1.0 TITLE: Modeling *Zostera marina* restoration potential in Barnegat Bay

QUALITY ASSURANCE PROJECT PLAN

Prepared by Jessie C. Jarvis, The Richard Stockton College of New Jersey, Galloway, New Jersey

Effective Date: June 1, 2013

Project Duration: August 1, 2012-May 31, 2014

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Barnegat Bay Partnership

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3.0 QAPP DISTRIBUTION LIST:

Signed copies of this Quality Assurance Project Plan (QAPP) and all subsequent revisions will be sent to the following individuals by electronic mail:

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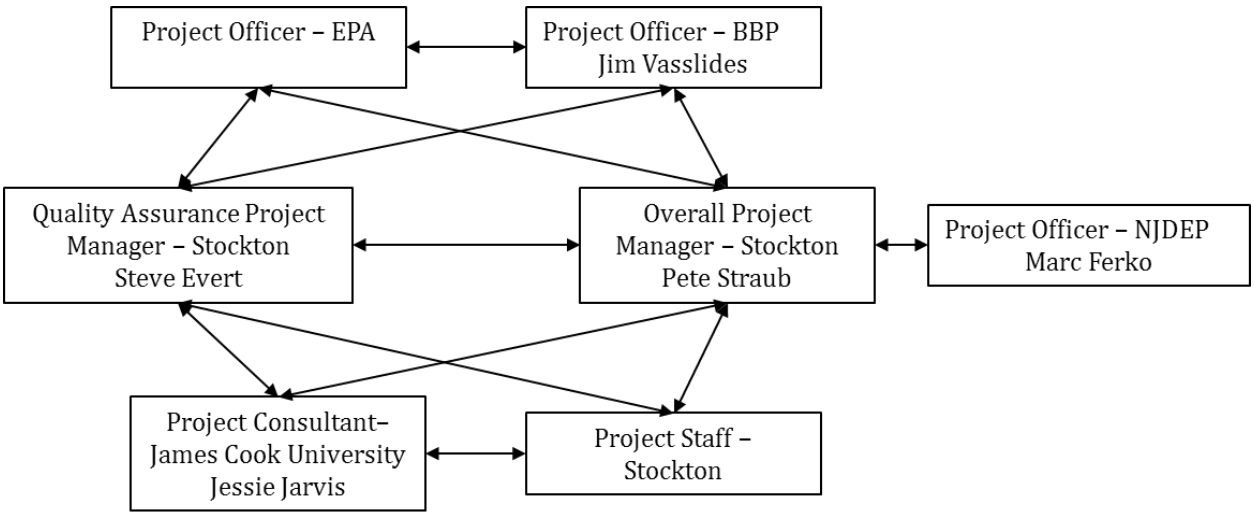
4.0 PROJECT TASK ORGANIZATION:

Overall project management will be the responsibility of Dr. Peter F. Straub. Dr. Straub will oversee the collection of samples by technical staff and will be responsible for laboratory analysis,

data processing and maintenance of the approved QA Project Plan. Dr. Jessie Jarvis will be responsible for data analysis, model calibration and development Steven Evert will provide quality assurance management, reviewing data acquisition and data analysis protocols, and ensuring compliance with all elements of the QA Project Plan. Dr. Straub will report to Jim Vasslides at the Barnegat Bay Partnership Program and the Region 2 EPA project officer.

Organizational Chart-Lines of Communication



5.0 SPECIAL TRAINING/CERTIFICATIONS:

All field crews and laboratory personnel that participate in this project must first successfully demonstrate team proficiency in each component of field sampling and data collection before they will be authorized to collect actual field data and samples. Stockton College personnel will conduct structured field training sessions for those field teams that are new to estuarine research projects, as well as, for any crew that requests a refresher course. During the training, crews will be instructed on sampling protocols and methods developed for the project, then they will actively participate in hands-on exercises conducted in the field during which all components of the field sampling will be covered. After the crew has developed proficiency in the core field activities, they will be observed and evaluated by the instructors on a pass/fail basis for each component as they conduct a full the NJ Coastal Bays field sampling scenario. The crews will be informed verbally by the reviewer as to whether they passed or failed the certification exercise. A list of certified individuals will be maintained in the laboratory notebook. All water quality testing shall be performed by a New Jersey laboratory certified under the requirements of N.J.A.C. 7:18 or laboratories which have formal approval from the NJDEP-Office of Quality Assurance. Certificates of formal approval are specific to the QAPP related analytical testing and are effective until June 30 of every year. Approvals and certifications must be renewed on an annual basis. Certificates can be found in Appendix 2.

5.1 Training procedures: Seagrass design modified from Kennish et al. (2008).

5.1.1 In situ:

An individual must be trained to identify the *Zostera marina* in situ from *Ruppia maritima* and macroalgae species. In addition, the worker must be able to collect single SAV shoots, use quadrats to sample (0.25 m^{-2} and 1 m^{-2}), operate a 22 cm diameter x 10 cm depth core device to remove the seagrass sample and a 10.4 cm diameter by 10 cm depth core device to remove the seed bank and sediment samples.

5.1.2 Laboratory:

A worker must be able to process and dry the aboveground and belowground portions of the seagrass, all macroalgal samples, and all epiphytes removed from the seagrass. Processing includes drying and weighing all samples. In addition, the worker must be able to remove epiphytes from seagrass blades and differentiate macroalgae from seagrass tissue. The worker must be able to process all seed bank samples including sieving, density counts, and tetrazolium staining for viability. Finally, the worker must be able to process all water quality samples for total suspended solids, chlorophyll *a*, and nutrients (NO_2 + NO_3. NH_4+NH_3, OPO_4) and sediment samples for percent organic content and pore-water nutrient analysis (NO_2 + NO_3. NH_4+NH_3, OPO_4).

6.0 PROBLEM DEFINITION/BACKGROUND:

6.1 Problem Definition:

In the Barnegat Bay-Little Egg Harbor Estuary (BB-LEH) *Z. marina* populations have declined significantly since 2004 with record lows recorded in 2010 (Lathrop and Haag, 2011). Restoration attempts in BB-LEH have increased in response to continued declines (Bologna and Sinnema, 2012); however, efforts have not been undertaken at the scale necessary to significantly increase *Z. marina* populations. In order to increase restoration efficiency, effectiveness, and success, a better understanding of bed resiliency to perturbations, as well as loss and recovery processes within established seagrass beds is required (Orth et al., 2006).

Ecological models are useful tools in quantitative analysis of complex ecosystems such as submerged aquatic vegetation (SAV) beds. The goal of this project is to refine and apply a *Z. marina* model developed by PI Jarvis to quantify SAV resiliency to perturbations through modeling loss and recovery processes within established SAV beds in NJ Coastal Bays. In order to apply the model in NJ Coastal Bays, collection of both abiotic (water column and sediment) and biotic data (density, biomass, seed bank characteristics) is necessary to refine and calibrate the model to local conditions.

Our specific objectives are to:

1. Refine and calibrate the model developed by PI Jarvis to project the response of *Z. marina* beds in NJ coastal bays to stressful environmental conditions.
2. Use the calibrated model to quantify the effects of environmental stress on *Z. marina* population maintenance and reestablishment in NJ coastal bays.
3. Determine suitability of three *Z. marina* sites along a nutrient loading gradient for restoration using the model and NJDEP comprehensive water quality data.

4. Quantify possible effects of reduced nutrient loading rates (i.e. present day, less 10%, less 30%) on seagrass survival and reestablishment in New Jersey coastal bays.

By focusing on interactions between SAV and their surrounding environment, the proposed model may be developed into a tool to select suitable SAV restoration sites in New Jersey coastal bays as well as to quantify impacts of proposed water quality changes (i.e. reduction of watershed nutrient loading) on SAV abundance and persistence. The final model will be available for coastal residents as well as for managers at local, state, and federal levels

6.2 Background:

Seagrass populations have declined globally over the last several decades (Orth et al., 2006; Waycott et al., 2009; Short et al., 2011). Losses have been linked to coastal development (Short and Wyllie-Echeverria, 1996), eutrophication (Burkholder et al., 2007), and climate change (Short and Neckles, 1999). Within the Mid-Atlantic and Northeastern United States, large-scale loss of the dominant seagrass species, *Z. marina*, has been attributed to chronic declines in water quality compounded by extreme episodic stresses from short term events such as tropical storms or high water temperatures (Bintz et al., 2003; Moore and Jarvis, 2008; Costello and Kenworthy, 2011). Loss of SAV from coastal habitats has significant impacts throughout the entire surrounding ecosystem due to the numerous ecosystem services provided by these populations (McGlathery et al., 2007; de Boer, 2007; Hasegawa, 2008). These include providing nursery and essential fish habitat and serving as a direct connection between benthic and pelagic habitats (Orth et al., 2006).

Since the mid-1970s, accelerated development in the BB-LEH watershed and atmospheric deposition from the overlying airshed has contributed greatly to the increasing eutrophication of the estuary (Kennish et al., 2007; Velinsky et al., 2011). As a result, benthic macroalgal blooms have increased leading to significant declines in *Z. marina* density and cover (Kennish et al., 2008; Lathrop and Haag, 2011). In response, the protection of the dominant seagrass species in NJ Coastal Bays, *Z. marina*, has been made a priority by both state (New Jersey Department of Environmental Protection) and federal agencies (Barnegat Bay Partnership, NJ Sea Grant). Despite these efforts, *Z. marina* populations in BB-LEH have continued to decline (Kennish et al., 2007; Lathrop and Haag, 2011). Restoration attempts in BB-LEH have increased in response to continued declines (Bolonga and Sinnema, 2012); however, efforts have not been undertaken at the scale necessary to significantly increase *Z. marina* populations. In order to increase restoration efficiency, effectiveness, and success, a better understanding of bed resiliency to perturbations, as well as loss and recovery processes within established seagrass beds is required (Orth et al., 2006).

Ecological models are useful tools in quantitative analysis of complex ecosystems such as SAV beds. Through models, the response of *Z. marina* to stressful environmental conditions such as low light, high nutrients, and high temperatures has been quantified under a variety of situations (Bach, 1993; Aveytua-Alcázar et al., 2008). While these models provide insight into the effects of environmental stressors on *Z. marina* production, the capacity to accurately model population responses to stressful conditions is limited by focusing solely on vegetative reproduction and ignoring sexual reproduction (van Lent, 1995). Recent research has shown that sexual reproduction plays a significant role in *Z. marina* bed recovery from large scale declines (Plus et al.,

2003; Greve et al., 2005); therefore, a key component of the bed loss and recovery dynamic may be missing from *Z. marina* production models when sexual reproduction is excluded. The *Z. marina* model developed by Jarvis (2009) is especially suited for application in areas marked by significant decline (i.e., NJ Coastal Bays) because it includes seed production, seed-bank density, seed viability, and germination. Information gathered from model simulations will provide a new approach for managers to assess areas for restoration or preservation of *Z. marina* in NJ Coastal Bays and support the Barnegat Bay Partnership Strategic Plan Habitat Priority to protect, restore and enhance habitats, especially submerged aquatic vegetation.

7.0 PROJECT DESCRIPTION:

This project will use direct abiotic and biotic measurements from two sites in BB-LEH (Barrel Island – BI N 39°33'22", W74°16'22"; Seaside Park - SS N39°47'53, W 74°05'31") to refine and apply the *Z. marina* model developed by PI Jarvis to quantify loss and recovery processes within established SAV beds in the NJ Coastal Bays region (Figure 1). Abiotic and biotic data will be collected independently at both sites from August 2012 – December 2013 (Table 1). Data collected from Barrel Island will be used to calibrate the model while data from Seaside Park will be used to verify the model.

Once verified, a second round of simulations will be run on the BI and SS sites to quantify possible effects of reduced nutrient loading rates (i.e. present day, less 10%, less 30%) on seagrass survival and reestablishment. The model will then be forced through simulations to determine the restoration suitability of three additional *Z. marina* sites selected along a nutrient loading gradient using secondary water quality data from NJDEP. For these simulations the model will be run with water quality data collected as part of the NJDEP comprehensive ambient water quality monitoring network in the Barnegat Bay from three sites along a gradient of declining nutrient loading to determine the suitability of potential restoration sites (Northern Barnegat NB; N 40.040556; W -74.054722; Waretown WT; N 39.791111; W -74.181944; and BI; Figure 2). For all additional model scenarios parameter values will be left unchanged for verification, but forcing functions will be updated to reflect site data.

Once the modeling scenarios have been completed the model will be published online to be used as a tool for *Z. marina* restoration site selection in BB-LEH. A public meeting for local residents and coastal resource managers/policy makers will serve to educate the community about SAV in nearby coastal waters and to explain the model, the purpose of the model, and the initial results. Once the final report is accepted by the Barnegat Bay Partnership the model will then be published online for public use.

7.1 Abiotic Data Collection Approach:

7.1.1 Sediment Characterization Analyses:

Sediment samples will be directly collected bi-monthly to monthly throughout the sampling period from BI and SS. The samples will be collected using a coring device and analyzed in the laboratory for percent organic content as well as for pore-water ammonium plus ammonia (NH_4 + NH_3), nitrite plus nitrate (NO_2 + NO_3), and orthophosphate phosphate (OPO_4).

7.1.2 Water Quality Parameters:

Direct water quality measurements will include in situ analyses of water in NJ coastal bays during the 2012-2013 study period. Water quality parameters (temperature, salinity, chlorophyll *a* and turbidity) will be measured at both sites using an automated YSI 6600 datasonde unit every 15 minutes throughout the entire sampling period at a uniform depth (~10 cm) above the sediment-water interface (Figure 3). Turbidity readings will not be collected by a certified laboratory, however quality control protocols to ensure data quality are maintained will be followed (Appendix 1.2.3). Additional water samples from both sites will be collected monthly and analyzed by staff at the Richard Stockton College of New Jersey for ammonium + ammonia ($NH_4 + NH_3$), nitrite plus nitrate ($NO_2 + NO_3$), and orthophosphate phosphate (OPO_4). The samples will be analyzed following the methods designated in standard colorimetric methods (Table 2; Appendix 1).

Secondary water quality data collected by the New Jersey Department of Environmental Protection from Barnegat Bay estuarine water quality monitoring sites and processed by the Leeds Point NJDEP certified laboratory will be used as data for forcing functions in the potential restoration site model scenarios (Figure 2). Parameters will include chlorophyll a, turbidity, and water column nutrient concentrations for ammonium + ammonia (NH_4+NH_3), nitrite plus nitrate ($NO_2 + NO_3$), and orthophosphate phosphate (OPO_4). In addition, water quality data collected and processed by the staff at MSEFS prior to the start of this project will be used as data for initial model calibration and validation. Parameters will include chlorophyll a, turbidity, and water column nutrient concentrations for ammonium + ammonia (NH_4+NH_3), nitrite plus nitrate ($NO_2 + NO_3$), and orthophosphate phosphate (OPO_4) and will be analyzed in accordance with all methods and standards described in Appendix 1.

7.2 Biotic Assessments:

Direct measurements will be made for SAV (*Z. marina* above and below ground biomass and seed bank), macroalgae, and epiphytes. SAV samples will be collected using a coring device and analyzed in the laboratory for vegetative and flowering shoot density and biomass. Samples will also be analyzed for total seed production, seed bank density, and seed bank viability. Epiphyte and macroalgal biomass will also be quantified in the lab following collection from both sampling sites (Appendix 1). In addition, biotic collected and processed by the staff at MSEFS prior to the start of this project will be used as data for initial model calibration and validation. Parameters will include SAV (*Z. marina* above and below ground biomass and seed bank), macroalgae, and epiphytes. SAV samples will be collected using a coring device and analyzed in the laboratory for vegetative and flowering shoot density and biomass. Samples will also be analyzed for total seed production, seed bank density, and seed bank viability. Epiphyte and macroalgal biomass will also be quantified in the lab following collection from both sampling sites (Appendix 1).

8.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA:

This project will use direct abiotic and biotic measurements from Barrel Island and Seaside Park to refine and apply the *Z. marina* model developed by consultant Jarvis to quantify loss and recovery processes within established SAV beds in the NJ Coastal Bays region. Abiotic and biotic data will be collected independently at both sites from August 2012 – December 2013 (Table 1).

Data collected from Barrel Island will be used to calibrate the model while data from Seaside Park will be used to verify the model. Once verified, a second round of simulations will be run on the BI and SS sites to quantify possible effects of reduced nutrient loading rates on seagrass survival and reestablishment. The model will then be forced through simulations to determine the restoration suitability of three additional *Z. marina* sites selected along a nutrient loading gradient using secondary water quality data from NJDEP. For all additional model scenarios parameter values will be left unchanged for verification, but forcing functions will be updated to reflect site data.

8.1 Accuracy and Precision:

The accuracy and precision of the abiotic and biotic data will vary with methodology (Table 2). In general, for all sites, replicate samples will be analyzed in triplicate and precision determined within stated parameters. Accuracy will be expressed as concordance of the measurement of a check standard or certified reference material (where available) for each run (within stated parameters). Acceptance criteria are found in Tables2 and 5. Corrections for data found outside of the acceptance criteria is found in Tables 3 and 5.

8.1.1 Sediment Characterization Parameters:

Sediment percent organic content measurement precision will be quantified by using the same method to make repeated measurements of the same sample. Sediment pore water ammonium plus ammonia ($NH_4 + NH_3$), nitrite plus nitrate ($NO_2 + NO_3$), and orthophosphate phosphate (OPO_4) sample precision will use the same instruments to make replicate analyses of the same sample (Table 2).

8.1.2 Water Quality Parameters:

To maintain precision for water quality measurements in the field two data sondes will collect data simultaneously for 30 minutes during datasonde switch-outs. This will ensure that a minimum of one measurement is overlapping and allow direct comparisons between data sondes. To increase precision of measurements of total available light data the same LI-190SA sensor will record light data during the entire sampling period. To increase precision when using the Secchi disk to make water clarity measurements, a specific crew member will be designated as the Secchi depth taker; taking all measurements from the shady side of the boat; and not wearing sunglasses when taking Secchi readings. In addition, for water quality laboratory analysis three separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures will be collected at each site for chlorophyll *a* and water column nutrients ($NH_4 + NH_3$, $NO_2 + NO_3$, and OPO_4) and run on the same analytical instruments (Tables 4 and 5). To ensure a high precision between water column total suspended solids measurements the same method will be used to make replicate measurements of the same sample (Table 3).

8.1.3 Biotic Parameters:

The precision of SAV, macroalgae, and epiphyte biomass measurements will be determined by using the same analytical scale to make repeated measurements (dry weight) on the same sample. For seed bank density and viability all samples will be processed with the same analytical instruments (Table 3).

8.1.4 Model Parameters:

The precision of all abiotic and biotic measurements incorporated into the model will be determined as stated in sections 8.1.1 – 8.1.3. Any additional secondary data incorporated into the model will only be incorporated if the standards described in the previous sections were met during data collection. Precision of model runs will be determined by using the same modeling software on the same computer for all modeling runs in all scenarios.

8.2 Bias:

The determination of any bias in the abiotic and biotic data will vary with methodology. Acceptable bias and corrections for data found outside the acceptance criteria is found in Tables 3 and 5.

8.2.1 Sediment Characterization Parameters:

Bias in sediment percent organic content measurements will be quantified by using the same method to make repeated measurements of the same sample. Sediment pore water ammonium + ammonia (NH_4 +NH3) and nitrite plus nitrate (NO_2 + NO_3) bias will be determined by analyzing a set of triplicate standards, reagent blanks and samples in triplicate and one set of samples in triplicate with added surrogate (to determine matrix effects) are run per analysis at 630 nm and 540 nm respectively. Sediment orthophosphate (OPO_4) samples will be run in triplicate with a set of triplicate standards including reagent blanks (0-25 µmol) at 880 nm.

8.2.2 Water Quality Parameters:

Bias in water quality measurements by all datasondes will be quantified through pre and post deployment calibrations according to YSI specifications (Appendix 1). Post-deployment, calibration checks of the Y.S.I. 6600 datasondes will be performed with standards of known measure to verify that the sensors were operating correctly during deployment (Table 3). In addition all datasondes will be compared to pre-deployment, mid-deployment (with a second instrument) and retrieval results. The QC checks should not require more than slight adjustments to bring the instrument into agreement. Failed calibration checks will initiate a thorough inspection of the unit for obvious sign of malfunction (e.g., loose connections, damaged probes, power source, fouling on DO membrane, etc.). After any maintenance to correct problems, the unit will be re-calibrated with documentation on the appropriate field data form. If the unit will calibrate within the guidelines, water column measurements can be continued. If one or more parameters remain suspect, the nature of the problem will be fully documented on the field form, and the situation will be reported to the Project QA Manager for resolution. If this situation occurs a backup instrument will be made available. Erroneous measurements and/or poor diagnostic values will warrant further scrutiny of the data collected and data outside of the accepted range for each probe will be flagged and removed prior to data analysis (Table 5; Section 12.2.2,Small et al. 2013).

To reduce bias with total available light data all sensors will be calibrated every two years and the datalogger will be calibrated to the LI-190SA sensor using company standards prior to deployment. All sensors will be wiped clean on all sampling dates. Data will be downloaded every 2 weeks and visually reviewed post-deployment. Erroneous measurements and/or poor diagnostic values will warrant further scrutiny of the data collected and data outside of the accepted range for each probe will be flagged and removed prior to data analysis (Table 5; Small et al. 2013). No field

calibration procedures are required for the Secchi disk. The disk must be clean, free of algae or other debris, and all surfaces white in color. All surfaces on the disk must be in good condition such that they are clearly visible. All project data including any data rejected as outliers, laboratory results flagged for not meeting quality control acceptance criteria or any other scientifically-based reason, even if the data is not used in the final research analysis will be retained and made available for review.

Bias of chlorophyll *a* samples will be determined by calibrating the fluorometer to a sample prepared from the primary dilution standard solution. In addition, the calibration standards will be verified with the analysis of a quality control sample (QCS). The QCS will be made from dilutions of a stock chlorophyll *a* standard of a different lot number from that used to prepare calibration solutions. If the determined value is not within the confidence limits established by project data quality objectives (Table 4), then the determinative step of this method is unacceptable. The source of the problem must be identified and corrected before continuing analyses.

Bias in water column nutrients will follow the same protocols defined for sediment nutrients in section 8.2.1. Bias in TSS samples will be determined by comparing replicate TSS samples from each site to one another and to a sample blank. To reduce bias in TSS samples non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter will be excluded from the sample. Acceptable bias in all measurements is found in Table 3. Corrections for data found outside of the acceptance criteria is found in Table 5.

8.2.3 Biotic Parameters:

Bias of SAV, macroalgae, and epiphyte biomass measurements as well as SAV seed bank density and viability will be determined by having a member of the laboratory crew, who did not process the original sample, visually determine if the samples were processed according to acceptable methods before the samples are placed in the drying oven. To reduce bias SAV and macroalgae biomass samples will be rinsed thoroughly with deionized water to remove any sediment or epiphytic algae prior to measurement. Epiphyte samples will be rinsed briefly to remove all sediments before processing. To determine bias in sieved seed bank density measurements a second laboratory crew member will look through all sieved materials to determine if any seeds have been missed. Bias in viability measurements will be determined by having a minimum of two laboratory crew members visually analyze seed embryos following tetrazolium staining.

8.2.4 Model Parameters:

The initial model simulation period will run 1.5 years (May 1, 2012 through October 31, 2013) with a time step (dt) of 0.125 days. The model will be calibrated to water column, sediment, and *Z. marina* data collected at bi-weekly to monthly intervals from April to October in 2012 and 2013 in site BI. Due to the lack of information on seedling production, parameter estimates for all seedling variables except density to carbon ratios will be assumed to be identical to vegetative shoots (Bintz and Nixon, 2001). Once calibrated, the model will be independently verified using data from a site located 31 km up estuary. Parameter values will be left unchanged for verification, but forcing functions will be updated to reflect SS data. Comparisons will be made between

computed and observed values on a monthly average basis to determine any bias. Acceptance criteria for both forcing functions and state variables are found in Table 3.

8.3 Representativeness:

8.3.1 Spatial Representativeness:

Barrel Island was selected to represent *Z. marina* beds in Little Egg Harbor and Seaside Park was selected to represent Barnegat Bay populations (Figure 1). These sites were also selected due to their persistent *Z. marina* populations (Lathrop and Haag, 2011). To ensure that all abiotic and biotic samples represented the spatial complexity of each site the maximum number of replicate samples will be collected for each parameter (Tables 3 and 5). The maximum number of samples for each parameter was determined based on cost, time to process samples, and damage to the ecosystem.

8.3.2 Temporal Representativeness:

To represent the temporal changes at both sampling sites, all abiotic parameters will be measured bi-weekly during the 2012 SAV growing season (May – October) and monthly during the 2013 SAV growing season. The reduction in sampling in 2013 reflects the reduced data requirements of the model during the second calibration year (Jarvis 2009). In addition, all abiotic samples will be measured monthly from November to March throughout the entire sampling period. Biotic measurements will be made bi-weekly during the 2012 SAV growing season and monthly during the 2013 growing season. The reduction in sampling in 2013 reflects the reduced data requirements of the model during the second calibration year (Jarvis 2009). Except for seed bank, no biotic samples will be collected from November to April due to prohibitive field conditions. Seed bank samples will be collected bi-monthly to monthly throughout the entire sampling period. Samples will be collected over two years to account for any inter-annual variation within each site (Table 1).

8.3.3 Model Representativeness:

Since the model is not spatially explicit it was determined that the model needed to represent BB-LEH seagrass beds found in both minimally developed (BI) and extremely developed (SS) regions of the system. A model that is calibrated and verified under both conditions would best represent the responses of BB-LEH seagrass populations to any changing environmental conditions forced through the model.

8.4 Comparability:

All abiotic and biotic sample collection and handling methods, sample preparation, and analytical procedures will follow methodologies in which the quantitative output can be directly compared to published literature values and existing NJDEP and EPA data. Water column and sediment nutrient samples will be processed using an analogous method compared to NJDEP. The discrepancy with nitrate analysis is due to a lack of access to an auto analyzer with a cadmium column (Table 4; Ringuet et al., 2011). Seagrass, macroalgae, and epiphyte sampling techniques follow those outlined in Coles et al. (2001) which is the standard for global seagrass methodology (Tables 3 and 5). In addition, biotic data will be reported in a format that can also be directly compared to Kennish et al. (2008).

8.5 Completeness:

Due to the high computational requirements of the *Z. marina* ecological model developed by PI Jarvis the completeness goal for all samples collected is 100%. Minimum requirements for abiotic and biotic sample collection compliance are 80% of samples per parameter per sampling date. If more than 20% of samples are not collected or lost during processing then a second sampling trip for that time period will occur to collect the missing data if feasible (Table 2).

8.6 Sensitivity:

8.6.1 Abiotic and Biotic Parameters:

Sensitivity of all abiotic and biotic measurements are defined in Table 6. The minimum level of detection varies with each parameter and method used (Table 6). Measurements for all parameters are within the expected range of concentrations for coastal estuarine systems as they apply to SAV ecosystems.

8.6.2 Model Parameters:

The sensitivity of base model conditions to all parameter estimates and forcing functions will be analyzed by sequentially varying values by ± 5, 10, and 20 %. The average percent change in all state variables between the base model and sensitivity simulations will then be calculated and tests that resulted in > 10 % change in state variable concentrations were considered to have the greatest impact on model results.

9.0 NON-DIRECT MEASUREMENTS (SECONDARY DATA):

Secondary water-quality data for use in the model simulations will be collected by the NJDEP as part of the annual state wide water quality monitoring program. All sample collection, processing and analyses will be conducted according to the New Jersey Department of Environmental Protection Division of Water Monitoring and Standards Bureau of Water Quality Standards and Assessment 2012 Integrated Water Quality Monitoring and Assessment Methods (http://www.state.nj.us/dep/wms/bwqsa/2012_final_methods_doc_with_response_to_comments.pdf). Analytical methods that were used are shown in Table 4. The rationale for selecting the NJDEP water-quality-monitoring data is that these data provide an extremely detailed analysis of water quality in NJ Coastal Bays, which is not available from any other source. In particular the data provided at both sites contains water column nutrient data as well as measurements recorded every 15 minutes which is necessary to due to the high data requirements of the model's forcing functions. In addition, the sites in Figure 2 were selected due to their proximity to areas where seagrass declines have been documented (Lathrop and Haag, 2011).

Secondary water-quality and biotic data for use in the model calibration and validation will be collected by the Richard Stockton College MSEFS staff as part of their existing SAV monitoring program at BI and SS. All sample collection, processing, and analysis will be conducted according to the methods and standards outlined in Appendix 1. The rational for using data collected prior to this project to help calibrate the model is that these data provide a necessary ecological record of abiotic (temperature, salinity, chlorophyll *a,* turbidity, Secchi depth, nutrients) and biotic (seagrass, macroalgae, epiphyte, and seed bank density and viability) data that can be used to more accurately

model Barnegat Bay seagrass bed response to environmental conditions. In particular the data provided at both sites contains water column nutrient data as well as measurements recorded every 15 minutes which is necessary to due to the high data requirements of the model's forcing functions.

If additional sources are identified during the course of the project, those data will be subject to same review of methods and values that the NJDEP data received. The sources of secondary data gathered during this investigation will be identified in all project deliverables. No data hierarchy will be administered; all water-quality data that meet the data-quality objectives will receive equal consideration.

10.0 FIELD MONITORING REQUIREMENTS:

10.1 Monitoring Process Design:

The field teams will collect abiotic and biotic response data at BI and SS within a 100 m x 50 m sampling area marked with PVC poles on each corner. Both sites will be sampled bi-weekly to monthly from August – October 2012 and from May – October 2013 for both abiotic and biotic measurements (Table 1). Seed bank measurements and abiotic measurements will be sampled once a month from November 2012 to March 2013 and again from November to December 2013. The crew will locate the sampling stations by use of on-board Global Positioning Satellite System (GPS). At the two sites, all sampling crews will uniformly collect samples following established sampling protocols and methods as outlined in this document. Field data samples include (these will be discussed in greater detail in following sections):

- Water quality parameters (temperature, salinity, pH, light attenuation, chlorophyll *a*, TSS, NO_2 + NO_3, NH_4+ NH_3, and OPO_4)
- Sediment Parameters (Percent organic content, Pore-water NO_2 + NO_3, NH_4+ NH_3, and OPO_4)
- Seagrass (vegetative + reproductive shoot density, biomass, number seeds per flowering shoot)
- Seed bank (density, viability)
- Epiphytes (biomass)
- Macroalgae (biomass)

Samples collected from the field will be taken to the Richard Stockton College for storage and analysis.

