CALFED BAY-DELTA PROGRAM

CALFED SCIENCE FELLOWS PROGRAM

Sea Grant California

In cooperation with the

California Sea Grant College Program

FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE ☒ Postdoctoral Researcher ☐ Ph.D. Graduate Student

PROJECT NUMBER _____

PROJECT TITLE Environmental Controls on the Distribution of Harmful Algae and their toxins in San Francisco Bay, California

FINANCIAL SUMMARY

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Will animal subjects be used? ☐ Yes ☒ No

APPROVAL DATE: _____ PROTOCOL #: _____ PENDING: _____

Does this application involve any recombinant DNA technology or research? ☐ Yes ☒ No

2009 California Sea Grant CALFED PROPOSAL
2. Proposed Research

A. Introduction/Question/Objectives

1. *Introduction and Identification of the Problem*

The major goal of work proposed here is to determine the distribution of harmful algae and their toxins in San Francisco Bay and characterize the environmental parameters that control toxin production by harmful algae with a specific emphasis on the invasive species *Microcystis aeruginosa*. This work is directly related to a few of CALFED's targeted topic areas. Specifically, work addresses issues related to research on **Trends and Patterns of Habitats** and **Populations and System Response to a Changing Environment**. Harmful algae are some of the less studied Bay and Delta species and their distribution, abundance and dynamics, as well as the conditions promoting their proliferation and toxin production are not well characterized. Research is needed to explore how these species currently respond to environmental conditions in order to establish a baseline for future comparison and for predicting changes associated with future climate and anthropogenic impacts. This is particularly important considering predicted future environmental change in temperature, stratification and nutrient and trace metal loading that can dramatically alter the abundance and toxic production of harmful algae. These changes are already starting to occur locally and globally [1, 2, 3] and more data is needed to determine if these are persistent trends. In addition a better understanding of the population and dynamics of harmful algae in the Bay and Delta system is needed for enhancing existing resource management and for developing new tools and decision support systems that improve management effectiveness that will ensure low risk associated with harmful algal blooms (HAB). The work also addresses the topic of **Aquatic Invasive Species**. *Microcystis aeruginosa* is an invasive bloom forming harmful algae that would be studied here and results will help predict the conditions under which this invasive species is successful and also under what conditions it produces toxins. The ultimate goal is to establish under which future scenarios including different water-management regimes, climate change, land use change, catastrophic events, and other potential changes harmful algal species including *M. aeruginosa* might bloom. Finally the work is also related to the topic of **Water Supply, Water Quality**; noxious toxins produced by harmful algae reduce the water quality and may impact the supply of clean water for drinking as well as the Bay and Delta water quality which directly impacts the livelihood of other species including several endangered species. The current global Water Crisis infers that it is crucial to gain a better understanding on the factors affecting water quality and Delta ecosystems that can limit the current and future management options and to develop sustainable solutions to minimize impacts to drinking water and to ecosystems. The Southwestern United States, and most particularly the San Joaquin-Sacramento Delta, ranks among the most vulnerable regions to the current global Water Crisis [4]. Indeed, models predict that water supply shortage will increase due to global warming and to overpopulation, while the contamination of water supplies is rising due to anthropogenic pollution and to the emergence of harmful and noxious toxin-producing algae blooms.

Harmful algal blooms are on the rise –

Over the past thirty years, harmful algal blooms (HABs) have increased in extent, frequency and types of organisms involved throughout the world, although the reasons for this remain unclear [5,6]. As the occurrence of HABs increases, their impacts also increase resulting in a plethora of acute, chronic and fatal illnesses in animals and humans [7, 8, 9, 10, 11, 12]. The economic impact of harmful algal blooms (HABs) on fisheries, recreation, human health, and the ecology of both marine and fresh water bodies in the United States is significant [13, 14]. Average annual economic losses in the United States

from HABs were approximated at $82 million [15]. Over the course of the last decade numerous scientific programs designed to increase the understanding of the fundamental processes underlying the causes and impacts of HABs have been initiated. Despite considerable progress made toward understanding and predicting HAB events and their impacts, the complexity of the problem has also become more apparent [16]. Indeed, a clear understanding of factors promoting algal growth and toxicity has remained elusive and hampers our ability to predict and prevent such events. It is therefore crucial to identify the drivers allowing the successful establishment and spread of harmful algae and their toxins under current conditions in order to predict the responses of these organisms in the context of a changing environment.

Factors which promote HABS and toxicity are not known –

HABs are diverse in terms of causative organisms, bloom dynamics and level of impact. Abundance and toxin production of the cyanobacterium *M. aeruginosa* suggest that they are influenced by a multitude of parameters including light, temperature, salinity, nitrogen, phosphate, and trace metals (Table 1). Nevertheless, most of the information concerning the environmental factors for toxin-producing cyanobacteria bloom development and persistence is compiled from freshwater lakes and reservoirs studies, less is known about the relative importance of environmental factors in estuaries, particularly nutrient-rich estuaries like San Francisco Estuary [17]. Laboratory experiments with various toxin-producing diatoms of the genus *Pseudo-nitzschia* spp. suggest that silicic acid limitation is the primary trigger of domoic acid (DA) production [18, 19, 20, 21, 22]. Field observations have corroborated this assumption [23, 24, 25]. However, other macronutrient limitations, including phosphate (P), and/or micronutrient supply [25, 26, 27] have also been shown to trigger DA production while nitrogen and light limitation have been shown to inhibit the production of DA [19, 29, 30, 31, 32, 33]. Similar complexity in controls over the growth and toxin production in various species of the dinoflagellate *Alexandrium* is also evident. In California, *Alexandrium catenella* has been associated with cold water conditions while *Alexandrium monilatum* the species abundant in Florida seems to grow best at high temperatures [34, 35]. Laboratory experiments show the enhanced toxin production in some *Alexandrium* strains under P limitation [36, 37, 38, 39, 40] and, in other toxic *Alexandrium* species N and P colimitation stimulate toxin production [41, 42] Blooms of another toxin producing dinoflagellate *Karenia brevis* have been linked to iron and nitrogen availability [43] yet this is not always consistent with field observations.

