Hypotheses

1) Phytoplankton and zooplankton species vary at high frequency spatial scales along the longitudinal axis of the estuary in relation to each other and to water quality variables in the estuary such that they form discrete patches with different abiotic and biotic characteristics.

2) Phytoplankton and zooplankton community composition vary at high frequency time scales (hours to weeks) at fixed locations in the delta in relation to each other and to changes in water quality variables.

3) The FlowCAM® is a more reliable and better indicator of real-time in situ phytoplankton community composition and biomass than the FluoroProbe which measures phytoplankton pigment concentration.

4) The FlowCAM® is an accurate and more rapid method for identification, enumeration and estimation of biomass for in preserved phytoplankton and zooplankton species samples than traditional microscopy.

3) Approach and Scope

General Summary - This study will conduct field studies and laboratory tests using the portable FlowCAM® and historical data analyses to 1) characterize the high frequency spatial and temporal variation of phytoplankton and zooplankton communities in the estuary, 2) evaluate state of the art technologies for in situ real-time measurement of phytoplankton community composition and biomass and 3) develop more rapid analysis of phytoplankton and zooplankton identification, enumeration and biomass estimates in preserved samples. Each task in this project will be is independent of the others.

Detailed study description by Task:

Task 2. Quantify the high frequency spatial variation of phytoplankton and zooplankton community composition along longitudinal gradients in the estuary.

Hypothesis - Phytoplankton and zooplankton species vary at high frequency spatial scales along the longitudinal axis of the estuary in relation to each other and to water quality variables in the estuary such that they form discrete patches with different abiotic and biotic characteristics.

Field study - High frequency phytoplankton and zooplankton species composition, density and biomass in conjunction with water quality conditions will be collected along the longitudinal axis of the estuary between Suisun Bay and Rio Vista on the Sacramento River and between Suisun Bay and Stockton on the San Joaquin River. These transects will be conducted each month between spring and fall (March through September) for two years. Transects will include different habitats included flooded islands, shoals and channel habitat. As many samples will be taken along these longitudinal transects as possible in a systematic fashion. Research demonstrated that equally spaced high frequency sampling along a longitudinal transect provided a good estimate of spatial variability for water quality variables and

chlorophyll a concentration in the estuary (Jassby et al. 1997). During the sampling run, water containing phytoplankton species will be transferred by diaphragm pump from the water column to a container linked to the FlowCAM® on board ship where the species will be identified and counted and the cell dimensions used to estimate biomass in situ. A water sample for phytoplankton species identification and enumeration by microscopy will also be preserved in Lugol's iodine solution (Utermohl 1958). Phytoplankton cells in the community will be measured with three objectives 20X (3 to 45 pm diameter cells), 10X (5 to 95 pm diameter cells) and 4X (10 to 250 pm diameter cells) or the most appropriate size range of the three for the sample area based on initial sampling. Zooplankton for species identification and biomass estimates will be collected simultaneously from the water column by diaphragm pump and concentrated on board ship by running the pumped water through a 153 pm mesh zooplankton net fixed in a vertical position. Zooplankton from the cod end of the plankton net will be immediately preserved in Lugol's iodine solution or 10% buffered formalin and then run through the FlowCAM® for real-time digital imaging to determine species composition, animal density and animal biomass once the phytoplankton sampling is completed. Zooplankton will be measured for small (<600-700 pm) and large (>1-3 mm) size categories. A replicate sample will be preserved in 10% buffered formalin for identification and enumeration in the laboratory by dissecting microscope combined with digital imagery processing for comparison.

