Grant Proposal

Phytoplankton communities in the San Francisco Estuary: monitoring and management using a submersible spectrofluorometer

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I. PROJECT PURPOSE AND BACKGROUND

A) **The Importance of Phytoplankton**

The base of food webs in estuaries is formed by organic materials transported into the estuaries with river flows and ocean tides and by organic materials produced in the estuaries themselves. The upstream, freshwater portions of estuaries such as the upstream portion of the San Francisco Estuary (SFE) known as the Sacramento-San Joaquin River Delta (Delta) (Figure 1), often receive large inputs of riverine organic matter, much of which is dead detrital material. While these large amounts of detrital material of riverine origin often lead to high microbial activity and net heterotiophic metabolism in these systems, phytoplankton-produced organic matter of riverine and estuarine origin is generally more readily bioavailable and nutritious for consumers and thus fuels. the consumer food web, including benthic and pelagic invertebrates and fish (Abril et al 2002). This has recently been confirmed for the Delta (Mueller-Solger et al 2002, Sobczak et al 2002 and in press). Declines seen in many zooplankton and fish species in the Delta, including the currently highly endangered Delta smelt, may be related to simultaneous declines in Delta-wide phytoplankton productivity (Jassby et al 2002). While phytoplankton tends to be more nutritious than detrital particles, the nutritional quality of phytoplankton for consumers also depends on the species composition of the phytoplankton community (Brett and Muller-Navarra 1997, Van Donk 1997, Sterner and Elser 2002, Anderssen et al 2003, Quigg 2003).

On a Delta-wide basis, phytoplankton production has decreased over the last thirty years (Jassby et al 2002), however, phytoplankton productivity and biomass in some parts of the Delta remains high (CDWR 2004) (Figure 2) and is generally thought to be regulated by local bathymetry, tidal amplitude, light availability, flows, and grazing as well as by transport-related mechanisms which regulate biomass distribution (Lucas et al 1999 and 2002, Jassby et al 2002). Excessive phytoplankton production can lead to adverse water quality conditions, including dissolved oxygen sags, taste and odor problems in drinking water, and formation of toxic or otherwise harmful algal blooms. Furthermore, algae can also interfere with the operation of water diversion and treatment operations, for example by clogging filters in water treatment plants, fish screens or channels. Finally, phytoplankton species also play an important role in the incorporation of elemental contaminants into the food web. These include several contaminants found at often high levels in the SFE, such as selenium (Riedel et al 1996, Stewart et al 2004) and mercury (Wang 2002, Wiener et al 2003). These processes and effects related to phytoplankton community composition, biomass, and production in the Delta are summarized in Figure 3.

Over the last decades, increases in harmful algal blooms (HAB) have been noted for both marine and freshwater systems (Nicholls 1995, Anderson and Garrison 1997). Often, only one or a few algal species are responsible for these adverse phenomena. Dissolved oxygen minima are commonly observed in. the south-central Delta along the deep, channelized reach of the San Joaquin River leading to the Port of Stockton. These

minima are at least in part related to phytoplankton blooms in the San Joaquin River and often lead to fish kills (Lehman et al 2004). Blooms of toxic cyanobacteria (blue-green algae) and dinoflagellates are increasing worldwide and along the west coast of the U.S. (Homer et al 1997, Chretiennot-Dinet 2001, Paerl et al 2001). Potentially toxic *Microcystis* sp. (cyanobacteria) blooms have been observed in the Sacramento-San Joaquin Delta in the last few years (Lehman and Waller 2003). While the exact extent, temporal dynamics, toxicity, and effects of these *Micro cystis* blooms as well as the mechanisms driving them are not yet known, one could speculate that these blooms might have at least been partially responsible for the apparent year-class failure leading to the lowest Delta smelt fall midwater trawl index ever observed in 2004 (G. Castillo, USFWS, personal communication), either directly through toxic effects on certain developmental stages of Delta smelt, or indirectly through food web effects.

In addition to toxins, several algal taxa also produce taste and odor causing compounds that can affect drinking water quality (Soeder and Siegel 2000, Paterson 2004). Twenty-two million Californians rely on the Delta as a source for drinking water, most of which is pumped from the southwestern area of the Delta (Figure 1). In July of 2003 and 2004, this area experienced "taste and odor events" caused by a bloom of the cyanobacteria *Oscillatoria* sp. The Jones Tract flooding of 2004 caused additional taste and odor problems (Jeff Janik, CDWR; personal communication). Since 2003, copper sulfate has been applied near the water intake facilities to manage this problem. Of particular concern for Delta drinking water quality is also the link between the formation of harmful drinking water disinfection by-products and organic carbon concentrations resulting from phytoplankton production in the Delta and from other carbon sources. Also, blooms of some common Delta algae such as the diatom species *Melosira varians* can clog filters in water treatment facilities and the South Bay Aqueduct usually receives regular copper sulfate applications from March to September to reduce the abundance of *Melosira varians.* If Delta waters become more transparent due to reduced sediment loads as has been predicted (Jassby et al 2002), toxic, taste and odor producing, filter clogging, or otherwise harmful algal blooms may become more frequent in the Delta.

B) Phytoplankton Composition and Distribution in the SFE

Estuaries and coastal regions fault the transition zone between freshwater riverine and saline marine environments. Due to the convergence of marine and riverine water, estuaries exhibit strong and often highly variable chemical, physical, and biological gradients (McLusky 1993). Along salinity gradients of estuaries, phytoplankton biomass and diversity often build up near the estuarine turbidity maximum due to tidal trapping of particles and decline in the oligo-mesohaline transition zone due to salinity stress and grazing mortality; blooms of marine species often occur at higher salinities (Muylaert and Sabbe 1999). This type of horizontal phytoplankton biomass distribution along the estuarine salinity gradient was observed in the San Francisco Estuary by Jassby et al during some of the 1995 MIDAS cruises from the Delta to South San Francisco Bay (Jassby et al 1997). Lehman (2000B) found more large phytoplankton species and diatoms at the landward than on the seaward edge of the low salinity zone.

In many turbid estuaries such as the SFE, phytoplankton production is limited by light and not nutrient availability, and phytoplankton communities are dominated by

riverine and marine diatoms and low-light adapted species such as cryptophytes. Dinoflagellates, chlorophytes, and cyanobacteria are also common in estuaries (Muylaert and Sabbe 1999, Lemaire et al 2002). In the Delta, the relative diatom density decreased over the last thirty years, while the relative density of smaller green, blue-green, and flagellated algae increased (Lehman 2000A, 2004). Jassby et al (2002) found that the high amount of interannual variability in Delta-wide phytoplankton production was driven by light availability, river flows, and grazing. These authors also suggested that turbid, nutrient-rich systems such as the Delta may be more prone to high phytoplankton variability across several temporal scales than clearer, less nutrient-rich estuarine systems due to dampened compensatory processes.

In addition to its east-west salinity gradient, the Delta is also characterized by a strong productivity gradient from north to south: while unproductive, turbid Sacramento River water dominates the northern and central portions of the Delta, more productive San Joaquin River water influences its southern portion (Figure 2) (Lehman 1996, Jassby and Cloem 2000, Jassby et al 2002). Flow paths and mixing of water sources in the Delta are mainly determined by river flows, tides, and operation of State and Federal Water Projects and affect phytoplankton community composition and biomass in Delta habitats, with great variability often observed across small spatial and temporal scales and among seemingly similar habitats. For example, Lucas et al (2002) found highly variable production and distribution of phytoplankton biomass within and between two nearby flooded island habitats in the Delta (Frank's Tract and Mildred Island, see Figure 1), which they attributed to variations in phytoplankton sources, sinks, and transport. Phytoplankton biomass monitored by the EMP over a two year period at a continuous monitoring station in Stockton (P8A in Figure 1) shows seasonal and spring-neap variation (Figure 4) as well as substantial daily fluctuations (Figure 5) which can rival the magnitude of longer-teiui. variations. These types of daily fluctuations have been observed at several sites in the Delta and upstream portions of the San Joaquin River and are likely related to daily fluctuations in algal physiology and cell division rates (Kiss 1996) and grazing (Dahlgren et al 2004, Lopez et al 2004) as well as tidal influences.

Phytoplankton production, biomass, and taxonomic composition are thus an important and highly variable determinant of water quality and the food supply for consumers in the SFE. Water resource management and ecosystem restoration conducted by the CALFED and other programs and agencies in the SFE can affect both the species composition and biomass of the phytoplankton community, with consequences for both water quality and the food web. Accurate monitoring of phytoplankton species composition and biomass is needed to (1) assess the spatial and temporal distribution of phytoplankton biomass and species in the highly dynamic SFE, including the potentially toxic *Microcystis* blooms, to (2) explore phytoplankton-related ecosystem processes; and to (3) forecast phytoplankton responses to management actions and other driving forces such as global warming. However, the challenges posed by the continuously and often rapidly changing conditions in the complex SFE combined with logistical limitations such as laborious sample analysis methods greatly affect the ability to accurately monitor phytoplankton biomass and especially phytoplankton community composition at relevant

temporal and spatial scales, and new approaches to monitoring methods and design are needed.

