Changing Nitrogen Inputs to the northern San Francisco Estuary: Science Opportunities and Constraints

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ABSTRACT:

Anthropogenic activities have resulted in elevated ambient nitrogen (N) and phosphorus (P) concentrations in many regions of the Sacramento-San Joaquin Delta and Suisun Bay (northern San Francisco Estuary, nSFE). The Sacramento Regional wastewater treatment plant (Regional San WWTP) currently acts as the largest N point source to the system, discharging 13,000-15,000 kg/d of ammonium-N (NH4-N) near the nSFE's northeastern boundary. By end of 2021, Regional San will complete major upgrades that will reduce its effluent dissolved inorganic nitrogen (DIN) loads by >65% and release the remaining DIN as predominantly nitrate ($NO3^-$). This major change in nitrogen inputs provides a unique opportunity to study ecosystem-scale responses to an altered nutrient regime. While, in general, the nSFE has not experienced some classic symptoms of nutrient over-enrichment typical in other estuaries —e.g., large phytoplankton blooms and hypoxia—other concerning nutrient-related impacts have been hypothesized, including: occurrence of harmful algal blooms (HABs) and the production of cyanotoxins; excessive growth of invasive aquatic vegetation; and declines in the abundance and nutritional quality of phytoplankton. These impacts have repercussions for the system's food web, and habitat quality, as well as the way we manage for transportation, recreation, water conveyance, and drinking water quality. This paper develops and applies a framework for i) identifying and examining ecosystem response scenarios to the forthcoming decreased N loads; and ii) identifying opportunities, and constraints or considerations, for investigating those responses, including key data needs or knowledge gaps. By applying this framework, we aim to provide a menu of plausible response scenarios and important considerations for use by regulators, managers, and the scientific community for prioritizing among, and then pursuing, scientific investigations that can address ecosystem management and ecosystem processes questions.

1. Introduction

Nutrient enrichment of aquatic ecosystems ranks among the most important water quality issues facing human-kind, in terms of its impacts worldwide on freshwater, estuarine, and coastal systems; the areal extent and severity of impacts; and the challenges and costs associated with mitigation (NAS, 200X; EPA, 200X; Cloern et al. 2001; Statham 2012; Pearl et al. 2014). Aquatic systems that drain urbanized or agricultural watersheds are often among the most substantially impacted, with excess nutrient loading leading to varied adverse responses, including eutrophication, hypoxia, harmful algal blooms (HABs), alteration of food webs, and reductions in fisheries (Bricker et al. 2008; refs).

The northern San Francisco Estuary (nSFE; **Figure 1**), including the Sacramento-San Joaquin Delta and Suisun Bay, receives large inputs of anthropogenic nutrients (Novick et al. 2015), resulting in ambient dissolved nitrogen (N) and dissolved phosphorous (P) concentrations that exceed levels linked to adverse impacts in many other freshwater and estuarine systems (Elser et al. 2007; Paerl 2009; Dahm et al. 2016). Although many habitats within the nSFE are nutrient-enriched, characteristics of the nSFE -- such as high turbidity, strong tidal mixing, and high benthic grazing rates -- have limited the classic eutrophication symptoms of nuisance phytoplankton blooms and hypoxia (Cloern 2001; Jassby 2007; Kimmerer and Thompson 2014), and large swaths of the nSFE are considered food-limited due to low phytoplankton production (Jassby et al., 2001; Jassby 2008). The nSFE's nutrient-enriched state nonetheless remains a major concern, with environmental managers examining potential linkages between excess nutrients and other ecological concerns (DSC 2013; CVRWQCB 2015; Cooke et al. 2018), including: harmful algal blooms (HABs), in particular the toxin-producing cyanobacteria *Microcystis* spp. (Lehman et al, 2005; Lehman et al 2017); rapidly spreading and dense invasive aquatic vegetation (IAV; Boyer and Sutula, 2015), which interferes with boating and water exports, traps sediment, and provides habitat for introduced predator fish species (Boyer and Sutula, 2016; Ta et al. 2017); and declines in phytoplankton blooms that have repercussions for the pelagic foodweb (e.g., Sommers et al. XXXX; Dugdale et al., 2007; Parker et al., 2012; Glibert et al, 2011) or highly-altered ratios of N:P (e.g., Glibert et al. 2011). To be effective in the long-term, nSFE management decisions must also weigh how plausible shifts in major physical forcings will affect nutrient "dose:response" relationships. For example, continuation of the decades-long decrease in sediment loads, and increasing nSFE water clarity (Kimmerer 2004; Cloern and Jassby 2012; Schoellhamer et al., 2013) could foster excessive phytoplankton production and low dissolved oxygen. Increases in light availability, along with anticipated increases in water temperature (Brown et al., 2016), may also leave the nSFE susceptible to more frequent and severe HAB events.

Currently, the Sacramento Regional Wastewater Treatment Plant (SRWTP), which discharges treated wastewater to the Sacramento River at the Delta's northeast boundary (**Figure 1**), is one of the largest nutrient sources to the nSFE, and the largest $NH4^+$ point source to the Sacramento River (**Figure 2**; Jassby 2008; Saleh and Domagalski, 2015; Novick et al. 2015). Regional San's nitrogen loads have increased 2-fold since the 1980s (Jassby 2008; **Figure 2**). Notably, Regional San is the largest source of nutrients to nSFE during non-winter months, when major tributaries' inputs are at their minimum. Upgrades to the WWTP, with expected completion by 2021, will substantially alter SRWTP's N inputs to the Delta by decreasing total dissolved inorganic N loads by >65%, with the remaining discharged N

occurring primarily as nitrate ($NO3^-$), as opposed to the current $NH4^+$-dominated release (**Figure 2**). Since nutrients have the potential to influence primary production, the upgrade could, in theory have many and major effects, some positive, some negative. However, how the system will actually respond is highly uncertain, in large part because of the many factors that influence responses. In addition, numerous factors that vary seasonally, interannually, and spatially, including physical connectivity, flow patterns, temperature, light availability, and foodweb interactions influence direct nutrient responses (Ward and Paerl, 2016). The nSFE is physically, biologically, and hydrologically complex (**Figure 1**), with a maze-like network of interconnected channels, sloughs, and wetlands; engineered or managed flows; and numerous inputs and outputs of water and nutrients. This complexity influences all biotic and abiotic characteristics of the estuary, including ambient nutrient concentrations and nutrient-related ecosystem responses.

This paper develops and applies a framework for i) identifying and examining ecosystem response scenarios to the forthcoming decreased N loads; and ii) identifying opportunities and constraints for investigating those responses, including key data needs or knowledge gaps. Through applying this framework, we aim to provide a menu of plausible response scenarios and important considerations for use by regulators, managers, and the scientific community for prioritizing among, and then pursuing, scientific investigations that can address ecosystem management and ecosystem processes questions.

2. Background and Approach

2.1 Background

The nSFE (**Figure 1**) serves as critical aquatic habitat and as a vital drinking water resource for 25 million Californians and irrigation water for the agriculturally-rich Central Valley (e.g., Jassby, 2008). It is also a physically, biologically, and hydrologically complex system, receiving flows from the Sacramento and San Joaquin Rivers, which drain approximately 40% of California and then move through and merge within the Delta, a maze-like network of interconnected channels and sloughs. The Delta has undergone physical alterations over the past 150 years that have altered the system's hydrology, terrestrial and aquatic habitats, and the ecosystem services these habitats provide (e.g., Whipple et al., 2012). A substantial portion of water entering the Delta (≥25% annually) is exported for irrigated agriculture and domestic water use (Jassby, 2008). The Delta also receives considerable inputs of treated wastewater effluent and agricultural runoff, both of which contribute the nutrients nitrogen (N) and phosphorous (P) (Novick et al., 2015) and other contaminants.

Analysis of long-term observational data demonstrate that the nSFE is in a state of severe ecological decline (Sommer et al. 2007; Thomson et al. 2011). Population collapses of several pelagic fish species, including the endemic Delta smelt, have received considerable scientific attention in terms of both extensive monitoring and targeted investigations, with results pointing to the combined impacts of multiple anthropogenic stressors contributing to the population declines (Sommer et al, 2007; Baxter et al. 2010; Hanak et al. 2013, Meyer et al. 2009 and NRC 2012), including: landscape alterations and habitat loss; species invasions; water withdrawals; declining food resources; and agriculturally- and wastewater-derived contaminants, including nutrients. While the 'endangered fish vs. water withdrawals' debate typically receives the most public dialogue and regulatory attention, there is also considerable evidence for negative impacts on habitat quality at lower trophic levels, such as harmful

algal blooms, invasive aquatic macrophytes, localized issues with low dissolved oxygen, and excessive anthropogenic nutrients.

Recent Efforts related to understanding the role of Nitrogen in the nSFE (and Bay): California's Central Valley Regional Water Quality Control Board (CVRWQCB) has been tasked with determining whether numeric water quality objectives for nutrients are warranted in the Delta to mitigate water quality issues associated with deleterious effects with the specific goal of protecting beneficial uses in the Delta. To inform that process, the CVRWQCB recently oversaw publication of five topic-specific white papers with several accompanying Knowledge Gap documents, along with a memo describing projected nutrient load reductions to the Delta (all documents available here). In parallel, the Delta Regional Monitoring Program has been working to synthesize existing information and augment current nutrient monitoring activities. Several recent State of Bay-Delta Science publications focused on the role of nutrients (see for example Dahm et al., 2016; Brown et al., 2016). More recently, information from these efforts was synthesized to develop a Delta Nutrient Research Plan (Cooke et al., 2018). The information generated by those efforts have been key determinants of this project's goals, to allow this current work to help inform the activities of management and regulatory efforts

The Regional San upgrade will substantially reduce nutrient loads to the nSFE. Studying ecosystem-scale responses to this altered nutrient regimes provides unique opportunities to inform adaptive management decisions in the nSFE and to advance our understanding of nutrient dynamics in freshwater and estuarine systems. However, given the nSFE's size and complexity and the diverse array of potential responses, decision-makers need a structured approach for systematically examining potential responses, to inform planning for science and monitoring activities. To address this need, we developed and applied a conceptual framework to i) identify and examine plausible ecosystem responses to the forthcoming decreased N loads; and ii) identify opportunities and constraints for investigating those responses; and iii) capture key data needs and knowledge gaps. Although developing, refining, and applying the framework comprised a major portion of this project's effort, the framework itself is merely a tool, or a means to an end. This project's main outputs are: the response scenarios; opportunities and constraints for studying those responses; and the data/knowledge gaps.

2.2 Approach

The framework consists of three major components (**Figure 3**):

- Identify the 'Universe' of Response Scenarios (**Figure 3A**),
- Capture Key Field-scale Observational/Experimental Considerations (**Figure 3B**)
- Distill observations down to a manageable set of Opportunities and Constraints for investigations (**Figure 3C**)

2.2.1 Universe of Response Scenarios

Four organizing principles shaped our approach to identifying the starting set of potential responses and their organization and linkages:

1. *Wide Net:* For this initial step, we took an inclusive approach to identifying potential responses, casting a wide net and gathering responses from a range of sources, including: scientific literature describing nutrient cycling and responses in diverse aquatic ecosystems (including nSFE); technical reports focused on nutrients dynamics in the nSFE; and other reports or guidance documents

related to nSFE nutrient management. Hereafter we refer to these potential responses as 'response scenarios'.

2. *Link to Upgrade:* Numerous factors influence ecosystem function in the nSFE, including both natural and human-influenced factors that originate as physical, biological, and chemical forcings or impulses. The analysis here focuses on upgrade-related response scenarios, with traceability back to the upgrade's nutrient load reductions serving as an essential inclusion criterion. Non-nutrient factors nonetheless play central roles in the discussions below because of potential influence of those factors on the upgrade-related responses (e.g., their magnitude, timing, spatial domain, likelihood of occurring, or measurability).
3. *Management Endpoints:* Nutrient-related management priorities, identified by regulators, managers, and stakeholders (e.g., Delta Nutrient Research Plan, CVRWQCB, 2018) are the primary motivation for this analysis, and thus serve as the structure's ultimate endpoints.
4. *Direct and Indirect Responses:* The changes in nutrient availability have the potential to induce both direct and indirect responses. Our overarching conceptual model adopts the basic premise that nutrients exert influence along "bottom-up" pathways, by directly influencing primary production (phytoplankton, macrophytes or aquatic vegetation) and the non-photosynthetic microbial community (heterotrophic, chemoautotrophic; referred to hereafter as microbes). Each indirect response must therefore occur through a series of physiological/ecological responses, initiated by one or more direct bottom-up responses.

Following this approach, we identified a diverse set of direct and indirect response scenarios (**Figure 4**). The response scenarios are organized into complete pathways, originating from the anticipated changes in nutrient loads (Tier 0), and extending to one of seven nutrient-related management priorities (T4), identified by regulators, managers, and stakeholders over the past decade (e.g., Delta Nutrient Research Plan, CVRWQCB, 2018), organized here into beneficial use categories and summarized in (**Table 2**; Consumptive Water Use; Recreation and Navigation; Habitat). Intermediate responses were grouped into three tiers: changes in ambient nutrient concentrations (T1); direct biological responses (T2); and higher-level biological responses, including food web interactions (T3).

Within T1-T3, we used conceptual models from a range of disciplines to identify and organize mechanistic responses, including important non-nutrient physical and biological factors. For each response, we also identified key dependencies - i.e., conditions that must be satisfied for the response to occur. Whenever possible, we utilized existing conceptual models from the nSFE's extensive catalogue (e.g., REF), and where needed augmented or refined those with insights from the broader scientific literature and recent studies in the nSFE. The full pathways cover a diverse range of relevant topics: estuarine physics, nutrient cycling, phytoplankton ecology, and zooplankton and fish physiology and ecology. Given this diversity and range, deep dives into each topic is both infeasible given length constraints, and would distract from this project's goals. Instead, the goals of this effort call for high-level overviews and synthesis across subject areas; for more details, the reader is referred to the original research and studies synthesizing that work.

While the network in **Figure 4** may at first appear unwieldy, the whole picture actually reduces down to something manageable. Working backward from T4, the pathways narrow down to a handful of distinct and fairly concrete endpoints, each tied to beneficial uses. Despite the nSFE's size and complexity, the ambient nutrient concentration responses (T1) can be distilled down to three major categories. Those T1

responses lead to three categories of direct biological responses (T2; phytoplankton, aquatic vegetation, microbial community). These intermediate-level responses (along complete pathways from T0-T4) are central to the major nutrient-related hypotheses. In addition, most of the paths leaving both Aquatic Vegetation and HABs point directly to T4 responses (beneficial uses). For those pathways, if robust studies can be designed and undertaken, linkages between nutrient management actions and beneficial use attainment may be more clearly detected than for pathways that are influenced by complex food web interactions.

Note: Since this study is focused exclusively on potential effects from the change in Regional San's nutrient loads, nutrient-related issues in areas outside Regional San's zone of influence are not considered here. In addition, although we use the term 'nutrients' throughout, the discussion focuses primarily on N, since N loads are the major planned change. Phosphorus (P) loads and fate were also considered, but P receives less attention in the text since P loads are not expected to change as a result of Regional San's WWTP upgrade.

2.2.2 Field and Experimental Considerations

We began by casting a wide net and yielded a large and diverse set of potential responses. While this served as a useful starting place, the goal of identifying opportunities and constraints for investigating responses led us to identify three criteria against which each response scenario could be evaluated in terms of its, loosely-dubbed, 'study-ability' (**Figure 3**, inset table): *1. Will the response be detectable (Detect)? 2. Will a link to the upgrade be testable (Test LInk)? 3. How likely is it that the response will occur (Occur)?* The criteria were used in two ways. First, we used the process of evaluating the response scenarios relative to criteria 1-3 to systematically identify hypotheses or mechanisms to test, important study requirements, and important data or knowledge gaps -- What components of a study would maximize the likelihood of detecting change(s)? What observations are needed to unambiguously determine whether upgrade-related nutrient decreases caused (directly or indirectly) the response? What work is needed to target unresolved science questions, competing hypotheses, or critical data gaps?

Second, the likelihoods with which response scenarios satisfied each criterion were scored categorically from Likely to Unlikely, based on a set of 9 basic considerations summarized in **Figure 3**'s inset table. (Consideration 1) For a study to test upgrade-related responses, spatial overlap is needed between zones where the upgrade alters ambient nutrient concentrations and, for example, regions where HABs have been prevalent or problem areas for invasive submergent or floating aquatic vegetation (iSAV or iFAV, respectively) exist. Predicting these overlapping regions is a common need across all response scenarios, and qualitative depictions of the spatial overlaps are presented for each response scenario. (Consideration 2) Suitable experimental or analytical methods that quantify key parameter(s) or process(es) are also essential. (Consideration 3) The likelihood of detecting changes and linking those changes to the upgrade hinges on the magnitude of the change, or its 'signal', being large (or quantifiable) relative to natural variations or 'noise' (**Figure 3B**). (Consideration 4) That signal:noise ratio could be sensitive to one or more of the following (**Figure 3B**): i) timing (seasonal) of data collection; ii) comprehensiveness of measurements, including ancillary parameters (e.g., non-nutrient factors); iiI) density of data collection (spatial and temporal); and iv) sufficient baseline data, pre-upgrade, to account for spatial, seasonal, and interannual variations. In addition, the anticipated lag between the

upgrade and when a response scenario would occur may also be influential (**Figure 3B**). Some responses will be evident almost immediately (e.g., hours to days post-upgrade), while other responses may lag the upgrade by months, years, or longer (see Boxes 1-5 for examples). With increasing lag time, the likelihood of unambiguously linking a response to the upgrade, or simply detecting it, will tend to decrease for several reasons. For example, temporal variations in non-nutrient factors over time may themselves affect response variables, necessitating longer pre-upgrade and post-upgrade observations to isolate upgrade-related change from seasonal or interannual variation. In an analogous manner, the potential for other anthropogenic activities within the nSFE to influence response variables also increases with increasing time. Lastly, on a practical note, long-term intensive studies to detect changes over years or decades may be difficult to sustain.

2.2.3 Opportunities & Constraints

In this final step, the results from the above work were distilled down to set of opportunities and constraints. A key aspect of this distillation was integrating across the individual response scenarios to identify shared data and knowledge gaps and synergies among study requirements. In addition to the integrated discussion, the key outputs for this step include two tables providing at-a-glance overviews, one compiling the relative likelihoods of successfully testing the full set of response scenarios, and the second capturing the compiled data and knowledge gaps.

3. Results and Discussion

This section is structured to allow readers to jump straight to the take-home messages by, consulting stand-alone technical Boxes (Boxes 1-6) for additional background or explanation, as needed. The main observations from Boxes 1-6 are captured in a set of summary graphics and tables (**Figure 4**, and **Tables 2-4**). **Table 2** and **Figure 4** summarize the response scenarios considered, with **Figure 4** also illustrating the mechanistic linkages between responses and their organization into tiers. As mentioned above, **Table 3** summarizes the assessments of response scenarios' "study-ability" (detectable? test link? occur?), and, **Table 4** gathers and presents the data and knowledge gaps across all response scenarios.

Boxes (B1-B5) identify and discuss a diverse set of response scenarios across the major biogeochemical and ecological response categories: Nutrient Cycling (B1); Phytoplankton Production and Biomass (B2); Harmful Algae Blooms (B3); Invasive Submerged and Floating Aquatic Vegetation (B4); Microbial Community (B5); and Food Web (B5). The boxes' follow similar structures: i) Basic Background, including relevance to nSFE ecosystem health and the upgrade; ii) Conceptual models, including mechanisms, nutrient and non-nutrient factors, key unknowns; iii) Response Scenarios overview: a summary table at the end of each box, describing the Response Scenarios, important dependencies (collected from all Boxes into **Table App.1**), evaluation of each response scenario against three 'study-ability' criteria (i.e., **Figure 3**, inset table); Relevant regions within the nSFE for studying response scenarios; and Characteristic response times.

3.1 Response Scenarios T1-T3

3.1.1 Major Observations

3.1.1.1 Tier 1: Changes to ambient Nutrients (T1: N1, N2, N3)

N1, N2: Decrease in water column DIN and NH4 concentrations: Predicting and measuring DIN and NH4 concentrations ([DIN], [NH4]) change due to the upgrade will be essential for all upgrade-related investigations (T1, T2, T3). In regions that are strongly influenced by the Sacramento River and thus Regional San effluent (i.e., where the upgrade's Zone of Influence (ZOI) will be greatest; see **Figure B1.6**), large changes directly related to the upgrade ($\triangle[DIN]_{direct}$ or $\triangle[NH4]_{direct}$) are expected (**Figure B1.3, B1.4**). In those regions, post-upgrade [DIN] will be roughly half pre-upgrade [DIN], and [NH4] will decrease from current concentrations of ~30 µM to near background (<1-2 µM) levels (**Figure B1.3)** . In addition, the chemical form of DIN will shift to predominantly NO3. These changes will be evident almost immediately, limited only by travel time (**Figure B1.6**).

