FINAL PROJECT REPORT

PROJECT TERM: 05/01/2006 to 6/30/2011
PROJECT TITLE: Phytoplankton communities in the San Franciso Estuary; monitoring and management using submersible spectrofluorometer
AMOUNT FUNDED:$159,158.00

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PROJECT SUMMARY

Estuaries form the transition zone between freshwater riverine and saline marine environments and are characterized by highly variable environmental conditions and dynamic ecosystem processes. While estuaries such as the San Francisco Estuary (SFE) receive large inputs of organic materials from upstream sources, phytoplankton (algae) provide the most important food resource for estuarine consumers such as invertebrates

and fish, including highly endangered SFE species such as Delta smelt. Some phytoplankton species can also adversely affect water quality by forming harmful blooms. Algal blooms, including blooms of a potentially toxic species, have become increasingly common in the upper SFE and affect water project operations. Water resource management and ecosystem restoration conducted by CALFED and other programs in the SFE can affect phytoplankton species composition and biomass, with consequences for bloom formation, water quality, and the food web. Accurate monitoring of phytoplankton species composition and biomass is needed to maximize the positive and minimize the negative effects of phytoplankton in the SFE ecosystem, but made difficult by the highly dynamic nature of the estuarine environment and estuarine phytoplankton communities. The primary purpose of this project was to evaluate a new high-frequency method for in situ monitoring of phytoplankton biomass associated with different taxonomic groups using a submersible spectrofluorometer. This evaluation was carried out in the laboratory with algal cultures as well as in the field to assess the need for calibrations. Instrument was also deployed in the field at stations and during vessel-based routine monitoring cruises and site visits. Secondly, this project applied the spectrofluorometric method to investigations of spatial and temporal phytoplankton group distributions, monitoring design optimization, and improved ecosystem restoration and management strategies for the SFE.

LIST OF TASKS AND ACTIVITIES PERFORMED

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

Project ID: SCI-05-C332
Multi-year time table, revised 5/2008 and 3/2010

Notes: *Project starts 6 months into State fiscal year. Long delay in year 1 due to difficulties purchasing instrument with non-competitive bid procedures.*
Stop-work order in effect during much of calendar year 2009. Subtask 2e was eliminated due to instrument safety concerns.

fiscal year #1 (January 2007 - June 2007): 6 project months
fiscal year #2 (July 2007 - June 2008): 12 project months
fiscal year #3 (July 2008 - June 2009): 12 project months
fiscal year #4 (July 2009 - June 2010): 12 project months
fiscal year #5 (July 2010 - June 2011): 12 project months

Proposal Task ID Number	SOW Task ID Number	Task Name	Year 1 (2007)				Year 2 (2008)				Year 3 (2009)				Year 4 (2010)				Year 5
		Fiscal year	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5
		Season	Wet Season		Dry Season		Wet Season		Dry Season		Wet Season		Dry Season		Wet Season		Dry Season		Wet S
		Quarter	Winter (Q1)	Spring (Q2)	Summer (Q3)	Fall (Q4)	Winter (Q5)	Spring (Q6)	Summer (Q7)	Fall (Q8)	Winter (Q9)	Spring (Q10)	Summer (Q11	Fall (Q12)	Winter (Q9)	Spring (Q10)	Summer (Q11	Fall (Q12)	Winter (Q13)
		Months	Jan-March	April-June	Jul-Sep	Oct-Dec	Jan-March	April-June	Jul-Sep	Oct-Dec	Jan-March	April-June	Jul-Sep	Oct-Dec	Jan-March	April-June	Jul-Sep	Oct-Dec	Jan-March
		Month	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	29-30	31-33	34-36	37-39	40-42	43-45	46-48	49-51
1	1a	Fluoroprobe acquisition, training, and project prep.																	
2	1b	Laboratory calibrations using cultures																	
3	1c	Laboratory calibrations using natural samples																	
4	2a	Short-term field evaluations																	
5	2b	Long-term field evaluations																	
6	2c	Vessel-based deployments																	
7	2d	Shallow-water and cross-channel transects (Cache Slough Region & Sac River)																	
8	2e	Frank's Tract long-term deployment - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible	eliminated - not feasible
9	2f	Non-routine sample analyses																	
10	2g	Data base, data entry, and data management																	
11	2h	Data analysis																	
12	3a	Reporting of results																	
13	3b	Project oversight and synthesis																	
14	3c	Project management																	
Product Due Dates		Database																	
		CALFED Annual Reports																	
		Final Project Report																	
		Presentations																	
		IEP Newsletter Articles																	
		Manuscript for peer review																	

Tasks were identified as part of the scope of work, but were revised during the June 30, 2011 contract extension agreement.

Task 1. Equipment Justification, Project Administration, and Project Oversight

The purchase price for the instrument included delivery and training and was based on a price quote received 12/16/2004. The instrument is owned by the California Department of Water Resources (DWR) and is being used for other DWR and interagency monitoring and research projects after the end of the study. Project oversight was provided by Charles Goldman of University of California at Davis and Anke Mueller-Solger of the Delta Council and Francine Mejia at DWR. Anke Mueller Solger was responsible for project administration and contract management with collaboration from Francine Mejia.

Tasks 2, 3, 4, 6,7,9, 11-14. See Attachment 1 (Final Report).

Tasks 5. Long term field evaluations

Task 5 was attempted, but soon deemed not feasible and eliminated due to the high potential for instrument fouling, as several attempts demonstrated. A newer model of the instrument that became available after the start of this project has a wiper because this problem was noted by many other scientists working with this instrument.

Task 8. Frank's tract long term deployment

Task 8 was eliminated due to the high potential for vandalism in Frank's tract. DWR has recently deployed and lost a number of water quality instruments in the Frank's tract vicinity. To compensate, we have expanded our vessel based deployment and we have included comparisons with another instrument, the FlowCAM. The FlowCAM is an imaging-in-flow instrument that automatically identifies, enumerates, and estimates biomass for

in situ phytoplankton and zooplankton species. For this study, the FlowCAM was used to estimate phytoplankton biomass. Results from both instruments were then compared. [

Spatial and temporal variations are key factors in assessing spatial phytoplankton distributions in the system. We have also conducted more shallow water and river transect surveys than originally intended by piggy-backing on other studies conducted by the IEP and SFSU in the Cache Slough area and in the lower Sacramento River. Some of these field surveys were conducted at no cost to this grant during the 2009 work stop period, but samples are still being processed.

Task 10. Database is available upon request.

ACHIEVED OBJECTIVES, FINDINGS, MAJOR CONTRIBUTIONS, AND LIMITATIONS

The main goal of this project was to evaluate a new multispectral fluorometer, the FluoroProbe, for phytoplankton monitoring and management in the SFE. Specific objectives included 1) comparing taxonomic algal groups by performing laboratory and field calibrations, 2) examining spatial patterns of high-frequency phytoplankton data among Delta habitats and along gradients from productive areas such as the southern Delta to more unproductive areas such as those near Rio Vista in the northern Delta, and 3) investigating high-frequency temporal phytoplankton patterns in contrasting regions. We intended to use the information gained in these investigations to make recommendations for improved phytoplankton monitoring and management in the upper SFE.

While laboratory calibrations of specific algae groups may not be needed because the overall good performance of the FluoroProbe and the variable nature of the phytoplankton community in the San Francisco estuary, field calibrations for yellow substances (colored dissolved organic matter), may be necessary prior to

the beginning of routine EMP sampling transects. Results from the field calibration exercise suggest that FluoroProbe manual's recommendation to calibrate instrument when chlorophyll a concentrations are low may need to be implemented. However, comparisons with a third independent method, such as microscopic enumeration should be completed before putting into practice this recommendation.

The FluoroProbe could be a useful tool for the Environmental Monitoring Program of the Interagency Ecological Program. Currently, the Environmental Monitoring Program only collects discrete phytoplankton samples for microscopic enumeration at two of the nine continuous monitoring stations in the Bay-Delta. Data from the FluoroProbe would allow resource managers to observe phytoplankton composition trends when discrete samples are not taken. Microscopic enumeration along with phytoplankton biomass data estimated from the FluoroProbe could provide useful information about the dominant species at fixed stations when trying to detect harmful algal blooms.

Either during deployments at fixed stations or during vessel deployments, one of the main limitations in this study was the inability to examine other phytoplankton samples microscopically than the EMP samples due to the contracting challenges that prevented us from sending already collected samples. Collecting and processing independent microscopic enumeration samples to compare Fluoroprobe results would help determine decisively whether this method is appropriate to use in the SFE.

Results from our comparison between the FluoroProbe and FlowCAM suggest that these two instruments are potentially useful for determining the general characteristics of phytoplankton community structure in situ when used concurrently. The FlowCAM is best suited for identification of larger phytoplankton species whereas the FluoroProbe strength is in detecting fluorescence signals of cells of all sizes, including small cells.

Another useful comparison would be with extracted pigments measured with High Pressure Liquid Chromatography (HPLC). This was, however, not included in the scope of work for this project and no HPLC capability was available at DWR.

Overall, the results of this project show that the use of in situ optical technology to distinguish algal group contributions to total algal biomass with fine spatial and temporal resolution can 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 San Francisco Estuary, and ultimately result in more science-based monitoring and environmental decision making.

PROJECT DELIVERABLES

Progress Reports:

1. CALFED Science Grant Agreement No.S-05-SC-054. 2007 Progress Report. December 20, 2007.
2. CALFED Science Grant Agreement No.S-05-SC-054. 2008 Progress Report. March 8, 2010.

Oral Presentations:

2007 - Update on Calfed Project: Using the bbe FluoroProbe to examine phytoplankton fluorescence by taxonomic group. Anke Mueller-Solger. IEP Estuarine Ecology Team December 4, 2007, Tiburon, CA

2008 - FluoroProbing Bay-Delta Phytoplankton: Beyond Chlorophyll a. Anke Mueller-Solger, IEP & CALFED, Scott Waller, DWR, Anne Liston, UCD-TERC, Mike Dempsey, DWR, Monika Winder, UCD-TERC, Darrell Kaff, DWR. Oral Presentation, CALFED Conference 2008, Sacramento, CA.

2009 - Phytoplankton Fingerprinting with the bbe FluoroProbe. Anke Mueller-Solger, IEP & CALFED and Francine Mejia, DWR with Scott Waller, DWR, Mike Dempsey, DWR, Tiffany Brown, DWR, Anne

Liston, UCD-TERC, Monika Winder, UCD-TERC. 2009 IEP Workshop Series, Workshop 5: Bay-Delta monitoring questions and tools for the 21st century. October 14-15, 2009. Sacramento, CA

2011 - Spatial and Temporal Patterns of Phytoplankton Communities in the San Francisco Estuary Using a Submersible Spectrofluorometer 2011. F. Mejia, DWR, E. Yu, DWR, S. Waller, DWR and Anke Mueller-Solger, IEP & CALFED. 2011. IEP Workshop, Folsom, CA.

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

Final Report

Francine Mejia[1], Edmund Yu[1], Anke Mueller-Solger[2], Scott Waller[1], Peggy Lehman[1] and Tiffany Brown[1]

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Introduction

Phytoplankton production, biomass, and taxonomic composition are an important and highly variable determinant of water quality and the food supply for consumers in the San Francisco Estuary (SFE). Water resource management and ecosystem restoration activities 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 assess the spatial and temporal distribution of phytoplankton biomass and species in the highly dynamic SFE, to explore phytoplankton-related ecosystem processes, and to forecast phytoplankton responses to management actions and other driving forces such as global warming. However, the challenges posed by the 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. Thus, new approaches to monitoring methods and design are needed.

Traditional approaches for monitoring phytoplankton biomass and community composition include microscopic examinations of samples (Utermoehl 1958) and chemical analysis

techniques for 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 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. More importantly, the temporal and spatial resolution of phytoplankton monitoring and studies using these techniques is limited when monitoring in dynamic systems such as the SFE. In turn, the advances in phytoplankton ecology are greatly limited 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, continuous sampling affords the ability to estimate constituent fluxes across regions of the estuary rather than mere concentrations at certain locations. From a management perspective, this ability is particularly important for load calculations and 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 for intense data collection over ecologically relevant scales, the calculation of mass fluxes, and provide powerful tools for bloom prediction and management when combined with forecasting techniques (Schofield 1999). However, until recent years,

most of the optical instruments commonly in use allowed 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 was only established during this past decade (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. Five spectral classes can be distinguished in mixed algal populations using five different diodes that emit light at 450, 525, 570, 590, and 610 nm: "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 was introduced in Europe for laboratory and in situ applications and has been successfully employed in environmental baseline (GKSS "ferrybox," see http://www.hzg.de/institute/coastal_research/structure/operational_systems/KOI/projects/ferrybox/001843/index_0001843.html) and harmful algal bloom monitoring (Leboulanger et al. 2002) in marine and freshwater systems. This instrument, the FluoroProbe (http://www.bbe-moldaenke.de) has been in use by several monitoring and research programs in the United States (US) and Canada (See et al. 2005; Ghadouani and Smith 2005; Twiss et al. 2010). 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. 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 can be a powerful tool for ecological research and environmental monitoring.

Since 1971, the Interagency Ecological Program's (IEP) Environmental Monitoring Program (EMP, http://www.water.ca.gov/iep/activities/emp.cfm) has consistently monitored water quality constituents and lower trophic 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 (DWR) and the US Bureau of Reclamation (USBR) to operate the State and Federal Water Projects. The EMP has undergone 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 the extent to which the EMP satisfied historical and current information needs, reformulated monitoring aims, reviewed the conceptual models that formed 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 (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. Although phytoplankton biomass and community composition can be highly variable in space and time, the current phytoplankton monitoring program is based on a discrete sampling scheme that does not adequately capture all of this spatial and temporal variability. Thus, continuous fluorescence measurements with the FluoroProbe combined with the discrete sampling carried out currently can help us better characterize the phytoplankton communities in the SFE and provide an early warning system for the detection of harmful algal blooms.

Project Goals and Objectives

The main goal of this project was to evaluate a new multispectral fluorometer, the FluoroProbe, for phytoplankton monitoring and management in the SFE. Specific objectives included 1) comparing taxonomic algal groups by performing laboratory and field calibrations, 2) examining spatial patterns of high-frequency phytoplankton data among Delta habitats and along gradients from productive areas such as the southern Delta to more unproductive areas such as those near Rio Vista in the northern Delta, and 3) investigating high-frequency temporal phytoplankton patterns in contrasting regions. We intend to use the information gained in these investigations to make recommendations for improved phytoplankton monitoring and management in the upper SFE.

Study Area

The SFE is the largest estuary on the Pacific Coast of the contiguous US. It is a transition zone formed by the Pacific Ocean and the freshwater runoff from small Coast Range streams and the Sacramento-San Joaquin watershed. The SFE exhibits strong gradients from the mostly marine central and southern San Francisco Bay to the freshwater Sacramento-San Joaquin Delta. For our study, continuous sampling ranged from San Pablo Bay east through the upper estuary to the mouths of the Sacramento and San Joaquin rivers (Figure 1). The discrete phytoplankton sampling consisted of 15 sites that are routinely sampled monthly. These sites characterize the range of aquatic habitats found in the estuary from narrow, freshwater channels in the Delta to broad, estuarine bays.

Methods and Data Analyses

Laboratory Calibrations (Task 2)

We conducted two laboratory calibrations. The first laboratory test, used eight species of green, blue-green, and cryptophyte algae, and was conducted at the UC Davis Tahoe Environmental Research Center (TERC). In addition to the DWR FluoroProbe, we simultaneously tested a FluoroProbe belonging to UC Davis-TERC, allowing for additional assessments of instrument performance. We obtained algal starter cultures from the University of Texas Culture Collection of Algae (UTEX). The algal cultures were grown in a temperature controlled room at the TERC on a shaker table using Wright's Chu #10 (WC) medium. On test day, cultures were microscopically checked for cell status and contamination. For each cultured species, we prepared three to five test solutions with target concentrations ranging approximately from 1 to 100 µg/L. In addition to the FluoroProbe measurements, we also measured in vivo chlorophyll fluorescence of all test solutions with a Turner 10 AU fluorometer and filtered subsamples for fluorometric measurement of extracted chlorophyll *a*.