10.2 Monitoring Methods:

10.2.1 Water Quality Sampling Methods:

Two sites (100 m x 50 m in area) in the Barnegat Bay-Little Egg Harbor Estuary, NJ (BI 39°33'22" N, 74°16'22" W; SS 39°47'53 N, 74°05'31" W) were selected for characterization based on historical seagrass cover and the development of the surrounding area (Kennish et al., 2008; Figure 1). Bottom water temperature (°C), salinity, chlorophyll a, (µg l^{-1}), and turbidity (NTU) will

be recorded at each site every 15 minutes during ice free periods from June 2012 to October 2013 with a Yellow Spring Instruments, Inc. model 6600 sonde deployed 4 cm above the sediment surface at both sites. Data sondes will be housed in anti-fouling PVC pipes per NERR set-up protocols. The PVC deployment pipes will be attached to one 4" round PVC pole that will be established at each site (Figure 3). All data sondes will be managed according to the National Estuarine Research Reserve (NERR) Central Data Management Office (CDMO) protocols for the calibration, deployment, and QA/QC of collected data developed for the NERR System-wide Monitoring Program (Appendix 1.2; Small et al. 2013). Stockton College has received QAPP specific laboratory approval for the *Barnegat Bay Ambient Water Monitoring Network* for the YSI 6000 datasondes (Appendix 2, DO, pH, specific conductance and temperature). Certifications for turbidity readings were not attained, however quality control protocols to ensure data quality are maintained will be followed (Appendix 1.2.3).Total available photosynthetically active radiation (PAR μE m^{-2} s^{-1}) will also be recorded every 15 minutes throughout the sampling period with a LI-COR, Inc. sensor (LI-190SA). LI-COR sensors and data loggers and will also be housed on the PVC pipes. Autonomously recorded data will be retrieved upon returning to port.

During each site visit, a second YSI 650 data sonde will be deployed following the same protocol as the 6600. This will serve as a bi-weekly QC check for all data sonde data. Secchi depth will be determined by using a 20-cm diameter white Secchi disc. The disc will be lowered to the depth, at which it can no longer be discerned, and then it will be slowly retrieved. Secchi depth will be reported in cm. In addition, three 500 mL water samples (amber bottles) and three 1000 mL water samples (clear bottles) will be collected by hand and refrigerated stored at 4 °C for less than 24 hours before analysis. All collection bottles will be rinsed with ambient surface water 3x prior to sampling. Filtered replicate samples (n = 3) will be analyzed for chlorophyll *a* (EPA 445), total suspended solids (USGS I-3765-85), and water column nutrients (NH_4+ NH_3 Sheldon and Weibe, 1997; NO_3 + NO_2 and OPO_4 Ringuet et al. 2011) using methods defined in Table 4 (see section 11.1.1).

10.2.2 Sediment Sampling Methods:

At both sites, sediment cores will be collected to quantify organic content and sediment pore water nutrients monthly. Nine clear acrylic cores (10.4 cm diameter by 10 cm depth) will be collected at each site, divided into three 2 cm horizontal sections (0-2 cm, 2-4 cm, and 4-6 cm), and placed in a plastic bag in a cooler on ice until taken back to the lab for processing. Nine additional clear acrylic cores (10.4 cm diameter by 10 cm depth) will be collected at each site and kept intact in a cooler with ice and site sea water until taken back to the lab for processing (See Section 11.1.2).

10.2.3 Biotic Sampling Methods:

Five *Z. marina* biomass cores (22 cm diameter, 10 cm depth) and five macroalgal biomass samples (0.25 m^2 quadrat) will be randomly collected monthly from both sites. Samples will be sieved (1.0 cm mesh box sieve) and washed clean of sediment in the field and all plant material will be immediately transported back to the lab in plastic bags for processing on ice (Short et al., 2001; Sidik et al., 2001). In addition, 15 individual *Z. marina* shoots will be randomly selected from each site and transported back to the lab in plastic bags to determine epiphyte biomass (Kendrick and Lavery, 2001). Seed bank density and viability will be quantified by collecting 15 sediment cores

(10.4 cm diameter by 10 cm depth) at each site. The cores will be divided into three 2 cm horizontal sections (0-2 cm, 2-4 cm, and 4-6 cm), and placed in a plastic bag in a cooler on ice until taken back to the lab for processing (McFarland and Shafer, 2011; See Section 11.1.3).

10.3 Field Quality Control (QC):

The diverse array of sampling and analytical requirements necessary in this investigation; therefore, sampling and QA procedures vary among the methods (Tables 3 and 5). If problems occur, such as lost, contaminated, mislabeled or improperly handled samples, these problems will be documented in the appropriate project record-keeping location (field or laboratory logs). If practical, replacement samples will be obtained. If differences between temporal or spatial characteristics of the replacement samples and the original samples have a bearing on calculations, modeling or other project activities or objectives, such differences will be noted in all subsequent documentation and products in which the replacement samples were used.

10.3.1 Field Calibrations:

To ensure that field measurements meet the accuracy goals, quality controls checks will be performed on a regular basis (bi-weekly to monthly) for all field equipment/instruments. When QC checks indicate instrument performance outside of acceptance criteria, the instrument will be calibrated (for those instruments that allow adjustments) against an appropriate standard to re-establish acceptable level of performance; the procedure will be documented on field data forms (Appendix 3). If the instrument cannot be re-calibrated, all associated data will be qualified accordingly.

Some instruments have fixed functions that cannot be adjusted under field condition (Li-COR total PAR sensors). In cases where these types of measurements fail the field-QC checks, the degree of variance will be documented in field records; if possible, the situation will be rectified by changing out the faulty equipment with a backup unit until the failed unit can be repaired. If no backup is available, depending on the relative importance of that particular measurement to overall success of the monitoring operation, the crew chief must decide whether to continue operations with compromised or deficient data or to suspend sampling until the situation is corrected. The field crew will contact the project QA Manager for guidance.

11.0 ANALYTICAL REQUIREMENTS:

Biotic samples will be analyzed at MSEFS. Biotic measurements will include:

- seagrass shoot density, aboveground and belowground biomass, vegetative + flowering shoot density, total number of seeds per flowering shoot
- seed bank density, viability
- epiphyte biomass
- macroalgal biomass

Primary water quality data used in this project will be collected and analyzed at the Richard Stockton College School of Natural Science and Mathematics laboratories (main campus). Water quality measurements will include

- chlorophyll *a*
- Total Suspended Solids
- Ammonium (NH_4+ NH_3)
- Nitrate + Nitrite (NO_2 + NO_3)
- Orthophosphate (OPO_4)

Secondary water quality data used in this project will be derived from the NJDEP marine water quality monitoring program. All in-state laboratory analyses have been conducted by the laboratories of NJDEP's Bureau of Marine Water Monitoring at Leed's Point. Only parameters for which the laboratory has certification from NJDEP's Office of Quality Assurance will be used. All of the information specified in this section is critical to the investigation, either as essential data for achieving the project objectives, or as quality-assurance parameters. None of the types of information mentioned are for information purposes only.

Sediment data used in this project will be collected and analyzed at MSEFS and the School of Natural Science and Mathematics laboratories (main campus). Sediment measurements will include:

- Percent organic matter (% LOI, MSEFS)
- Pore water ammonium + ammonia (NH_4 + NH_3, main campus)
- Pore water nitrate + nitrite (NO_2 + NO_3, main campus)
- Pore water orthophosphate (OPO_4, main campus)

11.1 Analytical Methods:

11.1.1 Water Column Nutrient Analysis:

During each site visit, three 500 mL water samples will be collected by hand, taken back to the lab on ice and refrigerated before analysis. Acid washed polyethylene collection bottles will be rinsed with ambient surface water 3x prior to sampling. Immediately after returning to the laboratory the water samples will be filtered through a 0.45 µm filer into pre-labeled sterile 50 mL polyethylene centrifuge tubes. The samples will then be frozen at -20 °C until analysis. During analysis replicate samples will be slowly defrosted by sitting at ambient lab temperatures and then analyzed for NH_4 + NH_3, NO_3 + NO_2, and OPO_4 by standard colorimetric methods (Zimmerman and Keefe, 1997; Zhang et al., 1997a; Zhang et al., 1997b, Sheldon and Wiebe, 1997) and adapted for a microplate reader (Ringuet et al., 2011; Table 4, Appendences 1.5-1.7). A Beckman-Coulter DTX 880 Multi-mode plate reader is used for the nitrate/nitrate analyses and a Shimadzu 1601 double beam UV/VIS spectrophotometer for the ammonium/ammonia and orthophosphate determinations. Certified reference material (CRM) from Sigma-Aldrich Scientific (TraceCERT), will be used in the assays to determine the accuracy of the ammonium/ammonia, nitrate/nitrate and orthophosphate methods (Table 5).

Determination of ammonium plus ammonia (NH_4 + NH_3) is accomplished using a colorimetric indophenol blue method [EPA 349] at 630 nm (Sheldon and Wiebe, 1997, Zhang et al., 1997b). This method has a working range of 0.5 µM-20 µM and uses a sample volume of 5 ml. For

each analysis a set of standards, a CRM, blanks, samples and one sample with added surrogate (to determine matrix effects) are run in triplicate.

Nitrate plus nitrite (NO3 +NO2) will be determined after enzymatic reduction using nitrate reductase (Patton and Kryskalla, 2011) and standard colorimetric assay [EPA Method 353.4] complexing with the azo dye N-(1-napthyl) ethylenediamine hydrochloride (NED) (Ringuet et al., 2011, Zhang et al., 1997a). This method utilizes nitrate reductase (AtNaR2) to reduce nitrate to nitrite and has been accepted as a comparable method to cadmium reduction of nitrate to nitrite in studies by the USGS National Water Quality Laboratory (Patton and Kryskalla, 2011). For each analysis a set of standards (0.5-10 µM), a CRM, blanks, samples and one sample with added surrogate (to determine matrix effects) are run in triplicate on a 96 well plate at 540 nm. The working range is 0.5-50 µM with a sample size of 100 µl.

Orthophosphate will be determined in a modification of a standard colorimetric assay based on a molybdenmum blue method [EPA 365.5] with absorbance quantified at 880 nm (Ringuet et al., 2011, Zimmerman and Keefe, 1997). For each analysis a set of standards (0.5-25 µM), a CRM, blanks, samples and one sample with added surrogate (to determine matrix effects) are run in triplicate at 880 nm. This method uses a sample volume of 200 µl and has a range of 0.5-25 µM

11.1.2 Water Column Chlorophyll Analysis:

In addition, during each site visit three 500 mL water samples will be collected by hand in amber bottles at an approximate depth of 0.5 m, taken back to the lab on ice, and refrigerated before analysis within 24 hours. All collection bottles will be rinsed with ambient surface water 3x prior to sampling. Water samples will also be filtered and analyzed for chlorophyll *a* using EPA method 445.0 (Arar and Collins, 1997; Table 8, Appendix 1). Chlorophyll in a measured volume of sample water will be concentrated by filtering the sample at low vacuum through a glass fiber filter. The pigments will be extracted from the phytoplankton in 90% acetone solution after the filters have been processed with a mechanical tissue grinder. Pigments will remain in the acetone solution for a minimum of 2 h, but no longer than 24 h, to ensure thorough extraction of the chlorophyll a. The filter slurry will then be centrifuged at 1000 g for 5 min to clarify the solution. An aliquot of the supernatant will then be transferred to a glass cuvette and fluorescence is measured with a Shimadzu RF-1501 Fluorescence Spectrophotometer before and after acidification to 0.003 N HCl with 0.1 N HCl. During sample processing one field duplicate and one laboratory reagent blank will be processed for each site per sampling date. Sensitivity calibration factors, which will be determined prior to analysis on solutions of pure chlorophyll *a* of known concentration using chlorophyll *a* from *Anacystis nidulans* algae, will then be used to calculate the concentration of uncorrected and corrected chlorophyll *a* in the water sample and will be reported as µg L^{-1} (Table 4, Appendix 1.3).

11.1.3 Water Column Total Suspended Solids Analysis:

During each site visit three 1000 mL water samples will be collected by hand in clear nalgene bottles, taken back to the lab on ice, and refrigerated before analysis within 24 hours. All collection bottles will be rinsed with ambient surface water 3x prior to sampling. A known volume of sample water will then be filtered through a pre-washed and weighed glass fiber filter at low

vacuum following protocols detailed in USGS I-3765-85 (Table 4, Appendix 1.4). Once filtering is complete, the filters will be dried at 103-105 °C for 24 hours and weighed. The drying process will be completed until a constant weight is obtained (weight loss < 0.5 mg). The concentration of total suspended solids in each water sample will be reported as mg L^{-1}.

11.1.4 Sediment Pore-water Nutrient Analysis:

At both sites, five sediment cores (10.4 cm diameter by 10 cm depth) will be collected to quantify sediment pore water nutrients monthly. Cores will be taken back to the lab and sectioned into 2 cm sections (0-2 cm, 2-4 cm, 4-6 cm) and each section will be divided into quarters. One quarter from each sub-section will be placed into a whirl-pak bag with 2M KCl and shaken for one hour. The sample will then be poured into a centrifuge tube and centrifuged for 6 minutes at 4000 RPM. Pore-water will be filtered through a 0.45 um Supor Acrodisk syringe filter into pre-labeled 2 oz whirlpak bag. All samples will be frozen until analysis using methods described in section 11.1.1 (Table 4, Appendices 1.8 - 1.10).

11.1.5 Sediment Total Organic Content Methods:

At both sites, nine sediment cores (10.4 cm diameter by 10 cm depth) will be collected to quantify sediment pore water nutrients bi-weekly to monthly and sectioned into 2 cm sections (0-2 cm, 2-4 cm, 4-6 cm). In the lab each sub-section will be cut in half and percent organic matter will be determined by drying the sediment sub-section at 50 °C until a constant dry weight is reached. Samples will then be weighed, combusted at 400 °C for eight hours, and weighed again. Percent organic matter will be calculated as the difference in weights (Schumacher revised 2002; Table 4, Appendix 1.11).

11.1.6 Seagrass Biomass Methods:

Seagrass will be removed from bag and placed in large plastic tray. The plants will be separated by species and into vegetative or flowering shoots. Shoots covered in epiphytes will be scraped with single edge razor blade held 90° to the leaf surface. The sample will then be rinsed deionized water. The total number of shoots (vegetative and flowering) and the total number of seeds per flowering shoot in the sample will be counted. The leaves will then be separated from the rhizome directly below the leaf sheath into aboveground and belowground biomass. The data sheet will be filled out with date, site, replicate, and species. The sample will be placed into the appropriate aluminum foil envelope and the weight recorded (different envelopes will be used for above and belowground biomass). All samples will be placed in an air circulating oven at 50°C for a minimum of 24 hours. Each sample will be weighed a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg) and all weights will be recorded on the appropriate data sheet. Biomass will be reported as g dry weight (DW) m^{-2}; (Duarte and Kirkman, 2001; Table 4, Appendix 1.12).

11.1.7 Macroalgal Biomass Methods:

All algae will be removed from sample bags placed in large plastic tray. The sample will then be separated by species and rinsed with distilled or deionized water. Species will be identified using guides and magnifying equipment. All data will be recorded on appropriate data sheets. Once the sample as been completely identified algae biomass will be placed into the appropriate aluminum foil envelope (by species) and the weight recorded. All samples will be placed in an air

circulating oven at 50°C for a minimum of 24 hours. Each sample will be weighed a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). Record all weights on the appropriate data sheet. Biomass will be recorded as g Dry Weight (g DW) m^{-2} (Sidik et al., 2001; Table 4, Appendix 1.13).

11.1.8 Epiphyte Biomass Methods:

The individual seagrass shoots will be removed from the sampling bag and placed in large plastic tray. The shoot will then be separated into individual leaves and rinsed with distilled or deionized water. Larger epiphytes will be removed from the leaves by hand or with forceps. Each individual leaf will be scraped single edge razor blade held 90° to the leaf surface. The entire length of each leaf will be scraped on both sides. All material scraped off of the leaves will be rinsed into pre-weighed aluminum pans. The total number of leaves will be counted and the leaf length and width will be measured for each leaf. All leaf data will be recorded on the data sheet along with date, site, replicate and sample wet weight. All samples will be placed in an air circulating oven at 50°C for a minimum of 24 hours. Each sample will be weighed a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). All weights will be recorded on the appropriate data sheet. Biomass will be recorded as g Dry Weight (g DW) m^{-2} (Kendrick and Lavery. 2001; Table 4, Appendix 1.14).

11.1.9 Seed Bank Methods:

A 0.5 cm sieve will be placed on top of an empty 5 gallon bucket. The seed bank sample bag will be emptied onto the sieve. All sediment will be rinsed through the sieve until only organic material remains. All contents remaining in the sieve will be carefully and systematically inspected visually. All seagrass seed will be removed and placed back into the sample bag with filtered seawater. Before rinsing sieve off a second member of the laboratory crew will observe the sieve to ensure all seeds have been collected. Seeds will be stored in aerated water at 18-24 °C.

The seed will be removed from the seed coat by cutting a small slit down the length of the seed with a single edge razor blade and then removing the seed with forceps. The seed embryo will be completely submersed in a pre-labeled cell in the 1% tetrazolium solution for 24-48 hours. The tray with the processed seeds will then be covered with aluminum foil and placed in the dark. After the soaking period the seeds will be removed from the tetrazolium solution and placed under a 10x magnification dissection microscope for analysis. At a minimum the cotyledon and the pumule need to be stained red. The total number of viable seeds will be recorded and viability will be presented as a percent of total seeds collected (McFarland and Shafter, 2010; Table 4, Appendix 1.15)

11.2 Model Description, Calibration and Validation:

11.2.1 Model Description:

An established perennial *Zostera marina* bed in the lower York River, Virginia USA was chosen as a basis for the production and reproduction model developed by Jarvis (2009; Jarvis et al. submitted). The base *Z. marina* productivity model was modified from the models of Madden and Kemp (1996), Buzzelli et al. (1999), and Cerco and Moore (2001) (Figure 4). This will be calibrated

using water quality, biological, and sediment data collected from site BI in the NJ Coastal Bays system.

Governing equations for *Z. marina* vegetative and seedling shoot biomass are balanced between gains through photosynthesis and losses due to mortality, respiration and translocation to roots and rhizomes (Table 7). Epiphytes are balanced similarly with the added loss of grazing but no loss due to translocation. Production terms for both epiphytes and *Z. marina* shoots are computed as the product of a temperature dependent maximum rate (*Pmax*) and a limiting factor:

$$PR = P\max^{*}\ MIN\left[PAR, DIN, DIP\right] \quad (1)$$

in which either nutrients (DIN, DIP) or light (PAR) is limiting (Madden and Kemp, 1996; Cerco and Moore, 2001). Maximum epiphyte production rates are currently taken from Buzzelli et al. (1999; Eq 2) and production rates for *Z. marina* were determined from Evans et al. (1986; Eq 3):

$$P\max_{epi} = 0.003 * T_w * \left[1.0 - \frac{(T_w - 25)}{20}\right] \quad (2)$$

$$P\max_{zm} = 0.0948 + 0.0309 * e^{\left(-0.5*\left(\frac{(T_w - T_{opt})}{3.2964}\right)^2\right)} \quad (3)$$

In both cases maximum production is related to ambient water temperatures (T_w) and optimum water temperatures (T_{opt}; Table 8). Maximum epiphyte production rates will be updated from data collected from NJ Coastal Bays as well as from literature values (Kennish unpublished data).

Zostera marina shoot and epiphyte production are limited by available light and nutrient concentrations. PAR is calculated similarly to Madden and Kemp (1996) where forced incident irradiance is reduced in successive stages. Initial light availability is reduced exponentially to depth (z) with a Beer Lambert equation (Kirk, 1983):

$$PAR_2 = PAR_1 * e^{(-K_d * z)} \quad (4)$$

Down-welling light attenuation coefficient (K_d) accounts for additive effects of chlorophyll *a*, TSS, and the water itself on light availability in the water column:

$$K_d = (0.054 * Chl^{0.667} + 0.0088 * Chl) + (0.0396 * TSS + 0.39) + 0.03 \quad (5)$$

(Madden and Kemp, 1996). Total light available to *Z. marina* leaves (PAR_1) is further attenuated by a simulated epiphyte layer based on the units of epiphyte biomass located on the leaf blade:

$$PAR_3 = PAR_2 * e^{(0.32 - (0.42 * 2.5 * C_{epi}))} \quad (6)$$

(Madden and Kemp, 1996). This simulated layer will be updated with information collected from NJ Coastal Bays. For both epiphytes and *Z. marina* light limitation of P_{max} values is calculated using Michaelis-Menten kinetics:

$$LTLIM = \frac{PAR}{(K_{PAR} + PAR)} \quad (7)$$

and a half saturation constant for light (Table 5; Madden and Kemp, 1996).

Nutrient limitation in epiphytes is computed similarly to light limitation with PAR substituted with water column nutrient concentrations (N_w) and the light half saturation constant with nutrient half saturation constants for epiphytes (Table 8). For *Zostera marina* multiple sources of nutrients (sediment and water column) are taken into account with a Monod-like function for nutrient limitation:

$$f(N) = \frac{(N_w + K^* * N_s)}{(K_{hw} + N_w + K^* * N_s)} \quad (8)$$

where N_w = water column nutrient concentrations (µmol l^{-1}) in the water column, Ns = sediment nutrient concentrations (µmol l^{-1}), K_{hw} = half saturation constant for nutrient uptake (µmol l^{-1}) by shoots (Madden and Kemp, 1999; Cerco and Moore, 2001), and:

$$K^* = \frac{K_{hw}}{K_{hs}} \quad (9)$$

where K_{hs} = half saturation constant for nutrient uptake (µmol m^{-3}) by roots (Madden and Kemp, 1999; Cerco and Moore, 2001).

Losses from epiphytes and *Z. marina* shoots are attributed to mortality (leaf sloughing in *Z. marina*) and respiration (Madden and Kemp, 1996; Buzzelli et al., 1999). *Zostera marina* had an additional loss term of translocation to the roots while epiphyte production was also lost to grazing (Buzzelli et al., 1999). Epiphyte mortality is a function of a density dependent mortality constant and the ratio of epiphyte and *Z. marina* shoot carbon:

$$M_{epi} = MR_{epi} * \frac{C_{epi}}{C_{zm}} \quad (10)$$

where MR_{epi} = the epiphyte mortality constant (Table 8; Buzzelli et al., 1999). *Zostera marina* mortality is a combination of a constant mortality term over time and a temperature dependent function:

$$M_{zms} = MR_{zms} + 0.0175 - 0.125 * \cos\left(\frac{2.0 * \pi * JD}{365}\right) * \left(\frac{(T_w - 23)}{10}\right) * C_{zms} \quad (11)$$

where MR_{zms} = constant mortality rate over time (day^{-1}, Table 5) and JD = Julian Day (Buzzelli et al., 1999).

Epiphyte respiration is temperature dependent where KtB_{epi} = constant epiphyte respiration rate and BMR_{epi} = epiphyte basal respiration rate:

$$R_{epi} = C_{zms} * BMR_{epi} * e^{(K_t B_{epi} * (T_w - T_{opt}))} \quad (12)$$

(Buzzelli et al., 1999). *Zostera marina* respiration, related to daily production via temperature, is held at 0 and only increased when water temperatures are greater than 14 °C (Nejrup et al., 2008):

$$R_{zms} = C_{zms} * PR_{zm} * \left[0.00317 * (T_w + 0.105) + e^{(0.135 * T_w - 10.1)}\right] \quad (13)$$

where R_{zms} = respiration and PR_{zm} = *Z. marina* production (day^{-1}; Buzzelli et al., 1999). In addition to mortality and respiration, production is also lost through translocation (T_d) to the roots at a constant rate (day^{-1}; Table 8). Epiphyte grazing rate is also held constant:

$$G_{epi} = C_{epi}^{\ 2} * K_{gepi} \quad (14)$$

where Gepi = epiphyte grazing and K_{gepi} = epiphyte grazing constant (day^{-1}; Buzzelli et al., 1999).

Zostera marina root and rhizome respiration was based on an Arrhenius relationship between respiration and water temperature (Bach, 1993). Respiration at an optimum temperature of 22 °C was scaled to daily temperatures with an Arrhenius constant (Θ_{zr}):

$$R_{zmr} = C_{zmr} * RR_{zmr} * \Theta^{(T_w - 22)} \quad (15)$$

(Buzzelli et al., 1999). Root mortality is computed as a constant fraction of biomass which increased after water temperatures became stressful (temperatures > 25 °C) to *Z. marina* in June of each model run (Setchell, 1929; Table 8).

11.2.2 Reproduction Model:

Seeds are produced via flowering shoots and a carbon to shoot density conversion ($Veg_{C:D}$) based on *Zostera marina* above ground biomass samples collected from site in Virginia in 2006 and 2007 (n = 560; Table 9). Flowering is limited by water temperature and Julian day and is currently based on the optimum conditions for *Z. marina* flowering observed in the Chesapeake Bay (Silberhorn et al., 1983). The optimum conditions for flowering in NJ Coastal Bays will be adjusted in the model based on data collected as part of this project. Currently, when water temperatures are < 21 °C and Julian Day is < 182 (July 1) then 10 % of total shoot density are converted to flowering shoots (Silberhorn et al., 1983). In addition, flowering is initiated only when vegetative

shoots dominate the above ground carbon pool as perennial *Z. marina* seedlings in the Mid-Atlantic region do not flower during their first year of growth (Silberhorn et al., 1983). Subsequent loss of flowering shoots is considered to be inherently included in the above ground biomass mortality term.

Seed-bank densities are derived from the product of total flowering shoot densities and the average number of seeds per reproductive shoot ($Seeds_{tot}$, Table 9). Once produced, seeds are then deposited into the sediment seed-bank. While in the seed-bank a portion of the seeds are removed via mortality and predation (Table 9; Fishman and Orth, 1996). The number of germinable seeds remaining in the seed-bank is further reduced by a loss of viability (V_{seeds}; Table 9). The seeds remain in the seed-bank until water temperatures decreased below 20 °C as this is when germination is initiated in Mid-Atlantic populations (Moore et al., 1993). Due to the transient nature of perennial *Z. marina* seed-banks those seeds that do not germinate by day 365 (December 31) are then lost from the system via seed mortality (Orth et al., 2000; Jarvis unpublished data). Germination of viable seeds ($Seeds_{via}$) is determined by a relationship between sediment organic content (SO, %) and seed burial depth (BD, cm) which is held constant at 3 cm:

$$Seeds_{germ} = \left(\frac{1}{\left(1 + e^{(-0.1432 + (1.1261 * BD) + (-1.3964 * SO))}\right)} \right) * Seeds_{via} \quad (16)$$

(Jarvis unpublished data). Once germinated, seedlings are then converted back to above and below ground carbon values (Table 9). When above ground *Zostera marina* biomass is < 0.44 g C m^{-2} then all above and below ground seedling biomass (T_{czmss}, T_{czmsr}, Table 9; Figure 4) is transferred to the vegetative shoot and root stocks. If vegetative shoot carbon is > 0.44 g C m^{-2} then seedling mortality is 100 %. This relationship is based on the inhibitory effect of shading by established vegetative above ground biomass on the survival of seedlings (Phillips et al., 1983; Robertson and Mann, 1984). Seedling above and below ground biomass is not tracked separately through the first year of growth due to a lack of information on all seedling parameters.

11.2.3 Model Scenarios:

Once the model is calibrated and verified, model scenarios will be run at both SS and BI with reductions in nutrient loading rates (present day, less 10%, less 30%) help quantify the possible impacts of water column nutrient reductions on BB-LEH seagrass survival and reestablishment. In addition, the model will be run with water quality data, specifically chlorophyll a, turbidity, and water column temperatures every 15 minutes, collected as part of the NJDEP comprehensive ambient water quality monitoring network in the Barnegat Bay from three sites along a gradient of declining nutrient loading to determine the suitability of potential restoration sites. For all modeling calibration, validation, and varying scenarios all water quality samples that are quantified to be below detection limits for any analytical method will be treated as the lowest reporting limit for that parameter in the model. The first potential restoration site, Northern Barnegat (NB; NJDEP site ID 01408167), will be in northern Barnegat Bay at Route 528 bridge at Mantoloking, NJ and will serve as the site with most significant amount of nutrient loading due to the high population density in the area and the lack of connectivity with the Atlantic Ocean. The second potential restoration site, near Waretown (WT; NJDEP site ID BB07a) will be located off the coast of

Waretown NJ near the Oyster Creek Channel and will serve as the intermediate site due to the high population density but greater connectivity with the Atlantic Ocean. The final potential restoration site will use the data collected as a part of this project at Barrel Island due to its location in a less populated area and greater connectivity with the Atlantic Ocean (Figure 2). The model scenarios will be run with water quality data from August 2012 through December 2013 using light and sediment nutrient data from Seaside for NB and WT model runs, BI will use light and sediment data recorded at that site. Initial modeling conditions will be set with 0 g *Z. marina* biomass, 0 g epiphyte biomass, and 0 g macroalgal biomass. Restoration scenarios will be based on seed broadcasting methodology and each site will be seeded with 50 seeds m^{-2}. Additional modeling scenarios will be run with 100, 250, and 500 seeds m^{-2} to determine if initial seed densities impact overall restoration success. All modeling runs will be run for a minimum of 3 years.

11.3 Analytical Quality Control:

The diverse array of analytical requirements necessary in this investigation; therefore, sampling and QA procedures vary among the methods (Table 5). If problems occur, such as lost, contaminated, mislabeled or improperly handled samples, these problems will be documented in the appropriate project record-keeping location (field or laboratory logs). If practical, replacement samples will be obtained. If differences between temporal or spatial characteristics of the replacement samples and the original samples have a bearing on calculations, modeling or other project activities or objectives, such differences will be noted in all subsequent documentation and products in which the replacement samples were used.

11.3.1 Laboratory Calibrations:

To ensure that analytical measurements meet the accuracy goals of established water quality projects, quality controls checks will be performed on a regular basis (bi-weekly to monthly or per analysis) for all instruments. When QC checks indicate instrument performance outside of acceptance criteria, the instrument will be calibrated (for those instruments that allow adjustments) against an appropriate standard to re-establish acceptable level of performance; the procedure will be documented on field data forms (Table 5). If changes, modifications, or additions are made to the QAPP, these items will be set in writing and sent to all of the project signatories for their review and approval prior to any and all changes being performed.