Over longer travel times (τ > several days; generally greater distances from the outfall) predicting $\triangle[DIN]_{direct}$ and $\triangle[NH4]_{direct}$ becomes increasingly difficult due to time- and space-varying N transformations rates and losses along flow paths (**Figure B1.4-B1.5**). The ZOIs for both DIN and NH4 will generally be smallest, and their gradients sharpest, during warm low flow periods, with a combination of slower transport and faster transformations tending to attenuate $\triangle[DIN]_{direct}$ and $\triangle[NH4]_{direct}$ (**Figure B1.3**). Seasonal variations in water withdrawals and flow routing (e.g., opening/closing the Delta Cross Channel) may result in important exceptions to this generalization. Uncertainties related to predicting $\triangle[DIN]_{direct}$ and $\triangle[NH4]_{direct}$ over space and time derive from the extremely limited field data on N transformation rates (e.g., nitrification; denitrification; uptake by phytoplankton, microbes and aquatic vegetation; mineralization of organic N) and on sediment:water fluxes. Coupled hydrodynamic-biogeochemical models, currently under development (Zhang et al., in prep), will be important tools for predicting $\triangle[DIN]_{direct}$ and $\triangle[NH4]_{direct}$, and their utility and accuracy will be directly related to the availability of appropriate observational data (transformation rates and high frequency concentration observations) for model calibration or tuning.

N3: Gradual decrease of labile sediment org-N (↓[sed-orgN], ↓sed-N-flux): We hypothesize that bioavailable N stored in bed sediments ([sed-orgN]), and N efflux from sediments (sed-N-flux), play important roles shaping current water column [DIN] and [NH4] concentrations in some nSFE regions (especially when τ is large), and will play a relatively larger role after the upgrade. Post-upgrade, [sed-orgN] and sed-N-flux are expected to gradually decrease. While in high ZOI regions [DIN] and [NH4] will show pronounced drops almost immediately, severely limited data makes it difficult to predict the rates of decrease for [sed-orgN] and sed-N-flux, and therefore [DIN] and [NH4] in large-τ regions. It is entirely possible that the response time scales (and post-upgrade new plateaus) for [sed-orgN] and sed-N-flux will dictate when, or if, some T2 response scenarios occur or can be reliably detected, either because sed-N-flux maintains [DIN] or [NH4] at sufficiently high levels (e.g., phytoplankton and HAB responses), or because [sed-orgN] remains at levels sufficient to support growth (SAV, FAV).

3.1.1.2 Phytoplankton Production (T2: P1a,P1b, P2a, P2b, P1,2slow)

P1a and P2a: ↓DIN, Decreased Phytoplankton Production, Community Composition Changes: To

produce new biomass, the phytoplankton community as a whole needs nitrogen (Box 2). In addition, some phytoplankton taxa grow better than others at low DIN levels. Based on these two basic points, decreased phytoplankton production and changes to the phytoplankton community, may at first seem like obvious and straightforward responses to $\triangle[DIN]_{direct}$. However, predicting the likelihoods of P1a or P2a occurring at ecologically-significant magnitudes or predicting their potential magnitudes is far from straightforward. For the upgrade to influence phytoplankton production, other often-dominant constraints on production (e.g., light, benthic grazing, residence time) must relax sufficiently to allow nutrient availability (i.e., concentrations reaching growth-limiting levels) to become the 'bottleneck' that slows phytoplankton growth (**Figure B2.3**). The same basic dependencies are relevant to phytoplankton community composition.

Will P1a or P2a occur in some locations or times? Will changes reach ecologically-significant levels? It seems quite plausible that, post-upgrade, N-limited growth will occur and be detectable at some locations or times. The fact that, under current conditions, blooms occur (albeit sporadic and generally short-lived; e.g., Wilkerson et al., 2006; Dugdale et al 2007, 2012) offers evidence that spatial-temporal windows favorable to phytoplankton growth can occur. If large enough $\triangle$[DIN] co-occurs with those windows, community-wide growth rates could slow due to DIN limitation, and some taxa may outcompete others for N (**Figure B2.3**). It is much less clear, however, whether ecologically-significant changes in phytoplankton production or composition will occur. This is not only because other factors like light, residence time or grazing may be the predominant controls on phytoplankton, but also because there may be feedbacks that buffer increases in phytoplankton growth. For example, increases in phytoplankton biomass may increase grazer populations, subsequently increasing phytoplankton grazing losses which could negate the effects of higher growth rates. Similarly, higher growth rates would increase demand for N and thus N-limiting conditions could occur more frequently. Thus, while a decrease in the DIN pool is not expected to *increase* phytoplankton production and biomass (CM1, Box 2), it is also conceivable that little change in production and biomass could occur.

While the above uncertainties could be interpreted as discouraging the pursuit of P1a and P2a, we actually reach the opposite conclusion. Despite phytoplankton's critical role in the nSFE food web role, our understanding of nSFE phytoplankton dynamics remains far from complete (Ward and Pearl, 2017; Cooke et al. 2018), including how a large decrease in an essential substrate might alter those dynamics - which argues for using the upgrade to, as much as possible, advance our understanding of phytoplankton dynamics. The strong non-nutrient controls and other complexities mean that carefully-designed, holistic studies will be needed to build upon our current understanding.

The complexities and uncertainties associated with links between DIN availability and both phytoplankton biomass and composition should highlight that our understanding of phytoplankton dynamics in the nSFE remains far from complete .

When-Where? See **Figure B2.5**. Based on phytoplankton's response rates, P1a and P2a could in theory be evident within days to weeks of observing $\triangle[DIN]_{direct}$ (**Figure B2.5**). In practice, however, multiple years of observations will likely be required to reach confident answers about these response scenarios, considering natural variations (tidal, seasonal, interannual) of other factors influencing phytoplankton production, biomass, and community composition. Relevant locations include those considered modest ZOI - long-enough $\boldsymbol{\tau}$ for nutrients to reach rate-limiting concentrations.

P1b and P2b: ↓NH4 leading to Increased Phytoplankton Production or Improved Food Quality
Hypotheses about ammonium's influence on phytoplankton growth and community have received considerable attention in the nSFE over the past decade (Dugdale et al., 2007; Parker et al., 2012; Glibert 2016). Those hypotheses also spurred considerable debate within the scientific community (see Ward and Pearl, XXX and references therein), and fundamental disagreements remain about whether the form of DIN (NH_4^+ vs. NO_3^-) has ecologically-significant influences on phytoplankton production or community composition. As a result, P1b and P2b response scenarios were assigned 'unknown' likelihoods of occurring (**Table 3B** and **Table B2.1**). Independent of viewpoint on these hypotheses, the upgrade, by drastically reducing NH_4^+ loads to the Sacramento River, offers opportunities to study P1b and P2b. To some degree, however, the same 'well-buffered-system' argument outlined above also applies here.

When-Where? Based strictly on phytoplankton response times, P1b and P2b could conceivably be detected within days-weeks of the upgrade. However, as noted above, natural variations in other factors will likely make necessary multiple years of observations necessary to confidently quantify changes. The field observations that led to the 'Ammonium Paradox' hypothesis, and monitoring data used to support the 'Ecological Stoichiometry' hypothesis were all from Sacramento River and Suisun Bay sites, including locations where changes to $\triangle[NH_4^+]_{direct}$ will be greatest (**Figure B2.5**), making site selection for some study types straightforward. Observed NH_4^+ concentrations (**Figure B1.2**), and sharper gradients in NH_4^+ than DIN with distance or $\boldsymbol{\tau}$, suggest qualitatively that the ZOI-NH_4^+ may occupy a smaller area than the ZOI-DIN.

P1,2slow: Changes in production or community due to ↓sed-N-flux In regions within the ZOI but at large $\boldsymbol{\tau}$, we hypothesize that sed-N-flux will bolster post-upgrade [DIN] or [NH_4^+], maintaining their levels above thresholds for phytoplankton response (P1a, P1b, P2a, P2b). To effectively investigate the above phytoplankton response scenarios, study designs and site selection will therefore also need to consider the importance of sed-N-flux, and the potential lag before sed-N-flux and water column N concentrations decline sufficiently to cause a measurable response.

3.1.1.4 Harmful Algae Blooms (P3A, P3B, P3slow)

The upgrade provides tractable opportunities to investigate HAB-nutrient linkages in the nSFE. Since their first documented appearance around 2000, harmful algal blooms (HABs) have become an increasingly concerning issue in the nSFE (Lehman et al., 2017). While all Tier 2 and Tier 3 responses (**Table 2**, **Figure 4**) have ecological health implications, HABs additionally pose serious human health risks due to the close proximity of the major HAB regions to critical water supply infrastructure in the nSFE. The HAB discussion (Box 3) focuses primarily on the most problematic harmful algae taxa in the nSFE, the cyanobacterium *Microcystis* spp, and the toxin it produces, microcystin (MCY).

P3A & P3B Decreasing HAB magnitude and toxicity due to $\triangle$[DIN] A relatively straightforward, plausible mechanistic case can be made for decreased HAB event magnitude (frequency, duration, density, area) and toxicity resulting from the upgrade $\triangle$[DIN]. *Microcystis* is non-diazotrophic, requiring bioavailable nitrogen to grow, especially to reach high densities over large areas. In addition microcystin (MCY) is a nitrogen-rich molecule, with the available evidence indicating that MCY gene transcription is down-regulated in response to decreasing N availability (Harke and Gobler, 2015). Nonetheless, several key issues and dependencies make it highly uncertain whether P3A or P3B will occur. First, $\triangle[DIN]_{direct}$ must be large enough in high-risk HAB areas for DIN to reach levels that moderate HA growth rates or

toxin production. In general high-risk HAB areas fall in the interior Delta (**Figure B3.1**), still within the ZOI but at moderate to large **τ**, where △[DIN] may be relatively small (**Figure B.3.4**). Second, multiple other factors strongly influence HAB occurrence and toxicity, and, in many cases the specific mechanisms and interactions between biotic and abiotic factors remain poorly characterized (see Box 3). Studies exploring the upgrade-HAB linkage will therefore need to measure, or otherwise control or account for, those other factors, in order to isolate and test for the role that nutrients play in HAB dynamics. Given phytoplankton response times, it is conceivable that a HAB response could be measured within days to weeks of post-upgrade SacRiver water reaching *Microcystis*-prone areas. In reality, multiple years of observations would likely be needed to observe conditions across a range of physical forcings.

P3slow Decreasing HAB magnitude and toxicity due to ↓[sed org-N] and ↓sed-N-flux Most of the nSFE's *Microcystis* impacted regions occur in areas with moderate to large **τ** (>10-15 days; **Figures B1.4** and **B3.4**). In these regions, $\triangle[DIN]_{direct}$ could be fairly small, due to both transformations along the flow path and continuation of sed-N-flux after the upgrade. To the extent that DIN availability plays an important role influencing HABs in the nSFE (frequency, magnitude, toxicity), it is plausible that sed-N-flux could satisfy HAB demand in these regions for some unknown lag period after the upgrade, and that HAB occurrences would decrease gradually, following the decrease in sed-N-flux (**Figure B3.4**).

3.1.1.5 Invasive Floating and Submerged Aquatic Vegetation (AV1, AV2, AV3, AV4, AVslow)

Invasive floating and submerged aquatic vegetation (iFAV, iSAV; Box 4) severely impact nSFE beneficial uses along diverse pathways (**Figure 4**). Although eradication of invasive vegetation from the Delta is not realistic, targeted management actions could mitigate some of their negative impacts (Santos et al. 2009); however, major knowledge and data gaps hinder progress along that front. The upgrade presents a unique opportunity to address some of these priority gaps, related specifically to the role of nutrients, as well as other insights into iAV physiology and factors that influence their ability to become established and spread.

AV1 Decreased iFAV biomass or spatial distribution due to $\triangle$**[DIN]$_{direct}$** On a mechanistic basis, it appears plausible that iFAV (invasive floating aquatic vegetation) biomass and spatial distribution will decrease due to the upgrade's direct influence on water column DIN concentrations ($\triangle[DIN]_{direct}$). The dense water hyacinth mats, for example, covering large swaths of the Delta must require substantial amounts of N to build and maintain that biomass. Eliminating a substantial portion of DIN influx to those swaths, therefore, has the potential to limit new growth or maintenance. Nonetheless, despite AV1 appearing quite plausible, multiple factors beyond water column N influence iFAV's ability to establish and spread, leading to AV1 being designated as likely to occur, but with low confidence (**Table 3** and **Table B4.1**). The upgrade offers solid opportunities to test the AV1 hypothesis, in the sense that changes in iFAV biomass could be readily detected, and to examine the linkage to the upgrade. Similar to other T2 response scenarios, spatial overlap between ZOI-DIN and iFAV problem areas will be essential for lined-effects to occur and be measurable. In addition, post-upgrade [DIN] must drop below growth-rate limiting concentrations. If $\triangle[DIN]_{direct}$ is a major influence on iFAV, responses could become evident within one to several years (**Figure B5.4**), influenced by how much of a summer's biomass survives into the subsequent spring/summer, and the relative rates of N recycling (including after settling to the sediments) versus N flushing from the system. Some iFAV (e.g., water hyacinth) can in some instances extend their roots into the benthos and directly access sediment-N, which, if important, would shift

response times closer to those for AV.*slow*.

AV3 Decreased iSAV biomass or spatial distribution due to $\triangle$[DIN]$_{direct}$ iSAV acquire much if not all of their nutrients from bed sediments via roots. Some species, however, can also incorporate dissolved nutrients directly from the water column (e.g., *Egeria*). For AV2 to occur, iSAV must be sufficiently reliant on [DIN] that anticipated $\triangle$[DIN]$_{direct}$ would curtail growth. While mechanistically plausible, in particular in terms of delivering a species some competitive advantage, we expect that large changes to iSAV biomass or spatial coverage would be more likely to come through decreased sed-orgN (below) than $\triangle$[DIN]$_{direct}$.

AV.slow: Decreased iFAV or iSAV biomass/spatial distribution due to $\triangle$[sed-orgN] If iSAV abundance does ultimately decrease, we posit that it would be most likely to occur after sufficient decreases in [sed-orgN]. Under this scenario, iSAV relevant response times would be on the order of years (**Figure B5.4)**, dictated by sed-orgN response times (N3). With multiple factors influencing iSAV prevalence, considerable uncertainty remains about whether decreases will in fact occur. However, if substantial decreases do occur, there is reasonable likelihood that those changes would be detectable and the upgrade-link testable (**Table 4**, **Table B4.1**). For iFAV, AV*slow* and AV1 have comparable likelihoods of being the causal pathway. In both cases, [DIN] must decrease to growth-rate limiting levels. Several key unknowns, though, prevent us from landing more confidently on one over the other; these include quantitative information about growth-rate limiting DIN concentrations for relevant AV species; linkages between water column N concentrations and sediment N stores; and sed-N-flux and its rate of change over time.

AV2, AV4: Changes to iFAV or iSAV community due to $\triangle$[DIN]$_{direct}$ Exploring these response scenarios essentially involved applying similar considerations as for AV1 and AV3 to multiple species, resulting in similar conclusions. While noted for sake of completeness, they are not discussed further.

3.1.1.6 Microbial Community Response Scenarios

The microbial community structure appears poised to change substantially in response to the upgrade, in ways that should be readily detectable and linkable to the upgrade. Nitrifiers and denitrifiers fuel their growth and metabolic activity by directly utilizing NH_4^+ and NO_3^- in energy-yielding biochemical reactions. It therefore stands to reason that nitrifier (*M1*) and denitrifier (*M2*) biomass and community composition (or diversity) will be substantially altered in regions experiencing large $\triangle[NH_4^+]_{direct}$ and $\triangle$[DIN]$_{direct}$, with some specific example hypotheses discussed in Box 5. To the extent that $\triangle$[DIN]$_{direct}$ leads to decreases in both phytoplankton production and fresh labile organic matter delivery, then heterotrophs (mineralizing communities) may also be affected (*M3*). Observing these changes, in both the surface sediments and water column, offers the potential to yield critical insights into how microbial communities respond to dramatic shifts in nutrient availability. Because studies of this kind usually focus on microbial responses to <u>increased</u> nutrients, this represents a truly unique and valuable research opportunity. In addition, a growing body of evidence indicates that non-photosynthetic microbes co-vary with phytoplankton, sometimes working symbiotically (Bertrand et al., 2015; Amin et al., 2015), other times antagonistically, but in both cases strongly influencing the others growth. With some studies postulating that these types of relationships are “key to understanding the controls on marine primary productivity” (Bertrand et al., 2015), there is good reason to consider examining whether analogous relationships could have ecologically-meaningful influences on phytoplankton productivity in the nSFE.

3.1.1.7 Food Web Responses, Higher Trophic Level

FW1a Changes in Pelagic fish abundances due to changes in food resources Studies setting out to detect nutrient-related pelagic fish responses, and definitively link those responses back to the upgrade, have a low likelihood of being successful. Major hurdles in detecting alterations to the pelagic fish community extend from their relatively long response times, numerous non-nutrient interactions and covariates whose importance vary with time; for example with Delta Smelt, the many non-nutrient yet potentially dominant factors that are unrelated to the food supply, e.g., entrainment, inflow and diversions, predation, position of the low salinity zone, and effects of iSAV on turbidity and other habitat parameters(see Baxter et al. 2015, Moyle et al. 2016).

FW1b Changes in food resources reaching target organisms Detecting lower food web responses, such as copepod abundance (FW1b), and linking those responses back to the upgrade seems more plausible than for fish, owing in part to copepods ability to respond rapidly (weeks) to changing conditions, such as changes in food availability. Detecting changes in copepod-related indicators (e.g., abundance, reproduction rates, growth, loss rates) could conceivably become evident shortly after the upgrade (weeks to months), within the context of, for example, a targeted mechanistic field study. Detecting changes over larger spatial scales (e.g., regional scale abundances) would likely require years for the signal of those changes to be detectable despite interannual variability.

FW2, FW3 Change in fish abundances due to ↓ HAB toxin exposure or ↓ predator habitat: Although toxins and predation are two very different stressors on pelagic organisms, clearly identifying FW2 and FW3 responses to the upgrade face similar challenges as FW1a, because of long response times and other confounding factors influencing survival.

3.1.2 Data/Knowledge Gaps and Opportunities: Response Scenarios T1-T3

Table 3 compiles major data and knowledge gaps for all response scenarios explored in Boxes 1-6 and mentioned above. Each Gap (row) is assigned categorical priority levels denoting its importance for investigating each response scenario (columns). G19-G22 were included as overarching needs for investigating all (or most) response scenarios. Gaps G1-G18 were added stepwise, after each was identified for an individual response scenario, after which its relevance or priority level was also evaluated across the other scenarios. Rows registering more (and darker) red address important gaps for broader range of scenarios. The summary below highlights priorities, major themes, and potential synergies, but does not walk through every combination.

Nutrients: G1, G2, and G3 in Table 3 were included as specific gaps of very high relevance to response scenarios N1, N2, and N3. G1-G3 also rank among the highest priorities gaps for Phytoplankton, HAB, Microbe, AV, Microbe, and FW response scenarios, emphasizing the need to quantify or predict $^{\triangle}[DIN]_{direct}$, $^{\triangle}[NH4]_{direct}$, sed-orgN, and sed-N-flux for exploring all of the response scenarios. Several other gaps, identified primarily for other response scenarios, emerge as additional high or moderate priorities for nutrient response scenarios (i.e., other red boxes in columns below N1-N3). These priorities reflect the important influences, or feedbacks, that some of the T2 responses are expected to have on N concentrations, including uptake by phytoplankton (G6) and AV (G13 and G14) and transformations by microbes (G10).

Phytoplankton: G4-G5 highlight the need for targeted (space, time) phytoplankton biomass and community composition data, both discrete and high-frequency, co-collected with nutrient and physical

data to better enable testing nutrient-related phytoplankton response scenarios. With potential phytoplankton community response times on the order of days, biomass and composition data are needed at comparable or higher space/time resolution to capture those changes, and foster efforts to quantitatively explore influential mechanisms. Some of that work, especially for composition, will require testing and validating new techniques that provide the necessary precision/accuracy, taxonomic resolution, and temporal resolution, including field instrumentation (e.g., in vivo fluorescence at multiple Ex/Em; radiometers), molecular techniques, and building capacity for remote sensing. Rate data are also needed (growth, grazing; G7-G8). In addition, a growing body of evidence indicates that phytoplankton and non-phototrophic microbes commonly live in consortia (e.g., contributing unique compounds needed by other organisms), and influence each other's abundance and succession patterns. Given that substantial changes to the microbial community are expected (Box 5), being able to characterize those changes in comparison with the phytoplankton community could prove extremely valuable.

HABs: HAB response scenarios, not surprisingly, share multiple priority data gaps with Phytoplankton. CyanoHAB data are not currently collected as part of routine Delta monitoring activities. Instead, much of the available cyanoHAB and toxin data are from event-driven observations (Lehman et al., 2008; Lehman et al., 2017; Otten et al., 2017). Routinely-collected cyanoHAB toxin measurements are therefore an important gap (G8). In addition, since methods commonly-used for phytoplankton sampling and enumeration often do not adequately quantify some cyanoHAB taxa, techniques currently being used should be evaluated and, if necessary be modified before the upgrade, so that baseline pre- and post-upgrade observations are using robust techniques (G21).

iSAV, iFAV: G13, G14, and G15 specifically address iFAV and iSAV data and knowledge gaps (Table 3). Growth-rate limiting N concentrations in the water column and sediments are critical unknowns, as are the abilities of species to utilize nutrients from both compartments (G13). While iFAV and iSAV undoubtedly act as N sinks, little is known about their quantitative influence on ambient concentrations (G14), which, if iFAV or iSAV abundance changes substantially, could influence downstream nutrient transport, and, by extension, influence downstream response scenarios. Lastly, while several studies have rigorously investigated iFAV and iSAV spatial distributions, and documented some changes over time, no routine iFAV or iSAV monitoring program exists (G15), which will be essential for documenting baseline conditions and changes over time. Shared priority knowledge gaps include those relevant to nutrient cycling and ZOI determination (G1-G3); interactions with or implications for wetland restoration (G18) and potential microbial-AV interactions; development of remote sensing capacity as an option for or component of sustained monitoring; and other jointly-beneficial work (G19-G21).

