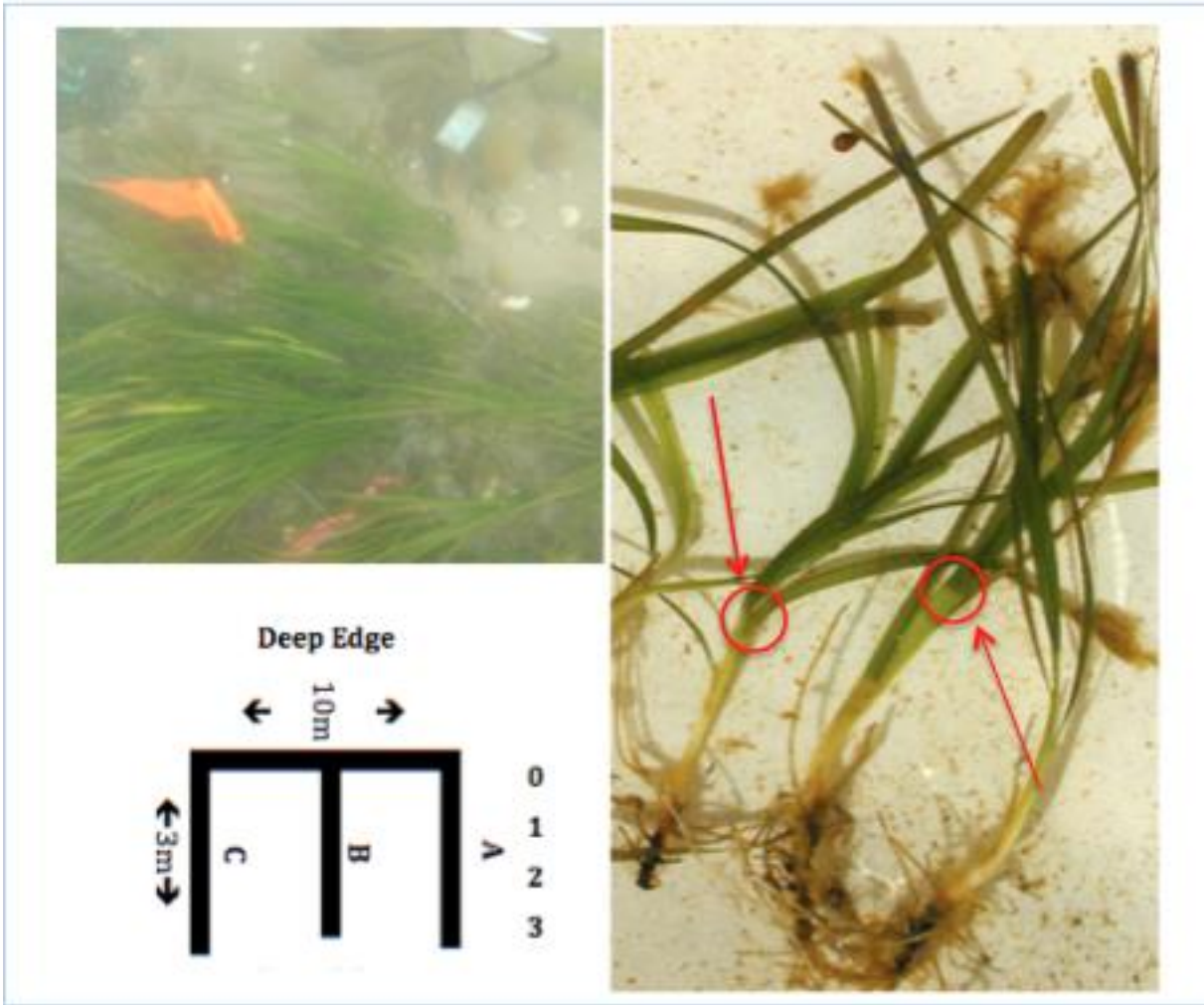

Figure 5: (A) Metal grid surrounding shoots punched for productivity measurements for easy retrieval. (B) *Zostera marina* shoots, arrows and circles indicate sheath bundle location for productivity punching (Image source: BioImages: The Virtual Field-Guide http://www.discoverlife.org/mp/20p?img=I_MWS26881&res=640) (C) Diagram of deep edge bed analysis, "A", "B", and "C" denote the 3m transect lines into the seagrass patch from the deep edge and #'s 0-3 display quadrat measurements taken every 1m along these transects.

Yearly monitoring

Analysis of the deep edge at each of the 5 seagrass sites will be conducted through transect dives on SCUBA along the deep edge of each seagrass area, performed by a pair of trained divers. The deep edge will be estimated from aerial and satellite imagery and visually confirmed before the HOBO data logger is deployed.

1. The divers will lay a 10m transect along the deep edge of the seagrass bed with a pre-labeled rope (flagging tape marker at 5m) for where intersecting transects will run perpendicular into the bed (Figure 5c). Stakes are placed at 0, 5, and 10m along the deep edge transect, labeled "A", "B", and "C". 3m lines are then attached to these stakes and run perpendicular into the bed. These lines are also pre-labeled with flagging tape at 0, 1, 2, and 3m for quadrat placement. This design allows for divers to easily locate quadrat measurement locations, especially in cases of poor visibility or high current. Quadrats are taken at 0, 1, 2, and 3m from the deep edge of the bed.

a. A 0.25m^2 quadrat will be used to determine approximate % seagrass cover and macroalgal cover.
b. A 0.0625m^2 quadrat will be used to determine shoot density within the larger quadrat, placed in the center of the 0.25m^2 quadrat.
c. Divers will take notice of grazing, wasting disease, root exposure, and epiphytic growth on the seagrass within each quadrat.
d. Site characteristics will also be taken note of: bathymetry, currents, wave action from boats, construction on land, or hardened shorelines, that may limit seagrass propagation to surrounding areas.

