MAM Activity Implementation Plan:
Lower Trophic Level Monitoring for Barataria Basin

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2.0 Acronyms

ACOLITE	Atmospheric Correction processor for coastal and inland waters
AFDW	Ash-Free Dry Weight
ANOVA	Analysis of Variance
CASM	Comprehensive Aquatic Systems Model
CFR	Code of Federal Regulations
CNS	Carbon, Nitrogen and Sulphur
CPRA	Coastal Protection and Restoration Authority
CRMS	Coastwide Reference Monitoring System
DO	Dissolved Oxygen
DOI	Department of Interior
DOM	Dissolved Organic Matter
DWH	Deepwater Horizon
EDA	*Exploratory Data Analysis*
EFM	Epifluorescence Microscope
EIS	Environmental Impact Statement
EPA	Environmental Protection Agency
ESA	European Space Agency
EV	Emergent Vegetation
EwE	Ecopath with Ecosim
FGDC	Federal Geographic Data Committee
FIMP	Fisheries Independent Monitoring Program
FWOA	Future without Action
FWOP	Future without Project
GF/F	Glass Fiber Filter
GIS	Geographic Information System
GRTS	Generalized Random Tessellation Stratified
HAB	Harmful Algal Bloom
HPLC	High-Performance Liquid Chromatography
HSI	Habitat Suitability Index
IRMS	Isotope Ratio Mass Spectrometry
ISO	International Organization for Standardization
LA	Louisiana
LDWF	Louisiana Department of Wildlife and Fisheries
LTL	Lower Trophic Level
MAIP	Monitoring and Adaptive Management Implementation Plan
MAM	Monitoring and Adaptive Management
MUMM	Management Unit of the Mathematical Model of the North Sea
NAP	Non-algal Particles
NEPA	National Environmental Policy Act
nMDS	Non-metric Multi-Dimensional Scaling
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NRDA	Natural Resource Damage Assessment
OM	Organic Matter

OPA	Oil Pollution Act
OW	Open Water: water column and submerged benthos
PAR	Photosynthetically Active Radiation
PC	Phycocyanin
PDARP	Programmatic Damage Assessment and Restoration Plan
PEIS	Programmatic Environmental Impact Statement
PERMANOVA	Permutated Multivariate Analysis of Variance
POC	Particulate Organic Carbon
POM	Particulate Organic Matter
QFT	Quantitative Filter Technique
RFU	Raw Fluorescence Unit
SAV	Submerged Aquatic Vegetation
SD	Standard Deviation
SIMPER	Similarity Percentage
SMART	Specific, Measurable, Achievable, Relevant, appropriate Timeline
SNAP	Sentinel Application Platform
SOP	Standard Operating Procedures
SWAMP	System-Wide Assessment and Monitoring Program
TIG	Trustee Implementation Group
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
TVS	Total Volatile Solids
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WCNH	Wetland, Coastal, and Nearshore Habitats
YOY	Young of the Year

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5.0 Introduction

The Deepwater Horizon (DWH) oil spill settlement in 2016 provides the Natural Resource Damage Assessment (NRDA) Trustees (Trustees) up to $8.8 billion, distributed over 15 years, to restore natural resources and services injured by the spill. As described in the DWH oil spill *Final Programmatic Damage Assessment and Restoration Plan* (PDARP) and *Final Programmatic Environmental Impact Statement* (PEIS; DWH NRDA Trustees, 2016), the Trustees selected a comprehensive, integrated ecosystem approach to restoration. The PDARP/PEIS considers programmatic alternatives, composed of Restoration Types, to restore natural resources, ecological services, and recreational use services injured or lost as a result of the DWH oil spill incident. As shown in the PDARP/PEIS, the injuries caused by the DWH oil spill affected such a wide array of linked resources over such an enormous area that the effects must be described as constituting an ecosystem-level injury. The PDARP/PEIS and information on the settlement with British Petroleum Exploration and Production Inc. (termed the "Consent Decree") are available at www.gulfspillrestoration.noaa.gov.

Given the unprecedented temporal, spatial, and funding scales associated with the DWH oil spill restoration effort, the Trustees recognized the need for robust Monitoring and Adaptive Management (MAM) to support restoration planning and implementation. As such, the following proposed data collection effort will provide essential information to develop and evaluate the programmatic goals established in the PDARP/PEIS to "Provide for Monitoring, Adaptive Management, and Administrative Oversight to Support Restoration Implementation" to ensure that the portfolio of restoration projects provides long-term benefits to natural resources and services injured by the DWH oil spill (Appendix 5.E of the PDARP/PEIS). The data collected by the proposed effort will allow the Trustees to evaluate ecosystem-wide impacts of restoration, address potential uncertainties related to restoration planning and implementation, and provide feedback to inform future restoration decisions. The integrated restoration portfolio emphasizes the broad ecosystem benefits that can be realized through coastal habitat restoration in combination with resource-specific restoration in the ecologically interconnected northern Gulf of Mexico ecosystem (DWH NRDA Trustees, 2016). The majority of the planned restoration projects identified in the integrated restoration portfolio (DWH NRDA Trustees, 2016) are concentrated in coastal Louisiana and in conjunction with the Louisiana Coastal Master Plan (Coastal Protection and Restoration Authority, 2017).

Lower Trophic Level (LTL) organisms are fundamental bioindicators of estuarine health, as evidenced by their extensive use by resource management agencies (e.g., the US EPA National Coastal Condition Assessment Framework; the European Water Framework Directive; and state and regional agencies including California, New Jersey, and the Chesapeake Bay). Changes in the ecosystem health of Barataria Basin over time, such as whether salinity changes or anoxia events had an ecosystem impact on fish and other aquatic resources, can be related to changes in the lower trophic level organisms that form the base of the estuarine food web. In estuarine settings like the Barataria Basin, system productivity of a truncated food web is dominated by LTL groups consisting of primary consumers (i.e., organisms at ~ trophic level 2) and the detrital pools and primary producers they consume (Rose et al. 2019, Lewis et al. 2021). However, the lack of information on these resources in Barataria Basin - even at a basic level,

such as whether or how the benthic community varies across the different salinity zones in the Basin - results in an inability to measure future changes with confidence.

This Monitoring and Adaptive Management (MAM) Activities Implementation Plan (MAIP) will enable data collection to fill known data gaps and address management needs related to Lower Trophic Level (LTL) organismal abundances, densities, biomass, and community composition in the Barataria Basin, information which is needed to develop Specific, Measurable, Achievable, Relevant, appropriate Timeline (SMART) Objectives for several of the fundamental objectives developed by the Louisiana Trustee Implementation Group (LA TIG) as described in the LA TIG MAM Strategy (Kiskaddon et al., 2021, 2022; LA TIG, 2021). This MAIP presents results of, incorporates, and builds off of the LA TIG Lower Trophic Level Monitoring for Barataria Basin planning (DIVER ID #269). The MAM activity described herein captures the current condition of the LTL in the Barataria Basin to assess ecosystem outcomes associated with restoration activities. In addition to combating land loss, restoration activities in Barataria Basin are intended to support ecosystem productivity and function to maintain Louisiana's contribution to the nation's fisheries. This MAM activity will also provide detailed information of LTL spatiotemporal variability, as well as variability relative to environmental conditions, which is crucial information needed to parameterize and validate new ecosystem models, or improve existing ones, used to evaluate restoration outcomes and adaptive management options.

Coordinating this MAM activity with other large-scale monitoring of vegetation and nekton provides a unique opportunity and the best chance to measure restoration benefits to ecosystem function while accounting for interannual variability noise in factors such as sediment accretion and emergent vegetation production.

6.0 Purpose of This Document

This MAIP describes a MAM activity to conduct an inventory sampling and analysis of key LTL groups (phytoplankton, microphytobenthos, zooplankton, and macroinfauna; Table 1) in the Barataria Basin. This MAM activity will collect the data that is needed to quantify the current condition of LTL organisms with which to evaluate the outcomes of LA TIG restoration activities for injured resources. This data can also be used to make improvements to existing ecosystem models (e.g., Ecopath with Ecosim [EwE], Comprehensive Aquatic Systems Model [CASM]) that have been used to evaluate food web structure and productivity in Barataria Basin and can be used in the future to assess ecosystem outcomes of NRDA restoration within and outside of Barataria Basin. This work will be used to develop SMART Objectives for several of the fundamental objectives developed by the LA TIG as described in the LA TIG MAM Strategy (LA TIG, 2021). This MAM activity is intended to support evaluation of regional outcomes within the Louisiana Restoration Area; perform data aggregation and data management; resolve critical information gaps and uncertainties for restoration planning; and inform restoration decision making. This document also describes the applicability of these activities to the PDARP/PEIS as well as their consistency with the Oil Pollution Act (OPA) and compliance with National Environmental Policy Act (NEPA).

Table 1. General definitions of key LTL groups relevant to this MAIP. The general definitions provided do not specifically exclude certain taxa unless indicated. Note: Due to the ubiquitous importance of detritus as an important driving variable for all key LTL groups included in this MAIP, it is not specifically identified in this table.

Key LTL Group	General Definition
Phytoplankton	Pelagic cyanobacteria and microalgae, including diatoms, dinoflagellates, and chlorophytes.
Microphytobenthos	Benthic diatoms, chlorophytes, flagellates, and cyanobacteria.
Zooplankton	Heterotrophic or mixotrophic plankton; inclusive of, but not limited to pelagic copepods, gelatinous zooplankton, and pelagic larval organisms.
Macroinfauna	Sediment-dwelling organisms > 500 µm in size; inclusive of, but not limited to: annelids (polychaetes), molluscs (gastropods and bivalves), and arthropods.

7.0 MAM Activity Overview: Lower Trophic Level Monitoring for Barataria Basin

This MAM activity addresses, or partially addresses, Cross Restoration Type and the Wetland, Coastal, and Nearshore Habitats (WCNH) Fundamental Objectives and SMART Objective/MAM Needs described in the LA TIG MAM Strategy (the full lists of objectives and needs are found in Tables 2 and 9 in LA TIG (2021) and are copied below as Table 2 and Table 3).

Fundamental Objectives addressed (LA TIG, 2021):

- WCNH #7: Provide benefits to estuarine-dependent fish and invertebrates (nekton and benthic) at a variety of life stages through habitat restoration
- Cross Restoration #1: Maximize the combined benefits of the various Restoration Types and approaches across the overall restoration portfolio (PDARP Section 5.5.1)
- Cross Restoration #2: Support injured species (trophic structure) via the estuarine food web structure (benthic and pelagic)

SMART Objective/MAM Needs addressed (LA TIG, 2021):

- WCNH #7b: Estimate the effects of changes in habitat availability and type, and other restoration actions, on estuarine community structure, food web, and population connectivity.
- Cross-Restoration #1b: Quantify wetland net ecosystem carbon balance at pre-DWH oil spill/post-DWH oil spill time scales and basin/sub-basin spatial scales, including export to nearshore Gulf of Mexico.
- Cross-Restoration #2a: Assess whether the DWH NRDA restoration portfolio supports LTL diversity, distribution, and productivity comparable to appropriate reference areas, when accounting for expected changes in environmental drivers (e.g., hydrology, water quality, conversion of shallow open water to wetlands)
- Cross-Restoration #2b: Develop approach to analyze and synthesize food web characteristics, identify and characterize appropriate reference ecosystems/basins
- Cross-Restoration #2c: Identify and develop approach to interpret and assess trophic linkages

Table 2. WCNH Restoration Type SMART Objectives. This table also highlights the associated MAM needs and MAM activities nested under this Restoration Type's High Level and Fundamental Objectives. The bolder text emphasizes the objectives addressed by this MAM Activity. Excerpted from Table 2 in LA TIG (2021).

High Level Objectives	Fundamental Objectives	MAM need to develop SMART objective	Suggested MAM activity to address MAM need	SMART Objectives
Marsh platform/ area is created, restored, or maintained (resilient / maintained over time)	1. Contribute to reduction in net marsh loss in coastal Louisiana	1.a. Quantify and assess historic, current, and future predicted emergent vegetated wetland habitat area in coastal Louisiana and determine appropriate quantification for implemented and long-term land area and from DWH NRDA restoration *(concurrent with* 3.a.)	1.a. Compile available historic emergent vegetated wetland loss and habitat datasets (e.g. CRMS, USGS), assess trends and limitations; develop coordinated/integrated approach to monitor and assess emergent vegetated wetlands across habitat types at necessary spatial and temporal scales, utilizing new technology, while allowing comparisons to older historic information *(concurrent with* 3.a.*)*	*Objective related to area of DWH NRDA created wetland to be developed based on current MAM need and activity* 1.a. *and* 3.a.
		1.b. Quantify and assess sediment volume availability for marsh creation projects in target basins, sub-basins, or HUC 12 watersheds	1.b. Synthesize available sediment volume data and, if needed, develop a plan for monitoring in target basins, sub-basins or HUC12 watersheds	*Objective related to sediment volume DWH NRDA created wetland to be developed based on current MAM need and activity* 1.b.
	2. Maintain elevational landscape sufficient to support wetland vegetation	2.a. Synthesize available data and/or quantify appropriate land elevation for different marsh vegetation types and develop approach for assessment and reporting on DWH NRDA projects to sustain a diversity of emergent marsh vegetation over the life of the restored marshes	2.a. Develop a technical report on available data and knowledge (e.g., CRMS data, and coastwide LiDAR data), supplementing with additional data sampling as needed within target coastal basins	*Objective related to DWH NRDA created wetlands elevation and ability to sustain a diversity of emergent wetlands to be developed based on current MAM need and activity* 2.a.
	3. Restore habitats injured by the spill in a range of salinity zones (fresh, intermediate, brackish, saline)	3.a. Quantify and assess historic, current, and predicted emergent vegetated wetland habitat area in coastal Louisiana and determine appropriate quantification for implemented and long-term vegetated marsh salinity community types from DWH NRDA restoration *(concurrent with* 1.a.*)*	3.a. Analyze and synthesize available historical data and numerical model predictions of future without action (CMP) to identify coastwide and basin specific marsh salinity community targets *(concurrent with* 1.a.*)*	*Objective related to DWH NRDA created wetlands vegetated marsh salinity community types to be developed based on current MAM need and activity* 3.a. *and* 1.a.
Barrier island habitat is created, restored, or maintained (resilient / maintained over time) to reduce land loss	4. Maintain protective function (wave attenuation) of barrier islands	4.a. Develop approach and plan for monitoring to quantify wave attenuation from barrier islands	4.a. Synthesize available data and develop a numerical model to assess change in wave climate/pattern within a basin or in the lee of a barrier island with different restoration options	*Objective related to wave attenuation by DWH NRDA created barrier islands to be developed based on current MAM need and activity* 4.a.
	5. Support natural processes of barrier island evolution (e.g., erosion, overwash that builds back-barrier platform, and longshore sediment transport within the littoral zone; barrier	5.a. Develop and document approach for assessing and characterizing restored barrier island response to natural processes (e.g., changes to dune morphology and island resistance or resilience to overwash and sea-level rise)	5.a. Synthesize available data (e.g., BICM, BISM) to establish baseline and assessment framework for natural processes in barrier island evolution including (overwash area; cross-shore and long-shore sediment transport volume, barrier island rollover [migration] rate, estuarine salinity gradient) using data synthesis, analysis, expert elicitation, and technical report	*Objective related to maintenance of natural processes of barrier island evolution to be developed based on current MAM need and activity* 5.a.

High Level Objectives	Fundamental Objectives	MAM need to develop SMART objective	Suggested MAM activity to address MAM need	SMART Objectives
	island rollover rate) through barrier island restoration projects			
	6. Maintain habitat heterogeneity to support resilient nearshore and coastal ecosystems	6.a. Develop and document approach to quantify and assess habitat heterogeneity in restored key barrier island habitat types	6.a. Collect and analyze data to report on habitat heterogeneity in a range of types of restored and reference barrier islands; such as BICM, project data including area by habitat and wetland type, identification of habitat complexes, topography, and aerial/CIR photo analysis	*Objective related to maintenance of habitat heterogeneity in barrier islands to be developed based on current MAM need and activity* 6.a.
Provide habitat and habitat complexes for Wetland Coastal Nearshore Habitats-dependent species and support species diversity for various life stages	**7. Provide benefits to estuarine dependent fish and invertebrates (nekton and benthic) at a variety of life stages through habitat restoration**	7.a. Develop reference ranges for density and relative abundance of target fish and invertebrate guilds or species, based on natural variability of relative abundance and density at appropriate reference sites; identify the distance from a restored area at which a restoration effect could be detected	7.a. Establish fixed area sampling stations, sample, and analyze with existing FIMP data, to develop reference ranges for densities and abundance, and distance and time at which effect could be measured	*Objective related to abundance of target fish and invertebrate guilds or species to be developed based on current MAM need and activity* 7.a.
		7.b. Estimate the effects of changes in habitat availability and type, and other restoration actions, on estuarine community structure, food web, and population connectivity	**7.b. Model [faunal diversity, richness, and/or diets] to forecast effects of estuarine restoration portfolio and recovery times *(informed by or performed in conjunction with Cross Restoration Type SMART Objective* 2.b.*)***	***Objective related to food web and habitat to be developed based on current MAM need and activity* 7.b. *(linkage to Cross Restoration Type SMART Objective* 2.b.*)*** ***Objective related to ecological connectivity of wetlands, coastal, and nearshore habitat restoration projects to be developed based on current MAM need and activity* 7.b. *(linkage to Cross Restoration Type SMART Objective* 2.b.*)***
		7.c. Within 5 years quantify habitat characteristics appropriate for target fish and invertebrate guilds or species	7.c. Develop a technical guidance document for restoration project design and monitoring to maximize habitat value for nekton, using data and knowledge at multiple spatial scales, supplementing with additional data sampling as needed within target coastal basins and SMEs as appropriate (relevant datasets may include: hydrologic connectivity, access, inundation, edge:interior ratio, vegetation, interspersion/features)	*Objective related to incorporation of habitat features into restoration approaches to be developed based on current MAM need and activity* 7.c.

Table 3. Cross-Restoration Type SMART Objectives. This table also highlights the associated MAM needs and MAM activities nested under this Restoration Type's High Level and Fundamental Objectives. The bolder text emphasizes the objectives addressed by this MAM Activity. Excerpted from Table 9 in LA TIG (2021).

High Level Objectives	Fundamental Objectives	MAM need to develop SMART objective	Suggested MAM activity to address MAM need	SMART Objectives
Contribute to maintaining and restoring ecosystem-scale condition and resilience at coastwide, basin, and sub-basin scales	1. Maximize the combined benefits of the various Restoration Types and approaches across the overall restoration portfolio (PDARP Section 5.5.1)	1.a. Evaluate the efficacy of various strategies in land creation/restoration (diversions, marsh platform creations, barrier island restoration, ridge restoration)	1.a. Identify appropriate time scale for evaluating the significant change or trajectory (every 5 years or it may be on a longer time scale?)	***Objective on efficacy of land creation/restoration strategies to be developed based on current MAM need and activity* 1.a.**
		1.b. Quantify wetland net ecosystem carbon balance at pre-spill/post-spill time scales and basin/sub-basin spatial scales, including export to nearshore Gulf of Mexico	1.b. Within the next 5 years, targeted numerical modeling based upon available/collected data to calculate carbon capture of flora, fauna, and soils, associated with restoration portfolio; synthesize as carbon budget and calculate carbon export to nearshore marine systems	***Objective related to coastal / basin carbon budget to be developed based on current MAM need and activity* 1.b.**
	2. Support injured species (trophic structure) via the estuarine food web structure (benthic and pelagic)	2.a. Assess whether the DWH NRDA restoration portfolio supports lower trophic level diversity, distribution, and productivity comparable to appropriate reference areas, when accounting for expected changes in environmental drivers (e.g., hydrology, water quality, conversion of shallow open water to wetlands)	2.a. Perform synthesis of available data and fill gaps with Lower Trophic Level/benthic inventory and analyze samples to establish pre-restoration baseline conditions for potential long-term monitoring of pelagic and benthic lower trophic levels (e.g., amphipods, small clams, zooplankton) as a basis for identifying change	***Objective related to lower trophic level biota to be developed based on current MAM need and activity* 2.a.**
		2.b. Develop approach to analyze and synthesize food web characteristics, identify and characterize appropriate reference ecosystems/basins	2.b. Ecosystem modeling to evaluate ecosystem function and drivers, improving confidence in ecosystem model outputs and parameters, and quantifying/ modeling the contribution of transient/ estuarine- dependent species to offshore food webs *(in conjunction with Wetland, Coastal, and Nearshore Habitat Restoration Type objective* 7.b.*)*	***Objective related to food web characteristics to be developed based on current MAM need and activity* 2.b.**
		2.c. Identify and develop approach to interpret and assess trophic linkages	2.c. Analysis of Ecosystem modeling (2.b.) to interpret and assess trophic linkages	***Objective related to trophic linkages to be developed based on current MAM need and activity* 2.c.**
	4. Provide for equivalent pre-spill baseline ecosystem communities and productivity	**4.a. Develop approach to understand and assess how the DWH NRDA restoration portfolio can maximize support to ecosystem communities primary and secondary productivity**	**4.a. Synthesize available data from different restoration techniques to identify relative benefits to ecosystem communities and productivity (project size, tidal flow, balance of elevation/resilience vs habitat, marsh creation scale ratio of edge to interior, oyster reef placement)**	***Objectives related to primary and secondary productivity to be developed based on current MAM need and activity* 4.a.**

7.1 MAM ACTIVITY DESCRIPTION

This MAIP focuses on the Barataria Basin, which experienced substantial heavy and persistent oiling impacts from the DWH oil spill (Nixon et al., 2016), and where significant investment is being made to restore for injured resources. The MAM activity described herein is a data collection and analysis effort to establish reference ranges of key LTL organisms' (phytoplankton, microphytobenthos, zooplankton, and macroinfauna) standing stock metrics (e.g., abundance, density, biomass), community composition/diversity, and trophic level information in the Barataria Basin. These data will help reduce uncertainty related to trends in nekton abundances/densities, and address data gaps that were identified by management stakeholders during planning (DIVER ID #269).

The work by Fry et al. (2003), Rozas & Minello (2011), Nelson et al., (2019), and others highlights the critical nature of the linkages between LTL organisms and key ecological LTL consumers, such as juvenile penaeid shrimp, blue crab, and Gulf menhaden, which are highly abundant in coastal Louisiana. Characterization of the population dynamics of primary and higher-level consumers (including species which are recreationally- and commercially-important to Louisiana) after significant restoration activities are completed will be enhanced by an accurate understanding of the current condition of LTL organisms known to drive the food web in this bottom-up estuarine system (Dynamic Solutions, 2016; Lewis et al., 2021). Reference ranges in LTL standing stock metrics (e.g., abundance, density, biomass), community composition/diversity, and accurate trophic position information would enable quantification of current spatiotemporal ranges in LTL abundance, density, biomass, community composition, and food web support across the estuarine gradient. This would provide a relevant LTL status against which to measure the ecosystem changes in Barataria Basin following the many NRDA and non-NRDA restoration activities that are expected to restore resources in the Barataria Basin over the next few decades. This proposed MAIP is also designed to meet natural resource management needs and fill known knowledge gaps related to LTL organisms in the Barataria Basin, and coastal Louisiana more broadly, as described in Kiskaddon et al. (2022).

This MAIP describes a data collection plan to reduce data gaps in order to adequately characterize the LTL current condition (Kiskaddon et al. 2022), establish appropriate metrics for restoration evaluation, and provide the data that would be needed to refine ecosystem models to support adaptive management. A quantified LTL status (developed under this MAM activity) in combination with a subsequent longer-term monitoring program would additionally enable the LA TIG Trustee agencies to answer the question "How has the aquatic ecosystem in the Barataria Basin changed as a result of restoration actions?" (Dixit et al., 1992). New data collection will also provide important field-based validation necessary to better represent LTLs in existing ecosystem models and enable application of emerging monitoring methodologies (e.g., remote sensing, machine learning) in place of standard methodologies that may reduce the cost burden of longer-term monitoring of LTL communities.

This activity's spatial domain coincides with those of existing ecosystem models that the proposed LTL monitoring activities would support to improve our understanding of the effects of these restoration

investments (for model information, see Dynamic Solutions, 2016, and Lewis et al., 2021). This MAM activity does not include ecosystem modeling, but has been designed to enable **future** improvement of existing ecosystem models, or provide LTL data to develop new models, to evaluate the ecosystem-wide impacts of collective restoration actions in the Barataria Basin, Louisiana. The sampling approach will provide the data needed to adjust LTL biomasses and potentially substantial parts of the Basin's estuarine food web in the existing (previously calibrated) models.

7.1.1 Intended Outcomes and Outputs

The data developed by this MAM activity will support multiple outcomes:

- Develop WCNH MAM SMART objective #7.b. (Table 2; LA TIG, 2021) using reference ranges for key LTL organisms for consideration and refinement by the LA TIG.
- Develop Cross-Restoration Type MAM SMART objective #2.a. (Table 2; LA TIG, 2021) for consideration and refinement by the LA TIG.
- Inform, in combination with additional datasets or activities, the development of MAM SMART objectives #1.b, # 2.b., and #2.c. of the Cross-Restoration Type MAM needs (Table 3; LA TIG, 2021).
- Support restoration planning and evaluation of restoration actions and associated benefits to fishes, mobile and sessile invertebrates, abiotic and biogenic estuarine habitats, and increased ecosystem services in Barataria Basin by supplying information on a critical portion of the complex estuarine food web.
- Develop information needed to describe ecosystem-level effects of DWH restoration projects holistically (not at an individual project scale), such as quantifying changes in key LTL community structure, population, and estuarine productivity.

The project has many outputs:

- Literature review summarizing existing $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values of key primary producers and LTLs by feeding guild and, where possible, specific higher trophic level consumers represented in Barataria Basin food web models (e.g., EwE and CASM).
- Detailed field and laboratory protocols, safety, data processing, and chain-of-custody processes
- At a minimum, five new datasets (one per LTL group [phytoplankton, zooplankton, microphytobenthos, and infauna] and one additional dataset for stable isotopes) and associated metadata that tabulate abundance, density, biomass, community composition/diversity, stable isotope values, and key environmental and habitat drivers (e.g., salinity, sediment type, land:water). The exact number of datasets may be larger as it may be necessary to format different data types in different ways for each LTL group.
- Summary report: sampling plan; results; analysis of spatiotemporal patterns in LTL standing stocks (e.g., abundance, density, biomass), community composition/diversity, and isotope values (including key primary producers); identification of key environmental and habitat drivers (e.g., salinity, sediment type, land:water) and of indicator species to evaluate estuarine condition; recommendations for future sampling and analysis methods.
- Up to six peer-reviewed publications describing the analytical findings.

- Reference ranges for key LTLs (to inform WCNH SMART objective 7.b.).
- Barataria Basin isoscape to validate ecosystem models and to demonstrate effect of environmental drivers on production of key LTLs.
- Long-term monitoring plan for data collection to evaluate future restoration outcomes in comparison to current condition quantified by this activity and incorporating efficiencies and other lessons learned from this activity.

7.1.2 Background

Coastal and nearshore habitats integrate and form a continuum within the nearshore ecosystem and contribute to an integrated, connected food web (Baillie et al., 2015; Boesch & Turner, 1984; Boström et al., 2011; Deegan, 1993; Deegan et al., 2000; Nelson et al., 2013, 2015). Estuarine open waters and wetlands in the Barataria Basin and along the Louisiana coast were among the most heavily oiled of the Gulf Coast shoreline in the aftermath of the 2010 DWH oil spill (Nixon et al., 2016). The PDARP/PEIS recognized the interconnected nature of habitats, organisms, and ecosystem benefits of habitat restoration; and also recognized that diverse types of restoration techniques (e.g., marsh creation, ridge restoration, sediment diversions, and submerged aquatic vegetation restoration) can be implemented in combination to increase overall benefits to other injured resources, such as fishes, mobile invertebrates, and shallow benthic communities. The LA TIG supports the commitments outlined in the PDARP/PEIS to report on progress towards meeting stated restoration goals and objectives at the project level and at the ecosystem level; and to inform future ecosystem-level project designs, implementation, and evaluation.