We also carried out a second set of laboratory tests using 16 individual species as well as 10 defined mixtures of green, blue-green, diatom, dinoflagellate, and cryptophyte algae. These tests were conducted in a similar fashion to those conducted earlier, and we again concurrently tested a FluoroProbe belonging to UC Davis-TERC, allowing for additional assessments of instrument performance.

Field Calibrations (Task 3)

Yellow substances are defined as CDOM. The FluoroProbe uses a ultraviolet (UV) light-emitting diode (LED) of 370 nm to measure yellow substances in the water. At 370 nm, it is possible to differentiate between algae (low signal) and yellow substances (high signal). The

result of this measurement is given in relative units and not in weight or volume because very different types of substances are detected. The result is mainly used to obtain a more accurate determination of algae classes, but it can also be used to determine the variations of the yellow substances.

The main objective of this exercise was to determine whether yellow substances have an effect on FluoroProbe algal categories readings when turbidity values are high and FluoroProbe percent transmission is less than 50%. Transmission is measured as a percent where 0 is 'clear water' and 100 is 'black water', and, similarly to turbidity, is used to measure particles in the water. It can be used to compensate for errors in the chlorophyll measurement caused by particles in the water which absorb either the excitation light or fluorescent light.

The FluoroProbe was calibrated by the manufacturer with the bbe standard algae types and yellow substances. However, the FluoroProbe manual recommends calibrating the offsets and/or the fingerprint of yellow substances when chlorophyll *a* concentration is low (< 10 µg/l, total chlorophyll *a* concentration) because yellow substances may interfere with the measurements due to the overlap in the excitation spectra with phytoplankton, particularly when chlorophyll *a* concentration is less than 10 µg/l. Jassby et al. (2002) reported a mean San Francisco Bay chlorophyll *a* concentration of 5.2 ± 0.7 µg/l Chl m^{-3}. This low mean concentration may suggest a need for instrument calibration.

Four 14 km long transects were conducted in Suisun Bay during July 19, 2011 to determine whether field calibrations were required for yellow substances during future EMP routine sampling events. Two transects were treated as controls (no calibrations) whereas the other two were calibrated for yellow substances with deionized water and ultra-filtered water.

Aside from the continuous data collected with the FluoroProbe and YSI 6600 instruments (algal categories from FluoroProbe, and chlorophyll *a*, water temperature, turbidity, specific conductance, and dissolved oxygen from the YSI sonde), we collected discrete samples at three sites. Sites were located roughly at the beginning, middle, and end of transects (Figure 2). At these discrete sites, we measured chlorophyll *a*, total suspended solids, volatile suspended solids, dissolved organic carbon (DOC), and total organic carbon (TOC). These water quality parameters were collected to examine whether differences observed between control transects and calibrated transects were due to water quality conditions rather than changes in algae composition.

All four transects were conducted within one tidal cycle to maintain environmental conditions as constant as possible. However, only data from the last two transects were included for comparisons due to the FluoroProbe malfunctioning. These two transects were conducted within 0.2 ft of the low slack tide.

Calibrations were carried out with deionized water and ultra-filtered water. For the filtered water, a 47-mm diameter glass-fiber filter with a 1.0 µm pore size was used to filter the water.

Short Term Field Evaluations (Task 4)

Sampling chlorophyll *a* and phytoplankton patterns at fixed stations

The FluoroProbe was deployed for approximately 24 hours at two of the nine DWR continuous monitoring stations in the upper SFE (Figure 3). The purpose of these short term deployments were twofold: to compare chlorophyll *a* measurements from the FluoroProbe against the YSI 6600 sonde at the stations, and to assess the functionality of the instruments under different

environmental conditions. Data were recorded using the 24-hour clock notation in Pacific Standard Time.

The FluoroProbe was first deployed at the Sacramento River at Hood (Hood) continuous monitoring station from September 29 at 0900 hrs to September 30, 2008, at 0700 hrs. Afterwards, it was deployed at the Rough and Ready Island (Stockton) continuous monitoring station from August 5 at 1000 hrs to August 6, 2009, at 1000 hrs. Both these stations were selected to represent different estuarine environments in the SFE system.

At both stations, continuous data for chlorophyll *a* were collected every minute using the FluoroProbe and every 15 minutes using the YSI 6600 sonde. Both instruments determined chlorophyll *a a* based on the spectral fluorescence signatures in the water, but the YSI 6600 sonde did not have the capability to break down the signals into different algal groups. In parallel, continuous data for water temperature, specific conductivity, turbidity, dissolved oxygen, and pH were recorded every 15 minutes using the YSI 6600 sonde. Water was collected at 1 m below the water surface for all measurements. Afterwards, data sets from both instruments were downloaded into local databases, checked for quality assurance and control, and combined into hourly averages.

Hourly flow and river stage data for the Hood station were obtained from the USGS Sacramento River at Freeport (Freeport) continuous monitoring station because flow and river stage data were not measured at the station. Similarly, hourly flow and river stage data for the Stockton station had to be obtained at another monitoring station since flow rates were unavailable at the Stockton station due to data logger issues. As a result, flow and river stage data at the Stockton station had to be obtained from the USGS San Joaquin River at Garwood Bridge (Garwood) continuous monitoring station.

Both Freeport and Garwood were chosen because the stations were closest to the Hood and Stockton stations. Hood is approximately 7.7 km away from Freeport, while Garwood is approximately 4.4 km away from Stockton (Figure 3). All flow and river stage data for each station were retrieved from the California Data Exchange Center (http://cdec.water.ca.gov/).

Chlorophyll *a* concentrations from a YSI 6600 sonde and FluoroProbe were compared using a two sample Mann-Whitney test to determine if the median concentrations of chlorophyll *a* from both instruments were significantly different from each other at each station. In addition, a correlation test using Spearman's Rho was conducted between chlorophyll *a* concentrations from each instrument to determine if the data sets were associated with each other at each station.

Correlation tests using Spearman's Rho were also conducted to determine if various environmental variables corresponded to chlorophyll *a* concentrations from each instrument. Correlation tests between an environmental variable and chlorophyll *a* from each instrument were used to determine the functionality of the YSI 6600 sonde and the FluoroProbe under different environmental conditions. If chlorophyll *a* values from both instruments produce similar correlations for an environmental variable at a station, then it suggests both instruments are functional under the environmental conditions. If the tests produce different correlations, then it suggests that one instrument may not be as functional when measuring for chlorophyll *a* under certain environmental conditions.

All statistical tests were conducted using the Minitab 15 Statistical Software package (Minitab, Inc. State College, Pennsylvania). For all tests, a p value of 0.05 or less was considered significant.

Vessel Based Deployments (Task 6 & 7)

Vessel based deployments during routine upper SFE monitoring cruises (Task 6)

Sampling took place during the 2010 upper SFE monthly water quality monitoring cruises to examine high-frequency patterns in spatial and temporal phytoplankton group variability. Data were collected at approximately high slack tide along transects in the main channel of the San Joaquin and Sacramento rivers. Water was pumped from a depth of approximately 1 m and directed for continuous readings (10 sec intervals) to a Sea-bird unit that measured specific conductance and temperature, a Turner 10 AU field fluorometer that measured chlorophyll fluorescence and turbidity, and a FluoroProbe unit that measured phytoplankton groups (categorized according to their fluorescence signals).

At EMP monitoring stations, discrete chlorophyll *a* and phytoplankton samples were taken for spectrophotometric analysis and for microscopic enumeration respectively. Subsets of the continuous data (0.5 & 1 km resolutions) were created for GIS (ESRI software, Redlands, California, USA) and multivariate analyses (PRIMER-E software, Plymouth Marine Laboratory, UK) that included analysis of similarities (ANOSIM), analysis selecting environmental variables that “best explain" community pattern, by maximizing a rank correlation between their respective resemblance matrices, BEST, and multi-dimensional scaling (MDS) plots.

Chlorophyll *a* and pheophytin *a* samples were collected with a submersible pump from 1 mbelow the water’s surface. Approximately 500 mL of water was passed through a 47 mm diameter glass-fiber filter with a 1.0 µm pore size at a pressure of 10 in of mercury. The filters

were immediately frozen and transported to Bryte Laboratory for analysis according to the spectrophotometric procedure from *Standard Methods* (APHA 1998). Samples were processed by mechanically grinding the glass-fiber filters and extracting the phytopigments with acetone. Chlorophyll *a* and pheophytin *a* pigment absorptions were measured with a spectrophotometer before and after acidification of the sample. Concentrations were calculated according to *Standard Method*'s formula (APHA 1998).

Phytoplankton samples were also collected using a submersible pump from 1 m below the water's surface. The samples were stored in 50 mL glass bottles. Lugol's solution was added to each sample as a stain and preservative. All samples were kept at room temperature and away from direct sunlight until they were analyzed. Phytoplankton were identified and enumerated by EcoAnalysts, Inc. according to the Utermöhl microscopic method (Utermöhl 1958) and modified procedures from *Standard Methods* (APHA 1998).

Phytoplankton species data from laboratory counts and phytoplankton groupings data from FluoroProbe continuous measurements were converted to cell carbon before comparison analyses. Phytoplankton cell carbon was calculated from cell dimensions based on simple geometrically appropriate shapes for each cell with an adjustment for the reduced plasma volume in diatoms (Menden-Deuer and Lessard 2000).

Contour plots for high frequency water quality data collected during the EMP monitoring cruises were made using the point kriging interpolation method in SURFER version 10 (Golden Software, Colorado, USA) to characterize spatial-temporal patterns from January 2010 to September 2010.

Comparisons of environmental and biological data during vessel deployments were computed using non-parametric statistical techniques. Patterns in phytoplankton community composition and their correlation with environmental factors were evaluated with PRIMER-E version 6 software. The first step included a MDS analysis to visualize the data. The MDS used a Bray Curtis dissimilarity index and was computed from the log+1 of phytoplankton biomass. The second step used an ANOSIM to test differences between regions (Figure 1). Significant differences produced by the ANOSIM test were determined by the value of the *R*-statistic. The *R*-statistic reflects the observed differences between groups. The last step used the BEST analysis to determine which environmental variables best describe the community patterns. BEST selects environmental variables that best explain these community patterns, by maximizing a rank correlation between their respective resemblance matrices. We used the BIOENV algorithm where all permutations of the trial variables are tried.

Comparing FlowCAM and FluoroProbe for identification of *in situ* phytoplankton communities (Task 7)

The FluoroProbe and FlowCAM were deployed simultaneously in July 2010 along transects of the Sacramento and San Joaquin rivers (Figure 4). Discrete samples for the FlowCAM analysis were collected approximately every 6 to 10 minutes and immediately processed through the machine. A total of 29 samples were taken in the Sacramento River and 32 in the San Joaquin River. The FluoroProbe was continuously deployed at 1 m depth. Three discrete phytoplankton samples were collected for microscopic analysis of species composition; however, these samples have not yet been analyzed. FlowCAM phytoplankton composition was categorized into the four FluoroProbe phytoplankton groups to carry out the statistical comparisons.

Results of the two instruments were compared with non-parametric statistical techniques using PRIMER-E version 6 software (Clarke and Gorley 2006). The spatial patterns in the data were first identified using the MDS ordination. The MDS ordination used a Bray Curtis dissimilarity index and was computed using percent (%) contribution of total phytoplankton biomass for each of the four phytoplankton groups. The same groups were used for each instrument. Differences between the MDS distributions were corroborated by using ANOSIM. Significant differences produced by the ANOSIM test were determined by the value of the *R*-statistic. Lastly, the SIMPER procedure was used to identify which groupings were responsible for the differences among samples within each MDS ordination. The SIMPER analysis also identifies which groups contributed most to the differences between MDS ordinations generated by each method. All statistical tests were deemed significant at the 0.05 level or less.

Comparisons between chlorophyll *a* counts and FluoroProbe readings were performed as well using the non-parametric routines in the PRIMER. ANOSIM was performed to test for differences between the two groups.

Results and Discussion

Laboratory Calibrations (Task 2)

Neither FluoroProbe exhibited any obvious problems and both instruments, the cuvette insert, and the software functioned as intended. Both instruments also produced comparable fluorescence readings for all algal species tested that day, although there were some small differences. During the first test, we were not able to include any algae belonging to the “brown” group.

Combining results from the 2007 and 2008 laboratory tests, the DWR FluoroProbe very accurately (91 to 100% correct) assigned chlorophyll fluorescence to individual green and blue-green species, while results for the brown group were more variable (39 to 100% correct) (Table 1). In mixtures of two to four species, the brown and cryptophyte signals tended to be somewhat underestimated while greens were overestimated. It is possible to reprogram the taxonomic group algorithms in the instrument to correct for these discrepancies, but because the overall good performance of the FluoroProbe in these lab tests and the very variable nature of the phytoplankton community in the SFE, we decided that we would maintain the factory default settings.

The FluoroProbe also predicted the biomass of the cultured algae expressed as chlorophyll *a* concentration well. Deviations from an overall 1:1 relationship between chlorophyll *a* measured by the FluoroProbe and chlorophyll *a* measured in culture extracts were due to differences in fluorescence responses by different algal species (Figure 5).

Overall, there was good agreement between the FluoroProbe and the Turner 10 AU in vivo fluorescence measurements (Figure 5) as well as between the FluoroProbe chlorophyll *a* estimates and chlorophyll *a* concentrations measured in extracted field samples (Figure 6). However, the FluoroProbe tended to underestimate the field chlorophyll *a* concentration, and there were also some fairly pronounced monthly and seasonal differences. This indicates the need for regular calibration against extracted chlorophyll *a* samples, as is the case for most in vivo fluorometers.

Field Calibrations (Task 3)

The MDS ordination showed strong differences between the control and calibration transects (Figure 7). The ANOSIM test also identified significant differences ($p < 0.01$) between the two transects (ANOSIM Global Rho=0.724, $p<0.01$). Additionally, SIMPER results determined that cryptophytes and blue-greens were responsible for about 68.2% of the differences between assemblages (Table 2).

Comparison of the phytoplankton groups quantified by each method revealed that the control samples had an average similarity of 94.1%. Calibration samples had an average similarity of 91.5%. Although there is a high degree of agreement within sampling groups according to the SIMPER results, the ANOSIM test suggests that there is a shift in fluorescence signals when calibration is performed. Comparison of the mean total chlorophyll *a* values between groups corroborated that there were significant differences between groups ($F= 4.42$, $p=0.04$) showing a mean concentration of 4.59 µg/L for control group vs. 4.32 µg/L for the calibrated group.

Because our study showed significant differences between methods, our next step is to compare the control and calibrated results with a third independent method, such as microscopic enumeration. This third method would allow us to further examine how calibration of the instrument can help describe phytoplankton patterns best. Judging from these preliminary results and the FluoroProbe manual's recommendation to calibrate the instrument when chlorophyll *a* concentrations are low, calibration should be conducted prior to sampling at the beginning of each transect.

Short term Field Evaluations (Task 4)

Comparisons between total chlorophyll *a* from the YSI 6600 sonde and the FluoroProbe

Hood

In 2008 at Hood, the hourly chlorophyll *a* averages from the YSI 6600 sonde ranged from a low of 0.8 µg/L at 15:00 to a high of 2.0 µg/L at 1800 hrs and 2100 hrs on September 29. In contrast, hourly chlorophyll *a* averages from the FluoroProbe ranged from a low of 0.4 µg/L at 1400 hrs to a high of 0.8 µg/L at 2100 hrs on September 29 (Figure 8). The majority of the chlorophyll *a* concentrations from the FluoroProbe was contributed by brown algae (78 to 100% of the chlorophyll *a* concentrations). For the entire period of deployment, Hood exhibited either no or low concentrations of other algal groups (0 to 22% of the chlorophyll *a* concentrations).

During the evaluation, diel variability in the chlorophyll *a* concentrations was not evident from either instrument. In addition, the YSI 6600 sonde returned higher values than the FluoroProbe as median concentrations for the entire evaluation were about 1.4 µg/L and 0.6 µg/L for each instrument.

The median concentration was equal to the mean concentration for both instruments. Thus, both data sets were evenly distributed around the mean. The difference between the median concentrations from both instruments was significant at alpha = 0.05 (Mann-Whitney W= 276.0, p= 0). Nevertheless, there was a significant correlation between chlorophyll *a* values from each instrument (Spearman's Rho= 0.557, p= 0.006).