11.4 Instrument and Equipment Testing, Inspection, And Maintenance:

Several pieces of equipment that may be utilized to collect or analyze environmental data for long term sampling should have periodic maintenance and calibration verification performed by manufacturer's representatives or service consultants. These procedures must be documented by date and the signature of the person performing the inspection:

- Meters (Li-COR PAR sensors)-biannual verification of calibration coefficient by manufacturer
- Datasondes – pre/post deployment calibration; annual certification of YSI datasondes by NJDEP. Temperature sensing devices will be checked on a quarterly basis,
- Analytical balances-daily calibration using ASTM traceable calibration weights, annual verification by service representative

- Analytical Instrumentation (Shimadzu RF 1501 Fluorescence Spectrophotometer, Shimadzu UV/VIS-1601 spectrophotometer and Beckman-Coulter Multi-mode DTX-880 Plate Reader) - as per need based on, passing startup self-diagnostic routines.

All other sampling gear and laboratory instrumentation will be maintained in good repair as per manufacturer's recommendations or common sense to ensure proper function.

12.0 SAMPLE HANDLING AND CUSTODY REQUIREMENTS:

Prior to all sampling dates all materials will be cleaned and an inventory of materials necessary for the field and in the laboratory will be completed and signed off by the project manager (Appendix 3). Prior to deployment a YSI 6600 and a YSI 650 datasonde will be calibrated and the calibration sheets will be copied and scanned with the hard and electronic copies stored in the data binder and on the MSEFS servers. All sample bottles, acrylic cores, acrylic spacers, core caps, and centrifuge tubes will be acid washed using 10% HCl, and rinsed 3 times with DI water to remove any remaining acid residue, and dried before each sampling date. All glassware, plastic trays, metal spatulas, metal slicers, and forceps used for water filtration, biomass, or sediment TOC analysis will be rinsed with DI water 3 times before each sampling date. All filters will be processed according to the appropriate SOP and stored in the appropriate labeled container.

12.1 Field Data:

12.1.1 Field Data Forms:

The project field crews will record most of their raw field data on hard copy data sheets. The field crews will also use instrumentation with self-contained data-logging capabilities (e.g., datasondes and LI-COR data loggers) that store values in electronic format which can be downloaded later as electronic files. The template for field data sheets will be the one designed for previous bioassessment field data acquisitions in similar estuaries and all field data sheets will be printed on Rite in the Rain© paper (Appendix 3). The field sheet will record site information (date, time, site, weather) as well as water quality data measured on site with YSI 650 datasonde. All sample bags and cores collected on site will also be recorded. Data on overall site % cover for seagrass (*Z. marina and Ruppia maritima*) as well as macroalgae and bare sediment will also be recorded although that data will not be used in this project (Appendix 3). All samples will be handled according to the appropriate SOP standards (Table 3; Appendix 1). Upon return to the field station all field data will be scanned into a digital format and then entered into an electronic spreadsheet format and saved on MSEFS servers.

12.1.2 Site/Sample Identification:

Sample ID numbers, month of data collection, and data parameters will be marked with permanent ink on the outside of the bag. Sampling packets for each site will be prepared prior to the sampling date by placing a complete set of field data forms and pre-labeled sample bags and cores into the appropriate dry box or cooler. Bag IDs for all field samples will be recorded for each site in situ using write-in-the-rain paper and pencils. Samples within all bags will be individually processed in the laboratory according to the appropriate SOP standards (Appendix 1). Results are then entered into database management software (excel) by the laboratory researcher.

12.1.3 Continuous Data Download:

At each site the deployed 6600 YSI will be switched out with a clean calibrated YSI 6600 bi-weekly or monthly depending upon the time of year (more frequent switch outs will be made in the summer if necessary). Once the switch out is complete the previously deployed YSI 6600 datasonde will be taken back to the lab in a 5 gallon bucket where the data will be downloaded using YSI EcoWatch software.

In addition to switching out YSIs data from the LI-COR LI 1400 datalogger will be downloaded on a toughbook with LI-1400 software while at the site. Once the data is downloaded, the battery level, PAR sensor calibration numbers, and overall set-up in the LI 1400 will be checked. Any necessary changes will be made before leaving the site. If the LI 1400 logger or the LI-190 quantum sensor has problems that cannot be addressed in the field the unit(s) will be swapped and sent to the manufacturer for repair.

12.2 Laboratory Data:

12.2.1 Non-continuous samples:

Prior to field sampling all aluminum foil envelopes and aluminum pans will be pre-labeled with pencil, weighed, and their weights will be recorded on the appropriate data sheets all samples will be processed within the time frame determined by each SOP (Table 4, Appendix 1). All biomass samples (seagrass, macroalgae, and epiphytes) as well as sediment seed bank and TOC samples will be placed immediately under a grow light in a cold table with recirculating water maintained at 4 °C. All water samples will be filtered and then either frozen or placed in the drying oven depending upon SOP requirements (Appendix 1). All sediment pore-water samples will be processed according to SOP requirements then frozen (Appendix 1).

12.2.2 Continuous samples:

All data downloaded from the YSI 6600 datasondes will be processed with the NERRQAQC macros as per NERR SWMP data management protocols (Small et al. 2013). During primary QAQC, data are flagged if they are out of sensor range or missing. All remaining data are flagged as having passed initial QAQC checks. If the temperature sensor malfunctions, then ALL data will be inaccurate and need to be rejected. If the conductivity sensor malfunctions or is badly calibrated then salinity, specific conductivity, and depth will need to be rejected. The only negative data that will be included in the data file(s) are from the temperature, depth or turbidity probes.

Once the primary QAQC has been accomplished the data will be saved and a secondary QAQC will be completed following NERR SWMP data management protocols (Small et al., 2013). During secondary QAQC flags and codes are entered directly into the dataset with the use of the NERRQAQC macro to provide additional data documentation. These QAQC flags and codes thus become metadata for the data, making this documentation more accessible to the user and reducing the amount of written documentation that must accompany the dataset. Secondary flags include:

> Rejected data: marked as *-3*, Used during secondary QAQC to indicate a rejected value. No data values are to be removed from the dataset under any conditions except for the removal of any pre- and post-deployment records if desired.

Missing data: marked as *-2*, Inserted by the CDMO during primary and secondary QAQC where a value is missing (not collected).

Optional SWMP supported parameter: marked as *-1*, Inserted by the CDMO to indicate an optional parameter that was not collected.

Suspect data: marked as *1*, Used during secondary QAQC to indicate a suspect value.

Corrected data: marked as *5*, Used during secondary QAQC to indicate a value that has been corrected or changed

All data will then be stored on the MSEFS servers and backed up on an additional hard drive.

12.3 Data Transfer:

Field information recorded on hardcopy must be transferred to an electronic format. The hardcopy field data will be transcribed within a week of collection to the electronic format. The electronic format will be a template similar to the hardcopy form; the same data will be entered to the electronic file that was recorded in the field. The Project Quality Assurance Manager will conduct QA/QC checks on the transfer of hardcopy data to the electronic format.

All electronic files created during field activities must be periodically backed up on disks. All QAPP related data and all associated raw data records (including chain of custody records, records of calibrations and calibration checks) shall reside indefinitely at MSEFS. If the facility cannot provide the required storage, the data shall be transferred for archival storage to the Richard Stockton College School of Natural Science and Mathematics.

12.4 Sample Transfer:

When the field crew returns to the dock or staging area, they will turn both the field samples and respective data forms over to their land-based support team (or designated recipient) who will again verify that all samples are accounted for by comparing actual sample containers against the field data forms (Appendix 3). Upon inventorying samples, the crew will then temporarily store the samples under designated conditions until processing (Table 10). In the event that a sample is missing, the person checking in samples will record the sample as missing on the inventory sheet. The boat crew responsible for the collection of that sample will be informed so that they may check the sample storage areas on the vessel. It may be that conditions in the field prevented the collection of a particular sample; in that situation, the reasons should have been recorded as a comment on the field data form. If the sample is not recovered, the crew chief will make the decision for corrective action, whether simply to re-sample while still in the area or to schedule a make-up sampling on a later date.

Once a complete set of field collected samples are received by a processing laboratory, a master list will be compiled of all sets of samples and where they reside (e.g., freezer A, refrigerator B, or storage shed Z). The master list should be filed in the general area where the samples are held. When samples are released to an analyst, the transfer will be documented on the master list by initial and date; the quantity of sample released should be recorded. If the sample or portions of it are returned to the central storage area, this should also be logged on the master list. When the

laboratory uses internal tracking codes, they must be indexed to the original sample ID code (both site and sample identifiers), and all analytical results will be reported using this code.

13.0 TESTING, INSPECTION, MAINTENANCE, AND CALIBRATION REQUIREMENTS:

Appendix 3 shows a list of field equipment and instruments used in this project. Both field and laboratory equipment and instruments require routine calibration checks to verify that their performance is within acceptable quality standards. The following sections will discuss the procedures and frequency for the various instrument calibrations that are key components in the collection of accurate environmental data.

13.1 Instrument/Equipment Testing, Inspection and Maintenance:

Several pieces of equipment that may be utilized to collect or analyze environmental data for long term sampling will have periodic maintenance and calibration verification performed by manufacturer's representatives or service consultants. These procedures will be documented by date and the signature of the person performing the inspection:

- Meters (LI-COR 1400 meter; LI-COR 190SA quantum sensor)-biannual verification of calibration coefficient by manufacturer
- Analytical balances-annual verification by service representative along with daily calibration using ASTM traceable calibration weights
- Analytical Instrumentation-
    - Beckman-Coulter Multi-mode DTX-880 –based on run of calibration sample (CRM) for each run.
    - Shimadzu RF-1501 Fluorescence Spectrophotometer –based on run of internal diagnostics at startup each use
    - Shimadzu 1601 UV/Vis Spectrophotometer –based on run of internal diagnostics at startup each use.
    - YSI 6600 Datasonde – based on pre/post deployment calibrations
- All other sampling gear and laboratory instrumentation will be maintained in good repair as per manufacturer's recommendations or common sense to ensure proper function.

13.2 Instrument/Equipment Calibration and Frequency:

13.2.1 YSI Datasonde Calibrations:

An SOP has been developed and will be followed closely while maintaining, calibrating and operating data sondes and associated equipment (Appendix 1).

Datasondes (dataloggers) (Yellow Spring International)
Vendor and Address:
YSI Incorporated, World Headquarters
Yellow Springs, Ohio
800-897-4151
Fax: +1-937-767-1058
Email: environmental@ysi.com

YSI Model used: 6600 datasonde (model #s6600 and 650) paired with a 650MDS display ("handheld") (model #650MDS)

The methods utilized to calibrate the probes on the 6600 and 650 datasondes are found in Appendix 1 and will be use both in pre and post deployment of all datasondes. These calibration procedures were approved as part of the NJDEP Barnegat Bay water quality monitoring program (Appendix 1). Spare probes will be maintained and stored according to factory directions at MSEFS.

13.2.2 LI-COR 190 Quantum Sensor Calibrations:

All LI-190 quantum sensors are calibrated in the factory and require recalibration every two years. A copy of the calibration certificate will be kept on record and all sensors will be recalibrated bi-annually. To ensure that all data collected with the LI-190 sensors is accurate the correct multiplier will be entered into the LI-1400 data logger prior to deployment. The multiplier is provided by the factory of part of the calibration process and is recorded according to the sensor serial numbers in the data log book and electronically. Spare LI-190 quantum sensors and LI-1400 dataloggers will be maintained at MSEFS.

13.2.3 Laboratory Calibrations:

Several pieces of equipment that may be utilized to collect or analyze environmental data for long term sampling will have periodic calibration verification performed laboratory analysis. These procedures will be documented by date and the signature of the person performing the inspection:

- Analytical balances – calibrated to level surface prior to each use; analyst will check that scale is at 0 prior to weighing all samples; scale will be zeroed as necessary
- Analytical Instrumentation
    - Beckman-Coulter Multi-mode DTX-880 – will be calibrated when startup diagnostics fail or there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier
    - Shimadzu UV-Vis-1601 Spectrophotometer –will be calibrated when startup diagnostics fail or there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier
    - Shimadzu RF-1501 Fluorescence Spectrophotometer – fluorometer will be calibrated when startup diagnostics fail or there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier
    - YSI 6600 Datasonde – calibrated pre and post deployment
- All other sampling gear and laboratory instrumentation will be calibrated (if possible) as per manufacturer's recommendations or common sense to ensure proper function.

Each piece of routine tools common to most laboratories (e.g., drying ovens, freezers, etc.) will have an assigned logbook in which the calibration or performance records are maintained. Other equipment such as sample drying ovens should be monitored on a routine basis during periods of use to ensure their performance.

13.3 Inspection/Acceptance of Supplies and Consumables:

All replacement probes for YSI datasondes and sensors for LI-COR measurements must be received directly from the appropriate company. Probes will only be accepted if they are shipped with a certificate of calibration, are not noted as damages after a visual inspection, and pass initial calibration tests (YSI) or reflect appropriate readings (LI-COR). Materials do not meet this criteria will be returned to the manufacturer and replaced.

An array of laboratory-based stoichiometric determinations will be conducted with a variety of environmental samples (Appendix 1). These analyses require extensive utilization of certified standards for instrument calibration, plus, many incorporate the use of standard reference materials (SRMs) as a routine QC samples. The analytical standards and reagents for all analyses will be provided by established, reputable suppliers and when available, only certified materials will be used; in cases where certified standards are not available, the analysts will obtain high purity (e.g., analytical or reagent grade) compounds to prepare in-house standards.

14.0 DATA MANAGEMENT:

The NJ coastal bays project will require that each data generating activity, both field measurements and laboratory analyses, be thoroughly documented. Data will be recorded in a variety of paper and digital formats. Specific formats for both written and electronically recorded data will be prescribed to document the field monitoring and pertinent steps of laboratory analyses (Table 10, Appendix 3). Once the field crews have returned to the lab all data sheets will be scanned electronically and the data will be entered manually into Microsoft Excel. All data sheets used in the laboratory will also be scanned and the data will be recorded in Excel. All data will be backed up on a hard drive and on the MSEFS servers.

A study file containing planning documents (QAPP), SOPS, field data sheets, laboratory notebooks or work sheets, study-related correspondence, records of peer reviews or QA assessments (reviews), and reports and publications will be maintained. These records will be permanently archived by Richard Stockton College of New Jersey

14.1 Field Activities:

Field crews will rely primarily upon hardcopy field data forms to record most field collected data; however, there are project components where self-contained dataloggers (e.g., datasondes) will be used to collect information that will be downloaded as electronic files. Standardized hardcopy forms will be used (Appendix 3). The core field indicators/data in this project will be recorded in an approved, uniform manner using the datasheets provided in Appendix 3. It is preferred that raw data are recorded by ballpoint pen on a real-time basis.

All core data recorded on field data sheets will be transcribed into the field computer system within a reasonable time following collection (target period, within a week). To ensure consistency, one person will be responsible for the data entry. Data entry will be straightforward and user friendly; the fields in the electronic format will closely resemble the hardcopy raw data forms. The hardcopy data forms filled out for a given station will be compiled into a "station data package" and photo-copied to provide in-house working copies for use by US EPA (study files). The

original field sheets will be archived, as well as backup disks for all electronic files. These raw data will be kept on file indefinitely at Richard Stockton College of New Jersey.

A systematic approach of sample tracking will be used to ensure accountability for the handling, storage, and transfer or shipment of the field collected samples. Chain-of-custody documentation (as per GLPs) will include the following basic components:

Sample Collection:

- A master inventory of all field samples that are expected to be collected (separate list(s) for each sample type and corresponding station IDs), with check off fields providing documentation of all samples that are collected (when, and by whom)
- Sample transfer information/invoice (where, what, to whom, and when, and by whom samples are transferred or shipped)

Sample Receipt (log-in):

- Documentation (sample log-in form) of the person receiving; when and what they receive; and general condition of shipment (e.g., breakage, thawed, etc)
- Reconciliation that what was reported shipped was in fact received
- Deposition/distribution of samples (e.g., where stored and holding conditions)
- Sample release to analysts

As in the case of the field data sheets, a sample tracking system will be followed verbatim (Appendix 3). If a sample is missing, the laboratory should then go through appropriate channels to contact the field team as soon as possible so that they may attempt to locate the sample at their end or possibly re-sample. A complete set of field data sheets, laboratory data sheets, and tracking documents will be submitted to the Project manager to be archived for at least a period of 7 years

14.2 Laboratory Analyses:

As with field collected data, the overall flow of data generated from laboratory analyses will follow the route established below:

Data Generator (raw data from laboratory)
↓
Project Manager (initial validation and formatting)
↓
Quality Assurance Officer (additional validation/verification, and formatting)
↓
The Richard Stockton College of NJ
↓
Public Website

The specific reporting requirements for each of the major laboratory activities are described in the following sections.

All analytical and processing laboratories used in this project will retain raw data files (e.g., primary standard certification, working standard preparations, instrument calibration records, results of QC check samples/measurements, instrument printouts, and final data calculations) for

each indicator and will be stored indefinitely at Richard Stockton College of New Jersey. Demonstration of laboratory approval is required. The contractor (Richard Stockton College) will review all data to verify that quality goals are satisfied. Upon issuing appropriate advance notification (i.e., minimum of 2 weeks), Barnegat Bay Partnership (BBP) and US EPA maintains the authority to access the active files and/or request copies of specific information at any time. In addition, the full set of data will be part of the study file of which BBP and US EPA will receive a copy at the completion of the project.

Whenever changes or updates are made to the QA Project plan, copies of the most current copy will be electronically transmitted to all persons on the distribution list specified in the QAPP distribution list. This will be the responsibility of the Project Manager (Peter Straub). Procedures that will be used for document and record keeping are described in sections 15 and 16.

15.0 ASSESSMENTS AND OVERSIGHT:

The following sections outline the structured data reviews and assessments of data quality planned for the project. Note: Routine audits will be conducted by the Quality Assurance Officer during the course of the project, and will include review of any project environmental data collection activity. EPA may implement, at their discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan.

15.1 Field Monitoring:

15.1.1 Field Crew Authorization:

Field teams will be responsible for the collection of environmental data and samples from the sampling sites. SOPs and guidelines are provided to all team members to help ensure that the data collected are of known quality. These guidelines allow for the use of different equipment (e.g., various YSI 6600 datasondes, work vessels, etc.) as long as the data generated meet acceptability criteria. Such performance-based QA/QC is a key factor to the project success in deriving comparable data from diverse participants. Prior to the start of the 2012 and 2013 field monitoring seasons, each field crew will be required to complete a 1-2-day field training to be authorized to collect actual field data and samples. Training will consist primarily of hands-on sessions during which field crew members will be instructed by the QA Manager (and MSEFS staff) on the sampling methods and protocols developed for the project. Members of the crews will conduct a certification exercise as they master each major component (e.g., seed bank core sampling), and then move on to the next. Crews that successfully demonstrate technical competence and a thorough appreciation of field QA/QC requirements will then be authorized to initiate field activities. If a crew fails to qualify on some aspect, the members will receive further instruction in the area of their deficiencies until they perform at an acceptable level.

15.1.2 Field Reviews:

Field reviews will be carried out once a month by the Project QA Manager or a member of the MSEFS staff. Any minor deficiencies observed during field surveillance (e.g., slight deviation from approved procedures, labeling irregularities, data reporting, etc.) should be immediately pointed out to the crew and corrective actions imposed on-the-spot. The evaluator will document

with a brief note on the checklist and no further write-ups are required. If significant deficiencies (i.e., data quality is seriously compromised) are observed, the evaluator will make the appropriate on-the-spot correction, and, if the case warrants, call a halt to the field activities until the problems are resolved to the satisfaction of the Project QA Manager. All cases of this nature will be documented through a written report submitted to the Project QA Manager. A completed checklist along with a copy of the completed field data forms, and sample checklist from each sampling date provides the basic documentation for an evaluation of the crew's overall performance on this sampling date. All field review data will be maintained as part of the study file (Section 14.0).

15.2 Laboratory Activities:

15.2.1 Laboratory Approval:

All samples will be processed at either MSEFS or Richard Stockton College School of Natural Science and Mathematics using the SOPs in Appendix 1. All instruments will be calibrated and maintained according to manufactures conditions and SOP standards (Section 13; Appendix 1). Records of calibrations will be kept for all instruments in both physical (data sheet in binder) and digital (scanned pdf) formats. All laboratory personnel will have to sign off all samples as they are checked in, when they are moved to storage, and when they are processed.

Prior to the start of sample analysis, all laboratory personnel will be required to complete laboratory training for individual SOPs to be authorized to processes samples collected from the field. Training will consist primarily of hands-on sessions during which laboratory analysis will be instructed by the Program Manager (and associates) on the SOPs developed/adopted for the project. Training for all laboratory personnel will culminate with an approval exercise in which crew members are observed and evaluated as they perform the full suite of laboratory analysis (i.e., process one plant, sediment, and water column sample). Individuals that successfully demonstrate technical competence and a thorough appreciation of laboratory QA/QC requirements will then be authorized to initiate lab activities. If an individual fails to qualify on some aspect, the individual will receive further instruction in the area of their deficiencies until they perform at an acceptable level.

15.2.2 Laboratory Reviews:

Laboratory reviews will be carried out once a month by the Project QA Manager or a member of the MSEFS staff. Any minor deficiencies observed during sample processing (e.g., slight deviation from approved procedures, labeling irregularities, data reporting, etc.) should be immediately pointed out to the analysts and corrective actions imposed on-the-spot. The evaluator will document with a brief note on the checklist and no further write-ups are required. If significant deficiencies (i.e., data quality is seriously compromised) are observed, the evaluator will make the appropriate on-the-spot correction, and, if the case warrants, call a halt to the field activities until the problems are resolved to the satisfaction of the Project QA Manager. All cases of this nature will be documented through a written report submitted to the Project QA Manager. A completed checklist along with a copy of the completed field data forms, and sample checklist from each sampling date provides the basic documentation for an evaluation of the crew's overall performance on this sampling date. All laboratory review data will be maintained as part of the study file (Section 14.0).

16.0 DATA REVIEW, VERIFICATION, VALIDATION AND USABILITY:

The data generated during the project will be systematically reviewed with varying levels of scrutiny at several junctures along the path from time of collection to final reporting; from quick, on-the-spot screening to in-depth evaluation against established criteria or standards. For much of the field collected data, the first level of validation, a cursory screening, will occur as data are recorded; persons conducting and documenting real-time observations should be aware of the range that constitutes realistic values for a specific measure. Certainly a water temperature of 40 °C should jump out as an obvious outlier and trigger an immediate response to find the source of the error. With other types of data, the initial validation may not occur in such an immediate time frame; for example, in the case of chlorophyll analysis, the analyst may first need to run several calculations to arrive at a meaningful result. Nonetheless, most data are amenable to some form of quick screening soon after being generated and the responsibility for this first- cut validation falls on the personnel performing the measurement. In addition, most laboratory analyses of the project samples will be monitored by a series of in-stream QC checks that indicate the general level of data quality for a given batch of samples. In addition, documented verifications are required to determine if data quality remains at a level acceptable for the program. The following sections outline the format and procedures to be used for evaluating and documenting data quality for the project and discuss how issues will be resolved when they occur.

16.1 Data Review, Verification, and Validation:

16.1.1 Review of Field Data:

A first review of field data occurs as the data are being collected by the field crews (e.g., are these data in the ballpark?). If the field personnel encounter situations where they question the validity of data they are collecting, they should immediately refer back to the appropriate SOP, attempt to isolate and resolve the problem; if they are unable to do so, then they must describe the situation in writing on the appropriate data sheet, and, as soon as possible, consult with their respective senior Field Manager or Project Manager for corrective actions.

The next level of review takes place as the Project Manager double checks that samples were properly checked in to the lab and consolidates and formats the field data. Most of the field crew will use hardcopy data sheets to record the bulk of field data; therefore, the data must be transcribed into an Excel spreadsheet. As soon as possible, upon return from the field, all raw data forms should be scanned and a digital PDF file created. In addition, all data from the YSI datasondes must be downloaded and a primary QAQC completed using the NERRQAQC macros as per NERR SWMP data management protocols (Small et al. 2013). Also, data downloaded on site from the LI-1400 data must be visually inspected to ensure that all data are within the instruments range of detection. All hard copy originals will then be placed in a secure file; and the electronic copies will be backed up on the MSEFS server and then be used for entering the data.

During the data entry process, the field data will be screened for missing or errant information based on instrument sensitivity (Table 10; Appendix 3). All field data will be subject to an evaluation of the relative frequency of transcription errors enacted going from hardcopy into the electronic format. To determine this, a randomly selected subset of at least 10% of the station packages (the entire set of field data sheets submitted for a given station) will be pulled and the

data (primarily, measurements or numerical values) manually compared against the electronic version on a field - by-field basis. Any errors will be listed in the data logbook and a final tally derived for the station. The total number of transcription errors for a complete set of data sheets should not exceed 5. If more than 5 transcription errors are found, the entire set of field data sheets will be pulled and re-examined for review and check for errors. If corrective actions are initiated (e.g., correcting a spelling error on the copied data form), the correction must be legible and the person who made the correction must document the alteration with their initial and date; a description of the correction must be noted in the bound log.

16.1.2 Verification of Field Data:

Measurements of water quality parameters taken directly in the field will be evaluated for accuracy by verifying the results of calibration and QC checks. These checks should be performed by the field crews as determined by the SOP (Appendix 1) and if the instruments are out of tolerance, they should be re-calibrated. Calibration and post-calibration of instruments will occur at the beginning/end of field sampling days. At the end of every month, copies of the field records for calibration and QC checks will be provided to the Project QA Manager and Project Manager for further review. Any data that was collected, when the instruments were out of compliance, will be flagged with a qualifier code.

16.1.3 Validation of Field Data:

Data will be validated by the Program Manager as frequently as possible. All YSI data will be analyzed using the NERRS SWMP QAQC protocols (Small et al. 2013) and any data that does not meet these criteria will not be used in any data analysis. To correct for missing data within the dataset the model will interpolate between the validated data on either side of the missing data. If more than 6 hours of data is missing the NJDEP water quality data from the nearest continuous monitoring station will be substituted. Light data will also be validated against the sensor detection limitations and all data that do not meet these validation criteria will be removed. To correct for missing data within the dataset the model will interpolate between the validated data on either side of the missing data. All sections with interpolated data will be clearly marked in the Excel spreadsheet.

16.1.4 Review of Laboratory Data:

All laboratory data generated for the project will be systematically reviewed and evaluated by both the Project Manager and the Project QA Officer. Upon receipt of a data set, a temporary file will be created and a series of error checks developed (checks will vary with SOP) will be performed to ensure the data: 1) are within specified ranges appropriate to each parameter measured, 2) contain all required fields, 3) have encoded valid values from constrained look-up lists where specified, and 4) are in the correct format (text in text fields and values in numeric fields, etc.).

A first review of laboratory generated data occurs as the data are being processed by the laboratory analysts (e.g., are these data in the ballpark?). If the analysts encounter situations where they question the validity of data they are collecting, they should immediately refer back to the appropriate SOP, attempt to isolate and resolve the problem; if they are unable to do so, then they

must describe the situation in writing on the appropriate data sheet, and, as soon as possible, consult with their respective senior MSEFS staff or Project Manager for corrective actions.

The next level of review takes place as the Project Manager double checks that samples were properly checked into the lab, stored, and checked out, that the proper number of QC samples was analyzed depending upon SOP (Appendix 1), as well as consolidates and double checks all calculations and formats all laboratory data. Most of the laboratory analysts will use hardcopy data sheets to record the bulk of the laboratory data; therefore, the data must be transcribed into an Excel spreadsheet. All hard copy originals will then be placed in a secure file; and the electronic copies will be backed up on the MSEFS server and then be used for entering the data.

During the data entry process, the field data will be screened for missing or errant information based on instrument sensitivity (Table 10). All laboratory data will be subject to an evaluation of the relative frequency of transcription errors enacted going from hardcopy into the electronic format. To determine this, a randomly selected subset of at least 10% of the station packages (the entire set of laboratory data sheets submitted for a given sampling date) will be pulled and the data, manually compared against the electronic version on a parameter by parameter basis. Any errors will be listed in the data logbook and a final tally derived for the parameter. The total number of transcription errors for a complete set of data sheets should not exceed 5. If more than 5 transcription errors are found, the entire set of laboratory data sheets will be pulled and re-examined for review and check for errors. If corrective actions are initiated (e.g., correcting a spelling error on the copied data form), the correction must be legible and the person who made the correction must document the alteration with their initial and date; a description of the correction must be noted in the bound log.

16.1.5 Verification of Laboratory Data:

Measurements of all parameters quantified in the laboratory will be evaluated for accuracy by verifying the results of calibration and QC checks. These checks must be performed by the lab analysts as determined by the SOP (Appendix 1) and if the instruments are out of tolerance, they must be re-calibrated. Calibration and post-calibration of instruments will occur as defined in section 13.2.3. At the conclusion of sample processing, copies of the laboratory records for calibration and QC checks will be provided to the Project QA Manager and Project Manager for further review. Any data that was collected, when the instruments were out of compliance, will be flagged with a qualifier code and not used in data analysis.

16.1.6 Validation of Laboratory Data:

The Project QA Manager and Project Manager will be responsible for conducting technical reviews of the data before the data are accepted for project assessments; certain aspects of these reviews may be delegated to other staff with final approval through the above quality management personnel. Data quality of a specific data set will be assessed by a critical comparison of the submitted QA/QC results to the quality criteria or standards established by the appropriate SOP (Appendix 1). If the evaluation indicates that the data, overall, meet the quality standards, with no or only minor deficiencies, then the data set will be acceptable for the project assessments without further qualification. If there are only a few, easily correctable errors, the changes will be made, according to SOP guidelines. If there are numerous errors or the corrections are difficult to

implement, the data will be removed from all further analysis only. All collected data, including data not used in analysis, will be retained in digital and hard copy records. Calculations specified by each SOP will be amended to reflect any loss of data

16.1.7 Model Calibration and Verification:

The initial model simulation period will run 1.5 years (June 1, 2012 through December 31, 2013) with a time step (dt) of 0.125 days using data collected from Barrel Island. Parameter values for epiphytes and *Zostera marina* variables will be selected from the literature and field data, and revised to increase model fit to calibration data within ecological limits defined as values ± 10% of the values given in Tables 8 and 9. The model will be calibrated to water column, sediment, and *Z. marina* data collected at Bi in bi-weekly to monthly intervals from August to October in 2012 and April to October 2013. The model will be considered calibrated once the overall standard of error between model measurements and observed state variable data is < 20%. Once calibrated, the model will be independently verified using data from Seaside Park located 31 km up estuary. Parameter values will be left unchanged for verification, but forcing functions were updated to reflect SS data. Comparisons will be made between computed and observed values on a monthly average basis. The sensitivity of base model conditions to all parameter estimates and forcing functions will be analyzed by sequentially varying values by ± 5, 10, and 20 %. The average percent change in all state variables between the base model and sensitivity simulations will then be calculated and tests that resulted in > 10 % change in state variable concentrations were considered to have the greatest impact on model results.