Collectively, information gained from this MAM activity will directly benefit LA TIG's ability to effectively evaluate Louisiana's estuarine food web within the broader context of future DWH WCNH and Cross-Restoration Type projects as described in the LA TIG MAM Strategy (LA TIG, 2021). Characterization of LTL organismal standing stocks (e.g., abundance, density, biomass) and community composition/diversity provides a window into the function and health of the estuarine ecosystem, can help assess broad impacts of restoration action in the Basin, and can be used to suggest if and when adaptive management actions should be considered.

Lower trophic level organisms have been established as excellent indicators of estuarine health, and standing stocks are known to vary with salinity regime (e.g., Brammer et al., 2007). Examples are listed below.

Species-specific estuarine health indicators that are evaluated spatially and temporally include

- Salinity and eutrophication indicated by **cyanobacteria** abundance (Henson et al., 2018; Mateo et al., 2015; Soltani et al., 2012)
- Salinity fluctuations and long-term hurricane effects indicated by the **bivalve** *Rangia cuneata* abundance (Poirrier et al., 2009; Poirrier & Caputo, 2015; Windham et al., 2019)
- Marsh restoration and oil spill effects indicated by **epifauna** taxa *Littoraria irrorata* abundance (Baumann et al., 2018; Deis et al., 2017; Fricano et al., 2020)

- Conversion of marsh to open water indicated by the **bivalve** *Ameritella versicolor* and the annelid *Alitta succinea* (C. Glaspie, unpublished)
- Invasive/damaging species indicated by the **bivalve** family Dreissenidae which includes the zebra mussel *Dreissena polymorpha* (C. Glaspie, unpublished)

Integrated indices of estuarine condition/health evaluated spatially and temporally include:

- **M-AMBI ('Multivariate-AZTI Marine Biotic Index'):** used within the U.S. EPA National Coastal Condition Assessment framework and as part of the European Water Framework Directive. This is an index based on abundance-weighted tolerance to pollution (proportion of disturbance-sensitive taxa), species diversity (Shannon index), and species richness (number of species) of benthic macroinvertebrates (Bald et al., 2005; Muxika et al., 2007; Sigovini et al., 2013)
- **Phytoplankton Index of Biotic Integrity** (Ren et al., 2017) – requires nutrients, chlorophyll a, and phytoplankton biomass by taxa.
- Other benthic indices:
    - **Relative Benthic Index** (Hunt et al., 2001) – requires identification of indicator taxa (negative indicator typically Oligochaeta/*Capitella capitata*; positive indicators typically include an amphipod, a bivalve, and a polychaete) and evaluation of total diversity, crustacean species richness, crustacean abundance, mollusk species richness
    - **Index of Biotic Integrity** (Thompson & Lowe, 2004)
    - **Benthic Response Index** (R. W. Smith et al., 2001) – requires identification of a disturbance vector for index calibration, which can be difficult to identify in estuarine systems
    - **Benthic Quality Index** (Rosenberg et al., 2004) – uses regression to identify the four most responsive indicators

Previous ecosystem modeling workshops have recognized that lower trophic level data are often limited and this condition constrains their explicit representation in ecosystem models of bottom-up forced systems where they are most needed (Townsend et al. 2008). Existing ecosystem models that analyze food webs and productivity for Barataria Basin rely on LTL information that is either extremely limited in value or adapted from other systems. For example, the CASM model zooplankton and small mollusk population parameterizations rely on generalized equilibrium densities, parameters for turnover rate, and size structure rather than on empirical observations from Barataria Basin (Sable 2007, Dynamic Solutions 2016). Furthermore, although the CASM benthic infauna equilibrium density and size structure were modeled on using empirical information from Barataria, these data suffer from spatiotemporal limitations (i.e., one assessment for spring and fall within one year from one or two locations for three salinity zones [intermediate, brackish, and saline]: Rozas and Minello 2011, 2015; Dynamic Solutions 2016). Similarly, recent EwE models that have been used to understand potential impacts of environmental management decisions also exhibit LTL parameterization deficiencies (de Mutsert et al. 2017, Lewis et al. 2021). For example, similar to the CASM, the EwE benthic crustacean parametrization

(de Mutsert et al. 2017) relies on the same spatiotemporally limited studies (Rozas and Minello 2011, 2015).

Given the current understanding of the importance of LTL (i.e., detrital pools, primary production, and primary consumers) for modeling ecological productivity in Barataria Basin (Rose et al. 2019, Lewis et al. 2021); it is imperative to improve the understanding of LTL and their spatiotemporal responses to changing habitat conditions, such as land:water ratio, habitat configuration, salinity regime, and nutrient regime. Lewis et al. (2021) pointed out that changes in environmental conditions can have strong and direct effects on the biomass-dominant LTL groups and thus food webs are liable to be variable and spatiotemporally responsive to changes in those environmental conditions. This MAM activity will collect the information needed to confirm, or refute, this assertion and provide the empirical observations needed to incorporate those conclusions within existing or as of yet undeveloped ecosystem models. This MAIP builds on the initial data review and synthesis (DIVER ID #269) of the LTL inventory project for the Barataria Basin, which concluded that significant data gaps exist related to the current understanding of the LTL standing stock metrics (Kiskaddon et al., 2021, 2022). This phase identified the management needs and associated knowledge gaps, which are summarized in Table 4, Table 5, and Table 6:

Table 4 . Data gap identification for key lower trophic level management need #1: Understanding lower trophic level standing stocks (abundance/density, biomass, diversity).

Knowledge Gap Related to Management Need #1	Suggested Data Types to Fill & Evaluate Knowledge Gap	Sufficient Existing Data	Data Gaps
(1a): Spatial & temporal variability of lower trophic level standing stock metrics.	Mean and variation of standing stock metrics of key lower trophic level groups in priority habitats across the salinity gradient and across seasons sampled from locations that reflect current conditions of the estuary.	Data spanning salinity zones **Detritus**: water column particulate, suspended, & total organic carbon (mg L^{-1}; % organic). **Phytoplankton**: density (cells L^{-1}), biomass (total Chl a, ug L^{-1}). Data spanning seasons **Detritus:** water column particulate, suspended, & total organic carbon (mg L^{-1}; % organic). **Phytoplankton**: biomass (total Chl a, ug L^{-1}).	Lower trophic level groups that lack sufficient contemporary data required to accurately quantify variability of standing stock metrics across all priority habitats, across the estuary's salinity gradient, and across seasons: phytoplankton (diversity/richness), microphytobenthos, microbes, zooplankton, meiofauna, bivalves, macroinfauna, select epifauna, and terrestrial insects.
(1b): Spatial & temporal variability of food webs in response to lower trophic level standing stock metrics (i.e., nutritional value, food web relationships and dynamics).	Assessment of estuary-wide food webs (i.e., CASM) integrating identified existing lower trophic level data into analysis (see **Need #3**). Diet reconstruction of higher consumers based on mixing model analysis incorporating stable isotope values ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) reflecting current conditions of the estuarine food web.	For food web model improvements, see **Need #3**. Currently existing stable isotope values ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were not summarized as part of this project.	

Table 5. Data gap identification for key lower trophic level management need #2: Understanding effects of restoration action.

Knowledge Gap Related to Management Need #2	Required Data Types to Fill & Evaluate Knowledge Gap	Sufficient Existing Data	Data Gaps
(2a): Specific effects of different types of restoration actions (dredging, diversions, hard infrastructure, etc.) on lower trophic level standing stocks	Mean and variation of standing stock metrics of key lower trophic level across seasons sampled from locations that are anticipated to be directly affected by different types of restoration action. Suggest data collection to occur for 2 years prior and 2 years after ecosystem modification, occurring in parallel with data collection efforts at unaffected locations (see ***1a*** above)	Data for the following metrics and lower trophic level groups exists for 100% of CASM polygons expected to be affected by restoration action as part of the Louisiana 2023 Coastal Master Plan: **detritus:** water column particulate, suspended, & total organic carbon (mg L^{-1}; % organic); **phytoplankton:** biomass (total Chl a, ug L^{-1})	Additional standing stock data are necessary for the following for key lower trophic level groups to characterize all CASM polygons with anticipated restoration action as part of the Louisiana 2023 Coastal Master Plan: **macroinfauna, microphytobenthos, zooplankton**
(2b): Specific effects of different types of restoration actions (dredging, diversions, hard infrastructure, etc.) on estuarine food webs.	Assessment of estuary-wide food webs (i.e., CASM) integrating identified existing lower trophic level data into analysis (see **Need #3**). Diet reconstruction of higher consumers based on mixing model analysis incorporating stable isotope values ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) sampled from locations that are anticipated to be directly affected by different types of restoration action. Suggest data collection to occur before and after ecosystem modification, occurring in parallel with data collection efforts at unaffected locations (see ***1b*** above).	For food web model improvements, see **Need #3**. Currently existing stable isotope values ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were not summarized as part of this project.	
(2c): Effects of the restoration portfolio (combined effects of many restoration projects in the estuary) on estuarine food webs / productivity of higher trophic levels	See required data types for knowledge gap ***2b***.	For food web model improvements, see **Need #3**.	

Table 6. Data gap identification for key lower trophic level management need #3: Estuary-wide reporting.

Knowledge Gap Related to Management Need #3	Required Data Types to Fill & Evaluate Knowledge Gap	Sufficient Existing Data	Data Gaps
(3a): Environmental factors that most directly drive estuarine health (including lower trophic level organisms).	Potential species-specific indicators and data for three potential indicator species were examined and summarized in Kiskaddon et al. (2022). A list of potential integrated indices is provided in Kiskaddon et al. (2022). Integrated indices require the following data types: macroinfauna density (indiv m^{-2}), annelid density (indiv m^{-2}), macroinfauna diversity (Shannon Wiener), and biomass (g m^{-2} ADFW) of *Rangia cuneata.*	No indicator species examined in this report (*Littoraria irrorata, Microcystis* spp., *Rangia cuneata*) could be characterized as having sufficient spatial (across the estuary-wide salinity gradient) or temporal (seasonal) density data to comprehensively describe current estuarine conditions in Barataria Estuary. Additional macroinfauna standing stock data, specifically in fresh and intermediate/brackish salinity zones, are necessary to evaluate ecosystem condition.	Additional species-specific data as well as macroinfauna standing stock data, specifically in fresh and intermediate/brackish salinity zones, are necessary to evaluate ecosystem condition.
(3b): Improved representation of lower trophic levels in ecosystem models to increase model accuracy.	Option #1) Update the existing CASM model using Barataria-specific daily Chl a (µg L^{-1}) and detritus data (see Appendix F). Option #2) Re-parameterize CASM primary producers (detritus – particulate organic carbon, phytoplankton, microphytobenthos, bacterioplankton). Option #3) Re-parameterize CASM growth assumptions of consumers based on seasonal and spatial (salinity, habitat – Land:Water) standing stock data (biomass) of zooplankton, oyster (spat & adults), benthic infauna (macroinfauna, inclusive of bivalve mollusks; discussed further in Kiskaddon et al. (2022)).	Option #1) Sufficient data were identified for **phytoplankton** biomass (total Chl a, ug L^{-1}) and **detritus** (water column particulate, suspended, & total organic carbon in units of mg L^{-1} and % organic). Option #2) Sufficient **macroinfauna** and **bivalve biomass** (g AFDW m^{-1}) data were identified from sources spanning 1971-2020. Option #3) Sufficient data was not identified from currently existing data.	Option #2) To re-parameterize CASM primary producers, additional biomass data is required for microphytobenthos, detritus (particulate organic carbon), and bacterioplankton (inclusive of open water microbes). Option #3) To re-parameterize CASM growth assumptions, additional biomass data is required for zooplankton, macroinfauna (inclusive of bivalve mollusks), and microphytobenthos particularly from priority habitat types.

Furthermore, the preliminary phase confirmed that field-validated trophic level information (informed by stable isotope values) is needed to understand how environmental conditions affect LTL distribution, primary production, and consumption of primary production via grazing by zooplankton, infauna, and epifauna (see Nelson et al., 2013, 2019). Collection of LTL standing stock (e.g., abundance, density, biomass), community composition/diversity, and trophic level information addresses several key management needs identified by natural resource managers in coastal Louisiana during the initial LTL inventory project planning phase, including: 1) Understanding LTL standing stocks (e.g., abundance, density, biomass) and community composition/diversity; 2) Understanding effects of restoration action; and 3) Reporting on Basin-wide ecosystem condition (see Kiskaddon et al., 2022).

Although ecosystem model improvements and calibration are not part of the funding request for this MAIP, the collected data will be compatible with **future** updates, if funded. There are two existing ecological food web models (i.e., EwE and CASM) that have thus far been used to characterize the energy flow and trophic level production of the Barataria Basin (Lewis et al., 2021), and to evaluate key species biomass changes to water resource and coastal habitat restoration projects in the estuary (de Mutsert et al., 2017; Dynamic Solutions, 2016). Both the EwE and the CASM currently use Chl *a* concentration as inputs to drive the primary production in the estuarine open waters and subtidal sediments that fuel the modeled food webs. The CASM describes how lower trophic levels are currently modeled within the estuarine food web, and how the lower trophic level data could be used for improving lower trophic level representation in the model because the CASM focuses on the bottom-up processes rooted in water quality that affect the food web. Incorporating stable isotope information [e.g., Audzijonyte et al. (2019)] in addition to standing stock information (e.g., abundance, density, biomass) and community composition/diversity data would improve ecosystem models such as CASM and EwE. While perhaps not solving all present limitations of applying ecosystem models to the management of these resources (Rose et al., 2019), improved understanding of environmental drivers of LTL and food web conditions will improve the utility of ecosystem models that incorporate this new information in that they will better characterize the food web structure and ecosystem condition as well as better project future food web and ecosystem conditions. Additionally, the lower trophic level standing stock data, when converted to the biomass units used in CASM (g m-2), could be used to improve lower trophic level food web modeling in three primary ways: 1) Simply adjusting CASM predicted daily biomasses or the lower trophic level parameters by modifiers to evaluate bottom-up changes to the modeled food web; 2) Changing the definition of lower trophic level taxa represented in the CASM and/or adding the identified lower trophic level groups to the existing model set; or 3) Add identified lower trophic level groups to the existing model set, with additional or alternative processes for directly simulating daily biomass dynamics and predator-prey interactions within the food web (Kiskaddon et al., 2022). Further data collection and analyses may identify important lower trophic level dynamics and processes that are not currently represented in the existing ecological models.

This MAM activity will be aligned both spatially and temporally with a recently funded MAM activity that includes new data collection for fish and invertebrate densities in marshes in Barataria Basin (DIVER Project ID #299, Monitoring the Effects of Coastal Wetland Restoration on Fish and Invertebrates). Additionally, this MAM activity will complement existing monitoring programs underway in the Barataria

Basin including the Coastwide Reference Monitoring System (CRMS; DIVER Project ID #249; Raynie et al., 2020) which collects vegetation (dominant species) and hydrology data (salinity, water temperature, and water level). Furthermore, this MAIP complements the Louisiana Department of Wildlife and Fisheries (LDWF) Louisiana Coastwide Fish and Shellfish Monitoring Program Fisheries Independent Monitoring Program (FIMP; DIVER Project ID #157; LDWF, 2019) and the System-Wide Assessment and Monitoring Program (SWAMP; Hijuelos et al., 2013; The Water Institute of the Gulf, 2019), by allowing the characterization of the potential prey base and/or life stages not adequately quantified by these existing LA TIG-funded monitoring programs and projects across the Basin. To the extent practicable, this MAM activity will also consider and integrate findings from the recently-funded Quantifying Changes in Wetland Area and Habitat Types in the DWH Louisiana Restoration Area 1985-Present with Remote Sensing (DIVER ID #307). This MAM activity will be cross-walked with the Mid-Barataria Sediment Diversion (DIVER #342) MAM plan to ensure that the two efforts are not duplicative. This MAM activity could likely benefit other efforts focused on describing carbon cycles within the region as well.

The combination of these MAM activities will allow a comprehensive ecological view of the Barataria Basin, which in turn will allow assessment of the restoration projects portfolio and a clearer understanding of the complex series of interconnected ecosystems that comprise the Barataria Basin.

7.1.3 Objectives

The objectives of this MAM activity in the Barataria Basin as they relate to MAM needs identified in the LA TIG MAM Strategy are outlined below:

- Characterize the density, relative abundance, biomass, community composition/diversity, and trophic level information of key LTL groups with consideration of natural spatiotemporal variability prior to implementation of the majority of DWH restoration actions in the Barataria Basin (WCNH #7.a., 7.b., 7.c.). These data will be used to develop draft SMART objectives related to standing stock status for the Barataria Basin (Cross-Restoration #2.a.) and will be used to inform *future* development of SMART objectives related to identifying appropriate time scales for evaluating ecosystem function (Cross-Restoration #2.b.), quantifying trophic linkages (Cross-Restoration #2.c.).
- Quantify habitat characteristics (e.g., salinity, sediment characteristics, water quality, and other abiotic conditions and drivers) appropriate for predicting key LTLs' standing stock within the Barataria Basin.
- Provide information to inform future development of a LA TIG MAM Strategy SMART objective associated with wetland net ecosystem carbon balance (Cross-Restoration Type #1.b.).

7.1.4 Task Summary

This MAM activity includes four tasks summarized below. These tasks are further elaborated upon in Section 7.1.5.

Task 1: Prepare for field sampling in the Barataria Basin (Year 1).

There are three goals of Task 1. The first is to assess the extent of existing data that can be used to quantify energy transfer from key LTLs to higher consumers, including a literature review summarizing existing $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values of primary producers and key LTLs by feeding guild and, where possible, specific higher trophic level consumers represented in Barataria Basin food web models (e.g., EwE and CASM). The second goal is to refine the originally selected sampling locations, if needed, based on field reconnaissance and/or to improve alignment with the fish and invertebrate sampling effort that will be occurring in Barataria Basin at the same time (DIVER Project ID #299). Co-location of at least some of the lower trophic level and fish and invertebrate sampling sites (i.e., aligning fixed-area monitoring locations to be identified under the Nekton MAIP DIVER #299 to the presently proposed LTL monitoring locations) would further enhance the overall value of both datasets. The third goal is to prepare for field sampling (e.g., sample site reconnaissance and logistics). The outputs of this task are one brief (< 10 page) technical addendum to Kiskaddon et al. (2021) summarizing the extent of existing $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope data relevant to the Barataria Basin the final selected sampling sites, and guides for field sampling and laboratory teams, including the detailed field and laboratory protocols (i.e., Standard Operating Procedures), safety, data processing (i.e., quality assurance/quality control), and chain-of-custody processes.

Task 2: Conduct three years of field sampling and up to another year of associated laboratory analyses in the Barataria Basin (Years 2–5).

The goal of Task 2 is to conduct three years of key LTL data collection, including up to another year of associated laboratory analyses, in the Barataria Basin (Years 2–5). Collected data include key LTL standing stock metrics (e.g., abundance, density, biomass), their community composition/diversity, and stable isotope values of primary producers and key LTLs species/groups ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$). The outputs of this task are field data and associated metadata submitted and uploaded to DIVER annually. While field sampling will be conducted in Years 2-4, the sorting, identification, and analysis of collected LTL samples will realistically continue into Year 5 of the project.

Task 3: Synthesize LTL data and produce a summary report characterizing LTL conditions within the Barataria Basin (Year 5).

Use data collected in Task 2 to characterize current conditions related to LTL organisms in the Barataria Basin. Data analysis will examine spatiotemporal patterns in LTL standing stocks (e.g., abundance, density, biomass), community composition/diversity, and isotope values; identify key environmental and habitat drivers (e.g., salinity, sediment type, land:water); and identify indicator species to evaluate estuarine condition. Additionally, power analyses will be conducted to identify potential future monitoring effort efficiencies that could be achieved. The output of this task will be a report that will detail the methods and results of this effort, discussion of results, and conclusions and recommendations for improving sampling and analysis methods during future data collection efforts (e.g., possible adjustment of sampling method, design, and/or gear types for future monitoring phases). Conclusions from the analysis will also include development of draft SMART objectives for LA TIG consideration and will provide the necessary standing stocks (e.g., abundance, density, biomass),

community composition/diversity, and trophic position values to inform future development of other LTL-related SMART objectives.

In addition, it is anticipated that up to six peer-reviewed publications will be produced describing the analytical findings of Tasks 1–3 to share the data and results of this monitoring effort more broadly with the scientific community. Of these six publications, four would focus on reporting the spatiotemporal trends and identified environmental drivers for each of the LTL groups monitored during this MAM activity: 1) phytoplankton, 2) zooplankton, 3) microphytobenthos, and 4) macroinfauna. Of the additional two publications, one (publication #5) would detail the results of relating remote sensing methods to spatiotemporal trends in phytoplankton. The final publication (publication #6) would detail the results of the isoscape analyses. The stable isotope data will be used to derive a spatial isotope contour map, or isoscape, that can be used to better understand the spatial and temporal patterns in stable isotopes and that can reveal areas of high potential biological productivity and elucidate LTL food web structure (see examples by James et al., 2022; Kendall et al., 2010; Matich et al., 2021).

Task 4: Develop long-term LTL monitoring plan for the Barataria Basin (Year 6).

Based on the established key LTL characteristics derived from Tasks 1-3, a long-term monitoring plan will be developed to collect data during and after future restoration implementation to compare against the initial characteristics established by this MAM activity. This long-term monitoring plan would be implemented to evaluate ecosystem impacts of future restoration actions in the Barataria Basin. The long-term monitoring plan would incorporate the knowledge gained from Tasks 1-3 including opportunities for reduced cost through implementation of emerging technologies (e.g., remote sensing and machine learning) and application of the power analysis results to maximize sampling efficiencies.

7.1.5 Activity Implementation Detailed Descriptions

A detailed description of each Task and intended outputs is provided below.

Task 1: Prepare for field sampling in the Barataria Basin (Year 1).

Purpose: Task 1 will provide the necessary information to guide stable isotope data collection from the Barataria Basin (see Kendall et al., 2010). Fieldwork planning will seek efficiencies (e.g., co-location of sampling sites) by aligning with the newly funded nekton and invertebrate sampling effort (DIVER Project ID #299).

Scoping existing stable isotope data: A literature review will summarize the extent of existing published and unpublished $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values identified as relevant to the Barataria Basin of Louisiana. Since the objective of this MAM activity is to characterize the Barataria Basin specifically, and stable isotope analyses are sensitive to basin-specific baselines (independent of diet/trophic ecology; M. Polito pers. comm.), data collection will be restricted to the Barataria Basin. Data from additional coastal areas may be considered but will not be the primary focus of this task. Literature review will focus on key LTL groups described in Table 1, detritus sources, and select consumers (e.g., those represented in EwE and CASM models and other species valued by Louisiana). Web-based search engines (e.g., Google

Scholar) and academic partner collaboration will be used to identify data and data sources. The product will serve as a framework with which to compare Barataria Basin-specific stable isotope values collected during Task 2; this literature review will enable characterization of stable isotope variability within the Barataria Basin and more broadly across Louisiana coastal regions.

- **Analysis:** $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values will be extracted from data sources and summarized (e.g., mean ± standard deviation [SD], min-max ranges) by within the Basin, stable isotope, feeding guild (e.g., Jumars et al. [2015]), taxonomic/trophic level grouping, habitat type, and salinity zone. Data visualization will include tables, bar charts, and stable isotope biplots.
- **Product**: A technical addendum to Kiskaddon et al. (2021) summarizing the current extent of existing stable isotope data ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), associated gaps relevant to key LTLs, and written methodology describing the development of a Barataria Basin isoscape to validate ecosystem models and inform monitoring programs (Kendall et al., 2010).

Refining field sampling plan: The sampling plan will be aligned with other planned work in the Barataria Basin in order to maximize the utility of datasets, including spatial and temporal alignment (e.g., co-location of sampling sites) with the nekton and invertebrate sampling effort (DIVER Project ID #299). Task 1 will also include logistics planning and preparation for field sampling (e.g., ordering supplies, reserving vessels, field reconnaissance of site accessibility) as well as development of detailed field and laboratory protocols (i.e., Standard Operating Procedures).

- **Products:** Final sampling plan, including detailed field and laboratory protocols, and documented processes for safety, data processing, and chain-of-custody.

Task 2: Conduct three years of field sampling and up to another year of associated laboratory analyses in the Barataria Basin (Years 2–5).

This task will implement three years of field data collection and up to another year of associated laboratory analyses in the Barataria Estuary Basin to collect new LTL data related to standing stock metrics (e.g., abundance, density, biomass), community composition/diversity, and stable isotopes.

- **Sampling Duration:** Three years of field sampling and up to another year of associated laboratory analyses is needed to adequately characterize temporal patterns of the Barataria Basin LTL groups' condition.
- **Site Selection:** The Task 2 sampling design was informed by the results and conclusions of power analyses summarized in Appendix A. New data collection will be confined to the Barataria Basin, Louisiana, in open water (OW; water column and submerged benthos) and emergent vegetation (EV) habitat types. Other habitat types such as submerged aquatic vegetation (SAV) and disarticulated oyster shell will be noted when encountered; SAV is not targeted specifically due to its ephemeral nature (Hillmann et al., 2019). This data collection effort includes sampling at both fixed and randomized probability-based sites within monitoring stations using a spatially-balanced generalized random tessellation stratified (GRTS) sampling design. Fixed sites are used

to assess long-term trends in standing stocks (e.g., abundance, density, biomass), community composition/diversity, and trophic position in specific regions of the Basin, whereas probability-based sampling sites are used to assess these metrics at different spatial scales. The sampling design was developed to align data collection activities with future ecosystem model improvements (see Appendix D for details). Further refinement of the monitoring plan (Task 1) to further align with other existing MAM activities may lead to modification of the plan detailed below. Vegetation mapping by Sasser et al. (2014), which reflects salinity zones on an inter-annual time scale (e.g., Visser et al. 2002), and the spatial polygons of the CASM were used to subdivide the Barataria Basin and assign three salinity zones defined as: fresh (salinity < 0.5 ppt, CASM polygons 0, 1, 15, and 18), intermediate/brackish (salinity 0.5–18 ppt, CASM polygons 2, 3, 4, 9, 10, 12, 14, 16, and 17), and saline (salinity 19–35 ppt, CASM polygons 5, 6, 7, 8, 11, 13, and 19; Figure 1). Soon to be completed updates to vegetation mapping could influence these assignments, and thus distribution of exact sampling locations, during Task 1 of project implementation.

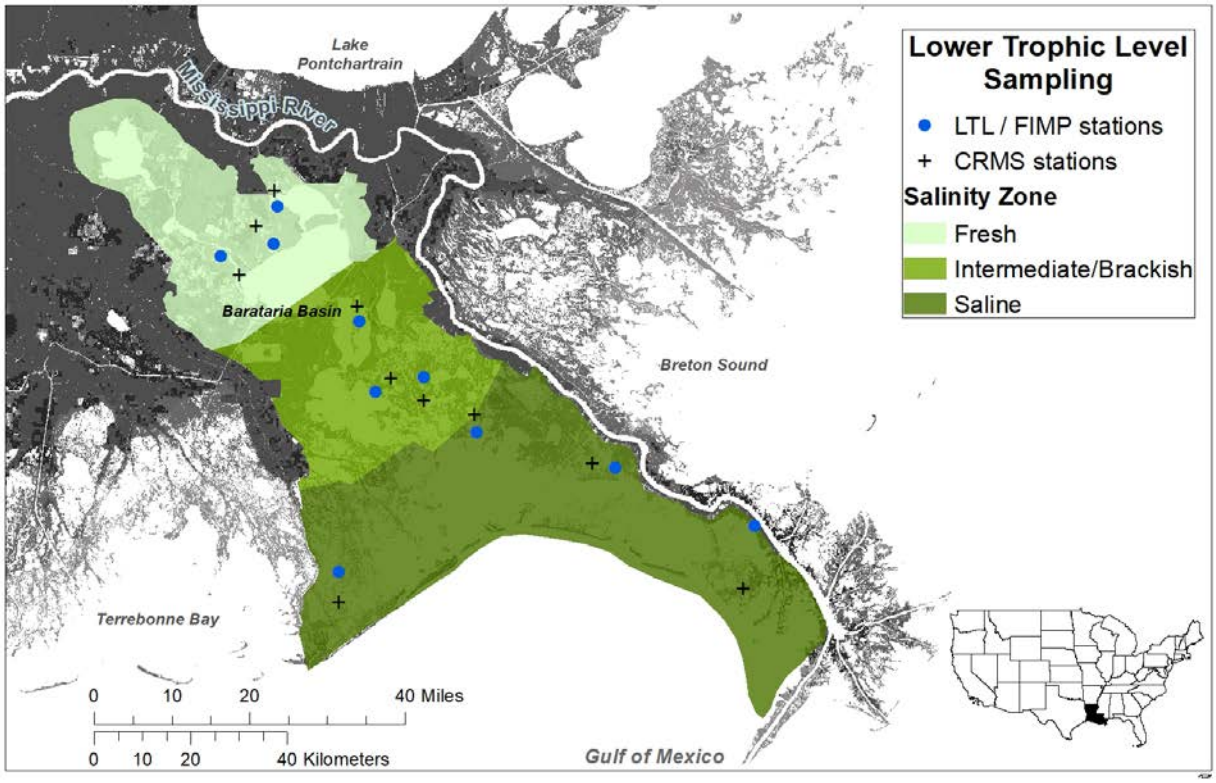

Figure 1. Locations of the ten LTL monitoring stations for Task 2 located within zones defined by salinity strata of the Barataria Basin, LA. These are intended to focus on areas of the basin with NRDA restoration projects, which are not planned in the upper reaches of the Basin. All LTL monitoring stations share a location with a FIMP 50-ft bag seine or electrofishing sampling location. The CRMS station closest to each LTL/FIMP station is also shown. General salinity zones are based on Sasser et al., (2014) aligned with modified CASM polygons (see Kiskaddon et al., 2021). A full list of coordinates for all LTL monitoring stations is provided in Appendix B.

