Nutrient Multi-Year Study Plan

Version 1.0
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Prepared By:

MLJ ENVIRONMENTAL

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LIST OF ACRONYMS

ACRONYM	DEFINITION
ANOVA	Analysis of Variance
ASC	Aquatic Science Center
BGC	Biogeochemical
chl-a	Chlorophyll-a
CSC	Cache Slough Complex
CVRWQCB	Central Valley Regional Water Quality Control Board

DCC	Delta Cross Channel
Delta RMP	Delta Regional Monitoring Program
DICU	Delta Island Consumptive Use
DIN	Dissolved Inorganic Nitrogen
DO	Dissolved Oxygen
DWR	Department of Water Resources
EMP	Environmental Monitoring Program
HAB	Harmful Algal Bloom
HPLC	High-Performance Liquid Chromatography
IAV	Invasive Aquatic Vegetation
IEP EMP	Interagency Ecological Program's Environmental Monitoring Program
N	Nitrogen
NH_4	Ammonium
NO_3	Nitrate
nSFE-BGCM	northern San Francisco Estuary Biogeochemical Model
O_2	Oxygen
P	Phosphorus
PO_4	Phosphate
POTW	Publicly Owned Treatment Works
RMA	Resource Management Associates
RMSE	Root Mean Square Error
SC	Steering Committee
SEP	Supplemental Environmental Project
SFE	San Francisco Estuary
SFEI	San Francisco Estuary Institute
Si	Silicon
TAC	Technical Advisory Committee
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
USGS	United States Geological Survey
WY	Water Year

LIST OF UNITS

cm	centimeter
L	liter
m	meter
mg	milligram
mL	milliliter
µg	microgram
µmol	micromole
s	second

1 INTRODUCTION

1.1 BACKGROUND

The Delta Regional Monitoring Program (Delta RMP) is developing a Multi-Year Nutrient Study Plan to guide long-term studies of the effects of nutrients on the ecology of the Delta. After discussion between the Delta RMP Steering Committee (SC) and the Nutrient Technical Advisory Committee (TAC), three primary questions (also referred to as focus areas) were developed to guide the development of the Study Plan.

1. Following a reduction in nutrient loading from different point and nonpoint sources, what ranges of nutrient concentrations are expected to occur throughout the Delta, and how might they be affected by climate change, wetland restorations, and water management and routing?
2. What are the thresholds for nutrients (nitrogen (N) and phosphorus (P) and their ratios) that can limit Harmful Algal Bloom (HAB) biomass and cyanotoxin accumulation to safe levels, limit the abundance and distribution of nuisance macrophytes, and support robust growth of desirable phytoplankton and macrophytes throughout the Delta?
3. How are the characteristics of harmful cyanobacteria blooms and cyanotoxins in the Delta changing (e.g., species, magnitude, geographic extent, and timing) and what factors contribute to these changes?

The Multi-Year Nutrient Study Plan addresses these three questions or focus areas using a combination of modeling, field/experimental studies, and monitoring. It is not the objective of this Multi-Year Nutrient Study Plan to completely address all three focus area questions. The intent of the studies included in this Study Plan is to begin a multi-year process that begins to address these questions with a hypothesis driven approach and prioritizing data gaps identified by the Steering Committee and Nutrient TAC.

1.2 DELTA RMP MANAGEMENT QUESTIONS

The Delta RMP has agreed upon a set of management questions that reflect specific concerns about multiple aspects of the Delta and the impacts of human activities.

Since each of the management questions is quite broad, it is important to first identify a set of more specific "assessment questions" to guide a future monitoring or special study design. **Table 1** lists the management questions that were developed by the SC and the assessment questions that were developed by the Nutrient Subcommittee in 2018. When the Delta RMP SC prioritized planning for a multi-year study plan, these questions were used as a starting point for the three primary questions or focus areas.

Table 1. Delta RMP management and assessment questions for nutrients.

TYPE	CORE MANAGEMENT QUESTIONS	NUTRIENT ASSESSMENT QUESTIONS
1. Status & Trends	Is there a problem or are there signs of a problem? 1a. Is water quality currently, or trending towards, adversely affecting beneficial uses of the Delta? 1b. Which constituents may be impairing beneficial uses in subregions of the Delta? 1c. Are trends similar or different across different subregions of the Delta?	1.1. How do concentrations of nutrients (and nutrient-associated parameters) vary spatially and temporally? A. Are trends similar or different across subregions of the Delta? B. How are ambient levels and trends affected by variability in climate, hydrology, and ecology? C. Are there important data gaps associated with particular water bodies within the Delta subregions?

Type	Core Management Questions	Nutrient Assessment Questions
2. Sources, Pathways, Loadings & Processes	Which sources and processes are most important to understand and quantify? 2a. Which sources, pathways, loadings, and processes (e.g., transformations, bioaccumulation) contribute most to identified problems? 2b. What is the magnitude of each source and/or pathway (e.g., municipal wastewater, atmospheric deposition)? 2c. What are the magnitudes of internal sources and/or pathways (e.g., benthic flux) and sinks in the Delta?	2.1. Which sources, pathways, and processes contribute most to observed levels of nutrients? A. How have nutrient or nutrient-related source controls and water management actions changed ambient levels of nutrients and nutrient-associated parameters? B. What are the loads from tributaries to the Delta? C. What are the sources and loads of nutrients within the Delta? D. What role do internal sources play in influencing observed nutrient levels? E. What are the types and sources of nutrient sinks within the Delta? F. What are the types and magnitudes of nutrient exports from the Delta to Suisun Bay and water intakes for the State and Federal Water Projects? 2.2. How are nutrients linked to water quality concerns such as harmful algal blooms, low dissolved oxygen, invasive aquatic macrophytes, low phytoplankton productivity, and drinking water issues? A. Which factors in the Delta influence the effects of nutrients on the water quality concerns listed above?

Type	Core Management Questions	Nutrient Assessment Questions
3. Forecasting Scenarios	3a. How do ambient water quality conditions respond to different management scenarios. 3b. What constituent loads can the Delta assimilate without impairment of beneficial uses? 3c. What is the likelihood that the Delta will be water quality-impaired in the future?	3.1. How will nutrient loads, concentrations, and water quality concerns from *Sources*, *Pathways*, *Loadings & Processes Question #2* respond to potential or planned future source control actions, restoration projects, water resource management changes, and climate change?
4. Effectiveness Tracking	4a. Are water quality conditions improving as a result of management actions such that beneficial uses will be met? 4b. Are loadings changing as a result of management actions?	4.1. How did nutrient loads, concentrations, and water quality concerns from *Sources*, *Pathways*, *Loadings & Processes Question #2* respond to source control actions, restoration projects, and water resource management changes?

1.3 THREE-YEAR PLANNING BUDGET

This section presents a comprehensive three-year planning budget. A nutrient budget of roughly $500,000 is allocated for each fiscal year (FY), for a total budget of $1,500,000 (FY 24-25, FY 25-26, FY 36-27). **Table 2** outlines how funds will be spent for each of the three focus areas over the course of three fiscal years. The planning budget allows for a 10% contingency ($150,000) as scopes and contracts are finalized in preparation of project implementation. The Delta RMP Annual Monitoring Workplan and final budget will include actual allocated funds for the upcoming fiscal year.

Table 2. Three-year budget for the Nutrient Multi-Year Study Plan focus areas.

Fiscal Year	Focus 1	Focus 2	Focus 3	FY Budget	FY Allocated Funds	Difference
FY 24-25	$167,500	$50,000	$150,000	$367,500	$500,000	$132,500
FY 25-26	$232,500	$265,252	$150,000	$647,752	$500,000	-$147,752
FY 26-27	$0.00	$225,252	$150,000	$375,252	$500,000	$124,748
Totals	**$400,000**	**$490,504**	**$450,000**	**$1,349,500**	**$1,500,000**	**$109,496**

2 FOCUS AREA #1

Biogeochemical (BGC) modeling efforts will be used to answer the following question by conducting a series of model scenarios based on hypothesis testing to address the following Focus Area #1 question:

- Following a reduction in nutrient loading, what ranges of nutrient concentrations are expected to occur throughout the Delta, and how might they be affected by climate change, wetland restorations, and water management and routing?

In pursuing the above question, the study targets a number of questions the Delta RMP has identified as priorities (**Table 1**), including: Management Questions 2a, 2b, 2c, 3a, and 3b; and Assessment Questions 2.1.A-F and 3.1.

2.1 PROJECT SUMMARY AND OBJECTIVES

The proposed project's high-level goals and approaches are summarized below, with details covered in subsequent sections.

High-Level Project Goals

1. Quantify the effects of nutrient source load reductions on nutrient concentrations throughout the Delta, including how those effects vary spatially, temporally (seasonally, interannually), and as a function of water management or flow-routing.
2. Evaluate in greater detail and provide visualizations of changes in nutrient concentrations within, or nutrient delivery to, regions of the Delta that are impacted by HABs and invasive aquatic vegetation (IAV).
3. Investigate additional priority hypotheses, including one or more of the following: a) Quantify the importance of remaining nutrient sources (beyond those included in scenarios) within HAB- and IAV- prone regions, including Delta non-point sources; b) Assess the relative importance of IAV on nutrient concentrations and cycling; and/or c) Characterize the relative importance of factors regulating phytoplankton biomass or productivity, including potential effects of decreased nutrient availability due to load reductions on 'beneficial production'.

Approach

To address these goals, the project will:

- Simulate hydrodynamics and biogeochemistry during water year 2022 (WY2022) and water year 2016 (WY2016), using actual nutrient loads during those years ('Base'), to predict nutrient transport, cycling, and concentrations throughout the Delta. The Base conditions include the nutrient loading rate that occurred in 2022 for both water years; see section **Model Updates, Calibration, and Validation** for more details.
- Re-simulate WY2022 and WY2016 biogeochemistry for a priority set of load reduction scenarios to forecast nutrient concentrations under each of those conditions.
- Quantify differences in nutrient concentrations between the Base and Scenario simulations, including their variability spatially (map-view concentrations), temporally (time-series at specific locations), and interannually.
- Test additional priority hypotheses (Project Goal #3) by undertaking targeted analyses of model output, including through, e.g., mass-budgets/control-volume analyses, numerical tracers (source-tracking, age/travel-time tracers), or sensitivity analyses.

Early work will include a set of tasks to extend hydrodynamic and biogeochemical simulations to WY2022 and improve model performance during low-flow conditions and reduced-load scenarios. The hydrodynamic and biogeochemical models will then be calibrated and validated for WY2022 and WY2016, and the updated model used for Base and Scenario simulations.

2.2 WHY IS THIS A PRIORITY?

Potential future regulation of N discharges could set allowable concentrations at levels meant to reduce or eliminate the proliferation of cyanobacteria and the production of cyanotoxins that are harmful to humans, companion animals, and wildlife. It is also anticipated that reductions of nutrients will lead to reductions in the growth of nuisance aquatic macrophytes. The desire is to determine if the anticipated outcomes will be realized without any adverse impacts such as decreases in the growth of desirable phytoplankton.

The goal of the modeling element of the Multi-Year Nutrient Study Plan is to identify the effects of changing Dissolved Inorganic Nitrogen (DIN) and Total Nitrogen (TN) concentrations from source areas inside and outside the Delta on the DIN and TN concentrations and chlorophyll-a (chl-a) concentrations at targeted areas in the Delta. The targeted areas include:

- Locations where beneficial algal production occurs, which can support zooplankton growth (an important food resource for zooplankton and native fishes, such as Delta Smelt) locally or distally through advection and dispersion including but not limited to:
    - Liberty Island and the North Delta
- Locations where Harmful Algal Blooms occur including but not limited to:
    - Stockton Waterfront
    - Discovery Bay
    - Franks Tract
    - Old and Middle Rivers

This project will model reductions in DIN and TN inputs to the Delta from various sources to determine if and how these reductions can affect the delivery of DIN and TN to or concentrations within regions of the Delta, in particular regions that experience HABs or IAV, and/or locations that are critical to the survival of pelagic fish in the Delta (primarily the north Delta). In pursuing the Focus Area #1 question, the study targets a number of Delta RMP priority Management Questions (2a, 2b, 2c, 3a, 3b) and Assessment Questions (2.1.A-F, 3.1) (Table 1) . The proposed work focuses in particular on the first half of the Focus Area #1 question, addressing knowledge gaps related to the relative contributions, or zones of influence, of nutrient sources within the Delta and the degree to which nutrient management options (individually, or in combination) could affect nutrient concentrations within or mass fluxes to priority management regions. There is also the potential for the modeling results to complement findings from the Focus Area #2 field studies in addressing the Focus Area #2 question. The proposed work will not directly investigate how load reduction scenario results would be influenced by factors like climate change, wetland restoration, or water management. Pursuing those topics would

require investigating additional layers of scenarios (e.g, climate change scenarios could include changes in temperature, sunlight (i.e., cloud-cover), flow, and sea-level-rise changes to flooded areas). However, some of those factors could be pursued through follow-up work that builds on this project's scenario results.