Microbial community G9, G10, and G11 explore mechanisms related to microbial abundances/community composition and transformation rates, contribution of microbes to the food web, and mechanistic exploration of interactions between microbes and primary producers, respectively. Shared priority gaps include those relevant to nutrient cycling and ZOI determination (G1-G3) and potential microbial-AV interactions; other specific (zoops) food quality or grazing information (G16,G7); and collection along with monitoring or on-going phytoplankton biomass and community investigations (G4-G5). Since there is currently a paucity of microbial community data in the

Delta, and these organisms may respond rapidly even to early upgrade steps, investigations need to be launched to establish baseline microbial community structure before the upgrade is on line.

Pelagic food web G15 and G16 relate to characterizing zooplankton populations and identifying key food resources that support them and were included specifically to address data needs for FW1b (the response scenario that envisions a change food resources reaching target organisms). Additional FW1b priorities align closely with nutrient cycling and phytoplankton production. For pelagic fish response scenarios (FW1a, FW2, FW3, FW*slow*), the likelihood of detecting a nutrient-related response and robustly testing its link to the upgrade through either enhanced monitoring or directed research are low. There are simply too many possible interactions and covariates to consider. Monitoring and research related to the upgrade are likely better focused on T1 and T2 responses: nutrient processes, primary producers and lower level consumers, primarily clams and zooplankton. Results from such studies could then be integrated with ongoing and future work on fishes, including Delta Smelt, conducted by IEP and others.

3.2 Other Important Considerations: Wetland Restoration and Drinking Water

3.2.1 Wetlands

Tidal wetlands influence, and are influenced by, nutrient concentrations, and are sometimes considered "hot spots" of nutrient cycling based on evidence suggesting that nutrient transformation rates, removal, storage, and release are augmented by increased plant and microbial densities (REFs). Under 'goldilocks' physical-exchange regimes, wetlands can experience high nutrient demand and turnover (Ensign et al. 2008; Loomis and Craft 2010; Gribsholt et al. 2005; Bowden 1987; Bowden et al. 1991; Loomis and Craft 2010) and receive sufficient nutrient inputs from adjacent water bodies via advective or tidal exchange. Changes in plant productivity along with changes in microbial activity resulting from altered nutrient regimes can influence the balance of soil organic matter production and decomposition rates, with possible consequences for soil stability and accretion rates, and therefore marsh integrity and resilience (Morris et al 2013). Lastly, effects of nutrient concentrations on vascular plant and algae production in tidal wetlands could also affect local food webs (see food web section).

Nutrient dynamics in tidal freshwater wetlands in general, and Delta wetlands in particular, are not as well studied as in salt marshes. The Regional San upgrade appears to provide opportunities to advance our understanding of these interactions, which is particularly relevant considering that large-scale Delta wetland restoration efforts are now underway (California Natural Resources Agency, 2017). The effects of nutrient changes on tidal wetlands may best be studied in existing wetlands and planned restoration projects closest to the outfall, including Delta Meadows, McCormack Williamson Tract, Cache Slough, Liberty Island, and Prospect Island. Interactions between the pelagic food web and the tidal wetland and SAV food webs are poorly characterized. The interaction with the tidal wetland food web is of interest because one of the proposed benefits of tidal wetland restoration is to create new habitat where pelagic species can feed and where excess production of phytoplankton, zooplankton, and other organic material can subsidize the pelagic food web (Herbold et al. 2014). To date, there is no evidence that tidal wetlands actually export zooplankton, but few studies have examined this question. Delta Smelt do consume prey other than zooplankton, including amphipods and insects (Whitley and Bollens 2014, Baxter et al. 2015), indicating they are not wholly dependent on the pelagic food web. Whitely and Bollens (2014) documented small numbers of Delta Smelt feeding in tidal wetland habitat.

3.2.2 Drinking water

The Delta supplies drinking water to over 25 million Californians. Although nutrient concentrations in Delta waters are well below levels considered toxic to humans, nutrients indirectly affect drinking water quality via the production of cyanotoxins, taste and odor compounds, and organic carbon. In addition, nutrients can fuel the production of aquatic macrophytes and phytoplankton which clog infrastructure, leading to additional costs associated with water conveyance, storage, and drinking water treatment (Delta Drinking Water Work Group, 2017). These issues occur not only in the Delta proper, but also during water conveyance and storage via aqueducts and reservoirs which results in long detention times (weeks to years), providing additional opportunity for organisms to grow. As with many of the other

nutrient-related responses discussed in this paper, links between nutrients and drinking water issues are complicated by a number of other abiotic (e.g., temperature, irradiance, flow) and biotic factors (e.g., phytoplankton abundance, community structure, physiological status) factors that affect how organisms grow and the compounds that they produce (**Figure 1**). Currently, the location, timing, and duration of cyanotoxins, taste and odor issues, and nuisance levels of phytoplankton and macrophytes in the Delta and in downstream conveyance and storage facilities occurs unpredictably, making it challenging to identify drivers of event initiation, duration, and attenuation, as well as predicting the fate of compounds of concern (Delta Drinking Water Work Group, 2017). As recommended by the Delta Drinking Water Work Group (2017), assessing whether nutrient reductions alone or in concert with other management actions will alleviate these occurrences will require continued monitoring, and would greatly benefit from modeling efforts.

4 Summary

[in progress]

References

[in progress]

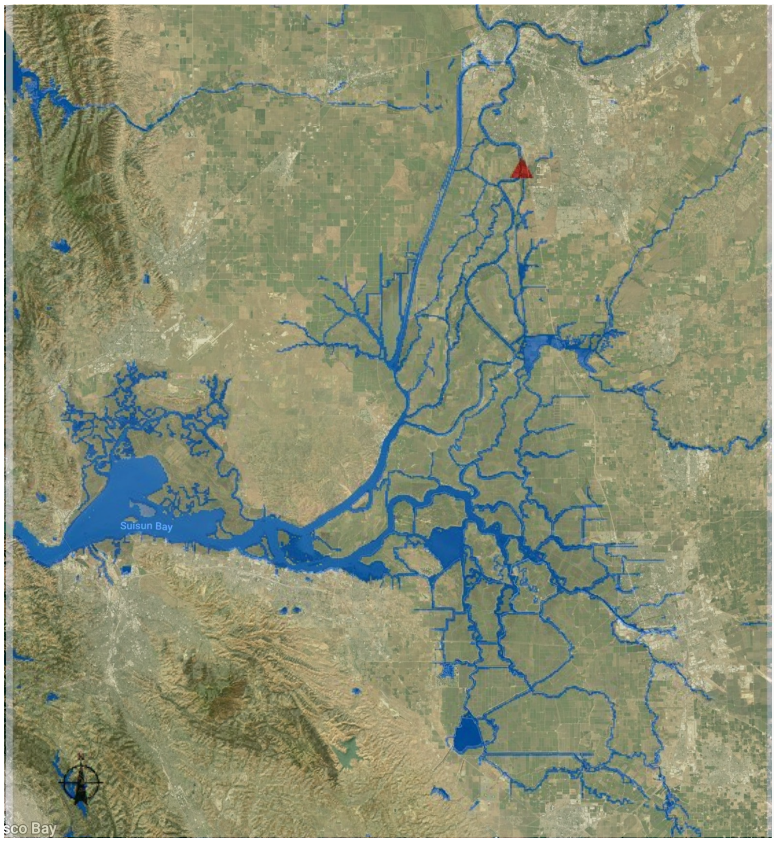


Figure 1 Map of the northern San Francisco Estuary, including the Delta and Suisun Bay. Red triangle: location of Regional San WWTP outfall, discharging to the Sacramento River.

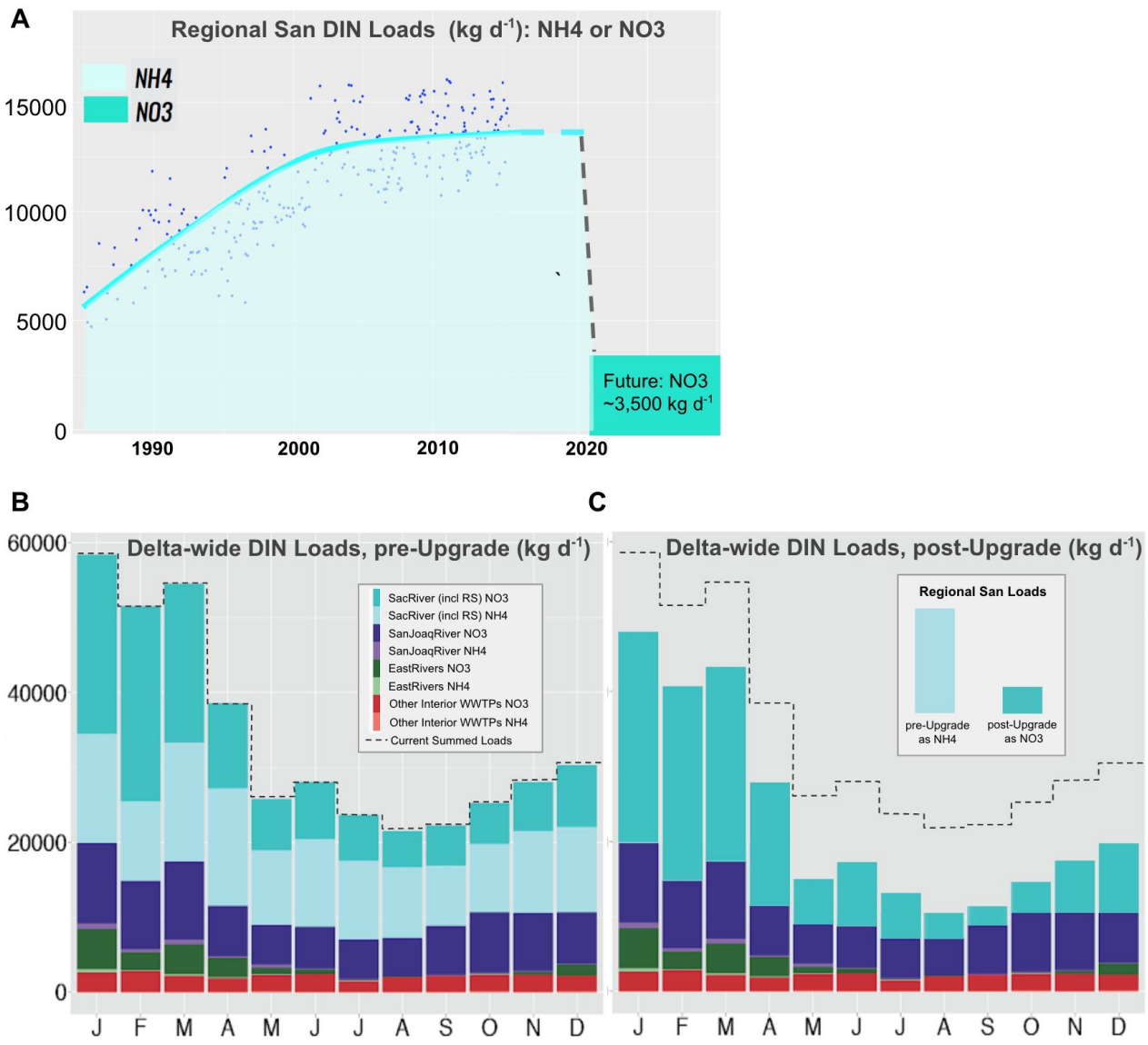
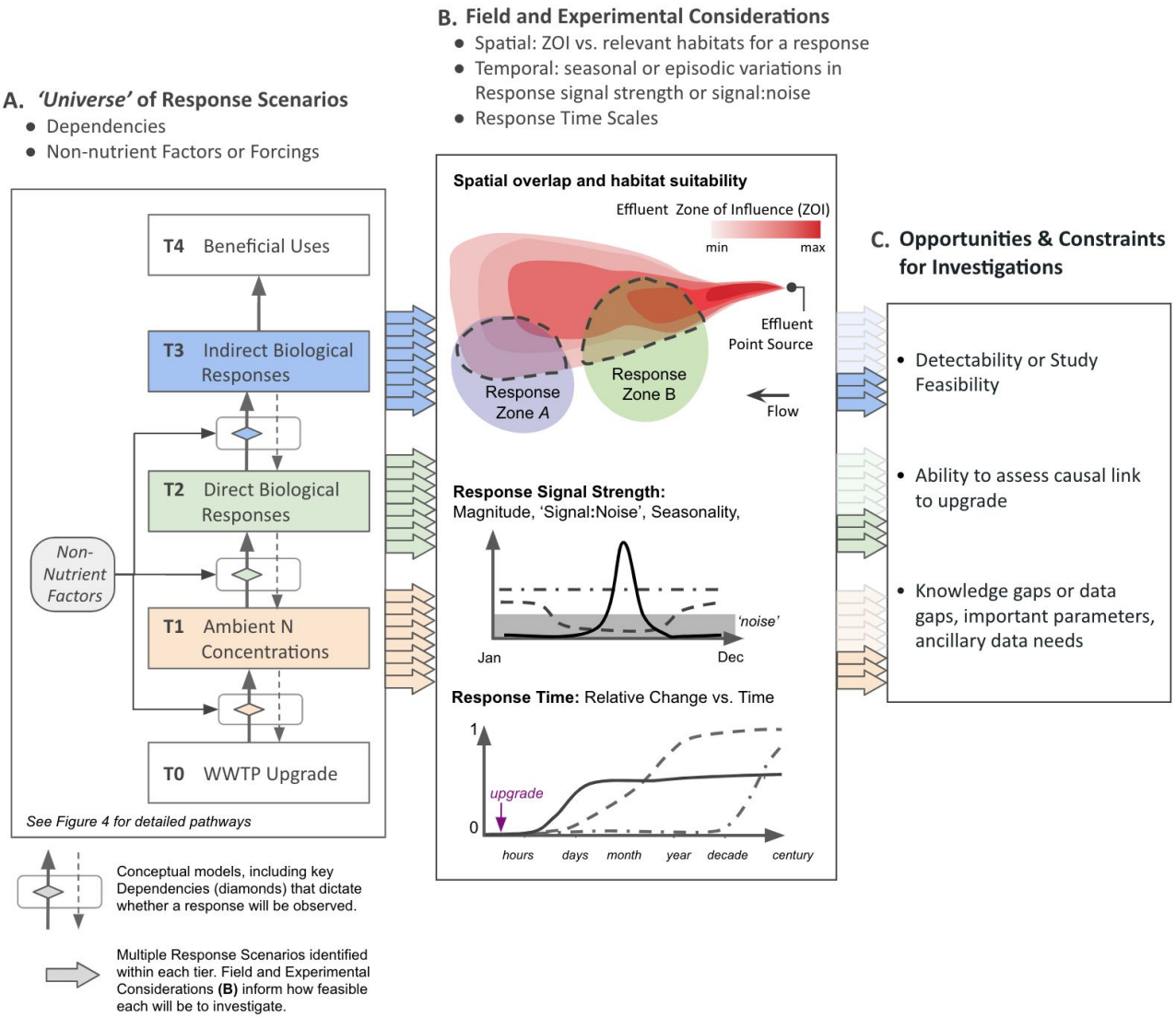


Figure 2: **A.** Time series of inorganic N loads from Regional San, ~1985-2016, and estimated post-upgrade loads. Points represent calculated load (Measured Concentration x Flow) based on monthly data (Data: pre-2005, Jassby 2008; 2008-2016, Regional San, pers. comm.); curve approximates annual average loads. Current N loads are predominantly in the form of ammonium (NH4). The upgrade will add nitrification and denitrification steps to the treatment plant, substantially decreasing dissolved inorganic N (DIN) loads and converting most of the NH4 to nitrate (NO3). **B**. DIN loads to the Delta as a function of month, depicting proportions entering as ammonium (NH4) and nitrate (NO3). Loads entering via the Sacramento, San Joaquin, and eastern tributaries (Mokulumne and Consumnes) were estimated using monthly flow rate and monthly NH4 and NO3 concentration data, with monthly averages estimated for the period 2007-2013 (see SFEI 2015). Sacramento River loads were estimated using a site downstream of Regional San's outfall (DWR-EMP station C3, ~4km downstream of outfall), and therefore include Regional San's contribution. Other interior WWTP loads (red) include inputs from Stockton and Tracey. **B.** Post-upgrade estimated DIN loads. Estimated by adjusting Sacramento River loads to account for Regional San's anticipated future inputs, assuming the following: Current loads: ~14000 kg N/d, ~100% of DIN as NH4; Future: ~3500 kg N/d, ~100% of DIN as NO3. These plots are presented as semiquantitative overviews. The current Sacramento River load estimates suggest that some NH4 loss had already taken place by the time water reached station C3 during summer months, which is consistent known nitrification rates (e.g., Kraus et al. 2017). To approximate future loads, DIN was assumed to behave conservatively over the stretch from the outfall to C3, and the difference between Regional San's current and future DIN loads (~10500 kg d^{-1}) was subtracted from Sacramento NH4 and NO3 loads.



Criteria	Major Considerations
Detect? Feasibility of detecting or observing a response (or lack thereof)	1. Analytical methods (sensitivity, specificity) 2. Spatial overlap between study site(s) and ZOI 3. Signal:Noise - Effect size vs. Natural variability 4. Data density (space, time, length of record)
Test Link? Ability to attribute a response (or lack thereof) to upgrade	5. Number steps (tiers) removed from the upgrade (T0) 6. Changes in other influential factors in the system 7. Response time
Occur? Likelihood that a hypothesized response will occur, and be substantial or important at an ecosystem-scale	8. Correct conceptual model, correct mechanisms and drivers; 9. Key dependencies satisfied, relative importance of processes

Figure 3 Framework for identifying and exploring Response Scenarios. The inset table summarizes the criteria by which Response Scenario were evaluated within Step B.

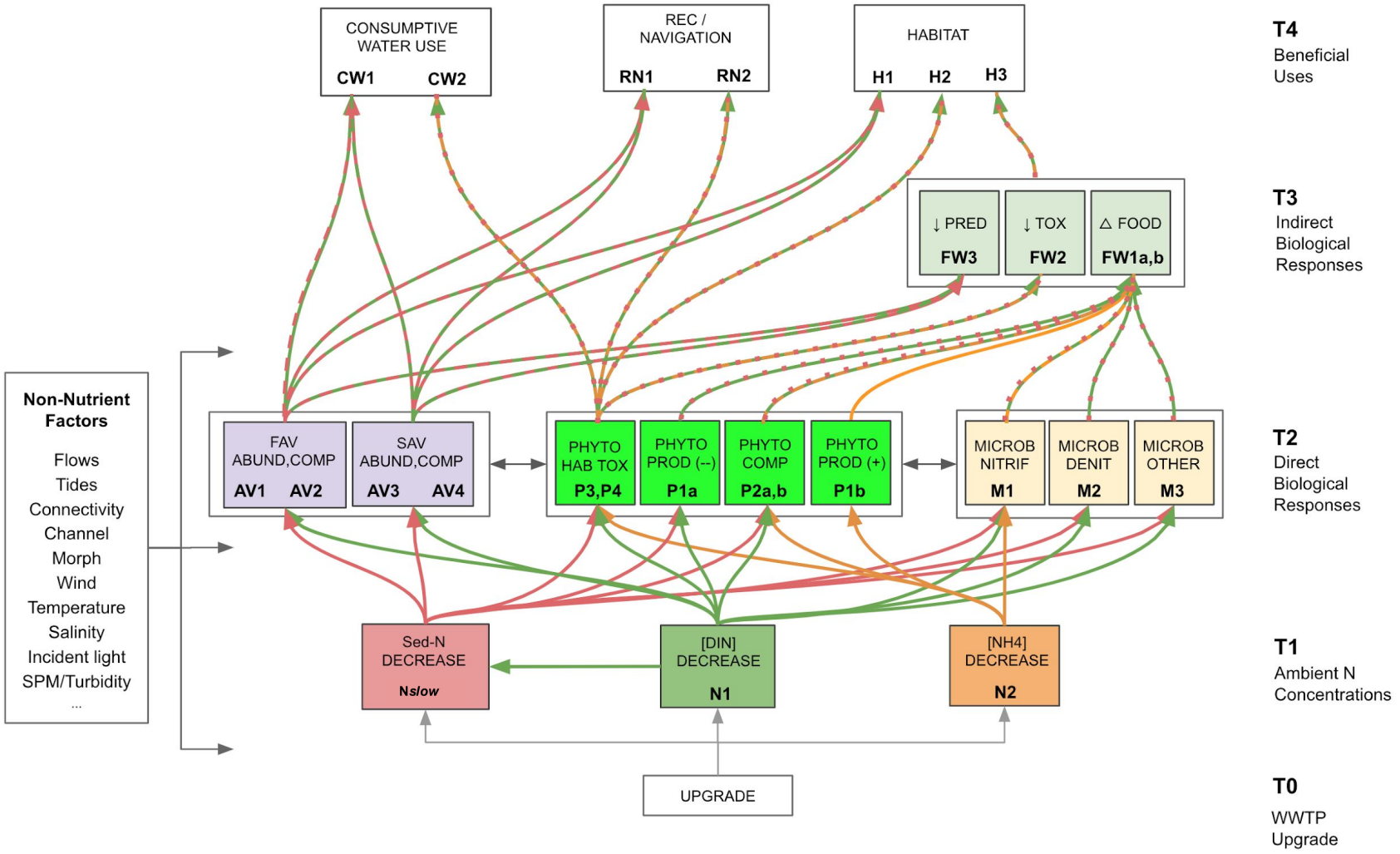
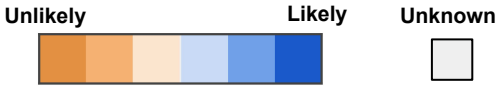


Figure 4 Response Scenarios (colored boxes; see Table 2 descriptions) organized along 'bottom-up' mechanistic pathways connecting the WWTP Upgrade (T0) with Beneficial Uses (T4). The pathways and the individual Response Scenarios along each path are all hypothetical. The likelihood that a response will occur, its magnitude (relative to natural variation), and its potential to trigger responses at higher tiers depends on an array of Non-Nutrient Factors that modulate biological and biogeochemical responses and other dependencies, as discussed in the text and summarized in Table 2. Arrow color represents a pathway's origin in T1 (N1=green; N2=orange; N*slow*=red; multi-colored = multiple potential origins). ↔ connotes interactions/feedbacks between T2 responses, P↔M, AV↔P, and AV↔M which are discussed in the text.