- **Site Selection (continued):** A total of ten LTL monitoring stations will be sampled as part of this field effort (Figure 1, Appendix B). Stations were identified spanning different CASM polygons

and the full salinity gradient of the Basin. Each station was selected to overlap with a subset of LDWF FIMP 50-ft bag seine and electrofishing sampling locations. This was done to align LTL data with the appropriate fish and invertebrate consumer species which heavily prey upon the LTL. The LDWF 50-ft bag seines were previously used to initialize and calibrate several key species biomasses in the Barataria Basin CASM and EwE models (De Mutsert et al., 2017; Dynamic Solutions, 2016), adding further value of collecting LTL data from a subset of these same locations. Two fixed sites and three randomized sites per habitat type (i.e., 5 OW sites and 5 EV sites, 10 total sites per station) will be sampled to ensure a spatially balanced GRTS sampling design. The LTL monitoring station and site coordinates are given in Appendix B.

- **Sampling Frequency:** Due to differences in relative turn-over rates between key LTL groups residing in the benthos versus the water column, data collection temporal regimes will differ to address the objectives of this field sampling effort. Sampling of macroinfauna, microphytobenthos, and stable isotopes will be conducted seasonally (fall, winter, spring, summer; Figure 2), whereas sampling of phytoplankton and zooplankton will be conducted bi-weekly (Figure 3).

- **Sampling Effort:** A suite of dedicated field sampling efforts are needed to match the sampling frequency described above and should be executed by field teams experienced with collecting these disparate LTL groups. Furthermore, the scope of collection activities would prevent a single field team from adequately collecting the samples in a way that maintains the sampling frequency as described above, especially with regards to collecting phytoplankton/zooplankton samples. A detailed description of data field sampling methodologies for each key LTL group and additional abiotic and biotic variables are described in Appendix C. In summary, samples will be collected from a boat (open water) or on foot, if accessible. Biweekly water samples will be collected by hand in buckets and bottles. Zooplankton samples will be collected biweekly using a diaphragm pump to sample water at a depth of 0.5 m. Quarterly samples of each dominant vegetation species will be collected by hand. Quarterly sediment samples and macroinfauna samples will be collected with acrylic hand push corer in EV sites, and with piston corer or long-handled push corer ((5 cm diameter and 5 cm depth).).

- **Laboratory Quantification:** A suite of laboratory sampling and analytical methods are necessary to adequately quantify the standing stock metrics (e.g., abundance, density, and biomass), community composition/diversity, and trophic level information of the disparate Barataria Basin key LTL groups (phytoplankton, microphytobenthos, zooplankton, and macroinfauna) from the field-collected samples. To adequately understand nutrient dynamics that drive productivity, substantial laboratory analyses of water samples is needed. Efficient characterization of phytoplankton and zooplankton communities will use analytical (i.e., High-Performance Liquid chromatography [HPLC]) and automated (i.e., zooscan) approaches; however, more labor-intensive microscopy will be strategically used to verify species identifications and standing stock metrics. Similarly, laboratory analysis of microphytobenthos samples will focus on analytical (i.e., HPLC) approaches by also employing targeted microscopy analyses for species/community confirmation. Quantifying macroinfauna from sediment cores requires considerable labor to sort and identify specimens. For all LTL groups, field collection will take

place over three years (years 2-4) but completion of the associated laboratory analyses is anticipated to occur during year 5 of the project. A detailed description of laboratory analysis methodologies for each key LTL group and additional abiotic and biotic variables are described in Appendix C.

- **Product (Task 2):**
    - Data and associated metadata will be delivered annually per requirements of DIVER annual project reporting.

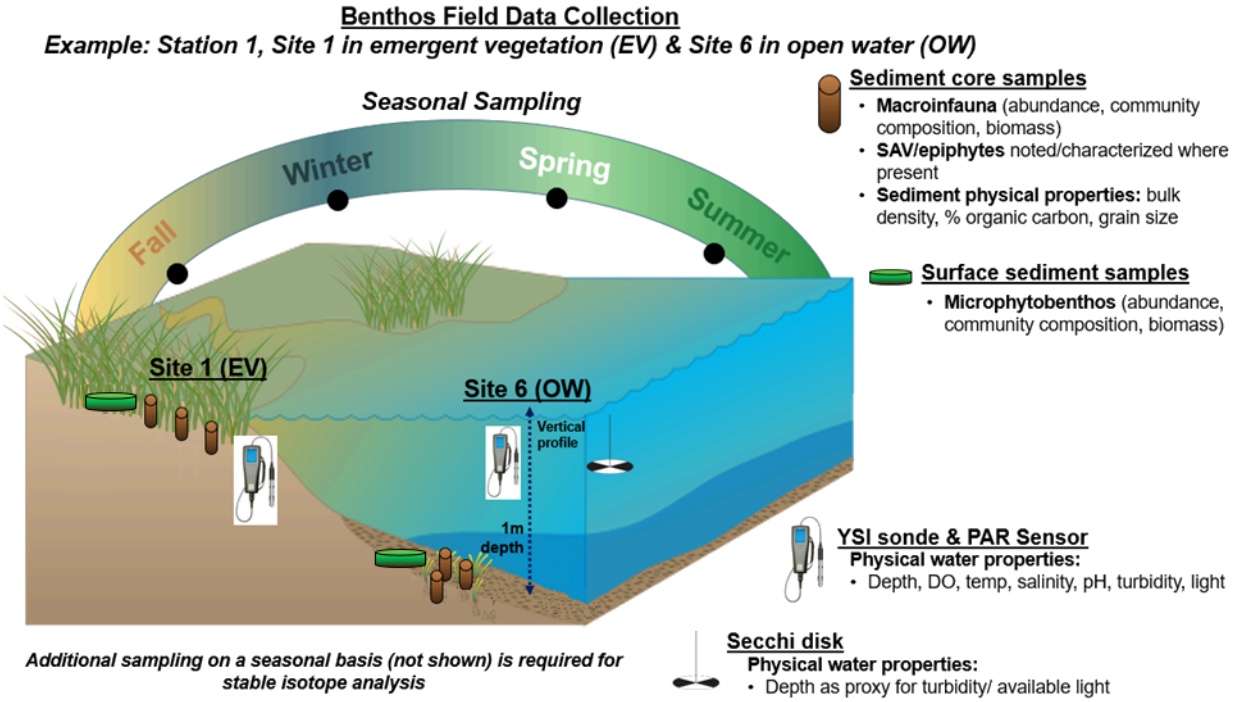

Figure 2. Schematic illustration of sample collection for benthos lower trophic levels (LTLs) in the Barataria Basin. Further detailed explanation of data collection is provided in Appendix C.

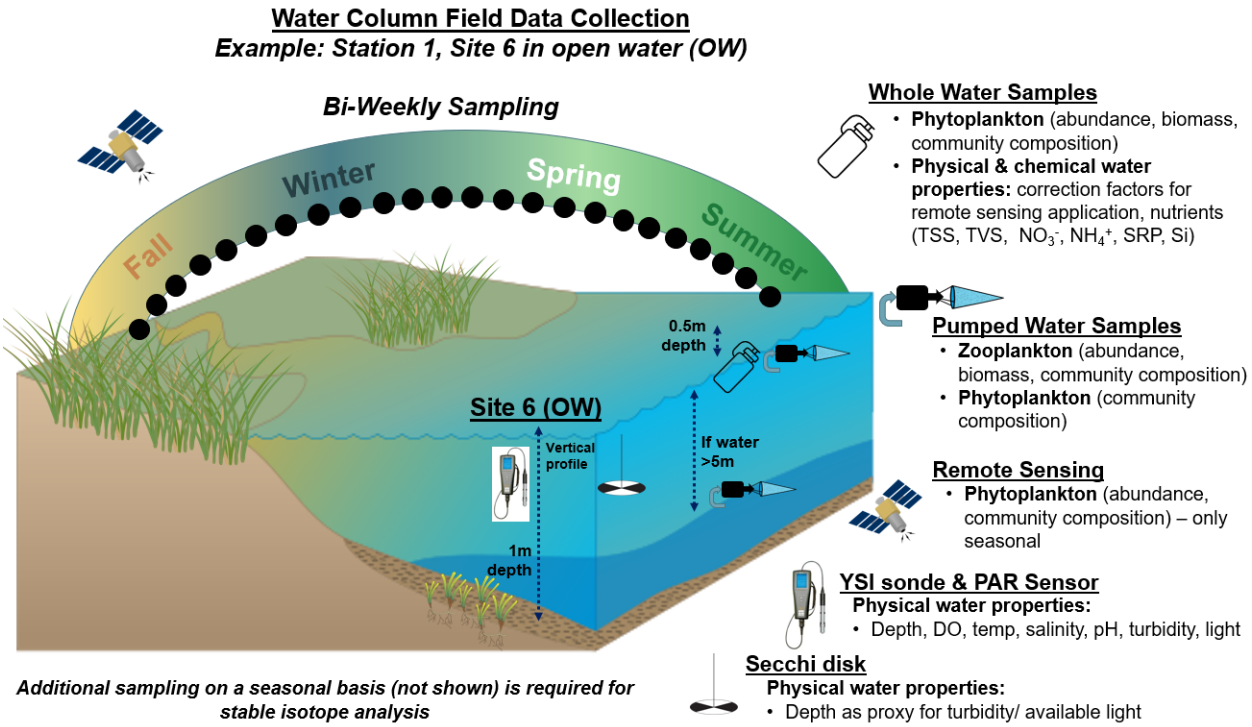

Figure 3. Schematic illustration of sample collection for water column lower trophic levels (LTLs) in the Barataria Basin. Further detailed explanation of data collection is provided in Appendix C.

Task 3: Synthesize LTL data, develop an isoscape, and produce a summary report characterizing LTL current conditions of the Barataria Basin (Year 5).

Purpose: The purpose of Task 3 is to evaluate the data collected in Task 2 to characterize conditions of key LTL groups in the Barataria Basin. Once current or "reference conditions" are established, they can be used to assess changes in Basin-scale conditions over time.

Methods for Standing Stock (i.e., abundance, density, biomass) and Community Composition/Diversity Analysis: Targeted data analysis will be conducted using the information gathered in Tasks 1 and 2 to address the specific goals and objectives of this MAM activity. Recommended analytical methods to characterize temporal and spatial patterns of key LTL groups in the Barataria Basin are provided, however exact analyses will be determined based on the structure and composition of the final dataset.

1) *Exploratory data analysis (EDA):* Due to the abundance of knowledge gaps related to key LTL data in the Barataria Basin, initial analysis will examine how standing stock (e.g., abundance, density, biomass), and community composition/diversity are spatiotemporally distributed. This analysis will identify general patterns in the data, including outliers and features of the data that might be unexpected. Graphical approaches for examining data distributions will include histograms, boxplots, cumulative distribution functions, and quantile-quantile (Q-Q) plots. Where appropriate, this summary will include consideration of metrics by functional (feeding) guild. Information gained through EDA will be used for determining appropriate analyses and confirming whether assumptions underlying particular methods are supported (e.g., normally

distributed residuals). These EDA will be flexible to be informed by the data so that any deviations from assumptions used during development of the sampling protocol may be incorporated into subsequent statistical analyses.

2) *Parametric and non-parametric statistical analyses:* Standing stock metrics (i.e., abundance, density, biomass) may require transformation for normality where needed for parametric statistical tests. Analysis of Variance (ANOVA) and/or regression analyses with post-hoc tests will be used to initially test for differences between sampling events (e.g., time series) and within and between salinity zones and/or continuous environmental parameters. To the extent practical, data analyses will incorporate the nested nature of the data collection where appropriate. Where assumptions of the ANOVA are not met by data transformation, non-parametric alternatives (e.g., Kruskal Wallis test, quantile regression) will be used. Regression analyses will also consider non-linear relationships between standing stock metrics and predictors or to investigate time series, where possible. Univariate community metrics (e.g., species richness, Shannon diversity index, and Pielou's evenness index) will also be calculated and similarly analyzed. Multivariate community analyses will employ zero-adjusted Bray-Curtis similarity (Clarke et al., 2006) using appropriate transformations (e.g., square root transformed abundance/density data) which will be used to run Cluster Analysis, Non-metric Multi-Dimensional Scaling (nMDS), Similarity Percentage (SIMPER), and Permutated Multivariate Analysis of Variance (PERMANOVA) to visualize and analyze the extents to which observed communities differ amongst season, habitat, and other categorical and continuous predictors. Other multivariate analyses (e.g., BIO-ENV procedure: (Clarke & Ainsworth, 1993)) will be used to examine correlations between the environmental parameters and the key LTL community structure metrics; this method will be used to define an optimal subset of environmental variables which best explains the observed key LTL community structures. Environmental parameters may require normalization/log transformation prior to analysis. These analyses will further guide identification of LTL indicator taxa that may be important for long-term monitoring or estuarine condition reporting.
3) *Power Analyses:* Additionally, power analyses will be conducted to identify potential future monitoring effort efficiencies that could be achieved. These power analyses will use the standing stock metrics (e.g., abundance, density, biomass) to identify minimal sample sizes that are suitable for detecting the effects of important spatiotemporal or environmental parameters effects.

Due to the likelihood of high variability in the final data, analysis will emphasize investigation of spatiotemporal patterns and trends (means and 95% confidence intervals) rather than strict interpretation of significance based on *p*-values (see Smith [2020], and Wasserstein & Lazar [2016]).

The results of statistical analyses will inform recommendations and lessons learned for development of a longer-term monitoring plan (Task 4); such recommendations may include modifications to the sampling design implemented in Task 2 (e.g., number of sites, frequency of sampling, application of lower-cost methods) to collect the necessary data to inform adaptive management decision-making and future evaluation of restoration impacts in the Basin.

Method for Isoscape Development: The stable isotope values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) of Particulate Organic Matter (POM), phytoplankton (by size fractions), microphytobenthos (including epiphytes

where present), zooplankton (by size fractions), bulk soil organic matter, macroinfauna (bivalves, annelids, arthropods), and live vegetation (C3 plants and C4 plants; epiphytic algae; SAV; including epiphytes where present)—also collected in Task 2—will be analyzed to characterize spatiotemporal variability of isotope values and determine key environmental drivers (e.g., temperature, salinity, nutrients, land:water, etc.) influencing them. The results of the LTL isotope analysis will include a Barataria Basin isoscape (James et al., 2022; Kendall et al., 2010; Matich et al., 2021) which will provide a means to validate the CASM, EwE, and Delft3D-WAQ models by demonstrating if and how Basin-wide drivers such as temperature, salinity, nutrients, and light availability/turbidity affect the production of the key LTLs. These responses or relationships between environmental drivers and isoscapes could then be used to modify or adjust the modeled LTL biomass or production (change in biomass per unit time) within the CASM, EwE, and Delft3D-WAQ, if future ecosystem model improvement work is funded. The isoscape is an effective modeling tool that complements the current habitat suitability index (HSI) modeling efforts and will support a future effort, if funded, to validate and re-parameterize ecosystem models (e.g., EwE and CASM). Development of the isoscape will also consider a means to identify efficiencies in sample collection for potential future stable isotope collection activities.

Product: A summary report will be developed). This summary report will include the following:

- Detailed sampling methods, procedures, and quantitative data analyses describing spatiotemporal trends and variability of key LTL standing stock (e.g., abundance, density, biomass), community composition/diversity, and trophic position data to be used to develop reference ranges.
- Characterization of key environmental and habitat drivers of LTL standing stock (e.g., abundance, density, biomass), community composition/diversity, and trophic position in the Basin.
- Assessment of remote sensing technologies and other emerging methods (i.e., FlowCam machine learning) for monitoring.
- High-level discussion of estuarine condition related to indicator species, cyanobacterial and harmful algal bloom (HAB) occurrences, and integrated indices.
- Draft SMART objectives 7.a., 7.b., and 7.c. related to characterizing standing stock (e.g., abundance, density, biomass), community composition/diversity, and trophic level descriptions of key LTLs in the Basin for LA TIG consideration.
- Data gaps, limitations, and lessons learned for corrective actions/adaptive management related to LTL data collection in Barataria Basin (e.g., possible adjustment of sampling method or gear types for future monitoring phases).

Results of data analyses will be documented in a digital database or spreadsheet using established DIVER data templates and following consultation with the DIVER data management team. It is anticipated that up toto six peer-reviewed publications that describe the analytical findings of Tasks 1–3 will be produced in accordance with the PDARP/EIS commitment to public communication of restoration information via published research, ensuring accessibility to and utility of this data for the scientific community.

Task 4: Long-term key LTL monitoring plan for the Barataria Basin (Year 6).

Purpose: Long-term collection of LTL standing stock (e.g., abundance, density, biomass), community composition/diversity, and stable isotope data can help detect change in ecosystem condition and be used to assess changes in the Barataria Basin over time as a result of restoration actions and other changing conditions.

Product: Based on the data synthesis and power analysis completed in Task 3, a long-term LTL monitoring plan will be developed for LA TIG consideration to enable evaluation of ecosystem impacts of NRDA DWH restoration actions in the Barataria Basin and will incorporate lessons learned and cost-saving methodology adjustments to streamline data collection and analysis.

7.2 BUDGET

Lower Trophic Level Monitoring for Barataria Basin, Louisiana Phase 2 Budget		
Cost Items	**Trustee**	**Cost Estimate**
Task 1: Prepare for field sampling	NOAA	$219,023
Task 2: Field sampling and associated laboratory analyse**s** – phytoplankton, water nutrients, remote sensing; zooplankton; sediment characteristics and macroinfauna; associated physical water quality	NOAA	Year 1: $1,266,748 Year 2: $1,266,748 Year 3: $1,266,748 Total: $3,800,244
Field sampling and associated laboratory analyse**s** – microphytobenthos HPLC and cell density, phytoplankton HPLC, and associated physical water quality (Task 2); synthesis and reporting (Task 3); and development of long-term LTL monitoring plan (Task 4).	DOI	Total: $1,662,223
Task 3: Synthesize LTL data/reporting/publications; develop draft SMART Objectives	NOAA	$367,529
Task 4: Develop long-term LTL monitoring plan for the Barataria Basin	NOAA	$168,023
MAM Activity Management, Oversight, Compliance, and Reporting	NOAA	$624,283
CPRA Labor Associated with development and review of draft SMART Objectives, review of deliverables, and alignment with MBSD MAM (Tasks 1, 3, 4)	CPRA	$70,000
Total MAM Activity Cost Without Contingency		$6,911,325
Contingency (10%)		$691,133
TOTAL ESTIMATED COST		$7,602,458

7.3 TIMELINE

The MAM activities described above will be conducted over a six (6) year project implementation period.

Task	Year(s)	Activities
1	1	Review literature and assess existing data to quantify energy transfer from key LTLs to higher consumers. Write technical addendum.
1	1	Coordinate sampling (6 months).
2	2, 3, 4, 5	Collect new LTL data in the Barataria Basin (3 years) with additional contingency for sample processing time. Upload field data and associated metadata annually.
3	5	Synthesize LTL data. Produce isoscape and summary report characterizing current condition of LTL organisms. Develop draft SMART objectives.
4	6	Write a long-term LTL monitoring plan for the Barataria Basin, based on LTL conditions established in previous tasks, and incorporating efficiencies and lessons learned.

7.4 IMPLEMENTATION ROLES

NOAA will be the Lead Implementing Trustee and will be responsible for implementing the work under Tasks 1, 2, 3, and 4. NOAA will provide overall direction and oversight for the MAM activity, including managing cooperator(s) agreement or contracts as needed, compliance, financial tracking, annual reporting, DIVER data management, and approval of deliverables. DOI will be a co-Implementing Trustee and will be responsible for work associated with planning, implementing, analyzing, and reporting microphytobenthos abundance and laboratory processing for phytoplankton community composition as well as assisting with development of a long-term LTL monitoring plan.

Funding withdrawal requests for Tasks 2, 3, and 4 will occur after NOAA and CPRA have worked together to identify opportunities to coordinate the implementation of this MAM activity with the lower trophic level pre-operations data needs for the Mid-Barataria Sediment Diversion MAM Plan and ensure there is no duplication between the two efforts. NOAA and CPRA will identify any additional MBSD project-level sampling stations or data types or other adjustments to the sampling plan for this MAM activity to address the needs of the MBSD MAM plan by June 15, 2023, so that those changes can be incorporated for the first year of sampling while allowing sufficient time for contracting and mobilization for field work. Once an agreement is reached on the allocation of effort between this MAIP and MBSD, the

budget of this MAIP will be reduced accordingly through resolution of the TIG. If MBSD LTL monitoring needs are unable to be identified by June 15, 2023, NOAA will proceed with a funds withdrawal request for implementation of Year 1 of lower trophic level sampling under Task 2 of this MAM activity, and additional coordination with CPRA related to MBSD lower trophic level sampling needs will occur prior to NOAA submitting a funds withdrawal request for Years 2 and 3 of sampling under Task 2 and the remaining project budget for Tasks 3 and 4. The LA TIG agencies will have the opportunity to provide feedback on the draft SMART objectives produced in Task 3 and the long-term LTL monitoring plan produced in Task 4. The SMART Objectives provided for Task 3 will not be considered finalized at the end of this project, but rather will serve as a starting point for further discussion and revision by the LA TIG.

7.5 DATA MANAGEMENT AND REPORTING

The DWH Trustees, as stewards of public resources under OPA, will inform the public on the MAM activity's progress and performance. Therefore, NOAA will report the status of the proposed activity via the Data Integration, Visualization, Exploration, and Reporting (DIVER) Restoration Portal annually, as outlined in Chapter 7 of the PDARP/PEIS (DWH Trustees, 2016). All reports and final datasets created as part of this activity will also be stored on the DIVER Restoration Portal within a year of completion, using DIVER templates, with properly documented FGDC/ISO metadata, a data dictionary (defines codes and fields used in the dataset), and/or a Readme file as appropriate. Data storage and accessibility will be consistent with the guidelines in Section 3.1.3 of the MAM Manual (DWH NRDA Trustees 2021). In the event of a public records request related to data and information that are not already publicly available, the Trustee to whom the request is addressed would provide notice to the other Louisiana TIG members prior to releasing any data that are the subject of the request. Some of the data collected may be protected from public disclosure under federal and state law (e.g., personally identifiable information under the Privacy Act) and therefore would not be publicly distributed.

8.0 LA TIG MAM Strategy Goals Addressed by this MAM Activity

Given the unprecedented temporal, spatial, and funding scales associated with the DWH oil spill restoration effort, the Trustees recognized the need for robust Monitoring and Adaptive Management (MAM) to support restoration planning, implementation, and performance. As such, one of the programmatic goals established in the PDARP/PEIS is to "Provide for Monitoring, Adaptive Management, and Administrative Oversight to Support Restoration Implementation" to ensure that the portfolio of restoration projects provides long-term benefits to natural resources and services injured by the spill (Appendix 5.E of the PDARP/PEIS). This framework allows the Trustees to evaluate restoration effectiveness, address potential uncertainties related to restoration planning and implementation, and provide feedback to inform future restoration decisions.

The DWH restoration projects constructed and planned in Barataria Basin create significant changes to the Basin, such as changes in hydrology and conversion of shallow open water to constructed marsh. Adaptive management requires more than simply documenting a high-level change (e.g., change in fish abundance/density or species composition); it requires understanding the causes and mechanisms of

change (e.g., changes in prey). For example, the ability to demonstrate the relationship between wetland restoration and fish productivity depends on sampling prey organisms to provide evidence for trophic linkages. The deliverables developed through this MAM activity will develop the information needed to describe ecosystem-level changes in the Barataria Basin, such as quantifying changes in community structure, population, and estuarine nekton productivity.

Therefore, this MAM activity will support the LA TIG commitment to report on progress towards meeting stated restoration goals and objectives at the project level and ecosystem level; and to inform future ecosystem-level project designs, implementation, and evaluation. Collectively, information gained from this MAM activity will directly benefit the LA TIG's ability to effectively predict and assess Louisiana's estuarine food web within the broader context of future DWH Wetlands, Coastal and Nearshore Habitats restoration projects. It will also support planning of the comprehensive, integrated ecosystem restoration approach described in the LA TIG's Strategic Restoration Plan and Environmental Assessment #3: Restoration of Wetlands, Coastal, and Nearshore Habitats in the Barataria Basin, Louisiana (LA TIG 2018). If combined with the long-term LTL monitoring plan that is delivered by this project, then it will also enable future evaluation of the DWH restoration program in Barataria Basin.

9.0 Consistency of MAM Activity with the PDARP/PEIS

This MAM activity is consistent with the DWH Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement (PDARP/PEIS) (DWH NRDA Trustees 2016). For injuries to coastal habitats in the northern Gulf of Mexico and resources that use these habitats (e.g., fish, invertebrates, and birds), the PDARP states this goal (PDARP 5.5.2.1): *Restore a variety of interspersed and ecologically connected coastal habitats in each of the five Gulf states to maintain ecosystem diversity, with particular focus on maximizing ecological functions for the range of resources injured by the spill, such as oysters, estuarine-dependent fish species, birds, marine mammals, and nearshore benthic communities* (PDARP 5.5.2.1, Goals of the Restoration Type).

The PDARP emphasizes the complex and interconnected food webs of nearshore habitats, stating, "Coastal and nearshore habitats integrate and form a continuum within the nearshore ecosystem and contribute to an integrated, connected food web." This complexity is a result of the interactions that occur among the different subsystems (e.g., salt marsh, oyster reef, and their associated communities) and a series of food webs that form connections among them. It also confirms that exposure of benthic fauna to sediments contaminated with DWH oil resulted in a series of adverse effects including death, reduced growth, and reduced reproductive success (PDARP 4.3.3.3).

As described in the PDARP (4.6.1.1.2), benthic organisms are a significant part of the estuarine food web and ecosystem:

- Various plants grow in the shallow water sediments (e.g., emergent and submerged aquatic vegetation, and benthic microalgae). Decomposing plant material is an important food in estuaries.

- Food and inorganic nutrients flow from the water column to the bottom and in the opposite direction.
- Benthic organisms filter water for food, and some move over and through sediments and take food from the sediment itself.
- Numerous other organisms also feed on the bottom, including many invertebrates (e.g., shrimp, crab), fish, and birds.
- The flow of energy from phytoplankton, detritus, and bottom sediments converges in upper trophic levels and upon top carnivores that are generalist feeders on various organisms. These top carnivores include many species of fish (e.g., sea trout, red drum, and flounder), birds (e.g., sea gulls, wading birds), and mammals (e.g., dolphins).

Recognizing this complexity, the PDARP emphasizes the potential for multiple restoration approaches to be implemented in combination to increase overall habitat benefits to other injured resources, such as fish and shallow benthic communities. For example, a goal of the Wetlands, Coastal, and Nearshore Habitats restoration type is to restore a variety of interspersed and ecologically connected coastal habitats[...] to maintain ecosystem diversity, with particular focus on maximizing ecological functions for the range of resources injured by the spill, such as oysters, estuarine-dependent fish species, birds, marine mammals, and nearshore benthic communities (PDARP 5.5.2.1). As such, this MAM activity is consistent with the PDARP/PEIS, including the Monitoring and Adaptive Management Framework, as described in Section 5.5.15.2.