Although laboratory experiments indicate that toxicity is species-specific and is induced by a broad range of predictable environmental factors, little is known about what stimulates growth and triggers toxin production *in situ* [44]. Indeed when laboratory and field observations were compared it was determined that HABs do not always occur when conditions seem appropriate [35]. Such discrepancies might result from reciprocal interactions among members of the plankton community [45]. Also, bacteria associated with toxin-producing algae can be factors in determining the production levels of the toxin and its elimination under particular circumstances [30, 45, 46, 47, 48, 49, 50, 51, 52, 53]. Additional factors that make a bloom likely in the natural environment are currently unknown and specifically, conditions inducing toxin production in San Francisco (SF) Bay have not been studied. This crucial yet missing understanding, which is key for predicting and controlling HABs, is best gained from direct field observations and manipulations of algae in their natural environment.

Research on harmful algae has intensified throughout the US and the world, however, the focus of this research has been for the most part restricted to areas where negative impacts (on economies and ecosystems) from HABs have been reported. It is certainly important to monitor and study areas where HABs frequently re-occur, however, we concur that there is much to be learned from settings where toxic species have been documented but deleterious effects on human and ecosystem health are not yet prevalent. A comparison between areas where HABs are common and areas where they have

not been occurring frequently, within a broad general region such as the west coast of the United States, may provide vital information to our understanding of trigger mechanisms of HABs in the natural environment. Moreover, such a comparison and the characterization of the specific conditions that preclude HABs from occurring at some sites could be used to protect these locations from future episodes of HABs by designing best management plans that reduce the risk of inducing such blooms (*e.g.* one can compare this aspect to preventative medicine).

HABs in California Coastal Systems and San Francisco Estuary –

HABs and their impact have been documented in California waters since 1793 [53]. There are six groups of harmful algae that have a strong presence on the West Coast of the United States. Blooms of the cyanobacterium (blue-green alga) *Microcystis aeruginosa* have been recorded in California estuaries since 1999 [55, 56, 17] and the presence of other potentially harmful cyanobacteria has been documented. *Microcystis aeruginosa* can produce a variety of toxins collectively called microcystins which are associated with both acute and chronic liver damage [57]. Exposure to microcystins has been linked to cancer in humans and wildlife [58] and reduced feeding success in zooplankton [59]. These hepatotoxins have been detected in the Delta and entered the foodweb [56, 17]. Also, the toxin-producing cyanobacterium *Cylindrospermopsis raciborskii* has been observed recently in the northern SF Estuary (Mueller-Solger, personal communication). This cyanobacterium was originally thought to be a tropical or subtropical alga but has been recorded as rapidly expanding in some temperate geographical areas and is regarded as an invasive species [59]. It is thought that its increased occurrence, rather than being just a recent invasion, is a combination of several factors such as improved water quality monitoring, availability of suitable habitat through climate warming and eutrophication. Some species of *Pseudo-nitzschia*, produce domoic acid, responsible for amnesic shellfish poisoning. *Alexandrium catenella* produces a suite of toxins responsible for paralytic shellfish poisoning symptoms. Diarrhetic shellfish poisoning is due to a milder suite of toxins which are produced by some species of *Dinophysis. Heterosigma* is responsible for massive finfish mortalities, and it has recently been observed in SF Bay and Los Angeles waters. Finally, *Lingulodinium polyedrum* and *Gonyaulax spinifera* have been identified in California as yessotoxin producers, which may cause problems for marine mammals and humans. Most of the wide spread massive blooms in California are associated with *Alexandrium* that produces paralytic shellfish poisoning or *Pseudo-nitzschia* that produces domoic acid poisoning [34, 60]. Recorded events of HABs are prevalent in the coastline around SF Bay (Marine coast south of Pt. Reyes and north Muir Beach, Monterey Bay, and Santa Cruz County), however, only few events of HABs have been reported within SF Bay. Since there is free exchange of water through the Golden Gate it is likely that harmful marine species are regularly introduced into the Bay from adjacent coastal waters. Moreover, with inflow of river water into the North Bay and the Delta, freshwater toxin producing cyanobacteria blooms such as *M. aeruginosa* can also develop [56, 17, 61]. HAB events have been documented in the last few years in SF Bay, possibly suggesting change in the conditions that dampened the occurrences of HABs in the Bay and in accord with the increase in occurrences in this region.