Table 1 Response Scenarios related to the Regional San WWTP upgrade. Figure 4 schematic organizes responses along bottom-up mechastic pathways.

Tier	Category	Response Scenario	
T1	Ambient Nutrient concentrations	**N1**	Decreased concentrations of dissolved inorganic nitrogen (↓[DIN])
		N2	Decreased concentration of ammonium (↓[NH4])
		N*slow*	Gradual decrease of labile sediment org-N (↓[sed org-N], ↓sed N flux)
T2	Phytoplankton Production	**P1a**	↓Phytoplankton primary production due to ↓[DIN]
		P1b	↑Phytoplankton primary production due to ↓[NH4] (Ammonium Paradox)
		P2a	Phytoplankton assemblage: changes due to ↓[DIN]
		P2b	Phytoplankton assemblage: ↑food quality due to ↓[NH4] (ecological stoichiometry)
		P12*slow*	One of above, but after years or decades, due to ↓[sed org-N] and ↓sed N flux
	Phytoplankton HABs	**P3**	Decreased severity (biomass, area) of HABs, due to ↓[DIN] or ↓[NH4]
		P4	Decrease in HA toxin production, due to ↓[DIN] or ↓[NH4]
		P34*slow*	One of above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux
	Microbial community	**M1**	Changes to nitrifier community (abundance, assemblage) due to ↓[NH4]
		M2	Changes to denitrifier community (abundance, assemblage) due to ↓[DIN]
		M3	Other changes to the heterotrophic microbial community due to ↓[DIN]
		Mslow	One of the above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux
	Aquatic Vegetation	**AV1**	↓FAV density, distribution, or areal coverage, due to ↓[DIN]
		AV2	Changes to FAV community composition, due to ↓[DIN]
		AV3	↓SAV density, distribution, or areal coverage, due to ↓[DIN]
		AV4	Changes to SAV community composition, due to ↓[DIN]
		AV*slow*	One of above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux
T3	Food Web	**FW1a**	Changes in pelagic target organisms' (fish) abundances due to changes in food resources
		FW1b	Changes in food resources reaching target organisms (e.g., primary consumers, copepods)
		FW2	Change in pelagic target organisms' abundances due to change in HA toxin exposure
		FW3	Decreased invasive predator habitat, indirect effects on target organisms (via the food web)
T4	Consumptive Water Use	**CW1**	Improvements to water operations due to decreased invasive aquatic vegetation
		CW2	Lower human exposure to HAB toxins
	Recreation and Navigation	**RN1**	Improved navigation and recreation due to reduction in invasive aquatic vegetation
		RN2	Decreased dermal exposure to toxins produced by harmful algae
	Habitat	**H1**	Improved physical habitat due to lower presence of invasive aquatic vegetation
		H2	Decreased impacts to biota from direct exposure to HAB toxins
		H3	Improved food supply, in particular within pelagic pelagic food web
		H4	Improved DO conditions, resulting from decreased production and metabolism

Table 2 Summary of scores for each response scenario across three criteria (See Figure 3 inset table): Detectable? Ability to Test Link? Likelihood of Occurring? See Boxes 1-6 for further discussion.



Detect	Test Link	Occur	RESPONSE SCENARIOS
			N1 Decreased concentrations of dissolved inorganic nitrogen (↓[DIN]direct)
			N2 Decreased concentration of ammonium (↓[NH4]direct)
			Nslow Gradual decrease of labile sediment org-N (↓[sed org-N], ↓sed N flux)
			P1a ↓Phytoplankton primary production due to ↓[DIN]
			P1b ↑Phytoplankton primary production due to ↓[NH4] (Ammonium Paradox)
			P2a Phytoplankton assemblage: changes due to ↓[DIN]
			P2b Phytoplankton assemblage: ↑food quality due to ↓[NH4] (ecological stoichiometry)
			P12slow One of above, but after years or decades, due to ↓[sed org-N] and ↓sed N flux
			P3 Decreased severity (biomass, area) of HABs, due to ↓[DIN]
			P4 Decrease in HA toxin production, due to ↓[DIN]
			P34slow One of above, but after years or decades, due to ↓[sed org-N] and ↓sed N flux
			M1 Changes to nitrifier community (abundance, assemblage), due to ↓[NH4]
			M2 Changes to denitrifier community (abundance, assemblage), due to ↓[DIN]
			M3 Other biological community changes, via microbes, due to ↓[DIN]
			Mslow: One of above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux
			AV1 ↓FAV density, distribution, or areal coverage, due to ↓[DIN]
			AV2 Changes to FAV community composition, due to ↓[DIN]
			AV3 ↓SAV density, distribution, or areal coverage, due to ↓[DIN]
			AV4 Changes to SAV community composition, due to ↓[DIN]
			AVslow One of above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux
			FW1a Changes in pelagic target organism abundances due to changes in food resources
			FW1b Changes in food resources reaching target organisms (e.g., copepods)
			FW2 Change in pelagic target organism abundance due to change in HA toxin exposure
			FW3 Indirect effects on target organisms via food web, improved physical habitat
			FWslow One of above, but after years or decades, due to ↓[sed org-N] or ↓sed N flux

	Data / Knowledge Gap	T1			T2																T3	
		Nutrients			Phytoplankton (incl., HABs)							Microbes				Invasive Aquatic Vegetation					FW	
		N1	N2	N slow	P1a	P1b	P2a	P2b	P12 slow	P 3 a, b	P3 slow	M1	M2	M3	M slow	AV1	AV2	AV3	AV4	AV slow	FW 1b	Necessary Gap-Fills, Dependencies
G1	Quantify ambient nutrient concentrations (higher spatial/temporal resolution, additional habitats)																					
G2	Quantify nutrient transformation rates, space/time (mineral., nitrif., denitrif., uptake)																					1,18
G3	Quantify sediment nutrient pools, availability and fluxes																					
G4	Phytoplankton Biomass: discrete + high frequency data; align with nutrient+physical data																					1,18
G5	Phytoplankton Community: High&low resolution (space,time): composition, densities, biovolume																					1,18
G6	Quantify phytoplankton primary production rates + nutrient requirements alongside other drivers																					1,18
G7	Quantify phytoplankton (and microbe) loss rates to grazers (planktonic, benthic)																					4,5
G8	Quantify HA abundance/toxins relative to nutrient field/other drivers, incl. in-situ+molecular																					1,18
G9	Characterize microbial assemblage (space,time) relation to nutrients, transformation rates, other drivers																					1,2,18
G10	Quantify contribution of microbial community to the foodweb.																					4-7,9
G11	Characterize interactions among primary producers (phyto, AVs, HABs, microbes)																					1-5,8-9,15,18
G12	Identify nutrient thresholds affecting AV growth by species (concentrations, form, timing)																					1,3
G13	Quantify AV nutrient demand to determine effects on water column nutrient concentrations																					1,2,3
G14	Monitor AV biomass and species composition over space/time																					
G15	Zooplankton sampling at relevant space/time freq, changes to carbon/energy delivery to food web																					1-7,18
G16	Characterize food source/quality consumed/assimilated by zooplankton and effects on abundance																					
G17	Quantify wetland nutrient demand, transformation rates, net exchange with adjacent habitats																					
G18	Collect physical data collection (e.g., temperature, light, salinity, water depth) and transport																					1-3,12-15,18
G19	Develop hydrodynamic/biogeochemical models: test hypotheses, exp'l design, synthesize results																					
G20	Maximize coordination: data + analysis across entities (monitoring, studies, modeling, synthesis																					
G21	Develop/Validate techniques to further enable cost-effective monitoring (discrete ↔*in situ* | HF↔RS)																					1,3-5,14,18

*AV, Aquatic Vegetation; HAB, Harmful algal blooms; phyto, phytoplankton; FW, Food web; HF, high frequency, RS: remote sensiting

Table 3 Data needs and knowledge gaps identified in the conceptual framework for potential nutrient-related responses to the Regional San WWTP upgrade. Cell color indicates the relevance of the data need or knowledge gap to each response. Note that data needs and knowledge gaps were identified for Tiers 1 and 2 only, with the exception of FW1b. The priorities (color) were assigned relatively for each Response (columns); relative priorities are therefore most pertinent within columns. While color across a row indicates that Data or Knowledge Gap is broadly applicable, the relative priorities are less meaningful. The far-right column (Necessary Gap-Fills, Dependencies) identifies other Data/Knowledge gaps (rows) upon which each row depends; i.e., for row 4, also pursuing rows 1 and 19 will make the data in row 4 most relevant.

Box 1 Ambient Nutrient Responses (T1)

- The nSFE receives elevated inputs of N and P from anthropogenic sources, including wastewater treatment plant (WWTP) effluent, agricultural runoff and urban runoff. N and P occur in a variety of chemical forms (Table B1.1), distributed between the water column, sediments, and living organisms. Long-term nSFE monitoring data show that DIN concentrations vary by ~100-fold over space and time (Figure B1.1).
- The Regional San upgrade will constitute a large shift in both the amount and form of N entering the nSFE (Figure 2A). Investigating upgrade-related biological responses (T2, T3) will require selecting study sites based on anticipated changes in N availability. While some T1 changes can be easily predicted, accurately predicting others -- the *where*, *when*, *what*, and *how much* - will require addressing important knowledge/data gaps.

Table B1.1 Major N and P Chemical Forms

NO_3^-	Nitrate
NH_4^+	Ammonium, primary form of ammonia at pH<8
DIN	Dissolved inorganic N (essentially NO_3^- + NH_4^+).
N_2	(di)Nitrogen gas
DON, PON	Dissolved and Particulate organic N
TN	DIN + DON + PON. In nSFE, 25-50% *TN = DIN
o-PO_4	orthophosphate (inorganic)
DOP, POP	Dissolved and Particulate organic P
✣-PO_4	Inorganic P bound to particles

Other common N forms in water: NO_2^- (nitrite), N_2O (nitrous oxide)

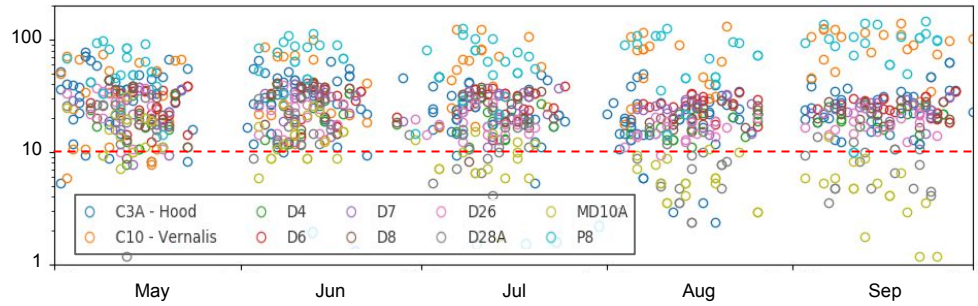


Figure B1.1 DIN concentrations (µM) vs. day-of-year (May-Oct) 2000-2015, at stations across the nSFE. Red line depicts 10*K_s (K_s, half-saturation constant, ~1 µM represents a central tendency value for diatoms, Kudela et al, 2010; see also Box 2). When DIN > ~10 µM, DIN is unlikely to limit phytoplankton growth rates See station locations in Figure B1.4A; D6, D7, and D8 are in Suisun Bay

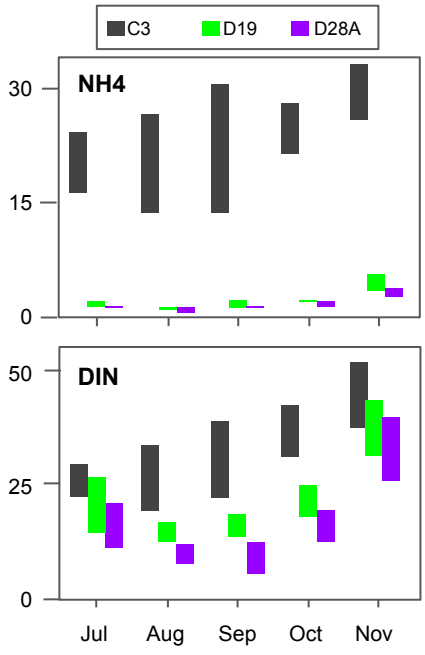
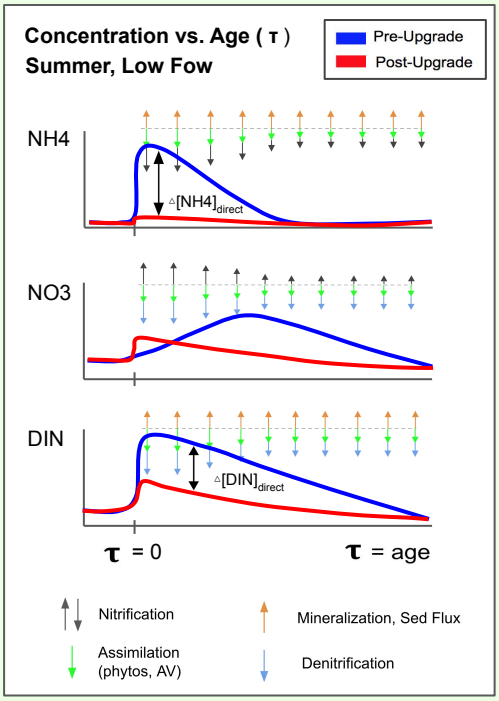


Figure B1.2 Interquartile range for NH4 (µM) and DIN (µM) during low flow months at three SacRiver dominated locations (1998-2011). See Figure B1.4 for locations.

How do N concentrations vary spatially? Regional San currently discharges N primarily as NH4 (~90% of TN), and, post-upgrade, will discharge N primarily as NO3. Upon entering the Delta, both NH4 and NO3 undergo reactions that alter their chemical form. Long-term monitoring data offer evidence of these transformations, and a sense of their quantitative importance at along the SacRiver's flow path (Figure B1.2). NH4 concentrations were >10-fold lower at downstream stations (D19, D28A) than near Regional San's outfall (C3). DIN concentrations were 2-4 fold lower. The large changes in NH4 and DIN result from the cumulative effects of biogeochemical transformations that occurred during transit, as the 'age' (τ) of the effluent-dosed river water increases (Figure B1.3). All three stations are dominated by SacRiver water during low flow months (Fig 1.4A). But, water parcels arriving at D19 and D28A were 15-20 days and >20 days old, compared to 2-3 days at C3 (Figure B1.4B). Thus, vigorous biogeochemical transformations along nSFE flowpaths strongly attenuate effluent nutrient signals. Predicting upgrade-related nutrient concentration changes will require: *1.* Accurate predictions of physical transport (water, chemicals) through the system; and *2.* Quantitative estimates of nutrient transformations during transport, including how rates vary temporally and spatially (i.e., along flow paths).

What can we say now about expected changes in DIN and NH4 concentrations, based on best available information?

Figure B1.3 Blue curves qualitatively depict pre-upgrade conditions, NH4, NO3, and DIN concentrations variations within a water parcel traveling along an idealized channel, subjected to transformations and losses (vertical arrows). The x-axis, tau (τ), represents water parcel age, or elapsed time since effluent addition. At $\tau = 0$, when the water parcel passes the WWTP outfall pipe, effluent mixes into the water parcel. Immediately downstream, nutrient concentrations are determined by effluent nutrient speciation and dilution in the river water (determined by instantaneous effluent and river discharge volumes), as well as upstream nutrient concentrations. As the parcel travels downstream, NH4, NO3, and DIN undergo transformations (arrows), which result in net changes in concentrations with increasing τ. Red curves depict post-upgrade concentrations, shortly after the upgrade (before changes to sediment fluxes are evident). Although the differences between post-upgrade and pre-upgrade concentrations ($\Delta[NH4]_{direct}$, $\Delta[DIN]_{direct}$) will be large under some conditions, they are expected to become small with increasing τ.



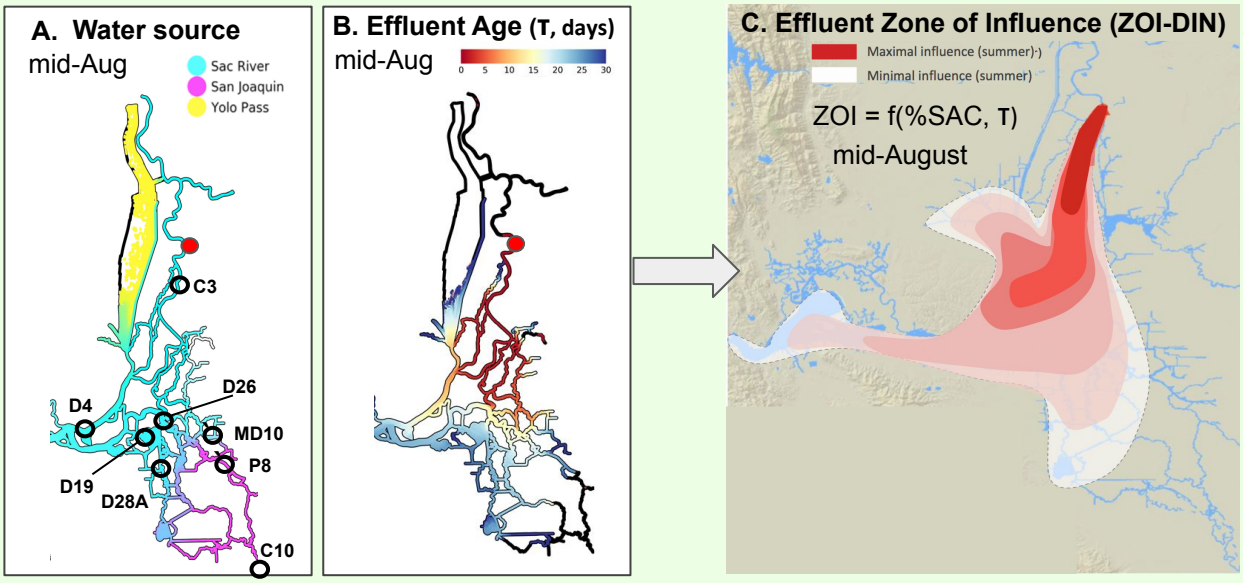


Figure B1.4 A. Water source, quantified using three conservative tracers introduced into a hydrodynamic simulation for WY2011; conditions depicted are for mid-August 2011. **B.** Water parcel age (τ) after receiving Regional San Effluent (outfall = red circle); results depicted here are for mid-August 2011, based on numerical age-tracer added at the location of the Regional San outfall. **C.** The WWTP upgrade's Zone of Influence (ZOI) for DIN during summer/low-flow periods, with maximal and minimal influence referring to largest and smallest direct changes to water column DIN concentration, $\Delta[DIN]_{direct}$ (see Figure B1.3). $\Delta[DIN]_{direct}$ will vary strongly in space and time, influenced by the proportion of SacRiver water (**A**) and water parcel age (τ; **B**). The ZOI gradations were approximated based on output from a simplified biogeochemical model output (SFEI, in prep), comparing results from simulations with pre- vs. post-upgrade N loads, which had similar patterns similar to τ (**B**), with maximal and minimal ZOI coinciding with shortest and longest τ. Predicting where, and by how much, nutrient concentrations are expected to change is critical for designing robust studies. In addition, some hypotheses or pathways are related to specific N forms (i.e., NH4). Field data suggest that ZOI-NH4 may be considerably smaller than ZOI-DIN (Figure B1.2), indicating that field site selection may differ depending on whether $\Delta[DIN]_{direct}$ or $\Delta[NH4]_{direct}$ is the most relevant change. Simulations: Zhang et al, in prep.