2.3 HYPOTHESES AND MODELING QUESTIONS

Nitrogen enters the Delta from point and nonpoint sources in the Sacramento Valley, San Joaquin Valley, and internal Delta from such sources as atmospheric deposition, agricultural discharges, urban runoff, and Public Owned Treatment Works (POTWs). Although regulatory actions are being considered to further reduce these inputs, it is currently unknown what the ecological responses of reduced N inputs will be. Decreased occurrences of HABs and IAV have been identified as plausible or hypothesized beneficial ecosystem responses to decreased Delta nutrient loads (Senn et al. 2020). However, thus far few studies (field or modeling) have directly investigated these potential responses. Since modules for mechanistically simulating cyano-HABs or IAV have not yet been incorporated into the northern San Francisco Estuary Biogeochemical Model (nSFE-BGCM), this project will focus on quantifying nutrient delivery to or predicted concentrations within priority management areas, and changes to those deliveries and concentrations in response to load reduction scenarios. Through continued discussions with Delta RMP stakeholders, additional priority hypotheses or management questions will be identified (example options summarized under **Analysis & Interpretation** below). Coupled with other studies and monitoring funded by the Delta RMP, it may be possible to estimate the amount of harmful cyanobacteria and cyanotoxins produced at low N concentrations throughout the Delta. These studies hopefully will access if the Delta responds similarly to other waterbodies and inform the development of nutrient regulation.

The Nutrient TAC will work with the Modeling Team to identify the most relevant set of load reduction scenarios to simulate. Approaches for establishing reduction scenarios include i) identifying a set of percentage reductions to DIN and TN from source areas and determine the relative impact on DIN, TN, and chl-a concentrations at locations in the Delta, and/or ii) establishing target DIN/TN concentrations at specific locations in the Delta and determining the percentage reduction and the location of the reductions needed to achieve the target concentrations.

Below are four initial hypotheses (null and alternative) that can be used to build model scenarios for testing the hypotheses. The hypotheses use the terminology of substantial change as a way to test the hypotheses; the Nutrient TAC will work with the modelers to define substantial (e.g., larger than background variation) as modeling is implemented and include this definition in the interpretation and reporting of model results.

2.3.1 BGC Model Hypothesis 1

H_0: Reducing the nonpoint and point source inputs of N from the Sacramento River to the Delta will have no effect on the concentration of DIN, TN, P, or chl-a at any targeted location or region in the Delta at any time during the year.

H_1: At least one targeted location/region in the Delta will experience a substantial change in the concentration of DIN, TN, P, or chl-a after reductions from point and nonpoint sources in the Sacramento Valley at some time during the year.

2.3.2 BGC Model Hypothesis 2

H_0: Reducing the nonpoint and point source inputs of N from within the Delta will have no effect on the concentration of DIN, TN, P, or chl-a at any targeted location or region in the Delta at any time during the year.

H_1: At least one targeted location in the Delta will experience a substantial change in the concentration of DIN, TN, P, or chl-a for a sustained period (e.g., days to weeks) after reductions from point and nonpoint sources in the Delta at some time during the year.

2.3.3 BGC Model Hypothesis 3

H_0: Reducing the nonpoint and point source inputs of N from the San Joaquin Valley will have no effect on the concentration of DIN, TN, P, or chl-a at any targeted location or region in the Delta at any time during the year.

H_1: At least one targeted location in the Delta will experience a substantial change in the concentration of DIN, TN, P, or chl-a for a sustained period (e.g., days to weeks) after reductions from point and nonpoint sources in the San Joaquin Valley at some time during the year.

2.3.4 BGC Model Hypothesis 4

H_0: Reducing the nonpoint and point source inputs of N simultaneously from the Sacramento River, the San Joaquin River, and internal Delta sources will have no effect on the concentration of DIN, TN, P, or chl-a at any targeted location or region in the Delta at any time during the year.

H_1: At least one targeted location/region in the Delta will experience a substantial change in the concentration of DIN, TN, P, or chl-a for a sustained period (e.g., days to weeks) after N reductions simultaneously from the Sacramento River, the San Joaquin River, and internal Delta sources at some time during the year.

2.3.5 BGC Model Scenarios

To test Hypotheses 1-4, the nSFE-BGCM will be used to simulate a series of load reduction scenarios (**Table 3**) during two proposed water years, WY2016 and WY2022. In the central and south Delta, nutrient concentrations in the winter and spring can be

higher than those in the summer and fall (Beck et al. 2018, Jabusch et al 2018). However, HABs typically occur in the summer through fall (Berg and Sutula 2015), so DIN reduction modeling scenarios were developed from Interagency Ecological Program (IEP) Environmental Monitoring Program (EMP) data collected July through October in 2022. The US EPA has recommended that states consider criteria of total N of 0.31 mg/L and total P of 0.047 mg/L for EcoRegion 1 which includes parts of Washington, Oregon, and California (EPA 2001). These concentrations are not directly related to the Delta but provide context for concentrations being evaluated for nutrient criteria in the Delta.

Internal nutrient concentrations were calculated as the difference in average DIN between Buckley Cove (1.1 mg/L-N) and Vernalis (0.36 mg/L-N) = 0.74 mg/L-N. The first two modeling scenarios reduce DIN from all sources to yield reduced concentrations (0.1 mg/L-N and 0.2 mg/L-N) that match those proposed in the Delta RMP N reduction bioassay study and reflect lowest observed concentrations detectable during the fall in the system (see section **3.3.2 N and P Reduction Bioassay Treatments** for more specifics). Scenarios 3 to 6 test percent DIN loading reductions to understand the importance of individual sources vs. a standard 20% reduction from all sources. The final scenario(s) evaluates nutrient concentrations based on the feasible limit of reductions in N loading from individual loading sources such as POTWs, municipal stormwater, and agriculture. A set of feasible N load reduction scenarios will be developed by Nutrient TAC and SC members with assistance from San Francisco Estuary Institute-Aquatic Science Center (SFEI-ASC).

Two potential phosphorus reduction scenarios may be added to the study. The Nutrient TAC will work with the modelers to identify scenario details and related hypotheses.

Table 3. Potential BGC modeling scenarios.

DIN CONCENTRATIONS IN 2022 (JULY-OCT)	DIN (MG/L-N)	REDUCTION
Sacramento River (Hood)	0.26	0%
San Joaquin River (Vernalis)	0.36	0%
Internal sources	0.74	0%

Model Scenario 1	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.1	61%
San Joaquin River (Vernalis)	0.1	72%
Internal sources	0.1	86%

Model Scenario 2	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.2	22%
San Joaquin River (Vernalis)	0.2	45%
Internal sources	0.2	73%

DIN CONCENTRATIONS IN 2022 (JULY-OCT)	DIN (MG/L-N)	REDUCTION

Model Scenario 3	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.13	50%
San Joaquin River (Vernalis)	0.36	0%
Internal sources	0.74	0%

Model Scenario 4	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.26	0%
San Joaquin River (Vernalis)	0.18	50%
Internal sources	0.74	0%

Model Scenario 5	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.26	0%
San Joaquin River (Vernalis)	0.36	0%
Internal sources	0.37	50%

Model Scenario 6	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	0.21	20%
San Joaquin River (Vernalis)	0.29	20%
Internal sources	0.59	20%

Model Scenario 7 (or more)	DIN (mg/L-N)	Reduction
Sacramento River (Hood)	Lowest feasible	TBD
San Joaquin River (Vernalis)	Lowest feasible	TBD
Internal sources	Lowest feasible	TBD

2.4 APPROACH

2.4.1 Model Overview

The core Modeling Team will consist of hydrodynamic and biogeochemical modelers from SFEI and Resource Management Associates (RMA).

Modeling work will be pursued using the nSFE-BGCM, a 3-D coupled hydrodynamic-biogeochemical model capable of simulating nutrient transport, nutrient cycling, and an array of relevant ecosystem responses (e.g., phytoplankton production). The hydrodynamic and biogeochemical models are described in detail elsewhere (SFEI 2019, 2020, 2021a), and are summarized briefly here. The SFE-BGCM uses the public-domain/open-source models D-Flow Flexible Mesh (DFM, Deltares 2019a) to simulate hydrodynamics; D-Water Quality (DWAQ; Deltares 2019b) to simulate water quality; and a suite of Python-based utilities to facilitate model setup and postprocessing. Two branches of the SFE-BGCM are maintained, emphasizing different regions of the San Francisco Estuary (SFE): the San Francisco Bay (SFB-BGCM); and the northern San Francisco Estuary (Delta, Suisun; nSFE-BGCM). The biogeochemical modules for each of the regional models have similar baseline capabilities, and refinements implemented within one regional model have been routinely transferred to other branches when relevant. For this Delta-Suisun focused analysis, the nSFE-BGCM model will be used. The model domain includes the Delta and San Francisco Bay, and extends into the Pacific Ocean to approximately Point Reyes to the north and Half Moon Bay to the south (**Figure 1**). The model has ~75,000 horizontal cells and 10 vertical layers (sigma layers). The nSFE-BGCM incorporates flows and nutrient loads from all known point sources (POTWs, refineries), along with flows and loads from upstream watersheds.

The nSFE-BGCM was developed to simulate the array of biogeochemical processes and state variables depicted in **Figure 2**, and summarized in the extended figure caption. Through recent Delta-Suisun focused projects (SFEI 2021a, 2021b) a number of substantial improvements were made, including (see also **Figure 3**): water column transformations and sediment diagenesis; adjustments to clam and zooplankton initial conditions and grazing rates informed by comparisons with biomass and grazing data from a complementary modeling effort; refining boundary conditions for nutrient loading from both freshwater sources and POTWs; developing spatially varying initial conditions for nutrient concentrations; calculating space-time varying light-attenuation coefficients using the network of high frequency turbidity sensors throughout the Delta; and developing a “global” calibration that performed well at predicting N, P, and silica (Si) concentrations across two water years with strongly differing physical conditions (WY2011, wet; WY2016, dry) and biogeochemical responses. Through those projects the Modeling Team have expanded capacity for processing model output, including establishing regional and sub-regional control volumes and quantifying mass budgets over

relevant time periods (**Figure 4**). Using this modeling framework, it is also possible to introduce several types of numerical 'tracer' techniques (conservative tracers, age tracers, habitat exposure tracers) and track their movement over space and time. These tracers can provide valuable information about, e.g., a point-source's zone of influence, and recent tracer applications in the Delta have demonstrated great promise at, for example, estimating rates of N loss in regions with dense growth of IAV, and detecting and tracking signals of ag-return-flow waters.

An important limitation of the prior version of the nSFE-BGCM also emerged during the WY2016 simulation, with results indicating that transport was under-resolved within the Cache Slough Complex (CSC) which affected the reliability of nutrient concentration predictions in the upper CSC. That issue will be remedied during this project by incorporating an improved bathymetry dataset and model grid within the CSC that were recently implemented as part of another related project (see below in section **Model Updates, Setup, and Calibration for WY2022 and WY2016**).

Model Updates, Calibration, and Validation

For this project, two water years will be simulated, WY2016 and WY2022. Of the two years that were previously calibrated (WY2016, WY2011), WY2016 is recommended because of the ample water quality monitoring data available for model calibration and validation (water quality moorings, high-resolution mapping, in addition to monthly discrete data). Key reasons for simulating WY2022 include: i) comparable or greater water quality monitoring as WY2016; and ii) the EchoWater Resource Recovery Facility's upgrades were completed and online during WY2022, allowing for a post-upgrade time-period to be included in model calibration/validation.

Model Updates, Setup, and Calibration for WY2022 and WY2016

Initial work will include incorporating several major improvements to the model grid and bathymetry (CSC, Suisun Bay, and portions of the Sacramento River, near the Delta Cross Channel (DCC)) (**Figure 5**). The grid and bathymetry improvements were developed through a recent project and have already been merged with the broader nSFE-BGCM domain. Remaining steps include updating model set-up scripts (re-plumbing boundary conditions or inputs into appropriate new grid cells) and post-processing scripts (analysis, plotting) to align with the altered grid, and incorporating any minor refinements that emerge during early test runs.

Hydrodynamic runs will be set up for WY2022 and WY2016, using the new grid and bathymetry. Hydrodynamic input files (boundary conditions, forcings) will be developed for WY2022, including river flows, point source flows, meteorological data, and gate and pump operations (WY2016 data already compiled). Model setup for WY2022 will also

require incorporating flow alterations at the Old River Drought Barrier (trial runs, iterations to fine-tune), During dry and critically dry years like WY2016 and WY2022, interior-Delta water withdrawals/returns (i.e., 'ag return flows', Delta Island Consumptive Use (DICU)) can affect both flow routing and biogeochemistry. The influence of interior-Delta flow withdrawals/returns will be estimated by incorporating spatially distributed daily flows from the Delta Channel Depletion dataset (DCDv1.0; CA DWR 2018). A major focus of the hydrodynamic calibration work will be on accurately representing discharge and water elevations at structures/drought-barriers, within the CSC, and within regions affected by HABs and IAV.

Biogeochemical model input files will be developed for WY2022, including (see SFEI 2021b): nutrient concentrations at model boundaries for estimating loads entering the Delta (e.g., Battey and Perry 2023); spatially and temporally (hourly to daily) varying light attenuation coefficients, estimated through interpolating turbidity data from the Delta's network of continuous turbidity sensors (DWR and USGS networks); and abundances of benthic (clam) (e.g., Wells et al., 2023; Zierdt et al. 2021); and pelagic (zooplankton) grazers (Burdi et al., 2023). Additional information on model boundary condition and forcing data can be found in SFEI 2021b. The Delta-focused biogeochemical model will be updated with relevant improvements made through recent Bay modeling work, including refinements to the sediment biogeochemical module (nutrient fluxes), and phytoplankton production and grazing modules. A major focus of effort will then be on developing an updated global biogeochemical calibration for water years 2022 and 2016.