The FlowCAM® identifies the phytoplankton and zooplankton by taxonomic group or species through libraries developed by the manufacturer and the user from the images obtained by the FlowCAM® at different locations. Once the libraries are created from initial cruise transects, the FlowCAM® software will allow for further analysis and pattern matching of future samples/runs with the FlowCAM®. Sample post-processing and Identifications are relatively fast once the libraries are developed and will not cause significant delay in the use of the system. Identification will be at least to the genus and species level if possible or binned into size classes for biomass estimates. Water quality conditions associated with phytoplankton and zooplankton communities along the longitudinal axis of each river will be obtained using a YSI 6600 (YSI Instruments Inc.) real-time water quality sonde (pH, dissolved oxygen, water temperature, specific conductance, NTU and chlorophyll fluorescence). The concentration of dissolved nitrate and ammonia nitrogen, dissolved soluble and total phosphorus, dissolved silica and chlorophyll a and phaeophytin concentration will be measured with selected discrete samples. Water for these samples will be pumped from the water column. Water samples for nitrogen and phosphorus will be filtered through 0.45 pm pore size Millipore HATF04700 nucleopore filters and frozen until analysis by colorimetric technique (US EPA, 1983). Silica concentration will be determined by the molybdate blue method (USGS, 1985). Water samples for chlorophyll a and phaeophytin concentration will be filtered through Millipore GF/F glass fiber filters and frozen until analysis. Pigments will be extracted in 90% acetone and analyzed for chlorophyll a (corrected for phaeophytin) and phaeophytin using spectrophotometry (method 10200H, APHA *et al.,* 1998). Because nutrients are rarely limiting, nutrient measurements will be made to confirm they are in excess. Comparisons of phytoplankton and zooplankton communities in relation to streamflow will be done using streamflow data from DAYFLOW data files (iep.water.ca.gov).

Data analysis - Correlation among phytoplankton and zooplankton community composition and water quality data will be determined statistically using multivariate statistical procedures such as canonical correlation and ARIMA time series analysis. Spectral analysis will be used to characterize the periodicity in the data. Similarity among phytoplankton and/or zooplankton groups will be determined by cluster analysis. The spatial patterns and associations in these

data will be compared with the historical data from monthly sampling at fixed stations analyzed in the same fashion as the high frequency data. Analyses will use the SAS statistical package (SAS 2004).

Value - This information will provide valuable information on the location, amplitude, variation and overlap of phytoplankton and zooplankton communities and their association with water quality conditions that may be important for real-time management of fishery resources. This information will also identify habitat needs for phytoplankton and zooplankton species. Such information is needed for long-term data analysis and predictive and mechanistic models. It may also suggest new approaches to field sampling and management of biological resources in the estuary.

Task 3. Quantify the high frequency temporal variation of phytoplankton and zooplankton community composition at fixed stations in the estuary.

Hypothesis - Phytoplankton and zooplankton species composition vary at high frequency temporal scales with regard to each other and to water quality variables.

Field study - The high frequency temporal (hours to weeks) variation of phytoplankton and zooplankton community composition and their association with water quality conditions will be measured at three continuous water quality monitoring stations operated by the Department of Water Resources. These stations routinely measure the water quality variables pH, water temperature, specific conductance, NTU and chlorophyll fluorescence at 15 min intervals using an in situ YSI 6600 water quality sonde. Weather measurements include air temperature, wind speed and direction and solar irradiance. Chlorophyll fluorescence is calibrated to laboratory extracted chlorophyll a concentration using techniques described in Hypothesis 1. The FlowCAM® will conduct real-time identification, enumeration and estimation of phytoplankton species composition for at least two weeks in the spring, summer and fall. Sample water will be pumped from 1 m depth in the water column into the station housing and then into a container attached to the FlowCAM®. Screening the pump and input port with a large mesh (>500 pm) will limit fouling of the input port from large particles. In addition, copper tubing will be applied to the intake port of the FlowCAM® to limit biofouling. For phytoplankton sampling a size range will be selected that best represents the community based on test runs. Companion water samples for microscopic analysis of phytoplankton species composition will be collected using an ISCO automatic water sampler. Sampling at the continuous monitoring station at Antioch will also allow evaluation of the ability of the FlowCAM® to conduct real-time monitoring of the *Microcystis* toxic bluegreen algal bloom. This work will be done in cooperation with a CALFED grant to Dr. Lehman to monitor *Microcystis* biomass in the estuary. Operation of the instrument will be monitoring daily during the sampling period. In a separate study, high frequency zooplankton species identification, enumeration and biomass estimates will be conducted at the same three continuous monitoring stations on concentrated zooplankton samples. The zooplankton will be pumped from the water column, concentrated using a zooplankton net (153 pm mesh) and preserved in 10% buffered formalin or Lugol's iodine solution before processing with the FlowCAM® as described in Hypothesis 1. Zooplankton will initially be preserved as a precaution against large zooplankton swimming against the FlowCAM® inflow current, but preservation will be eliminated if possible. Screening as described in Hypothesis I will limit the fouling by large particles on the input port to the FlowCAM®. The sampling will be systematic and include as many samples as possible during a diel period.