Forecasting upgrade-related changes to DIN and NH4 concentrations ([DIN], [NH4]): Predicting [DIN] and [NH4] under current loads is challenging because of N's complex biogeochemical cycle, combined with the nSFE's physical intricacies - multiple nutrient sources; woven network of channels; altered flow routing due to water withdrawals; and tidal influence. As a starting place, it is useful to first consider a plug, or parcel, of water traveling along an idealized uniform river channel that receives a constant flow of nitrogen-rich WWTP effluent (Fig. B1.3). Prior to reaching the WWTP outfall, the water parcel already contains some NO_3^- and NH_4^+ from upstream sources; as it passes the WWTP outfall, N-containing effluent quickly mixes uniformly over the river's cross-section (within several km), yielding a final well-mixed concentration. NH4, NO3, and some forms of organic-N are highly reactive compounds that readily change chemical form. Figure B1.6 captures the major transformation processes influencing N chemistry during transport through the nSFE, nearly all which are biologically-mediated, including biogeochemical transformations by the microbial community (Box 5). For this discussion, the most important transformations are nitrification, denitrification, assimilation by phytoplankton or aquatic plants, and mineralization, which occur within the water column, at the sediment interface, or within the sediments, and alter water column N concentrations as the travel time or age (τ) increases (Figure B1.4). Some N accumulates in the sediments through settling of particulate organic N; SAV root mass accumulation; and senescence of FAV. Sediments are a leaky repository, though, where microbes mineralize (degrade) sediment org-N, releasing dissolved NH_4^+ to the porewater, some of which gradually fluxes back to the water column as recycled NH_4^+ or NO_3^-. The rates and relative importance of these processes vary temporally and spatially. For example, high solar insolation and high temperature accelerate the above biological processes (i.e. faster rates). When river flow rates (and velocities) are lower, even if transformation rates stay the same, these processes progress further as the parcel travels the same distance (i.e, same distance, but higher τ). Under those conditions, the magnitude of the N-cycling processes acting on the water parcel can be large enough compared to DIN mass in the water column that only a small proportion of that DIN is actually the original N received from the outfall.

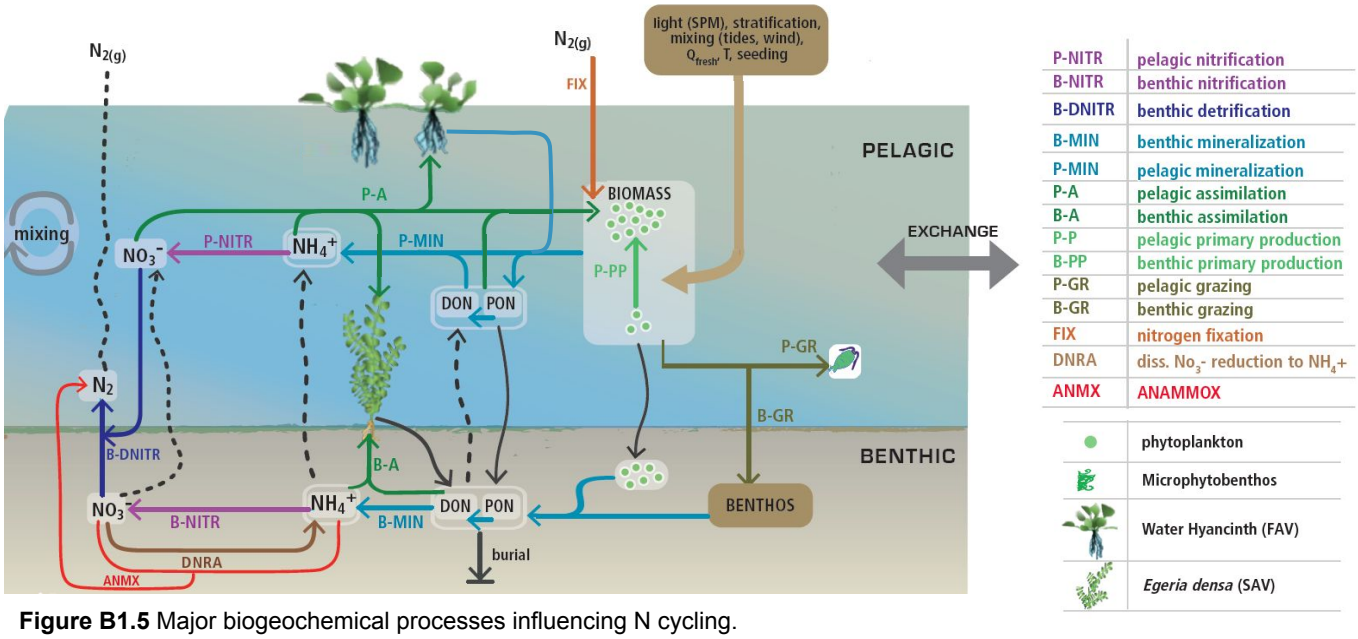


Figure B1.5 Major biogeochemical processes influencing N cycling.

The curves in Figure B1.3 qualitatively depict net effects of those processes on water column [NH4], [NO3], and [DIN], along a 1D flow path. Blue and red curves denote pre-upgrade and post-upgrade concentrations, respectively. The vertical differences between the blue and red curves, $\triangle[NH4]_{direct}$ and $\triangle[DIN]_{direct}$, represent the upgrade-related changes to [NH4] and [DIN] occurring immediately after the upgrade (i.e., time since upgrade $\geq \tau$), not including more gradual changes that will occur as sed-orgN decreases. Note that $\triangle[NH4]_{direct}$ and $\triangle[DIN]_{direct}$ decrease with increasing τ, due to transformations and N recycling along the flow path (e.g., similar to observed conditions, Figure B1.2). The upgrade 'Zone of Influence' (ZOI) for DIN, shown in Figure B1.3C (see caption), translates $\triangle[DIN]_{direct}$ into a 2D spatial view, for a specific set of flow conditions (typical August). The ZOI-DIN gradations from maximal to minimal correspond to maximum and minimum $\triangle[DIN]_{direct}$, respectively; the contours are not intended as quantitative prediction, but rather to paint an approximate or qualitative picture of variability. A comparison between ZOI-NH4 and ZOI-NH4 suggests that ZOI-NH4 has sharper gradients and the minimal zone extends over a smaller area. Although bottom sediments are areas of active N transformations, it is reasonable to expect that [sed-orgN] will respond slower to the upgrade than [DIN] or [NH4]. NH4 (or NO3) effluxes (sed-N-flux) to the water column will continue, causing [NH4] or [DIN] to remain elevated. Over time, [sed-orgN] and sed-N-flux will gradually decrease. Sparse data availability on sed-orgN and sed-N-flux makes it difficult to speculate about the rate with which they will change post-upgrade; however, response times on the order of months to years seem realistic first predictions

Det?	Link?	Occ?	Table B1.2 Nutrient Response Scenarios	Dependencies
			N.H$_0$ No change in N concentrations.	
			N1 Decrease in water column DIN concentrations	***dn1,dn2,dn3***
			N2 Decrease in water column NH4 concentrations	***dn1,dn2,dn3***
			N*slow* Gradual decrease of labile sediment org-N (↓[sed org-N], ↓sed N flux)	***dn1,dn2,dn3***

B3.1 Summary: Nutrient Response Scenarios

- **Occur?** High Confidence that water column DIN and NH4 concentrations will decrease substantially (N1, N2), Also High Confidence that sediment org-N levels will decrease (N*slow*), but the magnitude and response times of those changes are highly uncertain.
- **Detect? Link?** *N1, N2* Readily detectable and linkable in the near-field of the outfall and in SacRiver dominated areas; more difficult/uncertain with increasing distance or travel time from the outfall or where/when other water or nutrient sources contribute. N*slow* will be less straightforward, but still feasible.
- **Response times** (Figure B1.7): N1, N2: Near field (small τ): An almost immediate response (hours to days) would be expected (leftmost curve). Moderate to long τ: [DIN] and [NH4] may show early partial responses (dashed curve), but remaining changes could occur gradually (anywhere in shaded grey area). N*slow*: Sediment org-N, and sediment fluxes are expected to respond slowly (years to decades, right-most curve).
- **N1, N2:** *Key dependencies:* Changes will be most evident at locations/times within maximal or intermediate ZOI contours (Figure B1.5) The magnitude of [DIN] and [NH4] changes will vary greatly as a function of space and time, including among areas dominated by Sacramento River water, due to nutrient transformations along flow paths, and the age, or travel time (τ), of effluent-containing water will be a more relevant predictor than distance. In addition, other physical and biological characteristics of habitats (depth, vegetation, phytoplankton growth, etc.) will influence the detectability of concentration changes. Accurate assessments, or predictions, of [DIN] and [NH4] changes will require: sufficient pre- and post-ugrade data to account for temporal variability at multiple time-scales (interannual, seasonal, tidal); and data on transformation rates, which are currently poorly constrained.
- **N*slow*:** *Key dependencies:* Similar to above. In addition, the ZOI for sed-orgN may extend further than ZOI-DIN because continual recycling and benthic fluxes would allow for a larger effluent-related footprint. In areas where the sourcewater fingerprint changes substantially by season (e.g,, interior Delta, with San Joaquin River dominance during winter/spring), the influence of other sourcewaters could obscure the signal. Predictions of how much (and how fast) sed org-N will change are difficult to make because of the paucity of relevant data about sediments, including: magnitude of the current sediment N pool; the proportions of sedorg-N that are relatively bioavailabie vs refractory; and the major sources of orgN to the sediments (e.g., transport of particulate org-N by tributaries, internal production by phytoplankton; internal production by macrophytes).

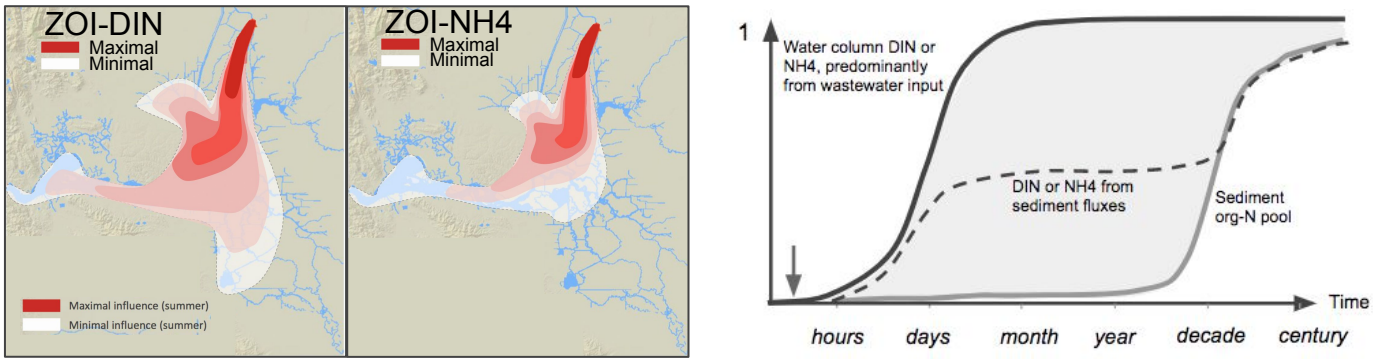


Figure B1.6 *left*: Qualitative depiction of spatial extents of ZOI-DIN and ZOI-NH4, for late-summer conditions (August). Contours based qualitatively on numerical simulations. *right:* Characteristic response times for Tier 1 nutrient responses.

Box 2 Phytoplankton Production

- Phytoplankton serve as a vital food resource at the base of nSFE food webs. Phytoplankton value as a food resource derives from both its quantity (biomass Mueller-Solger et al., 2001) and the nutritional-quality of the overall community composition (Cloern et al, 2015; Winder et al., REFS). The pre-1980s nSFE, despite its elevated nutrient levels, had relatively low phytoplankton production and biomass compared to other nutrient-enriched systems (REFS).
- Phytoplankton production and biomass (Figure B2.1) decreased dramatically throughout much (but not all) of the nSFE in the 1980s (Jassby et al., 2002; Jassby 2008), and remains low today. The loss of this critical food resource is considered to be among the factors contributing to declining fish populations (NAS 20XX; Sommers et al. 2007). Modest phytoplankton blooms still occur occasionally in the nSFE, and, even when biomass is low, the community often consists of compositions having overall have high nutritional value (Figure B2.2).
- N and P are essential ingredients for primary production. Therefore, substantially decreasing concentrations of one or both might therefore be expected to decrease phytoplankton production. However, how nSFE phytoplankton production will respond to the upgrade is far from obvious.

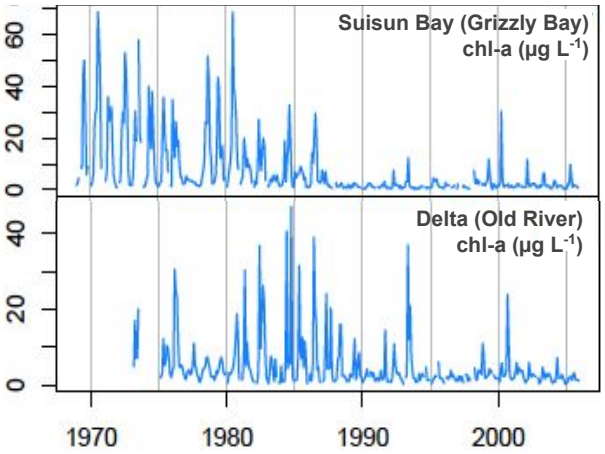


Figure B2.1 Phytoplankton biomass, based on chl-a concentration, from the 1970s-2005. Graphic: Modified from Jassby 2008

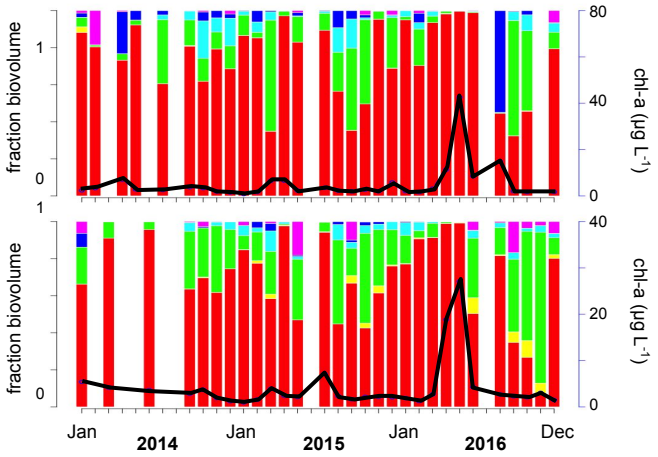


Figure B2.2 Monthly Chl-a (black) and phytoplankton community composition (fraction biovolume; stacked bars) in the Lower Sacramento River (top) and Suisun Bay (bottom). Source: SFEI 2017; Data: USGS

Relative food quality: Dinos>Cryptos>Diatoms>>Greens>Cyanos (Winder et al., 201X)

Multiple factors influence phytoplankton Growth, Biomass, and Community Composition (Figure B2.3)

- **Growth rate**: Temperature (+), light (+), stratification (+), nutrients (+), toxins (- ; xenobiotics, heavy metals)
- **Biomass**: Growth rate (+), grazing (-), settling (-), transport (±) , death (-), flushing rate (-)
- **Community composition**: Differences in growth requirements between taxa; selective grazing by consumers; Physiological/behavioral differences - e.g., motility; buoyancy regulation; different settling velocities.

In the nSFE, two factors have long been consided major controls on phytoplankton growth rates and biomass:

- High suspended sediment concentrations (SPM) maintain phytoplankton growth rates at low levels by limiting light penetration into the water column (Kimmerer and Thompson, 2014).
- Two introduced clams species have become densely established, one in freshwater regions (*Corbicula fluminea)* and the other in brackish regions (*Potamocorbula Amurensis*), and are believed to exert strong grazing pressure that draws down phytoplankton biomass (Kimmerer and Thompson, 2014).

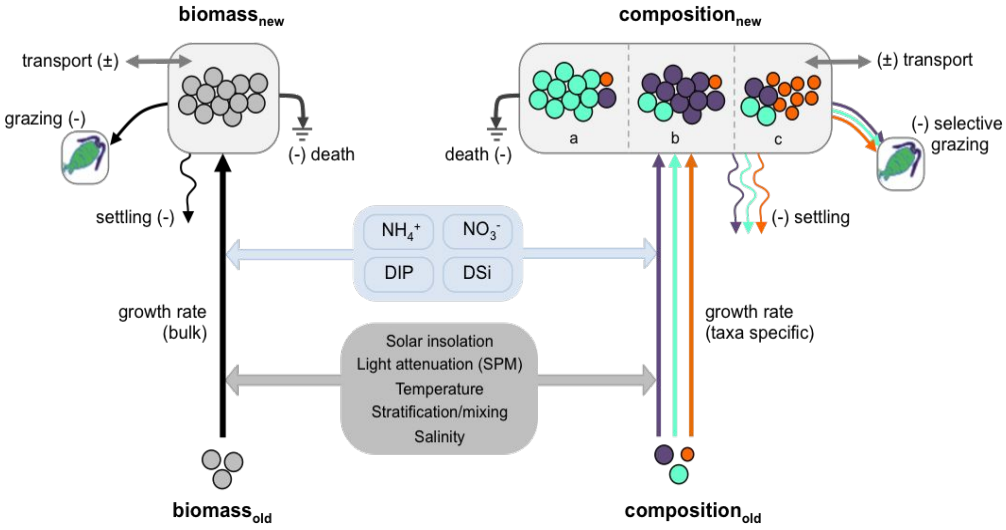


Figure B2.3 Factors influencing phytoplankton growth, biomass, and community composition. Many of the the same factors that influence bulk community growth and biomass also influence community composition, with different compositions emerging (a, b, c) because of inter-taxa variations in growth requirements or loss rates.

Classicl two basic criteria must be satisfied: i) N concentrations must fall to levels that substantially limit growth rates (Figure B2.4). Predicting where and when post-upgrade concentrations drop below growth-limiting levels will be essential for successful phytoplankton studies. ii) Any nutrient-growth-limiting effect must be substantial and sustained (over time) relative to other factors that dominantly influence phytoplankton dynamics (light attenuation by suspended sediment; grazing by invasive benthos).

The first criteria is illustrated qualitatively in Figure B2.4. Decreases in DIN will only influence phytoplankton growth if DIN drops below growth-rate limiting concentrations, [DIN] < ***B***, along the linear range of the curve. Currently, DIN seldom reaches growth-rate-limiting concentrations in routinely monitored areas (Figure B1.2). Given the large DIN load decrease, it is reasonable to expect that post-upgrade DIN will more frequently reach growth-rate-limiting concentrations. However, it remains uncertain how frequently the second criteria will be satisfied or changes will be measured, due to both the strength of those controls, and the dynamic relationship between hydrodynamics, light availability, phytoplankton production, clam and zooplankton grazing, and other factors. For example, both clam species are considered food limited (ref), meaning that phytoplankton biomass in the system may be “well-buffered’: i.e., if phytoplankton growth rates decrease, clam grazing rates could decrease accordingly (over months to years), having the net effect of offsetting any potential changes in biomass. Conversely, if production increases (i.e., NH4 paradox; see next page), clam abundance and grazing could increase in response to greater food availability, offsetting any phytoplankton biomass increases.

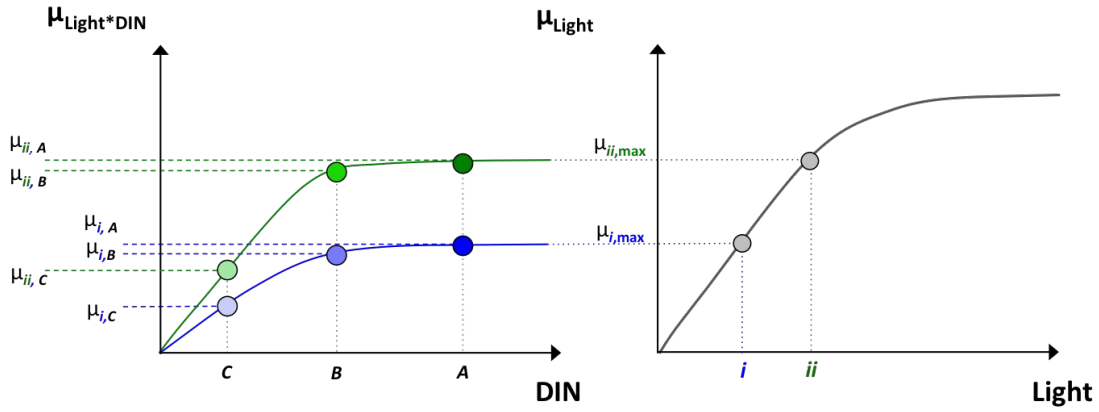


Figure B2.4 (right) Idealized phytoplankton growth rates as a function of light availability (when nutrients are present in excess). **(left)** Variations in growth rate as a function of DIN, at two light levels (**i** and **ii**). At high enough DIN levels, more DIN does not change the, growth rates. If DIN concentrations change from ***A*** to ***B***, growth rates will remain nearly the same. DIN must decrease further (e.g., to ***C***) for growth rates to drop substantially.