Model Validation

The hydrodynamic model will be validated for WY2016 and WY2022 by comparing time series of modeled and observed discharge, gauge height, salinity, and temperature at approximately 60 measurement stations across the Delta and Suisun Bay, and assessing performance using a suite of validation statistics (e.g., bias, root mean square error (RMSE), skill, r^2, tidal amplitude ratio, lag) (see SFEI 2019).

The biogeochemical model will be validated for WY2022 and WY2016 by comparing model-predicted concentrations of priority water quality parameters (e.g., nitrate, ammonium, phosphate, silica, chl-a) with observed data. For the above parameters, discrete monthly and semi-monthly data are available from 10-15 sites across the Delta and Suisun Bay (see **Figure 4**; Battey and Perry 2023]. Modeled nitrate and chl-a concentrations will be compared with the USGS's extensive network of moored monitoring stations in this region. Lastly, modeled nitrate and chl-a values will also be compared with data from USGS high-speed mapping surveys conducted during 2016 and 2022. Additional information on data sources for model validation can be found in SFEI 2021b.

2.4.2 Load Reduction Scenario Simulations

As noted in Section 2.3.1, the calibrated biogeochemical model will be used to simulate load reduction scenarios to assess the influence of load reductions on nutrient availability within the Delta. The final set of scenarios will be identified through consultation with the Nutrient TAC. In addition to the nitrogen-focused scenarios in **Table 3** at least one phosphorus reduction scenario will also be simulated.

Load reduction scenarios will be simulated and compared with 'Base' conditions. Two Base Cases will be established:

- $WY2022_{base}$: results from the WY2022 biogeochemical simulation, using actual loads during WY2022.
- $WY2016_{base}$: After model calibration (using actual WY2016 loads), WY2016 biogeochemistry will be re-simulated using post-upgrade loads at the EchoWater Facility, along with other upgrade-related changes to Delta point-source loads, with those model results serving as $WY2016_{base}$ (For nonpoint-source loads, WY2016 loads will be used).

Load reduction scenarios will be set-up and simulated as follows (for each scenario):

- *Scenario Load Estimates*: For each source that will be changed, nutrient concentrations or loads will be translated into a daily time-series.
- *Scenario Simulations:*
    - The updated load time-series will be substituted for the actual load time-series used for the Base case.
    - WY2022 and WY2016 biogeochemistry will be re-simulated using the scenario loads, with all other model inputs/boundary conditions/forcings the same as the base case, except as noted below.
    - Changes in nutrient concentrations will be quantified by comparing scenario conditions ($WY2022_{scenario}$, $WY2016_{scenario}$) with either $WY2022_{base}$ or $WY2016_{base}$.
- *Other Model Adjustments for Scenario Runs*: As needed, water column initial conditions (i.e., starting concentrations assigned throughout the domain) will be adjusted from the Base Case values. In some cases, adjustments to sediment conditions (and/or nutrient flux rates) may also be relevant to consider. The proposed approach to sediment-adjustments will involve: i) Assess the importance of sediment fluxes to water column nutrient concentrations or budgets (for Base case); ii) When necessary (i.e., flux is both quantitatively important and may overestimate fluxes under the scenario), a basic proportional adjustment to

sediment fluxes will be implemented. *Note*: More nuanced sediment-flux adjustments may be warranted for some cases, including considering the zones of influence of the altered sources, or the contribution of particulate nutrients from upstream (allochthonous organic matter) to the sediment nutrient pool.

The current budget is based on an assumption that four (4) load reduction scenarios will be explored, with each scenario simulated for WY2022 and WY2016 (8 year-scenario simulations).

2.4.3 Analysis & Interpretation

One of the primary outputs from this work will be the quantification of pre-/post-Scenario differences in N concentrations or fluxes (spatially, temporally), as described above. Where relevant, changes in P concentrations and fluxes will also be evaluated. Analysis of model output for the scenarios will include (for each scenario and water year):

- Delta-wide map-views of nutrient concentrations, at relevant times of the year:
    - Base concentrations, Scenario concentrations, Difference = Base-Scenario
    - Time period: plots can be developed for e.g., weekly- or monthly averages for representative times of the year, or daily average examples.
- For high-priority regions (e.g., HAB- or IAV-prone regions), changes in nutrient availability will also be investigated in greater detail.
    - Time-series of nutrient concentrations at specific stations or spatially averaged within areas of interest (Base, Scenario, Difference = Base-Scenario).
    - Changes in nutrient transport (mass flux, kg/d) into an area of interest (e.g., difference in the kg/d of DIN entering a region between $WY2022_{base}$ and $WY2022_{scenario}$).

Five of the seven priority regions (HAB- or IAV-prone regions) highlighted in **Section 2.2 Why is this a priority?** (Franks Tract, Old River, Middle River, Liberty Island, North Delta) are in the interior of the model domain, and are well-resolved by the grid. For each of these regions, load reduction scenarios will be examined by comparing DIN concentrations within, and mass fluxes into, the region. Both the Stockton Waterfront and Discovery Bay are positioned at or near the boundary of the model domain. For the Stockton Waterfront, the model grid extends along the majority of the Stockton Ship Channel (~2.8 km), but the region is not gridded at high-resolution, and data are relatively sparse for biogeochemical model validation. Discovery Bay is connected to the model grid at the boundary; however, the grid does not extend into Discovery Bay. For the Stockton Waterfront and Discovery Bay, the influence of load reduction scenarios will be examined primarily by characterizing changes in DIN mass fluxes into these regions (relative to the

Base simulations). These regions and relevant scenarios could be investigated further in follow-up modeling studies, informed by the results from this project.

The final technical report will include relevant graphics along with descriptive analysis of results. The current budget is based on the assumption that four load reduction scenarios will be simulated and analyzed for each water year (8 year-scenario simulations).

A second central aim of this work is to investigate one or more additional priority nutrient-related hypotheses, leveraging the same simulations, through using mass-budgets/control-volume analyses, numerical tracers, or other approaches. Examples include:

- Characterizing the relative importance of physical/biological/biogeochemical factors regulating phytoplankton biomass or productivity, including potential effects of lower nutrient availability due to load reductions.

Quantifying the importance of remaining nutrient sources (beyond those included in scenarios) within HAB- and IAV- prone regions, including Delta non-point sources; and/or assessing the relative importance of IAV on nutrient concentrations and cycling. This work could focus on regions where beneficial algal production occurs (Liberty Island, North Delta); or regions where HABs and/or IAV impact water quality and habitat quality (Stockton Waterfront; Franks Tract; Old and Middle Rivers). The specific combination of techniques used to investigate these issues will vary by topic, and may include some or all of the following: analysis of additional model output within regions of interest (e.g., changes to primary productivity or evidence of nutrient-limited growth rates within regions of interest); quantification of nutrient source contributions or additional nutrient losses within a region, using mass balance and various tracer approaches, The specific focus of this component of the project will be finalized with input from the Nutrient TAC. The current project cost estimate includes budget to pursue one of the above analysis-directions, with the potential to pursue additional hypotheses depending on their depth of analysis.

2.5 DATA DELIVERABLES AND REPORTS

2.5.1 Data Management & Data Deliverables

Modeling work is being conducted with open-source/public-domain tools, and all data, model output, and scripts.

2.5.2 Reporting

The primary deliverable will be a Technical Report, presenting the following:

1. Hydrodynamic and biogeochemical model validation, along with description of the model and relevant model updates.

2. Results of Base-Case and Scenario simulations, with the current budget based on an assumption of four scenarios (simulated during both water years). Analyses will include:
    a. Delta/Suisun-wide: analysis/interpretation of differences in nutrient concentrations, over space and time (seasonal, interannual).
    b. Additional focus or depth of analysis within priority regions, i.e., regions that are considered HAB-prone, IAV-prone, or where fostering beneficial production is a management priority.
3. Analysis/Interpretation of additional priority hypotheses or science/management questions.
4. For #3 (and potentially other components), the technical report may be written in the form of manuscript (time-permitting) for submission to a peer-reviewed journal, with additional analyses/figures/documentation that are relevant to the Delta RMP included in appendices as needed.

2.6 STUDY TIMELINE AND SCHEDULE

Project work will begin in July 2024, aligned with the start of the Delta RMP's FY2025. Assuming a 2-year project, work would proceed as follows (approximate):

- *0-6 months* hydrodynamic model setup and initial calibration work, initial biogeochemical model setup;
- *7-12 months* finalize hydrodynamic calibration and validation, setup/calibrate/validate biogeochemical model, and initial scenario/analysis work;
- *13-18 months* complete scenario simulations/visualizations and analysis;
- *19-24 months* report preparation.

2.7 BUDGET ESTIMATE

The cost estimates below (**Table 4**) are approximate and may vary depending on decisions related to the number of scenarios, and the breadth and depth of additional hypotheses to pursue.

Table 4. Cost estimates for hydrodynamic and biogeochemical modeling, and subsequent analysis, interpretation, and writing of a final technical report.

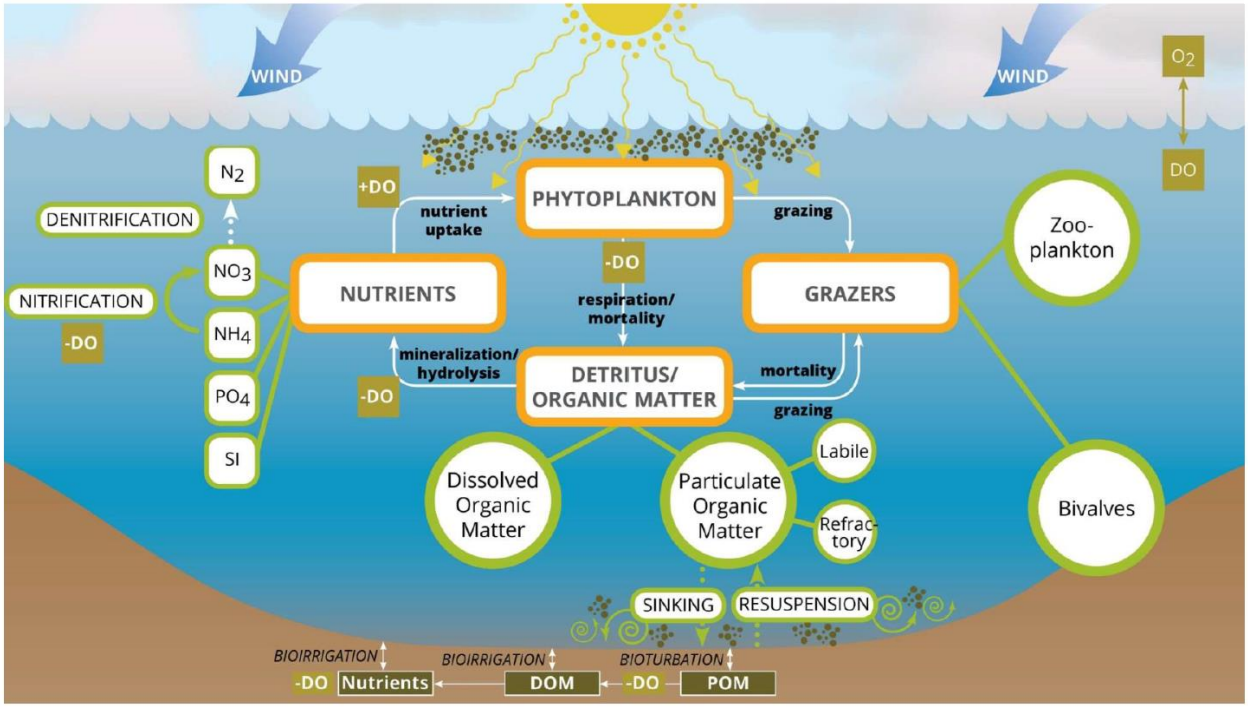
TASK	COST
1. Hydrodynamic: model updates, setup, calibration & validation	$90,000
2. Biogeochemical: model updates, setup, calibration & validation	$135,000
2a. Optional Phosphorus reduction scenarios	*$20,000*

TASK	COST
3. Analysis, Interpretation, Write-up	$155,000
Total	**$380,000**
Total with Optional Phosphorus reduction scenarios	***$400,000***

The above budget (**Table 4**) is based on an estimate of eight load reduction scenarios (8 scenarios simulated for two years each) being explored in depth, as opposed to all seven in **Table 3**, considering that there may be substantial information-overlap among some of the scenarios and not all will be required. Scenarios can be added as needed, at an estimated cost of $7,000-$10,000/scenario (for example, if only four scenarios are needed the cost would be reduced by approximately $30,000).

Figure 1. Model domain of the current nSFE-BGCM.

Figure 2. Schematic of state variables and processes simulated by the nSFE-BGCM.



Important water column and sediment-compartment processes include:

Water Column Processes

- Microbial: nitrification; respiration (dissolved oxygen [DO] consumption) and remineralization of organic matter (converting organic forms of nutrients, including dead phytoplankton, to inorganic forms).
- Phytoplankton: growth (production of new biomass), uptake/assimilation of nutrients, respiration, mortality.
- Grazers: grazing (consumption of phytoplankton), excretion of nutrients, growth (increased biomass), respiration, mortality
- Oxygen (O2) exchange between the water column and atmosphere.
- Light attenuation by suspended sediment and phytoplankton.