A survey of the abundance and distribution of toxic phytoplankton, based on a historical phytoplankton database of monthly phytoplankton sampling between 1992 and 1995, was conducted by Rodgers and colleagues [62]. They found over twenty algal taxa regarded as harmful, noxious, or toxin-producing in the Bay waters. The occurrence of these taxa ranged from common to rare and some reached moderately high concentrations [62]. Some species showed marked variation in abundance and frequency of occurrence among the different regions within the Bay. The abundance of certain algae was not always correlated with their distribution in the coastal waters outside the Bay. Specifically, *Alexandrium* cells were present at very high concentrations in the spring in South and Central SF Bay reaching densities of 10^5 cells/L but correlation with toxin levels in the coast was weak, suggesting within Bay growth. A bloom of *Pseudo-nitzschia* species with densities of 4.6×10^6 cells/L was recorded

in the Berkeley marina in July-August 1993 and freshwater cyanobacteria (*Oscillatoria* and *Anabaena* spp.) were documented in the North Bay. One of the questions posed by Rodgers et al. [61] is **why is there absence of acute HABs in the Bay and what has kept this system immune from HAB epidemic**. No time-series sampling program has been implemented to monitor the occurrence of toxin producing algae and toxin levels in the Bay and no answer to that question is available. Work proposed here will shed light on this paradigm. Establishing a coherent and consistent data set for the distribution and abundance of harmful algal and their toxins in SF Estuary in relation to the conditions within the Bay and Delta will constitute a baseline for determining future change and aid in identifying the parameters that are conducive to HABs and allow forecasting and managing HABs in the Bay and beyond.

HABs impact San Francisco Bay-Delta system, a new trend? –

The first bloom occurrence of the harmful algae colonial forming cyanobacterium *Microcystis aeruginosa* was documented in 1999 in the upper SF Bay Estuary [54]. Total cyanobacteria biomass has increased between 1975 and 1993 throughout the north SF estuary (NSFE) coincident with a decline in diatom biomass [4]. The single-celled form is currently a common cyanobacterium in NSFE but was not identified as a dominant genus in the phytoplankton community between 1975 and 1982 [62]. The colonial form of *M. aeruginosa* is also the first known introduced phytoplankton species with adverse impact to the estuary [55]. Since 1999 the distribution, biomass and toxicity of *M. aeruginosa* have been documented and indicate that the colonial form of *M. aeruginosa* is present throughout 180 km of waterways from freshwater to brackish water environments. The toxicity was highly variable spatially and temporally (*e.g*. between blooms) but is usually highest at low water temperature, low water transparency and low salinity [55, 16]. In 2000, one surface water sample collected in the NSFE contained microcystins concentration above the World Health Organization advisory level of 1$\mu g\ L^{-1}$ [54, 63]. Microcystins from blooms entered the food web and were present in both zooplankton and clam tissue. In 2007, the *M. aeruginosa* bloom was the worst on record in the Delta (P. Lehman, in prep.; 64). The center of distribution has shifted toward the West (near Antioch) as compared to previous years and might have affected invertebrates and fishes in the confluence and Suisun Bay regions of the upper estuary (P. Lehman, in prep.; 64). The toxicity and widespread distribution of *M. aeruginosa* in NSFE demonstrated the potential of this organism to negatively impact many beneficial uses in NSFE and suggested that an active and long-term monitoring forecasting and alert programs are needed. The potential adverse impact of this HAB on the estuary is large. Water from the northern region is used directly for drinking water and irrigation and the region is an important recreational area for sport fishing and water contact sports. The estuary is habitat for many anadromous commercial and recreational fish including striped bass and Chinook salmon and is a feeding ground for marine mammals. The estuary also contains many threatened or endangered aquatic organisms including the Delta smelt and Chinook salmon and many of these endangered fish species are declining [65, 66]. Some of these declines may be linked to the quantity and quality of the phytoplankton carbon available at the base of the food web. Indeed, *M. aeruginosa* blooms can reduce the growth of other phytoplankton impacting food quality and availability [55, 16].

Several HAB events have been documented in other parts of SF Bay as well. In September 2004, SF Bay had the largest red tide that U.S. Geological Survey scientists have observed since they began monitoring phytoplankton, nutrients, chlorophyll, and other water-quality indicators [66]. Some red tides are associated with phytoplankton that produce toxins, but fortunately for SF Bay the algal bloom dissipated within a week before any harmful effects occurred. The bloom was dominated by dinoflagellates in concentrations unprecedented in nearly three decades of observation. SF Bay is highly enriched in nutrients but has low summer-autumn algal biomass because wind stress and tidally induced bottom stress produce a well mixed and light-limited pelagic habitat. The bloom coincided with calm winds and record high air temperatures that stratified the water column and suppressed mixing long

enough for motile dinoflagellates to grow and accumulate in surface waters. This event followed a summer of weak coastal upwelling and high dinoflagellate biomass in coastal waters that apparently seeded the SF Bay bloom. This suggests that some blooms occur in response to changes in physical dynamics driven by large-scale atmospheric processes and operate over both the event scale of biomass growth and the antecedent seasonal scale that shapes the bloom community.

In 2005 phytoplankton species composition was determined by microscopic analyses of samples collected on Aug 18, Sept 13, Oct 19, and Nov 28 at a salt pond (Pond A18) in South SF Bay (SSFB) as part of a wetland restoration project (Cloern, personal communication). Six species of toxin-producing or harmful phytoplankton were abundant in samples taken in the pond. The cell counts of these harmful algae indicate much higher levels in the ponds than in open Bay water. These shallow ponds and other shallow bay lands (channels, marshes, etc.) may be regarded as high-productivity bioreactors that harbor harmful algae that could be introduced to the open Bay.