C) Adaptive Management and Monitoring

The mission of the CALFED Bay-Delta Program is "to develop and implement a long-term comprehensive plan that will restore ecological health and improve water management for beneficial uses of the Bay-Delta System." To accomplish this, CALFED has adopted a science-based, adaptive management strategy (Figure 6) focusing on maintaining and improving ecosystem processes and functions rather than on individual system components (CBDA 2001, Healy et al 2004). To be successful, this approach requires a solid data and information base about ecosystem processes and factors driving these processes.

Large scale, long-term ecological monitoring programs such as the San Francisco Bay and Delta monitoring programs conducted over the last three decades by the San Francisco Estuary Institute (SFEI) and the California Interagency Ecological Program (TFP) and smaller-scale, shorter-term monitoring of ecosystem restoration efforts such as those supported by the CALFED program can provide much of the needed data and information (Figure 6). However, many monitoring programs focus on tracking individual system components and are often limited to low-frequency observations, or observations at small spatial and temporal scales. While often extremely informative at local scales and for answering specific questions, the data gained in such monitoring efforts are often inappropriate for elucidating and tracking ecosystem patterns and processes that span multiple spatial and temporal scales and address multiple infounation needs. This problem is exacerbated in highly heterogeneous systems such as the SFE, and monitoring aimed at tracking system components as well as processes across multiple scales in this system presents an ongoing challenge.

To successfully address information needs such as the need for early HAB detection and for effective performance measures for ecosystem restoration and other management activities in the SFE, monitoring programs may need to adopt an adaptive strategy similar to the adaptive management strategy put forth for ecosystem restoration. This adaptive strategy emphasizes the connection between monitoring design and information needs for ecosystem management, and aims to maximize learning about system properties and functions rather than to merely detect trends in system components (Boyle 1998, Ringold 1999). According to this ***adaptive monitoring*** scheme (Figure 7), information needs determine monitoring goals, objectives, and specific questions. Conceptual models about how to address these aims using existing knowledge about the system form the basis for designing monitoring programs. As new information becomes available, information needs, monitoring aims, conceptual models and monitoring design are constantly reviewed and, if necessary, revised, while taking care not to unnecessarily disrupt valuable long-term data streams. Targeted research to refine monitoring questions, design, and data interpretation as well as pilot monitoring efforts play an integral role in this strategy. Unfortunately, agency monitoring programs tend to have limited (or no) funds for such studies and often need to seek outside research funds,

especially if costly purchases of innovative equipment or non-routine sample analyses are involved. This is the case for the study proposed here.

Since 1971, the IEP Environmental Monitoring Program (EMP, http://iep.water.ca.gov/emp/) has consistently monitored water quality constituents and . lower tropic level organisms, including phytoplankton community composition and biomass, in the Delta and northern San Francisco Bay. The EMP is mandated under State Water Right Decision 1641 (D-1641) which permits the California Department of Water Resources (CDWR) and the US Bureau of Reclamation (USER) to operate the State and Federal Water Projects. Figure 1 shows current discrete and continuous phytoplankton monitoring stations and additional D-1641 monitoring stations. Following the adaptive monitoring concept outlined above, the EMP recently underwent an in depth program review involving local experts, resource managers, and stakeholders, as well as a group of independent scientists from across the US. Reviewers investigated to what extent the EMP satisfied historical and current information needs, reformulated monitoring aims and the conceptual models forming the basis for this monitoring program, and recommended a modified monitoring design as well as a number of special studies targeted at refining the program and making better use of its large data base (IEP 2003). One of the high priority studies identified in this review concerned the spatial and temporal design of the EMP's phytoplankton monitoring element. Specifically, reviewers identified a need for conducting studies aimed at improving phytoplankton monitoring methods and providing information needed to devise a more representative, efficient phytoplankton monitoring design. The project proposed here follows directly from recommendations made by the EMP reviewers.

D) A New Phytoplankton Monitoring Method

Traditional approaches to monitoring of phytoplankton biomass and community composition include microscopic examinations of samples (Utermoehl 1958) and more recent chemical analysis techniques measuring pigments and other taxonomic markers (e.g., Lemaire et al 2002, Boscker et al 2005) in discretely collected samples. While the microscopic techniques deliver the greatest taxonomic resolution, large standard deviations are associated with estimating phytoplankton biomass from cell counts (Wilhem et al., 1991, Buchaca et al 2005). Microscopic analyses also often literally overlook small algal cells (Carrick and Schelske 1997). Pigment analysis using high pressure liquid chromatography (HPLC) delivers less taxonomic resolution but more reliable biomass conversions and. has become one of the most commonly used methods for estimating and characterizing phytoplankton biomass and community composition (Llewellyn et al 2005). However, both microscopic and chemical laboratory techniques remain labor intensive (to the point of `ruin of mind and body' through microscopic analyses according to Ernst Haeckel in 1890). Even more importantly for monitoring in dynamic systems such as the SFE, the temporal and spatial resolution of phytoplankton monitoring and studies using these techniques is necessarily limited which in turn greatly limits advances in phytoplankton ecology and management in highly dynamic systems such as estuaries. Moreover, the delay between sample collection and sample analysis prevents early detection, prediction, and effective management responses to harmful algal blooms (Schofield et al 1999, Leboulanger et al 2002). Finally, discrete sampling affords

only limited ability to estimate constituent fluxes across regions of the estuary instead of merely measuring concentrations at certain locations. From a management perspective, this is particularly important for load calculations and in the context of water management in the estuary, including water project operations.

In the past two decades, optical methods to measure phytoplankton biomass and productivity from satellites, airplanes, moorings, and moving vessels have become increasingly available and reliable. These methods allow intense data collection over ecologically relevant scales (Figures 4 and 5), calculate mass fluxes, and, in combination with forecasting techniques, provide powerful tools for bloom prediction and management (Schofield 1999). Unfortunately, most of the optical instruments commonly in use today only allow for measurement of bulk phytoplankton properties without giving any information about individual taxonomic groups.

Optical distinction of different algal groups based on *in vivo* fluorescence properties has been pursued since the 1970s (e.g. Yentsch and Yentsch 1979). However, a quantitative spectrofluorometric method for measuring chlorophyll concentrations associated with different algal groups *in situ* has only recently been established (Beutler et al 2002, 2003, 2004). This method makes use of characteristic fluorescence excitation spectra for different algal "spectral classes." These group-specific "finger prints" (norm spectra) result from phylogenetic differences in accessory pigments of the light harvesting complexes. Using five diodes emitting light at 450, 525, 570, 590, and 610 nm, five spectral classes can be distinguished in mixed algal populations: "green" (chlorophyta), blue (cyanobacteria and glaucophyta), "brown" (diatoms, chrysophyta, dinophyta, haptophyta), "red" (rhodophyta and some cyanobacteria), and "mixed" (cryptophyta). Additional sensors can measure and correct for interference by colored dissolved organic matter (CDOM), turbidity, and changes in temperature. A commercially available spectrofluorometer based on this method has recently been introduced in Europe for laboratory and *in situ* applications and has been successfully employed in environmental baseline (GKSS "ferrybox," see http://coast.gkss.de/projects/ferryboxf) and harmful algal bloom monitoring (Leboulanger et al 2002) in marine and freshwater systems. This instrument, the bbe FluoroProbe (Figure 8; http://www.bbe-moldaenke.de and http://www.bbe.us/) is now also in use by several monitoring and research programs in the U.S. and Canada, including reservoir monitoring by the Metropolitan Water District (MWD) of Southern California (R. Losee, MWD, personal communication). While this type of instrument does not provide the taxonomic resolution of microscopic algal enumerations, it is capable of providing real-time data with much greater spatial and temporal resolution. A field test of the FluoroProbe conducted in the Delta in December 2002 with an instrument loaned by the manufacturer showed promising and interesting first results (Figure 8). Combined with continuous measurements of hydrodynamic and water quality variables and strategically collected discrete samples for microscopic enumeration and size-fractionated chlorophyll measurements, this type of instrument may represent a very powerful tool for ecological research and environmental monitoring. Displayed properly on the internet (such as on the GKSS ferrybox website), the data and information collected with this instrument can also be used as an effective outreach and environmental education tool.