Differing Conceptual models for nSFE's low phytoplankton prodution: Across all the response scenario categories (Figure 4), Phytoplankton Production boasts the most diverse set of potential responses, including ones in direct opposition (e.g., P1a and P1b). That diversity results in large part from strongly diverent conceptual models (CMs) for the underlying causes of low phytoplankton productivity in the system. The different conceptual models are summarized below, and the related response scenarios are included in Table B2.1

***CM1. Light Limitation and Clams*:** Long-term monitoring data (since 1960s) indicates that the nSFE has long experienced relatively low phytoplankton productivity. The nSFE's highly turbid waters (from suspended sediments) severely restrict light penetration into the water column, and cause light availability to be a major factor limiting growth (Cloern 200x; Jassby 200x; Kimmerer and Thompson 2014). Even with the highly-turbid waters, nSFE phytoplankton production prior to the mid-1980s was much greater than today. For CM1, the hypothesized cause of dramatically lower post-1980s phytoplankton biomass levels in Suisun Bay and the western Delta is the introduction (ca. 1987) of a voracious filter-feeding clam, *Potamocorbula amurensis,* that now dominates nSFE salty and brackish benthic habitats (Kimmerer and Thompson 2014; Nichols et al., 19XX; NAS, 201x; Cloern et al,). A freshwater invasive clam (*Corbicula fluminea*, established *ca*. 1950s) is hypothesized to be a major factor impacting biomass in the Delta. *Corbicula,* however, cannot alone explain the post-1980s sharp decline phytoplankton biomass, because it was introduced much earlier. Based on long-term monitoring data (Figure B1.2), nutrient levels are hypothesized to substantially exceed growth-limiting thresholds (e.g., Jassby 2008), and therefore seldom restrict overall growth rates, or favor the growth of one taxa over another.

CM2. Nutrient-Centric: Two conceptuals models emerged over the past decade whose central hypotheses attribute declines in nSFE productivity to elevated NH4 levels. While these hypotheses have received considerable attention in nSFE adaptive management discussions over the past decade, there is substantial disagreement within the scientific community about their validity or quantitative importance.

*Ammonium Paradox (*AP; Dugdale et al., 2007; Parker et al 2012) i) Phytoplankton utilize only NH4+ (and not NO3) when NH4 levels exceed a threshold value (e.g., ~4 µM); ii) At the community level, phytoplankton grow slower when utilizing NH4 than NO3; iii) Elevated NH4 concentrations in the nSFE, primarily from WWTP loads, are therefore an important contributing factor to decreased primary productivity, and consequently decreased production at higher trophic levels.

Ecological Stoichiometry (ES; Glibert 2010; Glibert et al., 2011): i) Elevated NH4+ levels (or elevated N:P) select for phytoplankton taxa that have relatively low food quality. ii) Poor food quality at the base of the food web propagates to and negatively impacts higher trophic level organisms. iii) Elevated NH4 from WWTP discharges has caused a shift in phytoplankton food quality, and this bottom-up perturbation is a primary driver of declining fish populations.

***Counter Arguments**:*

- The broader phytoplankton ecology literature contains a large body of evidence demonstrating that, overall, phytoplankton taxa exhibit a diversity of relative growth rates on NH4 vs. NO3 (e.g., Berg et al., 2017; SFEI 2016; other papers).
- A field-scale experiment, conducted along the Sacramento River during a temporary hold of WWTP inputs found no clear evidence supporting the ammonium paradox (Kraus et al., 2015).
- Reexamination of the ES studies argue that the proposed 'community shifts' are actually artifacts introduced by problematic statistical methods (Cloern et al., 2011) and poor data quality (Senn 2016)
- While phytoplankton productivity and biomass in the nSFE have decreased, phytoplankton communities are still typically comprised of high-food-quality organisms on a biomass basis (e.g., Figure B2.2), suggesting that food limitation is an issue of quantity, not quality (Cloern et al., 2015).

Det?	Link?	Occ?	Table B2.1 Phytoplankton Production and Community Response Scenarios	Dependencies
			P1,2.H_0 No change in production rates, biomass, or assemblage	
			P1a: ↓Phytoplankton primary production (and ↓biomass) due to ↓ **[DIN]**	***dp1,2,3***
			P1b: ↑Phytoplankton primary production (and ↑biomass) due to ↓**[NH4]** (*NH4 Paradox*)	***dp4,5,2-3***
			P2a: Phytoplankton assemblage: changes due to ↓ **[DIN]**	***dp6,2,3***
			P2b: Phytoplankton assemblage: ↑food quality due to ↓**[NH4]** (*Ecological Stoichiometry*)	***dp5,7,2,3***
			P1,2.*slow* One of the above, but after years or decades, due to ↓ **[sed org-N]** and ↓ **sed-N-flux**	***dp8,9 + above***

The Phytoplankton Response Scenarios (Table B2.1) point to starkly different outcomes -- including direct opposites (P1a vs. P1b) -- and highlight important issues central to ongoing nSFE adaptive management discussions: how to increase lower food web productivity, and what effect (if any) will nutrient management actions have.

- **Occur?** Uncertain Although DIN and NH4 load reductions will be substantial, the numerous and complex factors controlling phytoplankton production prevent confident predictions about ecosystem-scale/ecologically-meaningful responses at this point.
- **Detect? Link?** The upgrade does present the opportunity to investigate or test conceptual models or hypotheses.
- **Response times** (Figure B2.5): Response times for phytoplankton productivity and assemblage to changes in water column [DIN] or [NH4] would be days to weeks. In habitats where sediment fluxes substantially influence [DIN] or [NH4], phytoplankton response times would follow those for sed org-N or sed N flux.
- **P1a, P2a:** *Key dependencies*: Other dominant factors do not limit growth (e.g., low turbidity); and [DIN] decrease to growth limiting levels, for overall community (P1a) or individual taxa (P2a). *Locations:* Habitats within the pre-upgrade ZOI, but with sufficiently large τ that post-upgrade [DIN] drops below growth-limiting levels ([DIN] along Sacramento River will likely exceed thresholds). Detecting P1a may be more straightforward than P2a because of the additional analytical challenges of quantitatively characterizing and interpreting community changes.
- **P1b, P2b**: *Key dependencies*: Hypothesized mechanisms need to be quantitatively important at ecosystem scales. *Locations*: Hypotheses emerged from Suisun Bay and the Sacramento River observations, placing those areas on the list of where to observe any changes. Assessing the hypothesized impacts on system-wide productivity requires studying this over broader areas, still within the ZOI, but at larger τ. Detecting P1b may be more feasible than P2b (similar to P1a vs. P2a, above).
- **P1,2*slow*** It is highly plausible that any of the above responses could be delayed in areas where N sediment fluxes are large enough to maintain sufficiently high [DIN] or [NH4].

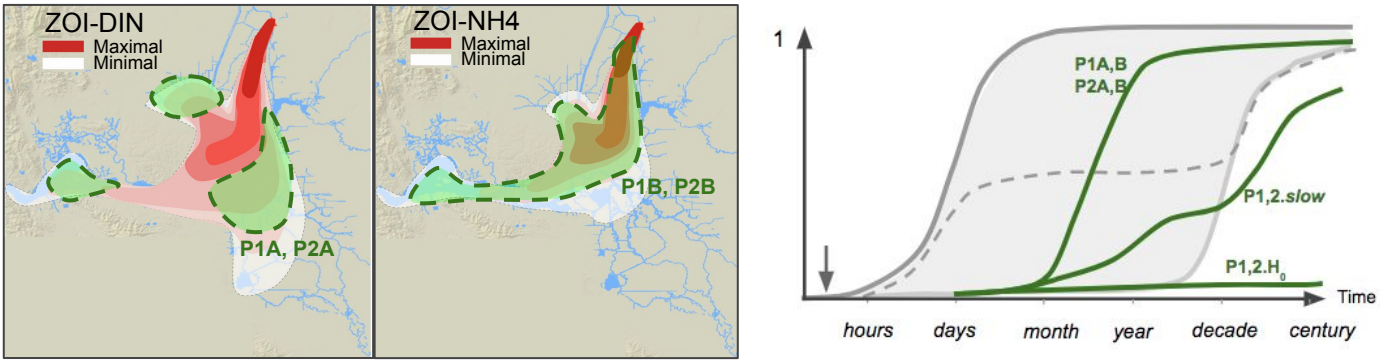


Figure B2.5 (left) Relevant zones for phytoplankton response scenarios, and their overlap with ZOI indicate potential areas where upgraded-related changes may occur. (right). Anticipated response times for phytpplankton response scenarios. (Grey curves and grey shaded area, are same as Figure B1.6, and denote ranges of response times for N1, N2, N*slow*, for comparison with phytoplankton response scenarios)

Box 3 Harmful Algal Blooms

- Harmful Algae Blooms (HABs) in the nSFE have become a growing problem since the early 2000s, with the potential of having diverse negative impacts (Figure 4 and Figure B4.1) including; toxins entering water supplies (CW2); toxicity to sensitive biota via the food web and direct exposure (H3, H2); and recreational and aesthetic impacts from dermal exposure to toxins and nuissance/noxious blooms (Dahm et al. 2016; Lehman et al. 2015).
- HAB events in the Delta are primarily related to cyanobacteria (cyanoHABs), and specifically *Microcystis* spp. and the toxin it produces, Microcystins (MCY). *Microcystis* were first documented in the Delta in 1999 (Lehman et al. 2005). Since then events have become fairly common (Lehman et al. 2005; Lehman et al., 2008; Lehman et al. 2017). Delta *Microcystis* blooms tend to occur during summer and early fall, and can be widespread; during events, MCY concentrations commonly exceed World Health Organization guidelines (humans and wildlife risks) (e.g., Lehman et al. 2005; Lehman et al., 2008).
- *Microcystis* blooms and MCY have the potential to negatively impact vital food web constituents (e.g., copepods) and sensitive fish species (Tseh et al., xxxx; Ger et al. 2010); limit recreational water uses (rashes from dermal exposure; ref); and threaten a critical drinking water supply for 25 million people.
- In addition to *Microcystis, o*ther potentially-harmful cyanobacteria have been observed in the Delta (REF). However, thus far *Microcystis* is the only confirmed toxin-producer (Otten et al., 2017; Lehman et al., 2017), and thus this section focuses primarily on *Microcystis* events.

- Currently there isn't a cyano-HAB monitoring program in the Delta. Instead, most available data on cyanoHABs have emerged from event-driven observations, which indicate that the the severe *Microcystis* events occur most frequently in the central/interior Delta (Figure B4.2), including proximal to Delta water withdrawals, and along San Joaquin River as it approaches the confluence with the Sacramento River

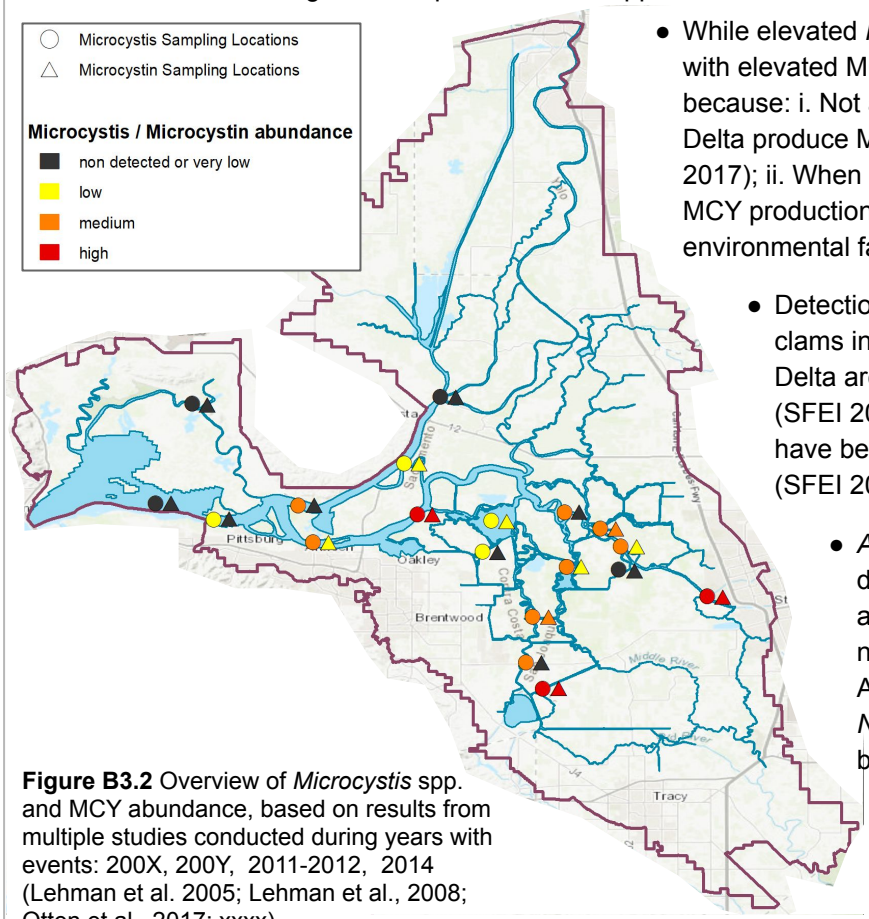


Figure B3.2 Overview of *Microcystis* spp. and MCY abundance, based on results from multiple studies conducted during years with events: 200X, 200Y, 2011-2012, 2014 (Lehman et al. 2005; Lehman et al., 2008; Otten et al., 2017; xxxx)

- While elevated *Microcystis* abundances often co-occur with elevated MCY, that is not always the case, likely because: i. Not all *Microcystis* strains found in the Delta produce MCY (Otten et al. 2017; Lehman et al., 2017); ii. When toxigenic strains are present, their MCY production rates vary depending on environmental factors (Figure B3.4)
- Detections of MCY in Suisun Bay filter-feeding clams indicate that MCY produced within the Delta are transported to down-estuary habitats (SFEI 2017). Particulate and dissolved MCY have been consistently detected in Suisun Bay (SFEI 2017; Peacock et al., 2018).
- *Aphanizomenon flos-aquae*, a diazotrophic organism (able to fix atmospheric nitrogen, N2), are the nSFE's most abundant cyanobacteria. At times *Dolichospermum* and *Nodularia*—also diazotrophs—also become abundant (Otten et al. 2017). While these genera do produce toxins in others systems, their toxin-producing ability has not yet been documented in the nSFE

Mulitple factors influence *Microcystis* blooms (Figure B3.2; Paerl and Otten, 2016; Lehman et al. 2017):

↑ *Microcystis*, ↑MCY ← ↑ T , ↑ Light, ↑ Residence time, ↓ Mixing/turbulence

In the nSFE, three of these factors (T, residence time, light) reach *Microcystis*-favoring levels in Jul-Sep, coinciding with time periods when blooms tend to be most severe (Lehman et al., 2017; Otten et al., 2017).

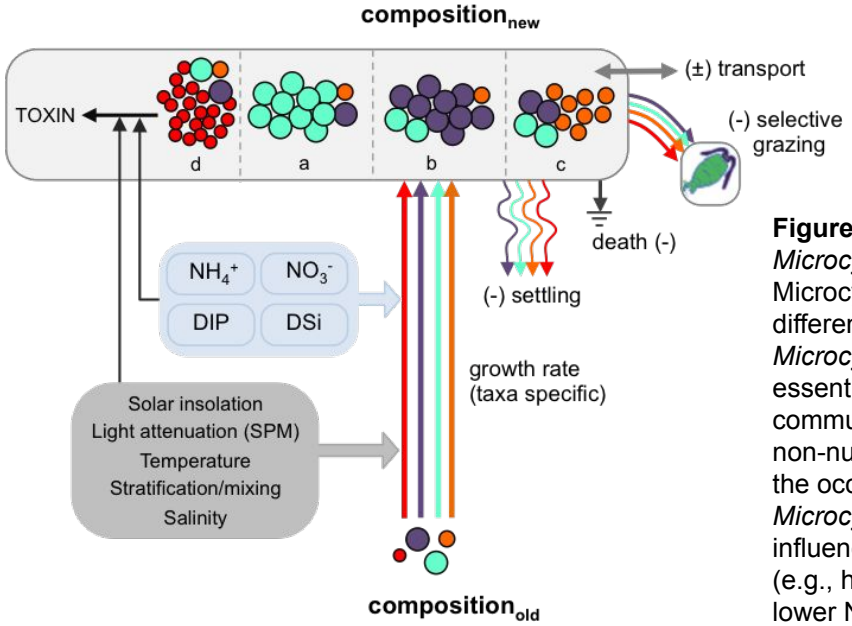


Figure B3.2 Factors influencing *Microcystis* growth, and production of Microcystins (MCY). Colored circles = different phytoplankton taxa; red circles = *Microcystis;* other c A HAB event is essentially a special case of phytoplankton community succession. In addition to non-nutrient and nutrient factors influencing the occurrence and magnitude of a *Microcystis* event, some factors may also influence toxin production (e.g., higher light levels → higher toxin; lower N → lower toxin).

Influence of DIN loads: While nutrient over-enrichment is expected to promote *Microcystis* blooms (Paerl and Otten 2013), N and P concentrations alone tend to be poor predictors of *Microcystis* bloom dominance and succession in environmental systems (Paerl and Otten, 2016; Otten et al., 2017). However, when other environmental factors (e.g., light, temperature, hydrodynamics, salinity; Figure B3.2) align to favor *Microcystis* growth, N and P availability can regulate the biomass or areal extent of a *Microcystis* event (REF). Therefore, decreasing DIN fluxes to the nSFE's *Microcystis*-bloom-prone areas has the potential to decrease the magnitude of HAB events. DIN availability can also influence bloom toxicity: toxin-producing *Microcystis* strains tend to dominate under replete N and P conditions compared to N- and P-limiting conditions (Vézie et al., 2002; Otten et al., 2012); cell toxin quotas (toxin per cell) are reduced (Horst et al., 2014); and MCY biosynthesis genes are downregulated under N-limiting conditions (Harke and Gobler, 2013). To the extent these observations point to mechanistic relationship(s) that would hold in the Delta, it is plausible that the toxicity of *Microcystis* events could also decrease following reduced N availability. For both bloom magnitude and toxicity, for a substantial change to occur will require a sufficiently large $\triangle[DIN]_{direct}$, or sufficiently large change in [DIN] once fluxes from the sediments decrease.

Influence of NH4 vs. NO3: Predicting the potential ecosystem-scale effects of discharging NH4 vs NO3 on *Microcystis* blooms is less straightforward than for changes in DIN (above). During summer months, the travel times, τ, from the WWTP outfall to *Microcystis* problem areas (Figure B3.1) are long (weeks). Under current conditions, NH4 concentrations in those areas are already relatively low during the summer, due to transformations and recycling of N during transport along flow paths (Figure B1.2). As a result, the expected upgrade-related NH4 concentration changes ($\triangle[NH4]_{direct}$) in these regions will be fairly small. Although there is some evidence to suggest that *Microcystis* growth rates or N-uptake rates can vary depending on the form of N utilized (REFs), *Microcystis* overall grows fairly well utilizing either NH4 or NO3 (REFs).

Taken altogether, the effects from changes in th DIN concentration rather than DIN form seem more likely to have effects on *Microcystis* blooms in the nSFE that are meaningful at the ecosystem scale, and can be detected and traced back to the upgrade. While this line of reasoning does not rule out effects related to NH4 changes, the HAB response scenarios (Table B3.1) focus on DIN responses.

Det?	Link?	Occ?	Table B3.1 Phytoplankton-HAB Response Scenarios	Dependencies
			P3.H_0 No change in HA occurrences: magnitude, severity, apatial extent, toxin concentrations	
			P3A: ↓HAB severity (biomass, area), due to ↓[DIN]	***dp11,12***
			P3B: ↓HA toxin production, due to ↓[DIN]	***dp13,11,12***
			P3.*slow* One of the above, but after years or decades, due to ↓[sed org-N] and ↓sed N flux	***dp14,15,11,12,13***

- **Occur?** Plausible, but uncertain. cyanoHABs clearly utilize and require nutrients. However, multiple other factors strongly influence when and where they occur, and their magnitude or severity.
- **Detect? Link?** The upgrade presents tractable opportunities to investigate HAB-related hypotheses, in part because it is such a large change in N inputs. Since cyanoHAB's occurrence and toxin production are influenced by numerous factors and, overall, poorly understood, studies will need to control or account for the role of those factors in order to examine the role of upgrade-related nutrient changes (e.g., through sufficient ancillary data collection, study over multiple years to capture interannual variations in those other forcings).
- **Response times** (Figure B3.4): If $\triangle[DIN]_{direct}$ is the primary nutrient driver (not sed-orgN or sedNflux), HAB response could, in theory, be rapid (weeks to months). In practice, though, the long transit times (large τ) during the narrow seasonal cyanoHAB window (Jul-Sep) would make it more likely to see a change from one year to the next. If sed-orgN or sediment N fluxes are important, responses would only begin becoming evident after years to decades.
- **P3A & P3B:** *Key dependencies*: i) Spatial overlap between cyanoHAB zones and zones with substantial $\triangle[DIN]_{direct}$. ii) N availability must be among the most important factors triggering HAB event and/or regulating their severity; iii) The change in ambient [DIN] caused by $\triangle[DIN]_{direct}$ must be large relative to other DIN sources (i.e., sed-N-flux, San Joaquin River). iv) Post-upgrade [DIN] must fall below thresholds that limit cyanoHAB growth, or limit the severity of events. v) (for P3B) [DIN] must fall below thresholds that would limit cyanotoxin production. *Locations:* While there cyanoHAB hotspot areas and ZOI overlap, much of that overlap occurs in at the ZOI's outer edges, where $\triangle[DIN]_{direct}$ may be smaller b/c of relatively longer τ. (Figure B3.4). To test cyanoHAB hypotheses, there will need to be excellent information about $\triangle[DIN]_{direct}$; best opportunities will be areas with smaller τ .
- **P3.*slow***: *Key dependencies*: i) Spatial overlap between cyanoHAB zones and zones where sedNflux is substantial source of DIN. ii) N availability must be among the most important factors triggering HAB event and/or regulating its severity; ii) DIN available to cyanoHABs from sedNflux must drop below thresholds that influence growth rate, magnitude of biomass, and/or cyanotoxin production. *Locations:* Areas still within the ZOI but at large τ .