Stockton

In 2009 at Stockton, the hourly chlorophyll *a* averages from the YSI 6600 sonde ranged from a low of 3.4 µg/L at 300 hrs on August 6 to a high of 5.4 µg/L at 1300 hrs on August 5. In comparison, the hourly chlorophyll *a* averages from the FluoroProbe ranged from a low of 3.0

µg/L at 1000 hrs to a high of 9.3 µg/L at 1400 hrs on August 5 (Figure 8). The majority of the chlorophyll *a* concentrations from the FluoroProbe was contributed by green algae (54 to 67% of the chlorophyll *a* concentrations). Blue-green algae, brown algae, and cryptophytes were also detected, but in lower concentrations (0.1 to 27% of the chlorophyll *a* concentrations). Unlike Hood, the effects of the diel cycle on the chlorophyll *a* concentrations from both instruments were more prominent. Both instruments recorded an increase in chlorophyll *a* as the afternoon approached and decreased as the evening approached on August 5. Afterwards, concentrations remained stable for the remainder of the evaluation.

Furthermore, the median concentrations of chlorophyll *a* for the YSI 6600 sonde and the FluoroProbe were about 4.1 µg/L and 3.9 µg/L. The mean and median were equal to each other for the data set from the YSI 6600 sonde, but not the FluoroProbe. For the FluoroProbe, the median concentration of 3.9 µg/L was lower than the mean concentration of 4.6 µg/L. Higher values from the FluoroProbe skewed the mean of chlorophyll *a* upward.

Overall, the YSI 6600 sonde returned higher values than the FluoroProbe when chl *a* conditions were low. However, when chl *a* conditions were high, the YSI 6600 sonde returned lower values than the FluoroProbe. Despite this, the difference between the median concentrations from both instruments was not significant at alpha = 0.05 (Mann-Whitney W= 709.0, p= 0.168). In addition, there was a significant correlation between chlorophyll *a* values from each instrument (Spearman's Rho= 0.474, p= 0.017).

Functionality of the FluoroProbe and the YSI 6600 sonde under different environmental conditions when measuring for chlorophyll *a*

Water Temperature

The FluoroProbe and the YSI 6600 sonde were both functional at different water temperatures when measuring for chlorophyll *a*. During the evaluations, Stockton was warmer than Hood. Hourly water temperature averages at Stockton ranged from 25.5 to 27.1°C (Figure 9). Water temperature was significantly correlated with the chlorophyll *a* from both the FluoroProbe (Spearman's Rho= 0.862, p= 0) and the YSI 6600 sonde (Spearman's Rho= 0.509, p= 0.009). Warmer water temperature corresponded to a higher concentration of chlorophyll *a*.

Moreover, cooler water temperature averages at Hood did not affect the functionality of both instruments to measure for chlorophyll *a*. Hourly water temperature averages at Hood ranged from 20.9 to 21.4°C (Figure 9). Chlorophyll *a* values from the FluoroProbe (Spearman's Rho= 0.016, p= 0.943) and the YSI 6600 sonde (Spearman's Rho= -0.054, p= 0.808) were not significantly correlated with water temperatures at Hood.

Specific Conductance

Specific conductivity is an estimation of salinity and it did not play a role on the functionality of the FluoroProbe or the YSI 6600 sonde when measuring for chlorophyll *a*. Chlorophyll *a* concentrations from both instruments produced similar relationships across different salinities. For the evaluations, Stockton was more saline than Hood.

Hourly averages of specific conductance at Stockton ranged from 397 to 467 µS/cm (Figure 10). Specific conductance was significantly correlated with the chlorophyll *a* from both the FluoroProbe (Spearman's Rho= 0.845, p= 0) and the YSI 6600 sonde (Spearman's Rho= 0.448, p= 0.025). Generally, specific conductance and chlorophyll *a* increased as the afternoon approached and decreased as the evening approached.

Furthermore, a less saline environment at Hood did not affect the functionality of both instruments to measure for chlorophyll *a*. Specific conductance ranged from 157 to 167 µS/cm (Figure 10). Chlorophyll *a* values from the FluoroProbe (Spearman's Rho= -0.153, p= 0.486) and the YSI 6600 sonde (Spearman's Rho= 0.002, p= 0.991) were not significantly correlated with the specific conductance at Hood.

Turbidity

The FluoroProbe and the YSI 6600 sonde were similarly functional at different turbidity levels when measuring for chlorophyll *a* . Turbidity levels were slightly higher at Stockton than at Hood. Turbidity at Stockton ranged from 7.6 to 12.0 NTU, while turbidity at Hood ranged from 3.5 to 6.3 NTU (Figure 11).

At Stockton, turbidity levels were not significantly correlated with the chlorophyll *a* concentrations from the FluoroProbe (Spearman's Rho= -0.083, p= 0.694) or the YSI 6600 sonde (Spearman's Rho= -0.291, p= 0.158). Similarly, turbidity levels at Hood were not significantly correlated with the chlorophyll *a* concentrations from the FluoroProbe (Spearman's Rho= 0.095, p= 0.668) or the YSI 6600 sonde (Spearman's Rho= 0.074, p= 0.737).

Dissolved Oxygen

There were differences in functionality between the FluoroProbe and the YSI 6600 sonde when measuring for chlorophyll *a* at different dissolved oxygen levels. At Hood, dissolved oxygen levels and chlorophyll *a* values from each instrument produced different statistical results. Chlorophyll *a* concentrations from the FluoroProbe and dissolved oxygen were not significantly correlated (Spearman's Rho= 0.238, p= 0.274), but chlorophyll *a* concentrations from the YSI 6600 sonde and dissolved oxygen were significantly correlated (Spearman's Rho= 0.602, p= 0.002).

However, the chlorophyll *a* values from the FluoroProbe and the YSI 6600 sonde both produced similar results at Stockton, where dissolved oxygen levels were lower than the dissolved oxygen levels at Hood. Hourly dissolved oxygen averages at Stockton ranged from 5.2 to 6.4 mg/L, while dissolved oxygen levels at Hood ranged from 6.4 to 7.2 mg/L (Figure 12). Dissolved oxygen at Stockton was significantly correlated with the chlorophyll *a* from both the FluoroProbe (Spearman's Rho= 0.572, p= 0.003) and the YSI 6600 sonde (Spearman's Rho= 0.539, p= 0.005).

pH

The FluoroProbe and the YSI 6600 sonde were both similarly functional when measuring for chlorophyll *a* at different pH environments. At Hood, pH showed very little variation in values as pH ranged between 7.3 and 7.4 (Figure 13). pH was not significantly correlated with chlorophyll *a* from the FluoroProbe (Spearman's Rho= -0.119, p= 0.587) or the YSI 6600 sonde (Spearman's Rho= 0.068, p= 0.76).

On the other hand, pH at Stockton showed more variation than at Hood as pH ranged from 7.3 to 7.6 (Figure 13). pH was significantly correlated with the chlorophyll *a* from both the FluoroProbe (Spearman's Rho= 0.567, p= 0.003) and the YSI 6600 sonde (Spearman's Rho= 0.478, p= 0.016).

River Stage

The FluoroProbe and the YSI 6600 sonde were both similarly functional at different river stages when measuring for chlorophyll *a*. Hourly river stage was higher at Freeport than at Garwood. Hourly river stage at Freeport ranged from 102.1 to 104.0 ft (Figure 14). In comparison, hourly river stage at Garwood ranged from 2.5 to 6.1 ft (Figure 14).

River stage at Freeport was not significantly correlated with chlorophyll *a* at Hood from the FluoroProbe (Spearman's Rho= 0.307, p= 0.155) or the YSI 6600 sonde (Spearman's Rho= 0.289, p= 0.181). In contrast, river stage at Garwood was significantly correlated with chlorophyll *a* at Stockton from the FluoroProbe (Spearman's Rho= -0.562, p= 0.003) and the YSI 6600 sonde (Spearman's Rho= -0.427, p= 0.033). Generally, river stage at Garwood was low when chlorophyll *a* at Stockton was high.

Flow

Flow rates did not appear to influence the functionality of the FluoroProbe or the YSI 6600 sonde when measuring for chlorophyll *a*. Hourly flow rates at Freeport ranged from 831 to 14,167 cfs (Figure 15). Flow at Freeport was not significantly correlated with the chlorophyll *a* at Hood from the FluoroProbe (Spearman's Rho= -0.024, p= 0.914) or the YSI 6600 sonde (Spearman's Rho= 0.109, p= 0.62).

In comparison, hourly flow rates at Garwood ranged from -3,404 to 2,746 cfs (Figure 15). Chlorophyll *a* values at Stockton from the FluoroProbe (Spearman's Rho= 0.1, p= 0.634) and the YSI 6600 sonde (Spearman's Rho= 0.243, p= 0.243) were not significantly correlated with flow rates at Garwood.

Comparability of chlorophyll *a* from the bbe FluoroProbe and the YSI 6600 sonde

The results suggest a relationship between chlorophyll *a* from both the FluoroProbe and the YSI 6600 sonde as the data sets were positively correlated with each other. In addition, the median concentration from the YSI 6600 sonde was higher than the median concentration from the FluoroProbe at both stations. However, chlorophyll *a* from both instruments did not always

produce similar values despite the correlation. Median concentrations of chlorophyll *a* were considered similar at Stockton, but not at Hood.

The discrepancy between the chlorophyll *a* values at Hood could be explained by the inability of the YSI 6600 sonde to differentiate between chlorophyll *a* and pheophytin *a* (Burks et al. 2008). As a result, chlorophyll *a* values from the YSI 6600 are normally higher than extracted values (Mike Dempsey, personal communication, 2011). On the other hand, the FluoroProbe tends to underestimate chlorophyll *a* when compared to extracted values (Rolland et al. 2010). Thus, it was not surprising to see higher values at Hood from the YSI 6600 sonde.

Nonetheless, the YSI 6600 sonde only returned higher chlorophyll *a* values when chlorophyll *a* conditions were low. When chlorophyll *a* conditions were high, the FluoroProbe returned higher chlorophyll *a* values than the YSI 6600 sonde (Figure 8). At Stockton, the median concentration from the YSI 6600 sonde was higher than the FluoroProbe, but the overall mean concentration from the FluoroProbe was higher than the YSI 6600 sonde.

The number of excitation wavelengths used to quantify chlorophyll *a* for each instrument could account for why the mean concentration of chlorophyll *a* from the FluoroProbe was higher than the concentration from the YSI 6600 sonde at Stockton. Chlorophyll *a* from the YSI 6600 sonde is only measured at one excitation wavelength, while chlorophyll *a* from the FluoroProbe is measured at five different excitation wavelengths. Thus, the YSI 6600 sonde is limited in its ability when quantifying chlorophyll *a* in systems with greater variation in phytoplankton composition. Stockton had higher chlorophyll *a* conditions than Hood and had more variation in phytoplankton composition (Figure 8). A similar conclusion was made by Gregor and Maršálek (2004) when explaining the variation in chlorophyll *a* between the FluoroProbe (five excitation

wavelengths) and the GENios fluorescence reader (one excitation wavelength) in different freshwater environments.

The higher chlorophyll *a* conditions at Stockton could have driven the statistical results from the Mann-Whitney median test and thus produce a different statistical result than that of Hood. Nevertheless, the chlorophyll *a* data from the YSI 6600 sonde and the FluoroProbe at both stations were both correlated with each other.

Functionality of both instruments across different environmental conditions

Not only was there a relationship between chlorophyll *a* from both instruments, but both instruments were also functional across different environmental conditions when measuring for chlorophyll *a*. There was only one instance where an environmental variable and chlorophyll *a* from each instrument did not produce similar correlation results.

In general, there were more correlations between environmental variables and chlorophyll *a* concentration at Stockton than at Hood. This could be explained by the longer residence time at Stockton (Brianne Noble, personal communication, 2011).
Longer deployments of the FluoroProbe are needed at the continuous monitoring stations in order to fully evaluate chlorophyll *a* from both instruments in terms of stability and reliability. Moreover, no strong conclusions could be made when comparing the chlorophyll *a* data from both instruments without looking at grab samples taken for spectrophotometric analysis. Unfortunately, no grab samples were taken during the short-term field evaluations.

Hood is currently one of the stations where discrete samples are taken for microscopic enumeration by the EMP. During the same period of the 2008 evaluation, a discrete sample was

taken for microscopic enumeration on October 1 at Hood. The results found that the brown algal group explained over 53% of the variation in phytoplankton composition (Brown et al. 2009). Similarly, the brown algal group from the FluoroProbe was also the most dominant group between September 29 and September 30 as it contributed 78 to 100% of the chlorophyll *a* concentrations (Figure 8).

Discrete samples were also taken on July 14, 2009 and August 13, 2009 at P8, a sampling station near Stockton (Brown et al. 2010). On July 14, 96% of the count data at P8 were contributed by the green algal group. Similarly, the green algal group from the FluoroProbe was the most abundant during the Stockton field evaluation as it contributed 54 to 67% of the chlorophyll *a* concentrations (Figure 8). However, when the EMP collected a discrete sample again on August 13 at P8, the dominant group was no longer the green algal group. Instead, the cryptophytes were the most dominant group as it explained 57% of the variation in phytoplankton composition (Brown et al. 2010). As a result, the FluoroProbe would be a useful tool to track the temporal variability of various algal groups.

Vessel based deployments (Tasks 6 &7)

Vessel based deployments during routine upper SFE monitoring cruises (Task 6)

Water quality

Results from the continuous water quality data are described in Figure 16. As predicted, water temperature had a strong seasonal gradient with cool temperatures in the winter and warmer temperatures in the summer. Warmest temperatures, near 27 ºC, were found in the San Joaquin River near Rough and Ready (Rough and Ready) in July and August. Specific

conductance as well as turbidity displayed a strong spatial gradient that increased from east to west. Highest specific conductance values occurred in January, August and September near San Pablo Bay. Turbidity also displayed a strong temporal variability showing high values from Suisun Bay to the Sacramento-San Joaquin rivers confluence in January and February, potentially due to high flows. High values were also observed in May around Suisun Bay. High turbidity values in Suisun Bay in the summer may be due to sediment re-suspension caused by high winds.

Chlorophyll *a* concentrations had localized high values in May in Suisun Bay and Rough and Ready areas. However, concentrations decreased as summer progressed.

Phytoplankton Communities

Although results from the GIS mapping effort showed spatial and temporal patterns (Figure 17), phytoplankton percent composition in January and April were fairly mixed and differences between regions were not significant during these two months (Figure 18). In January, greens made up over 50% of the total composition in all regions except in the Sacramento River (35%), blue-greens were also abundant (20% to31%) in all regions while browns only made up a significant portion of the total composition in the Sacramento River (23%). Cryptophytes composition was generally lower in all regions (2% to15%). Winter high flows likely facilitated the species mixing and helped move all phytoplankton groupings downstream to San Pablo Bay. Distribution patterns were more evident in April, but the differences between regions were still not significant (Figure 18). In April, greens made up 37% to 58% of the total phytoplankton composition in all regions. Browns contribution decreased from west to east, 42% in Grizzly Bay versus 18% in San Joaquin River and the interior Delta whereas cryptophytes contribution

increased considerably in the San Joaquin River and interior Delta, from 4% in Grizzly Bay to 24% in San Joaquin River.

As the year progressed, regional differences became significant except among the interior delta, San Joaquin River, and Sacramento River in June (ANOSIM Global Rho=0.298, $p < 0.01$) and in September between the San Joaquin River and interior delta (ANOSIM Global Rho=0.588, $p < 0.01$).

Similar to April, June's phytoplankton distribution was characterized by a large contribution of greens. This contribution made up over a third to almost a half of the total phytoplankton composition in all regions (31% to 47%). Browns percentage contribution was also significant in Grizzly Bay and the Sacramento River, 45% and 39% respectively. In addition, cryptophytes contribution was considerably higher in the San Joaquin River. Results from the BEST analysis with the 0.5 km resolution phytoplankton data suggested that turbidity and specific conductance explained 61% of the variation between regions (BEST Rho=0.614, $p<0.01$). Phytoplankton distribution in September followed a similar pattern to June's, with greens contributing the largest percentage in all regions followed by browns and cryptophytes. The major difference in September was the almost non-existent contribution of blue-greens to the total composition of phytoplankton in all regions. Results from the BEST analysis with the 0.5 km resolution phytoplankton data suggested that specific conductance and temperature explained 68% of the variation between regions (BEST Rho=0.676, $p<0.01$).