Sediment Processes

- Microbial: nitrification, denitrification, aerobic respiration (DO consumption), and mineralization of organic matter (converting organic forms of nutrients to inorganic forms).
- Benthic grazing: filtration/consumption of phytoplankton and detritus, excretion of nutrients, growth (increased biomass), reproduction, and death.
- Accumulation of organic matter (settling from the water column) and mixing/bioturbation of sediments.
- Sediment←→ Water: flux of Ammonium (NH4), Nitrate (NO3), Phosphate (PO4), and Si from the sediments to the water column, flux of NO3 and O2 from the water column to the sediments (denitrification and oxygen consumption, respectively, at the sediment-water interface).

Figure 3. Summary of major updates and improvements to the nSFE-BGCM incorporated during recent projects, including the recent DRMP-funded modeling project.
Additional biogeochemical model refinements (developed through on-going Bay modeling work) will be incorporated into the nSFE-BGCM during this project and applied to WY2022 and WY2016 simulations.

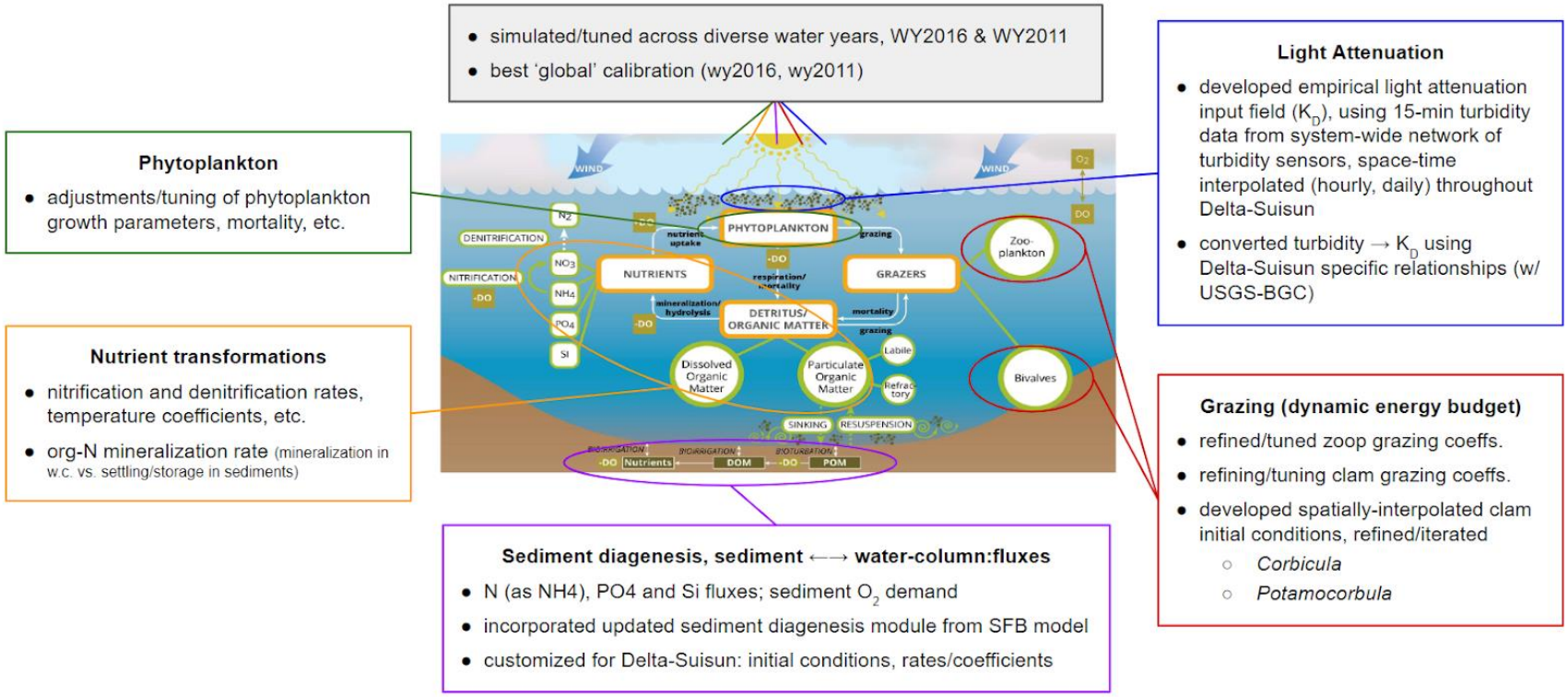


Figure 4. Regional control volumes used in nSFE-BGCM simulation for WY2016.
A set of finer resolution control volumes was also established (5-20 per region), allowing for more targeted analyses and interpretations. Simulations are run at full-spatial and full-temporal resolutions, and internal transformation rates are daily-integrated and spatially averaged. (Red circles indicate the locations of some of the monthly discrete monitoring stations used for model validation).

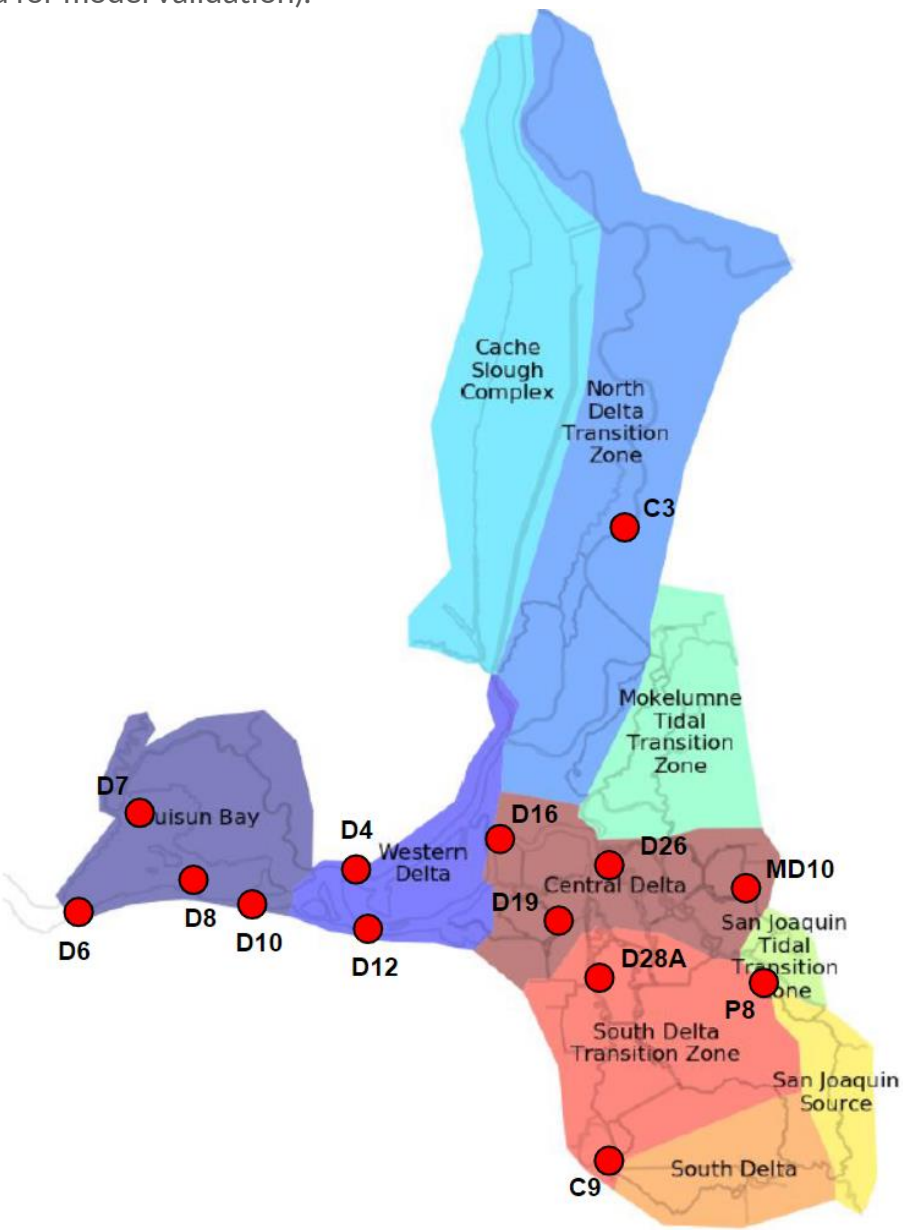
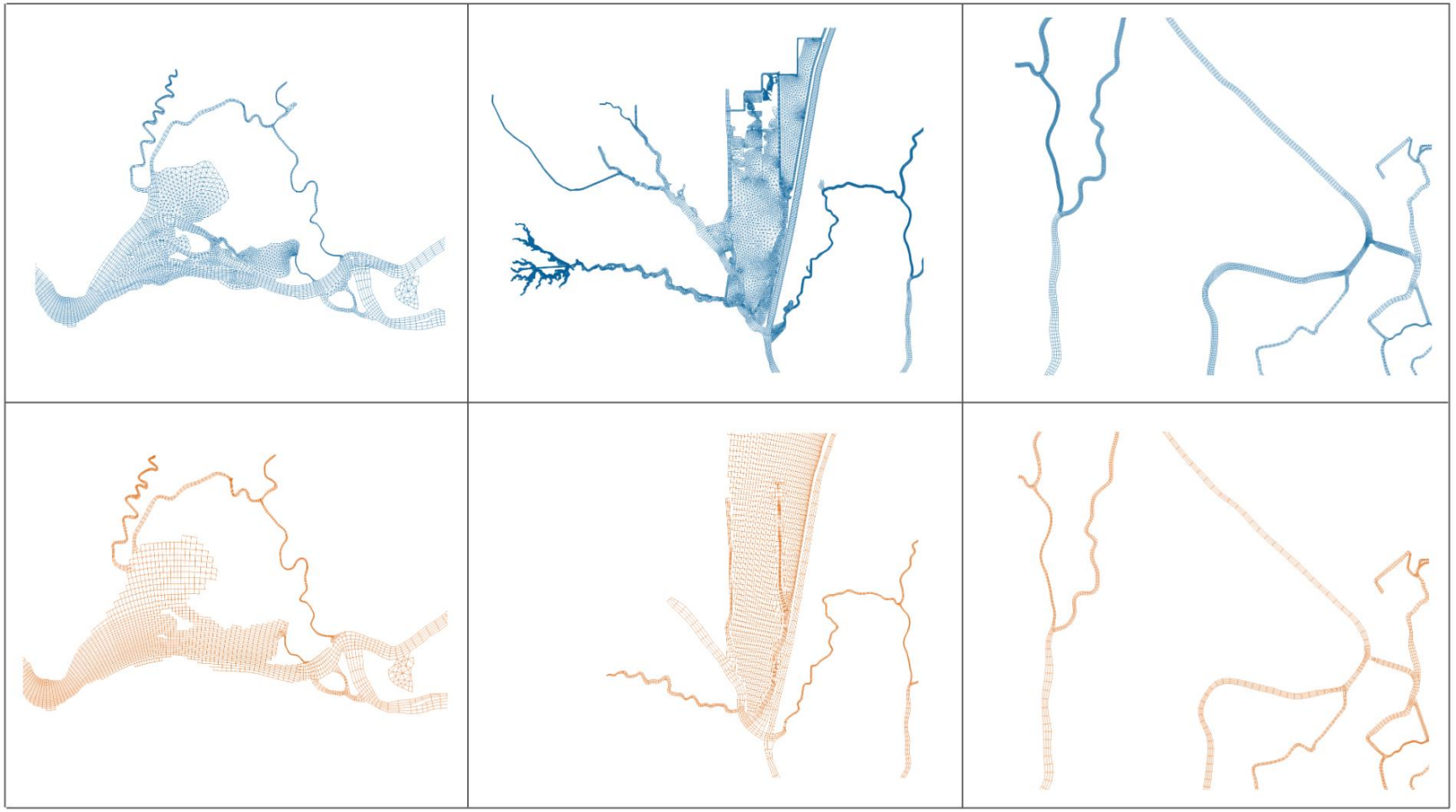


Figure 5. Current and new nSFE-BGCM grids.
Bottom: Subsets of grid used for prior nSFE-BGCM work; Top: Updated grid (Holleman, et al., in prep.), to be used for the proposed project. The grid updates have already been merged with the rest of the domain in **Figure 1**. The remaining work includes 're-plumbing' boundary conditions/forcings, and updating model set-up (control-volume or transect boundaries) and post-processing scripts (analysis, plotting), etc.



3 FOCUS AREA #2

To assist with understanding the ecological effects of nutrient reductions, a bioassay study will be used to answer the following Focus Area #2 question:

- What are the thresholds for nutrients (N and P and their ratios) that can limit HAB biomass and cyanotoxin accumulation to safe levels, limit the abundance and distribution of nuisance macrophytes, and support robust growth of desirable phytoplankton and macrophytes throughout the Delta?