E) Project Objectives

The primary objective of this project is to evaluate a new submersible spectrofluorometer, the bbe FluoroProbe, for phytoplankton monitoring and management in the SFE. Secondly, this project seeks to investigate high-frequency patterns in spatial phytoplankton group distributions among Delta habitats and along gradients from the productive southern Delta to the unproductive northern Delta and the increasingly saline western Delta and northern San Francisco Bay. A third aim of this project is to investigate high-frequency temporal phytoplankton patterns at fixed stations in contrasting Delta and Bay regions. Grantee intends to use the information gained in these investigations to make recommendations for improved phytoplankton monitoring and management in the upper SFE, including recommendations for a monitoring and rapid early-warning strategy for harmful algal bloom occurrences.

II. PROJECT DESCRIPTION

A) Hypothesis Tests and Methods

Objective 1: Method and instrument evaluation

Hypothesis 1: A commercially available multispectral fluorometer, the bbe FluoroProbe, can reliably detect chlorophyll a concentrations of different algal taxonomic groups in cultures of SFE phytoplankton species, in natural SFE samples, and during short- and longer-term *in situ* deployments under a wide range of different environmental conditions.

a) Laboratory Calibration using Cultures (Task lb): To test hypothesis 1, grantee intends to first compare the group-specific fluorescence "fingerprints" (norm spectra) provided by the FluoroProbe manufacturer with fluorescence spectra of laboratory cultures of at least five of the dominant algal species for each of the FluoroProbe "spectral groups" in the upper San Francisco Estuary. Calibrations will follow the procedures in Leboulanger et al (2002), Beutler et al (2002) and Beutler (2003). Grantee will also test the FluoroProbe's accuracy in detecting group-specific fluorescence signals when several of these cultures are mixed.

b) Laboratory Calibration using Natural Samples (Task lc): Chlorophyll *a* concentrations, "transmission" (turbidity) and "yellow substance" (CDOM) will be measured with the FluoroProbe and with conventional standard laboratory methods in laboratory standards and in natural water samples collected from the 20 discrete and continuous phytoplankton monitoring stations shown in Figure 1. These sites encompass the salinity and productivity gradients described above and are routinely sampled during monthly EMP monitoring cruises or routine station maintenance visits. Laboratory conditions will provide a more controlled environment for these initial tests than field deployments. For this study, samples will be collected at least once during the wet season when phytoplankton biomass is low and once during the dry season when phytoplankton biomass is higher. Samples will be transported to the UC Davis laboratory and used for calibrations and processed for discrete sample analyses as soon as possible after arrival. Microscopically determined phytoplankton densities and biovolumes will be compared with the FluoroProbe measurements. In addition to the routine phytoplankton medium-magnification enumeration method employed by the EMP, phytoplankton will also be enumerated using the high magnification technique (APHA 1998) to better account for small cells. Grantee will also test the potential influence of several additional variables measured in the sample water on the FluoroProbe measurements, including salinity, temperature, CDOM, total suspended solids, pheophytin *a* concentrations, chlorophyll *a* size fractions, particulate and dissolved organic carbon concentrations (POC and DOC), and nutrients (for analysis methods see below).

c) Short-term field evaluations (Task 2a): Grantee will compare *in situ* total chlorophyll a measurements made with the FluoroProbe against similar measurements made with other simultaneously recording *in situ* chlorophyll fluorometers (Yellow Springs Instruments and Turner Designs) and with other continuous measurements including flow, stage, temperature, salinity, turbidity, dissolved oxygen, and pH at the 9 continuous stations shown in Figure 1. Deployments at each site will last at least twenty-four hours. Grantee may also deploy the FluoroProbe alongside similar instrument packages proposed for deployment in wetlands in and surrounding the Delta, including

Suisun Marsh to the west of the Delta (Suisun Marsh Project, submitted for CALFED Science funding) and the Yolo Bypass, Liberty Island and a Cosumnes River floodplain north and east of Rio Vista (COYOTE Projects, submitted for CALFED ERP and CALFED Science funding), and other SFE wetlands (BREACH III), if funded. Collaborations may also occur with the SFEI Regional Monitoring Program (RMP), the Integrated Regional Wetlands Monitoring Program (IRWM), and the SFE National Estuarine Research Reserve (NERR). The objective is to conduct short-term side-by-side deployments of the FluoroProbe and existing continuous monitoring instrumentation in as many different estuarine environments as possible to assess its functionality across a range of salinities, temperatures, turbidity levels, CDOM concentrations, and phytoplankton biomass and community compositions. The proposed wetland monitoring sites and sites maintained by other programs would provide additional comparison opportunities. without adding extra costs. Further opportunities might arise through ongoing and proposed South Delta studies (IEP-CALFED South Delta projects) and in the San Joaquin River (R. Dahlgren, UC Davis). Deployments will take place at each site once during the wet season and once during the dry season, and FluoroProbe results will be statistically compared with measurements made with the other continuous instruments and in additional grab samples collected at the beginning and the end of the deployment period. Grab sample measurements will include the same variables measured under lb). Phytoplankton samples for microscopic enumeration collected at the beginning and at the end of each deployment will be pooled.

d) Long-term field evaluations (Task 2b): *Grantee* will deploy the FluoroProbe at three continuous phytoplankton monitoring sites with different productivity levels and sediment loads (stations D10, P8, and C3A) over periods of one to several weeks to evaluate its long-term recording stability and overall reliability, and potential fouling and other maintenance issues. Probe fouling by biofilms and sediment particles is a recurring problem for continuous monitoring in the San Francisco Estuary. Long-teen deployments will take place during the summer and fall months. Grantee will compare FluoroProbe maintenance requirements and data output with the requirements and data output of the other continuous instruments and investigate fouling susceptibility and maintenance strategies for the FluoroProbe. The FluoroProbe will be inspected every 2 to 4 days, and grab samples will be taken for laboratory analysis as above. Samples for phytoplankton enumeration will be combined into pooled samples spanning about one week.

e) Vessel-based deployments (Task 2c): In addition to the fixed station deployments, grantee will also test deployment of the FluoroProbe on board of the DWR/USBR research vessels, the RV San Carlos and the RV Endeavor, during routine monthly monitoring cruises. Both vessels are equipped with continuous monitoring instrumentation similar to the continuous monitoring stations, CTD instrumentation packages used for vertical profiling, and have on-board laboratories. Grantee will use the FluoroProbe to measure algal-group specific chlorophyll *a* levels along horizontal transects connecting the discrete sampling stations in Figure 1 (similar to the transects shown in Figures 2 and 8) and compare them with the chlorophyll and turbidity profiles recorded with the on-board (Turner) fluorometer and nephelometer. In addition, grantee will collect vertical chlorophyll fluorescence profiles at each discrete station (center channel) in combination with the vertical CTD drops. Vessel-based deployments will take place at least once in the dry and once in the wet season in combination with sample

collection for lb). Grantee will compare the FluoroProbe results with continuous and grab sample (collected as above at the monitoring stations) results from these monitoring cruises. Additional discrete chlorophyll samples will be taken at three to five locations between stations to enable more statistically powerful comparisons with the FluoroProbe measurements. Also, chlorophyll a concentrations will be measured in discrete water samples taken with a VanDorn water sampler from discrete depths for comparison with the vertical FluoroProbe measurements.

f) Additional considerations: Bbe-moldaenke offers both a submersible multispectral fluorometer, the bbe FluoroProbe, and a flow-through benchtop model, the bbe Algae Online Analyser. For this study, grantee intend to purchase the FluoroProbe because of its greater versatility. Grantee are experienced in building flow-through chambers for various submersible probes that allow bubble-free continuous immersion of probes in sample water pumped through these chambers, and intend to build and test such a chamber for the FluoroProbe. Grantee are also currently in the process of modernizing the transmission, storage, and distribution systems used for high frequency, multiparameter data collected at continuous monitoring stations throughout the upper SFE and will explore the integration of FluoroProbe data into these systems.

g) Instrumentation, sample analyses, and algal cultures (Tasks lb-2f): Details about the continuous instrumentation at the fixed monitoring stations and on the DWR/USBR research vessels can be found at http://iep.water.ca.gov/enip/Metadata/inetadata index.html. Details for station C9 can be found at http://wwwomwq.watenca.gov/AutoStationPage/index.cfm . Routine discrete water sample analysis for the EMP is carried out by the CDWR Bryte Chemical Laboratory which strictly follows standard laboratory methods and is certified by the US EPA. Details on routine discrete water quality and phytoplankton sample analyses can be found at http://iep.water.ca.gov/emp/Metadata/metadata index.html.

Non-routine sample analyses for this project include size-fractionated chlorophyll a measurements, high-magnification phytoplankton enumeration, CDOM, POC and DOC analyses. Water samples will be size fractioned using nitex screens with 30 µm and 5 µm mesh sizes to assess the contribution of nano- and pico-sized algae to total phytoplankton biomass (Carrick and Schelske 1997, Mueller-Solger et al 2002) and their possible differential impact on FluoroProbe measurements. Accurate assessment of smaller algal sizes is also the objective of the high-magnification phytoplankton enumerations which will be conducted by a subcontractor specializing in these analyses. CDOM concentrations will be determined fluorometrically using a Turner 10 AU fluorometer. POC and DOC concentrations will be analyzed by the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/) using published techniques (Cloern et al 2002). A side product of this project will be a comparison of the "volatile suspended solids" concentrations that have been part of the routine EMP monitoring program as a proxy for particulate organic carbon for the last three decades and POC concentrations measured during this study.