Several comparisons of microscopically enumerated phytoplankton data collected at discrete monitoring stations were made with FluoroProbe data collected concurrently in September 2010. Because discrete biovolumes were not available for the individual stations, the count data (organisms per mL) were used, along with average biovolume values. In all the

comparisons, the FluoroProbe failed to recognize at least one algal group, including sometimes the most common group (70% or more of the total count) in the discrete sample (in this case, failing to recognize cryptophytes, and calling everything in the sample either a diatom or a green alga). The inability of the FluoroProbe to recognize these groups at all when they are the dominant group in the discrete sample is troubling. However, these samples had low transmission values (below 50%), so it is possible that the FluoroProbe would have recognized the cryptophytes had the transmission been better. It is also possible that at least some phytoplankton taxa were misidentified during microscopic examination, but it is unlikely that cryptophytes would have been confused with either diatoms or green algae. It may also be due to the need for calibration of the FluoroProbe with natural water, or that some algal groups (like cryptophytes and dinoflagellates) with alternate feeding modes (mixotrophy or heterotrophy, respectively) cannot be accurately detected by the FluoroProbe. Because the FluoroProbe uses only fluorescence to categorize phytoplankton, it is more likely to miss mixotrophic taxa if they are not actively photosynthesizing, and will not detect heterotrophic taxa at all (photosynthetic dinoflagellates get lumped with diatoms and other brown-pigmented taxa).

In 2010, dinoflagellates were over 21% of the total phytoplankton biovolume (across all stations and months); over a third of that dinoflagellate biovolume (34.3%) were mixotrophic or strictly heterotrophic taxa. These are taxa that are likely to be missed by the FluoroProbe because of their feeding modes.

In addition, when comparisons were made using average values for Fluoroprobe carbon values and averaged pooled biovolumes from the microscopically enumerated phytoplankton, the Fluoroprobe estimates were over 10% either too high or too low (Figure 19).
Several potential reasons for the disagreements may be at play: the FluoroProbe data was selected for 4 months of 2010, whereas the EMP biovolume data includes the entire year

because biovolume data was not available on an individual sample basis. Also, transmission values measured by the FluoroProbe (indicative of the clarity of the water) were very low for most months and stations (below 50%). This may affect the accuracy of the readings, as non-algal particles in the water may accidentally be labeled as phytoplankton when transmission values are low, or may obscure fluorescing algal particles. The FluoroProbe is also limited to just 4 algal groupings in addition to yellow substances which are not included in the total chlorophyll a measurement: brown algae (including dinoflagellates and chrysophytes), green algae, bluegreen algae, and cryptophytes. While these groupings cover most common taxa, the FluoroProbe's inability to distinguish certain groups from each other (such as diatoms and dinoflagellates) limits its ability to identify the specific algal group in some cases (e.g. during a bloom dominated by one of these groups). Also, some photosynthetic ciliates (such as Mesodinium rubrum) will be mislabeled as cryptophytes due to their cryptophyte endosymbiont that gives them their photosynthetic capability.

Comparisons between FlowCAM and FluoroProbe for identification of *in situ* phytoplankton communities (Task 7)

The MDS ordination showed strong differences between the two methods for both river systems (Figure 20). The ANOSIM test identified a significant difference ($p < 0.01$) between results for the FlowCAM and FluoroProbe for both rivers as well as a significant difference for each river separately (Sacramento River ANOSIM Global Rho =0.611, $p<0.01$ and San Joaquin River ANOSIM Global Rho =0.659, $p<0.01$). Looking further, SIMPER results determined that in the Sacramento River, greens and bluegreens were responsible for about 72.4% of the differences between assemblages (Table 3) whereas in the San Joaquin River, greens and chryptophytes contributed about 64.5% to the differences between assemblages (Table 3).

Comparison of the phytoplankton groups quantified by each method revealed that the FlowCAM samples had an average similarity of 47.8% for the San Joaquin River and 56.9% for the Sacramento River. FluoroProbe samples had an average similarity of 74.4% for the Sacramento River and 84% for the San Joaquin River (Table 4). Overall, these values suggest that the FlowCAM samples are able to demonstrate more of the variability found in the system. One should assume that as species composition shifts, the fluorescence signal shifts as well unless the species shifts within the same phytoplankton group with the same fluorescence signal. The greater range of values demonstrated in the MDS ordinations for the FlowCAM suggested it was more successful at describing the range of plankton in the phytoplankton community that was present in July (Figures 20 and 21).

Because our study showed significant differences between methods, our next step is to compare the FluoroProbe and FlowCAM results with a third independent method, such as microscopic enumeration, that would allow us to further examine the community patterns of both systems and how the three methods fair when describing these patterns.

Studies comparing FluoroProbe and FlowCAM are few. See et al. (2005) compared both instruments in addition to HPLC chemical taxonomy analysis, light microscopy and epifluorescence microscopy for phytoplankton along the Texas Gulf coast. Their conclusion was that the FluoroProbe and FlowCAM are potentially useful for determining the general characteristics of phytoplankton community structure in situ when used in tandem. See et al. (2005) also found that FlowCAM is best suited for identification of larger phytoplankton species whereas the FluoroProbe is designed to detect fluorescence signals of small cells.

Overall Recommendations

- Laboratory calibrations of specific algae groups may not be needed because of the overall good performance of the FluoroProbe and the variable nature of the phytoplankton community in the SFE; however, field calibrations for yellow substances may be necessary prior to the beginning of routine EMP sampling transects. Results from the field calibration exercise suggest that FluoroProbe manual's recommendation to calibrate instrument when chlorophyll *a* concentrations are low may need to be implemented. However, comparisons with a third independent method, such as microscopic enumeration should be completed before putting into practice this recommendation.
- The FluoroProbe could be a useful tool for the EMP. Currently, the EMP only collects discrete phytoplankton samples for microscopic enumeration at two of the nine continuous monitoring stations in the upper SFE. Data from the FluoroProbe would allow resource managers to observe phytoplankton composition trends when discrete samples are not taken. Microscopic enumeration along with phytoplankton biomass data estimated from the FluoroProbe could provide useful information about the dominant species at fixed continuous stations when trying to detect harmful algal blooms. As a result, the FluoroProbe would be a useful tool to track the temporal variability of various algal groups. A continuously deployed FluoroProbe would, however, necessitate a wiper to prevent fouling (i.e. the newer model would have to be purchased).
- One of the main limitations in this study was the inability to examine other phytoplankton samples microscopically from the EMP samples due to the contracting challenges that prevented us from sending already collected samples. Collecting and processing independent microscopic enumeration samples to compare Fluoroprobe results would help determine decisively whether this method is appropriate to use in the SFE.

- Results from our comparison between the FluoroProbe and FlowCAM suggest that these two instruments are potentially useful for determining the general characteristics of phytoplankton community structure in situ when used concurrently. The FlowCAM is best suited for identification of larger phytoplankton species whereas the FluoroProbe strength is in detecting fluorescence signals of all cells, including that of small cells that may be missed or misidentified with the FlowCAM or microscopic methods.

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Table 1. Results of FluoroProbe laboratory tests with algal monocultures. Results are based on at least 30 measurements per species and test concentrations are given as average percentage of fluorescence detected in the correct algal FluoroProbe “group” category.

FluoroProbe Group	**Taxonomic Division**	**Test Culture Species** (3 - 5 concentrations each)	**% Correct group** (average)	**Main source of "pollution"**
Blue-Green	Cyanobacteria	*Microcystis aeruginosa (08)*	**99**	
Blue-Green	Cyanobacteria	*Microcystis aeruginosa (07)*	**91**	
Blue-Green	Cyanobacteria	*Synechococcus leopoliensis (08)*	**100**	
Blue-Green	Cyanobacteria	*Synechococcus leopoliensis (07)*	**92**	
Blue-Green	Cyanobacteria	*Anabaena sp.*	**98**	
Blue-Green	Cyanobacteria	*Cylindrospermum sp.*	**94**	
Green	Chlorophyta	*Ankistrodesmus angustus (09)*	**100**	
Green	Chlorophyta	*Ankistrodesmus angustus (07)*	**100**	
Green	Chlorophyta	*Chlamydomonas rapa*	**99**	
Green	Eustigmatoph.	*Nannochloropsis sp.* (only chl a)	**78**	**Brown**
Green	Chlorophyta	*Botryococcus braunii* (lipid matrix)	**48**	**Blue-Green**
Brown	Diatom	*Navicula sp.*	**100**	
Brown	Diatom	*Thalassiosira sp.*	**89**	**Blue-Green**
Brown	Diatom	*Pinnularia sp.*	**79**	**Cryptophyte**
Brown	Chrysophyta	*Synura sp.*	**75**	**Cryptophyte**
Brown	Dinophyta	*Prorocentrum sp.*	**85**	**Green**
Brown	Dinophyta	*Amphidinium sp.*	**75**	**Green**
Brown	Dinophyta	*Pyrocystis sp.*	**64**	**Green**
Cryptophyte	Cryptophyta	*Campylomonas reflexa (07)*	**80**	**Green**
Cryptophyte	Cryptophyta	*Campylomonas reflexa (08)*	**64**	**Green**
Cryptophyte	Cryptophyta	*Cryptomonas ovata*	**61**	**Green**
Cryptophyte	Cryptophyta	*Chroomonas sp.*	**39**	**Brown**

Table 2. Differences between control and calibrated transects according to SIMPER. Percent contribution of each spectral category to differences between transects.

Group	CONTROL	CALIBRATION				
Species	*Average Abundance*	*Average Abundance*	*Average Dissimilarity*	*Disimilarity/SD*	*Contribution %*	*Cumulative %*
Cryptophytes	0.44	0.21	4.82	1.62	36.1	36.1
Bluegreen	0.56	0.79	4.29	2.46	32.1	68.2
Green	1.24	1.16	2.19	1.51	16.4	84.7
Brown	0.59	0.51	2.05	1.46	15.4	100.0

Table 3. Differences between FlowCAM and FluoroProbe measurements according to SIMPER and percent contribution of each spectral category to differences between methods.

		Sacramento River		San Joaquin River	
Average Difference		58.1%		68.7%	
Species	%	Cum.%		%	Cum.%
Green	38.9	38.9	Green	40.9	40.9
BlueGreen	33.5	72.4	Cryptophyte	23.5	64.5
Brown	14.9	87.3	Bluegreen	23.5	88.0
Cryptophyte	12.7	100.0	Brown	12.0	100.0

Table 4. Similarity index, expressed as percentage (%) within methods for each river systems.

River	FlowCAM	FluoroProbe
Sacramento River	56.9	74.4
San Joaquin River	47.8	84.0

Figure 1. Map of the SFE. EMP route is indicated by the red line. Route was divided into four regions: Grizzly Bay (GB), Sacramento River (SAC), Franks Tract (FT), San Joaquin River (SJR).

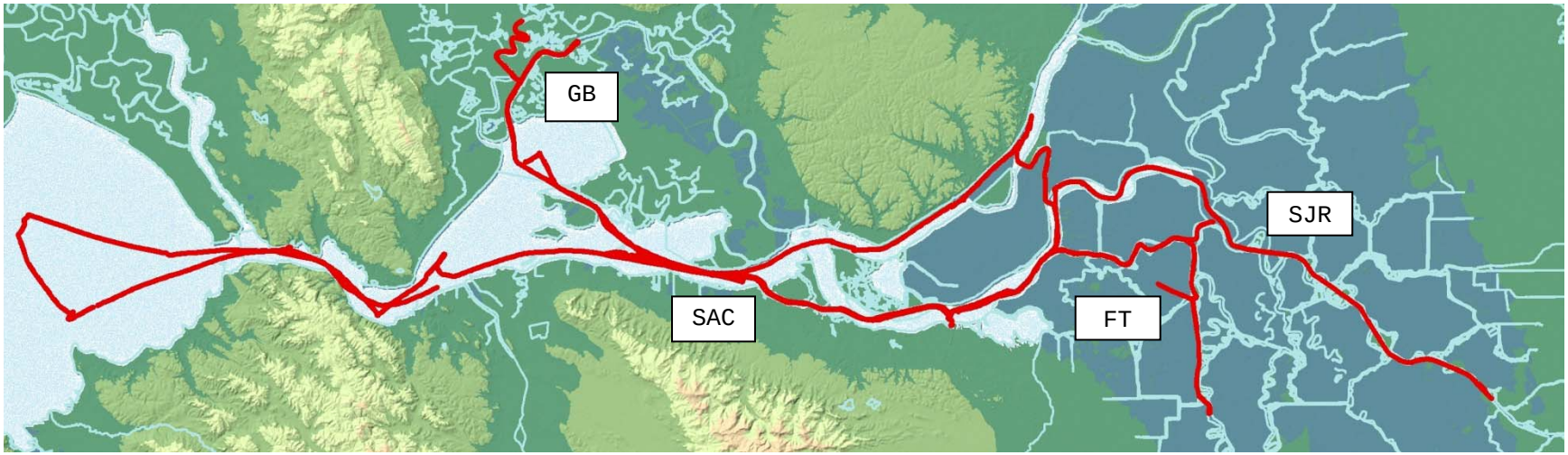


Figure 2. Field calibration transects and discrete sampling locations. Transects were located in the Suisun Bay vicinity.

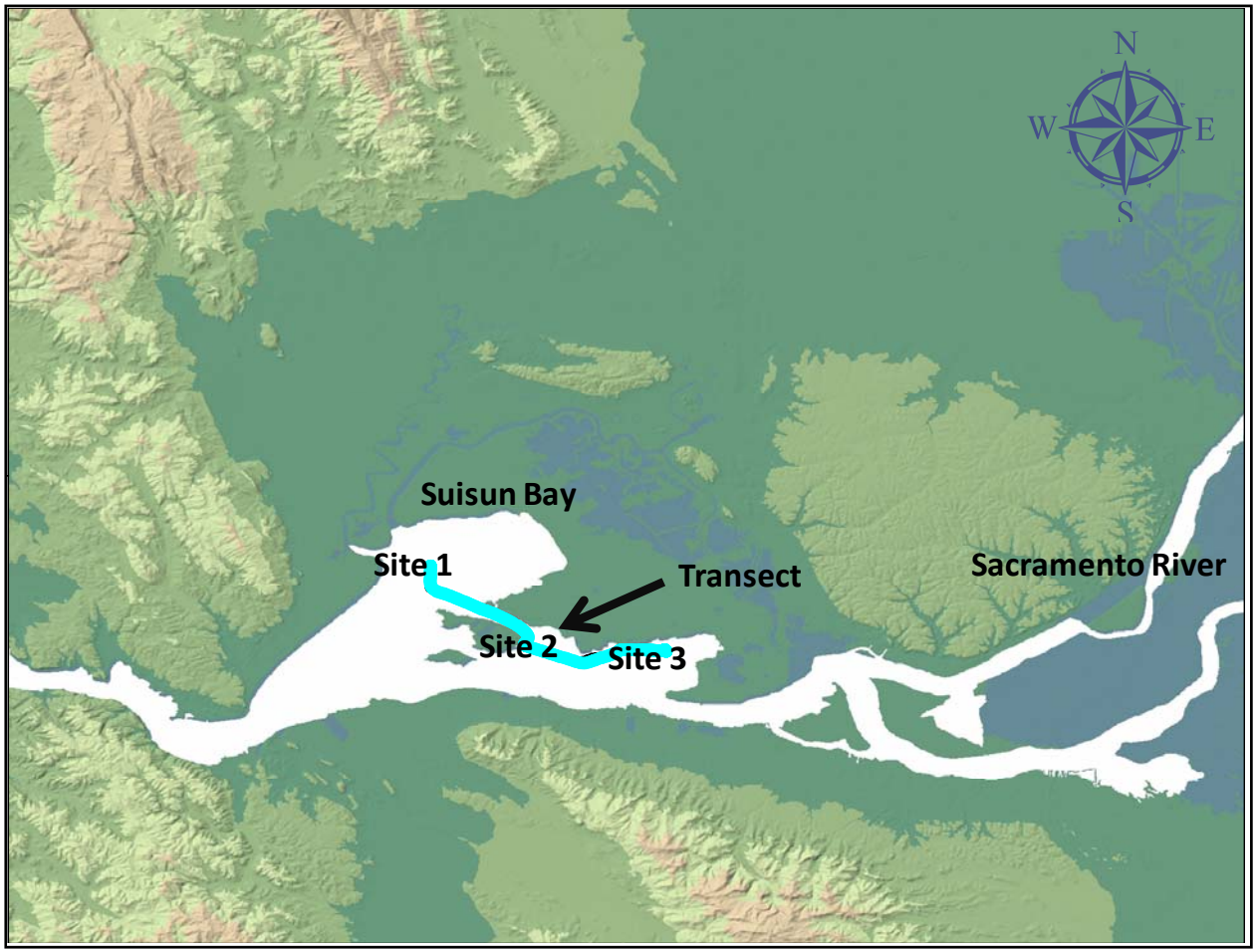


Figure 3. Locations of the sampling stations for the short-term field evaluations in the SFE.