3.1 STUDY OBJECTIVES

The study objective is to understand how reductions in N and P concentrations might affect phytoplankton species composition, biomass, and cyanotoxin production in the Delta and to identify if other environmental factors will influence phytoplankton growth at low N or P concentrations (potentially altering the outcomes of nutrient reduction actions). This study is designed to partially inform one of the management questions identified as a high priority by the Delta RMP SC. "What are the thresholds for nutrients (N and P and their ratios) that can limit HAB biomass and cyanotoxin accumulation to safe levels, limit the abundance and distribution of nuisance macrophytes, and support robust growth of desirable phytoplankton and macrophytes throughout the Delta?" It also follows a research recommendation in the Delta Nutrient Research Plan (Cooke et al. 2018), to perform a "Study of potential for changes in nutrients or physical drivers to reduce frequency and magnitude of harmful cyanobacteria blooms and toxins".

This study will use controlled and replicated bioassay experiments to investigate how phytoplankton sourced from the south Delta responds to limited N or P availability. Bioassay experiments simplify complex natural processes by controlling specific factors and can be used to test a hypothesis in a similar but controlled environment. However, there are limitations to bioassay experiments focused on phytoplankton communities, including unintended impacts of the study design such as: deleterious impacts of the enclosure on physiological performance of phytoplankton, potential changes in species composition, and the potential of inducing the limitation of other nutrients when adding another macronutrient (Beardall et al 2001). Results of these types of studies should be used in context of the limitations of the study design recognizing that they will not be a perfect representation of the Delta. More details regarding limitations of the bioassay design are included in section **Limitations of the Bioassay Design**.

Dilution bioassay studies allow the effects of low nutrient concentrations on phytoplankton to be evaluated in waterways where low nutrient concentrations rarely

occur. A recent bioassay testing Sandusky Bay water from Western Lake Erie determined that a 40% P reduction prevented the increase of microcystin concentrations in 3-day experiments compared to undiluted treatments where Microcystin concentrations roughly doubled (Barnard et al. 2021). These bioassay experiments also determined that in August low N concentrations limited chl-a production and production of the cyanotoxin anatoxin in Sandusky Bay water.

It is also important to recognize that the Delta is a complex ecosystem and many factors other than nutrient concentrations can influence phytoplankton growth, such light limitation, salinity, water temperature, nutrient competition with macrophytes, grazing losses to clams, and differences in water residence times. Therefore, a second set of mesocosm studies is proposed to examine how phytoplankton respond to nutrient reductions in combination with some of these other common environmental variables that can be manipulated in the bioassay containers (additional details are provided in the methods section). The goal for the second set of treatments is to determine if other environmental factors can have a large influence on phytoplankton community responses at low N concentrations. It is recommended that all of the multiple-factor treatments be conducted at a single low nitrate (DIN) concentration, such as 0.1 mg/L-N, allowing direct comparisons between treatments and control. If one of these environmental factors shows a strong effect on phytoplankton biomass, HAB biomass, or cyanotoxin production, compared to that of the low N concentration control, then the factor should be tested further in separate (future) sets of experiments across a range of low N concentrations. Future studies would be necessary to further understand how phytoplankton responses to a range of low nutrient reduction might differ in the presence of other common environmental factors.

This study is a first step in understanding how phytoplankton communities in the Delta can respond to low nutrient concentrations. It is currently unknown which species will dominate phytoplankton communities grown at low nutrient concentrations. The findings from this study will guide future research investigating the potential ecological effects of reduced nutrient loading into the Delta. Additional manipulative studies, comparisons to samples collected in the waterway, and biogeochemical and hydrological modeling are also needed to continue the development of nutrient objectives for the Delta and Suisun Bay.

The bioassay experiment is an exploratory study that seeks to inform the broad questions listed below using nutrient dilution assays.

- Would N or P reduction reduce HAB growth in the Delta? If so, what level of N or P reduction is needed to significantly reduce HAB growth and cyanotoxin concentrations to acceptable levels in the Delta?

- Would N or P reduction reduce the biomass of desirable phytoplankton in the Delta? If so, is the reduction significant and meaningful?
- Would N or P reduction significantly alter the growth of specific cyanobacteria that cause taste and odor problems for drinking water systems?
- How do other environmental factors, such as light limitation, aquatic plant growth, or clam grazing, alter the effects of N or P reductions on HABs and/or phytoplankton populations?

3.1.1 Why is this a priority?

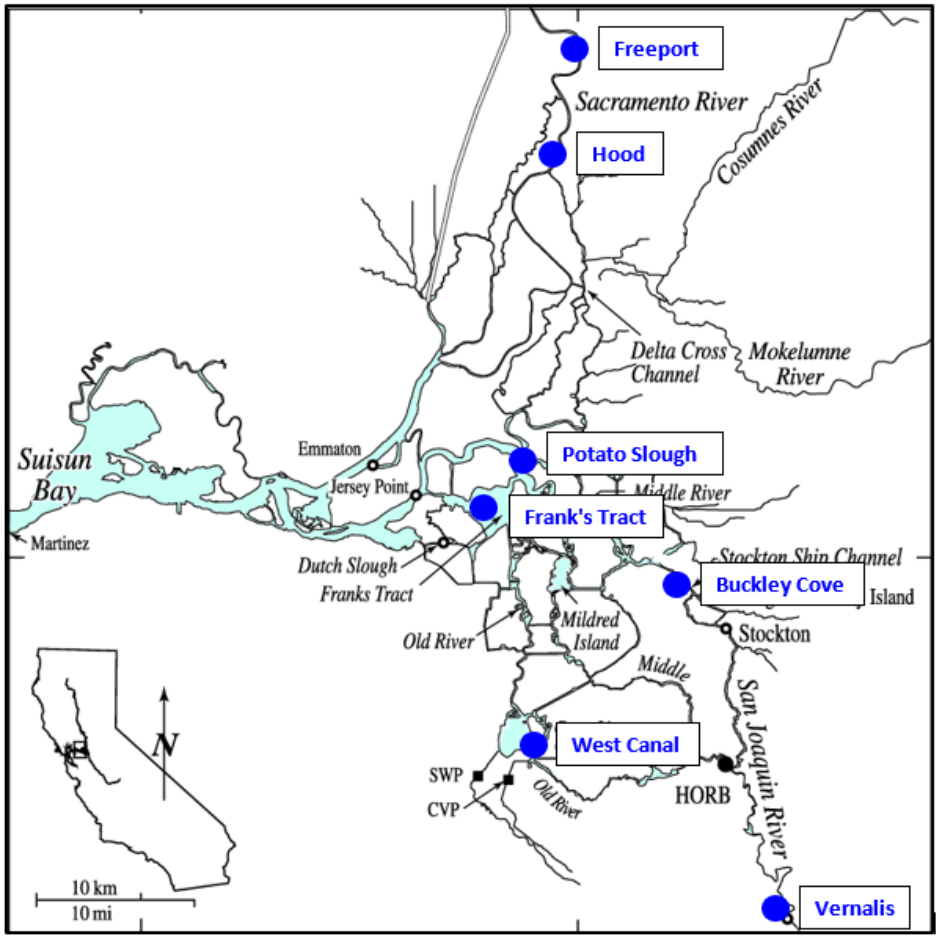
The Central Valley Regional Water Quality Control Board's (CVRWQCB or Central Valley Water Board) Delta Nutrient Research Plan identified research recommendations for further research to better address nutrient management questions in the Sacramento-San Joaquin Delta (Delta) (Cooke et al. 2018). The top-ranking special study recommendation was to determine the roles of nutrients and other drivers in controlling the growth rate, maximum biomass, and toxin production of HABs. The Central Valley Water Board noted that they anticipate the possible development of nutrient benchmarks and/or reduction goals during the Delta Nutrient Research Plan implementation. Accordingly, the Delta RMP Nutrient TAC has developed a study to evaluate the potential effects of nutrient reductions on phytoplankton in the Delta. Reduced nutrient concentrations in the Delta might help control the occurrence and severity of HABs, such as *Microcystis* sp., *Aphanizomenon* sp., and *Dolichospermum* sp. and reduce cyanotoxin concentrations, such as microcystin, anatoxin, saxitoxins, and cylindrospermopsin. However, nutrient reduction also has the potential to reduce the growth of desirable phytoplankton species, such as diatoms, which provide an important base to the Delta's pelagic food web.

Low concentrations of nutrients, including N and P, can limit phytoplankton and cyanobacteria growth. In the summer and fall periods of 2022, the average ratio of N:P in the Delta ranged from 6:1 to 24:2 (Battey and Perry 2023, Error! Reference source not found.), indicating that N supply might become depleted before P during phytoplankton blooms at some stations. If N is depleted in the early stages of a bloom, it might reduce the HAB biomass, shorten the HAB duration, and decrease the cyanotoxin concentration produced. Limiting nitrogen concentrations might also allow N-fixing cyanobacteria to become more prevalent in the Delta phytoplankton community, although N-fixing cyanobacteria can also supply N to other cyanobacteria species when they die (Molot et al. 2017).

Table 5. Average monthly N and P concentrations and standard errors at Delta locations measured by the IEP Environmental Monitoring Program between June and October 2022.
Samples with concentrations below the analytical minimum detection level were averaged using the detection limit. The less than symbol indicated that all sample concentrations in the data set are less than the analytical minimum detection level. Values are concentrations +/- (1 standard error). Standard errors were not calculated for stations where all values were below the analytical minimum detection level and are marked with "n/a".

Location	Station ID	Latitude	Longitude	DIN mg/L-N	Ortho-P mg/L-P	Total N mg/L-N	Total P mg/L-P	Total N µmol/L	Total P µmol/L	TN:TP molar ratio
Vernalis	C10A	37.679	-121.265	0.33 (0.07)	< 0.05 (n/a)	0.43 (0.08)	0.04 (0.004)	31.1 (5.8)	1.3 (0.1)	24.2
Hood	C3A	38.367	-121.521	0.24 (0.02)	< 0.05 (n/a)	0.31 (0.02)	0.03 (0.002)	22.3 (1.4)	1.0 (0.1)	23.1
West Canal	C9	37.830	-121.554	0.16 (0.03)	0.08 (0.007)	0.40 (0.07)	0.09 (0.009)	28.8 (4.9)	3.0 (0.3)	9.5
Buckley Cove	P8	37.978	-121.382	1.60 (0.51)	0.35 (0.03)	2.08 (0.51)	0.35 (0.03)	148.6 (36.7)	11.4 (1.0)	13.0
Frank's Tract	D19	38.043	-121.615	< 0.1 (n/a)	0.05 (0.002)	0.25 (0.01)	0.06 (0.003)	17.6 (0.7)	1.9 (0.1)	9.1
Potato Slough	D26	38.076	-121.567	0.13 (0.01)	0.05 (0.003)	0.26 (0.02)	0.10 (0.04)	18.7 (1.6)	3.1 (1.2)	6.1

Figure 6. A map of selected IEP EMP Monitoring Program station locations.



3.1.2 Background

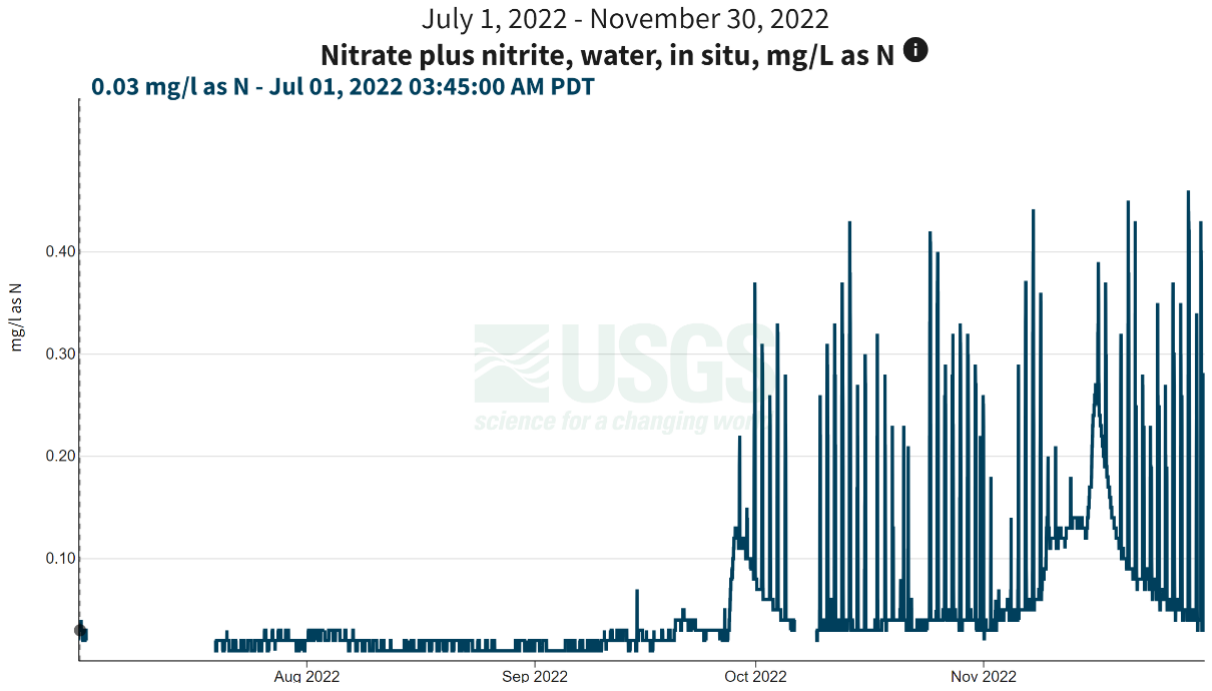
During 2022, California was in a prolonged drought and *Microcystis* sp. blooms were common in the South Delta from June to October (Battey and Perry 2023). Phytoplankton grew using the dissolved fraction of N in the water (DIN), which was mostly in the form of nitrate from June to October of 2022 (Battey and Perry 2023). Water quality monitoring (IEP EMP) in the Delta (at the stations indicated in **Figure 6**) determined that ammonium was often below the detection limit of 0.05 mg/L-N from June to October in 2022, with the only detectable ammonium concentrations of 0.06 and 0.07 occurring at Buckley Cove in September and October, respectively (Battey and Perry 2023).

