3. APPROACH AND SCOPE OF WORK

Tasks and Schedule

The scope of work has been split into 5 tasks, all of which extend throughout the 3 year (36 month) period and closely parallel our Scientific Objectives (see Project Purpose Section). We have explicitly chosen to focus on phytoplankton processes and not develop a large diffuse multi-disciplinary multi-institutional approach as this would not be appropriate at this point in time until some fundamental information for this trophic level is obtained. Task 1 is required by CALFED to be the administrative task of the scope of work. Tasks 2 and Tasks 3 could be done separately although interpretation of the results of one is heavily supported by the other, and by Task 4. Similarly, Task 4 is contingent on Tasks 2 and 3 to supply the samples. Task 5 could be done separately using previously obtained data but the predictive capability of the model for the POD habitat of the Delta region would be limited.

Task 1. Project Management
This represents project oversight, data handling, preparation of reports, communicating results and progress to CALFED and outreach activities. It involves most of the personnel.

Task 2. Suisun Habitat Description
This fieldwork task will assess the anomalous Suisun habitat (“Bad Suisun”) in context of the delta, upstream (into the Sacramento and San Joaquin Rivers) and downstream (a more oceanic "healthy" condition in Central Bay). Besides measuring gradients of bulk constituents (e.g. phytoplankton abundance as chlorophyll, nutrient concentration etc) which may not provide a very sensitive index, this task will focus on measuring physiological rates that respond more quickly to change (e.g. photosynthesis, nutrient acquisition, growth rate).

Task 3. Experimental Manipulations
ACcompanying field measurements of the "Bad" Suisun condition, experimental enclosures will be used to manipulate the possible drivers that are causing this unique habitat. These have been used effectively to compare Suisun Bay with San Pablo and Central Bays. Here they will be used to evaluate the relative response to changing conditions (drivers) of Suisun phytoplankton growth with those from upstream freshwater and downstream, oceanic populations.

Tasks 2 and 3 involve all personnel except algal taxonomist Ed Carpenter and include both field sampling in Years 1 and 2, with laboratory based biological and chemical analyses and presentation of the data at local and national meetings and workshops in all three years.

Task 4. Phytoplankton Condition Analysis
This is specifically to support Dr E Carpenter to use light microscopy to identify and enumerate the components of the Suisun phytoplankton community giving information (especially diatom numbers) about relative food sources for the food web of delta fishes. It will also evaluate the cellular condition (or health) and look for empty, dead or unhealthy looking phytoplankton cells.
**Task 3. Experimental Manipulations**

To supplement the characterization of the pelagic habitat description of Suisun Bay, water will be used to fill experimental enclosures (typically 20-L cubitainers incubated at 50% of surface irradiance and maintained at ambient temperature by flowing bay water on incubator tables), see Wilkerson et al. (2006), Parker (2004), Koch (2005) that are sampled daily after filling. Mesocosms experiments will be unmanipulated or manipulated experiments, in which conditions are changed and controlled, e.g. removing/replacing the phytoplankton community, changing light or nutrient conditions etc.

Enclosure experiments will be carried out monthly from March to August in conjunction with the field observations (Task 2) during years 1 and 2. In Year 1, each month water will be sampled from 4 locations ranging from oceanic (off RTC, Central Bay), to Suisun (USGS Sta 7), the Delta (USGS 649) and Sacramento River (USGS Sta 657 at Rio Vista) (Fig. 2) and used for unmanipulated mesocosms. San Joaquin River, JPT may be alternated with USGS 649. In year 2 manipulations will be employed based upon our findings in Year 1 although we will repeat and expand on the experiment reported in Fig. 1 where the resident phytoplankton are filtered from the water to be tested. The filtered water is inoculated with a mixture of cultured diatoms and cryptomonads that were originally isolated from the particular embayment and incubated and sampled for 5 days. We also plan to do a similar experiment but with a dilution series of the "bad" Suisun water to see if the inhibitory effects can be reduced or eliminated.

Water will be sampled for Task 3- enclosures- as close in time as feasible to the Task 2 but on separate cruises using the R/V Questuary as there would be insufficient sampling time and space for this on the Polaris and with a dedicated vessel the water can be quickly returned to RTC where the enclosures will be set up. The river water will be collected using a small boat in liason with the Questuary. Water will be sampled daily for temperature, salinity, turbidity, inorganic nutrients (NO$_3$, NO$_2$, NH$_4$, PO$_4$, Si(OH)$_4$), DIC, chlorophyll, cell size spectra using flow cytometry, primary productivity and nutrient uptake rate measurements using $^{15}$N/$^{13}$C. Analysis of these type of experiments will show whether the slow increase in phytoplankton growth rate anomaly in Suisun Bay (as demonstrated with elapsed time of sampling the enclosures) is a local or upstream phenomenon. Since DIC, PO$_4$ and Si(OH)$_4$ are always present in high concentrations in all parts of the bay, a lack of growth at a particular location water (Suisun in Fig. 1) points to a toxicity/inhibition problem. The geographic pattern should indicate the source, upstream or local.