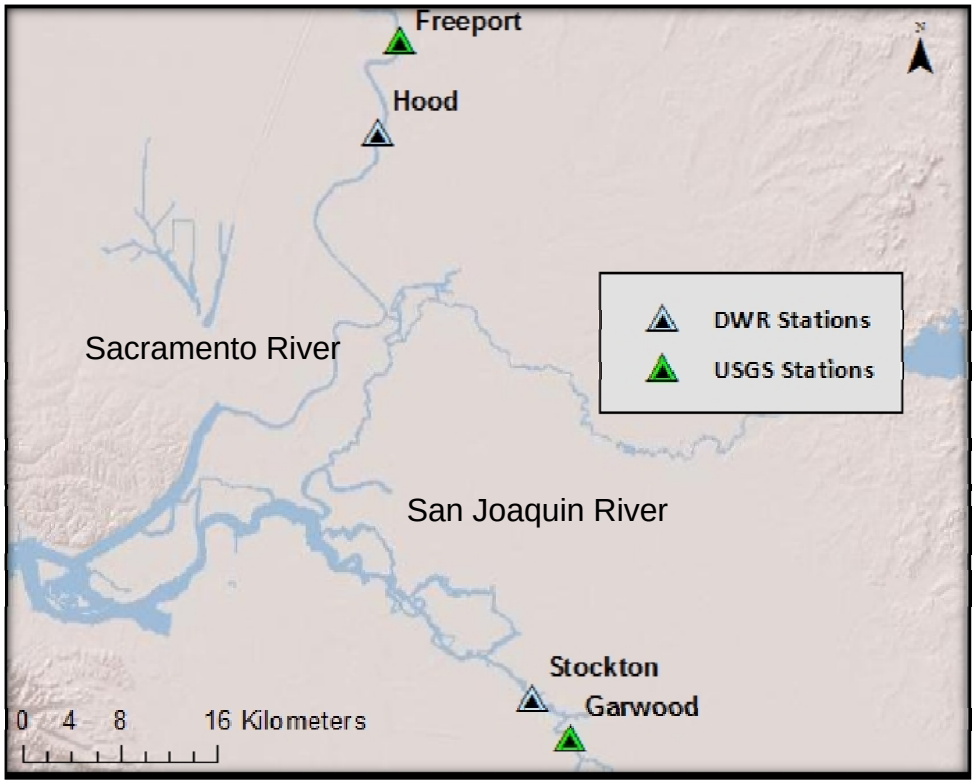


Fig. 4. Map of Sacramento-San Joaquin Delta indicating sampling transects for the Sacramento River (green circles) and the San Joaquin River (red circles).

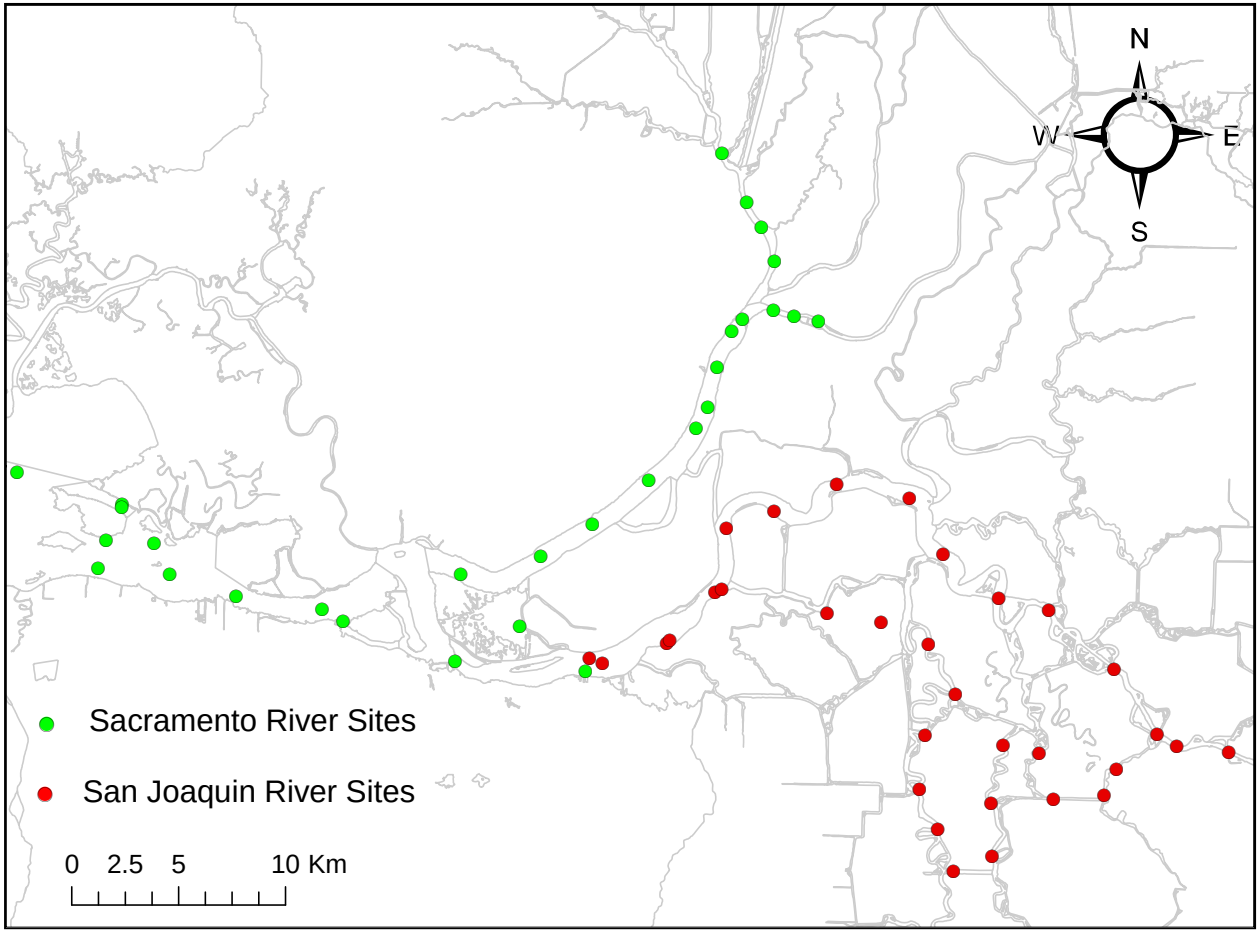


Figure 5. Chlorophyll *a* comparison between Turner 10 AU Fluorometer and FluoroProbe.

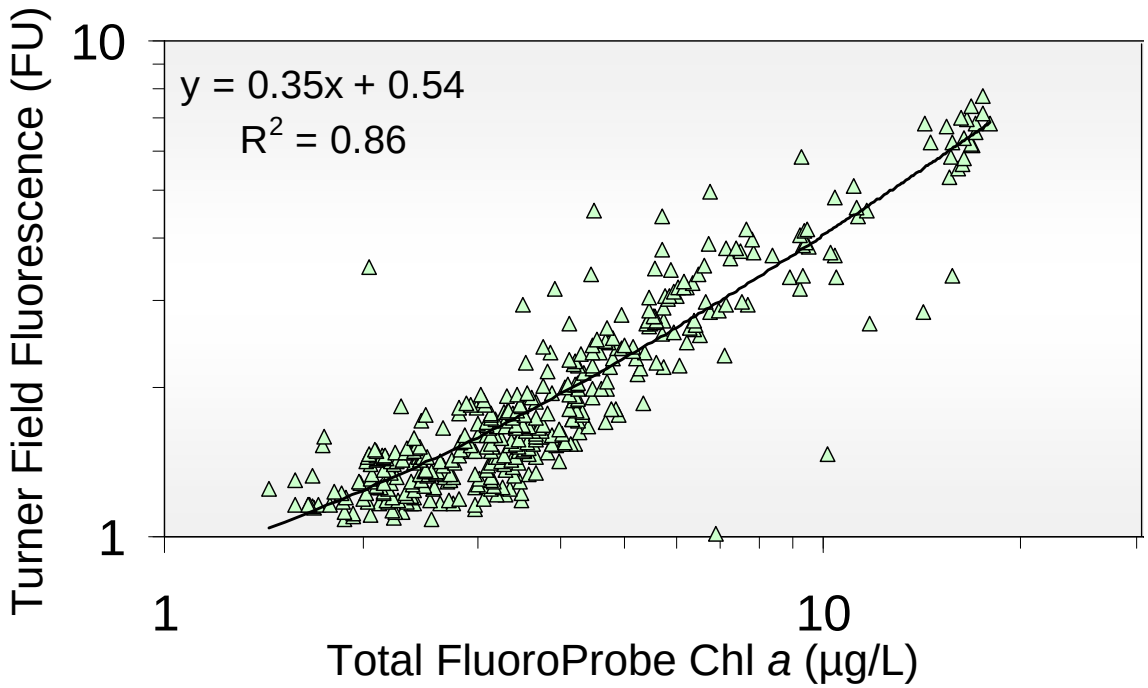


Figure 6: Chlorophyll *a* concentrations in algal monocultures measured by the FluoroProbe and in extracts of the same cultures. Symbols represent major algal groups and thin regression lines are for individual species.

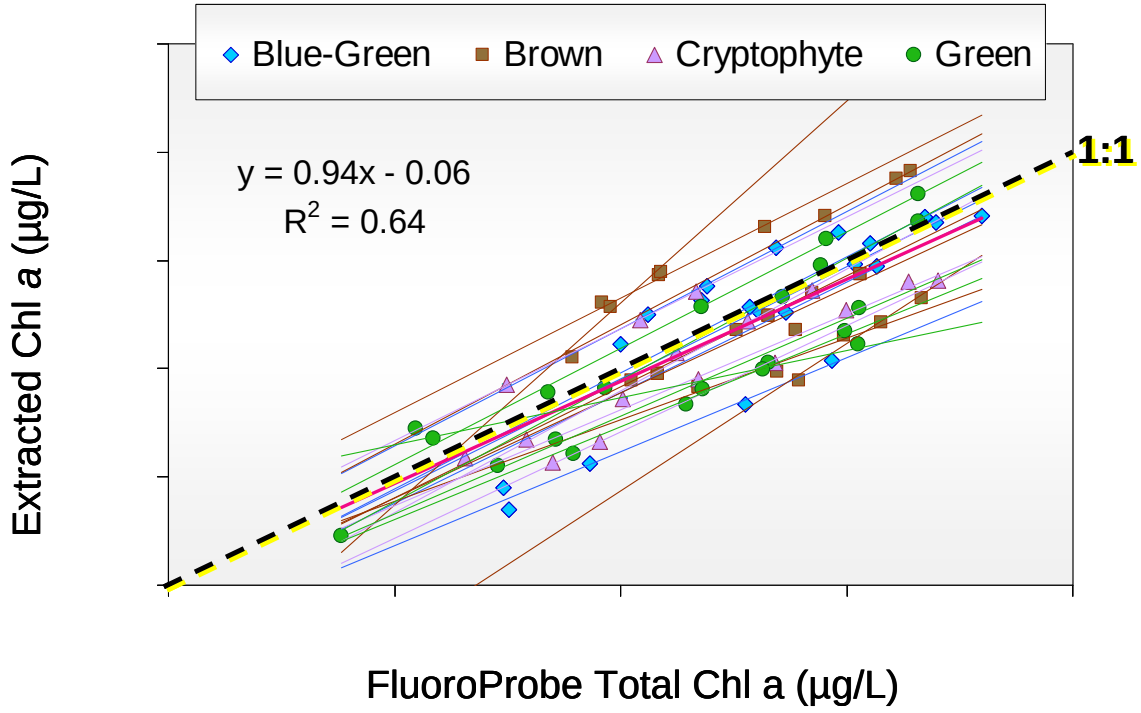


Figure 7. MDS ordination for samples collected along a Suisun Bay transect in July 2011. Control samples (green triangle) and calibrated samples (blue triangle).

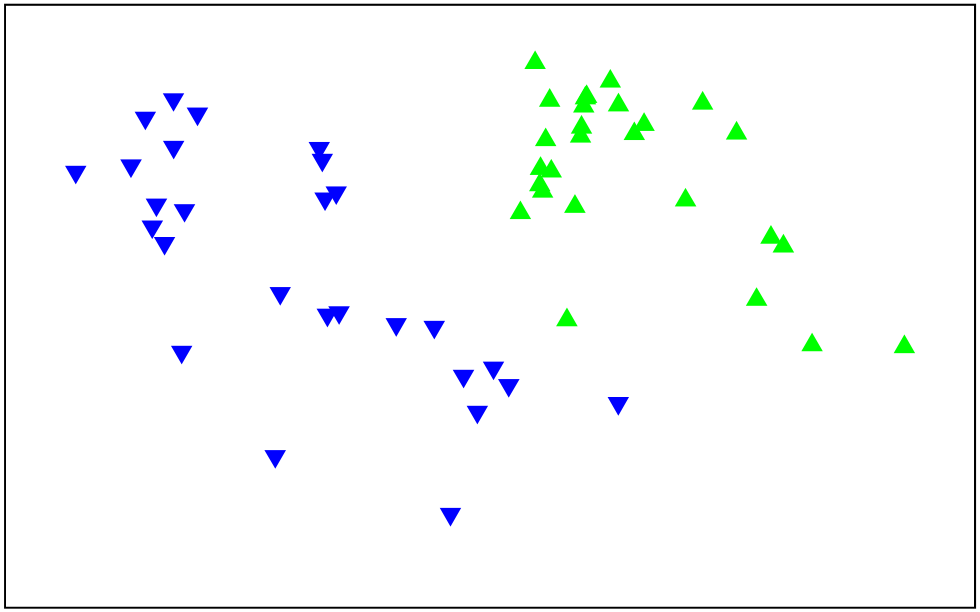


Figure 8. Hourly chlorophyll *a* averages from the FluoroProbe and the YSI 6600 sonde.