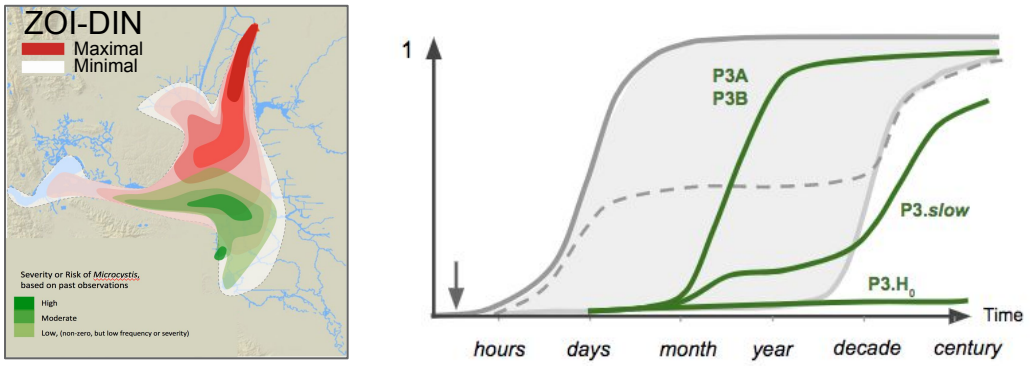


Figure B3.4 (left) Spatial extent of (green) regions in the Delta where *Microcystis* events occur most frequently or have been most severe; and (red) Zone of Influence (ZOI) for changes in dissolved inorganic nitrogen related to Regional San's upgrade. **(right)** Anticipated response times for HAB response scenarios. (Grey curves and grey shaded area, are same as Figure B1.6, and denote ranges of response times for N1, N2, N*slow*, for comparison with HAB responses).

Box 4 Invasive Aquatic Vegetation

- The spread of invasive submerged and floating aquatic vegetation (iSAV, iFAV) act along multiple pathways to severely impact nSFE ecosystem health (Figures 4 and B5.1, Table 2)
- Widespread and growing problem (Table B5.1, Figure B5.2)
  - Numerous species, broadly distributed throughout the Delta
  - Large increases in density and areal coverage, crowding out beneficial native emergent aquatic vegetation
  - Implications for protecting current high-value habitats, and for planned wetland restoration
- To date, iSAV and iFAV research studies in the nSFE have focused primarily on changes in coverage and distribution, and found large interannual variation for some taxa (Figure B5.2; e.g., Santos et al. 2016, Khanna et al. 2012, Ustin 2017).

Table B5.1 Important Invasive iSAV and iFAV Species

Sumberged Aquatic Vegetation (iSAV)	
Carolina fanwort	*Cabomba caroliniana*
Brazilian waterweed	*Egeria densa**
Eurasian watermilfoil	*Myriophyllum spicatum*
Crisped or curly-leaf pondweed	*Potamogeton crispus*
Floating Aquatic Vegetation (iFAV)	
Water hyacinth	*Eichhornia crassipes**
South American sponge plant	*Limnobium laevigatum*
Uruguay water primrose	*Ludwigia hexapetala**
Water primrose	*Ludwigia peploides**
Parrot's feather	*Myriophyllum aquaticum*

Meyerson et al. 2010; Khanna et al. 2012; Santos et al. 2016; Ustin 2017; Ta et al. 2017; Sutula and Boyer, 2015.

iSAV and iFAV have spread throughout the nSFE over the past ~20 years (Figure B5.2; Meyerson et al. 2010; Khanna et al. 2012; Santos et al. 2016; Ustin 2017; Ta et al. 2017). Impacts include:

Altered ecosystem function and habitat quality: physical and biogeochemical changes such as decreased water flow, temperature modifications, sediment retention, and reduced oxygen levels; modified lower food web structure, through crowding out native aquatic vegetation, reducing phytoplankton and benthic primary production; providing refuge to invasive predator fish;

Water resources management: increased costs for drinking water exports (operation and maintenance); impeded navigation (recreation, commercial); promotion of mosquito habitat; and degraded aesthetics.

(Brown et al. 2007; Yarrow et al. 2009; Hestir et al. 2015; Boyer and Sutula, 2015; Boyer et al. 2015; Conrad et al. 2016; Ta et al. 2017). Conversely, in the remaining nSFE areas where *native* aquatic vegetation still dominate, those communities deliver vital ecosystem services, such as stabilizing shorelines, attenuating flooding, and habitat provision to native animals (Vitousek 1997, Johnston 1991, McGonigal and Neubauer 2009, Smyth et al. 2013). To the extent that the nSFE's elevated N levels have contributed to the establishment or spread of iSAV and iFAV, it is conceivable that the SRWTP upgrade could lead to decreased iSAV or iFAV coverage.

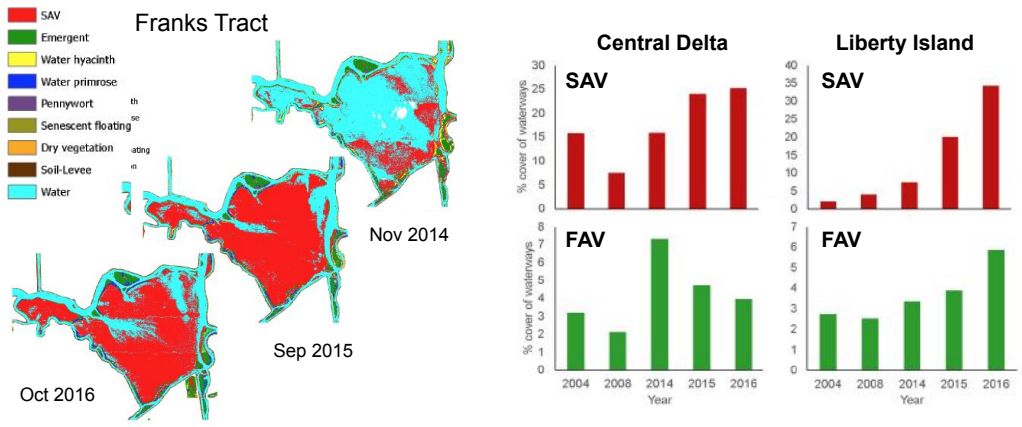


Figure B5.1 Changes over time in SAV and FAV spatial coverage. In both the Franks Tract spatial images and summary bar plots, the primary contributors to SAV and FAV were *Egeria* and water hyacinth, respectively. Graphics modified from Ustin et al. 2017 .

Key Factors regulating iSAV and iFAV (For details, see Boyer and Sutula 2015, Anderson 2008)

Abiotic and Biotic factors: Light, temperature, salinity, channel depth, hydrodynamics (water velocity, residence time, mixing energy), sediment substrate (quality, stability), dissolved oxygen, pH, nutrients.

- Ecosystem engineers: Growth alters surroundings to favor further growth, e.g., trap sediments, ↓ velocities.
- Influence of nutrients: iSAV and iFAV clearly need nutrients to grow. However, important mechanisms and nutrient-concentration thresholds relevant for growth are poorly understood and poorly quantified.
  - No nutrient-focused iFAV or iSAV studies have been conducted for the Delta. While aspects of the physiology or behavior of Delta-relevant iSAV and iFAV species have been studied in other systems, there is limited information on growth-limiting nutrient concentrations at ambient concentrations comparable to those observed in the Delta (Boyer and Sutula 2015). The concept of growth-limiting concentrations for AV is analogous to that for phytoplankton (see Figure B2.4).
  - Water column DIN vs. sediment N: While iFAV and iSAV are generally assumed to access different nutrient pools (water column and sediment, respectively), some FAV and SAV can utilize both. For example, water hyacinth can extend their roots into the sediment layer, allowing them to utilize sediment-N when water column DIN concentrations are low (ref). *Egeria*, a rooted SAV, can acquire N from sediments or the water column depending on relative abundance (Mony et al., 2007). Some studies have observed that *Egeria* utilizes NH4 before NO3 when both are present in the water column (Reddy et al. 1987); however, there is no information about whether that influences growth rates. In addition, much of the current understanding of N utilization by SAV and FAV is based on results from experiments using much higher water column N concentrations .
  - Some species can tolerate periods of low nutrient availability, making it challenging to study nutrient:AV relationships at the field scale, in particular given the large seasonal and interannual changes in Delta water column nutrient concentrations (Novick et al., 2015; Downing et al., 2017; Kraus et al. 2017; REFs).

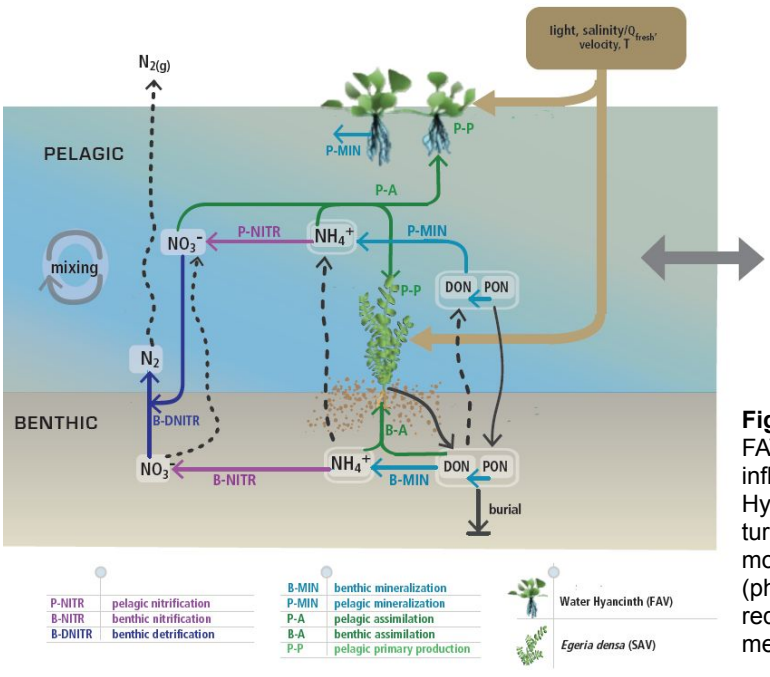
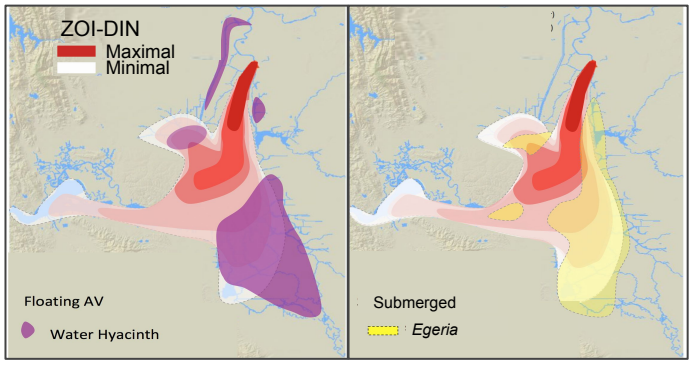


Figure B5.2 Conceptual diagram of factors influencing FAV and SAV growth. Important non-nutrient factors influencing growth include: Temperature; Hydrodynamics (eg., water depth, velocity, inundation, turbulence, residence time); Salinity; Channel morphology; Delivery of SPM; Sediment Properties (physical and chemical, e.g., bulk density, Porosity, redox potential, stability;Management Actions (control measures)

Det?	Link?	Occ?	Table B4 Invasive Aquatic Vegetation Response Scenarios	Dependencies
			AV-H_0 No change in FAV or SAV density, spatial distribution, or assemblage	
			AV1 ↓FAV biomass or altered spatial distribution, ↓[DIN]	*dAV1,2*
			AV2 Changes to FAV community, ↓[DIN]	*dAV1,2*
			AV3 ↓SAV biomass or altered spatial distribution, ↓[DIN]	*dAV1,2*
			AV4 Changes to SAV community, ↓[DIN]	*dAV1,2*
			AV.*slow* One or more of the above becomes evident after years or decades, as ↓[sed-orgN]	*dAV3,4,1,2*

- **Occur?** Highly plausible, but also highly uncertain whether iFAV and iSAV abundance could eventually decrease in response to the upgrade (AV1, AV3, AV.*slow*). It is reasonable to argue, at a basic level, that: i. System-wide, given their large (and expanding) biomass, iSAV+iFAV have substantial N demands; and therefore ii) Substantially decreasing N availability should curtail their production and biomass. However, the magnitudes and response times of AV1, AV3, and AV.slow strongly hinge on a few key dependencies (below). While iFAV and iSAV decreases seem plausible, it is highly unlikely that they would ever be eradicated. Changes to AV community composition are highly uncertain (AV2, AV4), and not discussed below.
- **Detect? Link?** Yes: The upgrade presents opportunities to investigate.
- **Response times** (Figure 5.4): If iFAV or iSAV species rely heavily on water column [DIN], timescales of year-years could be expected, as vegetation gradually dies off without new growth keeping apace. If sed-orgN (especially for SAV) or sediment N fluxes are important N sources, responses would only become evident after several years to decades when sediment N availability declines (Figure B1.6).
- **AV1:** *Key dependencies*: i) Spatial overlap of high-FAV and substantial [DIN] decreases. ii) Post-upgrade [DIN] must fall below growth-limiting thresholds, which are poorly known. *Locations:* Some high-FAV areas overlap with areas where large [DIN] decreases (small τ) could be expected. Most high-FAV areas lie at the ZOI's edges (Figure B5.4).
- **AV3**: *Key dependencies*: i) Spatial overlap of SAV and substantial [DIN] decreases. ii) Strong dependence of FAV species on water column [DIN], as opposed to sed-orgN. While this is possible (e.g., *Egeria*), it is poorly understand from a quantitative standpoint.
- **AV*slow*:** *Key dependencies*: i) sed-orgN or sedN-fluxes (and resulting [DIN]) decreasing enough over time to limit growth. AV*slow* emerges as the most likely of the AV response scenarios for several reasons: a) Although some SAV can take up water column [DIN, SAV would continue utilizing sed-orgN, which will likely decrease slowly. b) Most of the high-FAV area lies at the outer edges of the ZOI: [DIN] changes from the upgrade will be smallest there; sed-N-flux will continue supplying DIN to the water column, which may remain sufficient to support FAV growth. c) Some FAV (e.g., water hyacinth) can directly access sed-orgN via shoots. d) The current standing stock of FAV and SAV represents a large reservoir of N. As those plants die and accumulate in sediments, and slowly degraded, the liberated N will serve as a continued source to other plants.



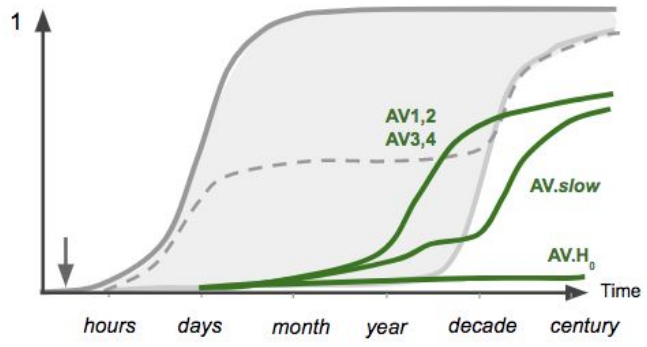


Figure B5.4 (left). Zones where two of the most problematic (areal extent, density, impact) iFAV (water hyacinth) and iSAV (*Egeria*) invasive vegetation species commonly occur. Areas of overlap with ZOI indicate potential areas where upgraded-related changes may occur. Note:specific habitat-types within these areas. (right). Anticipated response times for iAV response scenarios. (Grey curves and grey shaded area, are same as Figure B1.6, and denote ranges of response times for N1, N2, N*slow*, for comparison with iAV responses)

Box 5 Microbial Community

- The non-phototrophic microbial community (chemoautotrophs, heterotrophs) are key players in the nitrogen cycle, with nearly all of the most important (non-photosynthesis-related) transformations being biologically-mediated by microbes (e.g., Figure B1.5; nitrification, denitrification, mineralization, etc.; see Damashek & Francis, 2017).
- The nSFE microbial community will likely experience major changes in response to the upgrade due to the large decreases in NH_4^+ and NO_3^- concentrations, essential substrates fueling the chemical reactions that N-transformation specialists mediate to generate energy, build biomass, and produce compounds that other microbes and primary producers rely on for carrying out their own metabolic processes.
- The microbial community likely also has other important, but poorly characterized and poorly understood roles in the nSFE, from their role as a food resource to working synergistically (or antagonistically) with other microbes, phytoplankton, and other primary producers.

Microbes degrade both particulate and dissolved organic nitrogen, transforming it into more bioavailable DIN forms (mineralization); they also convert N between DIN species (nitrification, dissimilatory nitrate reduction to ammonium); and they convert dissolved N to the gas phase (denitrification) thereby providing a pathway for aquatic N removal. Together these process mediate net fluxes between the water column and the benthos, and N loss from the system as N_2 gas.

Microbes are believed play important roles in some nSFE food webs: e.g., breaking down organic matter and regenerating N, making organicN available for other organisms to utilize; and through their biomass serving as carbon and energy sources to primary consumers. Although important roles for microbes in the nSFE food web have been previously hypothesized, those roles and their quantitative importance relative to other food resources are poorly understood (refs; Durand et al. 2015; Dahm et al. 2016). Recent studies in other systems have found that phytoplankton and non-photosynthetic microbes not only coexist and co-vary, but do so while working in consortia, each carrying out essential biosynthetic reactions that another cannot (e.g., vitamin B_{12} production; Bertrand et al. 2015). While evidence continues to accumulate for covarations and consortia-like dynamics in freshwater and marine systems (Yang et al. 2017; Amin et al. 2012; Ambrust et al., Heal et al. 2018), much less information is available about such interactions within estuarine systems.

Considering that NO3 or NH4 act as critical substrate in energy yielding reactions mediated by nitrifiers and denitrifiers, it stands to reason that the abundance (or biomass) and community composition/diversity of N-cycling microbes will be substantially altered in regions experiencing large $\triangle[NH4]_{direct}$ and $\triangle[DIN]_{dirdirectect}$. Nearfield to the outfall, [NH4] will in general decrease by >90%, which in turn has the potential to fundamentally reshape the ammonia-oxidizing (nitrifying) community (M1, Table Box 5.1): since ammonia-oxidizing archaea (AOA) have much greater ability to assimilate low-level NH_4^+(i.e., low Km) than ammonia-oxidizing bacteria (AOB; Martens-Habbena et al., 2009), one might predict that the first step of nitrification would be driven primarily by an AOA-dominated community, and a shift in relative abundance from what may now be an AOB dominated community high [NH4] to a post-upgrade AOA dominated community. Since NO3 flux from the water to the sediments fuels denitrification within bed sediment anoxic layers (or NO3 from nitrification, at the sediment-water interface), the denitrifier community/activity may be impacted in ways analogous to the AOB to AOA description above (M2). To the extent that $\triangle[DIN]_{direct}$ leads to decreased phytoplankton primary production and decreased delivery of fresh labile organic matter, then heterotrophs (mineralizing communities) may also be affected (M3). Observing these changes, in both the surface sediments and water column, has the potential to provide critical insights into how microbial communities respond to dramatic shifts in nutrient availability. Because studies of this kind usually focus on microbial responses to increased nutrients, this represents a truly unique and valuable research opportunity . These types of changes (above) would begin happening immediately upon the upgrade coming on-line, necessitating pre-upgrade sampling to establish baseline information about community structure prior to load changes.

Det?	Link?	Occ?	Table B5 Microbial Community Response Scenarios	Dependencies
			M-H_0 No change in microbial assemblage	
			M1 ↓ nitrifier activity or abundance (or assemblage change) due to ↓[NH4] and ↓[DIN]	***dm1***
			M2 ↓ denitrifier abundance or change in assemblage due to ↓[NO3]	***dm2***
			M3 Other changes to biological communities (e.g., synergisms), via microbes, due to ↓[DIN]	***dm1,dm2,dm3***
			M.*slow* As sediment N pool changes, longer-burn, slow changes in assemblage may be expected.	***dm4***

- **Occur?** In regions that experience large $\triangle[NH4]_{direct}$ and large $\triangle[DIN]_{direct}$, the likelihoods of M1 and M*slow* occurring are high. In regions with moderate to high $\triangle[DIN]_{direct}$, M2 and M3 are highly plausible, but characterizing the specific types of change is less straightforward than M1.

- **Detect? Link?** M1: If $\triangle[NH4]_{direct}$ is large, high likelihoods for both detecting and linking back to upgrade. M2,M3,M*slow*: with sufficient baseline data, detections/successful-linking also appear moderately likely.

- **Response times** (Figure B5.3): In regions that experience large $\triangle[NH4]_{direct}$ or $\triangle[DIN]_{direct}$, microbial community changes, in the water column or in near-surface sediments, could be evident on time scales of days to weeks. For microbial communities deeper in sediments, response times will more closely align with sediment-N response times (years).