2. Sediment cores to 5cm depth will be taken outside of vegetation (deeper) and inside of vegetation (at deep edge) for sediment characteristics, % organic matter and grain size. Care is taken to avoid including seagrass, macroalgae, and large stones and instead noted. This will also be taken at non-seagrass sites, adjacent to the HOBO logger.
3. Sulfide sampling will occur at each site and will follow laboratory SOP, attached in the Appendix.

B3. Sample Handling and Custody

Samples taken in the field will be placed on ice and brought back to the laboratory and either analyzed or stored at appropriate temperature. Handling procedures for each parameter are described in Table 5. All samples taken in the field will be placed on ice until return to the laboratory where they will be placed in their appropriate location in the freezer or refrigerator. YSI handheld data measurements will be uploaded before next use with the YSI EcoWatch software, the data will then be added to a USB and uploaded to the Stony Brook University Electronic Repository.

Table 6. Sample container, transport, and holding times.

Parameter	Sample Container	Handling	Holding Time
NH_3, NO_2, NO_3, PO_4	20mL amber borosilicate glass vial with screw cap	Filtered and frozen at -20°C	30 days
Chlorophyll (Field collection and transport)	1L amber polyethylene wide-mouth Nalgene bottle	Kept on ice until filtration	8 hours (deviation from EPA method due to time constraints in the field based off of National Park Service protocol)
Chlorophyll (Laboratory Analysis)	20mL clear glass vial with screw cap	Kept at -20°C until analysis	<3.5 weeks
Total Suspended Solids	1L amber polyethylene wide-mouth Nalgene bottle	Cooled to 4°C	7 days
Colored Dissolved Organic Matter	20mL amber borosilicate glass vial with screw cap	Filtered and frozen at -20°C	6 months

Zostera marina shoots	1 gallon Ziploc bag	Cooled to 4°C	7 days
Sediment samples	1 quart whirl-pack bag	Frozen at -20°C	2 months

B4. Analytical Methods

All field samples gathered for further analysis will be brought back to the laboratory located on SUNY Stony Brook Southampton Campus in the Natural Science Building, Room 120.

1. Chlorophyll *a* Analysis: Chlorophyll whole water samples will remain on ice until filtration and be processed in order of collection upon return to the laboratory under subdued light. Chlorophyll-a samples are analyzed by filtering a thoroughly mixed 300mL subsample through a 47mm 0.7 GF/F filter with vacuum filtration not exceeding 20KPa to ensure no chlorophyll loss or damage. A 300mL filtration volume ensures that each filter retains sufficient chlorophyll for analysis, enough that the filter is slightly colored. Each water sample will be filtered onto two duplicate filters. The filter will be placed rough side up and the filter funnel will be attached in a manner that prevents leakage around the filter. The filter tower is rinsed three times with DI water between samples to ensure all chlorophyll has been captured on the filter. The sample is filtered completely through, but not dried completely to prevent filter damage. The filter is only touched with clean, stainless steel, forceps before and after filtration, and placed in a clear glass 20mL vial. The vials are then frozen in complete darkness at -20°C for <3.5 weeks before analysis to avoid chlorophyll loss. Procedure taken from the A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region, SOP 6 (Kopp and Neckles 2009). 5mL of 90% acetone is added to each vial, inverted, and placed back in -20°C until analysis, at least 24 hours and less than 2 weeks after addition. Each sample is analyzed using the Turner Trilogy Laboratory Fluorometer and Chlorophyll-a Non-Acidification snap-in module (Model 7200-046). The fluorometer is calibrated before each use with a stored calibration, calibrated using standards prior to sampling commencement. Each sample is added to a 2mL clear glass vial and wiped with a Kimwipe to ensure an accurate reading (modified EPA Method 445.0, laboratory SOP attached in Appendix). The 90% acetone used will be disposed of using Stony Brook University Environmental Health and Safety hazardous waste disposal procedure.
2. Total Suspended Solids Analysis: Total suspended solids (TSS) whole water samples will be analyzed within 1 week, typically within 24 hours following collection. Filters are rinsed with DI water and pre-combusted at 450°C for 1 hour to ensure no residue present. They are then weighed in pre-weighed, labeled, aluminum tins, and used in corresponding sample filtration. Duplicate samples for each site are mixed thoroughly before filtration. 500mL of each sample is filtered through and rinsed three times with DI water in approximately 20mL volume each rinse to remove salts. The filters are vacuumed until dried and placed in the drying oven at 103°C for at least 24 hours and re-weighed. The filters are then combusted for volatile suspended solids at 450°C for 4

hours and re-weighed. TSS procedure follows EPA Method 160.2 Residue, Non-Filterable (Gravimetric, Dried at 103-105°C).

3. Colored Dissolved Organic Matter Analysis: CDOM analysis will be performed using the Turner Trilogy Laboratory Fluorometer with the CDOM/NH4+ snap-in module (Model 7200-041), calibrated using a stored calibration before each analysis. Each replicate sample will be thawed to room temperature and added to a clear glass 2mL vial, wiped with a Kimwipe, and analyzed using the fluorometer within 6 months of sample collection, based on established laboratory protocol found in the Appendix.
4. Dissolved Inorganic Nutrient Analysis: DIN (nitrate, nitrite, ammonium) and DIP (orthophosphate) will be thawed to room temperature measured using the LaChat QuikChem spectrophotometer methods:
   a. Ammonia ($NH4^{+}$)- QuikChem method 31-107-06-Q, Determination of ammonia by flow injection analysis (Phenate method, citrate buffer, EPA Method 349.0)
   b. Nitrate/Nitrite (NO_2^-, NO_3^-) – QuikChem method 31-107-04-1-G, Determination of nitrate and/or nitrite in brackish or seawater by flow injection analysis colorimetry (high throughput method)
   c. Orthophosphate (PO_4^{3-})- QuikChem method 31-115-01-1-H, Determination of orthophosphate by flow injection analysis
5. Analysis of *Zostera marina* shoots: Terminal shoots are refrigerated on return to the laboratory and analyzed as soon as possible.
   a. Each shoot is measured for total and canopy length, and assessed for grazing, epiphytes, and wasting disease (Kopp and Neckles 2009).
   b. Each blade is measured for productivity (Tomasko and Dawes 1989) from the sheath bundle to the "punch" mark, the newest being the center, youngest leaf (1), and oldest being the outside leaf (Figure 6), usually about to slough off (~4-5 leaves, Drake et al.