In summary, this proposed MAM activity will support restoration planning and evaluation of restoration actions and associated benefits to fish, estuarine habitats, and increased ecosystem services in Barataria Basin by supplying information on a critical portion of the complex estuarine food web.

10.0 National Environmental Policy Act (NEPA) Review

The Trustees' approach to compliance with NEPA summarized in this section is consistent with, and tiers where applicable from, the PDARP/PEIS Section 6.4.14. Resources considered and impact definitions (minor, moderate, major) align with the PDARP/PEIS. Relevant analyses from the PDARP/PEIS are incorporated by reference. Such incorporation by reference of information from existing plans, studies or other material is used in this analysis to streamline the NEPA process and to present a concise document that briefly provides sufficient evidence and analysis to address the Louisiana TIG's compliance with NEPA (40 CFR 1506.3, 40 CFR § 1508.9). All source documents relied upon are available to the public and links are provided in the discussion where applicable.

10.1 NEPA REVIEW OF MAM ACTIVITY

Tasks 1, 3, and 4 of this activity (synthesize data, write long-term monitoring plan) are desk-top, data-based activities and as such would not cause adverse impacts to any resource category and do not require any additional environmental review, consistent with the previous evaluation in the PDARP/PEIS Section 6.4.14.

Task 2 of this activity includes field activities that would have minor short-term, adverse impacts and are necessary to complete the data-based activities. Temporary impacts to the biological and physical environment could include short-term, temporary disturbance of open water, intertidal, and subtidal coastal wetland habitat complexes (e.g., marsh, mangrove, oyster, submerged aquatic vegetation [SAV], and shallow mud habitat) and associated species. These minor impacts would be caused by the use of sampling gear (hand cores, piston cores, diaphragm pump, water bucket, syringes) and removal of water, sediment, and vegetation; these activities may temporarily disturb the marsh platform and benthic habitats (i.e., oyster and SAV) during sampling. The risk of entrapment of protected species (dolphins and sea turtles) while sampling is negligible given the sampling methods; however, protocols such as maintaining vigilant watches for protected species before deployment of sampling equipment will be followed to further minimize this risk. Descriptions of sampling methodology, instrumentation, and numbers of samples are detailed in Appendix C.

Other short-term, minor adverse impacts or disturbances could impact protected species or estuarine habitats due to the operation of small boats while conducting field sampling. To minimize or avoid these disturbances, best management practices will be used, such as operating at minimum safe speeds and maintaining vigilant watches while in transit by assigning designated individuals to observe for protected species. Field sampling will be conducted during daylight hours, thus maximizing the ability to observe potential interactions with protected species and habitats. Sampling will be conducted year-round, and thus a constant vigilance would be necessary for resident protected species, such as dolphins occurring in inshore waters.

Consistent with the analysis in Section 6.4.14 of the PDARP/PEIS, environmental consequences would be direct, short-term, minor impacts through the associated field work. Similar field work activities have been previously evaluated in DWH MAIPs including the project Monitoring the Effects of Coastal Wetland Restoration on Fish and Invertebrates (DIVER ID: #299). NOAA has previously developed specific protocols that must be adhered to should field operations lead to interactions with marine mammals, sea turtles, and Diamondback terrapins during sampling or other activities related to the execution of fieldwork.

10.2 NEPA CONCLUSION

After review of the proposed activities against those actions previously evaluated in the PDARP/PEIS, the Louisiana TIG determined that these activities are consistent with the PDARP/PEIS evaluation of preliminary phases of restoration (planning, feasibility studies, design engineering, and permitting activities) provided in Section 6.4.14 of the PDARP/PEIS. Therefore, no further NEPA analysis is required at this time.

11.0 Compliance with Environmental Laws and Regulations

The Louisiana TIG is working to complete technical assistance with the appropriate regulatory agencies for this project. Project Tasks 1, 3, and 4 consist of analysis of existing data and thus permits and consultations are not required. Task 2 of this project includes field sampling activities, and thus may require permitting and consultations with relevant state and federal agencies; where possible, existing permits and consultations will be reviewed to determine if they are sufficient to complete the work or if

additional compliance work is needed. For the status of reviews under Federal regulatory statutes, see the table below.

Federal environmental compliance responsibilities and procedures follow the DWH Trustee Council Standard Operating Procedures (SOP) (2016), which are laid out in Section 9.4.6 of that document. Following the SOP, the Implementing Trustees for each activity will ensure that the status of environmental compliance (e.g., completed vs. in progress) is tracked through the DIVER Restoration Portal.

Documentation of regulatory compliance will be available in the Administrative Record that can be found at the DOI's Online Administrative Record repository for the DWH NRDA (https://www.doi.gov/deepwaterhorizon/adminrecord). The current status of environmental compliance can be viewed at any time on the Trustee Council's website: http://www.gulfspillrestoration.noaa.gov/environmental-compliance/.

Table 7. Status of federal regulatory compliance reviews and approvals for the proposed project.

Federal Statute	Compliance Status
Bald and Golden Eagle Protection Act (USFWS)	In process
Coastal Barrier Resources Act (USFWS)	N/A
Coastal Zone Management Act	Under Evaluation
Endangered Species Act (NMFS)	In process
Endangered Species Act (USFWS)	In process
Essential Fish Habitat (NMFS)	Complete
Marine Mammal Protection Act (NMFS)	Complete
Marine Mammal Protection Act (USFWS)	In process
Migratory Bird Treaty Act (USFWS)	In process
National Historic Preservation Act	Under Evaluation
Rivers and Harbors Act/Clean Water Act	Under Evaluation
National Environmental Policy Act	Complete, NEPA analysis described in Section 5, above.

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Appendices

Appendix A. Analysis of Existing Data to Inform Sampling Effort

Purpose:
Existing data of key LTLs in the Barataria Basin were used to identify the approximate sampling intensity necessary to detect the influence of environmental change on these organisms in the Basin at multiple spatial and temporal scales. Power analysis was conducted using three salinity zones as a spatial component. Results of this analysis were used to provide an estimate of sampling effort for Task 2.

Methods:
A preliminary sample size estimate for detritus and phytoplankton is provided in Kiskaddon et al. (2022) based on functions readily available in R and a compilation of existing data. However, interpretation was limited due to insufficient data availability to conduct more intricate power analyses at that time, different assumptions around spatial scale, and statistical analysis based solely on t-tests.

A custom function was then created in R specifically for this MAIP. It bootstrapped three-way interactive analysis of variance (ANOVA) models by resampling available data for each group. compiled in Kiskaddon et al. (2022). This updated code was used to estimate sample sizes needed to detect differences in phytoplankton (Chl a, µg/L), macroinfauna (subtidal only due to data availability; indiv m^{-2}), and water column detritus (total suspended organic matter, mg/L) across the 3 three salinity zones in Figure 1. . For some LTL groups, additional analysis was not possible because the data that were amenable to run a power analysis were all collected from the same salinity zone. R code for each analysis is provided in Appendix D.

Datasets:
Power analysis was conducted for phytoplankton (Chl a, µg/L) and water column detritus (total suspended organic matter, mg/L) based on data compiled in Kiskaddon et al. (2022). For macroinfauna (subtidal only due to data availability; indiv m^{-2}), the power analysis was conducted using unpublished data from Tupitza & Glaspie, who investigated macroinfauna through field-based collections over three seasons, over two years (2021-2022), and in two of the salinity zones.

For sediment detritus, microphytobenthos, zooplankton, and emergent vegetation macrofaunal invertebrates, neither approach allowed for power analysis based on the spatial component of salinity zone.

Analysis:
In R statistical software, the data for each LTL group was first subset to only include data collected using a similar sampling protocol. The most prevalent protocol was chosen. From the subset data, combinations of salinity zones (Figure 1), seasons, and years were identified for which there were more than 10 data records in the dataset. Of the possibilities for salinity zone, season, and year, three salinity zones, three seasons, and three years were randomly selected for analysis for phytoplankton and water column detritus while two salinity zones, two seasons, and two years were randomly selected for analysis for subtidal macroinfauna based on the availability of the databased on the availability of the data.

For each LTL group, the data were resampled to represent a candidate sample size between 3 and 100 for each salinity zone, season, and year combination. Then the data were used to run an ANOVA with the full interaction between salinity zone, season, and year, when possible. ANOVAs were weighted based on sample size for a particular data record. *P-values* for main and interactive effects were

extracted from the ANOVA. This process was repeated 1,000 times for each candidate sample size between three and 100, providing a distribution of resampled *p-values* for each candidate sample size. From these distributions of *p-values*, the ideal sample size was calculated for each main and interactive effect as the sample size at which *p-values* were significant for 80% of the 1000 bootstrapped ANOVAs (representing a power of 0.8). Deviations from this procedure were necessary in some cases and are noted.

For each LTL group possible, these analyses resulted in a suggested sample size necessary to detect a salinity zone x season and/or a salinity zone x year interaction at $\alpha = 0.05$ and $\beta = 0.2$. R code for each analysis is provided in Appendix D.

Results:

Analysis of **phytoplankton** (units: total Chl *a* µg/L) was based on data provided in Schaeffer et al. (2012), Turner et al. (2019), Garcia et al. (2010), Ren et al. (2020), Liu et al. (2021), Wong et al. (2010), Ren et al. (2009), CPRA (2022), Powell (2018), Baustian et al. (2018), and Starr et al. (2017). The analysis resulted in a recommended sample size of 17-19 samples per salinity zone per season per year, allowing the subsequent analysis to capture a salinity zone x season and a salinity zone x year interaction, respectively, at $\alpha = 0.05$ and $\beta = 0.2$ (Figure 4). Thus, as a conservative estimate for monitoring across 3 salinity zones and 3 years, the required sample size would be on the order of 171 samples; adding a seasonal level, this would lead to 684 samples.

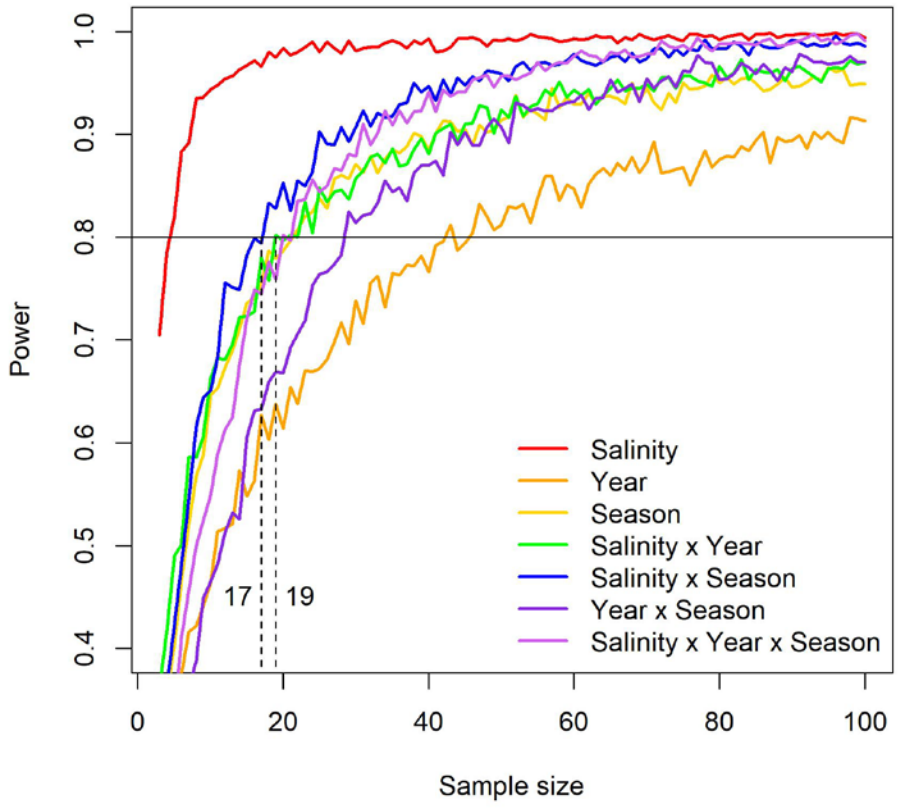

Figure 4. Calculated sample size (β = 0.2, power = 0.8) for phytoplankton, broken down by each term in a three-way, fully interactive ANOVA model. The term "salinity" indicates salinity zone.

Initial power analysis described in Kiskaddon et al. (2022) separated **macroinfauna** by habitat type (subtidal (i.e., OW) and EV habitat type); however, the data were deemed insufficient for conducting an EV habitat type analysis by salinity zone using the updated R code. Analysis of **subtidal macroinfauna** (units: number of individuals/m^2) was based on data provided in Tupitza and Glaspie (2020–2021, unpublished data.). The analysis was data-limited; therefore, it was not possible to examine the full interaction (i.e., salinity x season x year), but it was possible to examine the salinity zone x year interaction. The analysis identified a recommended sample size of 20 subtidal macroinfauna samples per salinity zone per season per year to see a salinity zone x year interaction at $\alpha = 0.05$ and $\beta = 0.2$ (Figure 5). Thus, for monitoring 3 salinity zones across 3 years, the required sample size would be on the order of 180 samples; adding a seasonal level, this would lead to 720 samples.

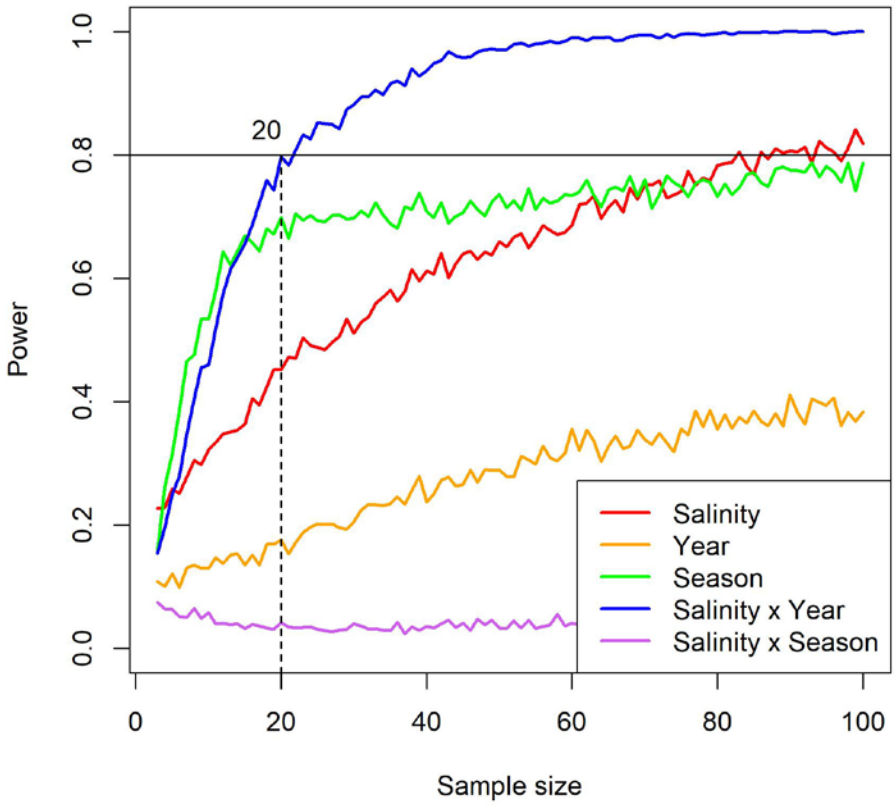

Figure 5. Calculated sample size (β = 0.2, power = 0.8) for subtidal macroinfaunal invertebrates, broken down by each term in a three-way, interactive ANOVA model. The term "salinity" indicates salinity zone.

Water column detritus (units: total suspended organic matter, mg/L) was based on data provided in Turner et al. (2019) and Baustian et al. (2018). This analysis resulted in a recommended sample size of 11-18 samples per salinity zone per season per year, allowing the subsequent analysis to capture a salinity zone x year and a salinity zone x season interaction, respectively, at $\alpha = 0.05$ and $\beta = 0.2$ (Figure 6). Thus, as a conservative estimate to ensure capturing the salinity x season interaction across 3 salinity zones and 4 seasons, the required sample size would be on the order of 216 samples; doing so across 3 years would lead to 648 samples.

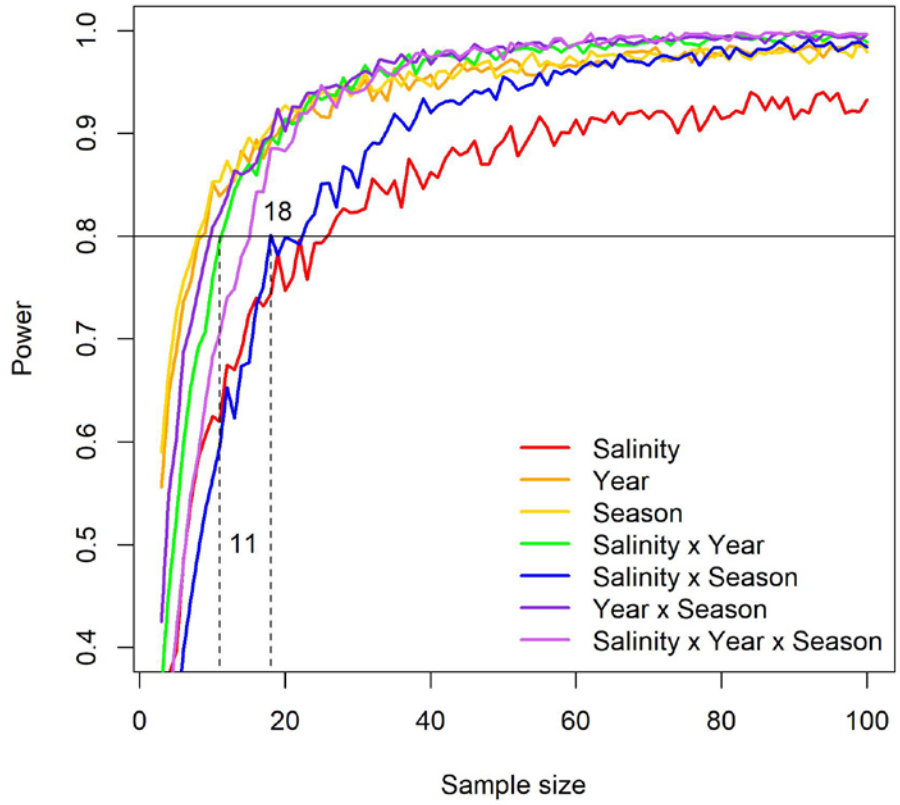

Figure 6. Calculated sample size (β = 0.2, power = 0.8) for water column detritus, broken down by each term in a three-way, fully interactive ANOVA model. The term "salinity" indicates salinity zone.

Conclusions:

Power analysis was conducted using existing data to inform the sampling effort included in this MAIP. Due to data limitations, this analysis was only possible for a subset of LTL groups and only for certain habitat types. As a result, these findings were used cautiously as a guide for determining the sampling design (Appendix B) and sampling methods (Appendix C). R code for each analysis is provided in Appendix D.

Appendix B. Sampling Design

The FIMP 50-ft bag seine sampling locations were used to haphazardly select seven LTL monitoring stations in intermediate/brackish and saline zones for the field collection activities under Task 2. The FIMP electrofishing sampling locations were used to also haphazardly select three LTL monitoring stations in fresh zone for a total of 10 stations across the Barataria Basin salinity gradient. The haphazard selection of LTL monitoring stations based on FIMP stations was done to encompass longitudinal differences across the Basin within each salinity zone without overburdening the sampling design by including as many stations as FIMP.

A spatially balanced GRTS sampling design is employed for this field sampling design. A 2 km × 2 km square area (approximately 4 km^2 area) was defined around each LTL monitoring station (also termed 'stratum') using the latitude and longitude coordinates as the center point for each area, which is fixed through time. Within each LTL monitoring station stratum, sampling will occur at 10 sites, 5 OW and 5 EV (Figure 7). For the first sampling event, all sites were selected at random and two sites per habitat type were designated as "fixed" for long-term monitoring whereas the other three sites were randomized for each sampling event.

Figure 7. Example of an LTL monitoring station stratum that contains randomized and fixed sampling sites by habitat type (OW and EV).

Due to the highly dynamic and spatially heterogeneous nature of the Barataria Basin, a strict GRTS protocol was modified for development of this MAM proposal to allow for flexibility in random site selection due to gear type and accessibility considerations:

- The OW sites should occur in water depths approximately 1 m, but it is known that water depth can change significantly across the Basin (e.g., deep channels, shallow mud flats). Upon visiting a designated site, if water levels are not appropriate for sampling the field team should identify the most immediate OW location with appropriate water depth for sampling this habitat type. The OW site location adjustments should be carefully documented by the field team. Protocols for this decision will be further detailed during Task 1 implementation.
- Similarly, sampling in EV sites should occur in areas of flooded vegetation. If the randomized site is not flooded at the time of sampling, samples should be collected from the closest location of flooded vegetation while traveling perpendicular to the vegetation/open water edge. Logistical planning for field expeditions should consider timing of sampling with high tide. Protocols for this recommendation will be further detailed during Task 1 implementation.

Coordinates are provided for each of the LTL monitoring stations in Table 8.

Table 9–Table 20 provide site information for each year, season (fall, winter, spring, and summer), and station of sampling that will be useful for the sampling of the benthos. The bi-weekly water column sampling will occur at one fixed OW site per station and thus OW site #1 could always be used for simplicity.

Table 8. The LTL monitoring station locations for the Barataria Basin that span salinity zones. Identified stations mirror LDWF FIMP 50-ft bag seine and electrofishing sampling locations. The closest CRMS site to each station, the associated FIMP station, the CASM polygon of each station, and salinity zone based on Figure 1 are also identified.

Proposed LTL Station Number	Latitude	Longitude	CASM Polygon	Salinity Zone	Nearest CRMS Site ID	FIMP Station Alignment
1	29.2414	-90.1697	6	Saline	CRMS0164	2069
2	29.4681	-89.9128	7	Saline	CRMS0224	2041
3	29.4108	-89.6553	11	Saline	CRMS0272	2045
4	29.3158	-89.3955	19	Saline	CRMS0163	2046
5	29.5339	-90.1012	10	Intermediate/ Brackish	CRMS0253	2011
6	29.5569	-90.0111	14	Intermediate/ Brackish	CRMS0251	2004
7	29.6478	-90.1308	2	Intermediate/ Brackish	CRMS4245	2002
8	29.7727	-90.2903	15	Fresh	CRMS0219	4329
9	29.75259	-90.38914	1	Fresh	CRMS3054	4335
10	29.83306	-90.28389	0	Fresh	CRMS3166	4155

Table 9. Year 1 Fall season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24346	-90.1731	Open Water	Random
	4	29.24232	-90.1672	Open Water	Random
	5	29.24065	-90.1618	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23801	-90.1714	Emergent Vegetation	Random
	9	29.24074	-90.1636	Emergent Vegetation	Random
	10	29.24149	-90.164	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.47503	-89.9123	Open Water	Random
	4	29.46313	-89.9186	Open Water	Random
	5	29.46546	-89.9094	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46089	-89.9214	Emergent Vegetation	Random
	9	29.46359	-89.9218	Emergent Vegetation	Random
	10	29.46507	-89.9118	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.41531	-89.6506	Open Water	Random
	4	29.40364	-89.6497	Open Water	Random
	5	29.41513	-89.6628	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41764	-89.6562	Emergent Vegetation	Random
	9	29.41709	-89.6456	Emergent Vegetation	Random
	10	29.40596	-89.6546	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.32111	-89.4046	Open Water	Random
	4	29.32164	-89.3996	Open Water	Random
	5	29.30954	-89.3973	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31339	-89.397	Emergent Vegetation	Random
	9	29.3117	-89.3888	Emergent Vegetation	Random
	10	29.32184	-89.3963	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed
	3	29.52811	-90.1048	Open Water	Random
	4	29.53292	-90.0997	Open Water	Random
	5	29.52835	-90.0924	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53739	-90.0912	Emergent Vegetation	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	9	29.54027	-90.101	Emergent Vegetation	Random
	10	29.5347	-90.1044	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.56225	-90.007	Open Water	Random
	4	29.56524	-90.0066	Open Water	Random
	5	29.55117	-90.0127	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.5649	-90.0175	Emergent Vegetation	Random
	9	29.55262	-90.006	Emergent Vegetation	Random
	10	29.54887	-90.0086	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.64014	-90.1309	Open Water	Random
	4	29.64616	-90.1222	Open Water	Random
	5	29.64639	-90.1297	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.65092	-90.1348	Emergent Vegetation	Random
	9	29.64808	-90.1379	Emergent Vegetation	Random
	10	29.64186	-90.1335	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.76694	-90.2963	Open Water	Random
	4	29.78117	-90.2827	Open Water	Random
	5	29.77566	-90.2921	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77213	-90.2905	Emergent Vegetation	Random
	9	29.76821	-90.2821	Emergent Vegetation	Random
	10	29.77847	-90.2966	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75233	-90.3903	Open Water	Random
	4	29.75176	-90.3966	Open Water	Random
	5	29.75633	-90.3954	Open Water	Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75547	-90.3947	Emergent Vegetation	Random
	9	29.75575	-90.3829	Emergent Vegetation	Random
	10	29.76043	-90.3809	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.84166	-90.2899	Open Water	Random
	4	29.83469	-90.2746	Open Water	Random
	5	29.83558	-90.283	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	8	29.8389	-90.2855	Emergent Vegetation	Random
	9	29.82873	-90.2884	Emergent Vegetation	Random
	10	29.83221	-90.2772	Emergent Vegetation	Random

Table 10. Year 1 Winter season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.23562	-90.1754	Open Water	Random
	4	29.24558	-90.1779	Open Water	Random
	5	29.24777	-90.1709	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23522	-90.179	Emergent Vegetation	Random
	9	29.24046	-90.1646	Emergent Vegetation	Random
	10	29.24814	-90.1647	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.47429	-89.9088	Open Water	Random
	4	29.46151	-89.9106	Open Water	Random
	5	29.46422	-89.9104	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46055	-89.909	Emergent Vegetation	Random
	9	29.46508	-89.9105	Emergent Vegetation	Random
	10	29.46629	-89.9093	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40728	-89.64766927	Open Water	Random
	4	29.4105	-89.65024828	Open Water	Random
	5	29.41062	-89.66265275	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41483	-89.65479365	Emergent Vegetation	Random
	9	29.41265	-89.65559601	Emergent Vegetation	Random
	10	29.40328	-89.65654104	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31927	-89.39952223	Open Water	Random
	4	29.31225	-89.40083739	Open Water	Random
	5	29.32425	-89.40093241	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	8	29.31274	-89.39230132	Emergent Vegetation	Random
	9	29.3216	-89.39620753	Emergent Vegetation	Random
	10	29.30731	-89.39492879	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed
	3	29.5361526	-90.110863	Open Water	Random
	4	29.5314084	-90.093868	Open Water	Random
	5	29.5279433	-90.092145	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.5401828	-90.094458	Emergent Vegetation	Random
	9	29.5370019	-90.1039	Emergent Vegetation	Random
	10	29.5377368	-90.108172	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55237	-90.0157	Open Water	Random
	4	29.56031	-90.02	Open Water	Random
	5	29.56106	-90.0073	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55897	-90.0134	Emergent Vegetation	Random
	9	29.56161	-90.0198	Emergent Vegetation	Random
	10	29.55525	-90.0042	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.65591	-90.1359637	Open Water	Random
	4	29.64176	-90.1312288	Open Water	Random
	5	29.65126	-90.1327037	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.65274	-90.136821	Emergent Vegetation	Random
	9	29.65138	-90.134461	Emergent Vegetation	Random
	10	29.64715	-90.1340196	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.775	-90.283554	Open Water	Random
	4	29.77087	-90.2963216	Open Water	Random
	5	29.76694	-90.2946754	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77595	-90.2826125	Emergent Vegetation	Random
	9	29.76828	-90.2874135	Emergent Vegetation	Random
	10	29.77414	-90.2967332	Emergent Vegetation	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75260302	-90.390539	Open Water	Random
	4	29.75554281	-90.387741	Open Water	Random
	5	29.75863033	-90.391085	Open Water	Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75069776	-90.390002	Emergent Vegetation	Random
	9	29.74828477	-90.392311	Emergent Vegetation	Random
	10	29.75871392	-90.393643	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.8304	-90.2903	Open Water	Random
	4	29.83858	-90.2842	Open Water	Random
	5	29.83369	-90.2827	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.82803	-90.2879	Emergent Vegetation	Random
	9	29.83214	-90.282	Emergent Vegetation	Random
	10	29.83923	-90.2884	Emergent Vegetation	Random