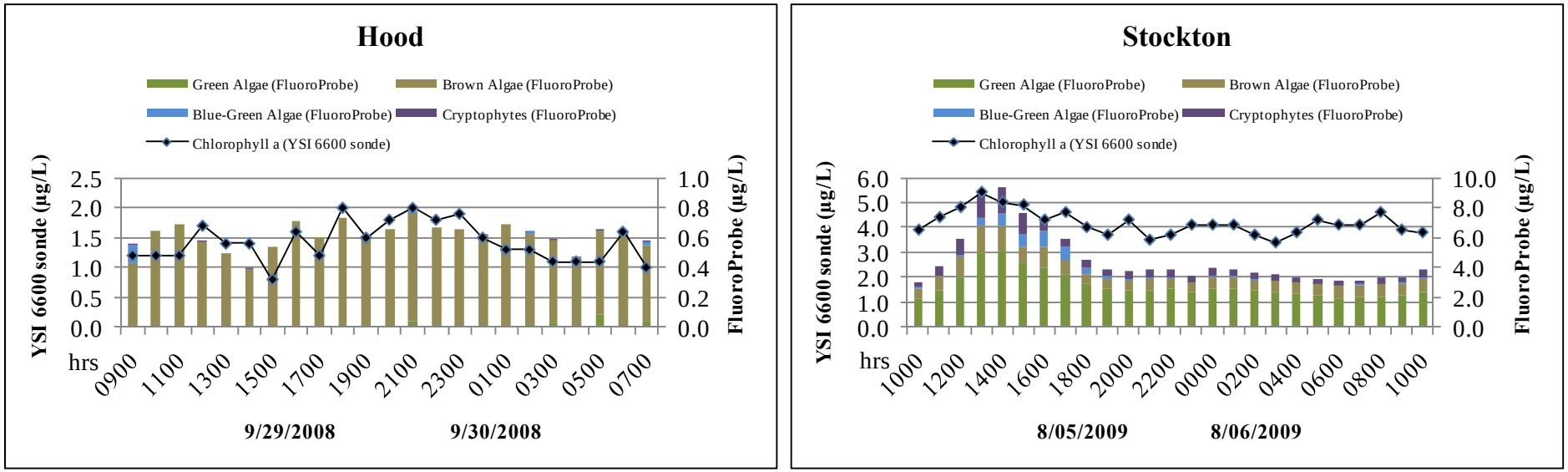
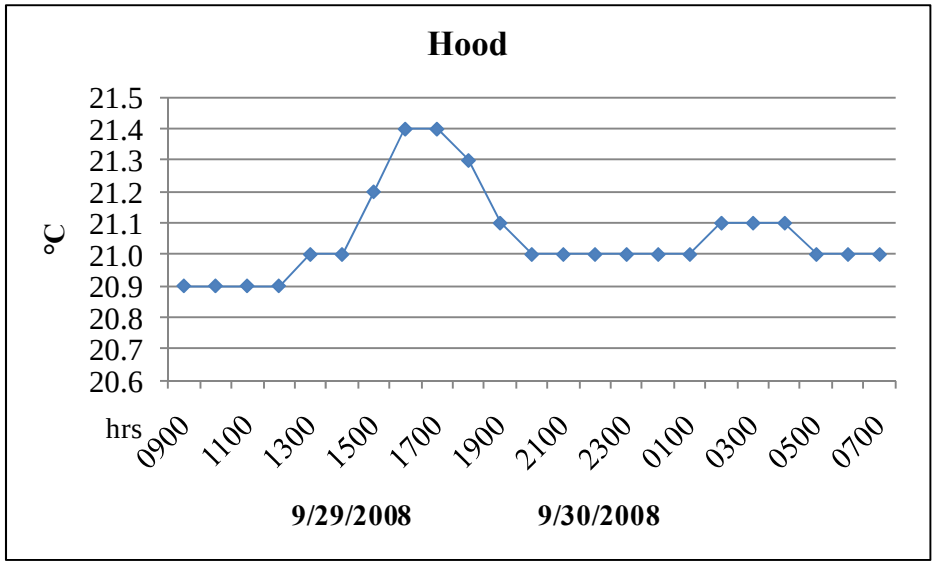


Figure 9. Hourly water temperature averages from Hood and Stockton.



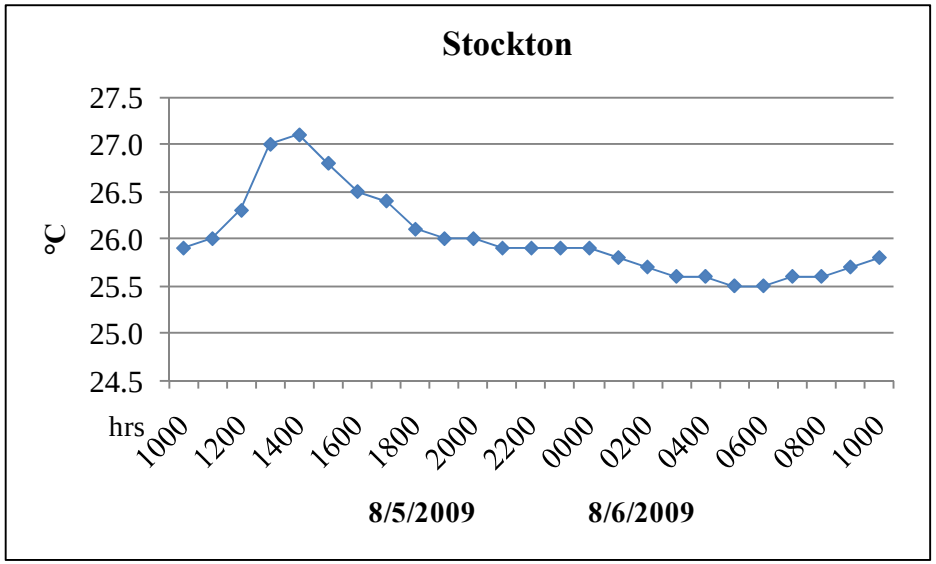
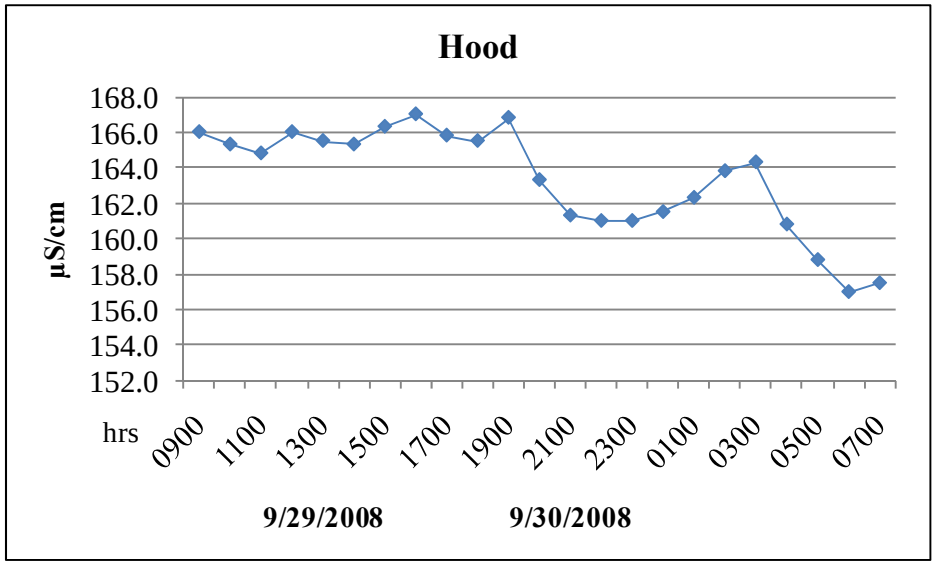


Figure 10. Hourly specific conductance averages from Hood and Stockton.



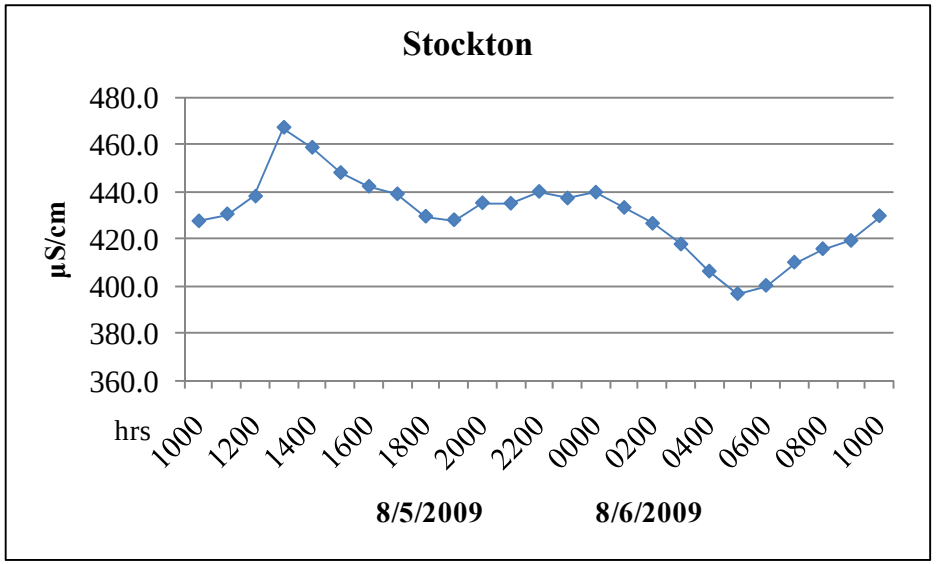
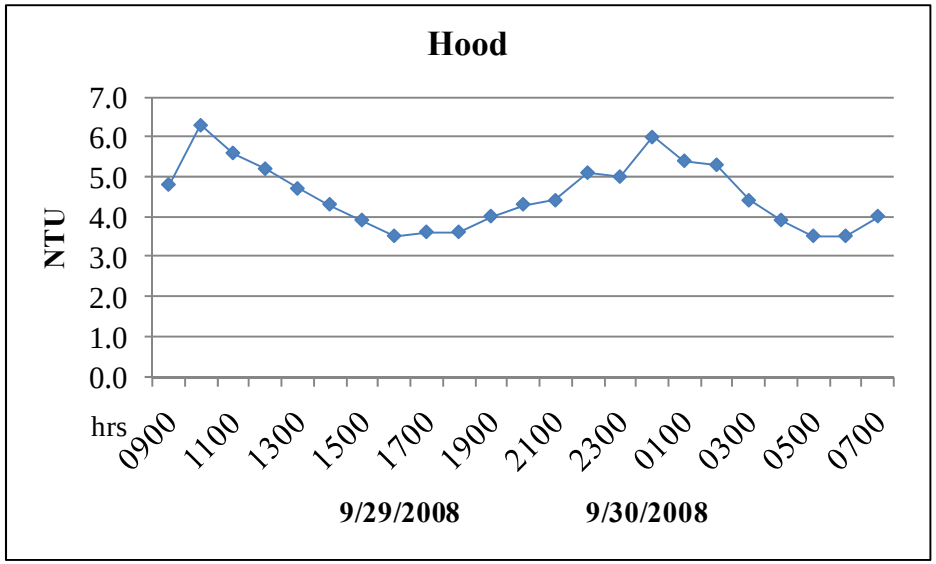


Figure 11. Hourly turbidity averages from Hood and Stockton.



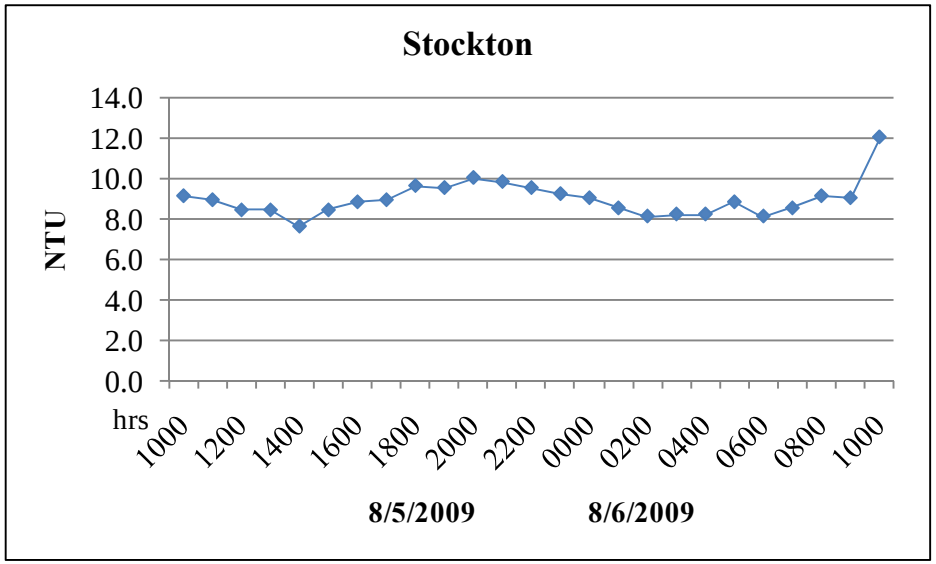
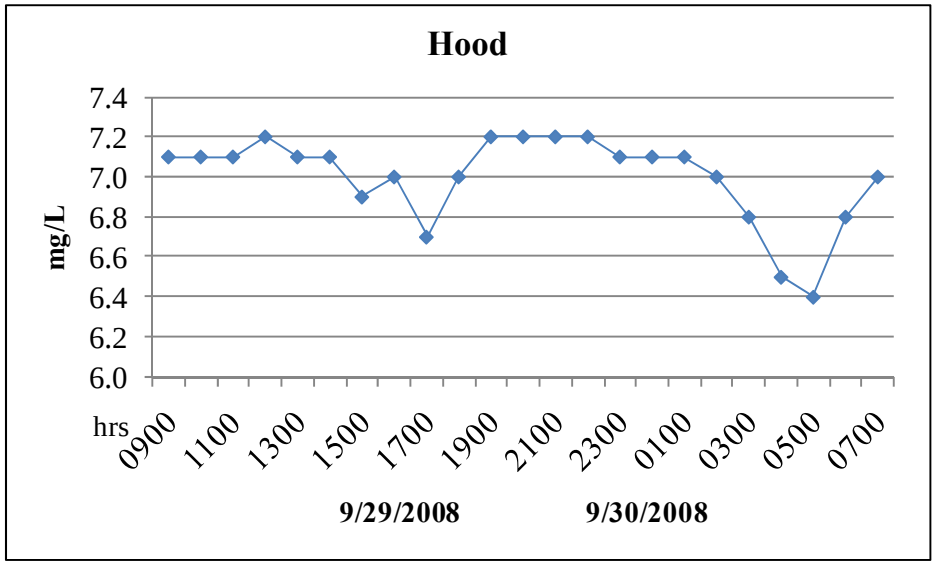


Figure 12. Hourly dissolved oxygen averages from Hood and Stockton.



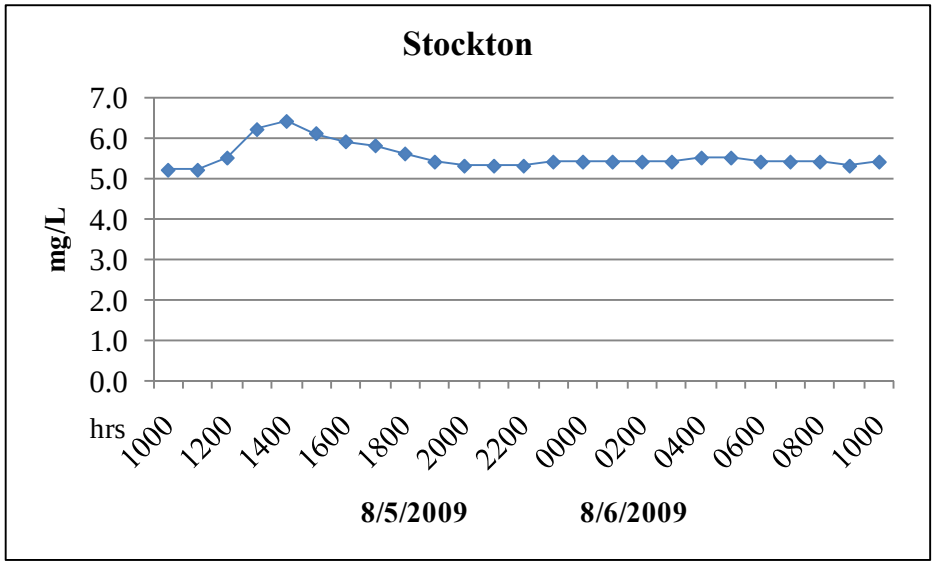
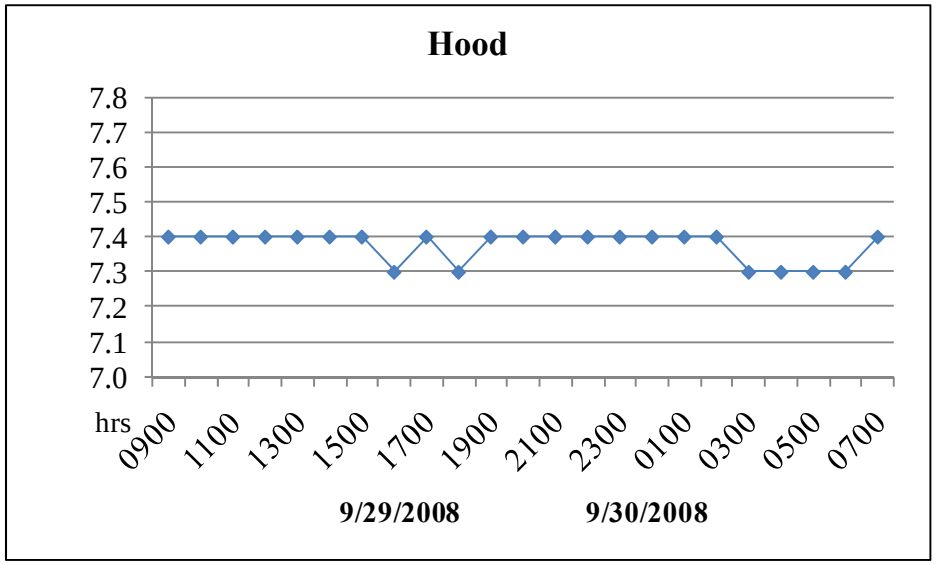


Figure 13. Hourly pH averages from Hood and Stockton.