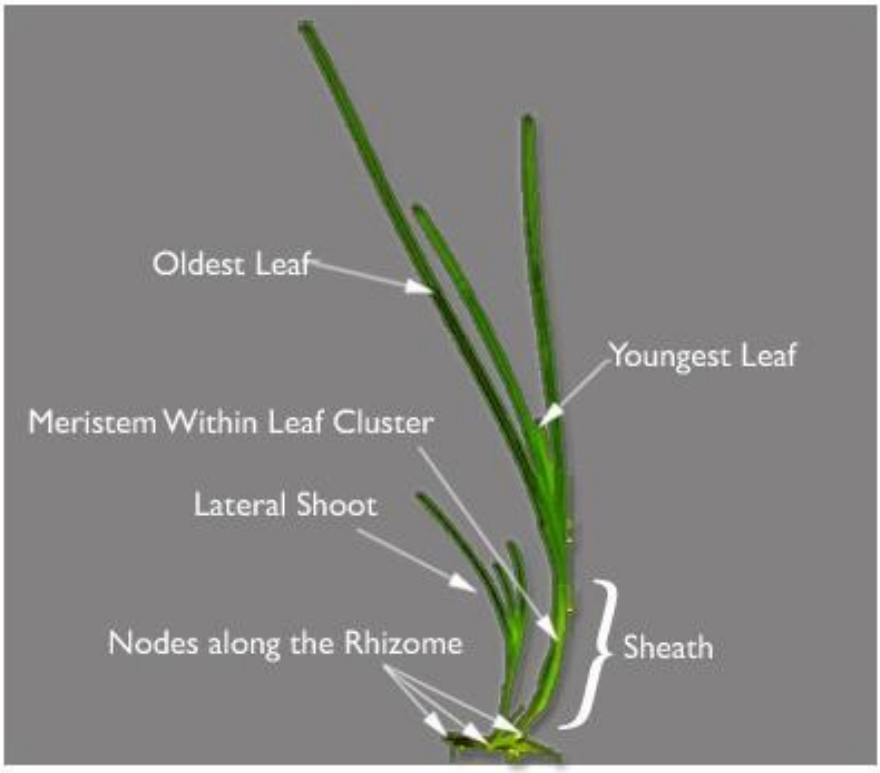

Figure 6: Parts of an Eelgrass shoot *(Zostera marina,* Image Source: http://www.seagrassli.org/ecology/eelgrass/a_sh/a_shoot_feb10.jpg)

2003).

c. The newest blade is taken from at least 3 shoots and dried over 48 hours in a labeled paper bag at 60°C for nutrient availability, as the newest leaf is a product of the nutrients available at the time when it is growing. These leaves are then ground into a fine powder and analyzed for total carbon and nitrogen contents via the Fisons NA Model 1500 CNS analyzer (Duffy et al. 2015).
d. Three shoots are scraped of epiphytes, dried to a constant weight at 60°C, and weighed for approximate epiphyte biomass (Ruesink 2016).

6. Organic Matter and Grain Size Analysis: Sediment cores are to be analyzed for organic matter and grain size within 6 months of collection. Each sample will be separated for organic matter and grain size simultaneously to ensure homogeneity between samples. Organic matter will be analyzed through loss on ignition analysis.
   a. Duplicate sub-samples will be homogenized and dried to a constant weight at 60° of at least 20g, pre-weighed and combusted at 400°C for 12 hours, then re-weighed for total matter loss (Schumacher 2002).
   b. Grain size will be analyzed through duplicate subsamples, each at least 40g. Each sample will then be weighed, dried at 60°C for 24 hours, placed in a desiccator to cool, and re-weighed for dry weight. The amount of salt in each sample can be calculated with the amount of water lost during drying and salinity at the site. The samples will then be partitioned into size classes, noting of larger non-sediment presence (clam shell fragments, plastic, etc.), using sieves corresponding to each class:
      i. Pebbles >2000 μm (10 mesh)
      ii. Very course and course sand 500-2000 μm (35 mesh)
      iii. Medium, fine, and very fine sand 63-500 μm (230 mesh)
      iv. Silt <63 μm

   After being partitioned the samples will be re-weighed according to their size classes (Poppe 2000).

B5. Quality Control

Personnel working with the water will be trained on all aspects of collection, filtration and analysis and monitored by Kaitlyn O'Toole while in the field and laboratory and overseen by Bradley Peterson. YSI and Li-COR measurements will be reiterated to the data recorder by trained personnel and overseen by Kaitlyn O'Toole. The data recorder will have been on several previous water sampling trips and understand water quality parameters taken to ensure accuracy. They will also record wind speed, direction, cloud cover, time, and unusual site conditions. Analysis of CDOM and chlorophyll will be performed by Kaitlyn O'Toole to ensure accuracy and consistency in use of the fluorometer. A technician who has been trained to use the LaChat QuikChem spectrophotometer will perform DIN and DIP analysis and duplicate samples of each site duplicate will be taken. Analysis of productivity and epiphyte biomass will be overseen by Kaitlyn O'Toole to ensure accuracy and consistency in measurements. Oversight includes being present during all shoot and epiphyte analysis. Triplicate epiphyte samples from each location and date will be taken from the shoots marked for productivity to avoid removing more shoots than necessary. At least 10 shoots will be punched for productivity and 3-4 weeks later, all shoots in the designated metal area (Figure 5A) will be taken back to the lab for analysis. Bradley Peterson will oversee, supervise, and review measurements. For initial quadrat and

transect sampling by other members of the dive team, % coverage and shoot density counts made by another team member will be verified upon visual inspection of quadrat by Kaitlyn O'Toole to ensure consistency.