Non-routine sample preparations and some of the analyses and the study element involving algal cultures will be carried out at the UC Davis Limnology laboratory which has facilities and equipment needed for water sample analyses and for maintaining batch

and continuous algal cultures, and staff experienced in various water analysis and algal culturing techniques. Starter cultures will be purchased from the Culture Collection of Algae at the University of Texas at Austin (UTEX, http://www.bio.utexas.edu/research/utex/).

li) ***Data handling, storage, and analysis*** (Tasks 2g and 2h): Data collected by the FluoroProbe is initially stored and displayed using the FluoroProbe software (Figure 8). After review for accuracy, completeness, and consistency, these data will be uploaded into the continuous EMP data base and made publicly available over the internet via the BDAT portal (http://www.bdat.ca.gov). All discrete data will be stored in the discrete EMP data base and also made available on the internet via BDAT. Data flagged as "bad" are retained in local files, and notes will be added to the data base. A standard data collection sheet for noting deployment observations will be developed for the FluoroProbe and used by all project members handling the instrument. Project metadata will be entered and linked to the actual data in the data base. All project members will undergo the initial on-site training in instrument and software use offered by the manufacturer (Task la).

For analysis of method comparison data grantee will use Deming regression and bias plots as described in Triboli et al (2003). Bias plots (Bland and Altman, 1999) are a graphical approach to explore how closely two methods agree (or by how much they differ) and yield a bias (= mean difference) and 95% "limits of agreement" with confidence intervals. Deming regression is a model II parametric regression technique that allows for error in both variables and statistically tests for method differences (Linnet 1998). Influences of other measured variables on FluoroProbe results will.be explored using ordinary regression techniques. FluoroProbe results, especially from longer-term deployments, will also be inspected for outliers. FluoroProbe fingerprint (noun spectra) development and verification with dominant SFE algae will follow the equations in Beutler et al (2002) and Beutler (2003).

Objective 2: Spatial Distribution of Phytoplankton Groups

Hypothesis2: The upper San Francisco Estuary can be divided into subregions and/or habitat types with distinct phytoplankton taxonomic group composition and temporal dynamics; this spatial distribution is associated with different water and phytoplankton sources, sinks, transport dynamics, and environmental conditions in these regions/habitat types.

a) Data collection: Data to test this hypothesis will come from the data collection efforts under lb) - e) and additional data collected with the FluoroProbe along transects through shallow water habitats including Frank's Tract, Mildred Island, Sherman Lake, and Honker Bay (Figure 1) and several cross channel transects (Task 2d). Shallow water habitats and channel cross-sections are historically undersampled by the EMP, but are important in assessing spatial phytoplankton distributions in this system. Shallow water areas also provide important habitat and may be sites for increased bloom development. Also, Frank's Tract is currently the focus of hydrodynamics studies aimed at water quality management in the Delta. Frank's Tract and Mildred Island were also targeted in the study of phytoplankton variability by Lucas et al (2002, see IB). Data collections will take place during the summer months when phytoplankton production is higher, and additional samples for laboratory analyses will be taken and analyzed-as above.

b) Data handling, storage, and analysis (Tasks 2g and 2h): All data will be handled and stored as described under objective 1. To analyze the high-resolution spatial data collected with the FluoroProbe, grantee will mostly use the statistical methods (including treebased modeling and multivariate techniques) described in Jassby et al. (1997) and Jassby and Cloern (2000). Also, project team members and EMP staff (Anke Mueller-Solger and Marc Vayssieres at CDWR) are currently engaged in a collaborative CALFED-funded (CALFED-ERP 2002) project focusing on regional phytoplankton biomass and production in the Delta which is led by Dr. Alan Jassby at UC Davis. Objectives of this project include using these statistical methods to evaluate phytoplankton monitoring strategies, approaches to estimating system-wide variance from discrete stations, and the relationship between system-wide variance and the number of necessary stations using existing EMP data. Other objectives are to quantitatively elucidate processes responsible for variability in phytoplankton production and related quantities and develop forecasting methods. Techniques developed and employed in this project will be immediately applicable to the FluoroProbe investigations of phytoplankton community composition described here, and this project will in effect extend the ongoing project led by Dr. Alan Jassby.

Objective 3: Temporal Distribution of Phytoplankton Groups.

Hypothesis 3: Phytoplankton taxonomic groups in the upper San Francisco Estuary vary at daily, tidal, and seasonal time scales; this temporal variability is associated with daily light and tidal fluctuations, spring-neap tide dynamics, and seasonal changes in environmental conditions, and differs between regions/habitat types.

a) Data collection: Data to test this hypothesis will come from the data collection efforts under 1b) - e) and additional data collected with the FluoroProbe in a longer-term deployment in Frank's Tract alongside continuously recording EMP instrumentation (Station D19, Figure 1) (Task 2e). Frank's Tract is an important, centrally located, shallow water body in the Delta, as described above. Additional samples for laboratory analyses will be taken as above.

b) Data handling, storage, and analysis (Tasks 2g and 2h): All data will be handled and stored as described under objective 1. To analyze the high-resolution temporal data collected with the FluoroProbe, grantee will adapt the methods described by Jassby et al (1997) to analyzing the variance in the temporal data related to daily and tidal time scales. This type of exploration is currently ongoing for the existing EMP continuous data sets in the collaboration with Alan Jassby mentioned under objective 2, and as for objective 2, techniques developed and employed in the collaborative project with Alan Jassby will be immediately applicable to the FluoroProbe investigations described here. Grantee will also compare the temporal variability in phytoplankton groups between the wet and dry season to gain an understanding of seasonal differences.

B) Timeline

This is a two-year project. The project schedule is summarized in Table 1 and assumes a March 2006 start date. The evaluation of the new method for high-frequency, phytoplankton-group specific chlorophyll a measurements is the primary objective of this project. The analyses of spatial and temporal distributions of phytoplankton groups

(objectives 2 and 3) are dependent on and inseparable from successful data collection under objective 1.

C) Products

(Task 3a) This evaluation will result in recommendations regarding use of the FluoroProbe in SFE phytoplankton monitoring and management, including early detection of potentially harmful algal blooms. Provided that the FluoroProbe provides usable results, grantee will also gain new insights into spatial and temporal distributions of phytoplankton groups in the SFE which can be used in forecasting and ecosystem management applications. Results will be published in reports, including a final report integrating all aspects of the project which will include recommendations for phytoplankton monitoring and management. Grantee will also publish shorter articles on aspects of this study in the IEP newsletter and in peer-reviewed journals. Grantee intends to submit at least one article for publication in the online *San Francisco Estuary and Watershed Science* in which Grantee may make use of the electronic foimat to include computer animations of spatial or temporal phytoplankton dynamics. Grantee will provide all data and information collected in this project via the EMP and BDAT gbsites. Grantee will also regularly inform the IEP Water Quality Project Work Team and the IEP Estuarine Ecology Teams during their ongoing bimonthly meetings and present results at regional conferences such as the CALFED Science Conference, the State of the Estuary conference, and the IEP Annual Meeting, and at the National Monitoring Conference and/or the Estuarine Research Federation's national conference. Grantee will also informally pass project results on to other regional monitoring project staff during ongoing professional interactions. Product due dates are indicated in Table 1.

Table 1: Task Table: task summary, schedule and deliverables

Task 1) Equipment acquisition, laboratory testing and training
Task 2) Field studies and data analysis
Task 3) Project management and reporting

Task Number	Task Name	Year 1				Year 2			
	Season	Wet Season		Dry Season		Wet Season		Dry Season	
	Quarter	Winter (Ql)I	Spring (Q2)	Summer (Q3)	Fall (Q4)	Winter (Q5)	Spring (Q6)	Summer (Q7)	Fall (Q8)
	Month	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24
la	Fluoroprobe acquisition, training, and proiect P reP.								
lb	Laboratory calibrations using cultures								
lc	Laboratory calibrations using natural samples								
2a	Short-term field evaluations								
2b	Long-term field evaluations								
2c	Vessel-based deployments								
2d	Shallow-water and cross-channel transects								
2e	Frank's Tract long-term deployment								
2f	Non-routine sample **analyses**								
2g	**Data** base, data entry, and **dat** mana gement								
2h	Data analysis								
3a	**Reporting** of results								
3b	Project **oversight** and synthesi								
3c	Project management								
Proddet Due Dates	Web-accessible database								
	CALFED Semiannual Report								
	Final Project Report								
	Presentations								
	IEP Newsletter Articles								
	Manuscript for peer review								

Deliverables:

- Semi-annual reports will be submitted every 6 months following the project start date;
- Presentation of results will be given at regional conferences such as the CALFED Science Conference, the State of the Estuary conference, and the IEP Annual Meeting, and at the National Monitoring Conference and/or the Estuarine Research Federation's national conference. Special presentations to CALFED staff will be given upon request.
- Final report will be submitted 24 months from the project start date.
- Draft manuscript or a project closure summary report will be submitted 24 months from the project start date.
- Copies of any journal articles resulting from this grant will be submitted immediately after publication.