As seen in the above summary, **while there is indication that harmful algae are present throughout the bay and that their abundance is increasing in recent years there is little systematic information about the occurrence, distribution and dynamics (*e.g.* controls on growth and toxicity and ecosystem impacts) of harmful algae and their toxins in SF Bay**. No coherent baseline estimates for these species' abundance and distribution exist for the Bay. Determining the distribution of and controls on harmful algae and their toxins in SF Bay in relation to physical, chemical and biological controls would shed light on the parameters that are conducive to HAB events and aid in forecasting and managing HABs in the Bay and beyond particularly in view of potential future climate and environmental change.

2. Overall Project Goal or Objective

The major goals of this project are to elucidate the role of physical (temperature, light, turbulence, salinity, hydrodynamics), chemical (inorganic and organic nutrients, dissolved organic carbon, trace metals) and biological (bacterioplankton abundance, phytoplankton species abundance and density, zooplankton community structure and grazing) drivers on the distribution, growth, and toxicity of harmful algae in SF Bay and to enhance our understanding of the relationships between these parameters and the occurrence and consequences of HABs. This information will aid in predicting and mitigating HAB events in the future.

Research proposed here is guided by the following motivation:

A combination of environmental factors (biotic and/or abiotic) has precluded toxic blooms from occurring frequently in SF Bay despite their prevalence in the adjacent coastal ocean. Future anthropogenic or natural climatic changes in the Bay (particularly warming and water stratification) may alter this unique situation resulting in more frequent blooms and negative impacts on human and ecosystem health (Figure 1).

The working hypotheses for the proposed research are:

Hypothesis 1: Increased temperatures, stratification and longer water residence time due to seasonal heat waves and droughts will favor dinoflagellates and cyanobacterial growth (in South Bay and the Delta respectively) over diatoms and will result in increased frequency in blooms of these species.

Hypothesis 2: Alleviation of light limitation due to stratification and decreased turbidity will increase the influence (control) of nutrient and trace metal availabilities and relative concentrations on toxin production, resulting in enhanced toxicity of the harmful algae in the Bay and Delta.

Hypothesis 3: Changing environmental conditions in the Bay over the last decade have and will continue to increase HAB frequency and toxicity. Spatial and temporal mapping of harmful algae and their toxins in the Bay will help elucidate the triggers for this change, provide a baseline for measuring future trends and enable forecasting and prediction.

We believe that determining the combination of parameters that enhance seeding, growth and toxicity of harmful algae in this system can be used to predict and prevent HABs from occurring and will serve as an important tool for managers and regulators. We will establish a baseline of conditions in the Bay, determine the above relationships and use existing and newly obtained data to design a set of *in situ* manipulative incubation experiments that will aid in determining the parameters that control growth and toxicity in the natural population of harmful algae, with a special focus on the invasive cyanobacterium *M. aeruginosa*. We will monitor the spatial and temporal variations of these factors to identify the sources and triggers of HABs within the SF Estuary. Data obtained could be used to develop a model that would enable forecasting and preventing HAB events. Such information is needed to reduce the likelihood for future negative impact of HABs on estuarine processes and for designing best management and preservation plans.

B. Approach/Plan of work

We propose a work plan that will combine monitoring and mapping of biological, chemical and physical components throughout the Bay and Delta along with controlled manipulations to examine specific parameters that are likely to control growth and toxicity in the natural population of harmful algae in SF Bay. Work proposed here is primarily a field-oriented research program. The work includes (a) monthly sampling throughout the Bay and Delta to map the spatial and temporal distribution of algae and toxins in relation to a suite of important environmental parameters (b) seasonal *in situ* enrichment incubation experiments to determine which targeted parameters (or combinations of parameters) induce growth and toxicity of harmful algae including a focused study on *Microcystis aeruginosa* in the Delta. The data will be used to shed light on the physical, chemical and biological factors conducive to bloom events and in the future could be used to construct a predictive model for HABs in SF Bay in the context of a changing environment.

Study Area – The SF Estuary ecosystem comprises a continuum of conditions with freshwater input in the Delta to hypersaline ponds in South Bay with an area influenced by communication with coastal seawater in Central Bay. This setting provides a range of conditions that will result in a range of HAB species and environmental controls on toxin production. In general the Bay can be described as two estuarine systems, each with different hydrodynamic and hydrologic regimes [68]. Northern SF Bay is a partially mixed estuarine environment with a high annual freshwater input. It includes San Pablo Bay, Suisun Bay, and Sacramento River/San Joaquin River Delta. The Sacramento – San Joaquin River Delta is a hydrodynamically complex system comprised of an intensely managed network of natural and human-made levees and lakes, diked agricultural fields, and relicts of tidal marshlands. The watershed comprises approximately 40% of the area of California [69]. Southern SF Bay is a tidally oscillating tributary lagoon with density-driven exchanges with Central Bay which communicates directly and extensively with coastal waters [70]. While salinities in North SF Bay may vary from 0 to 30 depending primarily on discharge of water through the San Joaquin/Sacramento Delta (and distance from the ocean), salinities in the South SF Bay generally remain between 26 and 33 with higher salinities in shallows and ponds [71].