Table 7: Accuracy, precision, and deviance between samples for water quality, shoot, and sediment analysis.

Parameter	Instrument	Accuracy Check	Precision Check	% Deviance between field duplicates
Chlorophyll	Turner Lab Fluorometer Chlorophyll-a Module	Pre-analysis calibration	Field duplicate	50
TSS	Mettler Toledo AG204 DeltaRange	Calibration scale checks	Field duplicate	50
CDOM	Turner Lab Fluorometer CDOM/NH4 Module	Pre-analysis calibration	Field and lab duplicates	50
Dissolved Inorganic Nutrients	QuikChem Analyzer	Pre-analysis calibration	Field and lab duplicates	50
Shoot Analysis (productivity, length, canopy height, width)	Ruler/transect tape	NA	Field duplicates (~10 shoots)	50% of mean
Epiphytes	Mettler Toledo AG204 DeltaRange	Calibration scale checks	Field triplicates	50% of mean
Sediment Grain Size	Stainless steel sieve	NA	Lab duplicates	50
Sediment Organic Matter	Combustion oven	NA	Lab duplicates	50

B6. Instrument/Equipment Testing, Inspection, and Maintenance

Laboratory Field Equipment Preparation and Maintenance

1. HOBO data loggers will be inspected for water damage, batteries replaced, and silicon O-rings greased with silicon prior to use. The loggers will be wiped of previous data with battery levels over 90%. The loggers will begin logging at least an hour before deployment. Time of deployment will be recorded. The HOBOs will be returned to monthly to retrieve data using the Onset HOBO Underwater Shuttle (U-DTW-1) and clean the HOBO of marine debris. The shuttle data will then be uploaded to the

laboratory computer to check battery levels and relieve potential memory overwriting. Upon retrieval the devices will be checked for saltwater damage, battery levels, and temperature shock, which may impact data.

2. Nalgene bottles used for whole water sample collection will be rinsed three times in the lab after sample has been filtered and allowed to air dry on rack. These bottles are then rinsed with ambient water in the field before collection (Kopp and Neckles 2009).
3. 20mL glass amber vials and 25mm 0.7µm GF/F filters will be pre-combusted at 450°C for at least 2 hours to ensure sterility for CDOM, DIN, and DIP collection.
4. GF/F filters are not used if they are torn or have wrinkles.
5. Syringes and luer-lock syringe tips used for CDOM/DIN/DIP collection are rinsed three times with deionized (DI) water at the laboratory and are dried while covered to avoid contamination. The tips are then pre-loaded with the filters at the laboratory for greater ease in the field. Individual filter tips are used for each sample (one for each replicate) and one syringe per site is implemented.
6. The syringe is first rinsed with ambient water three times before the syringe tip is added for filtration for the first replicate. The syringe is then rinsed three times with DI water, and then rinsed three times with ambient water before the syringe filter tip is added to filter the second sample. With each replicate, the cup used to take the sample is rinsed with ambient water three times.
7. Upon return to the laboratory the YSI probes will be cleaned with tap water and a small amount of water will be placed in the calibration cup until next use.
8. Li-Cor underwater sensor and frame will be rinsed with tap water upon return to the laboratory.
    a. If a significant amount of saltwater splashes on the handheld it will be cleaned with a moist towel and then dried.

Field Inspection and Testing

1. At every site the YSI 6600 multi-parameter sonde will be used, the travel cup is taken off before the boat leaves the dock to ensure easy transfer to the probe protecting cup and wrapped in a wet towel (DI water), the YSI is returned to the cup following docking.
2. At each site a QA/QC surface measurement is taken before lowering into water to ensure accuracy.
    a. The local DO is not to exceed +/-2% of 100.0%, if this occurs, re-rinse using DI water, re-calibration of the YSI is necessary if the value does not fall within this range.
3. YSI is rinsed with DI water after every site, taking care to rinse the conductivity port (Kopp and Neckles 2009), placed in a wet towel (DI water) to keep ports moist, and into an Action Packer for safe travel between sites.
4. The Li-COR will travel in a container big enough to hold the frame and rope, used to reduce cord tension and indicate depths of sampling, safely and a separate small bucket for the handheld to keep the surface connections dry.
5. The sensors will be kept covered with caps between sites.
6. Readings below the surface of the water will be under 100% incident PAR, and should decrease with increased depth. Underwater irradiance flux can fluctuate with depth

depending on cloud cover, so this should decrease with no clouds present, however, it may not if cloud cover changes during sampling (Kopp and Neckles 2009).

Laboratory Equipment Inspection, Testing, and Maintenance

1. The refrigerator and chest freezer will be maintained at 4°C and -20°C respectively for the duration of the sample holding period (until all the samples have been gone through).
    a. In case of power failure an alarm will sound and temperature changes will be ascertained, deterioration of temperatures will determine the next steps.
2. The Turner fluorometer will be not be moved for the duration of the sampling period and will remain out of the sunlight, for chlorophyll-a and CDOM analysis. Samples will be removed after each analysis to prevent corrosion from acetone vapors. The fluorometer will be calibrated before each season using standard methods and before each analysis using the stored calibration.
3. Electric balance will be calibrated before sampling season and checked monthly.