Table 11. Year 1 Spring season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24533	-90.1785	Open Water	Random
	4	29.24019	-90.1727	Open Water	Random
	5	29.24765	-90.1614	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23818	-90.1764	Emergent Vegetation	Random
	9	29.23399	-90.1738	Emergent Vegetation	Random
	10	29.2442	-90.1634	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46544	-89.9129	Open Water	Random
	4	29.46094	-89.9196	Open Water	Random
	5	29.46713	-89.909	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.4608	-89.9206	Emergent Vegetation	Random
	9	29.46488	-89.9101	Emergent Vegetation	Random
	10	29.46643	-89.9118	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.41239	-89.6619669	Open Water	Random
	4	29.4097	-89.6478583	Open Water	Random
	5	29.40411	-89.6508906	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.40668	-89.6573484	Emergent Vegetation	Random
	9	29.41273	-89.6495664	Emergent Vegetation	Random
	10	29.41611	-89.6599962	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31981	-89.3968	Open Water	Random
	4	29.31059	-89.3986	Open Water	Random
	5	29.32242	-89.3997	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31174	-89.3942	Emergent Vegetation	Random
	9	29.31694	-89.3935	Emergent Vegetation	Random
	10	29.30929	-89.3932	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53521	-90.0937	Open Water	Random
	4	29.53181	-90.1043	Open Water	Random
	5	29.53089	-90.1103	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53821	-90.0971	Emergent Vegetation	Random
	9	29.54226	-90.0928	Emergent Vegetation	Random
	10	29.53602	-90.1054	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.56144	-90.0193	Open Water	Random
	4	29.56371	-90.0041	Open Water	Random
	5	29.55671	-90.0165	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55523	-90.0039	Emergent Vegetation	Random
	9	29.55625	-90.0122	Emergent Vegetation	Random
	10	29.56414	-90.0101	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.6488	-90.12579982	Open Water	Random
	4	29.64107	-90.13132602	Open Water	Random
	5	29.65571	-90.13765336	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.65142	-90.13462723	Emergent Vegetation	Random
	9	29.65202	-90.13690678	Emergent Vegetation	Random
	10	29.64919	-90.14082067	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.78114	-90.2811	Open Water	Random
	4	29.77596	-90.2918	Open Water	Random
	5	29.77205	-90.2995	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77618	-90.2955	Emergent Vegetation	Random
	9	29.77653	-90.2823	Emergent Vegetation	Random
	10	29.76862	-90.2902	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75902	-90.3855	Open Water	Random
	4	29.75362	-90.3938	Open Water	Random
	5	29.75633	-90.3912	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.74975	-90.3926	Emergent Vegetation	Random
	9	29.75891	-90.3942	Emergent Vegetation	Random
	10	29.7513	-90.3846	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83416	-90.2816	Open Water	Random
	4	29.838	-90.2904	Open Water	Random
	5	29.8318	-90.2867	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83843	-90.285	Emergent Vegetation	Random
	9	29.83259	-90.2791	Emergent Vegetation	Random
	10	29.82726	-90.2917	Emergent Vegetation	Random

Table 12. Year 1 Summer season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.23952	-90.1703	Open Water	Random
	4	29.24173	-90.1748	Open Water	Random
	5	29.24758	-90.1694	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.24484	-90.1617	Emergent Vegetation	Random
	9	29.23925	-90.167	Emergent Vegetation	Random
	10	29.23648	-90.1793	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46263	-89.9217	Open Water	Random
	4	29.46792	-89.9134	Open Water	Random
	5	29.46854	-89.9113	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.4608	-89.9216	Emergent Vegetation	Random
	9	29.46718	-89.9118	Emergent Vegetation	Random
	10	29.46845	-89.9106	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40612	-89.6505	Open Water	Random
	4	29.41735	-89.6614	Open Water	Random
	5	29.41497	-89.6616	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41213	-89.6508	Emergent Vegetation	Random
	9	29.40284	-89.6546	Emergent Vegetation	Random
	10	29.41138	-89.661	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.32405	-89.4038	Open Water	Random
	4	29.31264	-89.4004	Open Water	Random
	5	29.32395	-89.3996	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31095	-89.3971	Emergent Vegetation	Random
	9	29.31473	-89.3963	Emergent Vegetation	Random
	10	29.30736	-89.3934	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53719	-90.1106	Open Water	Random
	4	29.53225	-90.0937	Open Water	Random
	5	29.53905	-90.0941	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.54151	-90.093	Emergent Vegetation	Random
	9	29.53765	-90.1087	Emergent Vegetation	Random
	10	29.53665	-90.1049	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55724	-90.0045	Open Water	Random
	4	29.562	-90.0156	Open Water	Random
	5	29.54874	-90.0092	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55306	-90.011	Emergent Vegetation	Random
	9	29.56047	-90.0124	Emergent Vegetation	Random
	10	29.56387	-90.0083	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.65467	-90.1317	Open Water	Random
	4	29.64472	-90.1312	Open Water	Random
	5	29.65613	-90.1354	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64858	-90.1361	Emergent Vegetation	Random
	9	29.65069	-90.1357	Emergent Vegetation	Random
	10	29.63917	-90.1403	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77419	-90.2999	Open Water	Random
	4	29.77973	-90.2896	Open Water	Random
	5	29.7662	-90.2936	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77426	-90.2825	Emergent Vegetation	Random
	9	29.76824	-90.2881	Emergent Vegetation	Random
	10	29.7771	-90.297	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75787	-90.3821	Open Water	Random
	4	29.76001	-90.3907	Open Water	Random
	5	29.74675	-90.3909	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75922	-90.3936	Emergent Vegetation	Random
	9	29.75132	-90.3888	Emergent Vegetation	Random
	10	29.7485	-90.3808	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83523	-90.2871	Open Water	Random
	4	29.84044	-90.2829	Open Water	Random
	5	29.83021	-90.2864	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83017	-90.2836	Emergent Vegetation	Random
	9	29.84163	-90.2857	Emergent Vegetation	Random
	10	29.83138	-90.2757	Emergent Vegetation	Random

Table 13. Year 2 Fall season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24183	-90.17	Open Water	Random
	4	29.24067	-90.1774	Open Water	Random
	5	29.24289	-90.1651	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23964	-90.1669	Emergent Vegetation	Random
	9	29.24051	-90.1748	Emergent Vegetation	Random
	10	29.2482	-90.1665	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46649	-89.9086	Open Water	Random
	4	29.46391	-89.919	Open Water	Random
	5	29.46929	-89.9122	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46662	-89.9122	Emergent Vegetation	Random
	9	29.46023	-89.9196	Emergent Vegetation	Random
	10	29.46513	-89.9113	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40485	-89.6509	Open Water	Random
	4	29.41107	-89.6628	Open Water	Random
	5	29.41597	-89.6621	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41117	-89.656	Emergent Vegetation	Random
	9	29.40871	-89.646	Emergent Vegetation	Random
	10	29.413	-89.6606	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31014	-89.3994	Open Water	Random
	4	29.31951	-89.3965	Open Water	Random
	5	29.31607	-89.3969	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31393	-89.3962	Emergent Vegetation	Random
	9	29.31124	-89.3939	Emergent Vegetation	Random
	10	29.32242	-89.3974	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53331	-90.1062	Open Water	Random
	4	29.53728	-90.1104	Open Water	Random
	5	29.53545	-90.0938	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.54099	-90.0925	Emergent Vegetation	Random
	9	29.54062	-90.1065	Emergent Vegetation	Random
	10	29.53624	-90.097	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.56024	-90.0078	Open Water	Random
	4	29.55464	-90.0149	Open Water	Random
	5	29.5591	-90.0166	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55648	-90.0055	Emergent Vegetation	Random
	9	29.56191	-90.0141	Emergent Vegetation	Random
	10	29.55247	-90.014	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.64762	-90.1308	Open Water	Random
	4	29.653	-90.1333	Open Water	Random
	5	29.64434	-90.1311	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64912	-90.1346	Emergent Vegetation	Random
	9	29.64261	-90.134	Emergent Vegetation	Random
	10	29.65071	-90.1338	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77501	-90.2998	Open Water	Random
	4	29.76631	-90.2919	Open Water	Random
	5	29.77635	-90.292	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.76914	-90.2851	Emergent Vegetation	Random
	9	29.77873	-90.2908	Emergent Vegetation	Random
	10	29.77175	-90.2925	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.74747	-90.3911	Open Water	Random
	4	29.75671	-90.3908	Open Water	Random
	5	29.7557	-90.386	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75618	-90.3894	Emergent Vegetation	Random
	9	29.75176	-90.385	Emergent Vegetation	Random
	10	29.75263	-90.3928	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83683	-90.2904	Open Water	Random
	4	29.83229	-90.2852	Open Water	Random
	5	29.83397	-90.2812	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.8285	-90.2886	Emergent Vegetation	Random
	9	29.83858	-90.2865	Emergent Vegetation	Random
	10	29.8321	-90.2827	Emergent Vegetation	Random

Table 14. Year 2 Winter season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24163	-90.1756	Open Water	Random
	4	29.24777	-90.1675	Open Water	Random
	5	29.24164	-90.1619	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.24433	-90.1632	Emergent Vegetation	Random
	9	29.24584	-90.1625	Emergent Vegetation	Random
	10	29.23803	-90.1697	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46764	-89.9088	Open Water	Random
	4	29.4645	-89.9206	Open Water	Random
	5	29.46894	-89.9116	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46862	-89.9098	Emergent Vegetation	Random
	9	29.47553	-89.9107	Emergent Vegetation	Random
	10	29.46084	-89.9224	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40493	-89.651	Open Water	Random
	4	29.41249	-89.6634	Open Water	Random
	5	29.40889	-89.6504	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.4123	-89.6552	Emergent Vegetation	Random
	9	29.41011	-89.6543	Emergent Vegetation	Random
	10	29.40632	-89.6616	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.32142	-89.3982	Open Water	Random
	4	29.31298	-89.3995	Open Water	Random
	5	29.30967	-89.3979	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.3189	-89.394	Emergent Vegetation	Random
	9	29.30958	-89.3926	Emergent Vegetation	Random
	10	29.31314	-89.3963	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53331	-90.1062	Open Water	Random
	4	29.53728	-90.1104	Open Water	Random
	5	29.53545	-90.0938	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.54099	-90.0925	Emergent Vegetation	Random
	9	29.54062	-90.1065	Emergent Vegetation	Random
	10	29.53624	-90.097	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55191	-90.0109	Open Water	Random
	4	29.5626	-90.0159	Open Water	Random
	5	29.56263	-90.0077	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55685	-90.0083	Emergent Vegetation	Random
	9	29.55361	-90.0084	Emergent Vegetation	Random
	10	29.56046	-90.0142	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.64832	-90.1306	Open Water	Random
	4	29.64109	-90.1311	Open Water	Random
	5	29.65395	-90.1357	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64198	-90.1343	Emergent Vegetation	Random
	9	29.64706	-90.1338	Emergent Vegetation	Random
	10	29.65149	-90.1384	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77501	-90.2998	Open Water	Random
	4	29.76631	-90.2919	Open Water	Random
	5	29.77635	-90.292	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.76914	-90.2851	Emergent Vegetation	Random
	9	29.77873	-90.2908	Emergent Vegetation	Random
	10	29.77175	-90.2925	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75933	-90.3818	Open Water	Random
	4	29.76017	-90.391	Open Water	Random
	5	29.75362	-90.3888	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75907	-90.3897	Emergent Vegetation	Random
	9	29.75169	-90.3902	Emergent Vegetation	Random
	10	29.75467	-90.3919	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83357	-90.2791	Open Water	Random
	4	29.83696	-90.2831	Open Water	Random
	5	29.82987	-90.2886	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.82763	-90.2931	Emergent Vegetation	Random
	9	29.84034	-90.2885	Emergent Vegetation	Random
	10	29.83231	-90.2788	Emergent Vegetation	Random

Table 15. Year 2 Spring season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24579	-90.1656	Open Water	Random
	4	29.23545	-90.174	Open Water	Random
	5	29.24148	-90.1671	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.24081	-90.1639	Emergent Vegetation	Random
	9	29.24549	-90.1639	Emergent Vegetation	Random
	10	29.24034	-90.1746	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46379	-89.919	Open Water	Random
	4	29.46917	-89.9094	Open Water	Random
	5	29.47504	-89.9113	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46647	-89.9123	Emergent Vegetation	Random
	9	29.47477	-89.9087	Emergent Vegetation	Random
	10	29.46202	-89.9204	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40578	-89.6509	Open Water	Random
	4	29.40574	-89.6604	Open Water	Random
	5	29.41443	-89.6621	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41328	-89.6521	Emergent Vegetation	Random
	9	29.4063	-89.6553	Emergent Vegetation	Random
	10	29.41318	-89.6607	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31286	-89.3995	Open Water	Random
	4	29.31515	-89.3993	Open Water	Random
	5	29.32218	-89.3998	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31608	-89.3932	Emergent Vegetation	Random
	9	29.31372	-89.3969	Emergent Vegetation	Random
	10	29.31125	-89.3888	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53276	-90.1032	Open Water	Random
	4	29.53557	-90.0923	Open Water	Random
	5	29.53479	-90.0975	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53775	-90.1045	Emergent Vegetation	Random
	9	29.53621	-90.1053	Emergent Vegetation	Random
	10	29.53759	-90.1068	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55201	-90.0096	Open Water	Random
	4	29.554	-90.0064	Open Water	Random
	5	29.56131	-90.0157	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.5623	-90.0086	Emergent Vegetation	Random
	9	29.56123	-90.0135	Emergent Vegetation	Random
	10	29.55709	-90.0137	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.65196	-90.1333	Open Water	Random
	4	29.64134	-90.1321	Open Water	Random
	5	29.64875	-90.1297	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64844	-90.1384	Emergent Vegetation	Random
	9	29.64059	-90.1392	Emergent Vegetation	Random
	10	29.65185	-90.138	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77103	-90.2966	Open Water	Random
	4	29.77227	-90.2838	Open Water	Random
	5	29.77539	-90.2997	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.7702	-90.291	Emergent Vegetation	Random
	9	29.7784	-90.2907	Emergent Vegetation	Random
	10	29.77618	-90.2989	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.751	-90.3838	Open Water	Random
	4	29.75678	-90.397	Open Water	Random
	5	29.7524	-90.3886	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.7483	-90.3897	Emergent Vegetation	Random
	9	29.75672	-90.3892	Emergent Vegetation	Random
	10	29.75156	-90.3872	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.84054	-90.2832	Open Water	Random
	4	29.82904	-90.2903	Open Water	Random
	5	29.83235	-90.2851	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83264	-90.2819	Emergent Vegetation	Random
	9	29.8283	-90.2882	Emergent Vegetation	Random
	10	29.83653	-90.2888	Emergent Vegetation	Random

Table 16. Year 2 Summer season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24007	-90.1657	Open Water	Random
	4	29.24832	-90.1688	Open Water	Random
	5	29.24124	-90.1733	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.2417	-90.1648	Emergent Vegetation	Random
	9	29.24523	-90.1634	Emergent Vegetation	Random
	10	29.23903	-90.1757	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46594	-89.9126	Open Water	Random
	4	29.46367	-89.919	Open Water	Random
	5	29.46898	-89.9079	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46178	-89.9202	Emergent Vegetation	Random
	9	29.46397	-89.9195	Emergent Vegetation	Random
	10	29.47536	-89.9109	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.41062	-89.6625	Open Water	Random
	4	29.4088	-89.6506	Open Water	Random
	5	29.40878	-89.6558	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41265	-89.6549	Emergent Vegetation	Random
	9	29.40501	-89.6529	Emergent Vegetation	Random
	10	29.41169	-89.6616	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31657	-89.3988	Open Water	Random
	4	29.32317	-89.401	Open Water	Random
	5	29.31911	-89.4036	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31141	-89.3975	Emergent Vegetation	Random
	9	29.31741	-89.3932	Emergent Vegetation	Random
	10	29.31055	-89.3914	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53666	-90.108	Open Water	Random
	4	29.53293	-90.1001	Open Water	Random
	5	29.53427	-90.0973	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53788	-90.0987	Emergent Vegetation	Random
	9	29.53754	-90.1081	Emergent Vegetation	Random
	10	29.53794	-90.1068	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55438	-90.0072	Open Water	Random
	4	29.55916	-90.0174	Open Water	Random
	5	29.55506	-90.0156	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.5571	-90.0145	Emergent Vegetation	Random
	9	29.55957	-90.0141	Emergent Vegetation	Random
	10	29.55668	-90.0093	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.65105	-90.1316	Open Water	Random
	4	29.64072	-90.1314	Open Water	Random
	5	29.64423	-90.1354	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64397	-90.1326	Emergent Vegetation	Random
	9	29.6529	-90.1382	Emergent Vegetation	Random
	10	29.6514	-90.1366	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.7663	-90.2967	Open Water	Random
	4	29.77302	-90.297	Open Water	Random
	5	29.7799	-90.289	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77507	-90.2907	Emergent Vegetation	Random
	9	29.76839	-90.2936	Emergent Vegetation	Random
	10	29.77242	-90.293	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75372	-90.3934	Open Water	Random
	4	29.746	-90.3909	Open Water	Random
	5	29.75443	-90.3912	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.74539	-90.3902	Emergent Vegetation	Random
	9	29.75119	-90.389	Emergent Vegetation	Random
	10	29.75805	-90.39	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83288	-90.2856	Open Water	Random
	4	29.83556	-90.2831	Open Water	Random
	5	29.82958	-90.2888	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.84125	-90.287	Emergent Vegetation	Random
	9	29.83641	-90.2857	Emergent Vegetation	Random
	10	29.82747	-90.2916	Emergent Vegetation	Random

Table 17. Year 3 Fall season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24156	-90.17	Open Water	Random
	4	29.24176	-90.1749	Open Water	Random
	5	29.2447	-90.1661	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23584	-90.1714	Emergent Vegetation	Random
	9	29.23753	-90.1774	Emergent Vegetation	Random
	10	29.24329	-90.1632	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46406	-89.9203	Open Water	Random
	4	29.46003	-89.9095	Open Water	Random
	5	29.46848	-89.9127	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46538	-89.9101	Emergent Vegetation	Random
	9	29.47577	-89.9094	Emergent Vegetation	Random
	10	29.46691	-89.9121	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40985	-89.649	Open Water	Random
	4	29.40652	-89.6523	Open Water	Random
	5	29.41125	-89.6623	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41206	-89.6537	Emergent Vegetation	Random
	9	29.40487	-89.6586	Emergent Vegetation	Random
	10	29.41099	-89.6554	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.32383	-89.3996	Open Water	Random
	4	29.31789	-89.3949	Open Water	Random
	5	29.30961	-89.3978	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31343	-89.3981	Emergent Vegetation	Random
	9	29.3194	-89.3945	Emergent Vegetation	Random
	10	29.31013	-89.3952	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53756	-90.1101	Open Water	Random
	4	29.53555	-90.1005	Open Water	Random
	5	29.54095	-90.1029	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53283	-90.0925	Emergent Vegetation	Random
	9	29.53761	-90.0986	Emergent Vegetation	Random
	10	29.53492	-90.1027	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55024	-90.0093	Open Water	Random
	4	29.55866	-90.0165	Open Water	Random
	5	29.5593	-90.008	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55645	-90.0087	Emergent Vegetation	Random
	9	29.55274	-90.0144	Emergent Vegetation	Random
	10	29.56387	-90.0169	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.65098	-90.1332	Open Water	Random
	4	29.64702	-90.1315	Open Water	Random
	5	29.63931	-90.1314	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64895	-90.139	Emergent Vegetation	Random
	9	29.6407	-90.1338	Emergent Vegetation	Random
	10	29.65138	-90.1348	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77304	-90.2975	Open Water	Random
	4	29.77061	-90.2963	Open Water	Random
	5	29.77728	-90.2881	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77895	-90.2905	Emergent Vegetation	Random
	9	29.77773	-90.2942	Emergent Vegetation	Random
	10	29.7686	-90.2938	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75513	-90.3854	Open Water	Random
	4	29.75498	-90.3832	Open Water	Random
	5	29.75237	-90.3889	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.76028	-90.3899	Emergent Vegetation	Random
	9	29.74599	-90.392	Emergent Vegetation	Random
	10	29.75499	-90.3887	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83234	-90.2846	Open Water	Random
	4	29.84068	-90.2838	Open Water	Random
	5	29.83547	-90.2903	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83233	-90.2793	Emergent Vegetation	Random
	9	29.82878	-90.2889	Emergent Vegetation	Random
	10	29.83801	-90.2853	Emergent Vegetation	Random

Table 18. Year 3 Winter season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24166	-90.1704	Open Water	Random
	4	29.24476	-90.1655	Open Water	Random
	5	29.24147	-90.1741	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23955	-90.1663	Emergent Vegetation	Random
	9	29.23874	-90.1673	Emergent Vegetation	Random
	10	29.24869	-90.1671	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46504	-89.9096	Open Water	Random
	4	29.46119	-89.9194	Open Water	Random
	5	29.46752	-89.9124	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.4611	-89.9092	Emergent Vegetation	Random
	9	29.4682	-89.9082	Emergent Vegetation	Random
	10	29.47506	-89.9093	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40459	-89.6556	Open Water	Random
	4	29.41295	-89.6618	Open Water	Random
	5	29.40664	-89.6542	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.4077	-89.6574	Emergent Vegetation	Random
	9	29.41212	-89.6549	Emergent Vegetation	Random
	10	29.41164	-89.6616	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31609	-89.3966	Open Water	Random
	4	29.31063	-89.3926	Open Water	Random
	5	29.32032	-89.3967	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.30942	-89.3961	Emergent Vegetation	Random
	9	29.30955	-89.3927	Emergent Vegetation	Random
	10	29.31252	-89.3917	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53479	-90.0982	Open Water	Random
	4	29.53352	-90.1067	Open Water	Random
	5	29.53711	-90.0959	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.54065	-90.0987	Emergent Vegetation	Random
	9	29.54063	-90.1002	Emergent Vegetation	Random
	10	29.53747	-90.1053	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55692	-90.0164	Open Water	Random
	4	29.55197	-90.0074	Open Water	Random
	5	29.5522	-90.0099	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55709	-90.013	Emergent Vegetation	Random
	9	29.55714	-90.0149	Emergent Vegetation	Random
	10	29.55242	-90.0117	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.64217	-90.1316	Open Water	Random
	4	29.64971	-90.1306	Open Water	Random
	5	29.64812	-90.1313	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64103	-90.135	Emergent Vegetation	Random
	9	29.64713	-90.1341	Emergent Vegetation	Random
	10	29.64522	-90.1334	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.7732	-90.2978	Open Water	Random
	4	29.76922	-90.2943	Open Water	Random
	5	29.77984	-90.2904	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77179	-90.2926	Emergent Vegetation	Random
	9	29.77811	-90.2877	Emergent Vegetation	Random
	10	29.77395	-90.2964	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.7539	-90.3934	Open Water	Random
	4	29.75353	-90.3859	Open Water	Random
	5	29.75841	-90.3907	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75806	-90.3898	Emergent Vegetation	Random
	9	29.75157	-90.3927	Emergent Vegetation	Random
	10	29.75055	-90.3923	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83973	-90.2833	Open Water	Random
	4	29.83339	-90.281	Open Water	Random
	5	29.83424	-90.288	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83622	-90.285	Emergent Vegetation	Random
	9	29.82871	-90.2892	Emergent Vegetation	Random
	10	29.83203	-90.2835	Emergent Vegetation	Random

Table 19. Year 3 Spring season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.242	-90.1682	Open Water	Random
	4	29.24007	-90.1748	Open Water	Random
	5	29.23967	-90.1704	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.23595	-90.1712	Emergent Vegetation	Random
	9	29.24662	-90.1625	Emergent Vegetation	Random
	10	29.23924	-90.1761	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46899	-89.9112	Open Water	Random
	4	29.46403	-89.9202	Open Water	Random
	5	29.46736	-89.9082	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.46855	-89.9102	Emergent Vegetation	Random
	9	29.462	-89.9203	Emergent Vegetation	Random
	10	29.46051	-89.9075	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40803	-89.6522	Open Water	Random
	4	29.41639	-89.6625	Open Water	Random
	5	29.40652	-89.6609	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.41308	-89.6553	Emergent Vegetation	Random
	9	29.40463	-89.6603	Emergent Vegetation	Random
	10	29.41077	-89.6561	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.31615	-89.3978	Open Water	Random
	4	29.31815	-89.3948	Open Water	Random
	5	29.30784	-89.3965	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31378	-89.3962	Emergent Vegetation	Random
	9	29.30995	-89.3964	Emergent Vegetation	Random
	10	29.31026	-89.3925	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53721	-90.0953	Open Water	Random
	4	29.53317	-90.1019	Open Water	Random
	5	29.53408	-90.1081	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.53399	-90.1058	Emergent Vegetation	Random
	9	29.53767	-90.0958	Emergent Vegetation	Random
	10	29.53734	-90.1072	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.56082	-90.0076	Open Water	Random
	4	29.55912	-90.0158	Open Water	Random
	5	29.55527	-90.0147	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55793	-90.016	Emergent Vegetation	Random
	9	29.55275	-90.0104	Emergent Vegetation	Random
	10	29.55487	-90.0083	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.64757	-90.1299	Open Water	Random
	4	29.64089	-90.1315	Open Water	Random
	5	29.65279	-90.1348	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.6539	-90.141	Emergent Vegetation	Random
	9	29.64515	-90.1334	Emergent Vegetation	Random
	10	29.64908	-90.1365	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77746	-90.2918	Open Water	Random
	4	29.77991	-90.2903	Open Water	Random
	5	29.77154	-90.2968	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77799	-90.2911	Emergent Vegetation	Random
	9	29.77207	-90.2906	Emergent Vegetation	Random
	10	29.77025	-90.2926	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.752	-90.3906	Open Water	Random
	4	29.75437	-90.3947	Open Water	Random
	5	29.75236	-90.388	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.74545	-90.3922	Emergent Vegetation	Random
	9	29.75352	-90.3878	Emergent Vegetation	Random
	10	29.74958	-90.3916	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83578	-90.2887	Open Water	Random
	4	29.83328	-90.2793	Open Water	Random
	5	29.83292	-90.2852	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.83523	-90.2844	Emergent Vegetation	Random
	9	29.83257	-90.2828	Emergent Vegetation	Random
	10	29.84132	-90.2849	Emergent Vegetation	Random

Table 20. Year 3 Summer season sampling locations.