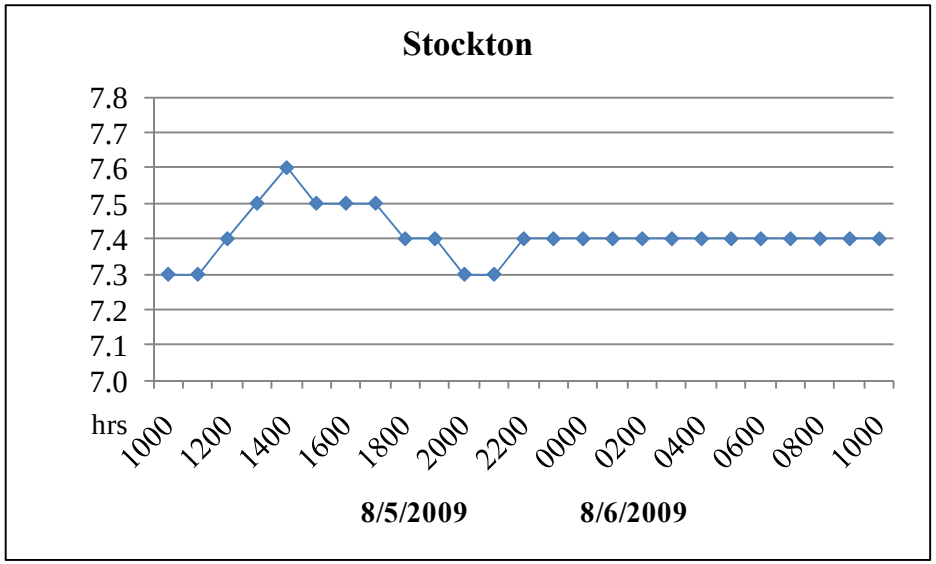


Figure 14. Hourly river stage from Freeport and Garwood.

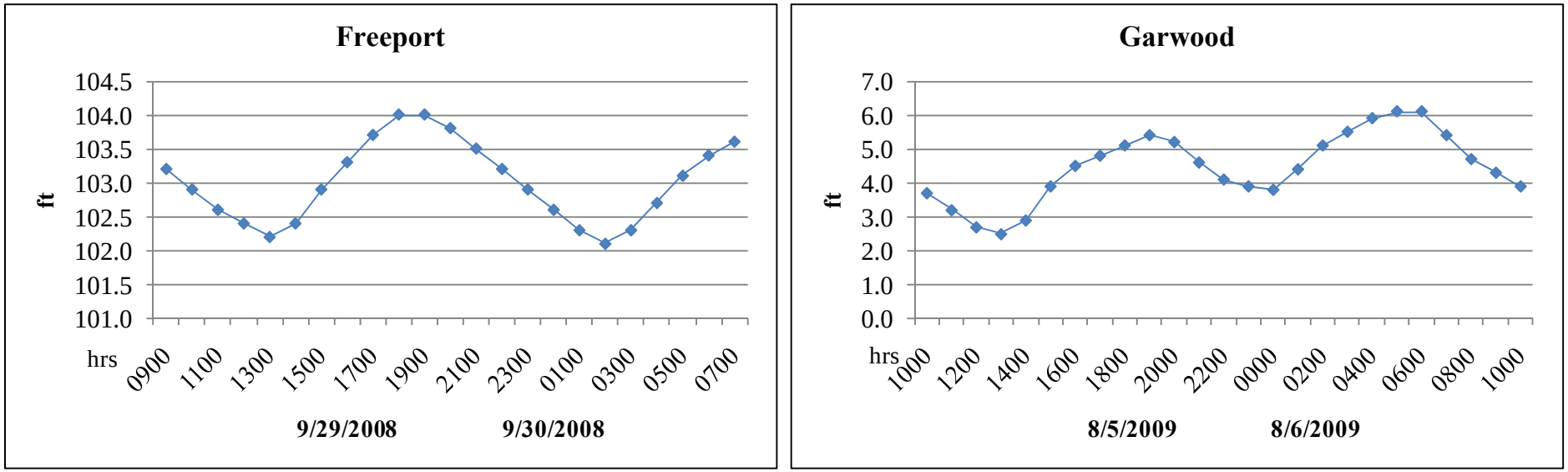
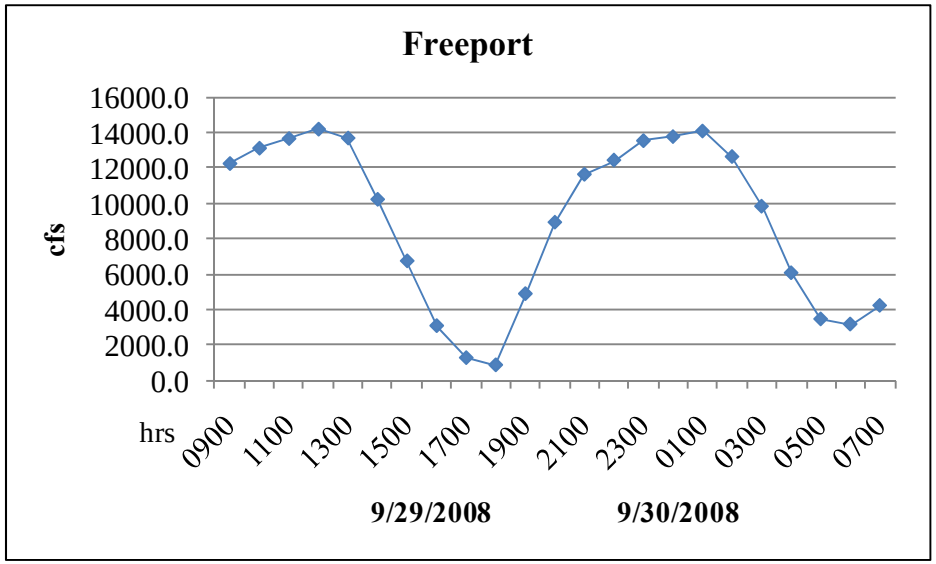


Figure 15. Hourly flow rates from Freeport and Garwood.



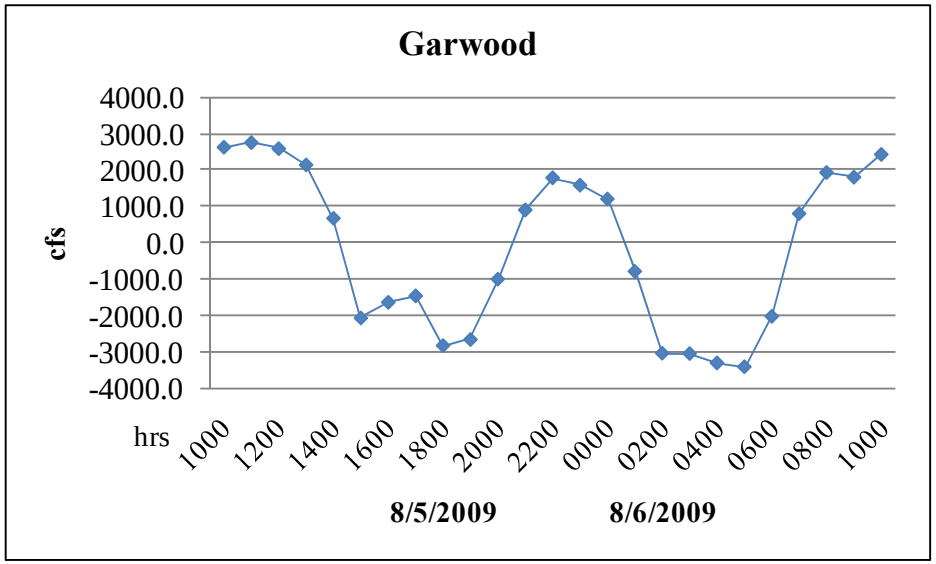


Figure 16. Continuous water quality parameters measured during routine monthly EMP monitoring cruises from January 2010 to September 2010. Cruises conducted over a 1-week period each month. X-axis is the longitude. Location markers: San Pablo Bay (SP), Carquinez Bridge (CB), Suisun Bay (SB), Antioch (AN), San Joaquin River (SJ) and Rough and Ready (RR).

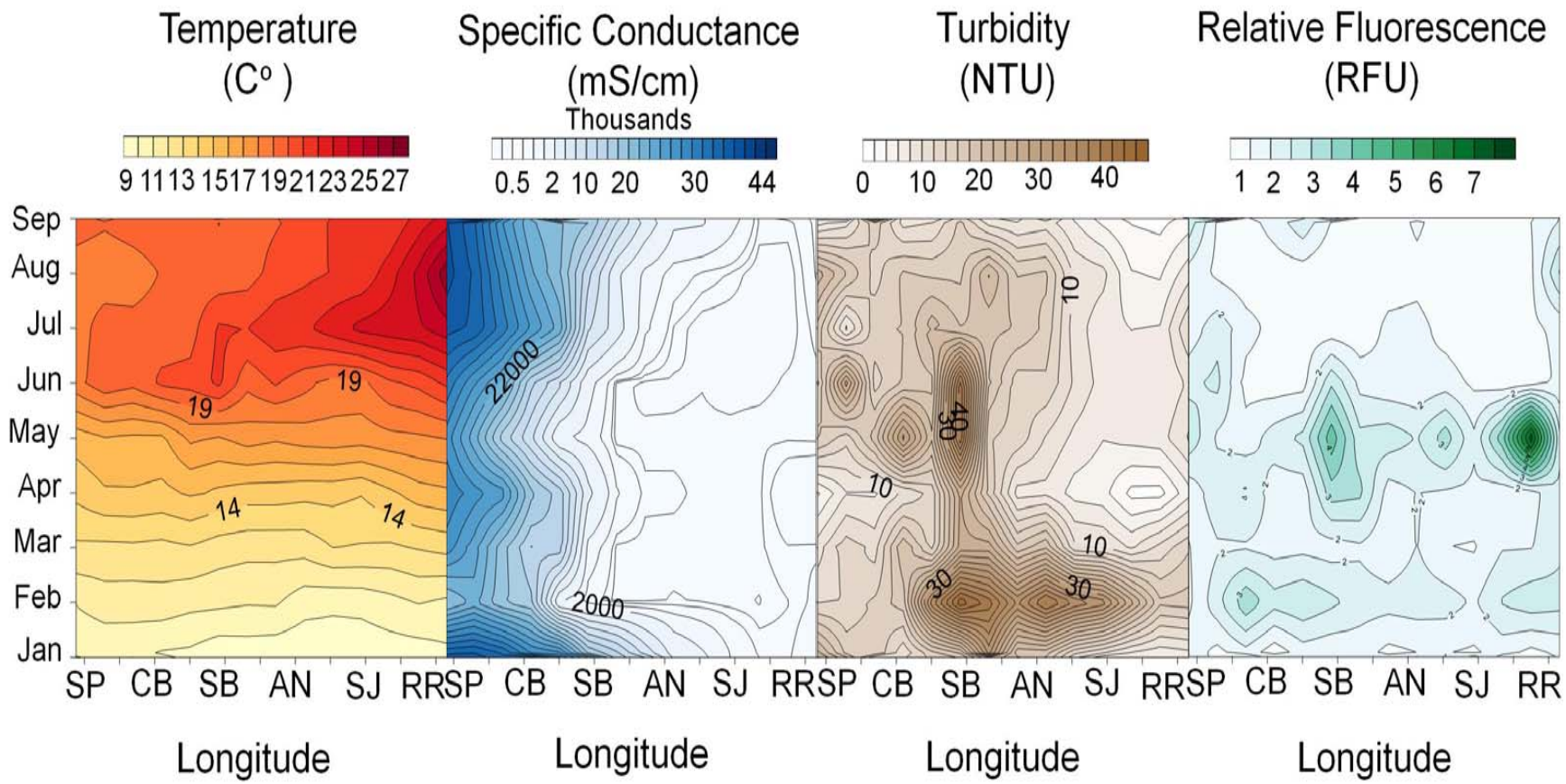


Figure 17. Spatial and Temporal Phytoplankton groupings distributions. Location markers: Grizzly Bay (GB), Sacramento River (SR), San Joaquin River (SJ), Interior Delta (ID).

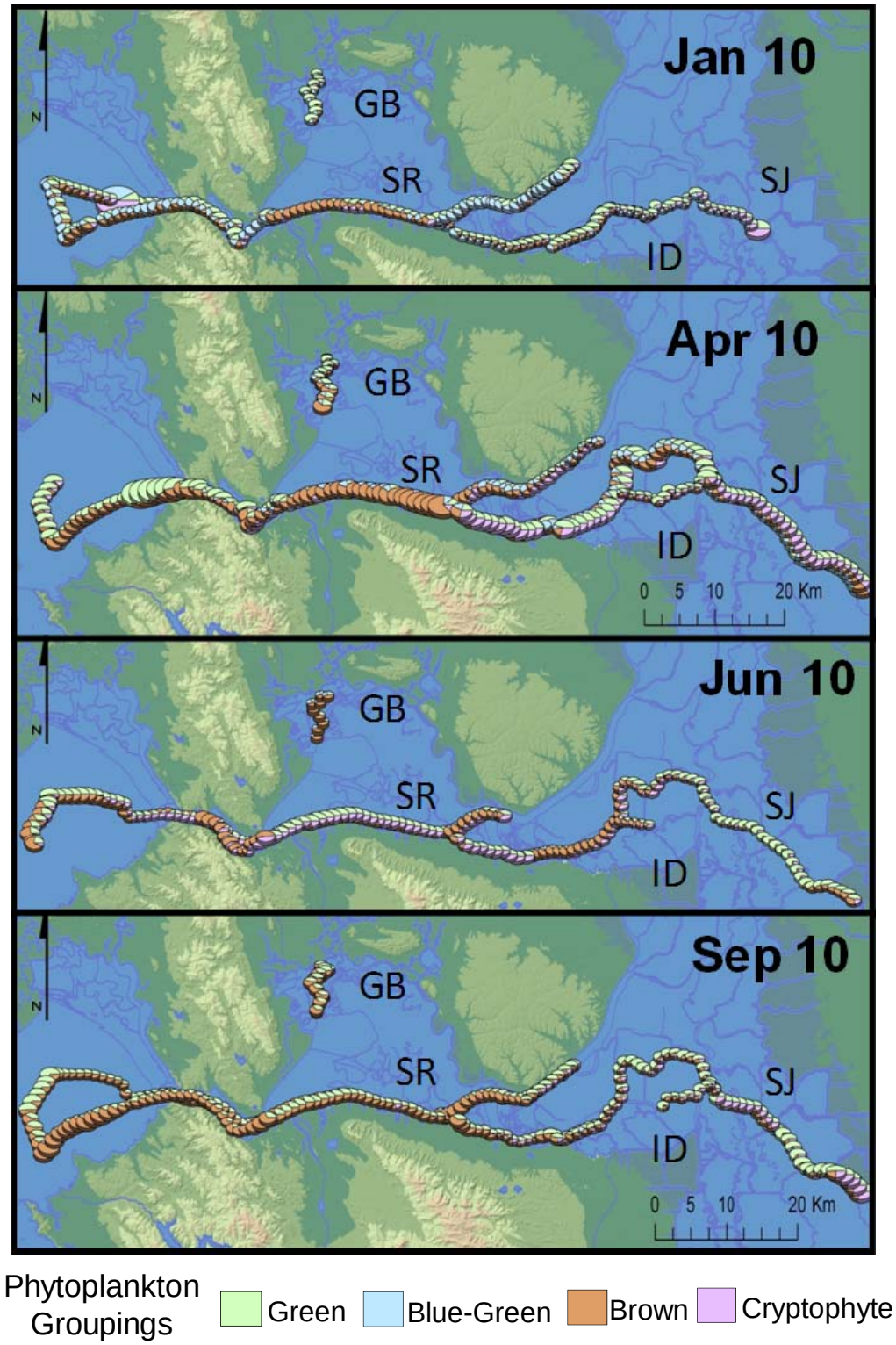


Figure 18. Relationship between water quality parameters (temperature --Temp, Specific Conductance --EC, and turbidity --Turb) and regional phytoplankton composition using stations every 0.5 km.