Data analysis - Periodicity in the species and water quality data will be quantified using spectral analysis. Correlation between phytoplankton and zooplankton species and water quality conditions will be determined using multivariate analysis such as canonical correlation analysis and ARIMA time series analysis. Similarity of phytoplankton and zooplankton groups will be determined using cluster analysis of nondimensional data. High frequency temporal patterns in phytoplankton biomass will be compared with continuous phytoplankton chlorophyll a concentration (YSI 6600 chlorophyll a fluorescence calibrated with laboratory extracted chlorophyll a concentration as described in Hypotheses 1) measured concurrently at the monitoring station using ARIMA (time series correlation) and spectral analysis (temporal pattern). Periodicity in the high frequency species and biomass data and their correlation with water quality will be compared with historical measurements of chlorophyll a concentration (fluorescence) and water quality variables at the continuous monitoring stations using spectral analysis and ARIMA. The influence of high frequency changes in species composition on carbon load compared with monthly data will also be evaluated using ANOVA. Analyses will use the SAS statistical package (SAS 2004).

Value - This information will provide valuable information on the influence of high frequency variation of phytoplankton and zooplankton communities on population trends that will assist development of mechanistic and predictive models and compute more accurate estimates of phytoplankton and zooplankton carbon load and the quality of phytoplankton food available to the estuary. It will also provide valuable information on the covariance of phytoplankton and zooplankton species composition and with high frequency changes in water quality conditions. This information could be used to better design continuous phytoplankton and zooplankton monitoring programs in the estuary for evaluation of fishery resources, impacts of harmful algal blooms and exotic species introductions.

Task 4. Compare the accuracy of FlowCAM® and FluoroProbe instruments for measurement of phytoplankton community composition and biomass.

Hypothesis - The FlowCAM is a more reliable and better indicator of in situ phytoplankton community composition and biomass than the Fluoroprobe which only measures phytoplankton pigment concentration.

Study - Comparison of FlowCAM® and bbe FluoroProbe (http://www.bbe-moldaenke.de and http://www.bbe.us) technologies for characterization of phytoplankton communities and their biomass will be tested for three of the DWR continuous monitoring stations: D10 in Suisun Bay, P8 at Stockton on the San Joaquin River and C3A at Hood on the Sacramento River. These stations were selected for the FluoroProbe in the CALFED grant to Dr. Mueller-Solger. High frequency samples will be collected for at least two weeks in spring, summer and fall with both instruments. Results will be compared between the two machines and with phytoplankton cell counts and biomass estimates from samples preserved with Lugols iodine solution and analyzed by the traditional inverted microscope technique (Utermohl 1958). Microscopy will count phytoplankton cells to the picoplankton level. Estimates of biomass will also be compared with chlorophyll fluorescence measured continuously by YSI 6600 sonde and calibrated with extracted chlorophyll a and phaeophytin pigment concentrations as described in Hypothesis 1 (method 10200H, APHA et al. 1998). The FluoroProbe work will be done in collaboration with Dr. Mueller-Solger who has a CALFED grant to evaluate its use for phytoplankton community sampling in the delta.