In general, the average DIN concentrations were lower in stations receiving Sacramento River water compared to those receiving San Joaquin River water (Error! Reference source not found.). Potato Slough N concentrations were lower than that supplied by Sacramento or San Joaquin Rivers, suggesting that nutrient drawdown had occurred before the water reached this station. The average concentration of N in July through August 2022 at Freeport Bridge in the Sacramento River was < 0.05 mg/L-N (**Figure 7**,

USGS 2023). The Freeport monitoring station is located just upstream of the Sacramento Area Sewer District's (SacSewer) discharge location, so reverse flows occurring in late September and November created short-term spikes in N. At the Hood monitoring station, which is downstream of SacSewer's discharge location, the effluent was well mixed with Sacramento River water and the average N was approximately 0.2 mg/L-N (Error! Reference source not found.). Therefore, the range in DIN occurring throughout the Delta in 2022, from 0.05 mg/L-N to 1.0 mg/L-N, provides a good range of DIN concentrations to evaluate phytoplankton response to differing N availability.

During this same period, the average of total P observed at monitored sites ranged from 0.03 to 0.35 mg/L-P, which serves as a benchmark for identifying a range of potential P reductions and N:P ratios suitable for evaluation along with DIN in nutrient reduction experiments.

Figure 7. Nitrate plus Nitrite concentrations at the USGS Freeport monitoring station on the Sacramento River from 7/1/2022 to 11/30/2022.
Concentration spikes in October and November are caused by Sacramento River flow reversals briefly transporting wastewater effluent to the Freeport Station.



3.2 HYPOTHESES

This study tests multiple N and P concentrations that occurred throughout the Delta during the 2022 drought when nutrient dilution was likely minimal. *Microcystis sp.* was common in the south Delta during this time (Battey and Perry 2023). The findings from this study should be compared to nutrient, chl-a, HAB, and phytoplankton enumerations

data collected from the Delta in 2022 to determine if similar chl-a concentrations and phytoplankton species occur in Delta locations with matching environmental parameters. Particularly strong interacting factors should be further investigated across a range of low N concentrations in future experiments.

The bioassay addresses an important question for nitrogen management; which phytoplankton species and how much phytoplankton biomass are likely to grow in the Delta at low N and P concentrations under ideal growing conditions? Phytoplankton (and HAB) management strategies also need to identify expected nutrient concentrations throughout the Delta under reduced nutrient loading (investigated by Focus Area 1) and how other factors known to reduce phytoplankton growth might interact with low dissolved inorganic nitrogen (DIN) to affect phytoplankton species biomass and occurrence.

Other important factors that should be studied in combination with nutrient limitation include light or silica limitation, reduced growth periods due to increased flows, temperature effects, herbicide effects, salinity effects, stratification, competition with macrophytes, grazing by herbivores, and mortality from disease and parasites. If a model can combine all the known outcomes of these interacting factors on phytoplankton growth, and estimate the biogeochemical nitrogen cycle, it should provide reasonable predictions for how phytoplankton would respond to nutrient loading reductions in the Delta.

The proposed bioassay study provides a useful first step by identifying the upper limit of phytoplankton biomass that might occur in the Delta at different low DIN and P concentrations in the absence of these other regulating factors. The study will also help evaluate if light limitation, competition with a submerged macrophyte, or grazing by clams might have substantial impacts on phytoplankton growth at low nutrient concentrations.

The findings from this study will help California State regulators and stakeholders estimate the upper limit of cyanobacteria biomass and cyanotoxins that can be produced at low N or P concentrations, under conditions promoting phytoplankton growth. This information will help California State regulators and stakeholders evaluate the level of nutrient reduction that might result in material reductions in cyanobacteria populations. The findings will also help determine if low N or P concentrations might limit the biomass of beneficial phytoplankton produced in the Delta. Chlorophyll-a concentrations above 10 µg/L have been shown to support maximal zooplankton growth rates (Müller-Solger et al. 2002). The study also provides an initial investigation into potential interactions between low N concentrations and other factors known to affect phytoplankton biomass in the Delta, including light limitation, nutrient competition with macrophytes, and grazing losses to clams, to assess the importance of combined effects. Detailed descriptions of these multi-factor treatments are provided in the methods section.

The proposed hypotheses to be tested are:

1. Low N or low P concentrations will prevent cyanobacteria from growing to nuisance concentrations and producing harmful concentrations of cyanotoxins.
2. Low N or low P concentrations will prevent beneficial phytoplankton species from growing to concentrations that support robust zooplankton growth (i.e., 10 µmol chl-a).
3. Low N or low P concentrations will reduce the biomass of specific planktonic cyanobacteria to concentrations that may not cause taste and odor problems for drinking water systems.
4. Phytoplankton species grown at a low N concentration and low light levels will differ from those grown at a low N concentration with moderate light levels.
5. At a low N concentration, nutrient competition with *Egeria densa* and its associated periphyton will result in lower cyanobacteria biomass, cyanotoxin concentrations, and beneficial phytoplankton biomass.
6. At a low N concentration, the presence of clams will reduce the accumulation of cyanobacteria, cyanotoxins, and beneficial phytoplankton biomass compared to phytoplankton grown at a low N concentration without clams.

3.3 MONITORING STRATEGY

The methods described here are provided for discussions of the study design which will be adjusted and refined by the Principal Investigator in close coordination with the Nutrient TAC members. All parameters and procedures will be adjusted to best evaluate the research questions and hypotheses. The final study design will be included in the Delta RMP Monitoring Workplan.

3.3.1 Pilot Scoping Studies

An initial set of pilot studies, testing different variables of the project design, such as sampling locations, water volumes, incubation duration, dilution water chemistry, N and P concentrations, salinities, light levels, clam biomass, and macrophyte biomass, should be performed prior to running the fully replicated study.

3.3.2 N and P Reduction Bioassay Treatments

Bioassay Treatments 1-8

Target N and P concentrations in the N reduction and P reduction bioassays (Treatments #1-8, **Table 6**) were selected to represent the range of average DIN and P concentrations occurring from June to October at different stations in the Delta in 2022. The highest average DIN and P concentrations occurred at Buckley Cove (roughly 1.6 mg/L-N and 0.35 mg/L-P, Error! Reference source not found.). The lowest DIN concentration occurred at Freeport (roughly 0.05 mg/L-N, **Figure 7**), while the lowest P concentration occurred at Hood (0.03 mg/L-P, Error! Reference source not found.). The N and P concentrations used in treatments #1-8 represent different levels of nutrient change, reduction or increase, at comparative stations across the Delta (**Table 7**). For example, a DIN value of 0.1 mg/L-N (treatment #3) would represent a 22% reduction at Potato Slough, a 58% reduction in average DIN at Hood, a 69% reduction at Vernalis, and a 94% reduction at Buckley Cove (**Table 7**). Similarly, a total P value of 0.03 mg/L-N (treatment #7) would represent a 70% reduction at Potato Slough, a 0% reduction in total P at Hood, a 25% reduction at Vernalis, and a 91% reduction at Buckley Cove (Table 6). The nitrate concentration in treatment #4 (0.05 mg/L-N) is representative of DIN concentrations in the Sacramento River from July through August upstream of SacSewer's discharge (**Figure 7**).

Treatment 9 is a river control treatment. Water will be collected directly from the source water location in the Delta during all three days of the experiment. This treatment will compare changes in phytoplankton biomass and assemblage occurring in the Delta waterway during the experimental period to those occurring in each of the bioassay treatments.

Treatment 10 is an ambient nutrient control treatment. Source water will be filtered with 100-200 µm Nitex screening, to remove large zooplankton and larval clams, and then be poured directly into the bioassay cubitainers without dilution or the addition of supplemental nutrients. This treatment will evaluate phytoplankton growth in the cubitainer environment at the ambient nutrient concentrations present in the source water.

Table 6. Treatments, bioassay descriptions, and target nitrate concentrations used in nitrogen reduction (#1-4), phosphorus reduction (#5-8), controls (9-11), and multi-factor (#12-14) bioassays. All treatments are tested in triplicate.

Treatment #	Treatment Category	Treatment Description	Nitrate (mg/L-N) or Phosphorus (mg/L-P)	Nitrate or Phosphorus (µmol/L)
1	N Reduction Bioassay	Nitrogen reduction, Unlimited P	0.4	29
2	N Reduction Bioassay	Nitrogen reduction, Unlimited P	0.2	14
3	N Reduction Bioassay	Nitrogen reduction, Unlimited P	0.1	7
4	N Reduction Bioassay	Nitrogen reduction, Unlimited P	0.05	4
5	P Reduction Bioassay	P reduction, Unlimited N	0.12	4
6	P Reduction Bioassay	P reduction, Unlimited N	0.06	2
7	P Reduction Bioassay	P reduction, Unlimited N	0.03	1
8	P Reduction Bioassay	P reduction, Unlimited N	0.015	0.5
9	Control	River control treatment	TBD	TBD
10	Cubitainer Control	Ambient nutrient control treatment	TBD	TBD
11	Duplicate Control	Duplicate of treatment 4 (control)	0.1	7
12	Multi-factor Bioassay	50% light reduction + nutrient reduction	0.1	7
13	Multi-factor Bioassay	*Egeria densa* addition + nutrient reduction	0.1	7
14	Multi-factor Bioassay	*Corbicula fluminea* addition + nutrient reduction	0.1	7

Table 7. The percent change in nutrient concentration reductions (negative values are reductions) per treatment (#1-8) based on average DIN and TP concentrations in the Delta from June to October in 2022 (Error! Reference source not found.**).**

Location	DIN Reduction				TP Reduction			
	Treatment							
	1	2	3	4	5	6	7	8
Frank's Tract	300%	100%	0%	-50%	100%	0%	-50%	-75%
Potato Slough	213%	57%	-22%	-61%	20%	-40%	-70%	-85%
West Canal	157%	28%	-36%	-68%	33%	-33%	-67%	-83%
Hood	68%	-16%	-58%	-79%	300%	100%	0%	-50%
Vernalis	23%	-39%	-69%	-85%	200%	50%	-25%	-63%

Location	DIN Reduction				TP Reduction			
	Treatment							
	1	2	3	4	5	6	7	8
Buckley Cove	-75%	-88%	-94%	-97%	-66%	-83%	-91%	-96%

Multiple-Factor Bioassay Treatments

The Delta is a complex ecosystem and many factors other than DIN and total phosphorus concentrations are known to influence phytoplankton growth, such as light limitation, nutrient competition with macrophytes, grazing losses to clams, and differences in residence times. This second set of mesocosm studies investigates how phytoplankton might respond to nutrient reductions in combination with other common environmental variables (summarized in **Table 6**). These experiments are intended to evaluate if other factors with the potential to regulate phytoplankton biomass and species composition might cause substantial changes to the experimental results. If these factors significantly impact phytoplankton growth in the bioassay, they also need to be considered in future phytoplankton management strategies.

- In low light, phytoplankton growth may be slower, but low light might also support phytoplankton species that are better adapted to living at low light levels.
- If nutrients are taken up by aquatic vegetation, then higher nutrient concentrations might be required to reach desirable levels of beneficial phytoplankton growth.
- Clam grazing might exclude some phytoplankton species, allowing a different phytoplankton assemblage to be dominant under nutrient-limited conditions in regions where clam grazing is common.

If phytoplankton in the multi-factor bioassays show substantial differences from the control, it is an indication that additional research on these factors will be necessary before the effects of nutrient reductions on phytoplankton biomass can be estimated for the Delta.

The goal is to determine if interactions between low N concentrations and any of these factors might significantly alter the outcome of nutrient reductions in phytoplankton communities. It is recommended that all multiple-factor cubitainer studies be conducted at the low nitrate concentration of 0.1 mg/L-N. A control treatment duplicating the conditions present in Treatment #4 should be conducted and used for comparisons in the multiple-factor studies. If one of these factors shows a strong effect on phytoplankton biomass, HAB biomass, or cyanotoxin production, then the interaction should be tested to a greater extent in a separate (future) sets of experiments across a range of low N concentrations.

The multiple-factor bioassay treatments will require 18 cubitainers per day, so the full study (Treatments 1 through 14) will use 42 cubitainers per day. The sampling frequency and test parameters used in the multiple-factor bioassay treatments will follow those listed in **Table 8**.

CONTROL (TREATMENT #11)

This treatment is a duplicate of treatment #4 and will be used for statistical comparisons with the other treatments in the multifactor bioassay treatments. Comparing the multifactor treatment results with treatment #4 would require a Bonferroni correction to be used in the Analysis of Variance (ANOVA) tests for each study.