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
1	1	29.24788	-90.1672	Open Water	Fixed
	2	29.23803	-90.1677	Open Water	Fixed
	3	29.24004	-90.1726	Open Water	Random
	4	29.24001	-90.1694	Open Water	Random
	5	29.24969	-90.1683	Open Water	Random
	6	29.24399	-90.1633	Emergent Vegetation	Fixed
	7	29.24007	-90.1712	Emergent Vegetation	Fixed
	8	29.24274	-90.1644	Emergent Vegetation	Random
	9	29.23881	-90.167	Emergent Vegetation	Random
	10	29.23767	-90.173	Emergent Vegetation	Random
2	1	29.46193	-89.9095	Open Water	Fixed
	2	29.46937	-89.9091	Open Water	Fixed
	3	29.46811	-89.9129	Open Water	Random
	4	29.46582	-89.909	Open Water	Random
	5	29.46047	-89.9068	Open Water	Random
	6	29.46856	-89.9103	Emergent Vegetation	Fixed
	7	29.47527	-89.9088	Emergent Vegetation	Fixed
	8	29.4756	-89.9093	Emergent Vegetation	Random
	9	29.4684	-89.9108	Emergent Vegetation	Random
	10	29.46191	-89.9206	Emergent Vegetation	Random
3	1	29.40933	-89.6499	Open Water	Fixed
	2	29.40641	-89.6597	Open Water	Fixed
	3	29.40303	-89.6521	Open Water	Random
	4	29.41155	-89.6624	Open Water	Random
	5	29.40924	-89.6511	Open Water	Random
	6	29.40778	-89.6577	Emergent Vegetation	Fixed
	7	29.41655	-89.661	Emergent Vegetation	Fixed
	8	29.40699	-89.6585	Emergent Vegetation	Random
	9	29.41229	-89.6551	Emergent Vegetation	Random
	10	29.41073	-89.6615	Emergent Vegetation	Random
4	1	29.31748	-89.396	Open Water	Fixed
	2	29.30861	-89.3982	Open Water	Fixed
	3	29.32333	-89.3993	Open Water	Random
	4	29.30849	-89.3968	Open Water	Random
	5	29.31957	-89.3956	Open Water	Random
	6	29.30888	-89.3945	Emergent Vegetation	Fixed
	7	29.31365	-89.3912	Emergent Vegetation	Fixed
	8	29.31353	-89.3973	Emergent Vegetation	Random
	9	29.30754	-89.394	Emergent Vegetation	Random
	10	29.31602	-89.3948	Emergent Vegetation	Random
5	1	29.53511	-90.1068	Open Water	Fixed
	2	29.53567	-90.0959	Open Water	Fixed

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	3	29.53612	-90.0963	Open Water	Random
	4	29.53856	-90.1064	Open Water	Random
	5	29.53866	-90.0992	Open Water	Random
	6	29.53786	-90.1067	Emergent Vegetation	Fixed
	7	29.53663	-90.0974	Emergent Vegetation	Fixed
	8	29.5387	-90.1024	Emergent Vegetation	Random
	9	29.53425	-90.1033	Emergent Vegetation	Random
	10	29.53598	-90.1067	Emergent Vegetation	Random
6	1	29.55255	-90.0082	Open Water	Fixed
	2	29.55917	-90.0166	Open Water	Fixed
	3	29.55872	-90.0083	Open Water	Random
	4	29.5616	-90.0151	Open Water	Random
	5	29.55622	-90.0141	Open Water	Random
	6	29.55384	-90.0137	Emergent Vegetation	Fixed
	7	29.55939	-90.0141	Emergent Vegetation	Fixed
	8	29.55704	-90.011	Emergent Vegetation	Random
	9	29.55716	-90.0144	Emergent Vegetation	Random
	10	29.55894	-90.0093	Emergent Vegetation	Random
7	1	29.65582	-90.1399	Open Water	Fixed
	2	29.64279	-90.1362	Open Water	Fixed
	3	29.6472	-90.1318	Open Water	Random
	4	29.64175	-90.1322	Open Water	Random
	5	29.65298	-90.1354	Open Water	Random
	6	29.64	-90.1323	Emergent Vegetation	Fixed
	7	29.65249	-90.1375	Emergent Vegetation	Fixed
	8	29.64263	-90.1331	Emergent Vegetation	Random
	9	29.651	-90.1341	Emergent Vegetation	Random
	10	29.65121	-90.1366	Emergent Vegetation	Random
8	1	29.76788	-90.2836	Open Water	Fixed
	2	29.77898	-90.292	Open Water	Fixed
	3	29.77853	-90.2919	Open Water	Random
	4	29.7726	-90.2897	Open Water	Random
	5	29.7674	-90.2946	Open Water	Random
	6	29.77643	-90.2893	Emergent Vegetation	Fixed
	7	29.77827	-90.2859	Emergent Vegetation	Fixed
	8	29.77213	-90.291	Emergent Vegetation	Random
	9	29.7701	-90.2933	Emergent Vegetation	Random
	10	29.77807	-90.2928	Emergent Vegetation	Random
9	1	29.75112	-90.3838	Open Water	Fixed
	2	29.75683	-90.3905	Open Water	Fixed
	3	29.75271	-90.3906	Open Water	Random
	4	29.7575	-90.3907	Open Water	Random
	5	29.75581	-90.3867	Open Water	Random

Station Number	Site	Latitude	Longitude	Habitat	Fixed or Random
	6	29.75709	-90.3923	Emergent Vegetation	Fixed
	7	29.74502	-90.3894	Emergent Vegetation	Fixed
	8	29.75189	-90.3879	Emergent Vegetation	Random
	9	29.74861	-90.3915	Emergent Vegetation	Random
	10	29.75741	-90.3897	Emergent Vegetation	Random
10	1	29.82951	-90.2938	Open Water	Fixed
	2	29.8354	-90.2854	Open Water	Fixed
	3	29.83746	-90.2888	Open Water	Random
	4	29.83175	-90.2845	Open Water	Random
	5	29.82955	-90.2879	Open Water	Random
	6	29.83099	-90.2831	Emergent Vegetation	Fixed
	7	29.83922	-90.2875	Emergent Vegetation	Fixed
	8	29.832	-90.2823	Emergent Vegetation	Random
	9	29.82921	-90.2875	Emergent Vegetation	Random
	10	29.83776	-90.2853	Emergent Vegetation	Random

Sampling Design by Key LTL Group:

- **Benthos (microphytobenthos, macroinfauna):** Due to the relatively slow turn-over rates and relatively immobile nature of sediment-associated organisms (Howes et al., 2003; USEPA, 2016), macroinfauna and microphytobenthos sampling is to occur seasonally (fall, winter, spring, summer) at all sites (OW and EV) and each station. Sampling and analytical methodologies are explained in greater detail in Appendices C.3 and C.5. Sampling of water quality (physical characteristics) and sediment characteristics will be performed concurrently with macroinfauna sampling (methods outlined in Appendix C.1). See the schematic provided in Figure 2. Existing estuarine monitoring programs including those occurring in the Chesapeake Bay sample benthos at a frequency of four to six times per year (Llansó & Zaveta, 2017); budgetary constraints are frequently cited as a significant factor in determining the frequency and extent of benthic data collection efforts.
- **Water column (phytoplankton, zooplankton):** More rapid turn-over rates of phytoplankton and zooplankton communities in the water column necessitate more frequent sampling compared to the benthos. This field plan includes bi-weekly sampling of standing stocks (e.g., abundance, density, biomass), community composition/diversity, and remote sensing validation at one OW site across all stations (methodology explained in greater detail in Appendices C.2 and C.4). Sampling of water quality (physical characteristics and nutrients and suspended solids) will be performed bi-weekly following methods outlined in Appendix C.1. See the schematic provided in Figure 3**.**
- **Stable isotopes**: Collection of samples for stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS) will be performed seasonally (concurrently with sampling of macroinfauna and microphytobenthos) or biweekly (concurrently with sampling of phytoplankton and

zooplankton). Sampling will occur across all LTL monitoring stations. Sampling and analytical methodologies are explained in greater detail in Appendix C.6.

Appendix C. Sampling Methods

This section provides an overview of the field methods, sample preparation, and necessary processing to obtain relevant biotic and abiotic data for Task 3 of this MAM activity. Field data collection will be conducted using four coordinated field campaigns: 1) macroinfauna, 2) microphytobenthos, and 3) water column (phytoplankton and zooplankton), 4) stable isotope samples not collected by the previous three efforts. The following descriptions provide an initial description of the methods and protocols to be used; these protocols will be further detailed during Task 1 implementation.

C.1. Environmental Variables

Water Quality

Physical Characteristics: Water column physical characteristics should be measured during all sample collection events including seasonal data collection for macroinfauna and microphytobenthos, as well as for bi-weekly data collection for phytoplankton and zooplankton. Water column physical characteristics should be examined before any biological samples are collected to prevent disturbance of ambient conditions. Data should be collected in the following order: 1) A Secchi disk depth measurement should be taken; 2) a photosynthetic active radiation (PAR) sensor should be lowered carefully to the bottom; and 3) a multiparameter sonde (e.g., YSI EXO) that measures depth, temperature, salinity, pH, turbidity, and dissolved oxygen should be lowered carefully to the bottom. Multiparameter water quality data will be collected at all OW and EV sites concurrent to each sampling event. Secchi disk and PAR measurements will be collected at OW sites concurrent to each sampling event; at EV sites, these measurements will be dependent on water depth, but effort should be made to evaluate these parameters of any standing water present.

Nutrients and Suspended Solids: Nutrients and suspended solids should be examined bi-weekly alongside phytoplankton and zooplankton samples. After physical characteristics of the water column are assessed in steps 1–3 above, one whole water sample should be collected at the same site as the phytoplankton/zooplankton and microphytobenthos samples are collected. Samples should be collected at the water surface (0.5 m depth) using a bucket to fill up a 2-L Nalgene bottle. Samples will be transferred to labeled bottles in the field and kept on ice. The samples can be sent to contracted laboratories for the following analyses: total suspended solids (TSS – organic and inorganic), total volatile solids (TVS, indicator of organic solids), nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (TN), total phosphorus (TP), phosphate (PO_4^{3-}), and silicate (SiO_2).

Sediment Characteristics

Physical Characteristics: Sediment samples will be collected from all LTL monitoring stations coinciding with seasonal sampling of macroinfauna. Sediment samples are necessary to characterize the local geophysical characteristics (bulk density; % organic carbon; grain size [% sand, % silt + clay]). One sample should be collected from each site across all stations. Samples should be collected using the same coring methods described for sampling macroinfauna. The top 5 cm of each sampled core should be sectioned

to retain the top 0–2 cm and bottom 2–5 cm depth fractions. These samples should then be placed in Whirlpaks and kept on ice until stored in a -20 °C freezer in the laboratory.

Habitat Characteristics

Habitat Characteristics: A qualitative assessment of habitat characteristics and type (e.g., EV, SAV, bare bottom, disarticulated oyster shell) will be conducted at all LTL monitoring stations coinciding with seasonal sampling of macroinfauna and microphytobenthos. The purpose of this assessment is to broadly characterize observed vegetation structure at each station for use alongside existing CRMS vegetation information.

Distance from edge: After all field sampling events, confirmed site coordinates will be imported into GIS to measure the shortest distance between each sampled site (OW and EV) and emergent vegetation edge.

C.2. Phytoplankton

Phytoplankton can be assessed by a multitude of methods ranging from highly labor-intensive (e.g., microscopy) to cost-effective emerging technologies (e.g., remote sensing). Due to the utility of emerging technologies and the programmatic goal of evaluating long-term restoration impacts to the Barataria Basin, this MAM data collection plan pairs field-based data collection with remote sensing technologies to validate use of emerging technologies which may offer a lower-cost approach for future phytoplankton monitoring efforts.

Field-Based Data Collection

Field Collection: Field collection of the phytoplankton LTL group will occur bi-weekly at all LTL monitoring stations (n = 10). Sampling of phytoplankton will occur at site 1 (a fixed OW site) for each station (see tables in Appendix B for site location information). One 1-L Nalgene bottle of water collected from 0.5 m depth at each station will be used to examine biomass (total Chl *a*), community composition (accessory pigments), and cyanobacteria (Phycocyanin:Chl *a*). Due to the highly patchy nature of phytoplankton in the Barataria Basin, additional spatial replication is not necessary (B. Stauffer and S. Bargu, pers. comm.). Upon collection, each whole water sample should be kept on ice and away from light until transported to the laboratory for filtration (ideally within 12 hours) and other analyses.

Sample Processing: When processing the 1-L sample for each OW site for analysis, 0.5 L should be reserved for biomass and composition (via high-performance liquid chromatography [HPLC]) and 0.5 L should be reserved for phycocyanin (PC) pigment analyses.

- **Biomass (Chl *a*) + Composition (accessory pigments):** Laboratory sample processing involves filtering a known volume (0.2 to 0.5 L) though a glass fiber filter (Whatman GF/F, porosity of 0.7 µm) via vacuum filtration (Van Heukelem et al., 1992; Wright et al., 1991). Filters are then put into disposable polypropylene microcentrifuge tubes (2-ml) and immediately frozen (-80 ℃). For long-term filter storage (6–12 months), samples should remain frozen at -80 ℃.
- **Cyanobacteria detection (Phycocyanin:Chl *a*):** Phycocyanin (PC) raw fluorescence units (RFUs) can be measured using the portable Turner CyanoFluor handheld fluorometer. The RFU values can be converted to PC concentrations (µg/L) using a standard curve created with laboratory-grade PC pigments (Sigma-Aldrich #P217210MG) dissolved in a phosphate buffer (see Bargu et al., *In Review*). Phycocyanin is a unique pigment to cyanobacteria and thus, a PC:Chl *a* ratio or index can help estimate what portion of the total phytoplankton population may be comprised of PC-containing cyanobacteria. Laboratory processing for PC:Chl *a* should occur immediately as samples arrive. To process a sample, a cuvette is filled with unfiltered sample water and the PC: Chl *a* ratio is recorded. A second reading is conducted on filtered water (dissolved organic matter [DOM] removed) so that a DOM correction can be applied (Turner Designs, 2022).

Analysis:

- **Biomass (Chl *a*) + Community Composition (accessory pigments):** Frozen sample filters will be analyzed by HPLC to provide data on total biomass (units: Chl *a* µg/L) and biomass of approximately 18 other major pigments (units: e.g., Fucoxanthin [diatoms], µg/L) used as indicators of phytoplankton community composition. Biomass values of pigments can be used to calculate the relative contribution of each phytoplankton group (units: %) to total Chl *a* with CHEMTAX software (Goela et al., 2014; Mackey et al., 1996; Seoane et al., 2011). **Note: community composition from HPLC pigment analysis will be validated against species level IDs collected via FlowCam analysis of microzooplankton (see section C.4. Zooplankton).**

Validation of Emerging Technologies: Remote Sensing

The field-based phytoplankton biomass and community composition data described above will be used to support remote sensing algorithm development and validate remote sensing data products for evaluating phytoplankton communities in the Barataria Basin. Pursuing validation of emerging technologies will enable lower-cost monitoring of the phytoplankton LTL group for future long-term monitoring efforts and therefore is a valuable component to this MAM activity.

Field Collection: To support the development and validation of remote sensing algorithms, other bio-optical in-situ measurements need to be collected concurrently with the above metrics (i.e., phytoplankton biomass and composition).

- **Phytoplankton Absorption Coefficient & Pigment Absorption Coefficient:** At each LTL monitoring station (n = 10), one additional surface water sample (2-L Nalgene bottle) should be collected to measure the phytoplankton absorption coefficient (a_{phy}; m^{-1}) and the pigment absorption coefficient (a_{pig}; m^{-1}). The surface water sample (2-L) should be stored on ice immediately and filtered within the same day (ideally within 12 hours).
- ***In-situ* Above-Water Reflectance (L_w, L_s, L_p):** Three above-water measurements of water-surface radiance (L_w), sky radiance (L_s), and plate radiance (L_p) should be collected using a GER 1500 512iHR spectroradiometer in the 350–1050 nm spectral range to validate the atmosphere-corrected remote sensing reflectance from satellite data ($R_{rs\_satellite}$). These measurements must be collected at each LTL monitoring station under clear-sky conditions. The spectroradiometer should be set to provide an average of four internal scans by considering the variability in reflectance and water conditions. Consequently, the final spectrum is an average of 12 spectra (3 samples with 4 internal scans per sample) at each station.

Field Sample Processing: Upon returning to the laboratory, the Phytoplankton Absorption Coefficient & Pigment Absorption Coefficient water samples (2-L bottles) should be filtered through a 0.7-µm Whatman GF/F filter, and the resulting filter pads (two filters per 2-L water sample) should be immediately frozen in liquid nitrogen or stored at -80 ℃ during sample processing. One filter pad will be used for Phytoplankton Absorption Coefficient analysis and the other for Pigment Absorption Coefficient analysis.

- **Phytoplankton Absorption Coefficient (a_{phy}):** The quantitative filter technique (QFT) is used to measure absorbance of particles (A_{total}) and non-algal particles (A_{NAP}) inside an integrating sphere at 1 nm intervals from 300 to 800 nm. The absorption coefficients of NAP (a_{NAP}), particles (a_{total}) and phytoplankton (a_{phy}) were calculated using the following equations:

$$a_{total} = 2.303 \times A_{total}$$

$$a_{NAP} = 2.303 \times A_{NAP}$$

$$a_{phy} = a_{total} - a_{NAP}$$

- **Pigment Absorption Coefficient (a_{pig}; in-vitro)**: Filter pads can remain stored in liquid nitrogen or a -80 ℃ ultracold freezer until transferred into 30-ml vials containing 10 ml cold 96% ethanol. The vials should be spun evenly in a centrifuge to ensure full exposure of the filter pad to the ethanol and then kept in the refrigerator (in the dark) overnight. The pigment solutions at room temperature are then poured off from vials into 1-cm cuvettes and measured on a PerkinElmer Lambda-850 UV–VIS spectrophotometer to obtain pigment absorption coefficients a_{pig}.

- ***In-situ* Above-Water Reflectance:** The measurements of water-surface radiance (L_w), sky radiance (L_s), and plate radiance (L_p) that were collected from each station will be converted to downwelling irradiance (E_d) and in-situ remote-sensing reflectance (R_{rs_insitu}) as follows:

$$E_d = \pi \times \frac{L_p}{\rho_p}$$

$$R_{rs\_insitu} = \frac{L_w - (\rho \times L_s)}{E_d}$$

  Skylight and residual corrections will be also applied to R_{rs_insitu}. Thereafter, the post-processed R_{rs_insitu} will be used to validate atmosphere-corrected $R_{rs\_satellite}$.

Satellite Data Processing: Satellite imagery will be analyzed seasonally (4x per year) using the semi-analytical inversion algorithm developed for Sentinel 3-OLCI (Liu et al., 2019, 2021). This algorithm will focus on all phytoplankton pigments (carotenoids, chlorophylls, and phycocyanin), and thus is different from the products produced by the NOAA HABs Branch efforts conducted in Lake Pontchartrain (https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/hab-forecasts/). The NOAA HABs Branch focuses only on Chl *a* and a cyanobacteria-based HAB index, whereas the data collection included in this MAM activity for the Barataria Basin will provide additional information about HABs and the phytoplankton community based on algorithms that can estimate all phytoplankton pigments. However, this MAM activity can coordinate with the NOAA HABs Branch to produce the same Composited Cyanobacteria Index for the Barataria Basin.

Imagery from Sentinel 3-OLCI (300 m), Landsat 8-OLI (30 m), and Sentinel 2-MSI (10 m) will be geometrically corrected and cropped/mosaiced to the Barataria Basin study area. Atmosphere correction will be further conducted using C2RCC package for Sentinel 3A/B-OLCI and ACOLITE package for Landsat 8-OLI and Sentinel 2A/B-MSI.

- **Sentinel 3A/B-OLCI:** The Ocean and Land Color Instrument (OLCI) on board the European Space Agency (ESA) Sentinel-3A/B satellite produces large swath width (~1,270 km) images of the entire coastal Louisiana area approximately every 3 days. The OLCI images are provided at 300 m spatial resolution which enables simultaneous monitoring of biogeochemical indicators across estuaries and coastal waters. Level 1 Sentinel 3-OLCI image at full resolution mode will be downloaded from ESA website (https://coda.eumetsat.int/#/home). Level-1 OLCI data will be preprocessed through Sentinel-3 Toolbox in Sentinel Application Platform (SNAP) and further corrected via Case 2 Regional Coast Color (C2RCC).
- **Sentinel 2-MSI and Landsat 8-OLI**: The Multi Spectral Instrument (MSI) on-board the Sentinel 2 satellite (Sentinel 2A/B-MSI) and the Operational Land Imager (OLI) on-board Landsat 8 can be obtained from Copernicus Open Access Hub (https://scihub.copernicus.eu/dhus/#/home) and USGS Earth Explorer (https://earthexplorer.usgs.gov/), respectively. ACOLITE, which is an atmospheric correction processor for coastal and inland waters developed by the Management Unit of the Mathematical Model of the North Sea (MUMM), will be used to conduct atmosphere correction.

Satellite Data Desktop Analysis: Ideally, 2–3 high quality cloud-free images are typically available per month. Imagery selected for analysis should coincide as closely as possible to field collection events (± 3 days) while taking into consideration avoidance of major atmospheric disturbance events (e.g., hurricanes). Paired atmosphere-corrected satellite image (R_{rs}) and field measurements (a_{phy} and phytoplankton biomass and composition via HPLC; see methods above) will be used to develop satellite algorithms for Basin-wide phytoplankton biomass (Chl *a*; µg/L) as well as for major phytoplankton groups (e.g., diatoms, dinoflagellates, chlorophytes, cyanobacteria, haptophytes) and phytoplankton size fractions (picoplankton, nanoplankton, and microplankton; Liu et al., 2021).

C.3. Microphytobenthos

Field Collection: It is common to take three to five sediment samples per station to account for the patchiness and variability of biomass (Chl *a*) and composition (accessory pigments) of microphytobenthos (Baustian et al., 2011; Miller et al., 1996) in various habitats. For this data collection effort, one surface sediment sample will be collected at each of the five EV sites and each of the five OW sites per LTL monitoring station on a seasonal basis. Quarterly sampling events will need to be coordinated with water column (phytoplankton and zooplankton) sampling in order to efficiently use water quality nutrient sampling for both sampling efforts; due to the well mixed nature of the waters being sampled, it is assumed that OW nutrient samples will be utilized during later analysis of both OW and EV microphytobenthos samples. Due to the cost associated with microphytobenthos, sampling this LTL component will be adaptively managed throughout the project.

- **EV (n = 5 sites [Year 1], 3 sites [Year 2 & 3]):** An acrylic hand push corer (7.6 cm diameter, 10 cm height) or a 50 cm^3 syringe with the tip removed can be used to collect the samples. The top 0.5 cm of soil from each sample is then removed with a spatula or spoon.
- **OW (n = 5 sites [Year 1], 3 sites [Year 2 & 3]):** The same acrylic cores (7.6 cm diameter) can be used with a piston corer (Fisher et al., 1992) to collect subtidal sediment samples. Overlying water should be carefully siphoned off with disposable plastic pipettes before the top 0.5 cm of sediment is removed with a spatula or spoon.

Sample Processing Steps in the Field:
The following steps should be followed sequentially to separate material for different analyses.

- **Step 1: Biomass & Community Composition (HPLC):** Each individual surface section (top 0.5 cm collected under "Field Collection" described above) should be placed into a Petri dish and homogenized (manually stirred). The homogenized sediment is then used to fill two cryovials (1.8 ml each) to obtain enough sediment to constitute one sample. In other words, one sample consists of two cryovials. These cryovials are subsequently stored in a liquid nitrogen Dewar in the field.
- **Step 2: Cell Density & Composition (microscopy):** The rest of the remaining homogenized sediment slurry sample (~17 ml) from Step 1 above should be carefully removed from the Petri dish and placed into a labelled 125 ml Nalgene plastic sampling bottle containing 1 ml of 50% glutaraldehyde. A squirt bottle filled with filtered ambient seawater can be used to add water to the 100 ml mark line and make a diluted glutaraldehyde solution of 0.5%. The 125 ml Nalgene plastic sampling bottles should then be placed on ice and taken to the laboratory where they can remain refrigerated until used for microscopic analysis (Baustian et al., 2011; Price & Rabalais, 2020).

Sample Processing Steps in the Laboratory:

- **Biomass & Community Composition (HPLC):** Cryovial samples should be kept in the -80 °C freezer (or liquid nitrogen dewars) until shipment to contract lab for sediment pigment analysis (Price et al., 2019).
- **Cell Density & Composition (microscopy):** Preserved samples in Nalgene bottles should be kept cold in a refrigerator to prevent decay until microscopic analysis.

Analysis:

- **Biomass & Community Composition (HPLC):** The HPLC will be used to determine the biomass (total Chl *a*) and community composition (accessory pigments) of microphytobenthos. Chl *a* is a common proxy for the total biomass, and accessory pigments (e.g., carotenoids, xanthophylls, and chlorophylls) can be used to identify major taxa groups. For example, fucoxanthin is a primary indicator pigment for diatoms but prymnesiophytes, raphidophytes, and some dinoflagellates can also contain this pigment (Baustian et al., 2011; Jeffrey et al., 1997). Various laboratories offer analytical services where the sediment samples (and water samples for phytoplankton) can be sent and the laboratory will extract the pigments, analyze, and produce available data for a fee per sample. For each sample, the following types of data could be expected: total Chl *a* (µg Chl *a* g dry sed^{-1}) and the various accessory pigments (~ 18), including as an example fucoxanthin (µg fucoxanthin g dry sed^{-1}). Examples of existing HPLC data in Barataria Basin (Fleeger & Riggio, 2016; Price et al., 2019) could be used to compare future results.
- **Cell Density & Composition (microscopy):** For microscopy analysis, 1–3 ml of preserved suspended microphytobenthos material from the 125 ml Nalgene sampling bottle should be used. Microphytobenthos is extracted following the method of Baustian et al. (2011) modified to extract only large autotrophs (8–63 µm). Once extracted, microscopic counts are made using an Olympus epifluorescence microscope (EFM) with blue and green excitation light and transmitted light. Samples should first be examined at 200x until 100 cells or 100 fields are reached. The magnification should then be changed to 100x and half of the same filter is scanned again for organisms not seen at the 200x count (this step allows for identification of large rare species). Diatoms should be classified into seven categories: 1) pennate diatoms < 90 µm, 2) pennate diatoms > 90 µm, 3) sliding/stacked pennate diatoms, 4) *Melosira* spp., 5) *Skeletonema* spp., 6) *Odontella* spp., 7) *Coscinodiscus* spp.; Price & Rabalais, 2020). Cyanobacteria should be enumerated either as chain-forming *Anabaena-type* (now known as *Dolichospermum*) or colonial *Merismopedia*. After microscopy is completed, the remaining sediment pellet that the microphytobenthos were extracted from should be dried and weighed. Thus, microphytobenthos cell density can be expressed as: cells g dry sed^{-1}. Microphytobenthos potential biomass (from cell density values) and composition has been most recently studied in Barataria Basin by Price and Rabalais (2020). Samples analyzed to confirm community

composition by microscopy analysis will be selected based on HPLC results, resulting in fewer analyzed samples for this labor-intensive technique.

C.4. Zooplankton

Field Collection: Field collection of the zooplankton LTL group will occur concurrently with phytoplankton sampling on a bi-weekly basis at one OW site per LTL monitoring station. Sampling for zooplankton should occur at the same site as the phytoplankton whole water sampling. A diaphragm pump (10 L/min flow rate or higher) will be used to sample water at a depth of 0.5m for both micro- and mesozooplankton. Due to difficulties sampling water less than 0.5 m depth with this method, sampling of zooplankton is recommended only in OW habitat (M. Sutor, pers. comm.).

Prior field sampling efforts with the diaphragm pump gear type indicate it causes less physical damage to organisms than other tow net gear types (M. Sutor, pers comm). Samples for microzooplankton will not be passed through mesh prior to collection to prevent damage to delicate structures.

- **Microzooplankton (~10 µm–72 µm body size; inclusive of everything from phytoplankton to ciliates):** With the pump activated, one 250 ml whole water sample will be collected from the pump intake (non-filtered) to obtain a sample from the appropriate 0.5 m depth. Microzooplankton samples should be preserved in 5% acid Lugols.
- **Mesozooplankton (> 72 µm, ~0.25 mm–2 cm body size; primarily copepods, nauplii, and ctenophores):** After the whole water sample has been collected from the pump discharge, sampling will start for mesozooplankton. Water will be pumped from the 0.5 m depth and passed through a 72-µm mesh collection net for 10 minutes (depending upon pump rate), processing a total volume of 1 m^3 for one sample at minimum. Mesozooplankton samples should be preserved in 10% buffered formalin.
- Should water depths exceed 5 m at any station, it is recommended that an additional sample be collected mid-water column to account for depth stratification of both microzooplankton and mesozooplankton.

Analysis: Samples of microzooplankton and mesozooplankton will be run through a FlowCAM to create a digital archive of plankton images. Due to the large volume of data generated, digital records will be stored on external hard drives until transferred to a central publicly-accessible data repository (e.g., SeaBASS https://seabass.gsfc.nasa.gov/ or EcoTaxa https://ecotaxa.obs-vlfr.fr/). Summary data and metadata will be submitted to DIVER. Subsamples will be processed until enough of the sample has been analyzed to ideally target 100 individuals of each target taxonomic group or the whole sample. Depending upon the composition and size spectra of the mesozooplankton samples, the sample may be analyzed with a Zooscan waterproof scanning system instead of the FlowCAM to create a digital archive of the sample. The Visual Spreadsheet software (FlowCAM) or Plankton Identifier software (Zooscan) will be used to analyze each sample for density (units: individuals/m^3), biomass (units: g C/m^3), and taxonomic composition (units: family, genus if possible). Note: FlowCam analysis of the microzooplankton fraction will also provide community composition for the phytoplankton LTL group. Methodology may require tweaking due to the abundance of detritus in samples.