Task 1 - Spatial and temporal distribution of harmful algae and toxins in SF Bay and Delta

In order to describe the spatial and temporal distribution (occurrence and abundance) of harmful algae and their toxins in SF Bay we will work closely with the US Geological Survey SF Bay monitoring program (see letter of support). This program includes regular measurements of water quality along a 145-Km transect spanning the length of the entire estuarine system. A series of fixed stations from Rio Vista (lower Sacramento River) to South Bay mouth of Coyote Creek are visited (Fig. 2A). Measurements along the entire Bay are done at least once each month. In addition, more intensive sampling in the South Bay is conducted during spring and fall, the seasons of rapid water quality changes

associated with the phytoplankton blooms. Sampling is done aboard the research vessel *Polaris.* Data are collected using a submersible instrument package (CTD) that concurrently measures multiple water quality parameters (depth, conductivity, temperature, suspended solids, chlorophyll, light penetration, and dissolved oxygen). Vertical profiles are obtained at each sample station resulting in a two-dimensional (longitudinal and vertical) description of water quality for each sampling date. In concert with the submersible instrument package sampling, during each cruise discrete water samples (from 14 stations throughout the Bay) are collected and processed for determination of dissolved oxygen, suspended particulate matter, chlorophyll a, and dissolved inorganic nutrients. Discrete samples are collected at the surface and 1 meter off the bottom. All of the ancillary data from this USGS water quality monitoring program will be available to this project at no extra cost. For details see the web page: http://sfbay.wr.usgs.gov/access/wqdata/index.html. Tidal currents speed, wind speed and other hydrodynamic and meteorological data will also be available from USGS and DWR. Details on collection, analytical methods and data quality control are available on the above web page.

In the Delta, we will monitor the spatial and temporal distribution of harmful algae and their toxins with a special focus on *Microcystis aeruginosa* Fig. 2B). Sampling will be conducted monthly by partnering with Dr. Anke Mueller-Solger (Department of Water Resources) and the Environmental Monitoring Program of the Interagency Ecological Program (http://www.baydelta.water.ca.gov/emp/). This program includes regular monitoring of water quality variables (conductivity, pH, dissolved oxygen, turbidity, dissolved chloride, chlorophyll fluorescence, water temperature, air temperature, wind speed and direction, solar radiation) as well as biological characteristics, such as phytoplankton and zooplankton community composition and biomass in the Sacramento-San Joaquin Delta, Suisun Bay, and San Pablo Bay. For more details, see: http://www.baydelta.water.ca.gov/emp/metadata_index.html.
All these ancillary data will be available to this project at no cost. Sampling will be done on board the DWR/USBR research vessels during the monthly routine monitoring cruises at the discrete stations (Fig. 2B). We will compare our results (see below) with Dr. Mueller-Solger's Fluoroprobe data. Additionally, we are partnering with Dr. Peggy Lehman (DWR) to contribute to an ongoing seasonal sampling program during *Microcystis aeruginosa* blooming season (June-September). Sampling will be done every two weeks at seven stations covering the Delta and in the Eastern region of the Suisun Bay. Variables measured during this study include nutrients concentration, DOC, POC, turbidity, temperature, salinity and toxin levels (microcystins, anatoxin A). Using molecular methods, the program will also monitor the toxin-producing chemotypes of *Microcystis* the expression of the gene involved with toxin production. Results from this study will shed light on the link between the SF Estuary *Microcystis* community structure and function and will have direct implications with respect to management of this invasive species. We will contribute to this seasonal program by monitoring the trace metal concentrations as well as complementary analyses such as saxitoxin monitoring and incubation experiments (see below).

Concurrent with the above monitoring programs in the Bay and Delta as part of this CALFED project we will collect samples for algal identification and enumerations and toxin (domoic acid, saxitoxins, microcystins) levels at all discrete sampling location in the Bay and Delta described above. We will also add analysis of dissolved organic carbon, total organic nitrogen, total organic phosphorus and trace metal concentrations (using trace metal clean sampling protocols) because these are not routinely monitored but may influence the growth and toxin production of harmful algae. Detailed sampling, processing, and analysis methods used for the above work are described in detail in appendix I (available upon request to Ms. Shauna Oh at the Sea Grant office: shaunaoh@ucsd.edu).

Task 2- In-situ incubation experiments

We will conduct enrichment bioassay incubation experiments seasonally to examine the individual and combined effects of nutrients, trace metals, temperature and light on growth of and toxin production by harmful algae. This is aimed at elucidating the underlying mechanisms that control the

distribution and toxicity of harmful algae and evaluate under what conditions algae growth is induced and toxic levels are expected to increase. Such "bottle experiments" allow getting a better resolution of the impact of chemical and physical variables on toxin production and harmful algae growth by excluding other parameters (*e.g.* grazing, mixing). Bioassays will be conducted at 3 sites in the Bay and 2 sites in the Delta (as determined by the assessment of field distribution of harmful algae). We will investigate the phytoplankton responses to the individual and combined effects of fertilization with inorganic macronutrients (N, P), organic nutrients (DON, DOP, DOC) and trace metals (Fe, Mn, Zn, Li, Cu) under environmental light and temperature conditions. The effect of increased temperature will also be assessed. Specific perturbations that we will initially test are based on existing data from previous work [17, 18, 20, 28, 29, 72, 73, 74] that describes potential influencing parameters on growth and toxicity of various harmful species. Following the initial experiment and based on accumulated data over the first 6 months of this project we will determine which parameters in the Bay are most strikingly different than observed in the adjacent coastal California and manipulate these specific parameters in following incubations. For example if we find that the relative ratios of certain nutrients (Si:N, N:P, Urea:P, etc.) is consistently different in SF Bay compared to the coastal ocean we will change these ratios and document the effects. Table 1 and Figure 1 illustrate some examples of possible manipulations and bioassays with Microcystis and other harmful algae species, which are based on previous work and will be tested here. For example it was suggested that the nitrogen and phosphorus sources as well as iron, light intensity and temperature may impact toxin production by *Microcystis* [75], thus these parameters will be tested in Delta waters.