LIGHT REDUCTION (TREATMENT #12)

Insufficient light is known to limit phytoplankton growth in the main channels of the Delta (Cloern 1987), where the water column is often turbid, deep, and well-mixed. The PAR levels in other treatments are intended to simulate the light available within the first meter of water depth and should be sufficient for rapid phytoplankton growth. However, it is important to also understand the combined effects of reduced N concentrations and limited light on phytoplankton growth. Lower light may allow slower-growing phytoplankton to become the competitively dominant species in the bioassays. Cubitainers in this treatment should be wrapped with an extra layer of neutral density screening to reduce light levels so they are at 50% of the PAR received in other treatments.

EGERIA DENSA ADDITION (TREATMENT #13)

Egeria densa (*E. densa*, also known as Brazilian waterweed) is an invasive submerged aquatic plant that is common throughout the Delta. *E. densa* stalks have whorls of leaves that create a bottle-brush appearance and can live free-floating (without roots) by absorbing nutrients directly from the water. The competition between *E. densa* and phytoplankton for scarce levels of nutrients has not been tested in the Delta. Periphyton can also grow on the surface of *E. densa* and take up additional nutrients. The study will not attempt to differentiate between the nutrients utilized by *E. densa* or its associated periphyton because they regularly occur together, and the goal is to understand their combined effect on phytoplankton biomass and species composition. It is also possible that some periphyton on *E. densa* stalks might propagate out into the suspended algae during the experiments.

E. densa stems will be harvested from the Delta and trimmed to a length of 25 cm from the stem tip. Stems should be gently rinsed with river water to remove built-up sediments. Stems should be visually inspected, and all macroinvertebrates removed (such as snails and insects). Non-branching stems that include at least one double node should be

selected to allow the cutting to grow into a new plant (GISD 2023). A double node consists of two single nodes separated by a greatly shortened internode. Two 25 cm-long *E. densa* stems (tip to cutting) should be added to each cubitainer in this treatment and moved between cubitainers during the daily water transfers. At the end of the experiment, the length of the cutting should be remeasured as an indicator of growth (measuring plant weights and marking the top leaves at the start of the experiment might also help measure and visualize plant growth, respectively). Under ideal light and nutrient conditions, *E. densa* can grow up to 3 cm per day.

CORBICULA FLUMINEA ADDITION (TREATMENT #14)

Small *Corbicula fluminea* (*C. fluminea*, 10-mm shell width) can be collected from the Delta using clam dredges (or by hand) the day preceding the experiment and transported to the collection site in chilled coolers, using an approved California Department of Fish and Wildlife scientific collecting permit. *C. fluminea* is resilient to brief periods of air exposure. In the 10L cubitainers, a single clam of this size represents a moderately-high clam biomass-to-water volume ratio for the Delta. The clam's filtering range will cover a larger proportion of the cubitainer than it would in a deep Delta channel, therefore the treatment only simulates phytoplankton growth in shallow water habitats inhabited by clams. *C. fluminea* frequently targets diatoms for consumption and may avoid ingesting cyanobacteria, which could promote cyanobacteria abundance in the phytoplankton community (Bolam et al. 2019). Diatoms might also sink to the bottom of the cubitainer in the relatively still water and be more easily grazed by clams compared to positively buoyant cyanobacteria, such as *Microcystis* sp. This would also promote a taxonomic shift to cyanobacteria in the bioassays.

3.4 SAMPLE COLLECTION FREQUENCY AND TIMING

Samples will be collected during each day of the study for each treatment as outlined in **Table 8**.

Table 8. Sample collection days for each test parameter. The initial day is listed as day 0 and will be evaluated with six replicate samples collected from the source water.

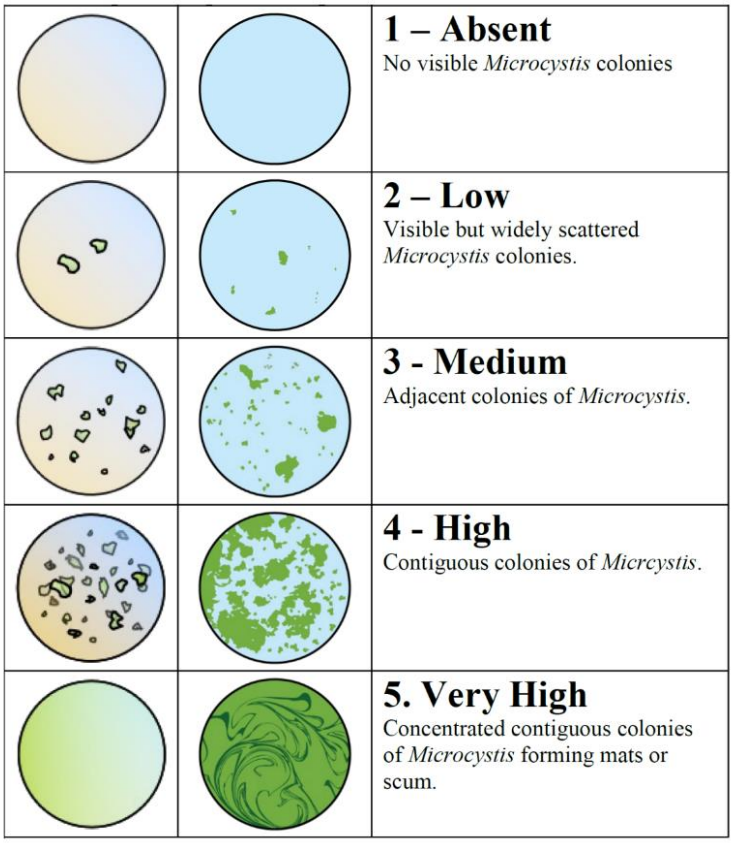
PARAMETER	MEASUREMENTS DAYS			
	0	1	2	3
Temperature	x	x	x	x
Dissolved oxygen	x	x	x	x
pH	x	x	x	x
Specific conductivity	x	x	x	x
Turbidity	x	x	x	x
Pesticides	x			
Nitrate + nitrite	x	x	x	x
Ammonium	x	x	x	x
Unfiltered Total Kjeldahl Nitrogen (TKN)	x	x	x	x
Dissolved TKN	x	x	x	x
Total phosphate	x	x	x	x
Dissolved Silica	x	x	x	x
Chlorophyll-a	x	x	x	x
High-Performance Liquid Chromatography (HPLC) phytoplankton pigment concentrations	x	x	x	x
Cyanotoxin concentrations	x			x
Taste and odor compounds	x			x
Phytoplankton enumeration	x			x

3.5 SAMPLING LOCATIONS AND METHODS

Water will be collected from one location in the south Delta where HABs are known to occur. Two collections will be made, the first occurring during the spring (March-April) before HABs develop and the second during the summer/fall, with water sources from an actively growing HAB bloom (July-August). If a bloom is not present in the late summer, then the second round of the study will be postponed, potentially until the following year. As these bioassay experiments are influenced by the starting conditions at the sampling location, it is recommended that the full experiment be repeated during the following year to evaluate how phytoplankton responses to N and P reductions change under different starting conditions.

Water samples will be collected from the Delta by boat, 0.5 m below the surface, at midday, at a location where *Microcystis* sp. populations are known to occur. If there is low cyanobacteria biomass, it is likely the experiment will be postponed. Sampling will be coordinated with Division of Boating and Waterways to avoid time periods when spraying is occurring within the Delta. Samples will also be collected in triplicate for pesticide analysis to understand background pesticide concentrations of the source water. During the late summer/fall collection, researchers should visually ensure that *Microcystis* sp. is at medium to high concentration at the time of collection based on the DWR visual assessment methodology (**Figure 8**, Flynn et al. 2022) or using another analytical method. All surface water samples should be collected from the same location on the same day during each sampling event. Source water should be filtered with 100-200 µm Nitex screening to prevent large zooplankton and clam veligers from being added to cubitainers. However, phagotrophic protists are likely to pass through the Nitex screening, due to their small cell sizes, and are expected to consume a substantial proportion phytoplankton's daily production in all treatments (Nogueira et al. 2014).

Figure 8. Microcystis scale for visual index of Microcystis sp., Aphanizomenon sp., and Dolichospermum sp. used by monitoring programs in the Delta (Flynn et al. 2022).



Surface water will be diluted to achieve the lowest N and P concentrations that are to be tested in the experiment (0.05 mg/L-N and 0.015 mg/L-P), using deionized water supplemented with other essential nutrients and major ions to match the initial river concentrations, following the methods of Barnard et al. (2021). Diluted river water should be mixed gently (to ensure that *Microcystis* sp. and other floating or sinking phytoplankton species are well distributed throughout the sample) and transferred to individual 10L containers. Nitrate and/or potassium dihydrogen phosphate (KH_2PO_4) will be added to cubitainers to achieve the initial target nitrate and phosphorus concentrations in each treatment (**Table 6**). Sodium bicarbonate ($NaHCO_3$) and Silica (Na_2SiO_3) should be added to the source water to prevent carbon or Si limitation from occurring during the incubation in the sealed containers. Cubitainers will also receive any other amendments required when they are filled, such as clams or macrophyte cuttings, as discussed below, in the Multiple-Factor bioassay treatments section. Cubitainers will be placed in flowing water baths for temperature control, either by utilizing laboratory water baths or by using floating enclosures attached to a dock located within the Delta waterway (**Figure 9**). Neutral-density screening should be used as enclosure covers to ensure there is moderate

illumination for phytoplankton growth. Light levels should approximately match the light present at 0.5 m depth at the collection location during the time of sampling (likely near 120 µmol photons m^{-2} s^{-1}) to prevent photo-inhibition. Measurements of photosynthetically active radiation (PAR) should be made using an underwater quantum sensor.

Figure 9. Example of 10L containers housed in floating encloses attached to a dock in the Delta. A neutral-density screening cover is shown on the right (Mussen et al., unpublished study).

3.5.1 Bioassay Monitoring Methods

The source water will be sampled (six times) prior to filling individual cubitainers, to avoid unnecessary duplication of samples. Triplicate samples should be collected from the water source on the following days of the experiment for comparison with the phytoplankton incubated in the cubitainers. Source water measurements should follow those shown in **Table 8**. The source water concentrations can be compared to those in the ambient treatment to understand how phytoplankton growth was affected by the cubitainer environment.

Initial nitrate and phosphorus levels in the cubitainers should match the concentrations outlined in **Table 6.** The ambient treatment will measure phytoplankton growth of filtered source water without any dilution, to determine how phytoplankton growth in the cubitainers compares throughout the experiment to the growth happening in the source water throughout the experiment. Three replicate cubitainers will be tested for each treatment. Water quality parameters should be measured during each day of the experiment, including temperature, dissolved oxygen, pH, specific conductivity, and turbidity. At low N concentrations, it is predicted that most of the available N will be taken up by phytoplankton after three days. Phytoplankton in treatments receiving higher N

concentrations are expected to have higher phytoplankton biomass at the conclusion of the experiment.

Measurements will also include discrete samples for nitrate + nitrite, ammonium, unfiltered total Kjeldahl nitrogen (TKN), dissolved TKN, total P, dissolved silica, and chl-a (**Table 8**). Water samples for phytoplankton enumeration (taxonomy, cell count, and biovolume) and cyanotoxins (microcystin, anatoxin, saxitoxins, and cylindrospermopsin) concentrations should be collected from the source water at the start of the experiment and from each cubitainer at the end of the experiment. Total N concentration can be calculated from TKN plus nitrate and nitrite. Each day, cubitainers will be mixed by turning them upside down and gently shaking, repeated three times, prior to sampling.

3.6 DATA DELIVERABLES AND REPORTS

3.6.1 Predictions and Evaluation Methods

Analysis of Variance (ANOVA) will be used to identify significant differences in the final chl-a concentrations, the biovolume of specific phytoplankton (such as HAB species, species producing taste and odor compounds, and diatom species), and cyanotoxin concentrations among the various treatments. Chlorophyll a is expected to reach relatively stable concentrations by the end of the experiment, with higher biomass occurring in treatments with higher N concentrations and P concentrations. Zooplankton population sizes are not expected to increase dramatically during the 3-day experiments. The final biovolume of HABs and cyanotoxins can be compared to established national and state thresholds. The final biovolume of diatoms, chlorophytes, and other desirable phytoplankton species should be compared to chl-a restoration targets in the Delta, which are intended to increase zooplankton populations as a food source for fish. The biomass of cyanobacteria known to cause drinking water taste and odor challenges (or taste and odor compounds such as geosmin and 2-methylisoborneol (MIB)) should be evaluated against known benchmarks of impairment (DNDWW 2017). However, many taste and odor-causing cyanobacterial species are epibenthic or periphytic, so they are unlikely to grow to high pelagic biomass in this experiment.