C.5. Macroinfauna

Field Collection: Field collection of the macroinfauna LTL group will occur at all LTL monitoring stations (n = 10 stations) at all OW and EV sites on a seasonal basis. Due to the high spatial variability in benthic infauna communities observed in the Barataria Basin, macroinfauna samples will be collected in triplicate from each habitat-type (i.e., OW or EV) site.

- **Community Composition, Density, and Biomass:** Three sediment core samples will be collected from each OW and EV site (total 30 samples per station per effort) for macroinfauna analysis. Sampling methods in both habitat types will involve sediment cores (5 cm diameter, 5 cm sediment depth). These three cores will be pooled to obtain one site-level community composition, density, and biomass per the sum of the area sampled by them (3*19.625 cm^2). Hand-held push cores are appropriate for EV habitat, whereas a long-handled piston core is necessary for OW sampling. Differences between hand-held push cores and long-handled push core gear types does not impact the resulting data as the cored volume remains constant (C. Glaspie, pers. comm.). Core samples should be gently sieved in the field using a 0.5-mm (500 µm) sieve. All material retained on the sieve should then be transferred to a plastic bag or jar and placed in a bucket with a prepared solution of 10% buffered formalin-rose Bengal solution for at least 48 hours to preserve the specimens, after which samples may be stored in 70% ethanol (Eleftheriou, 2013).

Sample Processing & Analysis:

- **Community Composition, Density, and Biomass:** Microscopy will be employed to extract or "pick" all preserved macroinfauna (stained pink) from the non-living material. Picked organisms will then be transferred to new vials of 70% ethanol. Once picked, organisms are to be identified and counted under the microscope to family level (genus and species, if possible, for more common taxa). Calculating biomass will include wet biomass of each taxonomic group; taken with counts of individuals, this can be used to derive an average individual organism wet weight. AFDW measurements can then be taken and compared to AFDW values derived from literature values (e.g., Philomena, 1983) to refine AFDW estimates. This approach retains samples for archival purposes rather than destroying them to obtain AFDW.

C.6. Stable Isotopes

As stated above, stable isotope data collection should occur on a seasonal basis. Due to spatial and temporal variability in stable isotope values obtained from coastal ecosystems (Nelson et al. 2015), a spatiotemporally balanced approach to stable isotope data collection was advised by subject matter experts during development of this MAIP. For some LTL groups (i.e., phytoplankton and zooplankton), this represents an increase in spatial sampling (number of sites per station per sampling event) but decreased temporal sampling (i.e., seasonal rather than biweekly). For other LTL groups (I.e., microphytobenthos and macroinfauna), the stable isotope sampling design aligns with the same temporal frequency but represents reduced spatial replication (e.g., fewer sites per station). For each protocol listed below, effort should be taken to prevent cross-contamination of samples by adequately preparing materials necessary for sample processing and storage: 1) all filters should be combusted (ashed) at 450 °C for 4 hours to remove any organic matter; 2) all sieves and filters should be weighed using an analytical balance before and after filtration; and 3) all combusted and pre-weighed filters should be stored individually to prevent cross-contamination. Consideration of these details requires specific analytical expertise and potentially additional laboratory benchwork to understand and troubleshoot difficult samples. It is recommended that stable isotope analyses be conducted by laboratories familiar with Louisiana estuarine waters to ensure accurate results.

Phytoplankton & Zooplankton Stable Isotopes

Field Collection: At least 1 L of whole water will be sampled from the water surface (0.5 m depth) at three OW sites per LTL monitoring station. Each 1L sample will be transferred to sample storage bottles in the field and kept cold on ice and away from light. Once returned to the lab, all samples must be filtered within 12 hours of collection.

Sample Processing: The following steps are to be completed sequentially in the order they are written.

1. **Extract Mesozooplankton:** Approximately 0.2 to 0.5 L of each water sample is to be filtered through a 0.25-mm sieve to remove mesozooplankton (primarily copepods and larger material) which will be retained for analysis (care will be used to ensure cross-contamination across samples does not occur). Material retained on the sieve should be weighed, dried at low temperature (60 °C), or freeze-dried, and stored in a desiccator prior to analysis. Retain the filtered water for the next step. Note, pre-filtering using a large mesh size such as 0.25-mm, may not guarantee there will be enough “zooplankton” organic matter for an effective stable isotope analysis. To ensure sufficient sample, more water may need to be filtered until the sieve is clogged and/or there is a lot of visible organic matter retained. The exact quantity of water will depend on how much material is present in the water column and difficult to ascertain in advance (M. Polito, pers. comm.). Three samples of mesozooplankton for stable isotope analysis will be collected from each station.
2. **Extract Phytoplankton/POM:** Following filtration of mesozooplankton, filter ~250 mL of water through a combusted 47-mm Whatman glass fiber filter (GF/F; 0.7 µm mesh). Combusted filters

should be individually weighed prior to filtration to allow for an accurate assessment of the mass of POM collected. Note: based on prior sampling in the marine and brackish zones of Barataria Basin, a volume of ~250 mL is typically the maximum possible to filter through a GF/F before it clogs; however, if there is less sediment and suspended POM in the water column, filtering a larger volume is recommended. In all cases, the volume of water filtered through each GF/F should be recorded (M. Polito, pers. comm.). This process should result in 4 phytoplankton/POM filters per whole water sample. All filters should be dried at low temperature (60 °C) or freeze-dried, weighed, and stored in a desiccator prior to analysis.

Analysis: Prior to analysis, all dried samples should be homogenized, encapsulated, and weighed again. Analysis of samples for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS is highly dependent on the amount of organic matter in the suspended POM and how much was captured on each filter, which can be highly variable (M. Polito, pers. comm.). With multiple filters per whole water sample, this may provide sufficient material. Often, C and N can be measured from a single filter in a single run, and S can be measured from another filter in a single run, and the remaining two filters are retained as back-up (M. Polito, pers. comm.).

Microphytobenthos Stable Isotopes

Field Collection: Due to the greater spatial variability in microphytobenthos isotopic signatures compared to zooplankton/phytoplankton POM, samples of microphytobenthos will span multiple habitat types at each LTL monitoring station. One surface sediment sample will be collected from each of three OW sites and each of the three EV sites at each station (n = 6 samples per station per season). Sampling will be conducted using acrylic cores (7.6 cm diameter) operated with a piston corer (Fisher et al., 1992). Once collected, overlying water should be carefully siphoned off with disposable plastic pipettes before the top 0.5 cm of sediment is removed with a spatula or spoon. Collected sediments should be homogenized before being placed into 50-ml Falcon tubes. All samples should be kept cold on ice and frozen upon return to the laboratory.

Sample Processing: Microphytobenthos should be isolated from sediments using density gradient centrifugation in colloidal silica as outlined by Bui and Lee (2014). In brief, sediment cores should be exposed to white fluorescent light for 16 hours to mediate vertical migration of microphytobenthos to the surface. The sediment surface (< 0.5 cm) should then be scraped, suspended in seawater, and then sieved through a 6-µm sieve to remove large detritus and nematodes. Next, the filtrate (that contains a mixture of seawater and microphytobenthos) should be centrifuged at 4400 rpm for 5 min, after which the supernatant that contains only seawater should be poured off. The remaining sediment “pellet” at the bottom of the centrifuge tube should then be divided into 5-ml aliquots in individual centrifuge tubes. Next, 40 ml of 30% Ludox colloidal silica (Sigma) is mixed into each 5-ml aliquot centrifuge tubes; this sediment/Ludox mixture is then centrifuged again at 4400 rpm for 5 min. At this point, the microphytobenthos should be a distinct layer suspended in the Ludox, which can be confirmed by microscopic examination. This layer should be collected from the tube, washed with distilled water to remove Ludox, filtered onto a combusted and pre-weighed 47-mm Whatman glass fiber filter (GF/F; 0.7 µm mesh), and then dried at 60°C, homogenized, weighed, and encapsulated for stable isotope analysis.

Analysis: Samples will be analyzed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS. See recommendations under "Analysis" of Phytoplankton & Zooplankton stable isotope samples above.

Macroinfauna Stable Isotopes

Field Collection: Additional "ad hoc" sediment core sampling will be conducted at three OW and three EV sites at each LTL monitoring station to enumerate sufficient macroinfauna tissue for stable isotope analysis. One sediment suction sample will be collected from each of the three sites, however high variability of macroinfauna across space may require additional effort so that 2.5 to 3.0 mg dry mass can be obtained for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ analysis. The volume of sediment processed to obtain sufficient biomass for each sample should be noted. Animals collected from suction samples will then be separated into targeted macroinfauna phyla (annelids, molluscs, and arthropods). Once collected from the suction sampler, animals will be placed into 50 ml falcon tubes and preserved in a 5% buffered formalin-Rose Bengal solution following methodology outlined in Gálvan et al. (2008).

Sample Processing: Following Gálvan et al. (2008), organisms should be prepared for stable isotope analysis within 2 weeks after fixation. All infauna organisms should then be rinsed with deionized water to remove external sediment and then dried at 70 ℃ for 24 hours for isotope analysis or immediately freeze dried. Samples should then be homogenized, weighed, and encapsulated.

Analysis: Samples will be analyzed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS. See recommendations under "Analysis" of Phytoplankton & Zooplankton stable isotope samples above.

Vegetation Stable Isotopes

One vegetation sample of dominant C3, C4 plants, and SAV will be collected concurrently with sampling for macroinfauna at each of the three EV sites per LTL monitoring station. For each of the vegetation types (C3 plants, C4 plants, and SAV) encountered at an EV site, a one-quart size Ziploc bag (about 1/2 full) should be filled with leaves/stems from multiple individual plants of the same dominant species (n = 3 samples of each vegetation type's dominant species per station per season, so up to 9 bags of each vegetation type per LTL monitoring station per season). Each sample should be oven dried (~60°C for 48 hours), ground, then homogenized and placed into separate vials.

Epiphytes should also be sampled at all three EV sites per station per season. At each EV site, epiphyte material should be separated from any attached plant stem and collected in one 15 ml falcon tube (at least ½ full, vegetated firmly packed) instead of a Ziploc bag. One falcon tube sample should be collected from each of the same three EV sites as the C3, C4, and SAV stable isotope samples (n = 3 samples of epiphytes per station per season). All samples should be stored frozen. Samples should be processed by defrosting the vegetation material, placing it in a water dish under a dissecting microscope to remove any remaining detritus, amphipods, and/or other plant material, freeze-dried, homogenized, and placed into separate vials for each sample. Samples will be analyzed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS. See recommendations provided under "Analysis" of Phytoplankton & Zooplankton stable isotope samples above.

Sediment Stable Isotopes

Surface sediment samples will be collected to characterize the $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS signatures across stations. Following stable isotope collection for microphytobenthos, one sample should be collected from three OW sites and three EV sites per station (n = 6 samples per station per season). Samples should be collected using the same coring methods described for sampling macroinfauna abundance. The top 0–2 cm of each core should be sliced off and transferred to a Falcon tube and kept cold on ice until frozen at the laboratory prior to further processing. See recommendations under "Analysis" of Phytoplankton & Zooplankton stable isotope samples above. Analysis will involve Isotope Ratio Mass Spectrometry (IRMS) and data post-processing (Marshall et al., 2021).

C.7. Data Collection Summary

The anticipated number of data points and the grand total of samples collected under Task 2 (field data collection effort) are summarized in Table 21 - Table 24. Where estimates of sample size requirements were available from power analyses (Appendix A), the estimates suggest that the sample sizes listed below are adequate to identify two-way interactions between salinity zone x season or salinity zone x year, and in some instances indicate three-way interactions between these main effects would be observable. The final sampling sizes were determined by balancing the power analysis indications of samples sizes required (Appendix A), the sampling design (Appendix B), and budgetary constraints. Adaptive management will be applied throughout the implementation of the LTL MAIP, including updated power analyses and consideration of potential further reductions in sample size that may allow for greater fiscal conservativism.

Table 21. Summary of data collection for water column LTL groups (phytoplankton and zooplankton) for Task 2. Anticipated data types and associated number of data points are summarized by effort (bi-weekly), year, and the three-year total. The total number of samples is also provided as multiple data types can be collected simultaneously from the same sample.

Key LTL Group	Metric	Total Data Points per Effort (bi-weekly)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
Phytoplankton	Biomass (Chl *a* µg/L) – *assessed by HPLC*	10 (OW) bi-weekly	260	780	780 samples (1 sample analyzed for both metrics via HPLC)
	Community composition (µg/L of major pigments; relative % abundance of major pigments) – *assessed by HPLC*	10 (OW) bi-weekly	260	780	
	Phycocyanin:Chl *a*	10 (OW) bi-weekly	260	780	780 samples
	Phytoplankton absorption coefficient (a_{phy})	10 (OW) bi-weekly	260	780	780 samples (1 sample analyzed for all metrics)
	Phytoplankton pigment absorption coefficient (a_{pig})	10 (OW) bi-weekly	260	780	

Key LTL Group	Metric	Total Data Points per Effort (bi-weekly)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
	Above-water reflectance (R_{rs})	10 (OW) bi-weekly	260	780	
	Satellite imagery analysis	3 per season	12	36	36
Zooplankton*	Density (individuals/m^3) – *micro- and mesozooplankton*	10 (OW) bi-weekly	260	780	780 samples for microzooplankton and 780 samples for meso-zooplankton (1 sample analyzed for all metrics)
	Biomass (g C/m^3) – *micro- and mesozooplankton*	10 (OW) bi-weekly	260	780	
	Community composition (family, genus if possible) – *micro- and mesozooplankton*	10 (OW) bi-weekly	260	780	
Water Quality (Physical Characteristics)	Secchi disk depth (m)	10 (OW) bi-weekly	260	780	No samples collected
	PAR profile (µmol m^{-2} s^{-1})	10 (OW) bi-weekly	260	780	
	Water column profile (depth, temperature, salinity, pH, turbidity, dissolved oxygen [DO])	10 (OW) bi-weekly	260	780	
Water Quality (Nutrients and Suspended Solids)	TSS, nitrate, ammonium, TN, TP, phosphate, silicate	10 (OW) bi-weekly	260	780	780 samples (1 sample analyzed for all metrics)
Habitat Characteristics (Distance from edge)	Distance of site from EV edge (m)	10 (OW) bi-weekly	260	780	No samples collected

*Note: values may be greater depending on the number of stations deeper than 5 m

Table 22. Summary of data collection for microphytobenthos benthic LTL group for Task 2. Anticipated data types and associated number of data points are summarized by effort (season), year, and the three-year total. The total number of samples is also provided as multiple data types can be collected simultaneously from the same sample.

Key LTL Group	Metric	Total Data Points per Effort (season)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
Micro-phytobenthos	Biomass (µg Chl *a* g dry sed^{-1}) – *assessed by HPLC*	100 (year 1: 50 OW, 50 EV), 60 (year 2 & 3: 30 OW, 30 EV) per season	400 (year 1), 240 (each year for years 2 & 3)	880	880 samples (1 sample analyzed for both metrics)
	Community composition (µg pigment g dry sed^{-1}) – *assessed by HPLC*	100 (year 1: 50 OW, 50 EV), 60 (year 2 & 3: 30 OW, 30 EV) per season	400 (year 1), 240 (each year for years 2 & 3)	880	
	Cell density (cells g dry sed^{-1}) – *assessed by microscopy*	20 per season	80	240	240 samples (1 sample analyzed for both metrics, decision will be based on samples collected for HPLC above)
	Community composition (dominant taxonomic groups) – *assessed by microscopy*	20 per season	80	240	

Key LTL Group	Metric	Total Data Points per Effort (season)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
Water Quality (Physical Characteristics)	Water column profile or standing water (depth, temperature, salinity, pH, turbidity, DO)	100 (year 1: 50 OW, 50 EV), 60 (year 2 & 3: 30 OW, 30 EV) per season	400 (year 1), 240 (each year for years 2 & 3)	880	No samples collected
Habitat Characteristics (Distance from edge)	Distance of site from EV edge (m)	100 (year 1: 50 OW, 50 EV), 60 (year 2 & 3: 30 OW, 30 EV) per season	400 (year 1), 240 (each year for years 2 & 3)	880	No samples collected

Table 23. Summary of data collection for macroinfauna benthic LTL group for Task 2. Anticipated data types and associated number of data points are summarized by effort (season), year, and the three-year total. The total number of samples is also provided as multiple data types can be collected simultaneously from the same sample.

Key LTL Group	Metric	Total Data Points per Effort (season)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
Macroinfauna	Density (indiv m^{-2})	300 (150 OW, 150 EV) per season	1,200	3,600	3,600 samples (1 sample analyzed for the three metrics)
	Community composition (family level)	300 (150 OW, 150 EV) per season	1,200	3,600	

Key LTL Group	Metric	Total Data Points per Effort (season)	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
	Biomass (g AFDW m^{-2})	300 (150 OW, 150 EV) per season	1,200	3,600	
Water Quality (Physical Characteristics)	Water column profile or standing water (depth, temperature, salinity, pH, turbidity, DO)	100 (50 OW, 50 EV) per season per metric	400	1,200	No samples collected; metric collected for each site
Sediment Characteristics	0-2 cm depth: Bulk density; % organic carbon; grain size (% sand, % silt + clay)	100 (50 OW, 50 EV) per season per metric	400	1,200	1,200 samples (1 sample analyzed for all metrics; each sample divided to appropriate depth gradient)
	2-5 cm depth: Bulk density; % organic carbon; grain size (% sand, % silt + clay)	100 (50 OW, 50 EV) per season per metric	400	1,200	
Habitat Characteristics (Distance from edge)	Distance of site from EV edge (m)	100 (50 OW, 50 EV) per season	400	1,200	No samples collected; metric collected for each site

Table 24. Summary of stable isotope data metrics ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and %CNS) for key LTL groups and other important environmental factors to be sampled seasonally from LTL monitoring stations.

Key LTL Group	Collection Effort	Total Data Points per Year	Grand Total Data Points for MAM Activity	Grand Total Samples for MAM Activity
Mesozooplankton	Phytoplankton/zooplankton	120 (1 rep from 3 OW sites per station)	360	360
Phytoplankton/POM	Phytoplankton/zooplankton	120 (1 rep from 3 OW sites per station)	360	360
Microphytobenthos	Microphytobenthos	240 (1 rep from 3 OW sites and 1 rep from 3 EV sites per station)	720	720
Epiphytic algae	Macroinfauna	120 (1 rep from 3 EV sites per station)	360	360
Macroinfauna (by major phyla)	Macroinfauna	240 (1 rep from 3 OW sites and 1 rep from 3 EV sites per station)	720	720
Vegetation (C4 plants)	Macroinfauna	120 (1 rep from 3 EV sites per station)	360	360
Vegetation (C3 plants)	Macroinfauna	120 (1 rep from 3 EV sites per station)	360	360
SAV	Macroinfauna	120 (1 rep from 3 OW sites per station, if present)	360	360
Surface Sediment	Macroinfauna	240 (1 rep from 3 OW sites and 1 rep from 3 EV sites per station)	720	720

Appendix D. R Code for Power Analysis

This section provides detailed R code for the power analyses summarized in Appendix A.

Phytoplankton

```
library(dplyr)
library(stringr)
library(pwr)

phytoplankton<-read.csv(file="phytoplankton_foranalysis.csv", header=T)

phytoplankton$Salinity_zone=NA
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==0]=1
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==1]=1
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==15]=1
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==12]=2
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==5]=3
phytoplankton$Salinity_zone[phytoplankton$CASM.polygon==7]=3
phytoplankton$Salinity_zone=as.numeric(phytoplankton$Salinity_zone)

phytoplankton=phytoplankton[!is.na(phytoplankton$SS.biomass),]

phytoplankton$Paper=as.factor(phytoplankton$Paper)
phytoplankton$CASM.polygon=as.factor(phytoplankton$CASM.polygon)
phytoplankton$Season=as.factor(phytoplankton$Season)
phytoplankton$Sampling.year[phytoplankton$Sampling.year=="2006-2007"]="2006"
phytoplankton$Sampling.year[phytoplankton$Sampling.year=="1972-1973"]="1973"
phytoplankton$Sampling.year[phytoplankton$Sampling.year=="1976-1978"]="1976"
phytoplankton$Sampling.year[phytoplankton$Sampling.year=="1973-1974"]="1973"
phytoplankton$Sampling.year[phytoplankton$Sampling.year=="Fall"]=NA

phytoplankton$Sampling.year=as.factor(phytoplankton$Sampling.year)
phytoplankton$SS.abundance=as.numeric(phytoplankton$SS.abundance)
phytoplankton$SS.biomass=as.numeric(phytoplankton$SS.biomass)
phytoplankton$SS.samples.n=as.numeric(phytoplankton$SS.samples.n)
phytoplankton$SS.abundance.units=as.factor(phytoplankton$SS.abundance.units)
phytoplankton$SS.biomass.units=as.factor(phytoplankton$SS.biomass.units)

summary(phytoplankton)

phytoplankton$Paper=as.factor(phytoplankton$Paper)
phytoplankton$CASM.polygon=as.factor(phytoplankton$CASM.polygon)
phytoplankton$Season=as.factor(phytoplankton$Season)
phytoplankton$Sampling.year=as.factor(phytoplankton$Sampling.year)
phytoplankton$SS.biomass.units=as.factor(phytoplankton$SS.biomass.units)
phytoplankton$SS.abundance.units=as.factor(phytoplankton$SS.abundance.units)
phytoplankton$SS.biomass=as.numeric(phytoplankton$SS.biomass)
phytoplankton$SS.abundance=as.numeric(phytoplankton$SS.abundance)
phytoplankton$Sediment.TOC=as.numeric(phytoplankton$Sediment.TOC)
phytoplankton$Sediment.TOC.units=as.factor(as.character(phytoplankton$Sediment.TOC.units))
phytoplankton$SS.samples.n=as.numeric(phytoplankton$SS.samples.n)

summary(phytoplankton)

#### Water Column Chl a ####

phytoplankton.bio=phytoplankton[phytoplankton$SS.biomass.units=="total Chl a ug/L",]
phytoplankton.bio=phytoplankton.bio[!is.na(phytoplankton.bio$SS.biomass),]

phytoplankton.bio.fall=phytoplankton.bio[phytoplankton.bio$Season=="Fall",]
phytoplankton.bio.spring=phytoplankton.bio[phytoplankton.bio$Season=="Spring",]
phytoplankton.bio.summer=phytoplankton.bio[phytoplankton.bio$Season=="Summer",]
phytoplankton.bio.winter=phytoplankton.bio[phytoplankton.bio$Season=="Winter",]

phyto.wc.tab.fall=with(phytoplankton.bio.fall, table(CASM.polygon, Sampling.year))
phyto.wc.tab.spring=with(phytoplankton.bio.spring, table(CASM.polygon, Sampling.year))
phyto.wc.tab.summer=with(phytoplankton.bio.summer, table(CASM.polygon, Sampling.year))
```

```
phyto.wc.tab.winter=with(phytoplankton.bio.winter, table(CASM.polygon, Sampling.year))

fall.polys=rowSums(phyto.wc.tab.fall>10)
names.fall.polys=names(fall.polys[fall.polys>1])
fall.years=colSums(phyto.wc.tab.fall>10)
names.fall.years=names(fall.years[fall.years>1])

summer.polys=rowSums(phyto.wc.tab.summer>10)
names.summer.polys=names(summer.polys[summer.polys>1])
summer.years=colSums(phyto.wc.tab.summer>10)
names.summer.years=names(summer.years[summer.years>1])

spring.polys=rowSums(phyto.wc.tab.spring>10)
names.spring.polys=names(spring.polys[spring.polys>1])
spring.years=colSums(phyto.wc.tab.spring>10)
names.spring.years=names(spring.years[spring.years>1])

winter.polys=rowSums(phyto.wc.tab.winter>10)
names.winter.polys=names(winter.polys[winter.polys>1])
winter.years=colSums(phyto.wc.tab.winter>10)
names.winter.years=names(winter.years[winter.years>1])

int.poly.1=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.spring.polys)))
int.poly.2=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.summer.polys)))
int.poly.3=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.winter.polys)))
int.poly.4=as.factor(intersect(as.numeric(names.spring.polys),as.numeric(names.summer.polys)))
int.poly.5=as.factor(intersect(as.numeric(names.spring.polys),as.numeric(names.winter.polys)))
int.poly.6=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.winter.polys)))

levels.poly=as.numeric(as.character(unique(c(int.poly.1,int.poly.2,int.poly.3,int.poly.4,int.poly
.5,int.poly.6))))

int.years.1=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.spring.years)))
int.years.2=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.summer.years)))
int.years.3=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.winter.years)))
int.years.4=as.factor(intersect(as.numeric(names.spring.years),as.numeric(names.summer.years)))
int.years.5=as.factor(intersect(as.numeric(names.spring.years),as.numeric(names.winter.years)))
int.years.6=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.winter.years)))

levels.years=as.numeric(as.character(unique(c(int.years.1,int.years.2,int.years.3,int.years.4,int
.years.5,int.years.6))))

levels.seasons=c("Fall","Spring","Summer","Winter")

levels.sal=as.numeric(c("1","2","3"))

phytoplankton.bio$CASM.polygon=as.numeric(as.character(phytoplankton.bio$CASM.polygon))
phytoplankton.bio$Sampling.year=as.numeric(as.character(phytoplankton.bio$Sampling.year))
phytoplankton.bio$Season=as.factor(phytoplankton.bio$Season)

sample.size=data.frame("Sample.size"=seq(from=3,to=100,by=1),"Salinity_zone"=NA,"Sampling.year"=N
A,"Season"=NA,"Salinity_zone:Sampling.year"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=N
A,"Salinity_zone:Sampling.year:Season"=NA)
for(m in 1:98)
{

  n=m+2

Fvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampling.ye
ar"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Season"=N
A)

pvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampling.ye
ar"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Season"=N
A)

  for(r in 1:1000)
  {
```

```
sample.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"SS.Biomass"=NA,"SS.sample
s.n"=NA)

    levels.sal.rand=as.factor(as.character(sample(levels.sal,size=3,replace=FALSE)))

phyto.sub.yr=unique(phytoplankton.bio$Sampling.year[phytoplankton.bio$Salinity_zone==levels.sal.r
and[1] | phytoplankton.bio$Salinity_zone==levels.sal.rand[2] |
phytoplankton.bio$Salinity_zone==levels.sal.rand[3]])
    years.choose=as.factor(intersect(as.numeric(phyto.sub.yr),as.numeric(levels.years)))
    levels.years.rand=as.factor(sample(years.choose,size=3,replace=FALSE))

phyto.sub.seas=unique(phytoplankton.bio$Season[phytoplankton.bio$Salinity_zone==levels.sal.rand[1
] | phytoplankton.bio$Salinity_zone==levels.sal.rand[2] |
phytoplankton.bio$Salinity_zone==levels.sal.rand[3]])

phyto.sub.seas2=unique(phytoplankton.bio$Season[phytoplankton.bio$Sampling.year==levels.years.ran
d[1] | phytoplankton.bio$Sampling.year==levels.years.rand[2] |
phytoplankton.bio$Sampling.year==levels.years.rand[3]])
    seasons.choose=as.factor(intersect(phyto.sub.seas,phyto.sub.seas2))
    seasons.choose2=as.factor(intersect(seasons.choose,levels.seasons))
    levels.seasons.rand=as.factor(sample(seasons.choose2,size=3,replace=FALSE))

    for(i in 1:length(levels.sal.rand))
    {
      for(j in 1:length(levels.years.rand))
      {
        for(k in 1:length(levels.seasons.rand))
        {


subset.phyto=phytoplankton.bio[phytoplankton.bio$Salinity_zone==as.numeric(levels.sal.rand[i])&ph
ytoplankton.bio$Sampling.year==levels.years.rand[j]&as.character(phytoplankton.bio$Season)==as.ch
aracter(levels.seasons.rand[k]),]
          subset.phyto=subset.phyto[!is.na(subset.phyto$SS.biomass),]
          subset.phyto$Salinity_zone=as.factor(as.character(subset.phyto$Salinity_zone))
          subset.phyto$Sampling.year=as.factor(as.character(subset.phyto$Sampling.year))
          subset.phyto$Season=as.factor(as.character(subset.phyto$Season))
          if(nrow(subset.phyto)==0) {
            samps.abund=rep(NA,length=n)
            samps.n=rep(NA,length=n)} else {
              samp.no=round(runif(n=n,min=1,max=nrow(subset.phyto)),0)
              samps.abund=subset.phyto$SS.biomass[samp.no]
              samps.n=subset.phyto$SS.samples.n[samp.no]          }

temp.df=data.frame("Salinity_zone"=rep(levels.sal.rand[i],length=n),"Sampling.year"=rep(levels.ye
ars.rand[j],length=n),"Season"=rep(levels.seasons.rand[k],length=n),"SS.Biomass"=samps.abund,"SS.
samples.n"=samps.n)
          sample.df=rbind(sample.df,temp.df)
        }
      }
    }

    if(nrow(sample.df)==0){

temp.Fvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampli
ng.year"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Seas
on"=NA)

temp.pvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampli
ng.year"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Seas
on"=NA)

    } else {
    sample.df$SS.samples.n[is.na(sample.df$SS.samples.n)]=1
    sample.df$SS.samples.n=as.numeric(as.character(sample.df$SS.samples.n))

    sample.df=na.omit(sample.df)

    sample.df$weights=sample.df$SS.samples.n/sum(sample.df$SS.samples.n)
```

```
    sample.df$Salinity_zone=as.factor(sample.df$Salinity_zone)
    sample.df$Sampling.year=as.factor(sample.df$Sampling.year)
    sample.df$Season=as.factor(sample.df$Season)


aov1=aov(SS.Biomass~Salinity_zone+Sampling.year+Season+Salinity_zone:Sampling.year+Salinity_zone:
Season+Sampling.year:Season+Salinity_zone:Sampling.year:Season,data=sample.df,weights=weights)

    sum.F.vec=summary(aov1)[[1]][["F value"]][1:7]
    sum.p.vec=summary(aov1)[[1]][["Pr(>F)"]][1:7]


temp.Fvalue.df=data.frame("Salinity_zone"=sum.F.vec[1],"Sampling.year"=sum.F.vec[2],"Season"=sum.
F.vec[3],"Salinity_zone:Sampling.year"=sum.F.vec[4],"Salinity_zone:Season"=sum.F.vec[5],"Sampling
.year:Season"=sum.F.vec[6],"Salinity_zone:Sampling.year:Season"=sum.F.vec[7])

temp.pvalue.df=data.frame("Salinity_zone"=sum.p.vec[1],"Sampling.year"=sum.p.vec[2],"Season"=sum.
p.vec[3],"Salinity_zone:Sampling.year"=sum.p.vec[4],"Salinity_zone:Season"=sum.p.vec[5],"Sampling
.year:Season"=sum.p.vec[6],"Salinity_zone:Sampling.year:Season"=sum.p.vec[7])

    Fvalue.df=rbind(Fvalue.df,temp.Fvalue.df)
    pvalue.df=rbind(pvalue.df,temp.pvalue.df)

    }

  }

  pvalue.df=na.omit(pvalue.df)

  sample.size[m,2]=sum(pvalue.df[,1]<0.05)/nrow(pvalue.df)
  sample.size[m,3]=sum(pvalue.df[,2]<0.05)/nrow(pvalue.df)
  sample.size[m,4]=sum(pvalue.df[,3]<0.05)/nrow(pvalue.df)
  sample.size[m,5]=sum(pvalue.df[,4]<0.05)/nrow(pvalue.df)
  sample.size[m,6]=sum(pvalue.df[,5]<0.05)/nrow(pvalue.df)
  sample.size[m,7]=sum(pvalue.df[,6]<0.05)/nrow(pvalue.df)
  sample.size[m,8]=sum(pvalue.df[,7]<0.05)/nrow(pvalue.df)

  # Fvalue.df=na.omit(Fvalue.df)
  #
  # sample.size[m,2]=sum(Fvalue.df[,1]<0.05)/nrow(Fvalue.df)
  # sample.size[m,3]=sum(Fvalue.df[,2]<0.05)/nrow(Fvalue.df)
  # sample.size[m,4]=sum(Fvalue.df[,3]<0.05)/nrow(Fvalue.df)
  # sample.size[m,5]=sum(Fvalue.df[,4]<0.05)/nrow(Fvalue.df)
  # sample.size[m,6]=sum(Fvalue.df[,5]<0.05)/nrow(Fvalue.df)
  # sample.size[m,7]=sum(Fvalue.df[,6]<0.05)/nrow(Fvalue.df)

  print(n)
}

sample.size.phyto.wc=sample.size
# write.csv(sample.size.phyto.wc,"sample.size.phyto_salinityzone.csv",row.names=FALSE)
# sample.size.phyto.wc=read.csv("sample.size.phyto_salinityzone.csv")

jpeg("phytoplankton water column anova power_salinityzone.jpg", width = 6, height =
6,units="in",res=300)

plot(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,2],xlab="Sample
size",ylab="Power",ylim=c(0.4,1),type='l',lwd=2,col="red")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,3],lwd=2,col="orange")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,4],lwd=2,col="gold")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,5],lwd=2,col="green")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,6],lwd=2,col="blue")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,7],lwd=2,col="blueviolet")
lines(sample.size.phyto.wc$Sample.size,sample.size.phyto.wc[,8],lwd=2,col="mediumorchid2")
abline(h=0.8,add=TRUE)
arrows(x0=17,y0=0.8,x1=17,y1=0,lty=2,length=0)
arrows(x0=19,y0=0.8,x1=19,y1=0,lty=2,length=0)

text(x=c(18,18),y=c(0.45,0.45),labels=c("19","17"),pos=c(4,2))
legend(x="bottomright",legend=c("Salinity","Year","Season","Salinity x Year","Salinity x
Season","Year x Season","Salinity x Year x Season"),
```

```
       col=c("red","orange","gold","green","blue","blueviolet","mediumorchid2"),lwd=2,cex=1,bty =
"n")

dev.off()
```