16.2 RECONCILIATION WITH USER REQUIREMENTS:

All data collected as part of this project must meet the QAQC standards defined by this QAPP. Missing data will be tracked with the sample log in systems both in the field and in the lab (Appendix 3). If possible missing samples will be replaced with additional samples. When that is not possible (e.g. missing continuous water quality data due to malfunctioning sonde) interpolations between missing data will be made (continuous data) or the data will be noted as missing in all excel datasheets. For both continuous and non-continuous data a completeness criteria of 80% is necessary to be able to meet the data requirements of the *Z. marina* restoration site selection model. If this requirement is not met the model will not be able to be run until additional data is collected.

During analysis not all data quality goals may be met. For example, if several sediment NH_4 samples are below the detection limit then levels will be set to 0 for the model for that month. If a trend of below detection samples continues, values from the literature for the particular parameter in question may be used in model calibration or verification. Another example may include the samples held longer in storage than defined by the SOP for that parameter. Due to the high frequency of sampling for this project samples will have to be processed quickly in order to free up equipment for additional sampling. However, if this did occur, the samples will be processed according to the appropriate SOP, the data will be flagged in the excel spreadsheet, and potential bias of the measurements will be quantified. If the bias is significant, then those samples will be removed from the analysis. Finally, due to the high amount of field data required as part of this

project it is possible that field conditions require that sampling procedures be changed significantly. Sampling during high wind or rain events will avoided as much as possible. In the event that conditions deteriorate as the sampling day progresses, remaining samples will not be collected and the field crew will return to the same sampling site the following day. A note about the extended sampling period (2 days instead of 1) will be noted on the field data sheets.

Data that pass the verification process will be used to calibrate and verify the *Zostera marina* restoration site selection model developed by PI Jarvis. All validated data will either be used as forcing functions (abiotic measurements) or as initial biomass stocks/calibration checks for state variables. To use the validated data generated as part of this project in the model it will first have to be analyzed. Average values for all parameters (except for continuous water quality and light data) will be calculated. Further data analysis will be undertaken in SAS, STELLA, SigmaStat and SigmaPlot Software Packages. The software package used will be dependent on the specific spatial, temporal question and the input data type. Point data (one specific point in time and space) will be analyzed using the SAS and SigmaPlot statistical software packages using a form of logistic regression. All model calibration, validation, and scenario functions will be made in STELLA. All output will be saved in an electronic format on MSEFS servers.

17.0 REPORTING, DOCUMENTS AND RECORDS:

All project documents and records will make use of a document notation system located in the footer region of all data sheets/reports. All documents will list the Project Name, Document Name and Revision number, Date, page number, and total number of pages in the document. All field and laboratory data will be maintained in hard copy in the data logbook, scanned copies of all sheets, and electronic versions of the data in Microsoft Excel will be maintained on Project Manager Jarvis's computer at the MSEFS as well as on the MSEFS servers (e.g. copies will be backed up on 2 servers). Once the model has been calibrated and verified a copy of the final model will be made public via the MSEFS website (http://intraweb.stockton.edu/eyos/page.cfm?siteID=105&pageID=94). All data (hard and electronic copies) will be maintained at the MSEFS for an indefinite period of time.

Sampling collection and handling records will be maintained by the Project Manager and checked by the QA officer after each sampling date. Analytical logbooks will be updated following the processing of each sample by the analyst and audited by the QA officer. All QC sample records and equipment calibration records will be maintained as part of the appropriate analytical logbook and will be observed with the logbooks. The project manager will prepare progress reports and submit them to the Barnegat Bay Partnership, as required by the contract between BBP and Richard Stockton College (Table 1). Data verification, analysis, completion of the modeling scenarios, and associated recommendations will be included in the final report to the Barnegat Bay Partnership which will be submitted in May 2014.

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TABLES:

Table 1. Project schedule of all major events or actions from August 2012 to May 2014.

Event	**Dates**
Bi -weekly sampling BI and SS (abiotic and biotic); sample processing	August - October 2012
Monthly sampling BI and SS (abiotic and seed bank); sample processing	November - December 2012
Monthly sampling BI and SS (abiotic and seed bank); sample processing	January - April 2013
Presentation of initial results at regional estuarine society meeting	March 2013
Monthly sampling BI and SS (abiotic and biotic); sample processing	May - October 2013
Financial status report to BBP with invoice due	July 31, 2013
Progress report due to BBP	July 31, 2013
Develop education and outreach plan with BBP	July 31, 2013
Progress report presentation to BBP Advisory Committee	July 31, 2013
Presentation of initial results at National estuarine society meeting	November 2013
Monthly sampling BI and SS (abiotic and biotic); sample processing	November - December 2013
Model Calibration and Verification	January - March 2014
Presentation of model scenario results at regional estuarine society meeting	March 2014
Public Informational Meeting on Model	April 2014
Model Website Published	May 2014
Article for Barnegat Bay Beat and BBP Website due	May 2014
Summary report detailing problems due	May 2014
Final Report to BBP Due	May 2014
Final Financial report to BBP due	May 2014

Table 2. Quality objectives and criteria for measurement data. Precision goals are expressed as relative percent difference (RPD) or relative standard deviation (RSD) between two or more replicate measurements. Bias goals are expressed either as absolute difference (± value) or percent deviation from the "true" value. Completeness goal is the percentage of expected results that are obtained successfully to maximize representativeness of the samples.

Indicator/Data Type	Maximum Allowable Precision Goal	Maximum Bias Goal	Completeness Goal
SAV			
Biomass	30%	10%	100%
Density	30%	10%	100%
SAV seed bank			
Seed Burial Depth	10%	0.02 M	100%
Density	30%	10%	100%
Viability	30%	10%	100%
Macroalgae			
Biomass	30%	10%	100%
Epiphytes			
Biomass	30%	10%	100%
Water Column Characteristics			
Temperature	10%	0.15 °C	100%
Salinity	10%	0.1 PSU	100%
pH	10%	0.2 units	100%
Depth	10%	0.018 m	100%
Turbidity	10%	10%	100%
Chlorophyll *a* (both)	15%	10%	100%
Secchi depth	10%	NA	100%
Total PAR	5%	5%	100%
TSS	5%	5%	100%
Ammonium/Ammonia	10%	10%	100%
Nitrate/Nitrite	10%	10%	100%
Orthophosphates	10%	10%	100%
Sediment Characteristics			
Ammonium/Ammonia	10%	10%	100%
Nitrate/Nitrite	10%	10%	100%
Orthophosphates	10%	10%	100%
Percent organic content	10%	10%	100%
Model Sensitivity			
Forcing functions	10%	10%	100%
State Variables	10%	10%	100%

Table 3. Field quality control summary and actions taken to resolve or reconcile variability with project objectives. Data quality indicators (DQI) are defined as P = precision, B = Bias, R = representativeness, C_1 = comparability, C_2 = completeness; S = sensitivity.

Analyte	DQI	Field QC Check	Frequency of Collection	Acceptance Criteria	Action(s) Taken
Temperature (YSI 6600)	$P/C_1/S$	Thermometer	Bi-weekly/monthly	± 1°C	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 1°C	Repair and replacement as needed
Salinity (YSI 6600)	$P/C_1/S$	Standard Seawater	Bi-weekly/monthly	± 0.2 ppt	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 0.2 ppt	Repair and replacement as needed
Turbidity (YSI 6600)	$P/C_1/S$	Second instrument	Bi-weekly/monthly	± 5%	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 5%	Repair and replacement as needed
Chlorophyll *a* (YSI 6600)	$P/C_1/S$	Second instrument	Bi-weekly/monthly	± 5%	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 5%	Repair and replacement as needed
Secchi depth	$P/C_1/S$	Second instrument	Bi-weekly/monthly	± 5%	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 5%	Repair and replacement as needed
Total PAR	$P/C_1/S$	Factory Calibrations	Bi-weekly/monthly	± 5%	Recalibration as specified
	P/B	Visual Fouling Check		< 10% fouled	Cleaning and maintenance
	C_2/R	Handheld display		± 5%	Repair and replacement as needed
Total Suspended Solids	$P/C_1/S$	Replicate samples	Bi-weekly/monthly	n = 3 per site	Replicate samples within 1 standard deviation by certified field crew
	B	Duplicate samples		n = 1 per site	Field Sample blanks
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples

Analyte	DQI	Field QC Check	Frequency of Collection	Acceptance Criteria	Action(s) Taken
Chlorophyll a (fluorometer)	P/C_1/S	Replicate samples	Bi-weekly/monthly	n = 3 per site	Replicate samples by certified field crew; acceptance criteria for standards
	B	Duplicate samples		n = 1 per site	Field Sample blanks
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Water Column Nutrients	P/C_1/S	Replicate samples	Bi-weekly/monthly	n = 3 per site	Replicate samples within accepted precision and accuracy by certified field crew
	B	Triplicate samples		n = 3 per site	Triplicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Sediment Percent Organic Content	P/C_1/S	Replicate samples	Bi-weekly/monthly	n = 9 per site	Replicate samples within accepted precision and accuracy by certified field crew
	B	Duplicate samples		n = 1 per site	Field Sample blanks
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Sediment Nutrients	P/C_1/S	Replicate samples	Bi-weekly/monthly	n = 9 per site	Replicate samples within accepted precision and accuracy by certified field crew
	B	Triplicate samples		n = 9 per site	Triplicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Seagrass Biomass	P/B/C_1/S	Replicate samples	Bi-weekly/monthly	n = 5 per site	Replicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Seagrass Seed Bank	P/B/C_1/S	Replicate samples	Bi-weekly/monthly	n = 15 per site	Replicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples

Analyte	DQI	Field QC Check	Frequency of Collection	Acceptance Criteria	Action(s) Taken
Macroalgaal Biomass	P/B/C_1/S	Replicate samples	Bi-weekly/monthly	n = 5 per site	Replicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples
Epiphyte Biomas	P/B/C_1/S	Replicate samples	Bi-weekly/monthly	n = 15 per site	Replicate samples
	R/C_2	Sample date		n = 1 or 2 per month	Bi-weekly/monthly re-measurement
	R/C_2	Sample location		Random sampling	Spatially composited samples

Table 4. Analytical methods. * denotes Analytical Methods that were used by the NJDEP to collect water-quality data that will be used as a source of secondary data in this investigation. References 0 = USGS; 1 = USEPA; 2 = Sheldon and Weibe, 1997; 3 = Ringuet et al., 2011; 4 = Small et al. 2013; 5 = LI-COR manual; 6 = Coles et al., 2001; 7 = McFarland and Shafer, 2011.

Analyte	Sample Matrix	Method	Analytical Method		Detection Limit	Reporting Limit
			Method	Reference		
Total Suspended Solids	Water on GFF filter	Non-filterable residue by drying oven	I-3765-85	0	4.0 mg L^{-1}	4.0 mg L^{-1}
Chlorophyll *a*	90% Acetone	Fluorescence	445	1	0.05 µg L^{-1}	0.11 µg L^{-1}
Ammonium + Ammonia	Water - DI water Sediment - 2M KCl	Indophenol blue	EPA 349	1/2	0.5 µM	1.0 µM
	Water - DI Water	Automated Phenate*	EPA 350.1 modified	1	9.49 µg L^{-1}	18.97 µg L^{-1}
Nitrate/Nitrite	Water - DI water Sediment - 2M KCl	Nitrate reductase complexing with N-(1-napthyl) ethylenediamine hydrochloride	USGS I-2547-11/EPA 353.4	0/1/3	0.5 µM	1 µM
	Water - DI Water	Automated Cd Red. Reduction*	353.3	1	11.25 µg L^{-1}	22.50 µg L^{-1}
Orthophosphate	Water - DI water Sediment - 2M KCl	Molybdemum blue	EPA 365.5	1/3	0.5 µM	1.0 µM
	Water - DI Water	Orthophosphate in Estuarine & Coastal Waters*	365.5	1	3.55 µg L^{-1}	7.09 µg L^{-1}
Sediment TOC	Sediment	% Loss on Ignition	EMASC-001	1	0%	0%
Data Sonde: Salinity	Water	Conductivity probe: Yellow Springs Incorporated (Y.S.I.) model #6560	NA	4	0 ppt	0 ppt
Data Sonde: Temperature	Water	Yellow Springs Incorporated Thermistor (model #6560)	NA	4	-5 °C	-5 °C

Analyte	Sample Matrix	Method	Analytical Method	Detection Limit	Reporting Limit	Analyte
Data Sonde: Turbidity	Water	Turbidity Probe: Yellow Springs Incorporated (Y.S.I.) (model #606136)	NA	4	0 NTU	0 NTU
Data Sonde: Chlorophyll a	Water	Chlorophyll Probe: Yellow Springs Incorporated (Y.S.I.) (model #606136)	NA	4	0 µg L^{-1}	0 µg L^{-1}
Total PAR	Air	LI-COR 190SA sensor	NA	5	0 µmol s^{-1} m^{-2}	0 µmol s^{-1} m^{-2}
Seagrass Biomass	Biomass	Dry weight by drying oven	NA	6	0 g DW m^{-2}	0 g DW m^{-2}
Macroalgal Biomass	Biomass	Dry weight by drying oven	NA	6	0 g DW m^{-2}	0 g DW m^{-2}
Epiphyte Biomass	Biomass	Dry weight by drying oven	NA	6	0 g DW m^{-2}	0 g DW m^{-2}
Seagrass Seed Bank (density)	Individual seeds	Density count	NA	6	0 seeds m^{-2}	0 g DW m^{-2}
Seagrass Seed Bank (viability)	Individual seeds	Tetrazolium staining	NA	7	0%	0%

Table 5. Analytical quality control.

Method/SOP	DQI	Lab QC Check	Frequency	Accept. Criteria	Corrective Action
Datasonde Calibration: Conductivity/Specific Conductivity	P	Duplicate machines	Pre/Post Deploy	± 0.001 mS cm^{-1}	Recalibrate machine
	B/S	Calibration to Standard	Pre/Post Deploy	± 0.001 mS cm^{-1}	Recalibrate machine
	C_1	Data QA/QC	Pre/Post Deploy	0 to 100 mS cm^{-1}	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde
Datasonde Calibration: Salinity	P	Duplicate machines	Pre/Post Deploy	0.1 ppt	Recalibrate machine
	B/S	Calibration to Standard	Pre/Post Deploy	0.1 ppt	Recalibrate machine
	C_1	Data QA/QC	Pre/Post Deploy	0 to 70 ppt	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde
Datasonde Calibration: Temperature	P	Duplicate machines	Pre/Post Deploy	0.15 °C	Recalibrate machine
	B/S	Calibration to Standard	Pre/Post Deploy	± 1.0 °C	Recalibrate machine
	C_1	Data QA/QC	Pre/Post Deploy	-5 to 45 °C	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde
Datasonde Calibration: Turbidity	P	Duplicate machines	Pre/Post Deploy	±0.3 NTU	Recalibrate machine
	B/S	Calibration to Standard	Pre/Post Deploy	±0.3 NTU	Recalibrate machine
	C_1	Data QA/QC	Pre/Post Deploy	0 to 1000 NTU	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde
Datasonde Calibration: Chlorophyll *a*	P	Duplicate machines	Pre/Post Deploy	± 5%	Recalibrate machine
	B/S	Calibration to Standard	Pre/Post Deploy	± 5%	Recalibrate machine
	C_1	Data QA/QC	Pre/Post Deploy	0 to 400 µg L^{-1}	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde

Method/SOP	DQI	Lab QC Check	Frequency	Accept. Criteria	Corrective Action
Total PAR	P/B/S	Factory Calibration	Pre/Post Deploy	± 5%	Recalibrate every 2 yrs
	C_1	Data QA/QC	Pre/Post Deploy	0 to 400 µg L^{-1}	Flag data out of range; remove data
	R/C_2	Download Data	Bi-weekly	80%	Make note in file; repair sonde
Total Suspended Solids	P/C_1	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples with same method (n = 3 per site)
	B/S	Sample Blanks	Bi-weekly/monthly	± 5%	Compare with filter weight (n = 1 per site)
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Chlorophyll a (fluorometer)	P	Replicate samples	Bi-weekly/weekly	± 5%	Replicate samples with same fluorometer (n = 3 per site)
	S	IDL/EDL	Per processing	± 5%	Compare with machine specs (n = 5 per processing)
	C_1	Calibration to Standard	Per processing	± 5%	Problem identified and corrected (n = 5 per processing run in triplicate)
	B	QCS	Per processing	± 5%	Problem identified and corrected (n = 5 per processing)
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Water Column/Sediment Nutrients: $NO_2 + NO_3$	P	Replicate samples	Bi-weekly/weekly	± 10%	Replicate samples with same instrument (n = 3 per site run in triplicate)
	S	LOD	Per processing	0.5 µM	Compare with lowest calibration standard
	C_1	Calibration to Standard	Per processing	± 10%	Problem identified and corrected (n = 5 per processing run in triplicate)
	B	Reagent blanks	Per processing	± 10%	Problem identified and corrected (n = 5 per processing run in triplicate)
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed

Method/SOP	DQI	Lab QC Check	Frequency	Accept. Criteria	Corrective Action
Water Column/Sediment Nutrients: NH_4 +NH_3	P	Replicate samples	Bi-weekly/weekly	± 10%	Replicate samples with same spectrophotomer (n = 3 per site run in triplicate)
	S	LOD	Per processing	0.5 µM	Compare with lowest calibration standard
	C_1	Calibration to Standard	Per processing	± 10%	Problem identified and corrected (n = 5 per processing run in triplicate)
	B	Reagent blanks	Per processing	± 10%	Problem identified and corrected (n = 5 per processing run in triplicate)
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Water Column/Sediment Nutrients: OPO_4	P	Replicate samples	Bi-weekly/weekly	± 10%	Replicate samples with same spectrophotometer (n = 3 per site run in triplicate)
	S	LOD	Per processing	0. 5 µM	Compare with lowest calibration standard
	C_1	Calibration to Standard	Per processing	± 10%	Problem identified and corrected (n = 9 per processing run in triplicate)
	B	Reagent blanks	Per processing	± 10%	Problem identified and corrected (n = 9 per processing run in triplicate)
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Sediment Total Organic Content	P/S	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples on same analytical balance (n = 9 per site)
	B/C_1	Triplicate measurements	Per processing	± 5%	Samples measured 3 times on same analytical balance
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed

Method/SOP	DQI	Lab QC Check	Frequency	Accept. Criteria	Corrective Action
Seagrass Biomass	P/S	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples on same analytical balance (n = 5 per site)
	B/C_1	Triplicate measurements	Per processing	± 5%	Samples measured 3 times on same analytical balance
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Macroalgal Biomass	P/S	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples on same analytical balance (n = 5 per site)
	B/C_1	Triplicate measurements	Per processing	± 5%	Samples measured 3 times on same analytical balance
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Epiphyte Biomass	P/S	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples on same analytical balance (n = 15 per site)
	B/C_1	Triplicate measurements	Per processing	± 5%	Samples measured 3 times on same analytical balance
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed
Seagrass Seed Bank	P/S	Replicate samples	Bi-weekly/monthly	± 5%	Replicate samples on same analytical balance (n = 15 per site)
	B/C_1	Second observer	Per processing	± 5%	Samples measured by a minimum of 2 observers
	R/C_2	Sample processing	Bi-weekly/monthly	80%	Collect additional samples as needed

Table 6. Lowest detection limit for all nutrient, TSS, and chlorophyll *a* samples.

Indicator/Data Type	Units	Minimum Reporting Level
SAV		
Biomass	g DW m^{-2}	0
Density	# shoots m^{-2}	0
SAV seed bank		
Seed Burial Depth	cm	0.1
Density	# seeds m^{-2}	0
Viability	# seeds m^{-2}	0
Macroalgae		
Biomass	g DW m^{-2}	0
Epiphytes		
Biomass	g DW m^{-2}	0
Water Column Characteristics		
Temperature	°C	-5
Salinity	PSU	0
Turbidity	NTU	0
Total Suspended Solids	mg L^{-1}	0
Chlorophyll *a* (datasonde)	µg L^{-1}	0
Chlorophyll *a* (fluorescence)	µg L^{-1}	0.11
Secchi depth	cm	0
Total PAR	µmol s-1 m-2	0
Sediment Characteristics		
Total organic content	percent	0.1
Water Column/Sediment Nutrients		
Ammonium/Ammonia	µM	0.5
Nitrate/Nitrite	µM	0.5
Orthophosphate	µM	0.5

Table 7. Governing equations for (1) epiphyte biomass ($C_{epi;}$ g C m^{-2}); (2) *Z. marina* vegetative shoot biomass ($C_{zms;}$ g C m^{-2}); (3) *Z. marina* vegetative root/rhizome biomass ($C_{zmr;}$ g C m^{-2}); (4) *Z. marina* seedling shoot biomass ($C_{zmss;}$ g C m^{-2}); (5) *Z. marina* seedling root/ biomass (C_{zmsr}; g C m^{-2}); (6) *Z. marina* seed-bank density (Zm_{seeds}; seeds m^{-2}); and (7) *Z. marina* seedling density (Zm_{sd}; seedlings m^{-2}). Terms include P = production; M = mortality; G = grazing; R = respiration; T_d = translocation down; T_{czmss} = transfer of seedling biomass to vegetative shoot biomass; T_{czmsr} = transfer of seedling root/rhizome biomass to vegetative root/rhizome biomass; $Seeds_{germ}$ = germinated seeds; $Seeds_{tot}$ = total seeds produced; $Seeds_{via}$ = viable seeds; P_{seeds} = seed predation; Zm_{sd} = germinated seedling density.

Differential Equations

$$C_{epi} = C_{epi}(t - dt) + (P_{epi} - M_{epi} - G_{epi} - R_{epi}) * dt$$

$$C_{zms} = C_{zms}(t - dt) + (P_{zms} + T_{czmss} - M_{zms} - R_{zms} - T_d) * dt$$

$$C_{zmr} = C_{zmr}(t - dt) + (T_d + T_{czmsr} - M_{zmr} - R_{zmr}) * dt$$

$$Zm_{seeds} = Zm_{seeds}(t - dt) + \left[\left(\left(Seeds_{tot} - M_{seeds} - P_{seeds}\right) - Seeds_{via}\right) - Seeds_{germ}\right) - M\right] * dt$$

$$Zm_{sd} = Zm_{sd}(t - dt) + (Seeds_{germ} - M_{zmsd}) * dt$$

$$C_{zmss} = C_{zmss}(t - dt) + (P_{zmss} + \left(T_{czmss} * Seeds_{C:DAG}\right) - T_{czmss} - T_d - M_{zms} - R_{zms}) * dt$$

$$C_{zmsr} = C_{zmsr}(t - dt) + (T_d + \left(T_{czmsr} * Seeds_{C:DBG}\right) - T_{czmsr} - M_{zmr} - R_{zmsr}) * dt$$

Table 8. Parameter estimates for the *Z. marina* production model. References: 1 = calibration, 2 = Buzzelli et al., 1999, 3 = Cerco and Moore, 2001, 4 = Madden and Kemp, 1996; 5 = Bach, 1993; 6 = Harwell and Orth, 1996.

Abbrev.	Description	Units	Value	Ref
JD	Julian Day	d^{-1}	0-365	
WD	Water Depth	m	0.5	
MR_{epi}	epiphyte mortality constant	d^{-1}	0.0085	1
K_{gepi}	epiphyte grazing constant	g C^{-1} day^{-1}	0.01	1
BMR_{epi}	epiphyte basal metabolic rate	day^{-1}	0.047	2
KtB_{epi}	epiphyte respiration constant	$ºC^{1}$	0.069	2
$Topt_{epi}$	epiphyte optimum temperature for metabolism	ºC	25	2
Khn_{epi}	epiphyte N half saturation constant	µmol N m^{-3}	1.79E-09	3
Khp_{epi}	epiphyte P half saturation constant	µmol P m^{-3}	7.14E-11	3
$Khnw_{zm}$	*Z. marina* N half saturation constant water	µmol N m^{-3}	7.14E-10	3
$Khns_{zm}$	*Z. marina* N half saturation constant sediment	µmol N m^{-3}	2.86E-09	3
$Khpw_{zm}$	*Z. marina* P half saturation constant water	µmol P m^{-3}	4.35E-10	3
$Khps_{zm}$	*Z. marina* P half saturation constant sediment	µmol P m^{-3}	7.14E-09	3, 4
$KPAR_{epi}$	epiphyte PAR half saturation constant	µE m^{-2} s^{-1}	90	4
$KPAR_{zm}$	*Z. marina* PAR half saturation constant	µE m^{-2} s^{-1}	57.5	3
MR_{zms}	*Z. marina* shoot mortality constant	d^{-1}	0.007	1
$Topt_{zm}$	*Z. marina* optimum temperature for metabolism	ºC	22.5	3
T_d	*Z. marina* shoot to root transfer	g C day^{-1}	0.3	3
MR_{zmr}	*Z. marina* root mortality constant Jan - July	d^{-1}	0.000085	1
	Z. marina root mortality constant July - Dec	d^{-1}	0.0095	1
RR_{zmr}	*Z. marina* root respiration at 20 ºC	d^{-1}	0.0005	1
$Θ_{zmr}$	*Z. marina* root respiration constant	unit-less	1.25	5
PR_{seeds}	Seed Predation	shoots d^{-1}	0.5	5
$MR_{zmseeds}$	Seed mortality constant	seeds d^{-1}	0.1	1
VR_{seeds}	Seed viability constant	seeds d^{-1}	0.4	1

Table 9. Parameter estimates for the *Z. marina* reproduction model. References: 1 = calibration, 2 = Silberhorn et al., 1983; 3. = Harwell, 2000; 4 = Fishman and Orth, 1996

Abbrev	Description	Units	Value	Ref
$Veg_{C:D}$	*Z. marina* shoot carbon to density	g C $shoot^{-1}$	0.0168	n = 560 shoots
$ZmRS_{den}$	reproductive shoot density	%	0-19	2
$Seeds_{tot}$	seeds per reproductive shoot	seeds $shoot^{-1}$	7 - 11	3
P_{seeds}	seeds predation rate	day^{-1}	0.33	4
M_{seeds}	seeds mortality rate	day^{-1}	0.1	1
V_{seeds}	seeds viability rate	day^{-1}	0.4	n = 100 seeds
$Seedling_{SD:C}$	*Z. marina* seedling density to shoots	g C $shoot^{-1}$	0.0374	n = 120 shoots
$Seedling_{RD:C}$	*Z. marina* seedling density t0o shoots	g C $shoot^{-1}$	0.0384	n = 120 shoots

Table 10. Sample collection and handling synopsis.