To conduct these incubations surface water from the 5 sites in the Bay and Delta will be collected. Four liters per sample of surface water will be pre-filtered over 100 µm nylon mesh to remove zooplankton grazers and collected into large (6 L) acid cleaned and sample rinsed, low-density, translucent polyethylene cubitainers (in triplicates). Water will be kept at *in situ* condition at all times except for the very short duration of filtration and nutrient/trace metal additions. Nutrient/metal additions will take place as soon as possible (pre-mixed aliquots will be prepared and added) and cubitainers returned to the water. An array of treatments will be tested; the specific details of these manipulations will be continuously evaluated and adjusted to reflect results from data accumulated in our monthly sampling. Following nutrient/metal additions, the cubitainers will be incubated for up to four days within the SF Estuary. The cubitainers will be secured to a peer or a buoy to ensure daily access. To simulate light impact the cubitainers will be deployed at different depths (or covered with mesh to lower light level) and for evaluation of the effect of increased temperature some incubations will be conducted on shore in the lab in controlled temperature chambers or at warmer shallow areas within the Bay. A full characterization (e.g. all the ancillary parameters such as temperature, salinity, oxygen, nutrients, etc.) of the ambient water used for the incubation experiments will be done. During the 4 days of incubation each cubitainer will be sampled daily for toxin levels, nutrients, trace metals, chlorophyll *a*, Fv/Fm, flow cytometery and cell identification and counts to monitor changes in these parameters. All reagent preparation and cleaning of culture ware and sampling bottles will be rendered trace metal clean via standard protocols established in the trace metal laboratory at UCSC. These incubation experiments will allow us to determine how various monitored parameters interact under ecologically relevant conditions to control the growth and toxicity of harmful algae.

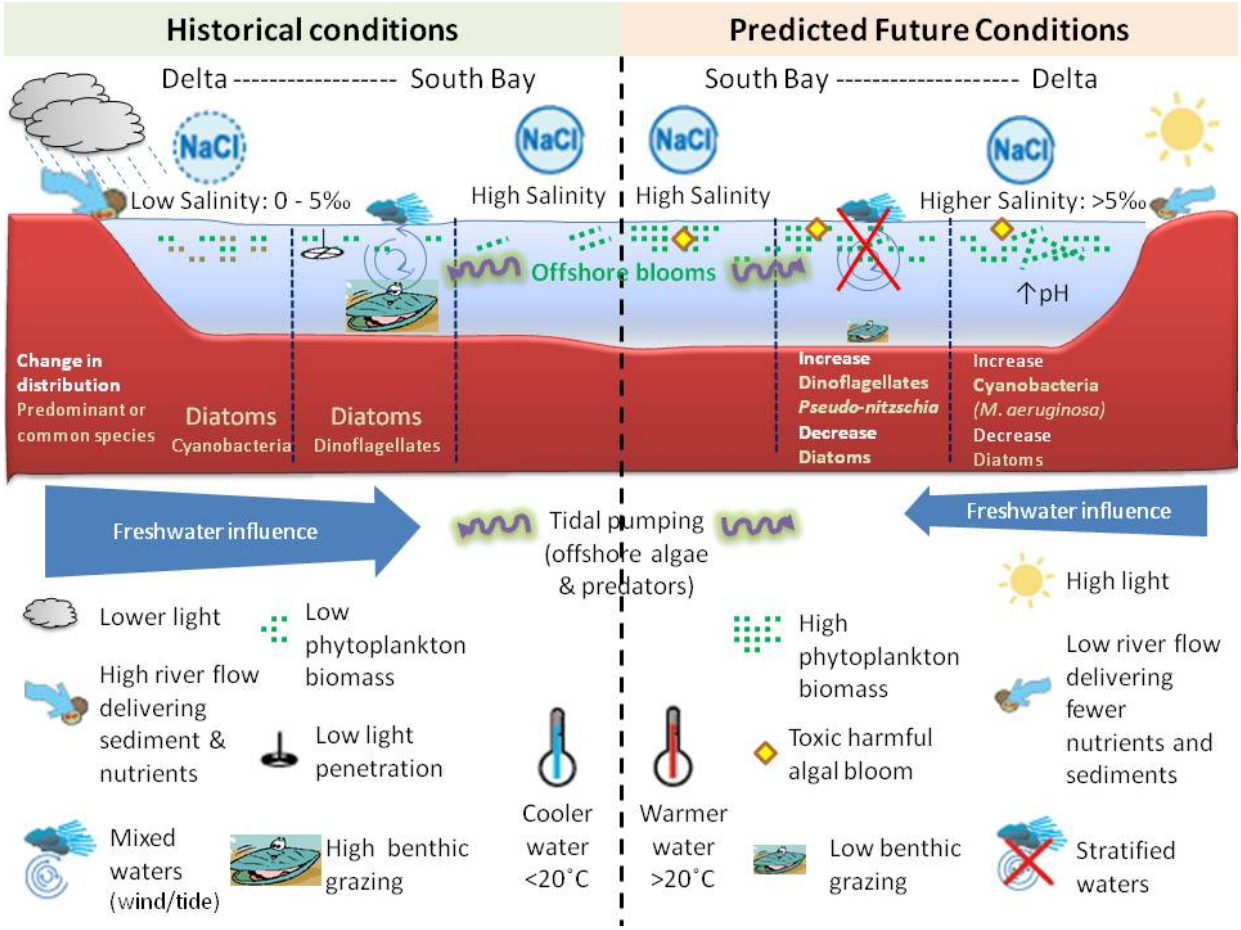
C. Output/Anticipated Products and/or Benefits