B7. Instrument/Equipment Calibration and Frequency

1. YSI 6600 multi-parameter sonde will be calibrated prior to field season by YSI. Throughout the season, the sonde will be calibrated for dissolved oxygen, conductivity, and turbidity bi-monthly following National Park Service, "A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region," calibration protocol (Kopp and Neckles 2009) by Kaitlyn O'Toole.
    a. Dissolved oxygen will be calibrated by placing an air-stone in a 5 gallon bucket and allowing the water to warm up to room temperature for at least an hour. The YSI is placed in the bucket for at least 10 minutes to equilibrate with temperature and oxygen pressure. The aerated water is assumed to be 100% saturated.
    b. Conductivity (10,000 µS) and turbidity (126 NTU) will be calibrated using YSI calibration standards.
    c. Turbidity and dissolved oxygen wipers will be observed to sit 180° from the optical windows of the probes.
    d. Post-calibration will take place at the close of the field season. This entails standard calibration procedures to monitor sensor drift over the season.
    e. Probe calibration performed by YSI every 2 years.
2. The Li-COR will be calibrated according to the National Park Service, "A Protocol for Monitoring Estuarine Nutrient Enrichment in Coastal Parks of the National Park Service Northeast Region," calibration protocol (Kopp and Neckles 2009). And will take place prior to the sampling season, re-calibrated before July 1, with a final post-calibration taking place after the field season closes.
    a. Sensor calibrations are sent to and performed by Li-Cor yearly.
3. The Turner Laboratory Fluorometer will be calibrated using a concentration curve prior to the field season. This curve is then saved as a calibration to be performed before every use. Each module will have a saved calibration prior to analysis.
    a. The chlorophyll module will be calibrated with concentrations according to SOP provided along with standards sent by Turner Designs yearly and will follow the

direct concentration non-acidification chlorophyll-a calibration procedure (http://www.turnerdesigns.com/t2/doc/manuals/998-7210.pdf).

b. The CDOM/NH4+ module will be calibrated using quinine sulfate and 0.1N acetic acid to create the concentration curve for CDOM (0.0, 2.5, 5.0, and 10.0 ppb).

4. The LaChat QuikChem spectrophotometer will be calibrated daily.
5. DI water filter changes occur when necessary to ensure non-contaminated water.
    a. Purifies the water to Type I quality through the use of carbon and deionization resins and provides a typical flow rate of 1.8 liters/minute.
6. The AG204 DeltaRange balance will be calibrated prior to field season and re-checked monthly for deviance.

Offsets and calibration data will be recorded on datasheets, which will then be scanned into the Stony Brook University electronic repository.

B8. Inspection/Acceptance of Supplies and Consumables

Supplies and consumables (Nalgene bottles, sampling vials, GF/F filters, syringes, syringe tips, DI water, Ziploc/Whirl-Pak bags, coolers, sediment corer) shall order through Stony Brook University from reputable sources and be inspected visually by Kaitlyn O'Toole prior to use. Standards purchased for sonde calibration are developed by YSI and are labeled with the date opened.

B9. Non-direct Measurements

Final selection of seagrass and water quality sites were chosen based off of Google Earth Pro Imagery, from 1995 to 2013 (depending on water clarity each year), and distance from river/creek runoff, bathing, and dredging areas. Historical seagrass locations from 1930 were determined from data acquired from Cornell Cooperative Extension and used in choosing western Peconic Bay sites in particular. Data showing changes of seagrass increase and decrease from 1990 to 2010 were used to pick water quality locations around Shelter Island and Eastern Peconic Bay/Western Gardiner's Bay. These 10 sites of previously vegetated areas that do not presently have grass are used as water quality locations. Five more sites that contain seagrass were picked from the areas that were known to have seagrass by CCE. They were chosen based on distance from one another (to get as much spread between as possible), size and density data acquired from CCE, no known groundwater influence (personal communication with CCE Marine Botany/Habitat Restoration Specialist Steve Schott and NYS DEC Seagrass Coordinator Soren Dahl), and ease of access by boat. Bathymetry data from 2015 will be obtained from NOAA DEM (1/9 arc second, ~3m horizontal resolution) LIDAR database.

B10. Data Management

Records from the field, water quality monitoring and deep edge analysis, will be kept on waterproof paper, formatted, and printed using a laser ink printer. The datasheets will be kept in an enclosed binder and taken out of the binder upon return to the laboratory and placed in a filing cabinet. The datasheets will then be entered by personnel, initials and date entered will be placed

at the header when complete, these entries will then be cross-checked by Kaitlyn O'Toole before analysis. The documents will be scanned and corresponding entered data will uploaded to the Stony Brook University Electronic Repository (SBUER). Hard copies will be kept by Kaitlyn O'Toole. Measurements made on the YSI 6600 will be uploaded via EcoWatch software and converted to .csv files. HOBO datalogger files will be transferred to the computer with the Onset HOBOware Underwater Shuttle (retrieved data from the dataloggers in the field) with HOBOware Pro software and converted to .csv files. All files will be duplicated onto a USB drive and uploaded to the SBUER.

Laboratory measurements of chlorophyll-a, TSS, CDOM, DIN, DIP, and sediment analysis will be recorded directly on an electric datasheet, later uploaded to the SBUER. Productivity and epiphyte measurements will be recorded on plain paper; they will be kept with other datasheets and uploaded the same manner as the field datasheets. All electronic data will have corresponding dates, location, and parameter values in the same format and unit. Calibration datasheets will be kept with the field datasheets in a separate folder, scanned, and uploaded to the SBUER.

C. ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

Assessments (e.g. data quality) can be scheduled for any time, which is mutually convenient for the user groups (PEP and EPA), QA staff, and our team. Bradley Peterson will oversee and provide regular checks including verifying field and laboratory procedures, throughout the season overseeing data analysis. Regular check in with the PEP according to the QAPP and contract agreement will occur quarterly.