Sample Matrix	Analyte(s)/ Parameters (s)	Total # samples	Sample Volume (per sample)	Type Container	Sample Preservation	Max. Allowable Holding Time
Site Water	TSS	6 samples site^{-1} month^{-1} (192 total)	1000 mL collected vol filtered varies	Clear Nalgene bottle with cap	Stored at 4 °C	< 48 hrs
		2 blanks site^{-1} month^{-1} (64 total)	1000 mL collected 200 mL filtered	Clear Nalgene bottle with cap	Stored at 4 °C	< 48 hrs
Site Water	Chl a	6 samples site^{-1} month^{-1} (192 total)	500 mL collected vol filtered varies	Amber Nalgene Bottle with cap	Stored at 4 °C	< 24 hrs
		2 blanks site^{-1} month^{-1} (64 total)	500 mL collected 50 mL filtered	Amber Nalgene Bottle with cap	Stored at 4 °C	< 24 hrs
Site Water	$NO_2 + NO_3$	6 samples site^{-1} month^{-1} (192 total)	500 mL collected* 50 mL filtered	Amber Nalgene Bottle with cap	Filtered, frozen at -20 °C polyethylene centrifuge tubes	< 1 month
Site Water	$NH_4 + NH_3$	6 samples site^{-1} month^{-1} (192 total)	500 mL collected* 50 mL filtered	Amber Nalgene Bottle with cap	Filtered, frozen at -20 °C polyethylene centrifuge tubes	< 1 month
Site Water	OPO_4	6 samples site^{-1} month^{-1} (192 total)	500 mL collected* 50 mL filtered	Amber Nalgene Bottle with cap	Filtered, frozen at -20 °C polyethylene centrifuge tubes	< 1 month
Sediment pore-water	$NO_2 + NO_3$	5 samples site^{-1} month^{-1} (160 total)	42 cm^3 collected 50 mL filtered	Amber Nalgene Bottle with cap	Extracted in 2 M KCl, filtered, frozen at -20 °C polyethylene centrifuge tubes bags	< 1 month

Sample Matrix	Analyte(s)/ Parameters (s)	Total # samples	Sample Volume (per sample)	Type Container	Sample Preservation	Max. Allowable Holding Time
Sediment pore-water	NH_4 +NH_3	5 samples site^{-1} month^{-1} (160 total)	42 cm^3 collected 50 mL filtered	Amber Nalgene Bottle with cap	Extracted in 2 M KCl, filtered, frozen at -20 °C polyethylene centrifuge tubes	< 1 month
Sediment pore-water	OPO_4	5 samples site^{-1} month^{-1} (160 total)	42 cm^3 collected 50 mL filtered	Amber Nalgene Bottle with cap	Extracted in 2 M KCl, filtered, frozen at -20 °C polyethylene centrifuge tubes	< 1 month
Biomass	Seagrass Biomass	5 samples site^{-1} month^{-1} (160 total)	380 cm^3	pre-labeled plastic bag	Sieved in field, stored at 4 °C under grow lamp	< 3 days
Biomass	Macroalgae Biomass	5 samples site^{-1} month^{-1} (160 total)	625 cm^3	pre-labeled plastic bag	Rinsed in field, stored at 4 °C under grow lamp	< 3 days
Biomass	Epiphyte Biomass	15 samples site^{-1} month^{-1} (480 total)	1 shoot (varies with shoot)	pre-labeled plastic bag	Stored at 4 °C under grow lamp	< 3 days
Sediment	Seed bank Density	15 samples site^{-1} month^{-1} (480 total)	167 cm^3	pre-labeled plastic bag	Stored at 4 °C	< 2 weeks
Sediment	Seed bank Viability	15 samples site^{-1} month^{-1} (480 total)	167 cm^3	pre-labeled plastic bag	Stored at 4 °C	< 2 weeks

FIGURES:

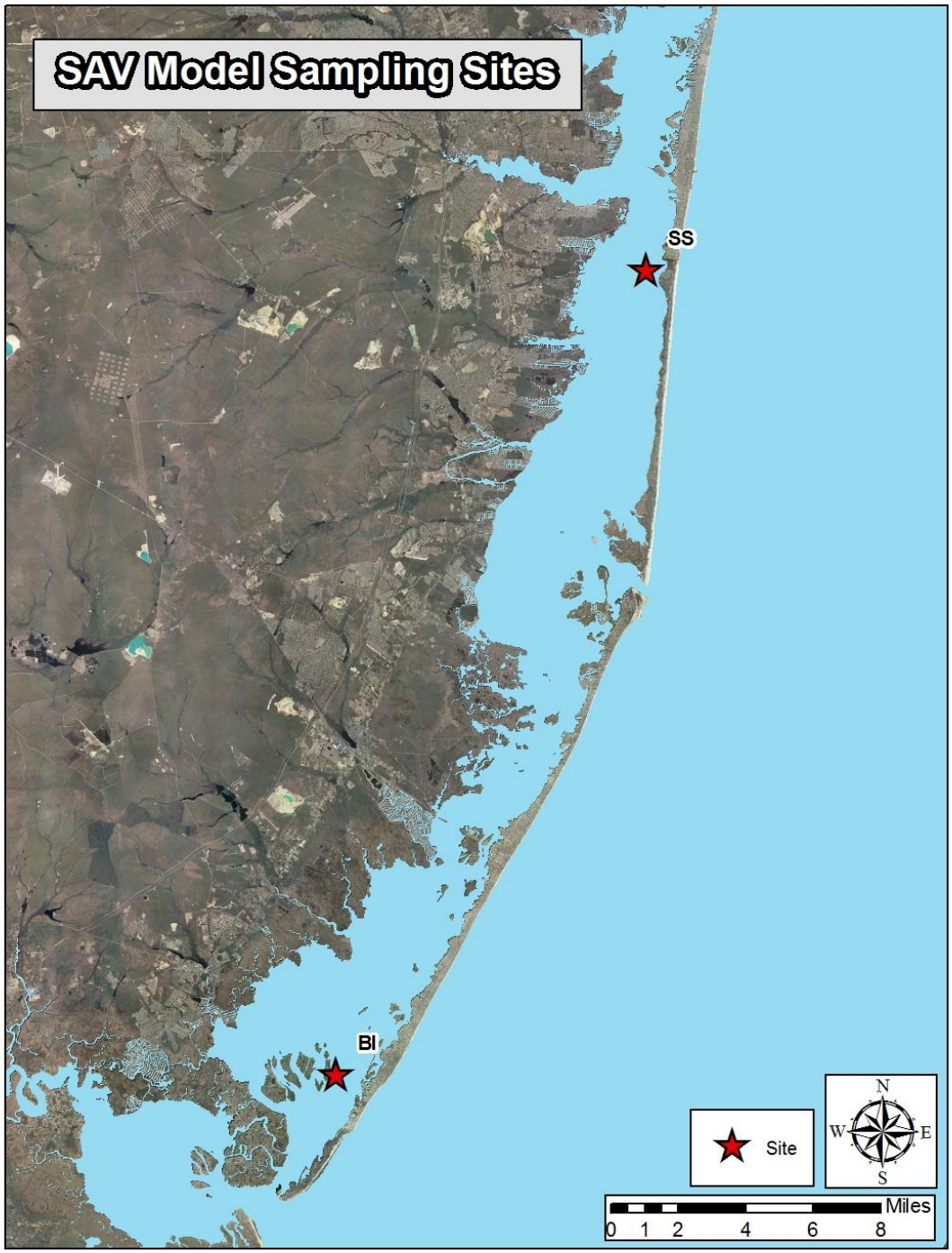


Figure 1. Map of sampling sites located in Little Egg Harbor (Barrel Island - BI) and Barnegat Bay (Seaside Park - SS).

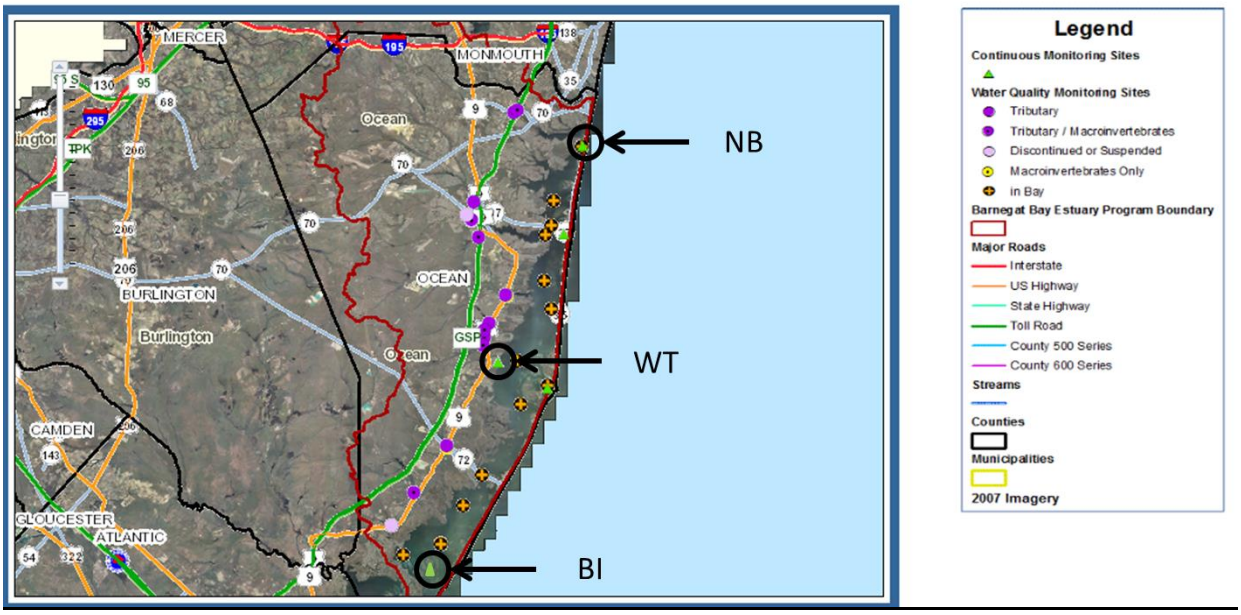


Figure 2. Map of continuous water quality monitoring stations used for model scenarios. Northern Barnegat – NB and Waretown – WT are stations maintained by NJDEP. BI – Barrel Island is a station maintained as a part of this project. Figure modified from http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm.

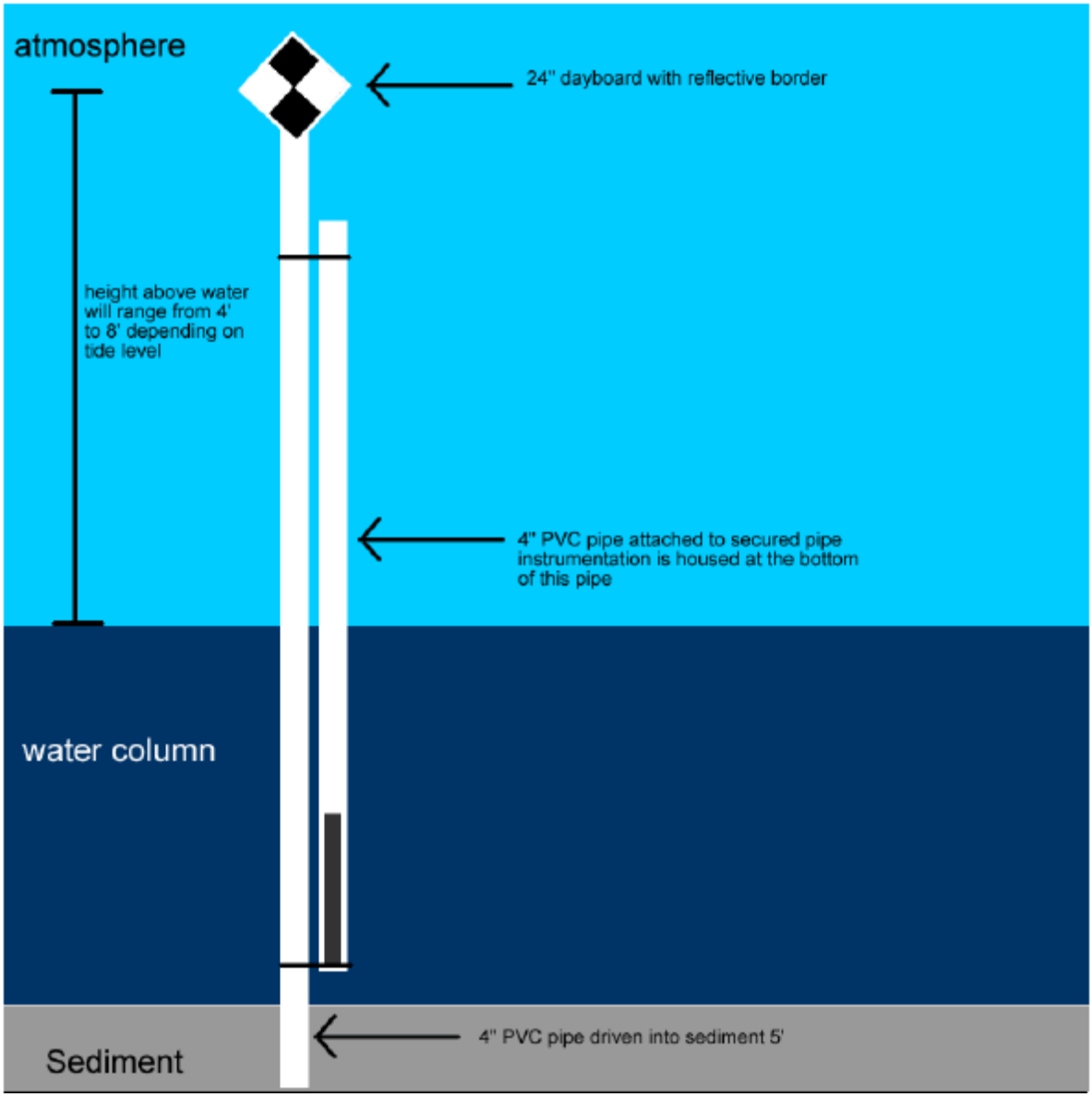


Figure 3. Schematic of datasonde deployment structure, showing 4" PVC pipe anchored into the sediment with a second 4" PVC pipe attached to house the scientific instrumentation.

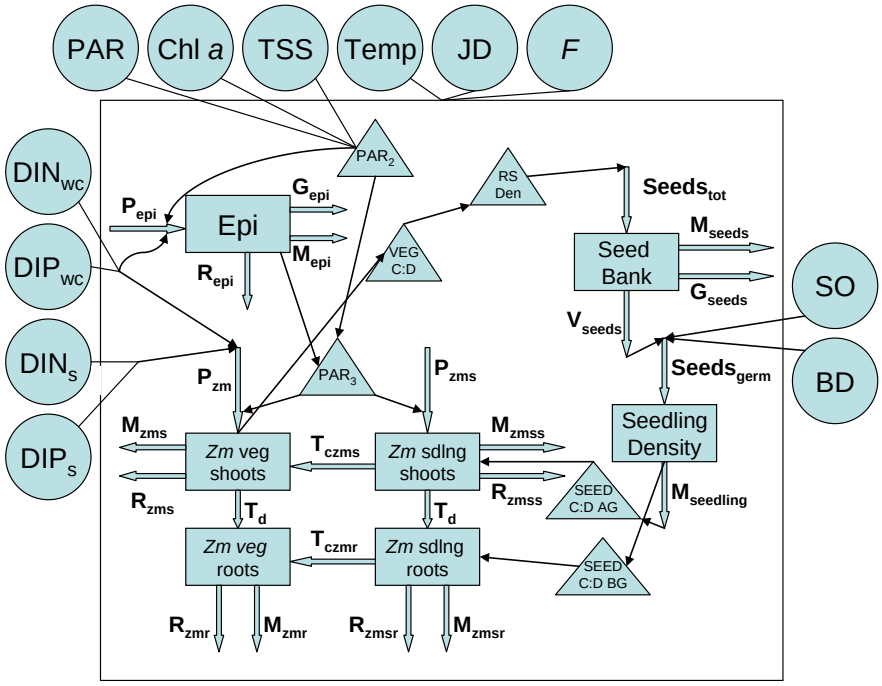


Figure 4. Conceptual diagram for *Z. marina* production and sexual reproduction model. Circles = forcing functions, triangles = modifiers, squares = state variables, thick arrows = flows, and thin arrows = iterations. Temp, JD, and F affect multiple processes so are not connected to minimize diagram complexity.

APPENDICES:

Appendix 1. Standard Operating Procedures for entire project

Appendix 1.1 Field Sampling:

Before Leaving the Dock:

1) Make sure that the following items are prepared and are on the boat or are ready in the laboratory once the sampling is complete. Check off each item as it is addressed.

General

a. Field sheets
b. Pencils
c. Sharpies
d. Plastic clipboard
e. Dry box
f. 2 - 5 gallon buckets
g. Dive gear (mask, snorkel, wet suit)
h. Camera w/case
i. Extra memory card for camera
j. Cell phone in waterproof case
k. Cooler for lunch w/ice and water
l. State boaters safety card
m. Sunscreen
n. Bug spray
o. Dive Flag for boat
p. In water dive flag
q. Boat key
r. Boat Log Book
s. Anchor
t. Diver Ladder
u. Extra dock line

Water Quality

a. Hand held YSI in bucket
b. YSI for switch out
c. Extra batteries for YSI
d. 1 cooler with ice
e. 6 TSS bottles (3 per site)
f. 6 Chl a bottles (3 per site)
g. 6 Nutrient bottles (3 per site)

Biomass

a. biomass corer
b. field sieve
c. 10 - 1 gallon pre-labeled Ziploc bags (5 per site) for Z marina
d. 10 - 1 gallon pre-labeled ziploc bags (5 per site) for macroalgae
e. 30 - 1 quart pre-labeled ziploc bags (15 per site) for epiphytes

Seed Bank Density

a. 1 cooler with ice
b. 7 - 2.25" diameter x 6" cores (15 per site, 5 per depth) for seed bank density (slice in field)
c. 14 plugs

d. Plunger
e. 90 labeled sample bags
f. 0-2 cm section
g. metal slicer

Sediment Analysis

v. 1 large cooler with water
w. 18 (3.25"x 6") cores for sediment analysis (9 per site)
x. 36 plugs
y. 54 labeled sample bags

Locating the Station:

1) "Tighten" the resolution on console GPS unit and motor over station (into wind/current, whichever is dominant)
2) Anchor nearby (as close as possible without interfering with diver in water), considering wind and current.
3) Record site location (transect and station), time (EST), and general weather conditions on field sheet.

Once at the Station: (for specific information about each protocol please see Appendix 2.2)

1) Deploy 6600 (if switching out units) and 650 YSI units - allow to contact bottom briefly, pull up approx 10cm from bottom and lash to cleat, allow to equilibrate for 2-3 minutes, record physico-chemical parameters on field sheet (refer to field sheet).
2) Replace 6600 YSI unit in permanent station. Download data from Li-COR data logger and replace logger in waterproof housing. Clean PAR sensor of any debris or fouling.
3) Obtain depth and Secchi values.
4) Obtain water column samples.
5) Diver enters water to put dive flag in a clear area to warn passing boaters of divers in the water.
6) Diver obtains sediment seed bank cores.
7) Diver obtains sediment organic content cores.
8) Diver obtains sediment cores from each site and transports them back to the boat. Technician on board sections core, places sample into the bags with appropriate label, and places in cooler.
9) Diver obtains macroalgal samples, places them in the appropriate bag and transports the samples back to the boat. Technician on board places sample in cooler.
10) Diver obtains epiphyte samples, places them in the appropriate bag and transports the samples back to the boat. Technician on board places sample in cooler.
11) Diver obtains seagrass core and places it on box sieve held by a second diver. The second diver sieves the sample removing excess sediment, places the in the appropriate bag and transports the samples back to the boat. Technician on board places sample in cooler.
12) Supervisor/delegated technician reviews field sheet and initials it if complete and approved.
13) Team retrieves dive float and moves on to next station.

Upon return:

Field Sheets

1) Field sheets are reviewed to verify that all stations and samples are accounted by the project manager.
2) Sheets are scanned and placed in designated physical and electronic folders/binders and placed in an approved location at MSEFS
3) Photocopied sheets are relocated to a secondary secure location (Richard Stockton College NAMS Office) and placed in the binder there.

Water Column:

1) Water column samples are cross-checked with field sheets to assure all samples are accounted for. The supervisor or senior technician should initial on a tracking sheet that all samples are accounted for. If any samples are missing, the site should be revisited at the next possible opportunity to obtain a replacement sample.
2) All water samples are processed (or "fixed") within 4-6 hours of collection. All samples that were "fixed" will be processed within 48 hours of sample collection.

Biomass:

1) Biomass samples are cross-checked with field sheets to assure all samples are accounted for. The supervisor or senior technician should initial on a tracking sheet that all samples are accounted for. If any samples are missing, the site should be revisited at the next possible opportunity to obtain a replacement sample.
2) Biomass samples from the day are placed in a larger, single bag (small or medium wastepaper basket bag) and placed in the "cold water table" in building 504 at MSEFS.
3) All biomass samples should be kept in the "cold water table" and processed within 72 hrs. If unable to process within 72 hrs, samples should be frozen along with the biomass samples.

Sediment:

Total organic content and Seed Bank Samples

1) Sediment total organic content and seed bank samples are cross-checked with field sheets to assure all samples are accounted for. The supervisor or senior technician should initial on a tracking sheet that all samples are accounted for. If any samples are missing, the site should be revisited at the next possible opportunity to obtain a replacement sample.
2) Sediment samples from the day are placed in a larger, single bag (small or medium wastepaper basket bag) and placed in the "cold water table" in building 504 at MSEFS.
3) All sediment samples should be kept in the "cold water table" and processed within 72 hrs.

Pore water Nutrients

1) Sediment porewater samples are cross-checked with field sheets to assure all samples are accounted for. The supervisor or senior technician should initial on a tracking sheet that all samples are accounted for. If any samples are missing, the site should be revisited at the next possible opportunity to obtain a replacement sample.

2) All sediment porewater samples are processed (or “fixed”) within 4-6 hours of collection. All samples that were “fixed” will then be frozen until processing.

YSI/CTD:

1) A post-calibration check of the YSI unit used to obtain physical parameters in the field must be performed to verify instrument/probe function and accuracy.
2) The YSI600 unit should be rinsed with fresh water and battery voltage assessed. If necessary, change batteries before next field day. The 650MDS display should be wiped down with a damp cloth and dried.

Other:

1) The field sheets should be transcribed into a digital spreadsheet as soon as possible.

Appendix 1.2 Water Column Data sonde Calibration *(RSC SOP):*

Prior to field deployment and immediately following retrieval all YSI 6600 and YSI 650data sondes will undergo the following calibration procedures:

1.2.1 Salinity/Conductivity

Scope of Work: In-situ measurements of specific conductance/salinity will be made in estuarine and near-coastal waters.

Sample Handling and Preservation: Not applicable. Conductivity/Specific Conductivity/Salinity measurements will be made in-situ.

Equipment: Conductivity probe: Yellow Springs Incorporated (Y.S.I.) model #6560

Parameter: Salinity

Units: parts per thousand (ppt)
Sensor Type: Calculated from conductivity and temperature
Range: 0 to 70 ppt
Accuracy: +/- 1.0% of reading or 0.1 ppt, whichever is greater

Resolution: 0.01 ppt

Temperature compensation is performed automatically by the instrument via pairing with a Y.S.I. thermistor (model #6560).

Reagents: Calibration standards required for specific conductance are purchased from Y.S.I. A standard of 10 mS/cm3 (p/n 060911) is used to calibrate for conductivity for sampling.

Prior to use, the accuracy of any conductivity probe utilized in this study will be verified by performing a two-point calibration check via immersion in the following standards: 0 mS/cm3 (de-ionized water) and 10 mS/cm3 (p/n 0660911).

The Conductivity cell constant is obtained during each calibration and recorded on the calibration sheet.

Only new standards (fresh from the bottle) are used for calibrations. Used standards are discarded.

Upon expiration standards are discarded and replaced with new standard.

Date of receipt, expiration dates, and date-of-first-use are recorded on each bottle of standard.

Maintenance: Probes are rinsed with fresh water between deployments and stored in a water-saturated air environment inside the sonde calibration/storage cup. If functionality/accuracy of the conductivity probe is in question, probes may be cleaned with a mild acetic acid solution to "shine" the terminals (as per the manufacturer's recommendations). If function is still questionable, the probe may be removed and replaced.

Calibration: Conductivity calibrations are performed by first rinsing the probe with the selected standard, followed by via immersion in the standard and accepting the reading after sixty seconds.

Procedure: Conductivity probes will be deployed using a Y.S.I. 600XL, 6920, or 6920V2 datasonde paired with a 650MDS "handheld" digital display unit, and measurements obtained, in-situ. A minimum of one-minute (60 seconds) will be allowed for acclimation/stabilization before the value is recorded for one-time sampling at each station

For extended deployments, Conductivity probes will be deployed on a 6600 or 660V2-4 datasonde for an extended period (up to 4 weeks-dependent on ice and/or fouling conditions) set to a pre-determined sample interval.

Quality Control: New conductivity probes are shipped by Yellow Springs Incorporated (Y.S.I.) with a certificate that the probe passed their QA/QC.

Calibrations of specific conductance are performed prior to each pre and post deployment. Detailed records of calibration procedures and diagnostic values are maintained. Diagnostic values (the cell constant) are recorded and considered to verify probe functionality as per the manufacturer's recommendations (the cell constant should be between 4.6 and 5.45). Immersion of calibrated probes in the conductivity standard post-calibration is performed to verify accuracy; these measured values are recorded as part of the calibration record.

1.2.2 Temperature

Scope and Application: In-situ measurements of Temperature will be made in estuarine and near-coastal waters.

Sample Handling and Preservation: Not applicable. Temperature measurements will be made in-situ.

Equipment: Yellow Springs Incorporated Thermistor (model #6560).

Parameter: Temperature

Units: Celsius (C)

Sensor Type: Thermistor
Model #: 6560
Range: -5 to 45 °C
Accuracy: +/-0.15 °C

Resolution: 0.01 °C

Reagent: N/A
Maintenance: Probes are rinsed with fresh water between deployments and stored in a water-saturated air environment inside the sonde calibration/storage cup.
Calibration Procedure: Calibration cannot be performed on Y.S.I. thermistors, but functionality/accuracy can be assessed. Thermistors will be checked against a fractional

thermometer as well as against a NIST certified thermometer at the NJ DEP Leeds Point Laboratory. Malfunctioning thermistors will be replaced with a functioning one and functionality/accuracy will be assessed on the replacement thermistor.

Procedure: Thermistors will be deployed (installed on a Y.S.I. 6600) datasonde paired with a 650MDS "hand-held" digital display unit in-situ. A minimum of one-minute (60 seconds) will be allowed for acclimation/stabilization before the value is recorded.

Quality Control: New thermistors are shipped by Yellow Springs Incorporated (Y.S.I.) with a certificate that the probe passed their QA/QC.

Verification of accuracy will be performed prior to pre and post deployment by comparing the sonde temperature readings to the readings of a Fisherbrand Red-Spirit Fractional Degree thermometer (0.1°C graduations) that has been placed in the same solution. Accuracy must be +/- .10°C.

Dated records of thermistor calibration checks will be maintained.

1.2.3 Turbidity

Scope of Work: In-situ measurements of turbidity will be made in estuarine and near-coastal waters. These measurements are not collected by a certified laboratory and therefore will only be used in this project and not posted on the web.

Sample Handling and Preservation: Not applicable. Measurements of turbidity will be made in-situ.

Equipment: Turbidity Probe: Yellow Springs Incorporated (Y.S.I.) (model #606136)

Parameter: turbidity

Units: NTU (Nephelometric Turbidity Units)

Sensor Type: optical
Model #: 606136
Range: 0 to 1000 NTU
Accuracy: ±2% of reading or 0.3 NTU, whichever is greater

Resolution: 0.1 NTU

Reagents: Calibration standards required for turbidity are purchased from YSI. A standard of 126 NTU is used to calibrate for turbidity for sampling.
Prior to use, the accuracy of any turbidity probe utilized will be verified by performing a two-point calibration check via immersion in the following standards: 0 NTU (de-ionized water) and 126 NTU (p/n 607300).

Only new standards (fresh from the bottle) are used for calibrations. Used standards are discarded.

Upon expiration standards are discarded and replaced with new standard.

Date of receipt, expiration dates, and date-of-first-use are recorded on each bottle of standard.

Maintenance: Probes are rinsed with fresh water between deployments and stored in a water-saturated air environment inside the sonde calibration/storage cup. Probe wipers are changed monthly, at minimum; more often if wear or fouling is present.

Calibration: A two-point calibration is employed for turbidity probe. Calibration is performed by first rinsing the probe with 0 NTU standard (which is then discarded), followed by immersion in fresh/new 0 NTU standard and accepting the reading when stable (a minimum of 60 seconds). The probe is then thoroughly dried and rinsed with fresh/new126 NTU standard, followed by immersion in fresh/new 126 NTU standard. Pre and post calibration values for both standards are recorded on the calibration sheet.

Procedure: Turbidity probes are deployed (installed on a Y.S.I. 6920V2, 6600, 6600V2-4) datasonde paired with a 650MDS "handheld" digital display unit, and measurements obtained, in-situ.

For extended deployments, turbidity probes will be deployed on a 6600 datasonde for an extended period (up to 4 weeks-dependent on ice and/or fouling conditions) set to a pre-determined sample interval.

Quality Control: New turbidity probes are shipped by Yellow Springs Incorporated (Y.S.I.) with a certificate that the probe passed their QA/QC.

Calibrations of turbidity are performed prior to each pre and post deployment. Detailed records of calibration procedures and pre/post values are maintained. The turbidity probe is immersed in 0 NTU and 126 NTU standards post-calibration to verify accuracy. These measured values are recorded as part of the calibration record.

1.2.4 Chlorophyll a

Scope of Work: In-situ measurements of chlorophyll will be made in estuarine and near-coastal waters.

Sample Handling and Preservation: Not applicable. Measurements of chlorophyll will be made in-situ.

Equipment: Yellow Springs Incorporated Chlorophyll a Probe (model # 606025)

Parameter: chlorophyll

Units: $\mu g\ L^{-1}$

Sensor Type: optical fluorescence

Model #: 606025
Range: 0 to 400 $\mu g\ L^{-1}$
Accuracy: ±2% of reading

Resolution: 0.1 $\mu g\ L^{-1}$

Reagents: Calibration standards required for chlorophyll are purchased from YSI. A Rhodamine WT standard is used to calibrate the chlorophyll sensor for sampling.

Prior to use, the accuracy of any chlorophyll probe utilized will be verified by performing a two-point calibration check via immersion in the following standards: 0 μg L^{-1} (de-ionized water) and a Rhodamine WT standard which varies with temperature.

Only new standards (fresh from the bottle) are used for calibrations. Used standards are discarded.

Upon expiration standards are discarded and replaced with new standard.

Date of receipt, expiration dates, and date-of-first-use are recorded on each bottle of standard.

Maintenance: Probes are rinsed with fresh water between deployments and stored in a water-saturated air environment inside the sonde calibration/storage cup. Probe wipers are changed monthly, at minimum; more often if wear or fouling is present.

Calibration: A two-point calibration is employed for the chlorophyll sensors. To calibrate set the units report output to display both the μg L^{-1} and RFU units. Mix the calibration standard and set aside so that both the Rhodamine WT standard and the sonde are at ambient temperature. Verify that the optics are clean. And finally, test the operation of the wiper. Confirm that the wipers on all optical probes park correctly and that the pads are in good condition before proceeding.

In the two-point calibration, the first point will be zero and the second point entry will come from the tables for the Rhodamine WT dye used to prepare the standard. Measure the temperature of the dye standard and use the corresponding μg L^{-1} value in the table. Enter this number and run the wiper. Wait at least 30 seconds before accepting the calibration point. Visually inspect the probe face to make sure it is bubble-free. Pre and post calibration values for both standards are recorded on the calibration sheet.

Procedure: Chlorophyll *a* probes are deployed (installed on a YSI 6600) datasonde paired with a 650MDS "handheld" digital display unit, and measurements obtained, in-situ.

For extended deployments, chlorophyll probes will be deployed on a 6600 datasonde for up to 4 weeks (dependent on ice and/or fouling conditions) set to a pre-determined sample interval.

Quality Control: New chlorophyll probes are shipped by Yellow Springs Incorporated (Y.S.I.) with a certificate that the probe passed their QA/QC.

Calibrations of chlorophyll are performed prior to each pre and post deployment. Detailed records of calibration procedures and pre/post values are maintained. The chlorophyll probe is immersed in 0 μg L^{-1} and a second standard value dependent on temperature post-calibration to verify accuracy. These measured values are recorded as part of the calibration record.