III. **PROJECT JUSTIFICATION**

A. Benefits to Science and Management

As described under I. and summarized in Figure 3, phytoplankton play a central role in the SFE. Of particular concern to managers may be the increasing occurrence of potentially harmful algal blooms such as the potentially toxic *Microcystis* blooms in the central Delta (Lehman and Waller 2003) and the taste and odor causing and filter clogging *Oscillatoria* and *Melosira* blooms in the southern Delta (Jeff Janik, CDWR, personal communication), as well as the overall decline in phytoplankton productivity in the Delta. Grantee expects that the use of in situ optical technology to distinguish algal group contributions to total algal biomass with fine spatial and temporal resolution will significantly increase early and accurate bloom detection and the general knowledge and understanding of phytoplankton distributions, factors driving these distributions, and processes affected by these distributions in the SFE, and ultimately result in more science-based monitoring and environmental decision making.

B. Relationship to CALFED Management Priorities

This project is aimed at improving phytoplankton monitoring and management of phytoplankton-related ecosystem processes in the SFE. Grantee expects to improve monitoring and gain a better understanding of phytoplankton-related ecosystem processes that relate to all goals of the CALFED Ecosystem Restoration Program (ERP), including management of at risk species such as the currently extremely endangered Delta smelt, management of harvestable species such a salmon, restoration of natural processes and habitats favoring a diverse phytoplankton community of good nutritional quality for consumers, reducing the impact of invasive or otherwise noxious species such as the previously rare *Microcystis*, and improving water and sediment quality, including drinking water quality in water drawn from the Delta. As outlined above, this project also touches on a large number of science and management needs, system components and processes targeted in the 2004 solicitation package for proposals to be funded by the CALFED Science Program. This includes adaptive management strategies, biological indicators, various estuarine habitats, human and ecosystem health, monitoring, natural resource management, performance measures, phytoplankton and primary productivity, trophic dynamics and food webs, water operations, and water quality management, to name only a few. As shown in Figure 6, monitoring can play a central role in CALFED's adaptive management strategy, and focused research studies such as the one proposed here are central to the ongoing adaptive optimization process of modem environmental monitoring programs (Figure 7).

C. Feasibility

This project is a collaborative effort between researchers and monitoring staff at UC Davis and CDWR. Project participants have successfully worked with each other in the past and bring a wealth of scientific and monitoring expertise to this project. Charles Goldman and Anke Mueller-Solger have been involved with conducting and administering long-teen monitoring and research projects in the San Francisco Estuary, Lake Tahoe and Castle Lake in California, and around the world. Both are currently

involved in CALFED-funded research projects. Anke Mueller-Solger is a regular participant in interagency and inter-institutional science and management activities, including acting as Program Co-chair for the 2004 CALFED Science Conference and working as an active member of the MP Management Team and as chair of the TFP Water Quality Project Work Team. Anke Mueller-Solger is also fluent in German which may facilitate interactions with the FluoroProbe manufacturer in Germany. Darrell Kaff and Michael Dempsey are experts on continuous water quality monitoring instruments and applications and long-time EMP staff members. Anne Liston is highly experienced with cultures of plankton organisms, including algae, protozoa, and zooplankton, as well as laboratory analyses of natural water and plankton samples. This project will also take advantage of and extend data analyses currently underway in a collaborative CALFED-funded (CALFED-ERP 2002) project led by Alan Jassby at UC Davis (see above). Further, this project will make use of the existing monitoring infrastructure in the upper SFE, including the continuous instruments at fixed stations and on research vessels, and routine sample analyses. A complete description of stations and instruments, and sample analyses is available at http://iep.water.ca.gov/emp/Metadata/metadata index.htrnl. Finally, preliminary evidence from a FluoroProbe test in the Delta conducted in December 2002 holds great promise for plausible and interesting results from FluoroProbe deployments (Figure 8).

Advantages of the collaborative project proposed here include the following: (1) Enhancement of project products and resulting monitoring and management recommendations through shared understanding of the system, shared monitoring experience, and shared learning; (2) Economy of scale: this project will take full advantage of the existing monitoring infrastructure and possibly also of other monitoring efforts currently proposed for CALFED funding, which will considerably simplify project logistics and reduce field and sample analysis costs; (3) Leverages: equipment, supplies, salaries and other costs will be substantially leveraged through the existing monitoring infrastructure. Leveraged funding will also include Charles Goldman's and Anke Mueller-Solger's salaries. Charles Goldman receives a full faculty salary from UC Davis and other projects, and Anke Mueller-Solger is a staff scientist with the EMP responsible for monitoring method development and data analyses such as those described in this project. State vehicles will be used to travel to and from field sites. The value of these leveraged costs is upwards of several million dollars.

D. Equipment Justification, Project Administration, and Project Oversight

Grantee intend to buy and test one bbe FluoroProbe for this project (Task la). The purchase price for this instrument also includes delivery and training and is based on a price quote received 12/16/2004. The instrument will be owned by CDWR and will be used for other CDWR and interagency monitoring and research projects after the end of the proposed study. US manufacturers do not yet offer this type of instrument. If similar instruments are available from US manufacturers by the project start date, Grantee will consider buying such an instrument instead of the bbe FluoroProbe. Project oversight will be provided by Charles Goldman of UC Davis and Anke Mueller-Solger and Darrell Kaff at DWR (Task 3b). Anke Mueller Solger will also be responsible for project administration and contract management (Task 3c).

E. Previously Funded Projects

An itemized budget has been entered into the CALFED PSP website. The proposed research has not been previously funded and no other sources of funding are currently being pursued. However, other sources of funding and existing monitoring infrastructure will be leveraged as described above.

SELECTION PANEL **REQUIRED** MODIFICATIONS

None.