C2. Reports to Management

Quarterly reports will be submitted, incorporating all of the sampling conducted to that point. Following the final quarterly report, a final report will be created that includes all of project data as well as spatial comparison of water optical properties. This final report will include all data and data analysis, and make appropriate interpretations as dictated by the Contract Agreement (Task 5-Reporting):

- Interim Progress Reports: quarterly reports will be made to the Peconic Estuary Program and shall describe all of the sampling and analyses conducted to that point.
    - Final Report: a draft final report will be submitted for review to the Peconic Estuary Program. This report will include:
        - An executive summary
        - A summary of all methods used, analytical techniques and results.
        - Implications of the work for comprehensive conservation and management plan implementation.
        - Recommendations for new management actions of modifications to existing priority actions.
        - Recommendations for future restoration and protection activities.

- Amend the draft final project report as necessary, in response to comments provided by the Department and PEP Program Director.
- Present a final report to the PEP Technical Advisory Committee and submit the final report in the form of five colored copies and five CDs containing a .pdf file of the final report and all associated data layers and files to the Department.

All reports will be subject to Peconic Estuary Program, with revisions made as appropriate. The final report will then be modified for publication in the primary scientific literature. Bradley Peterson will be responsible for all data reduction and data quality assurance. All data, after reduction and quality assurance, will be archived electronically and delivered to the Peconic Estuary Program. The data will be delivered to the Peconic Estuary Program with the final report. In addition to the electronic data files, metadata for all spatial and non-spatial data describing the history of where, when and why the data were collected, who collected the data and the methods used to collect and process the data will be provided with the final report. Prior to the submission of the Final Report, an oral presentation will be made to Peconic Estuary Program staff or other designees.

D. DATA VALIDATION AND USABILITY

D1. Data Review, Verification, and Validation

Data collected in the field (on datasheets and handheld devices) will be reviewed during collection, upon return to the laboratory, and again after data has been input electronically to ensure consistency. Data will be input by trained staff and crosschecked prior to analysis by Kaitlyn O'Toole. Inconsistent values will be removed before analysis, such as negative and otherwise impossible values. All data generated from field and lab work will be reviewed and analyzed by Kaitlyn O'Toole.

D2. Verification and Validation Methods

Data validation and verification will include checks on:

- Completion of all fields on data sheets; missing data sheets
- Completeness of sampling runs (e.g. number of sites visited/samples taken vs. number proposed, were all parameters sampled/analyzed)
- Completeness of QC checks (e.g. number and type of QC checks performed vs. number/type proposed)

D3. Reconciliation with User Requirements

At the conclusion of the sampling season, after all in-season quality control checks, assessment actions, validation and verification checks and corrective actions have been taken, the resulting data set will be compared with the program's data quality objectives (DQOs). This review will include, for each parameter, calculation of the following:

- Completeness goals: overall % of samples passing QC tests vs. number proposed in Section 7

- Percent of samples exceeding accuracy and precision limits
- Average departure from accuracy and precision targets.

After reviewing these calculations, and taking into consideration such factors as clusters of unacceptable data (e.g. whether certain parameters, sites, dates, volunteer teams etc. produced poor results), QA/QC Manager and PEP Technical Advisory Committee (TAC) members (as applicable) will evaluate overall program attainment of DQOs and determine what limitations to place on the use of the data, or if a revision of the DQOs is allowable.

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Appendix:

A. Sulfide Protocols for Seagrass Monitoring

I. Zinc acetate (0.05 M) preparation

a. Weigh and add 2.75 g of zinc acetate into 250 mL DI.
b. Mix solution and store in plastic screw top container. This reagent is stable and can be stored on a countertop.

II. Field sampling

a. Before sampling, label vials and add 500 uL of zinc acetate to all vials.
    i. Plastic 7 mL vials can be used as sampling, storage, and reaction vials.
b. In the field, draw sediment porewater as desired.
    i. Ideally, use a syringe and Rhizon sampler (these are somewhat delicate and expensive but render filtering unnecessary and carry the least risk of air exposure).
    ii. Alternatively, use a syringe and long, thin stainless steel cannula with mesh over the end to block large sediment particles from clogging the sampler.
    iii. Try to eliminate air intrusion. If using a three-way stopcock with the syringe, tubing and stopcock can be purged by drawing and discarding a small sample before drawing the main sample. The syringe with a sample inside can also be turned upside down and purged of any visible air bubbles. Try not to jostle the sample inside the syringe more than necessary. Minimize time between sampling and fixation.
    iv. If not using a Rhizon, the sample should be filtered. This can be done with an inline syringe filter or in the next step.
c. On a boat or land-based work area, transfer 250 uL of sample to the appropriate vial with zinc acetate.
    i. One method is to dispense sample into another 7 mL "transfer" vial and very quickly use a micropipette to transfer sample to the zinc acetate vial.
        1. If filtering at this step, attach the syringe to a reusable filter housing with a glass fiber filter inside and filter over the 7 mL transfer vial.
    ii. Alternatively, dispense a larger amount of sample from the syringe (with or without filter) into zinc acetate, e.g. 1 mL sample into 2 mL zinc acetate. This requires the additional step later of taking a 750 uL subsample and adding to a reaction vial before analysis.
    iii. If possible, use a vial rack that is heavy, e.g. wood, or weighed down.
d. Keep fixed samples in a cooler and freeze as soon as practical. These will keep ~1 month.

III. Diamine reagent preparation

a. Obtain dark plastic reagent bottle.
b. Use a fume hood, gloves, lab coat, and eye protection.
c. Mix 100 mL 6N HCl by adding 50 mL 12N HCl into 50 mL DI in the dark bottle.
d. Weigh 4.0 g anhydrous ferric chloride and 1.6 g N,N-dimethyl-p-phenylenediamine sulfate and add into dark bottle.

i. Both reagents are hygroscopic and toxic - be careful but work quickly.