Appendix 1.3 Water Column Chlorophyll a *(EPA Method 445.0):*

1. Materials and Equipment:
    a. Cooler with ice
    a. 6 pre-labeled amber 500 mL polypropylene bottles with lids
    b. Fluorometer
    c. Centrifuge, capable of 675 g.
    d. Glass tissue grinder
    e. Filters, glass fiber, 47-mm or 25-mm, nominal pore size of 0.7 µm
    f. Whirl-pak bags for transporting and storing sampled filters.
    g. Aluminum foil.
    h. Laboratory tissues.
    i. Tweezers or flat-tipped forceps.
    j. Vacuum pump
    k. Room thermometer.
    l. Assorted Class A calibrated pipets.
    m. Graduated cylinders, 500-mL and 1-L.
    n. Volumetric flasks, Class A calibrated, 25-mL, 50-mL, 100-mL and 1-L capacity.
    o. Glass rods.
    p. Pasteur type pipets or medicine droppers.
    q. Disposable glass cuvettes for the fluorometer.
    r. Filtration apparatus consisting of 1 or 2-L filtration flask, 47-mm fritted glass disk base and a glass filter tower.
    s. Centrifuge tubes, polypropylene or glass, 15-mL capacity with nonpigmented screw-caps.
    t. Polyethylene squirt bottles.
    u. Acetone, HPLC grade, (CASRN 67-64-1).
    v. Hydrochloric acid (HCl), concentrated (sp. gr.1.19), (CASRN 7647-01-0).
    w. Chlorophyll *a* free of chlorophyll *b*.
    x. Deionoized water
2. Solutions:
    a. ***0.1 N HCl Solution*** -- Add 8.5 mL of concentrated HCl to approximately 500 mL water and dilute to 1 L.
    b. ***Aqueous Acetone Solution*** -- 90% acetone /10% water. Carefully measure 100 mL of water into the 1-L graduated cylinder. Transfer to a 1-L flask or storage bottle. Measure 900 mL of acetone into the graduated cylinder and transfer to the flask or bottle containing the water. Mix, label and store.
    c. ***Chlorophyll Stock Standard Solution*** (SSS) -- Chlorophyll *a* from a commercial supplier will be shipped in an amber glass ampoule which has been flame sealed. This dry standard should be stored at -20 or -70EC in the dark and the SSS prepared just prior to use. Tap the ampoule until all the dried chlorophyll is in the bottom of the ampoule. In subdued light, carefully break the tip off the ampoule. Transfer the entire contents of the ampoule into a 50-mL volumetric flask. Dilute to volume with 90% acetone, label the flask and wrap with aluminum foil to protect from light. The concentration of the solution must be determined spectrophotometrically using a multiwavelength spectrophotometer. When stored in a light and airtight container at freezer temperatures, the SSS is stable for at least six months. The concentration of all dilutions of the SSS must be determined spectrophotometrically each time they are made.

   d. ***Laboratory Reagent Blank*** (LRB) -- A blank filter which is extracted and analyzed just as a sample filter. The LRB should be the last filter extracted of a sample set. It is used to assess possible contamination of the reagents or apparatus.
   e. ***Chlorophyll *a* Primary Dilution Standard Solution*** (PDS) -- Add 1 mL of the SSS to a clean 100-mL flask and dilute to volume with the aqueous acetone solution. If exactly 1 mg of pure chlorophyll *a* was used to prepare the SSS, the concentration of the PDS is 200 µg/L. Prepare fresh just prior to use.
   f. ***Quality Control Sample*** (QCS) -- Since there are no commercially available QCSs, dilutions of a stock standard of a different lot number from that used to prepare calibration solutions may be used.
3. Field Methods:
   a. At site each sample bottle will be rinsed 3x with site water.
   b. Once the bottle has been rinsed the sample will be collected at approximately 0.5 m depth and capped.
   c. This will be repeated 3 x per site. The samples will be placed on ice in a cooler and transported back to the lab.
4. Laboratory Procedure: Filtering
   a. Assemble the filtration apparatus and attach the vacuum source with vacuum gauge and regulator. Vacuum filtration should not exceed 6 in. Hg (20 kPa).
   b. Prior to drawing a subsample from the water sample container, thoroughly but gently agitate the container to suspend the particulates (stir or invert several times).
   c. Pour the subsample into a graduated cylinder and accurately measure the volume.
   d. Pour the subsample into the filter tower of the filtration apparatus and apply a vacuum (not to exceed 20 kPa). A sufficient volume has been filtered when a visible green or brown color is apparent on the filter.
   e. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.
   f. Remove the filter from the fritted base with tweezers, fold once with the particulate matter inside, lightly blot the filter with a tissue to remove excess moisture, wrap the filter with aluminum foil to protect the phytoplankton from light, and place it in the pre-labeled whirl-pak bag.
   g. Store the filters in the dark at -80 °C for analysis within 3.5 weeks.
5. Laboratory Procedure: Quality Control
   a. All of the procedures listed below should be performed prior to or during each time samples are processed
   b. **Instrumental Detection Limit (IDL)** -- Zero the fluorometer with a solution of 90% acetone on the maximum sensitivity setting. Pure chlorophyll a in 90% acetone should be serially diluted until it is no longer detected by the fluorometer on a maximum sensitivity setting.
   c. **Estimated Detection Limit (EDL)** -- Several blank filters should be extracted according to the procedure in Sect. 6, using clean glassware and apparatus, and the fluorescence measured. A solution of pure chlorophyll a in 90% acetone should be serially diluted until it yields a response which is 3X the average response of the blank filters.

d. **Quality Control Sample (QCS)** -- Verify the calibration standards and acceptable instrument performance with the analysis of a QCS. If the determined value is not within the confidence limits established by project data quality objectives, then the determinative step of this method is unacceptable. The source of the problem must be identified and corrected before continuing analyses.

e. **Extraction Proficiency** -- Personnel performing this method for the first time should demonstrate proficiency in the extraction of sampled filters Twenty to thirty natural samples should be obtained using the procedure outlined in section 4 of this method. Sets of 10 or more samples should be extracted and analyzed that provide midscale readings. Obtain response factors according to Sect. 6. The percent relative standard for chlorophyll *a* for each sensitivity setting as follows: deviation (%RSD) of uncorrected values of chlorophyll *a* should not exceed 15% for samples that are F = C /R approximately 10X the IDL. RSD for pheophytin *a* might typically range from 10 to 50%.

f. **Corrected Chl *a*** -- Prepare 100 mL of a 50 ppb chl *a* solution in 90% acetone. The new analyst should analyze 5-10 separate aliquots, using separate cuvettes, according to instructions in Section 6. Process the results according to Section 7 and calculate separate means and %RSDs for corrected and uncorrected chl *a*. If the means differ by more than 10%, then the stock chl *a* has probably degraded and fresh stock should be prepared. The %RSD for corrected chl *a* should not exceed 5%. If the %RSD exceeds 5%, repeat the procedure until the %RSD # 5%.

g. **Laboratory Reagent Blank (LRB)** – The laboratory must analyze at least one blank filter with each sample batch. The LRB should be the last filter extracted. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the IDL indicate contamination from the laboratory environment. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates must be analyzed after the contamination has been corrected and acceptable LRB values have been obtained.

h. **Calibration** -- Calibration must be performed bimonthly or when there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier. Prepare 0.2, 2, 5, 20 and 200 µg chl *a*/L calibration standards from the PDS. Allow the instrument to warm up for at least 15 min. Measure the fluorescence of each standard at sensitivity settings that provide midscale readings. Obtain response factors for chlorophyll a for each sensitivity setting as follows:

$$F_s = \frac{C_a}{R_s}$$

where:
F_s = response factor for sensitivity setting, S.
R_s = fluorometer reading for sensitivity s setting, S.
C_a = concentration of chlorophyll a.

6. Laboratory Procedure: Extraction of Filter Samples

a. Remove samples from the freezer but keep them in the dark. Set up the tissue grinder and have on hand tissues and squirt bottles containing water and acetone. Workspace lighting should be the minimum that is necessary to read instructions and operate instrumentation.

b. Remove a filter from its container and place it in the glass grinding tube. The filter may be torn into smaller pieces to facilitate extraction.

c. Push it to the bottom of the tube with a glass rod. With a volumetric pipet, add 4 mL of the aqueous acetone solution (Sect. 4) to the grinding tube. Grind the filter until it has been converted to a slurry.
    i. **NOTE:** Although grinding is required, care must be taken not to overheat the sample. Good judgement and common sense will help you in deciding when the sample has been sufficiently macerated.)
d. Pour the slurry into a 15-mLscrew-cap centrifuge tube and, using a 6-mL volumetric pipet, rinse the pestle and the grinding tube with 90% acetone. Add the rinse to the centrifuge tube containing the filter slurry. Cap the tube and shake it vigorously. Place it in the dark before proceeding to the next filter extraction.
e. Before placing another filter in the grinding tube, use the acetone and water squirt bottles to thoroughly rinse the pestle, grinding tube and glass rod. The last rinse should be with acetone. Use a clean tissue to remove any filter residue that adheres to the pestle or to the steel rod of the pestle.
f. Proceed to the next filter and repeat the steps above. The entire extraction with transferring and rinsing steps takes 5 min. Approximately 500 mL of acetone and water waste are generated per 20 samples from the rinsing of glassware and apparatus.
g. Shake each tube vigorously before placing them to steep in the dark at 4 °C. Samples should be allowed to steep for a minimum of 2 h but not to exceed 24 h. The tubes should be shaken at least once during the steeping period.
h. After steeping is complete, shake the tubes vigorously and centrifuge samples for 15 min at 675 g or for 5 min at 1000 g. Samples should be allowed to come to ambient temperature before analysis by letting them stand at room temperature for 30 min.
i. Recalibrate the fluorometer if the room temperature fluctuated ± 3 °C from the last calibration date.
j. After the fluorometer has warmed up for at least 15 min, use the 90% acetone solution to zero the instrument on the sensitivity setting that will be used for sample analysis.
k. Pour or pipet the supernatant of the extracted sample into a sample cuvette. The volume of sample required in your instrument's cuvette should be known so that the correct amount of acid can be added in the pheophytin *a* determinative step.
    i. For a cuvette that holds 5 mL of extraction solution, 0.15 mL of the 0.1 N HCl solution should be used.
    ii. Choose a sensitivity setting that yields a midscale reading when possible and avoid the minimum sensitivity setting. If the concentration of chlorophyll *a* in the sample is > 90% of the upper limit of the LDR, then dilute the sample with the 90% acetone solution and reanalyze.
    iii. Record the fluorescence measurement and sensitivity setting used for the sample.
l. Remove the cuvette from the fluorometer and acidify the extract to a final concentration of 0.003 N HCl using the 0.1 N HCl solution. Use a pasteur type pipet to thoroughly mix the sample by aspirating and dispensing the sample into the cuvette, keeping the pipet tip below the surface of the liquid to avoid aerating the sample.
m. Wait 90 sec before measuring fluorescence again.
    i. **NOTE:** Proper mixing is critical for precise and accurate results. Twenty-five to thirty-five samples can be extracted and analyzed in one 8 hr day.
n. Run a LRB and a QCS sample every 5 samples to determine if the fluorometer is still calibrated. Recalibrate as necessary.

7. Laboratory Procedure: Data Calculations
    a. For "uncorrected chlorophyll a," calculate the chlorophyll a concentration in the extract as:

$$C_{E,u} = R_b \times F_s$$

Where:
$C_{E,u}$ = uncorrected chlorophyll a concentration(μg L^{-1}) in the extract solution analyzed
R_b = fluoresence response of sample extract b before acidification
F_s = fluoresence respnse factor for sensitivity setting S.

    b. Calculate the "uncorrected" concentration of chlorophyll a in the whole water sample as follows:

$$C_{S,u} = \frac{C_{E,u} \times extract\ volume\ (L) \times DF}{Sample\ Volume\ (L)}$$

where:
$C_{S,u}$ = uncorrected chlorophyll a concentration (μg L^{-1}) in the whole water sample
Extract volume = volume (L) of extraction prepared before any dilutions
DF = dilution factor
Sample volume = volume (L) of whole water sample

    c. For "corrected chlorophyll *a*", calculate the chlorophyll *a* concentration in the extract as :

$$C_{E,c} = F_s \left(\frac{r}{r-1}\right)(R_b - R_a)$$

Where:
$C_{E,C}$ = corrected chlorophyll *a* concentration (μg L^{-1}) in the extract solution analyzed
F_s = response factor for the sensitivity setting S
r = the before-to-after acidification ratio of a pure chlorophyll *a* solution
R_b= fluorescence of sample extract before acidification
R_a = fluorescence of sample extract after acidification.

    d. Calculate the "corrected" concentration of chlorophyll a in the whole water sample as follows:

$$C_{S,c} = \frac{C_{E,} \times extract\ volume\ (L) \times DF}{Sample\ Volume\ (L)}$$

Where:
$C_{S,c}$ = corrected chlorophyll a concentration (μg L^{-1}) in the whole water sample
Extract volume = volume (L) of extraction prepared before any dilutions
DF = dilution factor
Sample volume = volume (L) of whole water sample

    e. Calculate the pheophytin *a* concentration as follows:

$$P_E = F_s \left(\frac{r}{r-1}\right)(rR_a - R_b)$$

$$P_S = \frac{P_{E,} \times extract\ volume\ (L) \times DF}{Sample\ Volume\ (L)}$$

Where:
P_E = pheophytin *a* concentration (µg L^{-1}) in the extract solution analyzed
P_S = corrected pheophytin a concentration (µg L^{-1}) in the whole water sample

Appendix 1.4 Water Column Total Suspended Solids *(USGS Method I-3765-85):*

1. Materials and Equipment:
    a. Cooler with ice
    b. 6 pre-labeled clear 1 L polypropylene bottles with lids
    c. Forceps
    d. Pre-washed and weighed Gelman glass fiber filters (0.47 cm) in labeled aluminum pans
    e. Graduated cylindar
    f. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
    g. Suction flask
    h. Vacuum pump
    i. Drying oven
    j. Desiccator.
    k. Analytical balance, capable of weighing to 0.1 mg.
2. Field Methods:
    a. At site each sample bottle will be rinsed 3x with site water.
    b. Once the bottle has been rinsed the sample will be collected at approximately 0.5 m depth and capped.
    c. This will be repeated 3 x per site. The samples will be placed on ice in a cooler and transported back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Preparation of glass fiber filter disc:
        i. Place the glass fiber filter on the membrane filter apparatus with wrinkled surface up.
        ii. While vacuum is applied, wash the disc with three successive 20 mL volumes of distilled water.
        iii. Remove all traces of water by continuing to apply vacuum after water has passed through.
        iv. Remove filter from membrane filter apparatus with forceps, place into a labeled aluminum pan, and dry in an oven at 103-105°C for one hour.
        v. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).
        vi. Weigh immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.
    b. Place pre-washed filter on filtering apparatus.
    c. Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.
    d. Shake the sample vigorously and measure out 100 mL of sample using a graduated cylinder and pour into filtering apparatus. Filter sample.
    e. Visually inspect the filter for any apparent color. If no color appears measure out an additional 100 mL and filter again. Stop filtering once color appears on the filter.

Remove all traces of water by continuing to apply vacuum after sample has passed through.

f. With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.
    i. NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, use an unweighed glass fiber filter of choice affixed in the filter assembly.
    ii. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five mL increments for timing are suggested.
    iii. Continue to record the time and volume increments until filtration rate drops rapidly.
    iv. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate.
    v. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.
g. Record volume filtered on datasheet.
h. Carefully remove the filter from the filter support. Dry at least one hour at 103-105°C in drying oven. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).
i. Repeat for all samples and one additional sample blank (repeat same procedure but replace sample with 100 mL DI water).
j. Calculate non-filterable residue as follows:
    i. where: $TSS\ mg\ L^{-1} = \frac{(A-B)\times 1{,}000}{C}$
    ii. A = weight of filter + residue in mg
    iii. B = weight of filter in mg
    iv. C = mL of sample filtered

Appendix 1.5 Water Column NO_2 + NO_3 (Ringuet et al., 2011):

1. Materials and Equipment:
    a. Cooler with ice
    b. 5 pre-labeled acid washed (10% HCl) amber 500 mL polypropylene bottles with lids
    c. potassium dihydrogen phosphate (KH_2PO_4)
    d. potassium hydroxide (KOH)
    e. ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$
    f. sulfanilamide
    g. HCL
    h. N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$
    i. nitrate reductase(AtNAR1)
    j. NADH
    k. Certified reference Material
    l. ultrapure reagent grade water
    m. 0.45 um filters
    n. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    o. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
    p. Suction flask
    q. Vacuum pump
2. Field Methods:
    a. At site each sample bottle will be rinsed 3x with site water.
    b. Once the bottle has been rinsed the sample will be collected at approximately 0.5 m depth and capped.
    c. This will be repeated 3 x per site. The samples will be placed on ice in a cooler and transported back to the lab.
3. Reagent and Standard Preparation:
    a. All reagents will be prepared using acid washed (10% HCl) lab ware and 2M KCl.
    b. Buffer (pH 7.6)
        i. 27.6 µM potassium dihydrogen phosphate (KH_2PO_4), 3.756 g L^{-1}
        ii. 25 µM potassium hydroxide (KOH), 1.403 g L^{-1}
        iii. 25 µM ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$, 0.0093 g L^{-1}
    c. Reagent 1.
        i. 58 µM sulfanilamide in 3.6 N HCl
        ii. 3.6 N HCL- dilute310.34 ml con HCL (11.6 N) to 1 L
        iii. 58 µM sulfanilamide $C_6H_8N_2O_2S$, 9.988 g L^{-1}
    d. Reagent 2
        i. 3.86 µM N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$, 1.00 g L^{-1}
    e. Mixed enzyme solution:

i. 9 ml buffer plus 1 ml nitrate reductase (AtNAR1) plus 0.5 ml NADH mixed immediately prior to use. Note enzyme and NADH part of NaR-PkAT reagent pack from Nitrate Elimination Corp (Lake Linden, MI.).

f. Primary stock standard solution
   i. 10 µM potassium nitrate (KNO_3); NOTE: Store at 4 °C. no more than 1 week

g. Secondary Stock standard solution
   i. 100 µM potassium nitrate (KPO_3); NOTE: Prepare fresh each day (1 ml primary + 999 ml H_2O)

h.

Standard	2M KCl	100 µM KNO_3	PPM Nitrate
0.0 µM	100 µl	0 µl	0
0.5 µM	99.5µl	0.5 µl	0.031
1.0 µM	99 µl	1 µl	0.062
2.5 µM	97.5 µl	2.5 µl	0.155
5.0 µM	95 µl	5 µl	0.31
10 µM	90 µl	10 µl	0.62
15 µM	85 µl	15 µl	0.93
25 µM	75 µl	25 µl	1.55
50 µM	50 µl	50 µl	3.10

4. Certified reference Material:
   a. TraceCERT, 1000 mg/L NO_3^- in H_20, Sigma Aldrch (Fluka)74246
   b. CRM is 1000 PPM or 16.12 mM.
   c. Working 1 PPM Nitrate CRM. dilute 1 ml TraceCERT, 1000 mg/L PO_4^{3-} to 1L with nanopure water.
   d. Use 100 µl for 1 PPM or 16.12 µM Nitrate (in triplicate)
5. Laboratory Procedure and Data Recording:
   k. Place 0.45 um filters filter on filtering apparatus.
   l. Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of ultrapure reagent grade water to seat it against the fritted support.
   m. Shake the sample vigorously and measure out 50 mL of sample using a graduated cylinder and pour into filtering apparatus. Filter sample. Remove all traces of water by continuing to apply vacuum after sample has passed through.
   n. Carefully remove the filter from the filter support and take apart filtering assembly.
   o. Pour filtrate into pre-labeled acid washed 50 mL centrifuge tubes and freeze at -20 °C until analysis.
   a. Defrost sample.
   b. Add 100 µl sample or standard or CRM (in triplicate) to wells of 96 well plate.
   c. Add 50 µl of freshly prepared mixed enzyme solution to each well, shake while reacting 60 min RT.
   d. Add 50 µl of reagent 1 and 50 µl of reagent 2 to each well, shake additional 10 min, RT.
   e. Read absorbance on Filter Max F-5 multimode plate reader at 540 nm.
   f. Absorbance will be transferred to Microsoft excel. The blank absorbance will then be averaged and subtracted from standard, sample and CRM absorbance values.

The average will be calculated for each standard value, plotted against the concentration and a linear regression analysis used to produce the best fit line. Sample and CRM values will be calculated from the regression and the mean and standard deviation of the 3 replicates reported.

Appendix 1.6 Water Column NH_4+NH_3 *(*Sheldon, J.E. and W.J. Wiebe (1997)):

1. Materials and Equipment:
    a. Cooler with ice
    b. 5 pre-labeled acid washed (10% HCl) amber 500 mL polypropylene bottles with lids
    c. ultrapure reagent grade water
    d. 0.45 μm filters
    e. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    f. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
    g. Suction flask
    h. Vacuum pump
    i. Sodium chloride
    j. Sodium hydroxide
    k. Clorox bleach
    l. Sodium nitroprusside ($Na_2Fe(CN_6)NO.2H_2O$)
    m. Phenol (C_6H_5OH)
    n. Sodium thiosulfate 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$
    o. ammonium sulfate $(NH_4)_2SO_4$
    p. Potassium Iodide (KI)
    q. TraceCERT, 1000 mg/L NH4+ in H20, Sigma Aldrich
    r. 25 ml glass test tubes
    s. Shimadzu 1601 spectrophotometer
2. Field Methods:
    a. At site each sample bottle will be rinsed 3x with site water.
    b. Once the bottle has been rinsed the sample will be collected at approximately 0.5 m depth and capped.
    c. This will be repeated 3 x per site. The samples will be placed on ice in a cooler and transported back to the lab.
3. Reagent and Standard Preparation:
    a. All reagents will be prepared using acid washed (10% HCl) labware and ultrapure reagent grade water
    b. Magnesium reagent
        i. Dissolve 45 g NaCl and 20 g $MgSO_4.7H_2O$ in about 200 mL water. Add 1 N NaOH dropwise until a slight precipitate forms. Add a few boiling chips and boil (to drive off ammonia) until < 200 mL. Cool and dilute to 200 mL. A slight precipitate will remain. Store at RT.
    c. Phenol/Nitroprusside solution
        i. Dissolve 0.2 g $Na_2Fe(CN)_6NO.2H_2O$ and 19 g C_6H_5OH in 500 mL water. Store in a dark bottle in refrigerator.
    d. Hypochlorite solution
        i. Dilute 750 mg available Cl^- as Clorox® bleach (see protocol below) to 500 mL with 0.5 N NaOH. Store in a dark bottle in refrigerator.
    e. Thiosulfate solution

i. Dissolve 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$ in 500 mL water. Store in refrigerator.

f. Potassium iodide
   i. Dissolve 0.5 g KI in 50 mL 1 N H_2SO_4. Make fresh each time.

g. 0.5 N Sodium hydroxide
   i. 10 g NaOH dissolve and dilute to 500 ml.

h. 1.0 N NaOH
   i. 4 g NaOH dissolve and dilute to 100 ml

i. Availible CL^-
   i. Make up potassium iodide solution and add 1 mL Clorox®. Titrate with thiosulfate solution until color is gone. 1 mL thiosulfate reacts with 3.54 mg available Cl^-, so (mL Clorox® needed to give 750 mg available Cl^-) = (750 ÷ 3.54) ÷ (mL thiosulfate used). Discard Clorox® if < 12 mL thiosulfate is used (Parsons, et al. 1984). Use regular or concentrated Clorox (no perfumes/additions)

j. Primary stock standard solution: 10 mM Ammonium Sulfate.
   i. Dry $(NH_4)_2SO_4$ overnight at 50 °C. Dissolve 0.6607 g and make up to 1 L with water. Store in refrigerator.

k. Working Stock standard solution: 1 mM Ammonium sulfate
   i. 1 mL Primary Standard + 9 mL water, or similar convenient amount. Make fresh each day.

l.

Standard	water	1 mM $[NH_4]_2SO_4$
Blank	5000 µl	0 µl
0.5 µM	4997.5 µl	2.5 µl
1.0 µM	4995 µl	5 µl
2.0 µM	4990 µl	10 µl
5.0 µM	4975 µl	25 µl
10.0 µM	4950 µl	50 µl
20.0 µM	4900 µl	100 µl

4. Certified reference Material:
   a. TraceCERT, 1000 mg/L NH_4^+ in H_20, Sigma Aldrch (Fluka)59755 CRM is 1000 PPM or Working 0.5 PPM Ammonium CRM.
   b. Working 5 µM Ammonium CRM. dilute 9.0 µl TraceCERT, 1000 mg/L NH_4^+ to 100 ml with nanopure water.
   c. A CRM will be evaluated as a sample set in each batch of ammonia determinations using 5.0 ml of 5.0 µM Ammonium CRM.
5. Laboratory Procedure and Data Recording:
   a. For each water sample (note salinity), triplicate 5.0 ml volumes are added to 25 ml glass test tubes. For each set, one of the samples in triplicate will be spiked with a methods surrogate (25 µl of 1mM NH_4^+ working standard).
   b. The standards/blank/CRM (5.0 ml each) are added in triplicate to 25 ml glass test tubes as well

c. Add 100 ml of magnesium reagent to each blank, standard, and sample with salinity <= 5 ppt and vortex.
d. To each tube add 250 μL nitroprusside/phenol, vortex, immediately add 250 μL hypochlorite, vortex, and cap (or parafilm). Keep tubes in a dark place until read at least 6 hours but not more than 30 hours later.
e. Absorbance determination: The Shimadzu UV/VIS 1601 spectrophotometer set at absorbance = 630 nm and warmed up for ~10 min. Use 1 ml of sample in a plastic micro cuvette 1 cm path. Zero both cuvettes with distilled water. Leave reference in back holder and sample in front cuvette. Handle carefully to avoid resuspending the precipitate. Rinse cuvette thoroughly between samples with distilled water. (Note if over highest standard absorbance dilute accordingly and reread).
f. Data Analysis:
    i. Corrections for salinity are obtained by running standards in waters of various salinities. For example, salt factors were obtained for 0, 5, 10, ... 34 ppt water made by mixing low nutrient seawater with 18 MΩ-cm water, and factors for other salinities were interpolated. Salt factor = 1 + (salinity in ppt) × 0.0062
    ii. Correct standards for dilution by magnesium reagent because not all samples will receive it (e.g. 20 μM standard is really 19.61 μM after addition of magnesium reagent). Unnecessary if all samples and standards receive reagent (ie: no FW samples).
    iii. Plot the corrected standard concentration vs. absorbance. The slope of this line is the calibration factor F. F should be approximately 55-60 for a 1 cm cell.
    iv. Correct sample calculations for dilution by magnesium reagent where necessary: Dilution factor = 1.02 if sample salinity was <= 5 ppt (i.e. magnesium reagent was added) or Dilution factor = 1 if sample salinity was > 5 ppt (i.e. no magnesium reagent).
    v. Spiked sample recovery. Recovery % is [(spiked sample conc.- sample conc.)/spike conc.]*100

Appendix 1.7 Water Column OPO_4 (Ringuet et al. 2001):

1. Materials and Equipment:
    a. Cooler with ice
    b. 5 pre-labeled acid washed (10% HCl) amber 500 mL polypropylene bottles with lids
    c. ultrapure reagent grade water
    d. 0.45 um filters
    e. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    f. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
    g. Suction flask
    h. Vacuum pump
    i. HCl
    j. ammonium molybdate
    k. sulfuric acid
    l. ascorbic acid
    m. antimony potassium tartrate
    n. potassium phosphate monobasic (KH_2PO_4)
    o. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch
    p. microcentrifuge tube
    q. 48 well microplates
    r. plastic microcuvette
    s. Shimadzu 1601 spectrophotometer
2. Field Methods:
    a. At site each sample bottle will be rinsed 3x with site water.
    b. Once the bottle has been rinsed the sample will be collected at approximately 0.5 m depth and capped.
    c. This will be repeated 3 x per site. The samples will be placed on ice in a cooler and transported back to the lab.
3. Reagent and Standard Preparation:
    a. All reagents will be prepared using acid washed (10% HCl) labware and ultrapure reagent grade water
    b. Reagent 1: 51 µmol ammonium molybdate
    c. Reagent 2: 4.9 N sulfuric acid
    d. Reagent 3: 0.1 µM ascorbic acid (make fresh daily)
    e. Reagent 4: 1.2 µmol antimony potassium tartrate
    f. Working Reagent:- make fresh daily- mix thoroughly after each addition
        i. 1.5 ml reagent 1
        ii. 5.0 ml reagent 2
        iii. 3.0 ml reagent 3
        iv. 0.5 ml reagent 4
    g. Primary stock standard solution:
        i. 10 mM potassium phosphate monobasic (KH_2PO_4); Store at 4 C. no more than 1 week

h. Secondary Stock standard solution:
    i. 100 µM potassium phosphate monobasic (KH_2PO_4); Prepare fresh each day (1 ml primary + 999 ml H_2O)

i.

Standard	water	100 µmol KH_2PO_4	PPM Phosphate
Blank	200 µl	0 µl	0
0.5 µM	199 µl	1µl	0.045
1.0 µM	198 µl	2 µl	0.09
2.5 µM	195 µl	5 µl	0.235
5.0 µM	190 µl	10µ	0.47
10 µM	180 µl	20 µl	0.95
15 µM	170 µl	30 µl	1.42
25 µM	50 µl	150 µl	2.37
35 µM	70 µl	130 µl	3.32

4. Certified reference Material:
    a. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch (Fluka)38364 CRM is 1000 PPM or 10.53 mM.
    b. Working 1 PPM Phosphate CRM.
    c. Dilute 1 ml TraceCERT, 1000 mg/L PO_4^{3-} to 1L with nanopure water.
    d. Use 200 µl for 1 PPM or 10.53 µM Phosphate (in triplicate)
5. Laboratory Procedure and Data Recording:
    a. Add 200 µl sample or standard or CRM (in triplicate)and 50 ml working reagent to 1.5 ml microcentrifuge tube. Vortex, incubate 30 min at RT.
    b. Transfer to a new plastic microcuvette
    c. Read absorbance at 880 nm on a Shimadzu 1601 spectrophotometer (reference cuvette water) collecting absorbance data in a Shimadzu data file.
    d. Data Analysis:
        i. Absorbance will be transferred to Microsoft excel. The blank absorbance will be averaged and subtracted from standard, sample and CRM absorbance values. The average will be calculated for each standard value, plotted against the concentration and a linear regression analysis used to produce the best fit line. Sample and CRM values will be calculated from the regression and the mean and standard deviation of the 3 replicates reported.