- **M1,M2,M*slow*:** *Key dependencies:* Short τ to ensure large $\triangle[NH4]_{direct}$ or large $\triangle[DIN]_{direct}$. For M1, this will increase the likelihood of major changes in nitrifier community by changing NH4 availability, a key ingredient for their energy production. Observations in the SFE (Damasheck et al., 2015, 2016) demonstrated that the nitrifier community varies seasonally and spatially due to factors other NH4 concentration (e.g., temperature, salinity). Therefore sufficient pre- and post-upgrade data will be needed to unambiguously distinguish upgrade-related changes from other factors. For M2, while $\triangle[DIN]_{direct}$ could influence the denitrifier community by decreasing its oxidant source (NO3), that community may also respond to other changes in conditions that foster denitrification: e.g., changes in phytoplankton primary production and subsequent settling of fresh organic matter to the sediments (substrate for denitrification), and thickness of sediment aerobic surface layer (since denitrification only occurs under anaerobic conditions), which are themselves related. Thus, changes to DIN could also indirectly influence the microbial community by altering phytoplankton production rates. M*slow*: Similar dependencies as for M1 and M2. In addition, to the extent that in some regions sed-N or sed-N-flux remain great enough immediately post-upgrade to satisfy microbial N demand (in water column or sediments), sed-N or sed-N-flux will need to decrease sufficiently in order for changes in nitrifier or denitrifier communities to become evident.

- **M3** *Key dependencies:* Similar dependencies as M1 and M2. While interactions between microorganisms, or between the microbial community and and other biota (microbe←→microbe; microbe←→phytoplankton; microbe←→AV; microbe←→animal), are poorly understood, recent field studies have begun documenting such interactions, including synergistic or antagonistic interactions. Therefore, while we cannot predict the nature of those interactions or changes or their ecosystem-scale importance, it is reasonable to argue that: i) it is quite plausible that they could occur; ii) the potential for some of those interactions to be ecologically-meaningful can not be ruled out.

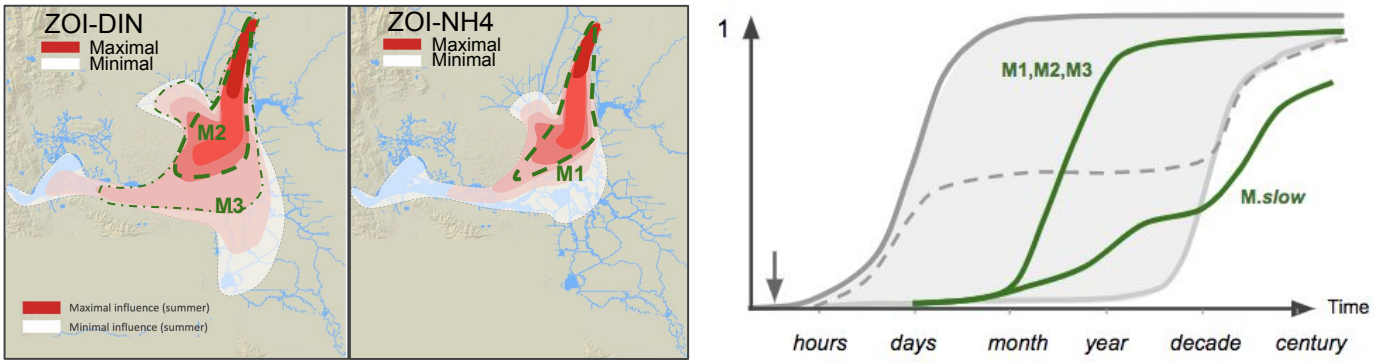


Figure B5.3 (left) Zones where microbial response scenarios may be most readily studied or observed. (right). Anticipated response times for microbial response scenarios. (Grey curves and grey shaded area, are same as Figure B1.6, and denote ranges of response times for N1, N2, N*slow*, for comparison with microbial responses).

Box 6 Higher Trophic Level Food Web Responses (Tier 3)

- Pelagic fish occupy a prominent role with regards to nSFE ecosystem management decisions, because of major declines in the abundances of several native and introduced (but recreationally important) fishes (Sommers et al., 200X; NAS xxxx ; Brown et al. 2016, Moyle et al. 2016). Numerous natural and anthropogenic factors can influence fish populations. A subset of those factors operate within or through the food web, including phytoplankton production and other pathways having potential mechanistic links/interactions with nutrients (Figure B6.1; Figure 4). Most prominent among plausible food web pathways is low phytoplankton productivity and resultant food limitation that may represent additional stressors to already struggling pelagic fish populations. As a result, productivity and overall health of the pelagic food web have also become a focus of nSFE management considerations.
- In this context, the Regional San WWTP upgrade is important because it will change N delivery to the nSFE ecosystem, with the potential for subsequent direct and indirect food web effects (see Boxes 1-5). The discussion below focuses on higher trophic level food web response scenarios, with a particular emphasis on the pelagic food web. The roles played by other influential factors are also covered through their potential influence on whether of nutrient-related response scenarios may occur or reach detectable levels.

Food webs describe the sources and flow of energy and biomass, from primary producers to consumer organisms, and ultimately to species of interest to humans (Figure B6.1). Since the 1980s, phytoplankton production throughout much of the nSFE has dropped substantially, decreasing phytoplankton biomass available for primary consumers, including copepods that are important food resources within the pelagic food web. A growing body of evidence suggests that Delta Smelt declines are due in part to declining availability of their zooplankton prey (Brown et al. 2016, Moyle et al. 2016), in particular nutritious copepods thought to derive much of their nutrition from phytoplankton produced within the system. Other proposed food-web-related pathways that may be affecting pelagic fish include: direct and indirect effects of toxins produced during HAB events (Box 3); and invasive SAV or FAV (Box 4). For simplicity, most of the subsequent discussion focuses on Delta Smelt and other pelagic fishes as the upper trophic level species of interest.

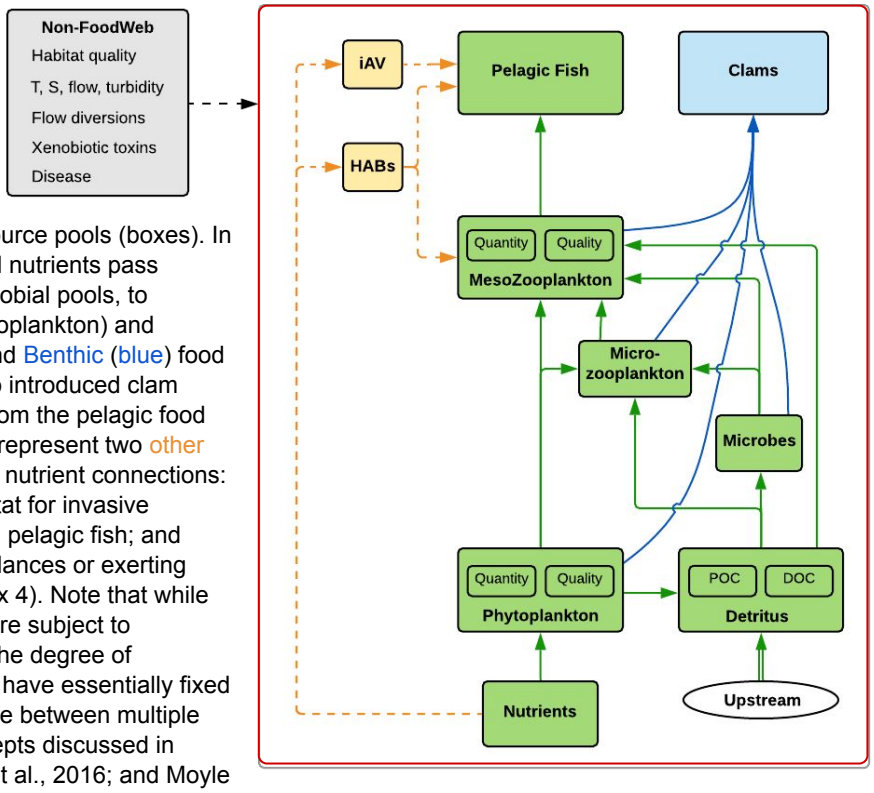


Figure B6.1 A simplified depiction of nSFE food webs. Pelagic fish abundances are influenced by multiple factors, including Non-Food Web factors (grey box) and food-web related factors (inside red box). Solid arrows depict the flow of energy and nutrients through resource pools (boxes). In the pelagic food web (green), energy and nutrients pass through phytoplankton, detritus, and microbial pools, to primary consumers (micro- and mezo-zooplankton) and ultimately to Pelagic Fish. The Pelagic and Benthic (blue) food webs are connected, with filtration by two introduced clam species siphoning off critical resources from the pelagic food web. Orange boxes and dashed arrows represent two other food web-related pathways with potential nutrient connections: SAV/FAV (Box 3) creating favorable habitat for invasive predators, causing top-down pressure on pelagic fish; and HAB toxins impacting zooplankton abundances or exerting direct toxicity impacts on pelagic fish (Box 4). Note that while all components of the pelagic food web are subject to transport, this depiction does not reflect the degree of connectedness between habitats. Clams have essentially fixed locations; however, pelagic fish may move between multiple distinct habitats. Graphic based on concepts discussed in Jassby et al. (2002); NAS 201X; Brown et al., 2016; and Moyle et al., 2016.

Potential Pelagic Food Web Outcomes: Delta Smelt are primarily dependent on the pelagic food web, feeding mostly on calanoid copepods (Baxter et al. 2015, Moyle et al. 2016), which rely on phytoplankton as an important part of their diet (Figure B6.1). When Delta management discussions turn to enhancing the pelagic food web's production, it is commonly assumed that increased primary production will enhance production of copepods and other zooplankton. While Delta Smelt and many other pelagic fishes are primarily dependent on the pelagic food web, the pelagic food web is not the only food web that needs to be considered when exploring tractable ways to augment pelagic fish food supply. Of particular importance is the interaction between the benthic and pelagic food webs through grazing effects of invasive bivalves on phytoplankton. Specifically, two species of introduced bivalve exert intense grazing pressure on small planktonic organisms (Figure B6.1). *Corbicula fluminea*, introduced in the 1940s, dominates the benthos in freshwater regions, and *Potamocorbula amurensis,* introduced in the mid-1980s, dominates brackish water regions (Cohen et al 1984, Lucas et al 2002, Lopez et al 2006; Cloern 2001, Kimmerer and Thompson 2014). The invasion of *P. amurensis* coincided with the decline in phytoplankton biomass and other pelagic food web changes in the nSFE's low salinity zone of the estuary (Cloern 2001, Kimmerer and Thompson 2014). *P. amurensis* has also been shown to feed on microzooplankton, including the early life stages of larger zooplankton that are eaten by Delta Smelt (Kimmerer et al. 1994, Kimmerer and Lougee 2015); thus, it has both direct and indirect effects on zooplankton eaten by Delta Smelt.

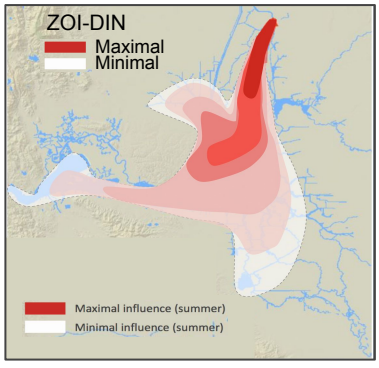
Even if the upgrade results in changes to phytoplankton production or food quality (1a, 1b, 2a, 2b; Figure 4), it is still plausible that little or no changes in phytoplankton biomass or copepod abundances would be detected. This outcome could occur if the light-limitation, phytoplankton-production, and grazing strongly buffer, or self-regulate phytoplankton biomass. Both C*. fluminea* (Foe and Knight 1985) and *P. amurensis* are believed to be food limited in the Delta; therefore, their populations could respond relatively rapidly to changes in phytoplankton production through increases or decreases in biomass (and grazing rates), or by shifts in spatial distributions away from lower productivity zones and toward higher productivity zones. In other words, shifts in clam biomass (higher, lower) could occur instead of changes to zooplankton biomass, which that supports pelagic fishes. Another often-overlooked factor in discussions of Delta Smelt food web support is the degree of spatial overlap between i) physical habitat most suitable for Delta Smelt (e.g., salinity, turbidity, and water temperature); ii) areas where zooplankton abundances might change; and iii) regions where phytoplankton productivity may change. Phytoplankton populations can respond to changing conditions on time scales of days, while the slower reproductive responses of zooplankton populations and pelagic fish populations will be on the order of weeks (Kimmerer et al. 2014) and months to years, respectively. The potential for spatial mismatches, combined with potential temporal mismatches, further detract from the feasibility of detecting changes in fish or copepod abundances that link back to the upgrade.

Lastly, numerous covariates could confound understanding of food web related outcomes from the upgrade. For example, Delta Smelt biology is influenced by numerous other potentially-dominant factors, most of them unrelated to food supply (see Baxter et al. 2015, Moyle et al. 2016), e.g., entrainment, inflow and diversions, position of the low salinity zone, and effects of SAV on turbidity.

All that said, it is reasonable to expect that windows will occur in space or time when phytoplankton dynamics and zooplankton dynamics align such that the influence of upgrade-related changes to phytoplankton production (increase, decrease) and their influence on zooplankton production can be studied. Moreover, some sustained changes may occur, for example, if the frequency or intensity of short duration spring blooms increased in the low-salinity zone before spring recruits of *P. amurensis* become established in shallow waters where bird predation decreases their abundance in late fall and winter. It is less clear, however, whether ecologically-meaningful changes will occur, or, if they do, whether they will be detectable and traceable back to the upgrade, considering the numerous challenges outlined above. In addition to appropriate alignment of phytoplankton and zooplankton dynamics, multiple other dependencies must be satisfied (see Table B6.1, and inherited dependencies from T1 and T2 responses). In summary, it will be difficult to confidently demonstrate direct higher-trophic level pelagic food responses to the upgrade, through either enhanced monitoring or directed research. There are simply too many possible interactions and covariates to consider. Monitoring and research related to the upgrade are likely best focused on T1 and T2 responses: nutrient processes, primary producers and lower level consumers, primarily clams and zooplankton. Results from such studies could then be integrated with ongoing and future work on fishes, including Delta Smelt, conducted by IEP and others.

Det?	Link?	Occ?	Table B6 Food Web Response Scenarios	Dependencies
			FW-H_0 No evidence of improved food web condition that can be traced back to N1-N*slow*.	
			FW1a: Changes in pelagic target organisms' abundances due to changes in food resources	***dFW.1,2,3,4,5,6,7*** *+ inherited*
			FW1b Changes in food resources reaching target organisms (e.g., change in copepod abundance)	***dFW.1,2,3,4,5,6,7*** *+ inherited*
			FW2: Change in pelagic target organisms' abundances due to ↓ HA toxin exposure	***dFW.8,9,7 +*** *inherited*
			FW3 Decreased invasive predator habitat, indirect effects on target organisms (via the food web)	***dFW.10,11 +*** *inherited*
			FW.*slow* One of the above, but after years or decades, due to ↓[sed org-N]	*see above*

- **Occur?** Possible, but all are highly uncertain. Numerous dependencies, in particular the numerous other factors that influence wildlife responses. Even if a nutrient-related factor changes, other dominant controls may prevent a meaningful Food Web response from occurring.
- **Detect? Link?** Detecting lower food web responses (FW1b; e.g., copepods) and linking back to the upgrade seems plausible, given the potential of these organisms to respond rapidly (weeks). If large changes occurred via FW1a, FW2, and FW.slow, detection is plausible but highly uncertain; with anticipated response times of years, the 'noise' from interannual variability will make detections more challenging, however ongoing intensive long-term biological monitoring in the nSFE will be a valuable resource for detecting such changes. For most of these response scenarios, the likelihoods of confidently linking changes back to the upgrade are low, because of the relatively long response times and the other strongly-influential factors that will vary over time.
- **Response times** (Figure B6.4): FW1b Changes in copepod abundances or other indicators (e.g., reproduction rates) could become evident shortly after the upgrade (weeks to months), within the context of, for example, a targeted mechanistic field study. Detecting changes over larger spatial scales (e.g., regional scale abundances) would likely require years for changes to be evident relative to interannual variability. Fish abundances (FW1a, FW2, FW3) at the population level would respond with time scales of years, and longer for those changes to be detectable above interannual variability. Any responses originating with [sed org-N] will respond relative to that lag.
- **FW1a, FW1b**: Key dependencies: Inherit all relevant Phytoplankton Production dependencies (Box 2); and Food quantity/quality among the most important factors currently impacting target organism(s); Changes in food bypasses benthic food web and reach pelagic food web; Temporal overlap (life-stages) and spatial overlap between zones with changed food production (or quality) and fish or copepod populations.
- **FW2:** Key dependencies: Inherit all relevant HAB dependencies (Box 3); and Toxin exposure among the most important factors currently impacting target organism(s); Substantial change in toxin production and exposure
- **FW3:** Key dependencies: Inherit all relevant iAV dependencies (Box 4); and Habitat for invasive predators (iAV) among most important factors currently impacting target organism(s), and sufficient change to predation rate



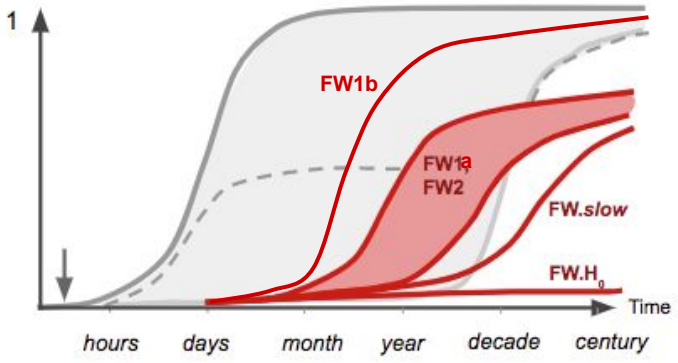


Figure B6.4 (left) Relevant Food Web response zones overlain with ZOI-DIN. (**right***)* Anticipated response times for Food Web response scenarios. The range of potential response times for FW1a and FW2 are represented by the red shaded area. (Grey curves and grey shaded area are same as Figure B1.6, and denote ranges of response times for N1, N2, N*slow*, for comparison with FW responses)

Table App.1 Dependencies influencing the likelihood or ecological-significance of response scenarios.

dn1	Locations are within areas that are strongly influenced by effluent (maximal or intermediate ZOI, DIN or NH4)
dn2	Within the broadly defnied ZOIs, specific habitat or location has small or moderate τ.
dn3	Sufficient resolution of nutrient data to (space, time) to detect changes above interannual and seasonal variations
dp1	[DIN] fall below a threshold(s) at which overall community growth rates decline substantially.
dp2	N-related effects (DIN or NH4) are quantitatively important relative to non-nutrient factors
dp3	Spatial or temporal windows in which benthos minimally influence phytoplankton biomass.
dp4	NH4-inhibition mechanism occurs and is quantitatively important at the field scale.
dp5	[NH4] fall below a relevant threshold (NH4-inhibition hypothesis, ecological stoichiometry hypotheses)
dp6	[DIN] falls below growth-limiting thresholds, leading to competition and nutrient-driven community succession.
dp7	Ecological stoichiometry mechanism(s) occur and are quantitatively important at the field scale.
dp8	N fluxes from the sediments sustain DIN or NH4 above relevant thresholds after the upgrade (t~months to years)
dp9	N fluxes from sediments decrease over time, and [DIN] or [NH4] fall below relevant thresholds.
dp11	[DIN] or [NH4] concentrations are important factors contributing to the occurrence and severity of HA events
dp12	[DIN] or [NH4] decrease below relevant thresholds for HA growth or toxin production.
dp13	Microcystin production is strongly regulated by N availability.
dp14	N fluxes from the sediments sustain DIN or NH4 above relevant thresholds for HA growth or toxin production
dp15	N fluxes from sediments decrease over time, [DIN] or [NH4] fall below thresholds for HA growth or toxin production
dav1	[DIN] is a quantitatively important factor (ecosystem scale) contributing to FAVs or SAVs spread, density, distribution
dav2	[DIN] decreases below growth-limiting thresholds for FAV or SAV
dav3	N fluxes from the sediments sustain [DIN] above relevant thresholds for FAV or SAV growth
dav4	Bioavailable forms of N in the sediments decrease to levels that restrict SAV growth; or N fluxes from sediments decrease such that [DIN] eventually drops below growth-limiting thresholds for FAV
dm1	[NH4] decrease sufficient to cause changes within the nitrifier community.
dm2	[NO3] decrease sufficient to cause changes within the denitrifier community
dm3	Large enough changes in availability of oxidant (NO3) or organic matter to drive a change among heterotrophs
dm4	Sufficient changes in labile sediment organic matter stores to drive a change in the benthic microbial assemblage.
dfw1	Food quality is among the important factors that are currently influencing zooplankton and fish populations.
dfw2	The changes in food quantity/quality are not substantially affected by the benthic food web.
dfw3	Densities or spatial coverage of invasive clams do not increase in response to new food resources.
dfw4	Ecologically-meaningful change in food quality/quantity production rates, sufficient to affect target pelagic organisms
dfw5	Spatial overlap/connectedness between target pelagic organisms' habitat and regions having improved food quantity/quality
dfw6	Temporal overlap (seasonal) between food quantity/quality changes and organisms' food requirements (life cycle)
dfw7	Other factors influencing target micro- and macrofauna populations remain relatively constant (or can be controlled for statistically), i.e., lower 'noise' allowing better chance of identifying any nutrient-related signals
dfw8	HA toxin exposure is among the important factors that are currently influencing zooplankton and fish populations.
dfw9	Decreased toxin exposure: sufficient magnitude of change to influence population level success of target organisms
dfw10	Physical habitat is among the quantitatively important factors influencing zooplankton and fish populations.
dfw11	The magnitude and locations of changes to physical habitat are sufficient to have a measurable affect.