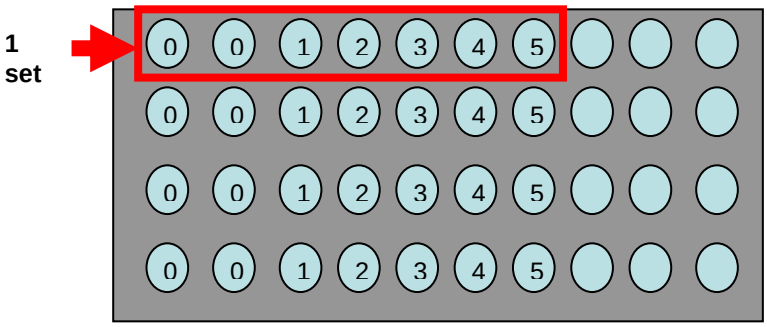
e. Cap dark bottle and shake. Solution should be orange. Store in refrigerator. Good for ~ 1-2 months.

IV. Making H_2S standards

a. Attach regulator to N_2 tank and set up tubing.
   i. An ideal tubing setup is split twice. First split is to switch N_2 flow between bubbling of liquids with Pasteur pipettes and a stopcock that can be used to blow directly into standard vials. Second split is to allow bubbling of two liquids at once, one for distilled water (DI) and one for the sulfide stock solution.
   ii. Testing is highly recommended. If setup is not done properly, N_2 flow could be blocked and result in rapid, dangerous pressure buildup that damages the regulator. If flow is too high for stopcock vs. liquids, liquids can "explode" when flow is switched from stopcock to liquids.

b. Label multiple sets of sample vials for H_2S standards. One set contains 7 clean vials (0, 0, 1, 2, 3, 4, 5).

c. Place labeled sample tubes in sample tray holder making sure that labeled vials line up (Fig. 1)



   i. **Fig. 1**.

d. De-aerate two flasks of DI by bubbling N_2 gas into water.
   i. Adjust gas to allow for continuous "boiling" type bubbles. Cover most of the way with Parafilm to allow gas exchange but minimize air re-intrusion.
   ii. Use as precise an amount of DI as possible in Flask 1 for the sulfide stock solution (see below about making sulfide stock solution). The amount of DI in Flask 2 for de-aerated DI (DDI) is not really important.
   iii. Consider the tradeoffs between using small flasks and making direct removal of liquid with pipettes easier vs. needing to use a very small amount of sodium sulfide in Flask 1.

e. Allow to de-aerate for about 15 min or longer.

f. While de-aerating, calibrate pipettes using analytical balance if desired (options: 1000 uL, 900 uL, 750 uL, 500 uL, 250 uL 100 uL).
   i. Calibrate pipettes by adjusting volume of DI in pipette until it is equal to 1.000 g, 0.900 g, 0.750 g, etc.)
   ii. Having 6 set pipettes is more convenient than switching quickly during making of standards.

g. Pipette 500 uL of zinc acetate into all standard sample vials.

h. Consider flushing all standard vials with N_2 and capping to ensure a minimum of oxygen within the vials.

i. Once DDI in Flask 2 is ready, put the following volume of DDI into standard vials using calibrated pipettes and cap:

H2S Standards

Label	DDI (ml)	H2S soln (ml)	Zinc Acetate (ml)	Total Volume (ml)
0	1	0	0.5	1.5
0	1	0	0.5	1.5
1	0.9	0.1	0.5	1.5
2	0.75	0.25	0.5	1.5
3	0.5	0.5	0.5	1.5
4	0.25	0.75	0.5	1.5
5	0	1	0.5	1.5

j. Make sulfide stock solution in Flask 1.
   i. Aim for a ~ 500 uM stock solution.
   ii. If using Na_2S-$3H_2O$, use Excel sheet to calculate weight of sodium sulfide and volume of water required.
      1. e.g. 0.01 - 0.02 g Na_2S-$3H_2O$ to make 200 mL stock solution.
   iii. If using Na_2S-$9H_2O$, original protocol calls for 0.1 - 0.14 g Na_2S-$9H_2O$ to make 100 mL of solution in a volumetric flask, then diluting 5 mL into a 50 mL volumetric flask.
      1. This requires careful flushing with N_2 and can be messy.
   iv. Weigh sodium sulfide and add to Flask 1. Swirl until all crystals are dissolved. Keep bubbling with N_2.
   v. **RECORD THE WEIGHT** of sodium sulfide used and calculate the target concentrations in the standard vials so the calibration curve can be evaluated later.

k. Starting with standard sample vials labeled "1", add 100 uL of H_2S solution to all vials labeled "1".
   i. Use one hand for stopcock blowing N_2 and one hand for uncapping and pipetting. Blow N_2 into stock solution flask, uncap vial, blow N_2 into vial, pipette from stock solution flask to vial, blow N_2 into vial, recap, blow N_2 into stock solution flask.
   ii. If the flask is too large to allow direct pipetting, flush a 20 mL vial with N_2 and transfer some stock solution to that container before starting.

l. Repeat procedure for standard vials "2-5" using the table:

H2S Standards

Label	DDI (ml)	H2S soln (ml)	Zinc Acetate (ml)	Total Volume (ml)
0	1	0	0.5	1.5
0	1	0	0.5	1.5
1	0.9	0.1	0.5	1.5
2	0.75	0.25	0.5	1.5

3	0.5	0.5	0.5	1.5
4	0.25	0.75	0.5	1.5
5	0	1	0.5	1.5

m. Once complete, shake vials gently, separate standard vials into sets, and package each set into plastic bags in the freezer.
    i. Label with date, name, "H_2S standards", and weight of sodium sulfide.
n. Testing a set immediately using the protocol below is highly recommended.
o. Standards are good for ~ 1 month.