Appendix 1.8 Sediment Pore-water NO_2 + NO_3 (Ringuet et al., 2011):

1. Materials and Equipment:
    a. clear acrylic acid washed (10% HCl) cores (10.4 cm diameter by 10 cm depth)
    b. 10.4 cm diameter red lids
    c. cooler with ice
    d. Gloves
    e. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
    f. metal plate
    g. metal spatula
    h. 5 gallon bucket
    i. clear acrylic cores cut to 2cm as sediment sub-section depths
    j. De-ionized (DI) water for cleaning
    k. Large plastic tray
    l. analytical balance capable of 3 decimal places
    m. Potassium chloride (KCl; granular, certified ACS)
    n. 4 oz whirlpak bags (pre-labeled)
    o. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
    p. graduated cylinders
    q. shaker table
    r. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    s. Centrifuge
    t. 30 mL BD syringe with luerlok tip
    u. Acrodisc 25 mm syringe filters with 0.45 □m supor membrane
    v. 2 oz. Whirlpak bags (pre-labeled)
    w. potassium dihydrogen phosphate (KH_2PO_4)
    x. potassium hydroxide (KOH)
    y. ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$
    z. sulfanilamide
    aa. HCL
    bb. N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$
    cc. nitrate reductase (AtNAR1)
    dd. NADH
    ee. TraceCERT, 1000 mg/L NO_3^- in H_20, Sigma Aldrch (Fluka)74246
    ff. CRM is 1000 PPM or 16.12 mM
    gg. ultrapure reagent grade water
2. 2M KCl preparation for extractable nutrients:
    a. Prepare 2M KCl solution by dissolving KCl in DI water. To do this, mix 149.1 g of KCl (FW=74.55) with DI water to a total volume of 1 L. For example, for a 2.5 kg bottle of KCl, add entire bottle of KCl to a large carboy and use DI water to help dissolve and break-up KCl in the bottle. After adding all 2.5kg of KCl, add DI water to carboy until total volume of solution is 16.77 L.
    b. To extract nutrients from the sediment, the volume of 2M KCl used is based on 2:1 KCl to sediment volume ratio. Thus, determine the total volume of sediment for each

sediment depth interval to be collected and multiply by 2 for the volume of 2M KCl required.

i. **Note:** 2 M KCl volume should be a minimum of 30 mL to provide sufficient volume for nutrient analysis. If more KCl is needed, a larger volume of sediment should be collected (e.g., 2 quarter sections of the sub-core).

c. Pre-label whirlpak bags with appropriate depth sections, core #, and site name and fill with the pre-determined volume of KCl for the specific sediment depth interval using graduated cylinders. The whirlpak bag should have sufficient size to hold both the KCl and sediment that will be added during sampling.

d. Weigh each whirlpak bag of KCl solution.

3. Field Methods:
   a. Select sampling sites based on needs of study.
   b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
   c. Secure the red lid on top of the corer and carefully remove the core. Cap the bottom of the core with a second red lid and hold upright with a minimum of 2 cm of water above the sediment surface.
   d. Repeat coring procedure until 5 cores are collected per site. Store all samples on ice in cooler until the samples are taken back to the lab.
4. Immediate Laboratory Procedure and Data Recording:
   a. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
   b. To sub-core, place 2 cm core section on top and push sediment to top of sub-core. Insert metal plate between cores to separate it. Place sediment sub-section into the appropriate large plastic tray. Divide the core into two more sections following the same protocol.
   c. Divide each sub-section into quarters and place one quarter into pre-weighed, pre-labeled whirlpak bag with 2M KCl, weigh, and place on shaker table for 1 hour. Pour sample into centrifuge tube and centrifuge for 6 minutes at 4000 RPM. Filter (0.45 um Supor Acrodisk syringe filter) 25 mL into pre-labeled 2 oz whirlpak bag (later to be analyzed for NH_4^+, NOx, and PO_4^{3-}).
   d. Put whirlpak bags in labeled ziplock bag and place in freezer.
   e. Discard remaining sediment sections in 5 gallon bucket.
   f. Rinse acrylic sub-cores, metal plates and metal spatulas with DI water between core sampling.
6. Reagent and Standard preparation
   a. All reagents will be prepared using acid washed (10% HCl) lab ware and 2M KCl.
   b. Buffer (pH 7.6)
      i. 27.6 µM potassium dihydrogen phosphate (KH_2PO_4), 3.756 g L^{-1}
      ii. 25 µM potassium hydroxide (KOH), 1.403 g L^{-1}
      iii. 25 µM ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$, 0.0093 g L^{-1}

c. Reagent 1.
    i. 58 µM sulfanilamide in 3.6 N HCl
    ii. 3.6 N HCL- dilute310.34 ml con HCL (11.6 N) to 1 L
    iii. 58 µM sulfanilamide $C_6H_8N_2O_2S$, 9.988 g L^{-1}
d. Reagent 2
    i. 3.86 µM N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$, 1.00 g L^{-1}
e. Mixed enzyme solution:
    i. 9 ml buffer plus 1 ml nitrate reductase (AtNAR1) plus 0.5 ml NADH mixed immediately prior to use. Note enzyme and NADH part of NaR-PkAT reagent pack from Nitrate Elimination Corp (Lake Linden, MI.).
f. Primary stock standard solution
    i. 10 µM potassium nitrate (KNO_3); NOTE: Store at 4 °C. no more than 1 week
g. Secondary Stock standard solution
    i. 100 µM potassium nitrate (KPO_3); NOTE: Prepare fresh each day (1 ml primary + 999 ml H_2O)
h.

Standard	2M KCl	100 µmol KNO_3	PPM Nitrate
0.0 µM	100 µl	0 µl	0
0.5 µM	99.5µl	0.5 µl	0.031
1.0 µM	99 µl	1 µl	0.062
2.5 µM	97.5 µl	2.5 µl	0.155
5.0 µM	95 µl	5 µl	0.31
10 µM	90 µl	10 µl	0.62
15 µM	85 µl	15 µl	0.93
25 µM	75 µl	25 µl	1.55
50 µM	50 µl	50 µl	3.10

7. Certified reference Material:
    a. TraceCERT, 1000 mg/L NO_3^- in H_20, Sigma Aldrch (Fluka)74246
    b. CRM is 1000 PPM or 16.12 mM.
    c. Working 1 PPM Nitrate CRM. dilute 1 ml TraceCERT, 1000 mg/L PO_4^{3-} to 1L with nanopure water.
    d. Use 100 µl for 1 PPM or 16.12 µM Nitrate (in triplicate)
8. Final Laboratory Procedure and Data Recording:
    p. Place 0.45 um filters filter on filtering apparatus.
    q. Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of ultrapure reagent grade water to seat it against the fritted support.
    r. Shake the sample vigorously and measure out 50 mL of sample using a graduated cylinder and pour into filtering apparatus. Filter sample. Remove all traces of water by continuing to apply vacuum after sample has passed through.
    s. Carefully remove the filter from the filter support and take apart filtering assembly.
    t. Pour filtrate into pre-labeled acid washed 50 mL centrifuge tubes and freeze at -20 °C until analysis.
    a. Defrost sample.

b. Add 100 µl sample or standard or CRM (in triplicate) to wells of 96 well plate.
c. Add 50 µl of freshly prepared mixed enzyme solution to each well, shake while reacting 60 min RT.
d. Add 50 µl of reagent 1 and 50 µl of reagent 2 to each well, shake additional 10 min, at room temperature.
e. Read absorbance on Filter Max F-5 multimode plate reader at 540 nm.
f. Absorbance will be transferred to Microsoft excel. The blank absorbance will then be averaged and subtracted from standard, sample and CRM absorbance values. The average will be calculated for each standard value, plotted against the concentration and a linear regression analysis used to produce the best fit line. Sample and CRM values will be calculated from the regression and the mean and standard deviation of the 3 replicates reported.

Appendix 1.9 Sediment Pore-water NH_4+NH_3

(Sheldon, J.E. and W.J. Wiebe (1997):

1. Materials and Equipment:
    a. clear acrylic acid washed (10% HCl) cores (10.4 cm diameter by 10 cm depth)
    b. 10.4 cm diameter red lids
    c. cooler with ice
    d. Gloves
    e. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
    f. metal plate
    g. metal spatula
    h. 5 gallon bucket
    i. clear acrylic cores cut to 2cm as sediment sub-section depths
    j. De-ionized (DI) water for cleaning
    k. Large plastic tray
    l. analytical balance capable of 3 decimal places
    m. Potassium chloride (KCl; granular, certified ACS)
    n. 4 oz whirlpak bags (pre-labeled)
    o. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
    p. graduated cylinders
    q. shaker table
    r. 50 mL centrifuge tubes
    s. Centrifuge
    t. 30 mL BD syringe with luerlok tip
    u. Acrodisc 25 mm syringe filters with 0.45 □m supor membrane
    e. 2 oz. Whirlpak bags (pre-labeled) Cooler with ice
    f. 5 pre-labeled acid washed (10% HCl) amber 500 mL polypropylene bottles with lids
    g. ultrapure reagent grade water
    h. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    i. Sodium chloride
    j. Sodium hydroxide
    k. Clorox bleach
    l. Sodium nitroprusside ($Na_2Fe(CN_6)NO.2H_2O$)
    m. Phenol (C_6H_5OH)
    n. Sodium thiosulfate 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$
    o. ammonium sulfate $(NH_4)_2SO_4$
    p. Potassium Iodide (KI)
    q. TraceCERT, 1000 mg/L NH4+ in H20, Sigma Aldrch
    r. 25 ml glass test tubes
    s. Shimadzu 1601 spectrophotometer
2. 2M KCl preparation for extractable nutrients:
    a. Prepare 2M KCl solution by dissolving KCl in DI water. To do this, mix 149.1 g of KCl (FW=74.55) with DI water to a total volume of 1 L. For example, for a 2.5 kg bottle of

KCl, add entire bottle of KCl to a large carboy and use DI water to help dissolve and break-up KCl in the bottle. After adding all 2.5kg of KCl, add DI water to carboy until total volume of solution is 16.77 L.

b. To extract nutrients from the sediment, the volume of 2M KCl used is based on 2:1 KCl to sediment volume ratio. Thus, determine the total volume of sediment for each sediment depth interval to be collected and multiply by 2 for the volume of 2M KCl required.
    i. **Note:** 2 M KCl volume should be a minimum of 30 mL to provide sufficient volume for nutrient analysis. If more KCl is needed, a larger volume of sediment should be collected (e.g., 2 quarter sections of the sub-core).
c. Pre-label whirlpak bags with appropriate depth sections, core #, and site name and fill with the pre-determined volume of KCl for the specific sediment depth interval using graduated cylinders. The whirlpak bag should have sufficient size to hold both the KCl and sediment that will be added during sampling.
d. Weigh each whirlpak bag of KCl solution.

3. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
    c. Secure the red lid on top of the corer and carefully remove the core. Cap the bottom of the core with a second red lid and hold upright with a minimum of 2 cm of water above the sediment surface.
    d. Repeat coring procedure until 5 cores are collected per site. Store all samples on ice in cooler until the samples are taken back to the lab.
4. Immediate Laboratory Procedure and Data Recording:
    a. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
    b. To sub-core, place 2 cm core section on top and push sediment to top of sub-core. Insert metal plate between cores to separate it. Place sediment sub-section into the appropriate large plastic tray. Divide the core into two more sections following the same protocol.
    c. Divide each sub-section into quarters and place one quarter into pre-weighed, pre-labeled whirlpak bag with 2M KCl, weigh, and place on shaker table for 1 hour. Pour sample into centrifuge tube and centrifuge for 6 minutes at 4000 RPM. Filter (0.45 um Supor Acrodisk syringe filter) 25 mL into pre-labeled 2 oz whirlpak bag (later to be analyzed for NH_4^+, NOx, and PO_4^{3-}).
    d. Put whirlpak bags in labeled ziplock bag and place in freezer.
    e. Discard remaining sediment sections in 5 gallon bucket.
    f. Rinse acrylic sub-cores, metal plates and metal spatulas with DI water between core sampling.
5. Reagent and Standard Preparation:
    a. All reagents will be prepared using acid washed (10% HCl) labware and ultrapure reagent grade water

b. Magnesium reagent
    i. Dissolve 45 g NaCl and 20 g $MgSO_4.7H_2O$ in about 200 mL water. Add 1 N NaOH dropwise until a slight precipitate forms. Add a few boiling chips and boil (to drive off ammonia) until < 200 mL. Cool and dilute to 200 mL. A slight precipitate will remain. Store at RT.
c. Phenol/Nitroprusside solution
    i. Dissolve 0.2 g $Na_2Fe(CN)_6NO.2H_2O$ and 19 g C_6H_5OH in 500 mL water. Store in a dark bottle in refrigerator.
d. Hypochlorite solution
    i. Dilute 750 mg available Cl^- as Clorox® bleach (see protocol below) to 500 mL with 0.5 N NaOH. Store in a dark bottle in refrigerator.
e. Thiosulfate solution
    i. Dissolve 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$ in 500 mL water. Store in refrigerator.
f. Potassium iodide
    i. Dissolve 0.5 g KI in 50 mL 1 N H_2SO_4. Make fresh each time.
g. 0.5 N Sodium hydroxide
    i. 10 g NaOH dissolve and dilute to 500 ml.
h. 1.0 N NaOH
    i. 4 g NaOH dissolve and dilute to 100 ml
i. Availible CL^-
    i. Make up potassium iodide solution and add 1 mL Clorox®. Titrate with thiosulfate solution until color is gone. 1 mL thiosulfate reacts with 3.54 mg available Cl^-, so (mL Clorox® needed to give 750 mg available Cl^-) = (750 ÷ 3.54) ÷ (mL thiosulfate used). Discard Clorox® if < 12 mL thiosulfate is used (Parsons, et al. 1984). Use regular or concentrated Clorox (no perfumes/additions)
j. Primary stock standard solution: 10 mM Ammonium Sulfate.
    i. Dry $(NH_4)_2SO_4$ overnight at 50 °C. Dissolve 0.6607 g and make up to 1 L with water. Store in refrigerator.
k. Working Stock standard solution: 1 mM Ammonium sulfate
    i. 1 mL Primary Standard + 9 mL water, or similar convenient amount. Make fresh each day.
l.

Standard	2 M KCL	1 mM $[NH_4]_2SO_4$
Blank	5000 µl	0 µl
0.5 µM	4997.5 µl	2.5 µl
1.0 µM	4995 µl	5 µl
2.0 µM	4990 µl	10 µl
5.0 µM	4975 µl	25 µl
10.0 µM	4950 µl	50 µl
20.0 µM	4900 µl	100 µl

6. Certified reference Material:
    a. TraceCERT, 1000 mg/L NH_4^+ in H_20, Sigma Aldrch (Fluka)59755 CRM is 1000 PPM or Working 0.5 PPM Ammonium CRM.
    b. Working 5 µM Ammonium CRM. dilute 9.0 µl TraceCERT, 1000 mg/L NH_4^+ to 100 ml with 2 M KCl.
    c. A CRM will be evaluated as a sample set in each batch of ammonia determinations using 5.0 ml of 5.0 µM Ammonium CRM.
7. Laboratory Procedure and Data Recording:
    a. For each water sample (note salinity), triplicate 5.0 ml volumes are added to 25 ml glass test tubes. For each set, one of the samples in triplicate will be spiked with a methods surrogate (25 µl of 1mM NH_4^+ working standard).
    b. The standards/blank/CRM (5.0 ml each) are added in triplicate to 25 ml glass test tubes as well
    c. Add 100 ml of magnesium reagent to each blank, standard, and sample with salinity <= 5 ppt and vortex.
    d. To each tube add 250 µL nitroprusside/phenol, vortex, immediately add 250 µL hypochlorite, vortex, and cap (or parafilm). Keep tubes in a dark place until read at least 6 hours but not more than 30 hours later.
    e. Absorbance determination: The Shimadzu UV/VIS 1601 spectrophotometer set at absorbance = 630 nm and warmed up for ~10 min. Use 1 ml of sample in a plastic micro cuvette 1 cm path. Zero both cuvettes with distilled water. Leave reference in back holder and sample in front cuvette. Handle carefully to avoid resuspending the precipitate. Rinse cuvette thoroughly between samples with distilled water. (Note if over highest standard absorbance dilute accordingly and reread).
    f. Data Analysis:
        i. Corrections for salinity are obtained by running standards in waters of various salinities. For example, salt factors were obtained for 0, 5, 10, ... 34 ppt water made by mixing low nutrient seawater with 18 MΩ-cm water, and factors for other salinities were interpolated. Salt factor = 1 + (salinity in ppt) × 0.0062
        ii. Correct standards for dilution by magnesium reagent because not all samples will receive it (e.g. 20 µM standard is really 19.61 µM after addition of magnesium reagent). Unnecessary if all samples and standards receive reagent (ie: no FW samples).
        iii. Plot the corrected standard concentration vs. absorbance. The slope of this line is the calibration factor F. F should be approximately 55-60 for a 1 cm cell.
        iv. Correct sample calculations for dilution by magnesium reagent where necessary: Dilution factor = 1.02 if sample salinity was <= 5 ppt (i.e. magnesium reagent was added) or Dilution factor = 1 if sample salinity was > 5 ppt (i.e. no magnesium reagent).
        v. Spiked sample recovery. Recovery % is [(spiked sample conc.- sample conc.)/spike conc.]*100

Appendix 1.10 Sediment Pore-water OPO_4 (Ringuet et al. 2001):

1. Materials and Equipment:
    a. clear acrylic acid washed (10% HCl) cores (10.4 cm diameter by 10 cm depth)
    b. 10.4 cm diameter red lids
    c. cooler with ice
    d. Gloves
    e. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
    f. metal plate
    g. metal spatula
    h. 5 gallon bucket
    i. clear acrylic cores cut to 2cm as sediment sub-section depths
    j. De-ionized (DI) water for cleaning
    k. Large plastic tray
    l. analytical balance capable of 3 decimal places
    m. Potassium chloride (KCl; granular, certified ACS)
    n. 4 oz whirlpak bags (pre-labeled)
    o. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
    p. graduated cylinders
    q. shaker table
    r. 50 mL centrifuge tubes
    s. Centrifuge
    t. 30 mL BD syringe with luerlok tip
    u. Acrodisc 25 mm syringe filters with 0.45 □m supor membrane
    v. 2 oz. Whirlpak bags (pre-labeled)
    w. ultrapure reagent grade water
    x. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
    y. HCl
    z. ammonium molybdate
    aa. sulfuric acid
    bb. ascorbic acid
    cc. antimony potassium tartrate
    dd. potassium phosphate monobasic (KH_2PO_4)
    ee. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch
    ff. microcentrifuge tube
    gg. 48 well microplates
    hh. plastic microcuvette
    ii. Shimadzu 1601 spectrophotometer
2. 2M KCl preparation for extractable nutrients:
    a. Prepare 2M KCl solution by dissolving KCl in DI water. To do this, mix 149.1 g of KCl (FW=74.55) with DI water to a total volume of 1 L. For example, for a 2.5 kg bottle of KCl, add entire bottle of KCl to a large carboy and use DI water to help dissolve and break-up KCl in the bottle. After adding all 2.5kg of KCl, add DI water to carboy until total volume of solution is 16.77 L.

b. To extract nutrients from the sediment, the volume of 2M KCl used is based on 2:1 KCl to sediment volume ratio. Thus, determine the total volume of sediment for each sediment depth interval to be collected and multiply by 2 for the volume of 2M KCl required.
    i. **Note:** 2 M KCl volume should be a minimum of 30 mL to provide sufficient volume for nutrient analysis. If more KCl is needed, a larger volume of sediment should be collected (e.g., 2 quarter sections of the sub-core).
c. Pre-label whirlpak bags with appropriate depth sections, core #, and site name and fill with the pre-determined volume of KCl for the specific sediment depth interval using graduated cylinders. The whirlpak bag should have sufficient size to hold both the KCl and sediment that will be added during sampling.
d. Weigh each whirlpak bag of KCl solution.

3. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
    c. Secure the red lid on top of the corer and carefully remove the core. Cap the bottom of the core with a second red lid and hold upright with a minimum of 2 cm of water above the sediment surface.
    d. Repeat coring procedure until 5 cores are collected per site. Store all samples on ice in cooler until the samples are taken back to the lab.
4. Initial Laboratory Procedure and Data Recording:
    a. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
    b. To sub-core, place 2 cm core section on top and push sediment to top of sub-core. Insert metal plate between cores to separate it. Place sediment sub-section into the appropriate large plastic tray. Divide the core into two more sections following the same protocol.
    c. Divide each sub-section into quarters and place one quarter into pre-weighed, pre-labeled whirlpak bag with 2M KCl, weigh, and place on shaker table for 1 hour. Pour sample into centrifuge tube and centrifuge for 6 minutes at 4000 RPM. Filter (0.45 um Supor Acrodisk syringe filter) 25 mL into pre-labeled 2 oz whirlpak bag (later to be analyzed for NH_4^+, NOx, and PO_4^{3-}).
    d. Put whirlpak bags in labeled ziplock bag and place in freezer.
    e. Discard remaining sediment sections in 5 gallon bucket.
    f. Rinse acrylic sub-cores, metal plates and metal spatulas with DI water between core sampling.
5. Reagent and Standard Preparation:
    a. All reagents will be prepared using acid washed (10% HCl) labware and ultrapure reagent grade water
    b. Reagent 1: 51 µM ammonium molybdate
    c. Reagent 2: 4.9 N sulfuric acid
    d. Reagent 3: 0.1 mM ascorbic acid (make fresh daily)

e. Reagent 4: 1.2 µM antimony potassium tartrate
f. Working Reagent:- make fresh daily- mix thoroughly after each addition
    i. 1.5 ml reagent 1
    ii. 5.0 ml reagent 2
    iii. 3.0 ml reagent 3
    iv. 0.5 ml reagent 4
g. Primary stock standard solution:
    i. 10 µM potassium phosphate monobasic (KH_2PO_4); Store at 4 °C no more than 1 week
h. Secondary Stock standard solution:
    i. 100 µM potassium phosphate monobasic (KH_2PO_4); Prepare fresh each day (1 ml primary + 999 ml H_2O)

i.

Standard	2M KCl	100 µmol KH_2PO_4	PPM Phosphate
Blank	200 µl	0 µl	0
0.5 µM	199 µl	1µl	0.045
1.0 µM	198 µl	2 µl	0.09
2.5 µM	195 µl	5 µl	0.235
5.0 µM	190 µl	10µ	0.47
10 µM	180 µl	20 µl	0.95
15 µM	170 µl	30 µl	1.42
25 µM	50 µl	150 µl	2.37
35 µM	70 µl	130 µl	3.32

6. Certified reference Material:
    a. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch (Fluka)38364 CRM is 1000 PPM or 10.53 mM.
    b. Working 1 PPM Phosphate CRM.
    c. Dilute 1 ml TraceCERT, 1000 mg/L PO_4^{3-} to 1L with nanopure water.
    d. Use 200 µl for 1 PPM or 10.53 µM Phosphate (in triplicate)
7. Laboratory Procedure and Data Recording:
    a. Add 200 µl sample or standard or CRM (in triplicate)and 50 ml working reagent to 1.5 ml microcentrifuge tube. Vortex, incubate 30 min at RT.
    b. Transfer to a new plastic microcuvette
    c. Read absorbance at 880 nm on a Shimadzu spectrophotometer (reference cuvette KCl) collecting absorbance data in a Shimadzu data file.
    d. Data Analysis:
        i. Absorbance will be transferred to Microsoft excel. The blank absorbance will be averaged and subtracted from standard, sample and CRM absorbance values. The average will be calculated for each standard value, plotted against the concentration and a linear regression analysis used to produce the best fit line. Sample and CRM values will be calculated from the regression and the mean and standard deviation of the 3 replicates reported.

Appendix 1.11 Sediment Total Organic Content *(Loss on Ignition: EMASC-001)*

1. Materials and Equipment:
    a. clear acrylic cores (10.4 cm diameter by 10 cm depth)
    b. 10.4 cm diameter red lids
    c. cooler with ice
    d. Gloves
    e. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
    f. metal plate
    g. metal spatula
    h. 5 gallon bucket
    i. clear acrylic cores cut to 2cm as sediment sub-section depths
    j. De-ionized (DI) water for cleaning
    k. Large plastic tray
    l. pre-weighed/numbered aluminum foil envelopes (use pencil to number envelope)
    m. pre-weighted/numbered aluminum pans (use pencil to number pan)
    n. analytical balance capable of 3 decimal places
    o. aluminum baking pans
    p. drying oven set at 50°C
    q. muffle furnace capable of maintaining 400°C
    r. Desiccator with desiccant
    s. Tongs
    t. Heat resistant gloves
2. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
    c. Secure the red lid on top of the corer and carefully remove the core. Cap the bottom of the core with a second red lid and hold upright with a minimum of 2 cm of water above the sediment surface.
    d. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
    e. To sub-core, place 2 cm core section on top and push sediment to top of sub-core. Insert metal plate between cores to separate it. Place sediment sub-section into the appropriate plastic bag.
    f. Divide the core into two more sections following the same protocol.
    g. Repeat coring procedure until 9 cores are collected per site. Store all samples on ice in cooler until the samples are taken back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Place sample from plastic bag into clean large plastic tray. Divide the sample in half placing one half into pre-weighed, numbered foil envelope using the metal spatula.
    b. Weigh and record weight, date sample was collected, and sediment core #, site from where it was collected, depth section on datasheet.

c. Discard remaining sediment sections in 5 gallon bucket.
d. Rinse acrylic sub-cores, metal plates and metal spatulas with DI water between core sampling.
e. Place foil envelopes with sediment in drying oven (50°C) for a minimum of 24 hrs. Place in aluminum baking pan standing up, slightly open to allow sufficient drying. Do not pack foil envelopes too tightly in aluminum baking pan because it may cause the foil envelopes to form holes and leak.
f. Weigh each sample a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). Record all weights on the appropriate data sheet.
g. Once the sample as reached a constant dry weight pour a sub-sample (10 – 15g) into the appropriate pre-weighed and labeled pan. Weigh each sub-sample and record all weights on the appropriate data sheet.
h. Place all sub-samples into aluminum baking pan and place into muffle oven at 400 °C for a minimum of 8 hours.
i. After combustion, remove samples from muffle oven with tongs and heat resistant gloves, and place samples into desiccator to cool. Once cool weigh samples and record weights.
j. Total organic content is presents as a percent and is calculated using the following equation:

$$\%\, TOC = \frac{(pre - combustion\ weight - post\ combustion\ weight)}{pre - combustion\ weight} \times 100$$

Appendix 1.12 Seagrass Biomass (Duarte and Kirkman, 2001):

1. Materials and Equipment:
    a. Local area map and tide tables
    b. Small boat for visiting sites
    c. GPS (if available)
    d. Snorkel, mask, and wetsuit for intertidal sampling
    e. Cooler with ice for storing samples
    f. 5 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
    g. Waterproof data sheets and a clipboard
    h. Sampling corer (22 cm diameter) of light and durable material (e.g. PVC) with hole and stopper on lid
    i. Box sieve (1.0 cm mesh)
    j. Large plastic tray
    k. Single edge razor blades
    l. Distilled/deoionized water
    m. Preweighed and numbered aluminum envelopes
    n. Digital scale capable of reading 3 decimal places
    o. Drying oven
2. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
    c. Place the stopper in to the hole on top of the corer (make sure no leaves are floating out of the top of the corer) and carefully remove the core.
    d. Place the sediment and plants removed with the core into the mesh box sieve and rinse with site water.
    e. Place all plant material into pre-labeled plastic bag with site water to keep plants moist.
    f. Collect 5 cores per site. Store all samples on ice in cooler until the samples are taken back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Remove seagrass from bag and place in large plastic tray. Make sure that the entire sample has been removed from the bag. Set bag aside for cleaning and reuse.
    b. Separate all plants by species and into vegetative or flowering shoots.
    c. Scrape leaf blades with Single edge razor blades held 90° to the leaf surface. Be careful to not remove any leaf epidermis.
    d. Rinse seagrass with distilled or deionized water.
    e. Count the total number of shoots in the sample.
    f. Separate leaves from the rhizome directly below the leaf sheath into aboveground and belowground biomass.

g. Fill out data sheet with date, site, replicate, and species and place sample into appropriate aluminum foil envelope and record the weight (different envelopes will be used for above and belowground biomass).
h. Place all samples in an air circulating oven at 50°C for a minimum of 24 hours.
i. Weigh each sample a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). Record all weights on the appropriate data sheet.
j. Biomass is measured as g Dry Weight (g DW). To determine the final sample dry weight subtract the weight of the aluminum foil envelope from the last dry weight of the sample.
k. Biomass is converted from g Dry Weight (DW) cm^{-2} to g DW m^{-2} by using the following equation: $\frac{x\ g\ DW}{380\ cm^2} \times \frac{10{,}000\ cm,^2}{1\ m^s} = x\ g\ DW\ m^{-2}$

Appendix 1.13 Macroalgae Biomass (Sidik et al., 2001)

1. Materials and Equipment:
    a. Local area map and tide tables
    b. Small boat for visiting sites
    c. GPS (if available)
    d. Snorkel, mask, and wetsuit for intertidal sampling
    e. Cooler with ice for storing samples
    f. 5 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
    g. Waterproof data sheets and a clipboard
    h. Sampling quadrat (25 cm x 25 cm) of light and durable material (e.g. PVC)
    i. Dive knife
    j. Large plastic tray
    k. Distilled/deoionized water
    l. Identification guides for macroalgae
    m. Pre-weighed and numbered aluminum envelopes
    n. Hand lens or magnifying device
    o. Digital scale capable of reading 3 decimal places
    p. Drying oven
2. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to toss quadrat. Make sure that the quadrat is placed completely flush with the sediment surface.
    c. Carefully cut around the interior edges of the quadrat and scrape holdfasts away from substrate
    d. Carefully remove macroalgae from quadrat and place into pre-labeled plastic bag.
    e. Collect 5 samples per site. Store all samples on ice in cooler until the samples are taken back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Remove algae from bag and place in large plastic tray. Make sure that the entire sample has been removed from the bag. Set bag aside for cleaning and reuse.
    b. Separate all algae by species
    c. Rinse algae with distilled or deionized water.
    d. Identify species using guides and magnifying equipment
    e. Fill out data sheet with date, site, replicate, and species.
    f. Place sample into appropriate aluminum foil envelope and record the weight.
    g. Place all samples in an air circulating oven at 50°C for a minimum of 24 hours.
    h. Weigh each sample a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). Record all weights on the appropriate data sheet.
    i. Biomass is measured as g Dry Weight (g DW). To determine the final sample dry weight subtract the weight of the aluminum foil envelope from the last dry weight of the sample.

j. Biomass is converted from g DW cm^{-2} to g DW m^{-2} by using the following equation:
$\frac{x\ g\ DW}{25\ cm^2} \times \frac{10{,}000\ cm,^2}{1\ m^s} = x\ g\ DW\ m^{-2}$

Appendix 1.14 Seagrass Epiphyte Biomass (Kendrick and Lavery, 2001)