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FIGURES

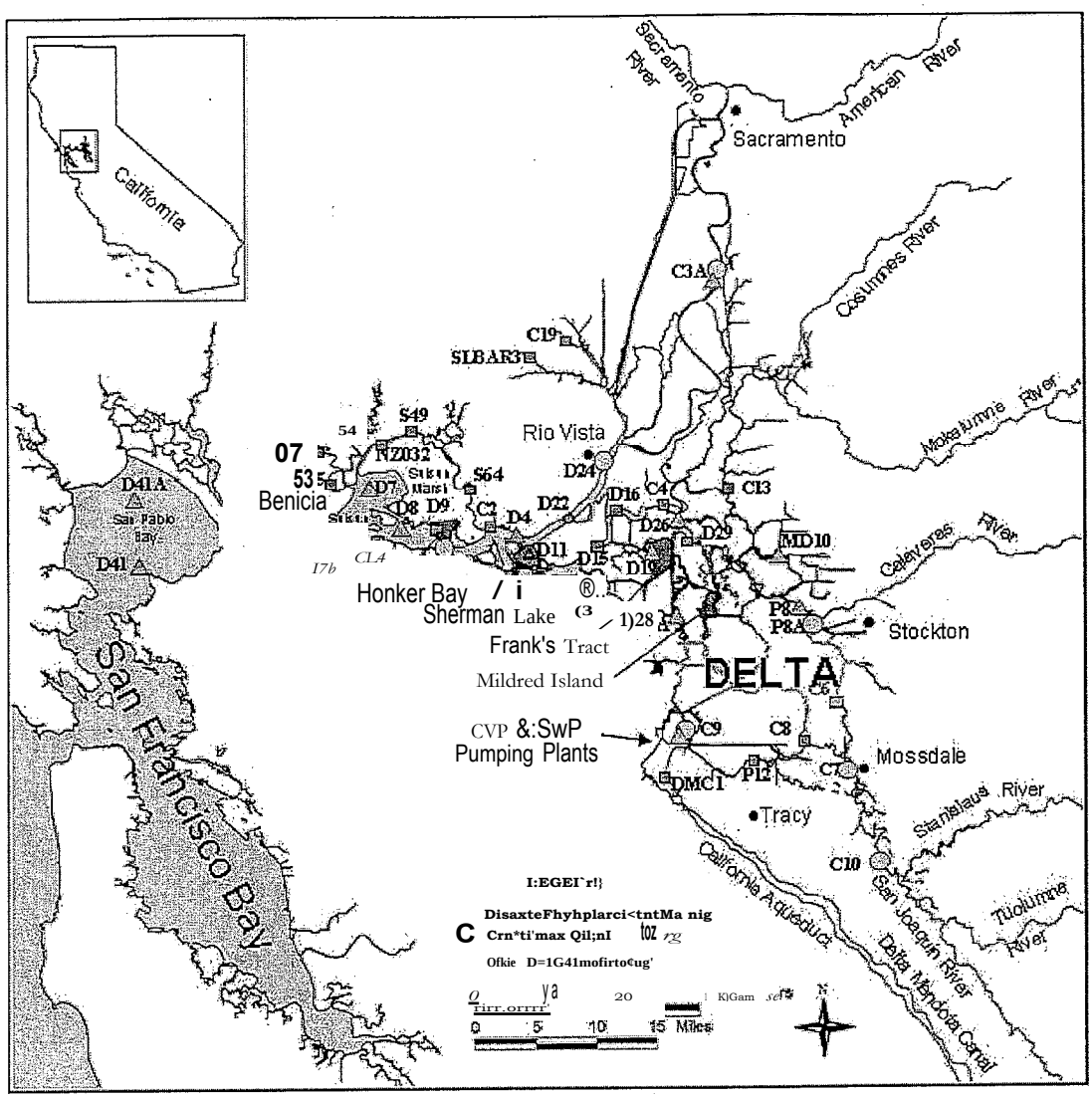

Figure 1. Discrete and continuous phytoplankton and other monitoring mandated under Water Right Decision 1641 in the upper San Francisco Estuary. Teal areas: Shallow-water habitats targeted in this study.

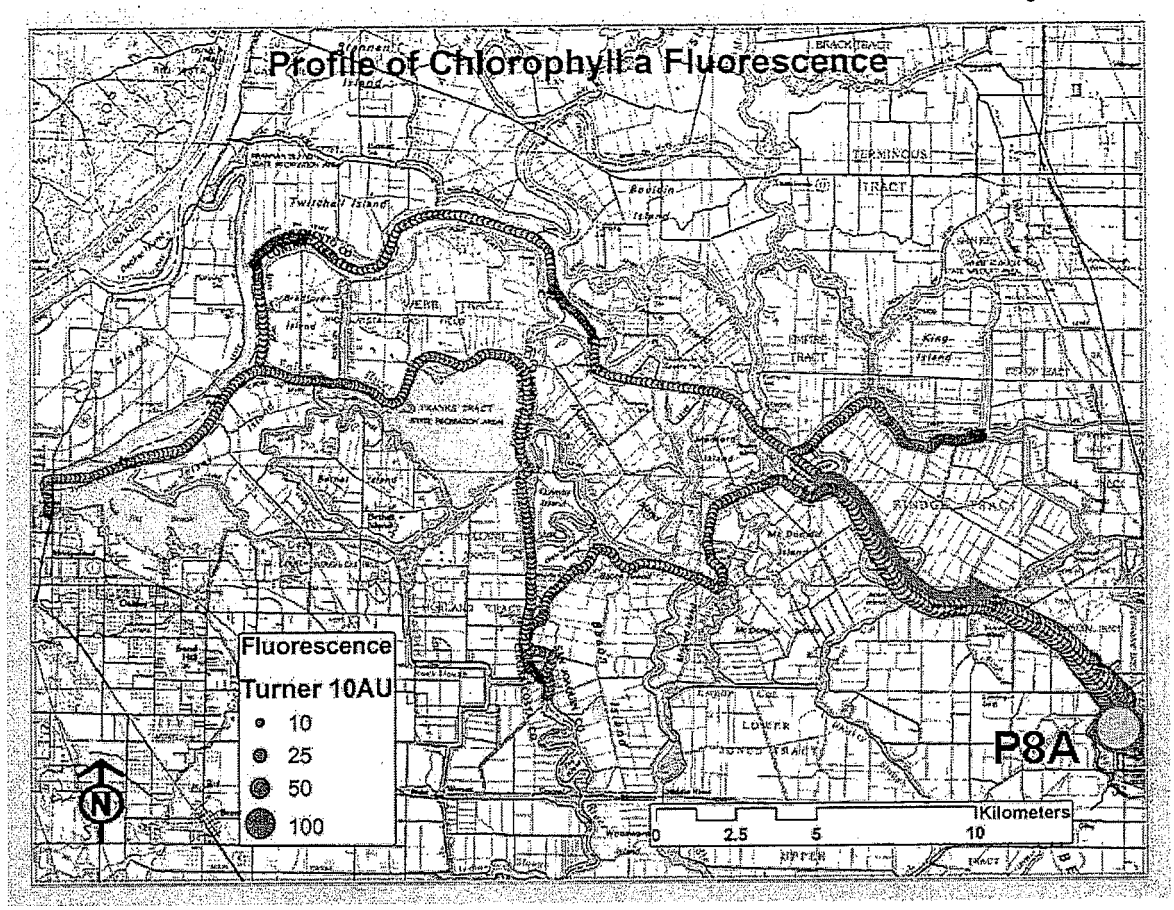

Figure 2. Results of a horizontal chlorophyll a fluorescence profile study conducted on December 4, 2002, on board the RV San Carlos using a Turner 10 AU fluorometer. The size of the overlapping green circles indicates the magnitude of chlorophyll fluorescence. Red arrow: location of a simultaneous FluoroProbe profile shown in Figure 8. Pink Circle: Continuous Monitoring Station P8A. Note the strong north-south gradient in chlorophyll fluorescence.

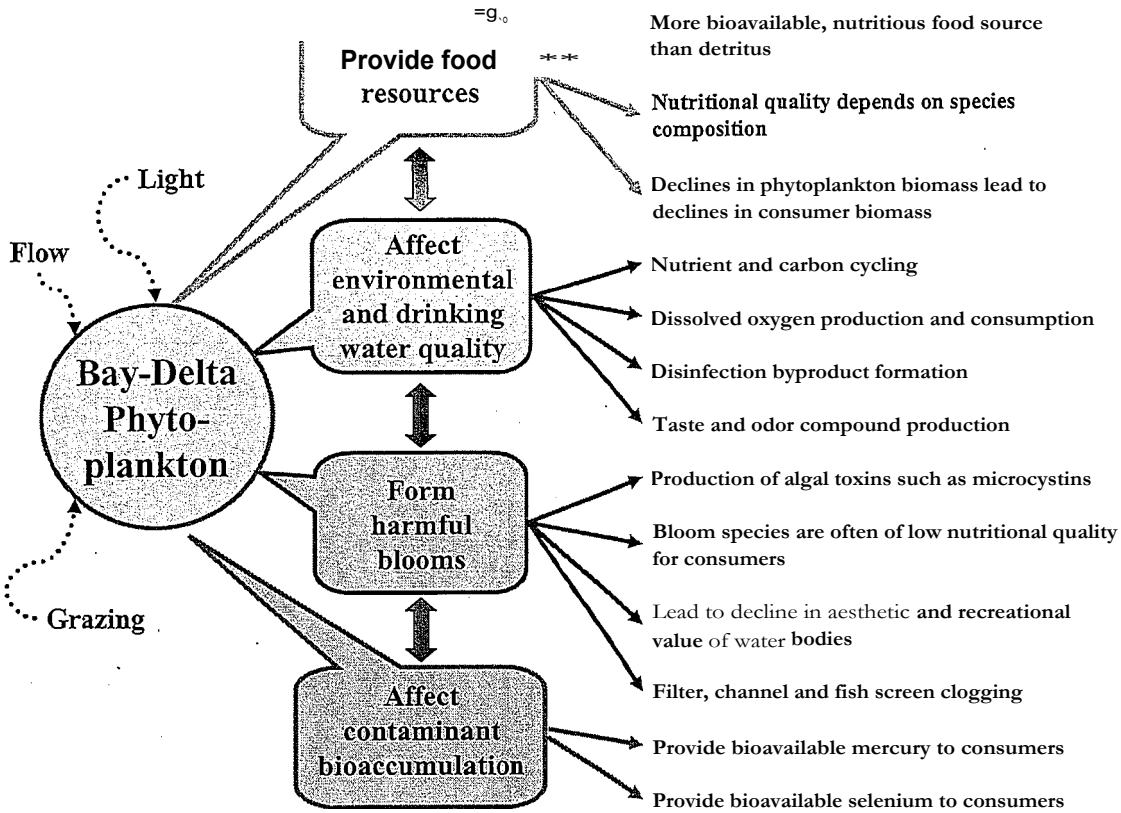

Figure 3: Summary of processes and ecosystem effects related to phytoplankton community composition and biomass in the Bay and Delta (solid, colored arrows) and factors affecting Delta phytoplankton production and biomass (broken black arrows) described in the proposal text.