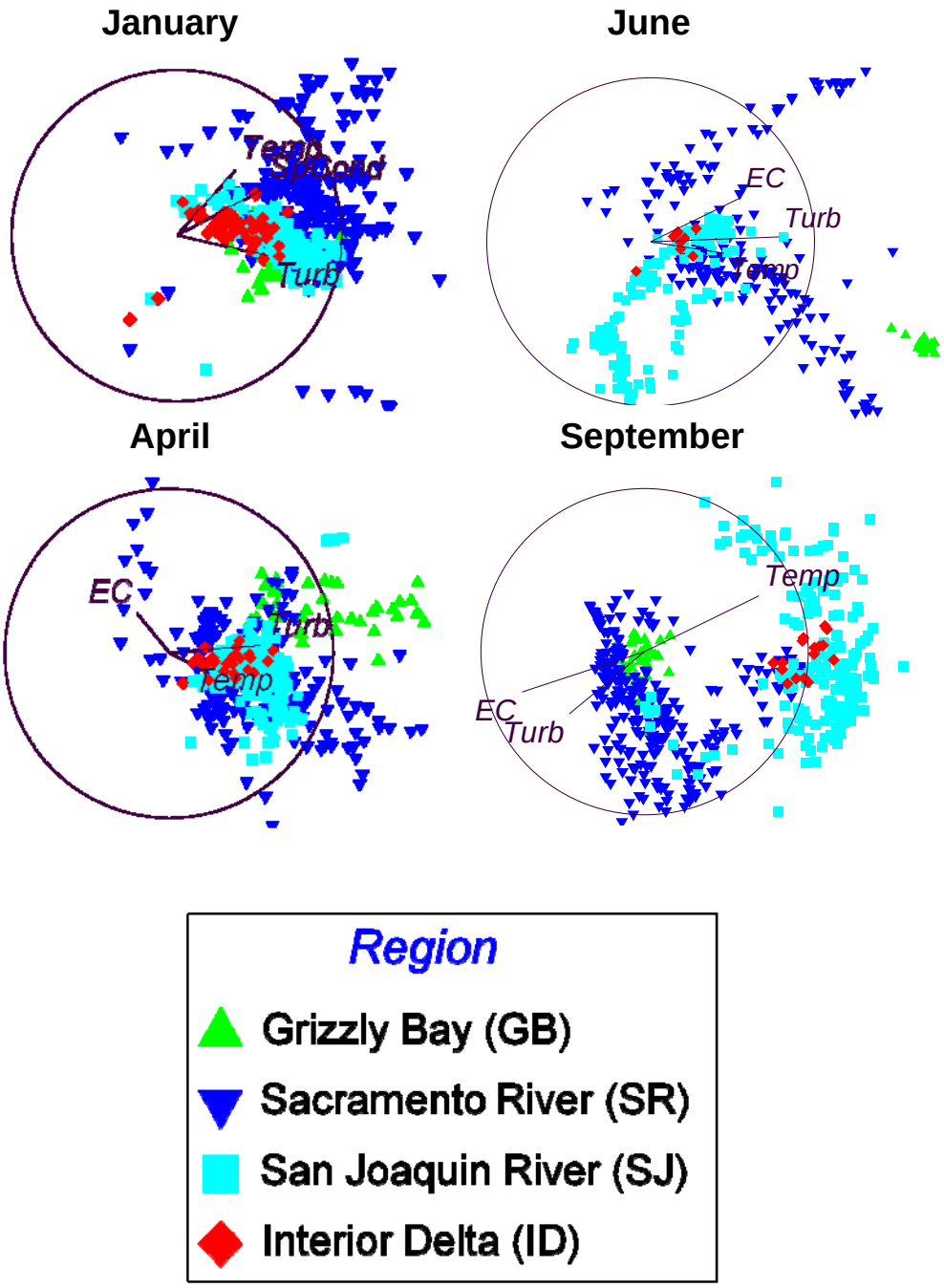


Figure 19. Comparison between phytoplankton carbon biovolumes (2010 12 month average values) and FluoroProbe biomass estimates (2010, 4 month average)

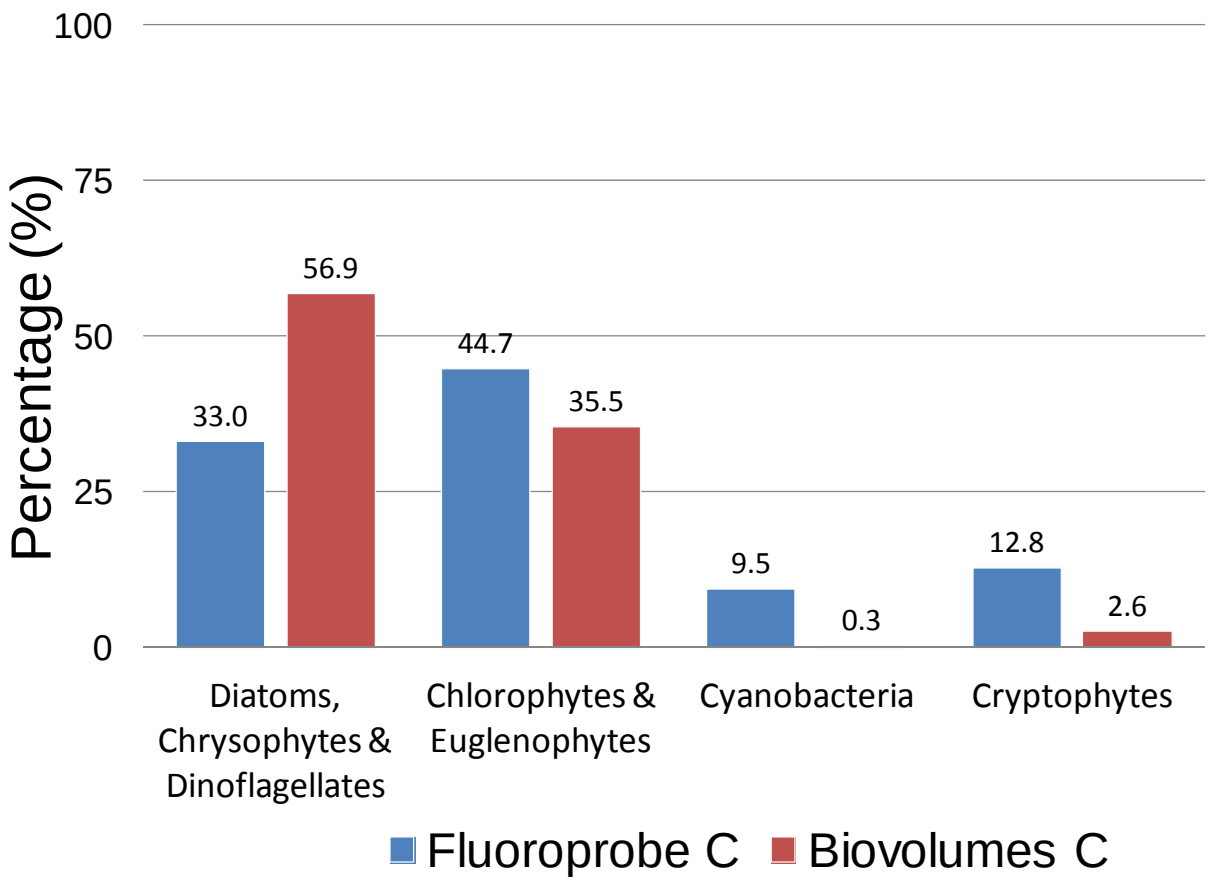


Figure 20. MDS ordinations for samples collected along July transects for the Sacramento and San Joaquin Rivers by the FlowCAM (green triangle) and the FluoroProbe (blue triangle).

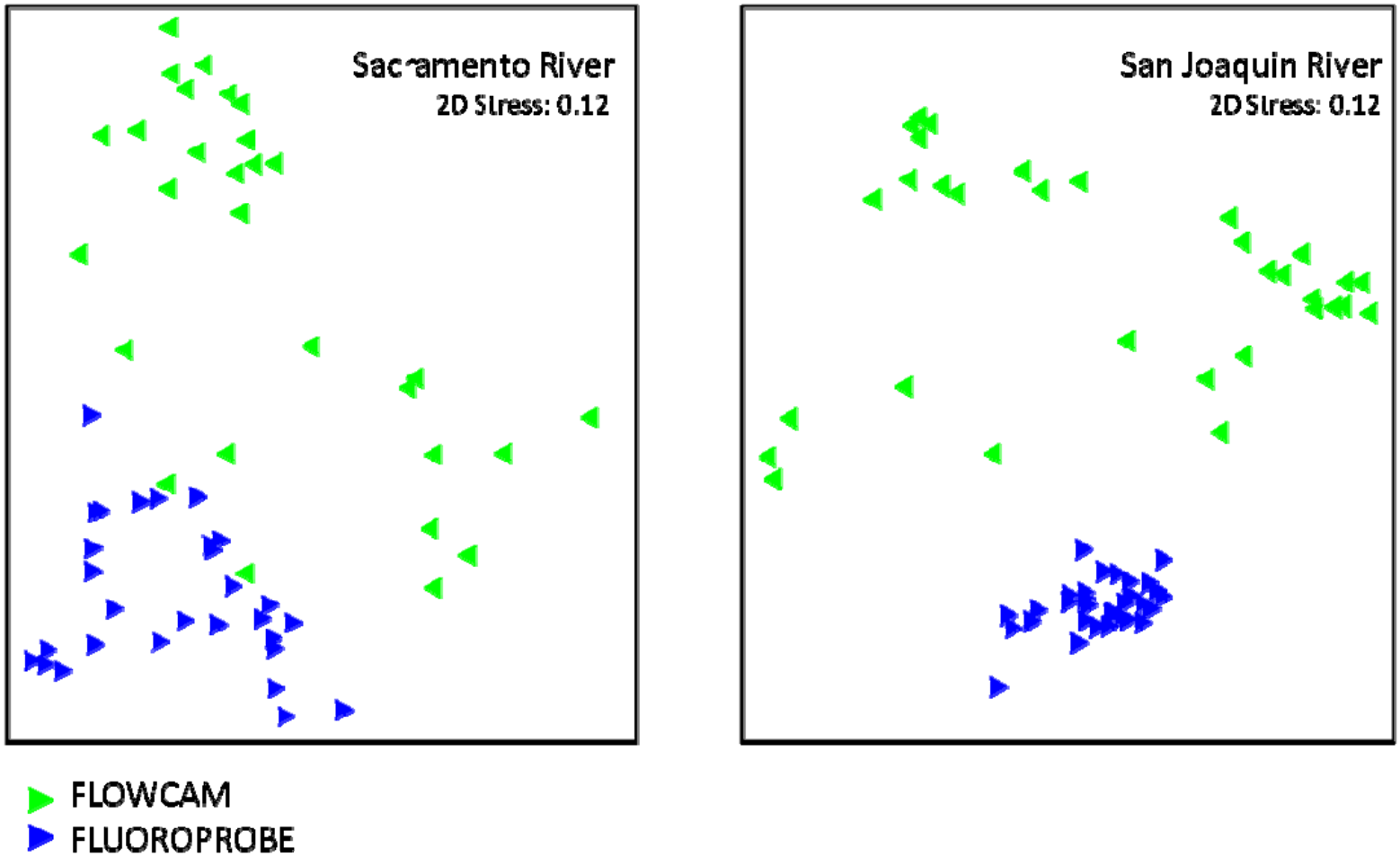
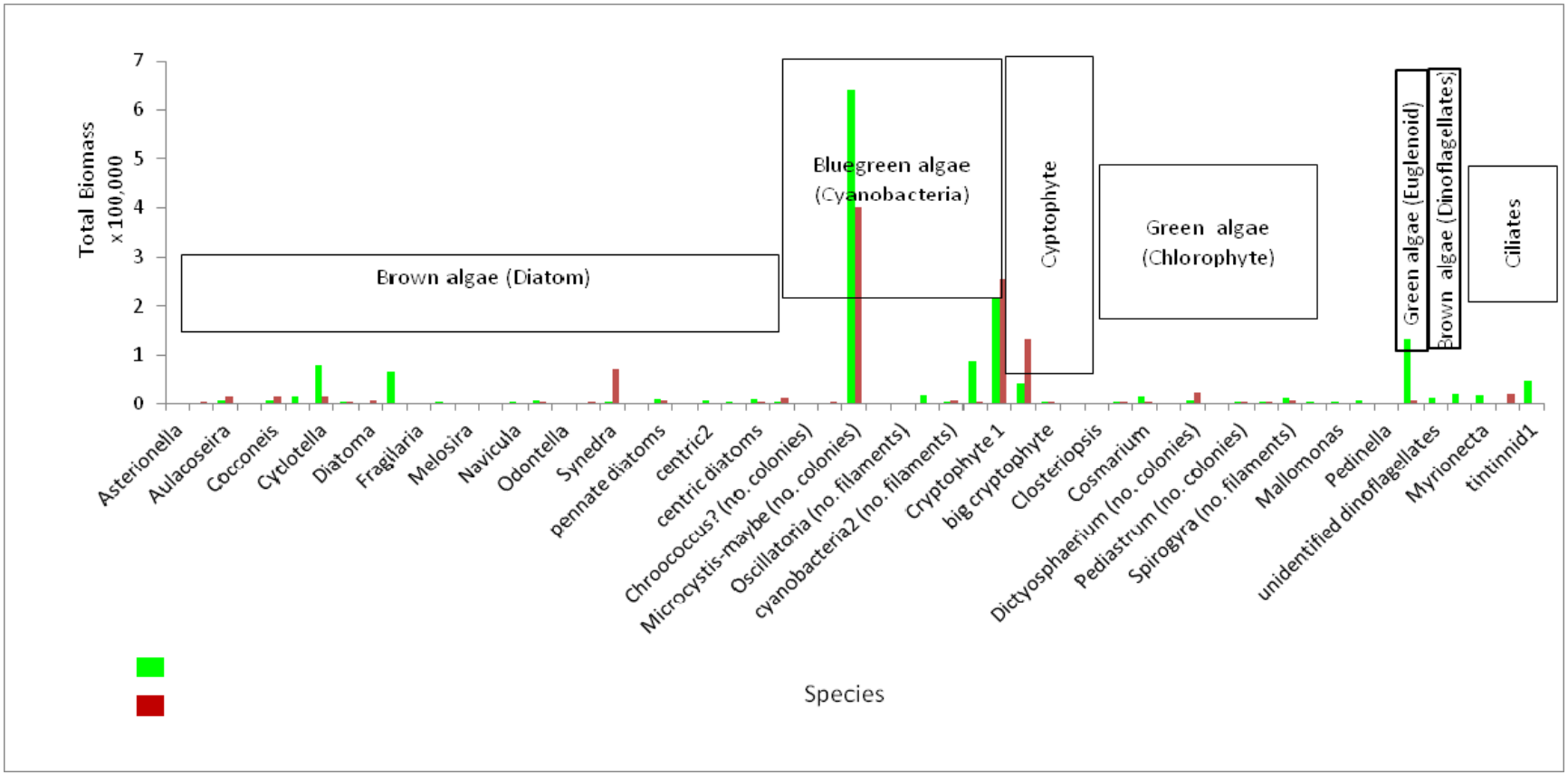



Figure 21. Biomass of phytoplankton genera among taxa identified by the FlowCAM collected in July along the Sacramento (green bar) and San Joaquin rivers (red bar). Boxes represent phytoplankton groups used for comparison.