**Task 4. Phytoplankton Condition Analysis**

As part of Tasks 2 and 3, the size spectra of fluorescent cells (-phytoplankton) will be made using a CytoSense flow cytometer that is designed for studying larger eukaryotic cells. But this does not give species composition data or observations of cellular status. During Tasks 2 and 3, water will be collected for phytoplankton identification, enumeration and cellular observation. Surface samples (from stations in Fig. 2 or the enclosures) will be collected directly into 500 ml amber glass bottles, and preserved and stained with Lugol's solution. Phytoplankton will be identified to species using the Utermohl techniques (Utermohl, 1958; Hasle, 1978) and an inverted phase contrast microscope (Nikon Type 180). In doing counts, at least 200 of the commonest species will be counted for good statistical estimation.

In addition the cellular condition of the cells will be documented. In particular we have noticed phytoplankton cells from Suisun often to be coated with particles compared with other SFE phytoplankton samples. Particular care will be made to document if cells are empty, shreveled or look abnormal in any way.
Uptake of NO$_3$ and NH$_4$ and primary production will be estimated with dual labeled $^{15}$N/$^{13}$C stable isotope tracer techniques (Slawyk et al., 1977, Legendre and Gosselin, 1996) to yield simultaneous N and C uptake rates from a single sample. $^{13}$C estimates of primary production are reliable and consistent with the $^{14}$C method in estuaries (Parker, 2004). Water will be dispensed into 150 ml incubation bottles and inoculated with either Na$^{13}$CO$_3$ and K$^{15}$NO$_3$, or Na$^{13}$CO$_3$ and NH$_4$Cl(99 at%). Additions will be made to approximately 10% of ambient concentration to avoid substrate enhancement effects. Immediately following inoculation, one sample will be filtered onto a precombusted GF/F filter (450°C, 4-hr) by gentle vacuum to determine the initial PON and POC concentration and isotopic filter blank. Incubations at ambient temperature and 50% surface light will last 24 hours and will be terminated by gentle vacuum filtration onto precombusted GF/F filters. N and C concentration and isotopic composition will be determined using a Europa 20/20 mass spectrometer. N and C uptake rates will be calculated based on isotopic enrichment according to Dugdale and Wilkerson (1986).

Equipment and Facilities

Dugdale and Wilkerson each have an analytical chemical/biological research laboratory at RTC with appropriate small equipment. These include muffle ovens, spectrophotometers, fluorometers, balances, high precision micropipettes, filtration racks, pumps and all reagents needed for analyses of inorganic nutrients and phytoplankton biomass (chlorophyll, PON, POC etc) and isotopic abundance in samples from shipboard studies. One has a designated radioactive area for $^{14}$C, H and $^{32}$Si uptake analyses. Small boats and the R/V Questuary are available for water sampling of SF Bay, and jointly shared equipment (image processing and production lab, microscopes, scintillation counters etc) are available at RTC. We have extensive water table and plexiglas incubator facilities. RTC monitoring site SFBeams (CTD, weather, fluorometer, transmissometer, PAR) is available with real-time data.

At RTC, the following major equipment owned mostly by Dugdale and Wilkerson will be used:

- Bran and Luebbe Technicon 11 AutoAnalyzer for nutrient measurements
- RTC-Joint Use PDZ Europa 20/20 mass spectrometer for measuring $^{15}$N and $^{13}$C enrichments and PON and POC biomass
- DIC Analytical System (duplicate of U Delaware/MBARI system)
- Barnstead NanoPure water system
- Cytobuoy Flow Cytometers designed for applications with a dynamic range for pico to micro-plankton sizes. Used to obtain size spectra and fluorescence characteristics of phytoplankton
- Zeiss inverted microscope for phytoplankton enumeration with settling chambers etc.

Expected Deliverables

Specific to CALFED: Semi-annual report(s) every 6 months and a final report, one page project summaries for public audience at start and end of project, presentation(s) at CALFED Science Conferences and at the request of CALFED Science Program staff and copies of all published material resulting from the grant

WebSite: Data and analyses will be available on a dedicated page on our lab website (http://online.sfsu.edu/~phytopl/)