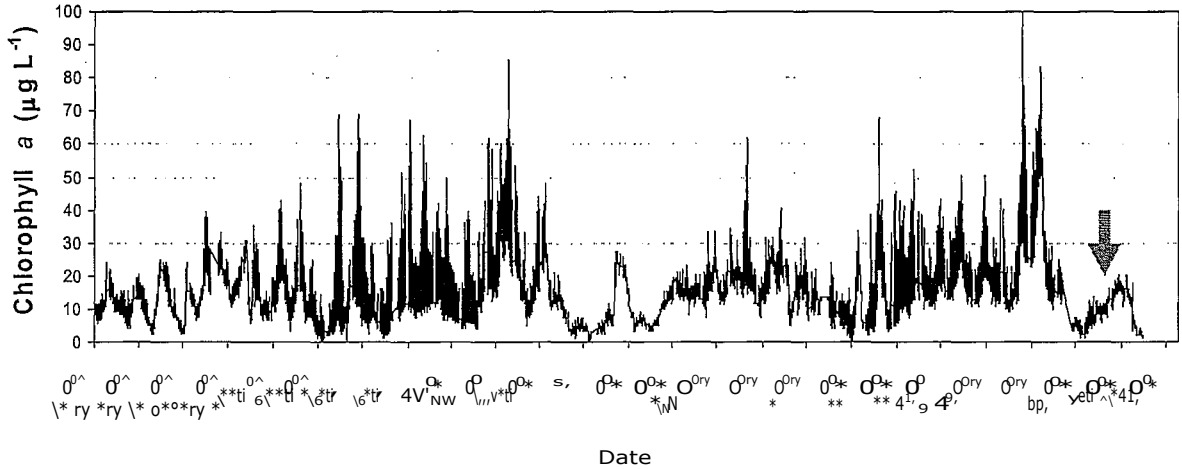

Figure 4. Time series of chlorophyll a concentrations measured at the continuous monitoring station P8A (Rough and Ready Island near Stockton, see Figures 1 and 2) with a Turner 10 AU fluorometer from January 2001 to December 2002. The red arrow denotes the date during which the horizontal profile studies shown in Figures 2 and 8 were conducted. Note the fortnightly (spring-neap tidal) and seasonal variations.

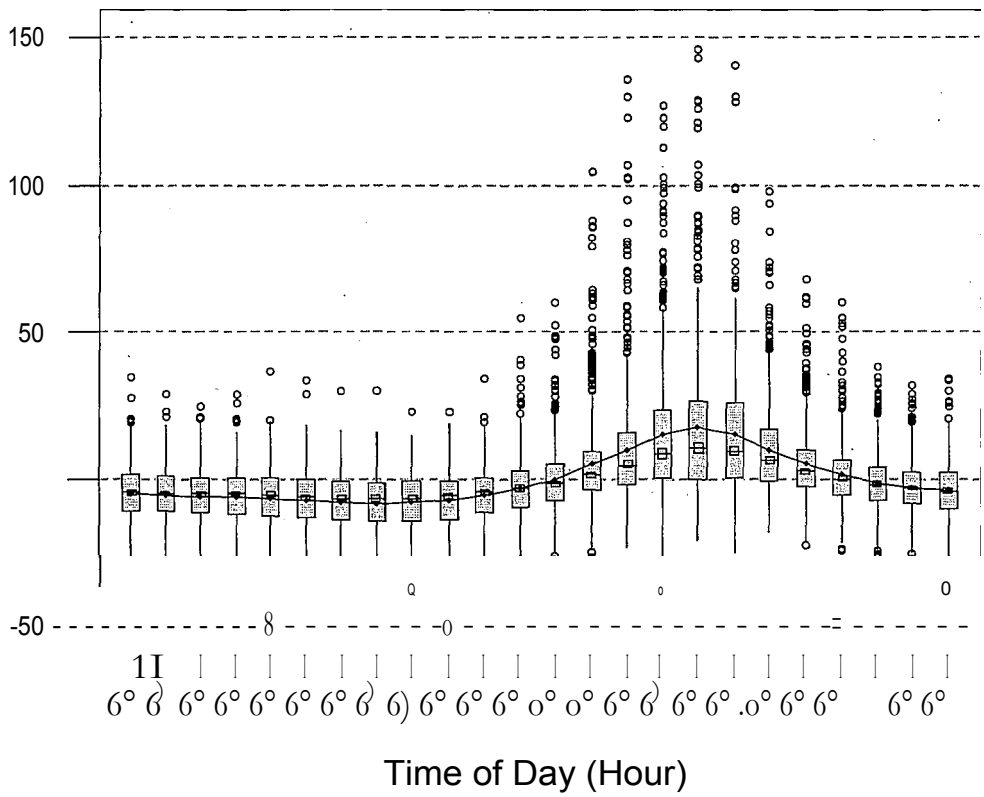

Figure 5. Box plots of diel differences between hourly and daily fluorescence means in percent of the daily mean for the chlorophyll fluorescence time series data shown in Figure 4. Note the minimum in the early morning hours and the maximum at about 4:00 pm. Also note the substantial magnitude in differences between hourly and daily means. *(Box plots:* Mean differences for each hour are connected with a line connecting the box plots and median differences are denoted with a line and an associated 95% confidence interval inside the boxes. The bottom and tops of each yellow box are the first and third quartile values, respectively. Whiskers and points outside the boxes show data that falls outside of this interquartile range.)

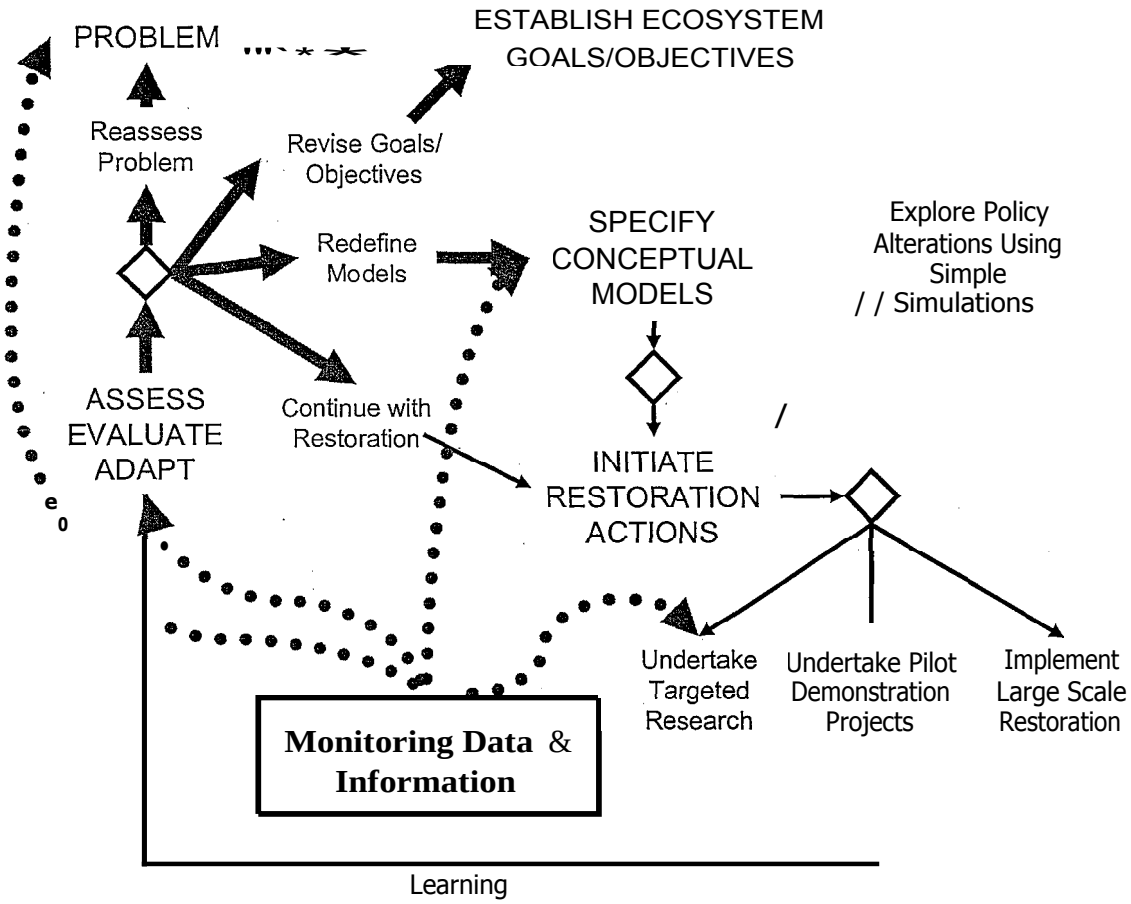

Figure 6. Adaptive management process redrawn from Figure 1 of the CALFED Draft Stage I Implementation Plan (CBDA 2001). The red broken arrows show where monitoring data and information can become part of this process and how this affects the next steps in this process (solid arrows)