Recent reports such as that published by the U.S. Commission on Ocean Policy have highlighted the severity of the problem of HAB events and have stressed the importance to develop new management approaches (http://www.oceancommission.gov). Effective HAB management and regulatory interventions are stymied by lack of an integrated understanding of the physical, chemical and biological parameters that induce, sustain and regulate blooms and toxin production. Results from

this project include: (a) description of current status of harmful algae and related toxins in SF Bay-Delta system and development of a baseline dataset, and (b) better understanding of the mechanisms underlying the occurrence and toxicity levels of harmful algae in SF Bay and Delta. Data obtained through this project will be compiled to produce some simple distribution matrix (model) that assesses the impact of various parameters on the algal abundance, growth and toxin production in SF Estuary. These data could be used in the future to develop a prediction model that will have the ability to forecast various algae densities and toxin concentrations in SF Bay under various climate and development scenarios. Predictive model suitable for forecasting bloom occurrences are important tools for controlling and reducing the impact of HABs. The accuracy and predictive capability of such a model however dependents on the understanding and formulating the biotic and abiotic processes which are included in the models. Thus insight into the relation between algal abundance, toxin levels and various environmental parameters obtained through our proposed study is critical for the development of accurate and precise models. Data and understanding from this project will be relevant for environmentalists, regulators, local governments, and industries as well as environmental managers and policy makers interested in protecting the San Francisco Estuary and its watershed.

Specific outputs of the proposed project include research results (publications, presentation). We will disseminate the results broadly. Public outreach effort targeting resource managers and decision makers as well as stakeholders and the general public via open house lectures, workshops, newsletters, K-12 education initiatives and websites will be planed and executed. We will create monthly report cards describing the health of the Bay on our website and we will report elevated levels of toxins or toxic algae each month in a HAB alert section. Our website will be linked to the CALFED and USGS webpages. We will also partner with educators to develop research based education materials. All of the above outputs directly address the California Sea Grant CALFED priorities described in this solicitation: provide information needed to prevent, control and mitigate blooms of invasive species; examine the relationships between the primary production and the habitat quality by connecting nutrient and trace metal loading, HABs, and food web dynamics in a changing environment; investigate how the SF Bay ecosystem will respond to future conditions (e.g. raising water temperature, changes in salinity and turbidity); provide information that will enhance current HAB monitoring and forecasting efforts; help facilitate bloom prevention through an advanced understanding of the controlling factors.

This proposal brings together a multidisciplinary team (PI and collaborators) with expertise on hydrodynamic modeling, nutrient and trace metal dynamics, phytoplankton biology, HABs, and the SF Bay-Delta system to shed light on the occurrence, distribution and governing environmental parameters of harmful algae and their toxicity. While Dr. Paytan will be my principal research mentor, we will collaborate with Dr. Lisa Lucas' and Dr. Cloern's group (USGS), Dr. Mueller-Solger and Dr. Lehman (DWR) as well as Dr. Silver and Dr. Kudela (UCSC). Dr Mioni, the applicant, and Dr Paytan, as the PI and research mentor, will be responsible for the overall oversight of the project and the field work. Dr Lucas and Dr Cloern will provide knowledge on SF Bay and help provide access to boat facilities and monitoring data. Dr Lucas, an experienced modeler has agreed to assist with the interpretation and modeling of hydrodynamic and physical variables. Both Dr Mueller-Solger and Dr Lehman are involved in phytoplankton community and HAB monitoring in the SF Delta. They will provide knowledge and access to research vessels and monitoring data in the Delta. Both Kudela and Silver are involved in HAB research in California and will help identify target parameters for incubation experiments. Advantages of the collaborative research project include: (a) Enhancement of current monitoring programs and resulting management recommendation through complementary analyses as well as sharing of monitoring experience; (b) Economy of scale: this project will take full advantage of preexisting monitoring infrastructures and will focus on enhancing rather than duplicating the current programs and measurements.



	Microcystis aeruginosa	*Pseudo-nitzschia spp.*	*Alexandrium catenella*	*Akashiwo sanguinea*
Temperature (˚C)	>20 [16,76,77]	10 – 25 [84]	10 – 25 [90,91]	10 – 30 [92]
Light ($\mu E\ m^{-2}\ s^{-2}$)	>26 [75]	45 –200 [85]	120 [90]	14.4 – 114 [92]
Salinity (‰)	0 .1 – 10 [16] (survive up to 18‰)	15 – 40 [84, 86]	30 – 35 [90]	10 – 40 [92]
NO_3 (mM)	> 1.27 [75]	>0.055 [87,88]	0.22 – 8.83 [90]	Mixotrophic [93] (algal predator)
NH_4 (µM)	0 – >1,000 [78,79,80]	55 – 200 [87,88]	25 – 200 [90]	
Urea (µM)	0 – >1,000 [80]	200 [88]	–	
PO_4 (µM)	> 100 [75]	>3 [21]	40 – 60 [90]	
$Si(OH)_4$ (µM)	0 – 0.68x10^6 [81]	>100 [21,85]	100 – 540 [90]	
N:P	10 – <30 [76,82]	15 [88]		
Si:N	20 – 50 [16]	1:8 [85]		
Fe_T (µM)	>10 [75]	0.15 [89]	70 – 110	
Cu (M)	< 10^{-12} [83]	<10^{-9} – $10^{-10.5}$ [83,89]	$10^{-8.3}$–$10^{-7.4}$ [83]	< 10^{-11} [83]