The multifactor cubitainer treatments will provide initial insight into understanding how other environmental factors might alter phytoplankton responses to nutrient reductions. Analysis of Variance (ANOVA) will be used to identify significant differences among the treatments in final chl-a concentrations, the biovolume of specific phytoplankton (such as HAB and diatom species), and cyanotoxin concentrations among treatments. Phytoplankton biomass is expected to be lower in treatments with lower nutrient concentrations. Reduced light levels might reduce the increase in phytoplankton biomass over time or allow a different phytoplankton species to dominate the bioassay. The

presence of *E. densa* has the potential to reduce phytoplankton growth due to nutrient competition, shading, and the release of allelochemicals. *C. fluminea* is expected to have a strong grazing effect on the phytoplankton in the test chambers. These clams can deplete diatom abundance while potentially excluding cyanobacteria through selective filtration or consumption (Bolam et al. 2019). A refuge from clam grazing may also be established at the top of the cubitainer for motile or positively buoyant phytoplankton species (including *Microcystis* sp) because the filtering range of *C. fluminea* is unlikely to extend to the top of the 10L cubitainers. This study is intended to provide a starting point for understanding how phytoplankton in the Delta would respond to low N or to low P concentrations. Low N concentrations were rare in the system historically so the phytoplankton species that grow at low N concentrations are unknown. The study can inform how cyanobacteria in the Delta respond relative to low N concentrations, which might suppress the magnitude and duration of HABs and cyanotoxins production, and the growth of cyanobacteria causing taste and odor issues. The results also help evaluate if beneficial phytoplankton species biomass will grow to sufficient levels to support the Delta's food web at low N concentrations. Importantly, this study helps to determine if other environmental factors might significantly alter phytoplankton responses to nutrient reductions and if they should be included in nutrient management strategies. It is believed that the results from this study will guide future research questions and models predicting the outcome of N and P reduction management strategies in the Delta. However, it is not expected that the study findings will directly establish thresholds for nutrient benchmarks because other environmental effects, such as water flow rates (residence times), temperature, light availability (influenced by water and water depth), nutrient competition with macrophytes, and grazing effects from invertebrates should all be accounted for in the development of nutrient thresholds.

3.6.2 Limitations of the Bioassay Design

The findings from this study should be interpreted cautiously as many environmental conditions in cubitainers are different from those present in the Delta waterways. Cubitainers are beneficial for isolating and evaluating mechanistic effects of environmental factors, but they may not accurately represent phytoplankton growth under natural conditions in the Delta. Additionally, phytoplankton responses to N and P reductions are likely to differ due to interactions with other organisms and environmental variables. Future field monitoring studies may be needed to investigate and verify the bioassay findings of this proposed study.

It is assumed that the phytoplankton community is healthy at the start of the bioassay and that the water does not contain high levels of herbicides or other contaminants that can

inhibit phytoplankton growth. It is particularly important that all essential nutrients and major ions required for phytoplankton growth are included in the constructed dilution and exchange water. High phytoplankton production should be confirmed in pilot studies using constructed dilution water and tested using a high nitrate addition (and non-limiting P) prior to running the full bioassay testing low N and low P concentrations. Water in Delta channels is frequently turbid, well-mixed, and unlikely to stratify compared to that in the containers. Heavy particulate matter is likely to settle out of suspension in the cubitainers, increasing water clarity and potentially allowing the water to thermally stratify. Frequent rotation of the cubitainers should reduce the potential for stratification. Relatively stable water conditions in the cubitainers might select for the growth of motile phytoplankton species that are adapted to calmer environments than are typical for Delta channels. Tidal currents in the Delta also exchange water with shallow wetlands, which likely affects phytoplankton growth, grazing losses, and residence time in the waterway.

This experiment does not evaluate the taxonomy and biomass of invertebrates that develop when phytoplankton are grown at low N and low P concentrations, so the effects of N and P reduction on other trophic levels must be estimated using other techniques. Zooplankton can reduce phytoplankton biomass through grazing and zooplankton grazing might promote cyanotoxin production. The bioassay design also does not fully account for shading or nutrient competition with floating macrophytes, which are likely to impact phytoplankton growth at low N and/or P concentrations in the Delta.

This bioassay does not evaluate salinity effects on phytoplankton growth, but salinity can be a highly regulating factor for many phytoplankton and cyanobacteria species. Salinity is elevated in the Delta where river water mixes with Pacific Ocean water, and also in some backwater sloughs with minimal tidal exchange, where salinity is elevated due to evaporation. Water temperature also regulates phytoplankton growth and community composition. Therefore, the findings from this study will only represent phytoplankton growth at the salinity and water temperature that were present in the bioassay which generally are more ideal growth environments than the actual conditions outside of a cubitainer.

Effective nutrient management strategies need to be based on a strong scientific understanding of the mechanisms regulating phytoplankton growth and biomass in the Delta. Identifying the phytoplankton species, and predicted biovolumes, that will grow at different low N and P concentrations, the quantity of cyanotoxin or food resources generated, and interactions with other common environmental factors, will provide a solid foundation for future nutrient management discussions.

3.7 STUDY TIMELINE AND SCHEDULE

It is anticipated that the pilot study will be conducted in late summer / early fall of 2024 to allow for the first bioassay experiment to occur in spring of 2025 and the second bioassay experiment to occur in fall of 2025.

3.8 COST ESTIMATE

This experiment will require approximately 2,400 discrete samples to be collected and analyzed, plus those included in the pilot study. A rough estimate of the study's total cost is $490,504 as described in **Table 9**, which includes the costs of discrete sample analysis, field sampling and monitoring equipment, labor for conducting the experiment and reporting the findings, and overall project management and interactions with the Delta RMP Nutrient TAC and SC. The list of samples from each set of experiments and the total number of samples are shown in **Table 10**.

Table 9. Estimated nutrient reduction bioassay study costs (this estimate is for a pilot study in 2024 and two experiments conducted in the spring and late summer of 2025).

TASK	COST
Pilot Study	$40,000
Discrete samples	$262,080
Materials and equipment	$8,424
Operations	$90,000
Reporting	$60,000
Project management	$30,000
Total	**$490,504**

Table 10. Discrete analyte counts for one bioassay in one season. Treatments share initial data for the water source, with six replicates collected per analyte.

Discrete samples	Sample days	Treatments	Replicates	Initial	Total
Nitrate + nitrite	3	14	3	6	132
Ammonium	3	14	3	6	132
Unfiltered TKN	3	14	3	6	132
Dissolved TKN	3	14	3	6	132
Total phosphorus	3	14	3	6	132
Dissolved silica	3	14	3	6	132
HPLC phytoplankton pigment concentrations	3	14	3	6	132
Chlorophyll-a	3	14	3	6	132
Cyanotoxin concentrations	1	14	3	6	48
Taste and odor compounds	1	14	3	6	48
Phytoplankton enumeration	1	14	3	6	48

4 FOCUS AREA #3

The Delta RMP will look for opportunities to collaborate or leverage funding to address the question for Focus Area #3:

- How are characteristics of harmful cyanobacteria blooms in the Delta changing over time including the status of cyanobacteria blooms and cyanotoxins in the Delta and factors that affect their magnitude, geographic extent, and timing?

The Delta RMP will explore partnerships and funding opportunities with existing monitoring programs such as Department of Water Resources for Environmental Monitoring Program's discrete phytoplankton monitoring and regular fixed monitoring station maintenance crews, California Department of Fish and Wildlife for Interagency Ecological Program fish trawls and the Fish Restoration Program, and USGS Water Science Center studies. The Delta RMP is receptive to providing funds toward sample supplies, laboratory analyses, and shipping to add cyanotoxins and cyanobacteria to existing efforts.

4.1 STUDY OBJECTIVES

The study objective is to support the collection of data to better understand changes in cyanobacteria status and risks in the Delta. There is no comprehensive monitoring of cyanotoxins currently in place in the Delta. The Delta RMP has effectively contributed to HABs science by adding funding to studies led by others. The Delta RMP has added Focus

Area #3 into the Nutrient Multi-Year Study Plan with the objective to monitor cyanobacteria blooms and toxins by collaborating with, and/or augmenting other data collection efforts, or funding Supplement Environmental Project (SEP) studies (pending the ability for Delta RMP to use SEP funds in the future). Cyanotoxin analyses are relatively expensive and bloom conditions vary significantly over space and seasons. Therefore, leveraging Delta RMP funds by collaborating with other efforts is important to expand the scope of information that will be gained. Likely methods include collecting water and/or passive sampler media for analyses of cyanotoxins. Other analytes (water samples) could include chl-a, phytoplankton community composition, and genetic analyses for cyanotoxin production potential. An ideal study would measure multiple factors potentially affecting HAB blooms such as water temperature, salinity, depth, light availability, turbidity, water column mixing and flows, dissolved oxygen, pH, nutrient concentrations, and zooplankton abundance and assemblage. These are desirable factors to be measured but may not always be included in a study design; the Delta RMP may decide to fund studies to supplement factors measured to better understand HAB bloom mechanics.

4.1.1 Why is this a priority?

The Delta RMP SC identified status and trends of HABs as a priority area as part of the long-term planning process. This priority aligns with the Central Valley Water Board Delta Nutrient Research Plan special study recommendations to determine the roles of nutrients and other drivers in controlling the growth rate, maximum biomass, and toxin production of HABs, as mentioned above. Focus Area #3 works to address status and trends questions outlined by the Delta RMP in a set of management and assessment questions for nutrients (Table 1). The priority is to support studies looking to gain additional information to help understand what can be done to prevent and/or minimize harmful algal blooms.

4.2 HYPOTHESIS

There is no predetermined hypothesis for Focus Area #3. Hypothesis testing will be determined based on the specific project(s) funded for study.

4.3 MONITORING STRATEGY

Monitoring strategies could include but are not limited to collection of water, sediment, biota, and/or passive sampler media for analysis of cyanotoxins. Priority study areas include the impact of sediment resuspension, light and turbidity effects, HAB cyanotoxin concentrations and potential impacts, and transport (such as residence time effect on

HAB growth or movement of HABs across locations). Studies could focus on genetic analysis of cyanotoxin production potential, molecular assays, phytoplankton community compositions, and chl-a concentrations.

4.4 MONITORING STUDY PLAN REQUIREMENTS

Following the Delta RMP Data Management Plan and proposal or study plan requirements established by the Delta RMP, each project must include details specific to the monitoring design, including hypotheses to be tested, sample collection locations, sampling frequency, sample collection and analytical methods, data deliverables and data management, project schedule, and budget. Study plans approved by the Delta RMP will be incorporated into the Annual Monitoring Workplan which requires the following study design information:

a. Specific hypothesis(es) to be tested;
b. Sample locations;
c. Sample collection frequency;
d. Sample analytes;
e. Analysis methods;
f. Preliminary data deliverables;
g. Planned reports to summarize results;
h. Timeline and schedule for all the study design elements to be complete.

As described in the Data Management Plan, associated data management and quality assurance documentation will also be required and approved prior to implementation. The components of the study design should be implemented in a timeline that compliments the other studies included within this Study Plan and meets the objectives of Focus Area #3.

4.5 SAMPLE COLLECTION METHODS, ANALYTES AND ANALYTICAL METHODS

Specific sample collection methods, analytes, and analytical methods will be included with the study plan and/or proposal and evaluated to ensure that the study plan meets the objectives of Focus Area #3. Analytes for Focus Area #3 could include factors potentially affecting HAB blooms such as water temperature, salinity, depth, light availability, turbidity, water column mixing and flows, dissolved oxygen, pH, and nutrient concentrations. Analytes could also include chl-a, phytoplankton community composition, and genetic analyses for cyanotoxin production potential.

4.6 DATA DELIVERABLES AND REPORTS

4.6.1 Data Management

Data management associated with the study plan or proposal must follow the outlined requirements for Collaborative Studies in the Delta RMP Data Management Plan.

4.6.2 Data Deliverables and Reporting

Data deliverables and reporting must meet the requirements of Resolution R5-2021-0054 and the Data Management Plan. This includes data being publicly available and reporting of data within the timelines prescribed by Resolution R5-2021-0054.

4.7 STUDY TIMELINE, SCHEDULE, AND BUDGET

The study plan or proposal must include a project timeline, schedule, and budget. There is no prescribed timeline for project completion for Focus Area #3 and studies may range from months up to 3 years. It is most likely that these collaborative studies will be funded on an annual basis to correspond with the Delta RMP fiscal year Annual Monitoring Workplan timeline; however, it is possible that the Delta RMP would commit to multiple years of collaborative funding depending on the project, if the project's objectives align with the overall Nutrient Multi-Year Study Plan.

4.8 PROJECT APPROVAL PROCESS

The Delta RMP's Data Management Plan outlines the current proposal process which will be followed when reviewing project proposals. The process includes review of the initial proposal with the entity proposing the project and the Delta RMP Program Manager to ensure that the project meets the objectives of Focus Area #3, the overall Nutrient Multi-Year Study Plan objectives, is consistent with the Data Management Plan, and includes enough details to meet the Delta RMP study design requirements. The next step is for the Nutrient TAC to review the proposal using the proposal process outlined in the Data Management Plan. The Nutrient TAC review of the proposal will be provided to the Steering Committee for review and recommendation to the DRMP Board of Directors (BOD). The DRMP BOD will decide if they wish to fund the project. Once the project is funded, it will be integrated into the Annual Monitoring Workplan and associated data management documentation will be developed.

In cases where multiple proposals are being presented, the Steering Committee may ask to review pre-proposals to determine which projects should move forward into a complete proposal for review by the Nutrient TAC. This may require a joint discussion of the Steering Committee and Nutrient TAC.

The DRMP BOD is allocating approximately $150,000 a year for projects that fall within Focus Area #3 with a total amount of $450,000 over three years (**Table 2**).

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