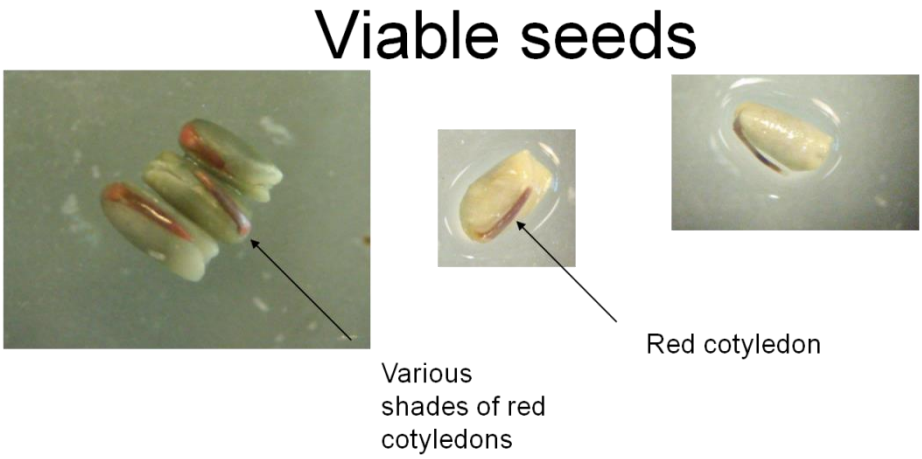
1. Materials and Equipment:
    a. Local area map and tide tables
    b. Small boat for visiting sites
    c. GPS (if available)
    d. Snorkel, mask, and wetsuit for intertidal sampling
    e. Cooler with ice for storing samples
    f. 15 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
    g. Waterproof data sheets and a clipboard
    h. Fine forceps
    i. Large plastic tray
    j. Single edge razor blades
    k. Distilled/deoionized water
    l. Pre-weighed and numbered aluminum pans (label pans with pencil)
    m. Digital scale capable of reading 4 decimal places
    n. Drying oven
2. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select 1 single shoot to sample. Remove shoot from rest of pant at the first rhizome increment.
    c. Place all plant material into pre-labeled plastic bag with site water to keep plants moist.
    d. Repeat random shoot collection 15 times per site. Store all samples on ice in cooler until the samples are taken back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Remove seagrass from bag and place in large plastic tray. Make sure that the entire sample has been removed from the bag. Set bag aside for cleaning and reuse.
    b. Separate shoots into individual leaves.
    c. Remove larger epiphytes from the leaves by hand or with forceps.
    d. Scrape leaf blades with a Single edge razor blades held 90° to the leaf surface. Scrape the entire length of the leaf on both sides. Be careful to not remove any leaf epidermis.
    e. Rinse all material scraped off of the leaves into pre-weighed aluminum pans.
    f. Count the total number of leaves and measure the length and width for each shoot.
    g. Record all leaf data on data sheet along with date, site, replicate and sample wet weight.
    h. Place all samples in an air circulating oven at 60°C for a minimum of 24 hours.
    i. Weigh each sample a minimum of 3 times using the same scale or until the sample reaches a constant dry weight (weight loss < 0.5 mg). Record all weights on the appropriate data sheet.
    j. Calculate the leaf area for all leaves in each sample and then sum the areas. This will equal the total area of epiphytes sampled *l.*

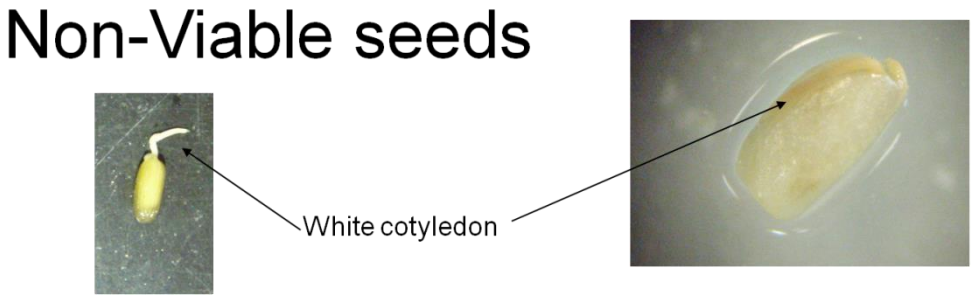
k. Biomass is measured as g Dry Weight (g DW). To determine the final sample dry weight subtract the weight of the aluminum pan from the last dry weight of the sample.
l. Biomass is converted from g DW cm^{-2} to g DW m^{-2} by using the following equation:
$\frac{x\ g\ DW}{l\ cm^2} \times \frac{10{,}000\ cm,^2}{1\ m^s} = x\ g\ DW\ m^{-2}$

Appendix 1.15 Seagrass Seed Viability (McFarland and Shafer, 2010)

1. Materials and Equipment:
    a. Local area map and tide tables
    b. Small boat for visiting sites
    c. GPS (if available)
    d. Snorkel, mask, and wetsuit for intertidal sampling
    e. Cooler with ice for storing samples
    f. Clear acrylic cores (10.4 cm diameter by 10 cm depth)
    g. core plugs
    h. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
    i. 0-2 cm clear aslicing section
    j. metal slicer
    k. 90 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
    l. 0.5 cm mesh sieve (large enough to fit over 5 gallon bucket)
    m. Waterproof data sheets and a clipboard
    n. Fine forceps
    o. Large plastic tray
    p. Single edge razor blades
    q. Distilled/deoionized water
    r. 1 % tetrazolium solution
    s. Acid washed labeled ice cube trays
    t. Aluminum foil
2. Field Methods:
    a. Select sampling sites based on needs of study.
    b. At site randomly select area to core. Place core flush with the sediment surface and push down to a minimum depth of 10cm.
    c. Place the stopper the top of the corer and carefully remove the core. Cap the bottom of the core and hold upright with a minimum of 2 cm of water above the sediment surface.
    d. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
    e. To sub-core, place 2 cm core section on top and push sediment to top of sub-core. Insert metal plate between cores to separate it. Place sediment sub-section into the appropriate plastic bag.
    f. Divide the core into two more sections following the same protocol.
    g. Repeat coring procedure until 15 cores are collected per site. Store all samples on ice in cooler until the samples are taken back to the lab.
3. Laboratory Procedure and Data Recording:
    a. Place 0.5 cm sieve on top of an empty 5 gallon bucket.
    b. Empty the sample bag onto the sieve and rinse bag out dumping contents onto sieve.
    c. Rinse sediment through the sieve until only organic material remains.

d. Carefully and systematically visually inspect all contents removing all seagrass seeds and placing them back into the sample bag with filtered seawater.
e. Before rinsing sieve off have a second individual observe sieve to ensure all seeds have been collected.
f. Store seeds in aerated water at 18-24 °C. Keep out as many organics as possible.
g. Prepare a 1% tetrazolium solution with DI water.
    i. Mix 1 g of 2,3,5-triphenol tetrazolium chloride in 100 ml of DI water (see AOSA Handbook on Tetrazolium Testing; AOSA 2000). Keep tetrazolium solution refrigerated in a dark container.
h. Remove the seed from the seed coat by cutting a small slit down the length of the seed with a single edge razor blade and then removing the seed with forceps.
    i. It is extremely important that no damage occur to the embryonic axis when a seed is cut.
i. Completely submerse the seed embryo in a pre-labled cell in the 1% tetrazolium solution for 24-48 hours. Record total number of seeds in each cell. Cover entire tray with aluminum foil and place in the dark.
j. After the soaking period the remove seeds from the tetrazolium solution and place under a 10x magnification dissection microscope for analysis. At a minimum the cotyledon and the pumule need to be stained red. Non stained tissue is dead tissue (see figure below).





Appendix 2. Copy of NJDEP certification of YSI 6600 for Richard Stockton College MSEFS.

State of New Jersey
Department of Environmental Protection
Office of Quality Assurance
Certifies That

Richard Stockton College of New Jersey

having been found compliant with the technical standards given in the

Regulations Governing the Certification of Laboratories and Environmental Measurements N.J.A.C. 7:18 et. Seq.

is hereby approved to analyze for the parameters

Dissolved Oxygen, pH, Specific Conductance, & Temperature

in support of the

Barnegat Bay Ambient Water Monitoring Network

Expiration Date June 30, 2013

Joseph F. Aiello, Manager
Office of Quality Assurance

Appendix 3. Project check lists and data sheets

Appendix 3.1 Checklist for field sampling

General

1. field sheets
2. dive slates
3. pencils
4. sharpies
5. plastic clipboard
6. dry cooler
7. 5 gallon bucket
8. dive gear
9. Camera w/case
10. Extra memory card
11. Cell phone in waterproof case
12. Cooler for lunch w/ice and water
13. State boaters safety card
14. Sunscreen
15. Bug spray
16. Dive Flag
17. In water dive flag
18. Weight belt
19. Truck key
20. Trailer Box
21. Boat key
22. Boat Log Book
23. Anchor
24. Diver Ladder
25. Extra dock line

Water Quality

26. YSI 650 in bucket with read out
27. YSI 6600 for switch out
28. Extra batteries for YSI
29. 1 cooler with ice
30. 8 TSS bottles (3 per site. 2 blanks)
31. 8 Chl a bottles (3 per site, 2 blanks)
32. 6 Nutrient bottles (3 per site)

Biomass

33. biomass corer
34. field sieve
35. 10 1 gallon pre-labeled Ziploc bags (5 per site) for *Z marina*
36. 10 1 gallon pre-labeled ziploc bags (5 per site) for macroalgae
37. 30 1 quart pre-labeled ziploc bags (15 per site) for epiphytes

Seed Bank Density/Viability

38. 1 cooler with ice
39. 7 clear acrylic acid washed (10% HCl) cores (10.4 cm diameter by 10 cm depth); 15 per site
40. 14 -10.4 cm diameter red core caps
41. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
42. metal plate for slicing core
43. metal spatula

44. 5 gallon bucket
45. clear acrylic cores cut to 2cm as sediment sub-section depths
46. 90 labeled sample bags for seed bank density
47. 27 labeld sample bags for sediment TOC
48. 10 acid washed (10% HCl) cores (10.4 cm diameter by 10 cm depth)
49. 20 - 10.4 cm diameter red core caps

Appendix 3.2 Checklist for laboratory analysis:

Water Column Chlorophyll a:

1. Fluorometer
2. Centrifuge, capable of 675 g.
3. Tissue grinder
4. Filters, glass fiber, 47-mm or 25-mm, nominal pore size of 0.7 µm
5. Whirl-pak bags for transporting and storing sampled filters.
6. Aluminum foil.
7. Laboratory tissues.
8. Tweezers or flat-tipped forceps.
9. Vacuum pump
10. Room thermometer.
11. Assorted Class A calibrated pipets.
12. Graduated cylinders, 500-mL and 1-L.
13. Volumetric flasks, Class A calibrated, 25-mL, 50-mL, 100-mL and 1-L capacity.
14. Glass rods.
15. Pasteur type pipets or medicine droppers.
16. Disposable glass cuvettes for the fluorometer.
17. Filtration apparatus consisting of 1 or 2-L filtration flask, 47-mm fritted glass disk base and a glass filter tower.
18. Centrifuge tubes, polypropylene or glass, 15-mL capacity with nonpigmented screw-caps.
19. Polyethylene squirt bottles.
20. Acetone, HPLC grade, (CASRN 67-64-1).
21. Hydrochloric acid (HCl), concentrated (sp. gr.1.19), (CASRN 7647-01-0).
22. Chlorophyll *a* free of chlorophyll *b*.
23. Deionoized water

Water Column Total Suspended Solids:

1. Forceps
2. Pre-washed and weighed Gelman glass fiber filters (0.47 cm) in labeled aluminum pans
3. Graduated cylindar
4. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
5. Suction flask
6. Vacuum pump
7. Drying oven
8. Desiccator.
9. Analytical balance, capable of weighing to 0.1 mg.

Water Column NO_2 + NO_3:

1. potassium dihydrogen phosphate (KH_2PO_4)
2. potassium hydroxide (KOH)
3. ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$
4. sulfanilamide
5. HCL
6. N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$

7. nitrate reductase(AtNAR1)
8. NADH
9. Certified reference Material
10. ultrapure reagent grade water
11. 0.45 um filters
12. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
13. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
14. Suction flask
15. Vacuum pump
16. Spectrophotometer/plate reader

Water Column NH_4+NH_3:

1. ultrapure reagent grade water
2. 0.45 um filters
3. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
4. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
5. Suction flask
6. Vacuum pump
7. HCl
8. Sodium chloride
9. Sodium hydroxide
10. Clorox bleach
11. Sodium nitroprusside ($Na_2Fe(CN_6)NO.2H_2O$)
12. Phenol (C_6H_5OH)
13. Sodium thiosulfate 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$
14. ammonium sulfate $(NH_4)_2SO_4$
15. Potassium Iodide (KI)
16. TraceCERT, 1000 mg/L NH4+ in H20, Sigma Aldrch
17. 25 ml glass test tubes
18. Shimadzu spectrophotometer

Water Column OPO_4:

1. ultrapure reagent grade water
2. 0.45um filters
3. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
4. Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
5. Suction flask
6. Vacuum pump
7. HCl
8. ammonium molybdate

9. sulfuric acid
10. ascorbic acid
11. antimony potassium tartrate
12. potassium phosphate monobasic (KH_2PO_4)
13. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch
14. microcentrifuge tube
15. 48 well microplates
16. plastic microcuvette
17. Shimadzu spectrophotometer

Sediment Pore-water NO_2 + NO_3:

1. Gloves
2. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
3. metal plate
4. metal spatula
5. 5 gallon bucket
6. clear acrylic cores cut to 2cm as sediment sub-section depths
7. De-ionized (DI) water for cleaning
8. Large plastic tray
9. analytical balance capable of 3 decimal places
10. Potassium chloride (KCl; granular, certified ACS)
11. 4 oz whirlpak bags (pre-labeled)
12. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
13. graduated cylinders
14. shaker table
15. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
16. Centrifuge
17. 30 mL BD syringe with luerlok tip
18. Acrodisc 25 mm syringe filters with 0.45 μm supor membrane
19. 2 oz. Whirlpak bags (pre-labeled)
20. potassium dihydrogen phosphate (KH_2PO_4)
21. potassium hydroxide (KOH)
22. ethylendiaminetetracetic acid (EDTA) $C_{10}H_{14}N_2Na_2O_8.H_2O$
23. sulfanilamide
24. HCL
25. N-(1-napthyl)ethylenediamine dihydrochloride (NED) $C_{12}H_{16}C_{12}N_2$
26. nitrate reductase (AtNAR1)
27. NADH
28. TraceCERT, 1000 mg/L NO_3^- in H_20, Sigma Aldrch (Fluka)74246
29. CRM is 1000 PPM or 16.12 mM
30. ultrapure reagent grade water
31. spectrophotometer/plate reader

Sediment Pore-water NH_4+ NH_3:

1. Gloves
2. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
3. metal plate
4. metal spatula
5. 5 gallon bucket
6. clear acrylic cores cut to 2cm as sediment sub-section depths
7. De-ionized (DI) water for cleaning
8. Large plastic tray
9. analytical balance capable of 3 decimal places
10. Potassium chloride (KCl; granular, certified ACS)
11. 4 oz whirlpak bags (pre-labeled)
12. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
13. graduated cylinders
14. shaker table
15. 50 mL centrifuge tubes
16. Centrifuge
17. 30 mL BD syringe with luerlok tip
18. Acrodisc 25 mm syringe filters with 0.45 μm supor membrane
19. 2 oz. Whirlpak bags (pre-labeled) Cooler with ice
20. 5 pre-labeled acid washed (10% HCl) amber 500 mL polypropylene bottles with lids
21. ultrapure reagent grade water
22. Acid washed (10% HCl) 50 mL centrifuge tubes with caps
23. Sodium chloride
24. Sodium hydroxide
25. Clorox bleach
26. Sodium nitroprusside ($Na_2Fe(CN_6)NO.2H_2O$)
27. Phenol (C_6H_5OH)
28. Sodium thiosulfate 7.9 g $Na_2S_2O_3$ or 12.4 g $Na_2S_2O_3.5H_2O$
29. ammonium sulfate $(NH_4)_2SO_4$
30. Potassium Iodide (KI)
31. TraceCERT, 1000 mg/L NH4+ in H20, Sigma Aldrch
32. HCl
33. TraceCERT, 1000 mg/L NH4+ in H20, Sigma Aldrch
34. 25ml glass test tubes
35. Shimadzu spectrophotometer

Sediment Pore-water OPO_4:

1. Gloves
2. rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole
3. metal plate
4. metal spatula
5. 5 gallon bucket
6. clear acrylic cores cut to 2cm as sediment sub-section depths

7. De-ionized (DI) water for cleaning
8. Large plastic tray
9. analytical balance capable of 3 decimal places
10. Potassium chloride (KCl; granular, certified ACS)
11. 4 oz whirlpak bags (pre-labeled)
12. container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker)
13. graduated cylinders
14. shaker table
15. 50 mL centrifuge tubes
16. Centrifuge
17. 30 mL BD syringe with luerlok tip
18. Acrodisc 25 mm syringe filters with 0.45 μm supor membrane
19. 2 oz. Whirlpak bags (pre-labeled)
20. ultrapure reagent grade water
21. Acid washed (10% HCl) 50 mL centrifuge tubes with caps.
22. HCl
23. ammonium molybdate
24. sulfuric acid
25. ascorbic acid
26. antimony potassium tartrate
27. potassium phosphate monobasic (KH_2PO_4)
28. TraceCERT, 1000 mg/L PO_4^{3-} in H_20, Sigma Aldrch
29. microcentrifuge tube
30. 48 well microplates
31. plastic microcuvette
32. Shimadzu spectrophotometer

Sediment TOC:

1. Large plastic tray
2. pre-weighed/numbered aluminum foil envelopes (use pencil to number envelope)
3. pre-weighted/numbered aluminum pans (use pencil to number pan)
4. analytical balance capable of 3 decimal places
5. aluminum baking pans
6. drying oven set at 50°C
7. muffle furnace capable of maintaining 400°C
8. Desiccator with desiccant
9. Tongs
10. Heat resistant gloves

Seagrass Biomass:

1. Large plastic tray
2. Single edge razor blades
3. Distilled/deoionized water

4. Preweighed and numbered aluminum envelopes
5. Digital scale capable of reading 3 decimal places
6. Drying oven

Macroalgal Biomass:

1. Large plastic tray
2. Distilled/deoionized water
3. Identification guides for macroalgae
4. Pre-weighed and numbered aluminum envelopes
5. Hand lens or magnifying device
6. Digital scale capable of reading 3 decimal places
7. Drying oven

Epiphyte Biomass:

1. 15 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
2. Waterproof data sheets and a clipboard
3. Fine forceps
4. Large plastic tray
5. Single edge razor blades
6. Distilled/deoionized water
7. Pre-weighed and numbered aluminum pans (label pans with pencil)
8. Digital scale capable of reading 4 decimal places
9. Drying oven

Seed Bank Density/Viability:

1. 90 pre-labeled plastic bags (with a zipper, and date, site identification code, and sample number written on bag in permanent ink) for storing samples
2. 0.5 cm mesh sieve (large enough to fit over 5 gallon bucket)
3. Waterproof data sheets and a clipboard
4. Fine forceps
5. Large plastic tray
6. Single edge razor blades
7. Distilled/deoionized water
8. 1 % tetrazolium solution
9. Acid washed labeled ice cube trays
10. Aluminum foil

Appendix 3.3 Field data sheet

Date: _____ Time: _____ Site: _____

Temp © _____ DO (%) _____

Sp Cond _____ DO (conc) _____

Salinity _____ Turb (NTU) _____

pH _____ Depth (sonde) _____

Depth (stick) _____ Secchi

Biomass Bag #s _____ Seed bank bag #s _____

Sediment Core #s _____ Chl *a* bottle #s _____

TSS bottle #s _____

% Cover *Zostera*	_____	_____	_____
	_____	_____	
% Cover *Ruppia*	_____	_____	_____
	_____	_____	
% Cover macroalgae	_____	_____	_____
	_____	_____	
% Cover *other*	_____	_____	_____
	_____	_____	
5 blade lengths (mm) *(Zostera* only)	_____	_____	_____
	_____	_____	

Comments: _____

Supervisor Initials _____

Appendix 3.4 Datasonde Calibration data sheet

CALIBRATION WORK SHEET

Date of Calibration: _____ Sonde ID: _____
Technician: _____

RP DO membrane changed? Y N
RP DO membrane o-ring changed? Y N

Note: Wait 3 to 6 hours before calibrating for unattended deployments; run in Discrete mode for 10 minutes to accelerate burn in. (Rapid Pulse DO Only)

Turbidity wiper changed? Y N Chlorophyll wiper changed? Y N
ROX DO wiper changed? Y N BGA-PE wiper changed? Y N
BGA-PC wiper changed? Y N Rhodamine wiper changed? Y N

Note: If parking problems occur with optical probes having a serial number 07L (Dec 07) or older, be sure the firmware is 3.06 or later. Parking issues with optical probes having a serial number prior to 07L may be related to a dirty wiper body or pad.

Record sonde battery voltage: _____ (if applicable)

Record the following diagnostic numbers after calibration.

6560 Conductivity cell constant _____ Range 5.0 ± .45
Integrated conductivity cell constant _____ Range 5.0 ± .70
pH mv Buffer 7 _____ Range 0 ± 50 mv
pH mv Buffer 4 _____ Range +180 ± 50 mv*
pH mv Buffer 10 _____ Range -180 ± 50 mv *

***Note:** Millivolt span between pH 4 and 7 should be ≈ 165 to 180 mv
Millivolt span between pH 7 and 10 should be ≈ 165 to 180 mv

DO charge (RP only) _____ Range 25 to 75
DO gain _____ Range 0.7 to 1.4
ODO gain _____ Range 0.85 to 1.15

Turbidity standard used in calibration _____
Manufacturer and part number _____

Barometric Pressure: _____mmHg
DO % Calculated – (BARO mmHg divided by 7.6) = % saturation
Example: 760 ÷ 7.6 = 100.0%

Record Calibration Values

Standard	Pre Cal / Post Cal
Temperature _____	____Sonde
Conductivity _____	____/____
pH 7 _____	____/____
pH 4 _____	____/____
pH 10 _____	____/____
ORP _____	____/____
Turbidity _____	____/____
Turbidity _____	____/____
Turbidity 0.5_____	____/____
Chlorophyll _____	____/____
Chlorophyll _____	____/____
DO RP _____	____/____
DO ROX _____	____/____
BGA PE/PC _____	____/____
BGA PE/PC _____	____/____
Rhodamine _____	____/____

Depth Calibration - If zero was entered, record barometric pressure at time of calibration _____mmHg
Depth Calibration - If offset depth was entered, record value _____ meters/feet and pressure _____ mmHg
Depth Calibration (Vented) – Acceptable calibration constant: 0.0 psig ± 0.15 _____

Notes:

Appendix 3.5 Water Column TSS/Chl a data sheet

BB H2O Column

Name _____

Date _____

Water Column TSS

Pan #	Site	Filter Wt g	Dry Wt g	Dry Wt g	Dry Wt g	Combst Wt g	Amt Filt. mL
	BI 1						
	BI 2						
	BI 3						
	BI Blank						
	SS 1						
	SS 2						
	SS 3						
	SS Blank						

Water Column Chl a

Site	Chl a B. Acid	Chl a A. Acid	Amt Filt. mL
BI 1			
BI 2			
BI 3			
BI Blank			
SS 1			
SS 2			
SS 3			
SS Blank			

Water Column Nuts

Site	Amt Filt. mL
BI 1	
BI 2	
BI 3	
BI Blank	
SS 1	
SS 2	
SS 3	
SS Blank	

Appendix 3.6 Sediment TOC data sheet

Barnegat Bay

Name _____

Date _____

Sediment Organic Matter

Env #	Site	Depth mm	Env Wt g	Wet Wt g Env + Sed	Dry Wt g Env + Sed	Dry Wt g Env + Sed	Dry Wt g Env + Sed	Pan #	Pan Wt g	Final DW g Pan+Sed	Combst Wt g Pan + Sed
600	BI-1	0,2									
601		2,4									
602		4,6									
603	BI-2	0,2									
604		2,4									
605		4,6									
606	BI-3	0,2									
607		2,4									
608		4,6									
609	BI-4	0,2									
610		2,4									
611		4,6									
612	BI-5	0,2									
613		2,4									
614		4,6									
615	SS-1	0,2									
616		2,4									
617		4,6									
618	SS-2	0,2									
619		2,4									
620		4,6									
621	SS-3	0,2									
622		2,4									
623		4,6									
624	SS-4	0,2									
625		2,4									
626		4,6									

Appendix 3.7 Seagrass biomass data sheet. Spp/VF = species (Z. marina or R. maritima); AG/BG = above ground/below ground, WW = wet weight, DW = Dry weight.

BB Zmarina Model Biomass Data Sheet

Date	Site	Rep	Env #	Env. Wt g	Spp/VF	AG/BG	WW g 1	DW g 1	DW g 2	DW g 3	Comments
			1								
			2								
			3								
			4								
			5								
			6								
			7								
			8								
			9								
			10								
			11								
			12								
			13								
			14								
			15								
			16								
			17								
			18								
			19								
			20								
			21								
			22								
			23								
			24								
			25								
			26								
			27								
			28								
			29								
			30								

BB Z marina Biomass
Supervisor Int. _____

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Appendix 3.8 Macroalgae biomass data sheet

BB Macroalgae Model Biomass Data Sheet

Date	Site	Rep	Species	Env #	Env. Wt g	WW g	DW1 g	DW2 g	DW3 g
				1					
				2					
				3					
				4					
				5					
				6					
				7					
				8					
				9					
				10					
				11					
				12					
				13					
				14					
				15					
				16					
				17					
				18					
				19					
				20					
				21					
				22					
				23					
				24					
				25					
				26					
				27					
				28					
				29					
				30					

BB Macroalgae Biomass
Supervisor Int. _____

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Appendix 3.9 Epiphyte biomass data sheet

BB Epiphyte Biomass Data Sheet

Date	Site	Rep	Pan #	Pan Wt g	WW g 1	DW g 1	DW g 2	DW g 3	Comments
			1						
			2						
			3						
			4						
			5						
			6						
			7						
			8						
			9						
			10						
			11						
			12						
			13						
			14						
			15						
			16						
			17						
			18						
			19						
			20						
			21						
			22						
			23						
			24						
			25						
			26						
			27						
			28						
			29						
			30						

BB Epiphyte Biomass
Supervisor Int. _____

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Appendix 3.10 Epiphyte area data sheet

BB Epiphyte Biomass Data Sheet

Date	Site	Rep	Pan #	# shoots	L/W	L/W	L/W	L/W	L/W	L/W	L/W	L/W	L/W	L/W
			1											
			2											
			3											
			4											
			5											
			6											
			7											
			8											
			9											
			10											
			11											
			12											
			13											
			14											
			15											
			16											
			17											
			18											
			19											
			20											
			21											
			22											
			23											
			24											
			25											
			26											
			27											
			28											
			29											
			30											

BB Epiphyte Area
Supervisor Int._____

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Appendix 3.11 Sediment Seed Bank data sheet

Name ____________________
Date ____________________

BB 2012 Core Seed Data

Date	Rep	Site	Species	Depth mm	Tray Position	# Seeds	# Viable

BB 2012 Seed Viability
Supervisor Int.____________ Page 1 of 1 7/9/2012

Appendix 3.12 Field Sample inventory sheet

Sample Inventory Sheet Date _____ Site _____

Sample ID	Logged In
WATER SAMPLES	
TSS 1	
TSS 2	
TSS 3	
TSS Blank	
Chl 1	
Chl 2	
Chl 3	
Chl Blank	
Nut 1	
Nut 2	
Nut 3	
BIOMASS SAMPLES	
SG BM 1	
SG BM 2	
SG BM 3	
SG BM 4	
SG BM 5	
MA BM 1	
MA BM 2	
MA BM 3	
MA BM 4	
MA BM 5	
EPI BM 1	
EPI BM 2	
EPI BM 3	
EPI BM 4	
EPI BM 5	
EPI BM 6	
EPI BM 7	
EPI BM 8	
EPI BM 9	
EPI BM 10	
EPI BM 11	
EPI BM 12	
EPI BM 13	
EPI BM 14	
EPI BM 15	
SEED BK SAMPLES	
SB 1	
SB 2	
SB 3	
SB 4	
SB 5	

Sample ID	Logged In
SEED BK SAMPLES	
SB 6	
SB 7	
SB 8	
SB 9	
SB 10	
SB 11	
SB 12	
SB 13	
SB 14	
SB 15	
SB 16	
SB 17	
SB 18	
SB 19	
SB 20	
SB 21	
SB 22	
SB 23	
SB 24	
SB 25	
SB 26	
SB 27	
SB 28	
SB 29	
SB 30	
SB 31	
SB 32	
SB 33	
SB 34	
SB 35	
SB 36	
SB 37	
SB 38	
SB 39	
SB 40	
SB 41	
SB 42	
SB 43	
SB 44	
SB 45	

Sample ID	Logged In
SED TOC SAMPLES	
%SO 1	
%SO 2	
%SO 3	
%SO 4	
%SO 5	
%SO 6	
%SO 7	
%SO 8	
%SO 9	
%SO 10	
%SO 11	
%SO 12	
%SO 13	
%SO 14	
%SO 15	
%SO 16	
%SO 17	
%SO 18	
%SO 19	
%SO 20	
%SO 21	
%SO 22	
%SO 23	
%SO 24	
%SO 25	
%SO 26	
%SO 27	
SED NUT SAMPLES	
SN Core 1	
SN Core 2	
SN Core 3	
SN Core 4	
SN Core 5	

PM Signature _____

Appendix 3.13 Sample Location inventory sheet

WATER SAMPLES

Sample ID	Date Collected	Location	Date(s) Processed	Analysist Initials
TSS 1				
TSS 2				
TSS 3				
TSS Blank				
Chl 1				
Chl 2				
Chl 3				
Chl Blank				
Nut 1				
Nut 2				
Nut 3				

BIOMASS SAMPLES

Sample ID	Date Collected	Location	Date(s) Processed	Analysist Initials
SG BM 1				
SG BM 2				
SG BM 3				
SG BM 4				
SG BM 5				
MA BM 1				
MA BM 2				
MA BM 3				
MA BM 4				
MA BM 5				
EPI BM 1				
EPI BM 2				
EPI BM 3				
EPI BM 4				
EPI BM 5				
EPI BM 6				
EPI BM 7				
EPI BM 8				
EPI BM 9				
EPI BM 10				
EPI BM 11				
EPI BM 12				
EPI BM 13				
EPI BM 14				
EPI BM 15				

Appendix 4. Supporting material for nutrient analysis.