Figure 1 – Conceptual diagram detailing the main factors that determine HABs and characteristics impacting growth and distribution of harmful algae species sighted in the SF Bay

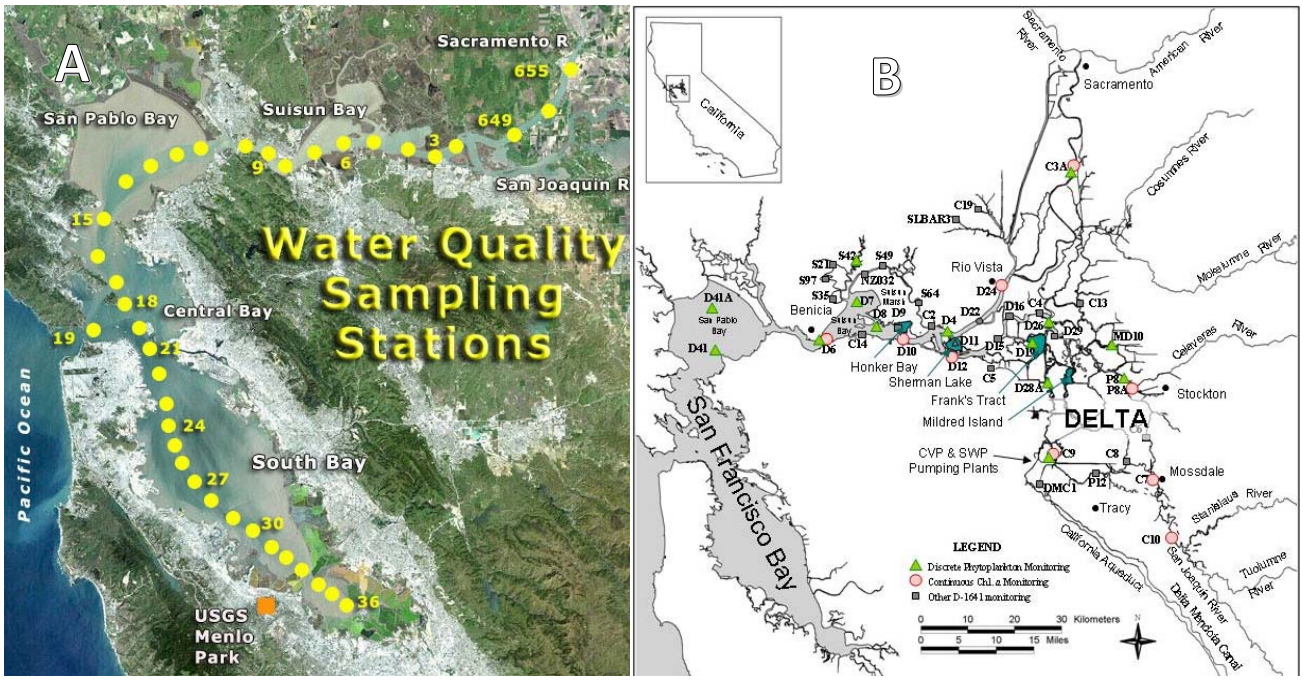


Figure 2 – Map of sampling stations and discrete sample sites in the San Francisco Bay (A) and in the upper San Francisco Estuary (B)

Table 1 - Environmental drivers associated with *Microcystis aeruginosa* growth and bloom development and with microcystin production. *In Situ* values represent observed values reported in the literature for the San Francisco Estuary and Delta Systems. In vitro values represent values reported in the literature for laboratory experiments. Model values represent statistically-significant predicted values for the formation of a toxic bloom of *Microcystis aeruginosa* [74].

Drivers	Growth/Bloom development	Microcystin production
Physical drivers		
Light intensity ($\mu E\ m^{-2}\ s^{-1}$)		
In situ	2000 [55]	
In vitro	80 (μmax) [94]	80 (MCmax/cell) [94]
Model	**>26** [75]	
Temperature(˚C)		
In situ	> 20 [16]	20 – 25 [16]
In vitro	28 – 32 (μmax) [77]	20 – 24 [94,95]
Model	**>18.8** [75]	
Streamflow ($m^3 s^{-2}$)		
In situ (SJR)	28-32 [16]	–
Chemical drivers		
Salt tolerance (‰)		
In situ	0.1 – 18 [55]	<5 [16,55]
In vitro	<7[96,97], <10[98,99], <14[100]	<10 [99]
[NH_4^+] (μM)		
In situ	0.56 – 1.67 [16]	
In vitro	0 – 1000 [80]	0 [79,102]
[NO_3^-] (μM)		
In situ	3 – 5.8 [16]	
In vitro	variable	350 – 5,800 [101]
Model	**>1270** [75]	
Inorganic Phosphate(μM)		
In situ	0.63 – 1[16]	
In vitro	14.4 [100] – 176 [82]	14.4 –143.5 [101]
Model	**>100** [75]	
Si:N		
In situ	20 – 50 [16]	
In vitro	–	–
N:P		
In situ	10 – 15 [16]	≤5 [76]
In vitro	18 – 51 [82]	16 – 46 [82]
Iron (μM)		
In situ		
In vitro	10 – 100 [101]	10 [79, 101]
Model	**>10** [75]	

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