Analysis - Differences between the total and group biomass measured by each machine, microscopy and chlorophyll a concentration will be analyzed by analysis of variance. Analyses will use the SAS statistical package (SAS 2004).

Task 5. Evaluate the speed and accuracy of the FlowCAM ® to identify, enumerate and compute phytoplankton and zooplankton in preserved samples.

Hypothesis - The FlowCAM® is an accurate, reliable and more rapid method for identification, enumeration and estimation of biomass for phytoplankton and zooplankton species than traditional microscopy for preserved samples.

Study - The accuracy and efficiency of analyzing preserved phytoplankton and zooplankton samples in the laboratory with microscopy and FlowCAM ® technology will be compared for samples collected at 10 stations for one year at monthly intervals by the EMP monitoring program. For phytoplankton, comparisons will be made of the species composition at least to genera, cell density and biomass values obtained by traditional inverted microscope analysis of samples preserved with Lugol's iodine solution (Utermohl 1958) enhanced to capture picoplankton and the FlowCAM® run for three objectives: 20X (3 to 45 pm cell diameter), 10X (5 to 95 pm cell diameter) and 4X (10 to 275 pm cell diameter). For zooplankton samples preserved with 10% buffered formalin or ethanol, comparisons will be made between species composition at least to genera, animal density and biomass estimates using the FlowCAM ® and standard dissecting microscope techniques (zooplankton metadata, www.iep.water.ca.gov. The FlowCAM® will be run at two magnifications to quantify large (>1-3 mm) and small (>600-700 pm) zooplankton in the samples. The time and cost necessary to analyze each sample will be recorded for an efficiency comparison.

Analysis - Comparison of phytoplankton or zooplankton species density and biomass and the sample processing time between the FlowCAM® and microscopy will be compared statistically with ANOVA (t-test). Analyses will use the SAS statistical package (SAS 2004).

Task List

Task 1 Management of contracts, subcontracts, accounting, personnel and reporting. January 2007 - December 2008.

Task 2 Conduct high frequency phytoplankton and zooplankton spatial variation study. June 2007 - September 2008.

Task 3 Conduct high frequency phytoplankton and zooplankton temporal study. June 2007 - September 2008.

Task 4 Compare FlowCAM and Fluoroprobe for estimating phytoplankton community 'composition and biomass. June 2007 - September 2008.

Task 5 Evaluate the accuracy and efficiency of using the FlowCAM for analyzing phytoplankton and zooplankton species composition compared with microscopy. June 2007 - December 2008.

Task 6 Technical assistance with field sampling, data analysis and journal paper and report writing. January 2007 to December 2008

EXHIBIT A:ATTACHMENT 4
TASK TABLE

Task	Task Title	Start month	End month	Personnel	Description
1	administration	1	30	Lehman	Administation of the grant, budgeting, semi-annual progres reports, final reporrts, presentation at local and nation meetings, manuscripts and presentations
2	High frequency data collection (spatial focus)	14	30	Lehman, Dahlgren, DWR Staff,	deploy and recovere instrumentation for establishing spatial variability in phytoplankton and zooplankton communities
3	high frequency data collection (temproal focus)	16	30	Lehman, Dahlgren, DWR Staff,	deploy and recovere instrumentation for establishing temporal variability in phytoplankton and zooplankton communities
4	comapre flowCAM and fluoroprobe sampling	24	30	Lehman, Dahlgren, Mueller-solger, DWR Staff	comparison of data collected with companion devices and interpretation of results
5	F1owCAM accuracy evaluation	15	30	Lehman, Dahlgren, DWR Staff,	Determine ability of flowCAM to analyze laboratory samples
6	Technical assistance with sampling program	13	30	Poulton	Provide technical oversight and assistance for sampling and analysis with flowCAM
7	Report preparation and presentation	1	30	Lehman, Dahlgren, Mueller-solger, DWR Staff	provide progress reports, journal manuscript and conference presentations