V. H_2S analysis

a. Turn on spectrophotometer, make sure it is set to 670 nm, and wait for warmup (usually 15 min - 1 hr depending on machine).
b. Take out one set of H_2S standards and your samples from the freezer. Allow to thaw.
c. Arrange samples in desired order in an adequately sized vial holder.
d. Consider whether dilution of samples is required, since the calibration range on a typical spectrophotometer using this protocol is ~ 0-500 uM.
e. Add DI to SAMPLE VIALS ONLY with a calibrated pipette so that a total volume of 1.5 mL (i.e. 0.500 mL zinc acetate, 0.250 mL sample, 0.750 mL DI) is obtained.
    i. Standard and sample volumes should be equal.
f. Take out diamine reagent from refrigerator and pour a small amount into a small beaker. Put diamine reagent back in refrigerator.
g. Add 50 uL of diamine reagent to each standard and then to all sample vials.
h. Cap and mix gently, manually or on a mixing platform at a low speed. Wait 20 minutes.
i. Add 4 mL of DI to all standards and samples in the same order as the diamine was added.
j. Cap and mix gently, manually or on a mixing platform at a low speed. Wait 15 minutes.
k. Obtain datasheets and/or a computer.
l. Obtain clean analysis cells.
    i. For plate-reading spectrophotometer, this will be a 96-well plate.
    ii. For single-sample spectrophotometer, this will be cuvettes with caps.
        1. Make sure there are cuvettes with DI for blanks.
m. Fill cells with standard or sample solution.
    i. For well plate, start each plate with standards and plate all samples in duplicate.
    ii. For single-sample, use appropriate volume for cuvette.
n. Follow directions for operating machine.
    i. For plate-reader, find or make sulfide protocol on computer, read, and save output as text, csv, or Excel file. Make sure to have a diagram relating cells to sample IDs.
    ii. For single-sample, repeat as needed for standards and samples and record data.

1. Make sure there are no air bubbles or smudges on cuvettes, as this can alter readout.
2. Make sure cuvettes are in proper orientation.

o. When finished, make sure all wastes are put in properly labeled hazardous waste container (all liquids, e.g. zinc acetate, diamine reagent, blue colored analytical solution, can go in the same container).

Reference:

Cline JD. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnology and Oceanography 14: 454-458.

B. Peterson Lab Chlorophyll Analysis

Preparation:

1. Add 5mL of appropriate quantity of 90% acetone to each storage vial with filters.
2. Replace the storage vials in the freezer for 24 hours.
3. After 24 hours, shake and tap the bottom of the storage vials.
4. Pipette out 1.8mL from the storage vial into the 2mL vials.
5. Click Chl-NA on the fluorometer (since No Acid was added).
    a. Hit calibrate
    b. Use the most recent stored calibration
6. Wipe the glass vial with a Kimwipe and place in the flurorometer.
7. Click measure chlorophyll-NA
8. Enter 300mL for amount of the seawater filter (unless different)
9. Enter 5mL for solvent volume
10. Hit ok
11. Record chlorophyll concentration.
12. After the analysis has been read, empty acetone into acetone waste container and throw empty glass vials into the broken glass waste box.

Peterson Lab CDOM Calibration and Analysis

Calibration:

1. Create the 0.1N acetic acid solution (need 2 L for Stock A and 200 mL for Stock B and C) to dilute stock solution concentrations:
    a. To make 1L of .1N acetic acid solution (57.5mL acetic acid per 942.5mL DI to make 1 N):
        i. Add 25mL of DI water to a flask, then add 5.742mL glacial acetic acid, add remaining 69.258 mL DI water to create 100mL of 1N acetic acid solution
        ii. Take this 100mL of 1N acetic acid solution and dilute with 900mL of DI water to create 1000mL of 0.1N acetic acid solution
2. To make 3L of 0.1N acetic acid solution add 75mL of DI water to a flask, then add 17.226 mL acetic acid, then add remaining 207.78

   a. 75mL DI water + 17mL acetic acid + 205mL DI water= ~300mL of 1N acetic acid
   b. Take 300mL of 1N acetic acid solution to dilute with 2700mL of DI water for 3L of 0.1N acetic acid
3. Create calibration concentrations
   a. Solution A/Stock Solution (10 ppb solution):
      i. First make a 10 ppm solution (1L):
         1. Measure out 0.1000 g of Quinine Sulfate
         2. Add 500mL of 0.1 N Acetic Acid to a flask
         3. Add Quinine Sulfate and swirl until dissolved
         4. Add 500mL of 0.1 N Acetic Acid more and swirl
   b. Second make a 10 ppb solution (1L)
         1. Add 1mL of of the 10.0 ppm solution to 1000 mL of 0.1 N acetic acid to create 10.0 ppb solution, this is solution A and the stock solution for creating solution B&C
4. Solution B (5 ppb solution):
   a. Take 50mL of solution A (10.0 ppb) and add to 50mL of 0.1 N acetic acid to create a 5.0 ppb solution
5. Solution C (2.5 ppb solution):
   a. Take 25mL of solution A (10.0 ppb) and add to 75mL of 0.1 N acetic acid to create a 2.5 ppb solution
6. Solution D/blank (0 ppb solution):
   a. 0.1 N acetic acid solution (no quinine sulfate)

Analysis:

1. Defrost the CDOM vials to room temperature.
2. Pipette out 1.8mL from the storage vial into the 2mL vials.
3. Click CDOM/NH_4^+ on the fluorometer
   a. Hit calibrate
   b. Use the most recent stored calibration
4. Wipe the glass vial with a Kimwipe and place in the flurorometer.
5. Click measure CDOM.
6. Hit ok
7. Record CDOM values.
8. Throw empty glass vials into the broken glass waste box.