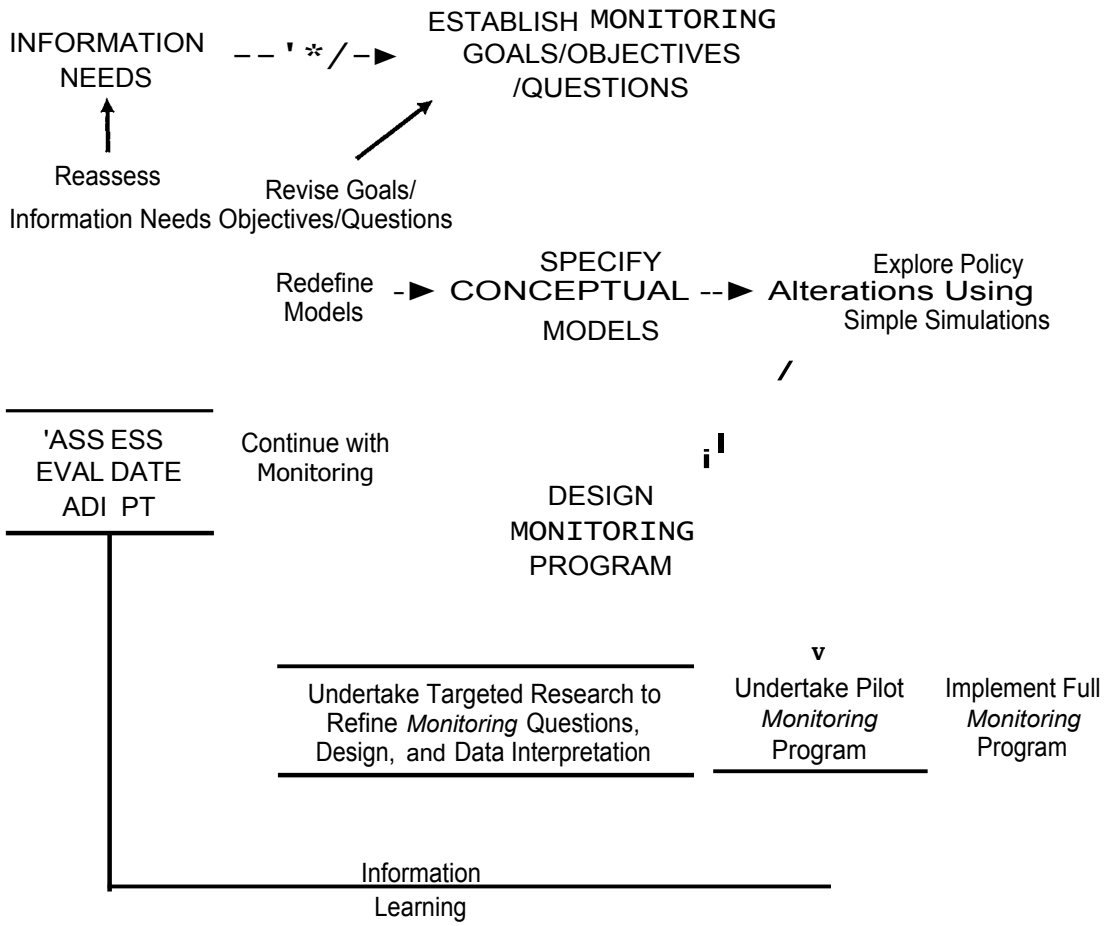

Figure 7. Adaptive monitoring process. The project proposed here falls into the two process components outlined in red (Targeted Research and Pilot Monitoring). The purpose of this project is closely aligned with the blue box.

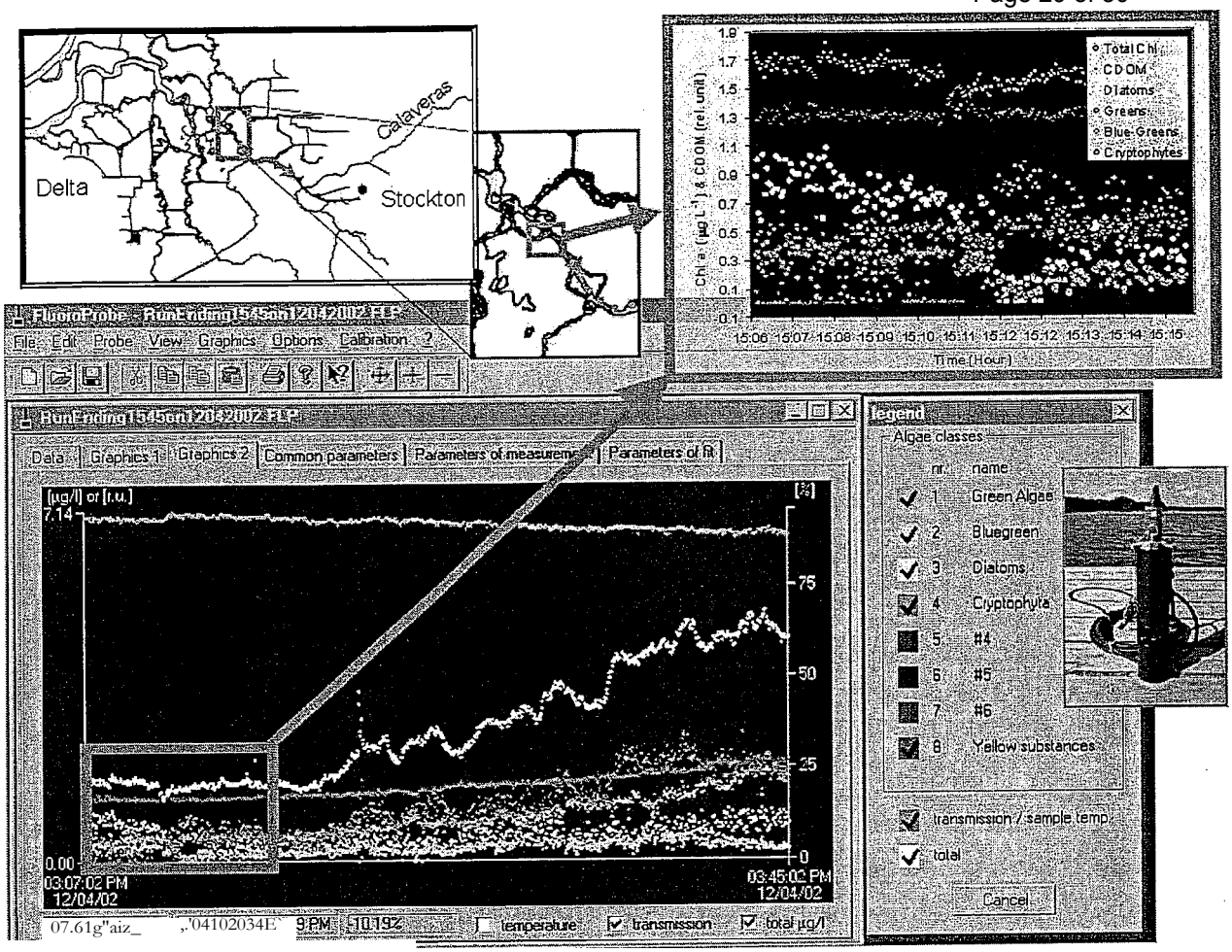

Figure 8. Results of a horizontal chlorophyll a fluorescence profile test deployment of the bbe FluoroProbe conducted on December 4, 2002, on board the RV San Carlos. The instrument is shown on the right. The geographic location and direction of the profile along the San Joaquin Ship Channel is shown on top (red arrow) and in Figure 2. The bottom graphic is a screen shot of results* displayed with the FluoroProbe software. Note the similarity with Figure 2 for total chlorophyll a fluorescence (white points). The top right graph shows the portion of the FluoroProbe data outlined by the grey box which corresponds to the small map insert. Note the abrupt change from diatom to cryptophyte dominance and the decrease in cyanobacteria (bluegreen) chlorophyll while total chlorophyll levels (initially) stayed the same. This abrupt shift in phytoplankton community composition happened as the RV San Carlos left Empire Cut and started heading up the San Joaquin Ship Channel and is likely associated with the different water sources and hydrodynamics prevailing in these areas which are affected by Delta water management. This pattern is not apparent in Figure 2 and represents the first observation of this type of abrupt change in phytoplankton composition in the Delta. Hydrodynamic studies of source water distributions have shown similar abrupt changes between source water influences at certain junctions in the Delta. Linking this type of high-resolution biological infounation with hydrodynamic and other water quality information may provide a powerful tool for better water and ecosystem monitoring and management.

* Chlorophyll a results reported in p.g L^{-1}, but FluoroProbe was not calibrated, and results are really fluorescence units.