Open Water Detritus

```
detritus<-read.csv(file="detritus_foranalysis.csv", header=T)

detritus$Salinity_zone=NA
detritus$Salinity_zone[detritus$CASM.polygon==0]=1
detritus$Salinity_zone[detritus$CASM.polygon==1]=1
detritus$Salinity_zone[detritus$CASM.polygon==15]=1
detritus$Salinity_zone[detritus$CASM.polygon==12]=2
detritus$Salinity_zone[detritus$CASM.polygon==5]=3
detritus$Salinity_zone[detritus$CASM.polygon==7]=3
detritus$Salinity_zone=as.numeric(detritus$Salinity_zone)

detritus$SS.samples.n[detritus$SS.samples.n=="?"]=NA
detritus$SS.samples.n[detritus$SS.samples.n=="36?"]=36
detritus$SS.samples.n[detritus$SS.samples.n=="many (exact number not given)"]=NA

detritus$Season[detritus$Month.s.=="June"|detritus$Month.s.=="July"|detritus$Month.s.=="August"]=
"Summer"
detritus$Season[detritus$Month.s.=="September"|detritus$Month.s.=="October"|detritus$Month.s.=="N
ovember"]="Fall"
detritus$Season[detritus$Month.s.=="December"|detritus$Month.s.=="January"|detritus$Month.s.=="Fe
bruary"]="Winter"
detritus$Season[detritus$Month.s.=="March"|detritus$Month.s.=="April"|detritus$Month.s.=="May"]="
Spring"
detritus$Season[detritus$Season=="Fall-Fall"]="Fall"
detritus$Season[detritus$Season=="Spring-Spring"]="Spring"
detritus$Sampling.year[detritus$Sampling.year=="1970, 1971"]=1970
detritus$Sampling.year[detritus$Sampling.year=="1972-1973"]=1972
detritus$Sampling.year[detritus$Sampling.year=="1996, 1997"]=1996
detritus$Sampling.year[detritus$Sampling.year=="2012-2013"]=2012
detritus$Sampling.year[detritus$Sampling.year=="2013-2014"]=2013
detritus$Sampling.year[detritus$Sampling.year=="2014-2015"]=2014
detritus$Sampling.year[detritus$Sampling.year=="2015-2016"]=2015
detritus$Sampling.year[detritus$Sampling.year=="2019-2020"]=2019
detritus$Sampling.year[detritus$Paper=="Gosselink and Kirby, 1974"]=1974

detritus$Paper=as.factor(detritus$Paper)
detritus$CASM.polygon=as.factor(detritus$CASM.polygon)
detritus$Season=as.factor(detritus$Season)
detritus$Sampling.year=as.factor(detritus$Sampling.year)
detritus$SS.biomass.units=as.factor(detritus$SS.biomass.units)
detritus$SS.abundance.units=as.factor(detritus$SS.abundance.units)
detritus$SS.biomass=as.numeric(detritus$SS.biomass)
detritus$SS.abundance=as.numeric(detritus$SS.abundance)
detritus$Sediment.TOC=as.numeric(detritus$Sediment.TOC)
detritus$Sediment.TOC.units=as.factor(as.character(detritus$Sediment.TOC.units))
detritus$SS.samples.n=as.numeric(detritus$SS.samples.n)

summary(detritus)


#### Water Column OM ####

detritus.wc=detritus[detritus$SS.abundance.units=="total suspended organic matter (mg/L)",]
detritus.wc=detritus.wc[!is.na(detritus.wc$SS.abundance),]

detritus.wc.fall=detritus.wc[detritus.wc$Season=="Fall",]
detritus.wc.spring=detritus.wc[detritus.wc$Season=="Spring",]
detritus.wc.summer=detritus.wc[detritus.wc$Season=="Summer",]
detritus.wc.winter=detritus.wc[detritus.wc$Season=="Winter",]

det.wc.tab.fall=with(detritus.wc.fall, table(CASM.polygon, Sampling.year))
```

```
det.wc.tab.spring=with(detritus.wc.spring, table(CASM.polygon, Sampling.year))
det.wc.tab.summer=with(detritus.wc.summer, table(CASM.polygon, Sampling.year))
det.wc.tab.winter=with(detritus.wc.winter, table(CASM.polygon, Sampling.year))

fall.polys=rowSums(det.wc.tab.fall>10)
names.fall.polys=names(fall.polys[fall.polys>1])
fall.years=colSums(det.wc.tab.fall>10)
names.fall.years=names(fall.years[fall.years>1])

summer.polys=rowSums(det.wc.tab.summer>10)
names.summer.polys=names(summer.polys[summer.polys>1])
summer.years=colSums(det.wc.tab.summer>10)
names.summer.years=names(summer.years[summer.years>1])

spring.polys=rowSums(det.wc.tab.spring>10)
names.spring.polys=names(spring.polys[spring.polys>1])
spring.years=colSums(det.wc.tab.spring>10)
names.spring.years=names(spring.years[spring.years>1])

winter.polys=rowSums(det.wc.tab.winter>10)
names.winter.polys=names(winter.polys[winter.polys>1])
winter.years=colSums(det.wc.tab.winter>10)
names.winter.years=names(winter.years[winter.years>1])

int.poly.1=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.spring.polys)))
int.poly.2=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.summer.polys)))
int.poly.3=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.winter.polys)))
int.poly.4=as.factor(intersect(as.numeric(names.spring.polys),as.numeric(names.summer.polys)))
int.poly.5=as.factor(intersect(as.numeric(names.spring.polys),as.numeric(names.winter.polys)))
int.poly.6=as.factor(intersect(as.numeric(names.fall.polys),as.numeric(names.winter.polys)))

levels.poly=as.numeric(as.character(unique(c(int.poly.1,int.poly.2,int.poly.3,int.poly.4,int.poly
.5,int.poly.6))))

int.years.1=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.spring.years)))
int.years.2=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.summer.years)))
int.years.3=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.winter.years)))
int.years.4=as.factor(intersect(as.numeric(names.spring.years),as.numeric(names.summer.years)))
int.years.5=as.factor(intersect(as.numeric(names.spring.years),as.numeric(names.winter.years)))
int.years.6=as.factor(intersect(as.numeric(names.fall.years),as.numeric(names.winter.years)))

levels.years=as.numeric(as.character(unique(c(int.years.1,int.years.2,int.years.3,int.years.4,int
.years.5,int.years.6))))

levels.seasons=c("Fall","Spring","Summer","Winter")

levels.sal=as.numeric(c("1","2","3"))

detritus.wc$CASM.polygon=as.numeric(as.character(detritus.wc$CASM.polygon))
detritus.wc$Sampling.year=as.numeric(as.character(detritus.wc$Sampling.year))
detritus.wc$Season=as.factor(detritus.wc$Season)

sample.size=data.frame("Sample.size"=seq(from=3,to=100,by=1),"Salinity_zone"=NA,"Sampling.year"=N
A,"Season"=NA,"Salinity_zone:Sampling.year"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=N
A,"Salinity_zone:Sampling.year:Season"=NA)
for(m in 1:98)
{

  n=m+2

Fvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampling.ye
ar"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Season"=N
A)

pvalue.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"Salinity_zone:Sampling.ye
ar"=NA,"Salinity_zone:Season"=NA,"Sampling.year:Season"=NA,"Salinity_zone:Sampling.year:Season"=N
A)

  for(r in 1:1000)
  {
```

```
sample.df=data.frame("Salinity_zone"=NA,"Sampling.year"=NA,"Season"=NA,"SS.Abundance"=NA,"SS.samp
les.n"=NA)

    levels.sal.rand=as.factor(as.character(sample(levels.sal,size=3,replace=FALSE)))
    det.sub.yr=unique(detritus.wc$Sampling.year[detritus.wc$Salinity_zone==levels.sal.rand[1] |
detritus.wc$Salinity_zone==levels.sal.rand[2] | detritus.wc$Salinity_zone==levels.sal.rand[3]])
    levels.years.rand=as.factor(sample(levels.years,size=3,replace=FALSE))
    det.sub.seas=unique(detritus.wc$Season[detritus.wc$Salinity_zone==levels.sal.rand[1] |
detritus.wc$Salinity_zone==levels.sal.rand[2] | detritus.wc$Salinity_zone==levels.sal.rand[3]])
    det.sub.seas2=unique(detritus.wc$Season[detritus.wc$Sampling.year==levels.years.rand[1] |
detritus.wc$Sampling.year==levels.years.rand[2] |
detritus.wc$Sampling.year==levels.years.rand[3]])
    seasons.choose=as.factor(intersect(det.sub.seas,det.sub.seas2))
    seasons.choose2=as.factor(intersect(seasons.choose,levels.seasons))
    levels.seasons.rand=as.factor(sample(seasons.choose2,size=3,replace=FALSE))


    for(i in 1:length(levels.sal.rand))
    {
      for(j in 1:length(levels.years.rand))
      {
        for(k in 1:length(levels.seasons.rand))
        {


subset.det=detritus.wc[detritus.wc$Salinity_zone==as.numeric(levels.sal.rand[i])&detritus.wc$Samp
ling.year==levels.years.rand[j]&as.character(detritus.wc$Season)==as.character(levels.seasons.ran
d[k]),]
          subset.det=subset.det[!is.na(subset.det$SS.abundance),]
          subset.det$Salinity_zone=as.factor(as.character(subset.det$Salinity_zone))
          subset.det$Sampling.year=as.factor(as.character(subset.det$Sampling.year))
          subset.det$Season=as.factor(as.character(subset.det$Season))
          if(nrow(subset.det)==0) {
            samps.abund=rep(NA,length=n)
            samps.n=rep(NA,length=n)} else {
              samp.no=round(runif(n=n,min=1,max=nrow(subset.det)),0)
              samps.abund=subset.det$SS.abundance[samp.no]
              samps.n=subset.det$SS.samples.n[samp.no]          }

temp.df=data.frame("Salinity_zone"=rep(levels.sal.rand[i],length=n),"Sampling.year"=rep(levels.ye
ars.rand[j],length=n),"Season"=rep(levels.seasons.rand[k],length=n),"SS.Abundance"=samps.abund,"S
S.samples.n"=samps.n)
          sample.df=rbind(sample.df,temp.df)
        }
      }
    }

    sample.df$SS.samples.n[is.na(sample.df$SS.samples.n)]=1
    sample.df$SS.samples.n=as.numeric(as.character(sample.df$SS.samples.n))

    sample.df=na.omit(sample.df)

    sample.df$weights=sample.df$SS.samples.n/sum(sample.df$SS.samples.n)

    sample.df$Salinity_zone=as.factor(sample.df$Salinity_zone)
    sample.df$Sampling.year=as.factor(sample.df$Sampling.year)
    sample.df$Season=as.factor(sample.df$Season)


aov1=aov(SS.Abundance~Salinity_zone+Sampling.year+Season+Salinity_zone:Sampling.year+Salinity_zon
e:Season+Sampling.year:Season+Salinity_zone:Sampling.year:Season,data=sample.df,weights=weights)

    sum.F.vec=summary(aov1)[[1]][["F value"]][1:7]
    sum.p.vec=summary(aov1)[[1]][["Pr(>F)"]][1:7]


temp.Fvalue.df=data.frame("Salinity_zone"=sum.F.vec[1],"Sampling.year"=sum.F.vec[2],"Season"=sum.
F.vec[3],"Salinity_zone:Sampling.year"=sum.F.vec[4],"Salinity_zone:Season"=sum.F.vec[5],"Sampling
.year:Season"=sum.F.vec[6],"Salinity_zone:Sampling.year:Season"=sum.F.vec[7])
```

```
temp.pvalue.df=data.frame("Salinity_zone"=sum.p.vec[1],"Sampling.year"=sum.p.vec[2],"Season"=sum.
p.vec[3],"Salinity_zone:Sampling.year"=sum.p.vec[4],"Salinity_zone:Season"=sum.p.vec[5],"Sampling
.year:Season"=sum.p.vec[6],"Salinity_zone:Sampling.year:Season"=sum.p.vec[7])

    Fvalue.df=rbind(Fvalue.df,temp.Fvalue.df)
    pvalue.df=rbind(pvalue.df,temp.pvalue.df)

  }

  pvalue.df=na.omit(pvalue.df)

  sample.size[m,2]=sum(pvalue.df[,1]<0.05)/nrow(pvalue.df)
  sample.size[m,3]=sum(pvalue.df[,2]<0.05)/nrow(pvalue.df)
  sample.size[m,4]=sum(pvalue.df[,3]<0.05)/nrow(pvalue.df)
  sample.size[m,5]=sum(pvalue.df[,4]<0.05)/nrow(pvalue.df)
  sample.size[m,6]=sum(pvalue.df[,5]<0.05)/nrow(pvalue.df)
  sample.size[m,7]=sum(pvalue.df[,6]<0.05)/nrow(pvalue.df)
  sample.size[m,8]=sum(pvalue.df[,7]<0.05)/nrow(pvalue.df)

  # Fvalue.df=na.omit(Fvalue.df)
  #
  # sample.size[m,2]=sum(Fvalue.df[,1]<0.05)/nrow(Fvalue.df)
  # sample.size[m,3]=sum(Fvalue.df[,2]<0.05)/nrow(Fvalue.df)
  # sample.size[m,4]=sum(Fvalue.df[,3]<0.05)/nrow(Fvalue.df)
  # sample.size[m,5]=sum(Fvalue.df[,4]<0.05)/nrow(Fvalue.df)
  # sample.size[m,6]=sum(Fvalue.df[,5]<0.05)/nrow(Fvalue.df)
  # sample.size[m,7]=sum(Fvalue.df[,6]<0.05)/nrow(Fvalue.df)

  print(n)
}

sample.size.det.wc=sample.size
# write.csv(sample.size.det.wc,"sample.size.det.wc_salinityzone.csv",row.names=FALSE)
# sample.size.det.wc=read.csv("sample.size.det.wc.csv")

jpeg("detritus water column anova power_salinityzone.jpg", width = 6, height =
6,units="in",res=300)

plot(sample.size.det.wc$Sample.size,sample.size.det.wc[,2],xlab="Sample
size",ylab="Power",ylim=c(0.4,1),type='l',lwd=2,col="red")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,3],lwd=2,col="orange")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,4],lwd=2,col="gold")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,5],lwd=2,col="green")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,6],lwd=2,col="blue")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,7],lwd=2,col="blueviolet")
lines(sample.size.det.wc$Sample.size,sample.size.det.wc[,8],lwd=2,col="mediumorchid2")
abline(h=0.8,add=TRUE)
arrows(x0=c(11,18),y0=c(0.8,0.8),x1=c(11,18),y1=c(0,0),lty=2,length=0)
text(x=c(19,10),y=c(0.8,0.5),labels=c("18","11"),pos=c(3,4))
legend(x="bottomright",legend=c("Salinity","Year","Season","Salinity x Year","Salinity x
Season","Year x Season","Salinity x Year x Season"),
       col=c("red","orange","gold","green","blue","blueviolet","mediumorchid2"),lwd=2,cex=1,bty =
"n")

dev.off()
```

Subtidal Macroinfauna

```
library(dplyr)
library(stringr)
library(pwr)

macroinfauna<-read.csv(file="community.csv", header=T)
macroinfauna$Latitude=as.numeric(substr(macroinfauna$Coordinates..DMM.,
3,4))+(as.numeric(substr(macroinfauna$Coordinates..DMM., 6,11))/60)
macroinfauna$Longitude=as.numeric(substr(macroinfauna$Coordinates..DMM.,
16,18))+(as.numeric(substr(macroinfauna$Coordinates..DMM., 20,25))/60)

macroinfauna$abundance=rowSums(macroinfauna[,5:113],na.rm=TRUE)
```

```
macroinfauna=macroinfauna[,c(115:143,152:153)]
macroinfauna=macroinfauna[,-13:-25]
colnames(macroinfauna)[11]="Season"

macroinfauna$samples.n=rep(1,nrow(macroinfauna))
macroinfauna$Year=NA

macroinfauna$Year[macroinfauna$Year.1==2020]=1
macroinfauna$Year[macroinfauna$Year.1==2022]=2
macroinfauna$Year[macroinfauna$Year.1==2021&macroinfauna$Season=="Spring"]=1
macroinfauna$Year[macroinfauna$Year.1==2021&macroinfauna$Season!="Spring"]=2

macroinfauna$Salinity_zone=rep(1,nrow(macroinfauna))
macroinfauna$Salinity_zone[macroinfauna$Latitude>29.4]=2

macroinfauna$Salinity_zone=as.factor(macroinfauna$Salinity_zone)

summary(macroinfauna)

levels.poly=as.factor(as.character(unique(macroinfauna$Salinity_zone)))

levels.years=as.factor(as.character(unique(macroinfauna$Year)))

levels.seasons=as.factor(as.character(unique(macroinfauna$Season)))

sample.size=data.frame("Sample.size"=seq(from=3,to=100,by=1),"Salinity_class"=NA,"Year"=NA,"Seaso
n"=NA,"Salinity_class:Year"=NA)
for(m in 1:98)
{

  n=m+2

Fvalue.df=data.frame("Salinity_zone"=NA,"Year"=NA,"Season"=NA,"Salinity_zone:Year"=NA,"Salinity_z
one:Season"=NA)

pvalue.df=data.frame("Salinity_zone"=NA,"Year"=NA,"Season"=NA,"Salinity_zone:Year"=NA,"Salinity_z
one:Season"=NA)

for(r in 1:1000)
{

sample.df=data.frame("Salinity_zone"=NA,"Year"=NA,"Season"=NA,"abundance"=NA,"samples.n"=NA)

levels.poly.rand=sample(levels.poly,size=2,replace=FALSE)
levels.years.rand=sample(levels.years,size=2,replace=FALSE)
levels.seasons.rand=sample(levels.seasons,size=2,replace=FALSE)

for(i in 1:length(levels.poly.rand))
{
  for(j in 1:length(levels.years.rand))
  {
    for(k in 1:length(levels.seasons.rand))
    {

subset.macro=macroinfauna[macroinfauna$Salinity_zone==as.character(levels.poly.rand[i])&macroinfa
una$Year==levels.years.rand[j]&macroinfauna$Season==as.character(levels.seasons.rand[k]),]
      subset.macro=subset.macro[!is.na(subset.macro$abundance),]
      if(nrow(subset.macro)==0) {
        samps.abund=rep(NA,length=n)
        samps.n=rep(NA,length=n)} else {
        samp.no=round(runif(n=n,min=1,max=nrow(subset.macro)),0)
        samps.abund=subset.macro$abundance[samp.no]
        samps.n=subset.macro$samples.n[samp.no]
      }

temp.df=data.frame("Salinity_zone"=rep(levels.poly.rand[i],length=n),"Year"=rep(levels.years.rand
[j],length=n),"Season"=rep(levels.seasons.rand[k],length=n),"abundance"=samps.abund,"samples.n"=s
amps.n)
      sample.df=rbind(sample.df,temp.df)
    }
```

```
  }
}

sample.df$samples.n[is.na(sample.df$samples.n)]=1
sample.df$samples.n=as.numeric(as.character(sample.df$samples.n))

sample.df=na.omit(sample.df)

sample.df$weights=sample.df$samples.n/sum(sample.df$samples.n)

sample.df$Salinity_zone=as.factor(sample.df$Salinity_zone)
sample.df$Year=as.factor(sample.df$Year)
sample.df$Season=as.factor(sample.df$Season)

aov1=aov(abundance~Salinity_zone+Year+Season+Salinity_zone:Year+Salinity_zone:Season,data=sample.
df,weights=weights)

sum.F.vec=summary(aov1)[[1]][["F value"]][1:5]
sum.p.vec=summary(aov1)[[1]][["Pr(>F)"]][1:5]

temp.Fvalue.df=data.frame("Salinity_zone"=sum.F.vec[1],"Year"=sum.F.vec[2],"Season"=sum.F.vec[3],
"Salinity_zone:Year"=sum.F.vec[4],"Salinity_zone:Season"=sum.F.vec[5])
temp.pvalue.df=data.frame("Salinity_zone"=sum.p.vec[1],"Year"=sum.p.vec[2],"Season"=sum.p.vec[3],
"Salinity_zone:Year"=sum.p.vec[4],"Salinity_zone:Season"=sum.p.vec[5])

Fvalue.df=rbind(Fvalue.df,temp.Fvalue.df)
pvalue.df=rbind(pvalue.df,temp.pvalue.df)

}

pvalue.df=na.omit(pvalue.df)

sample.size[m,1]=n
sample.size[m,2]=sum(pvalue.df[,1]<0.05)/nrow(pvalue.df)
sample.size[m,3]=sum(pvalue.df[,2]<0.05)/nrow(pvalue.df)
sample.size[m,4]=sum(pvalue.df[,3]<0.05)/nrow(pvalue.df)
sample.size[m,5]=sum(pvalue.df[,4]<0.05)/nrow(pvalue.df)
sample.size[m,6]=sum(pvalue.df[,5]<0.05)/nrow(pvalue.df)

# Fvalue.df=na.omit(Fvalue.df)
#
# sample.size[m,2]=sum(Fvalue.df[,1]<0.05)/nrow(Fvalue.df)
# sample.size[m,3]=sum(Fvalue.df[,2]<0.05)/nrow(Fvalue.df)
# sample.size[m,4]=sum(Fvalue.df[,3]<0.05)/nrow(Fvalue.df)
# sample.size[m,5]=sum(Fvalue.df[,4]<0.05)/nrow(Fvalue.df)
# sample.size[m,6]=sum(Fvalue.df[,5]<0.05)/nrow(Fvalue.df)

print(n)
}

sample.size.mac.abund=sample.size
# write.csv(sample.size.mac.abund,"sample.size.mac.abund_salinityzone.csv",row.names=FALSE)
# sample.size=read.csv("sample.size.mac.abund_salinityzone.csv")

jpeg("macroinfauna anova power_Tupitza.jpg", width = 6, height = 6,units="in",res=300)

plot(sample.size$Sample.size,sample.size[,2],xlab="Sample
size",ylab="Power",type='l',lwd=2,col="red",ylim=c(0,1))
lines(sample.size$Sample.size,sample.size[,3],lwd=2,col="orange")
lines(sample.size$Sample.size,sample.size[,4],lwd=2,col="green")
lines(sample.size$Sample.size,sample.size[,5],lwd=2,col="blue")
lines(sample.size$Sample.size,sample.size[,6],lwd=2,col="mediumorchid2")
abline(h=0.8,add=TRUE)
arrows(x0=20,y0=0.8,x1=20,y1=-.1,lty=2,length=0)
text(x=c(18),y=c(0.8),labels=c("20"),pos=c(3))
legend(x="bottomright",legend=c("Salinity","Year","Season","Salinity x Year","Salinity x
Season"),
       col=c("red","orange","green","blue","mediumorchid2"),lwd=2,cex=1)

dev.off()
```