CALFED BAY-DELTA PROGRAM

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California Sea Grant College Program

FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE [x] Postdoctoral Researcher [ ] Ph.D. Graduate Student

PROJECT NUMBER

PROJECT TITLE CALFED Fellows Program

FINANCIAL SUMMARY

First Year CALFED Funds Requested:	$82,475
Total CALFED Funds Requested:	$164,931
Duration:	2 years
Proposed Start/Completion Dates:	1/15/2009-1/14/2011

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Will animal subjects be used? [ ] Yes [x] No
APPROVAL DATE: PROTOCOL #: PENDING:

Does this application involve any recombinant DNA technology or research? [ ] Yes [x] No

Proposed Research **K. Ali Ger**

Trophic Impacts of *Microcystis* on the crustacean zooplankton community of the Delta

Introduction

The recent decline in the pelagic foodweb of the upper San Francisco Estuary (SFE) has caused widespread concern in management agencies and the public. Abundances of several pelagic fish species have declined to historically low levels, and the apparent slide to extinction of the Delta smelt, along with the consideration of longfin smelt for listing, raise serious worries that the entire pelagic ecosystem may be affected. This broad decline across the foodweb has led to new ecological studies on multiple stressors and the consideration of several synergistic processes such as hydrographic changes, water quality, species introductions, food limitation, habitat quality and connectivity, and fisheries ecology (Sommer et al 2007). An ambitious multiagency research group has been studying the potential causes of the decline (POD Management Team 2007).

The conceptual model used to investigate the potential causes of decline has four main components; 1) effect of previous abundance on juvenile fish production; 2) effects of habitat and water quality on estuarine species; 3) top-down effects due to predation and entrainment; and 4) bottom-up effects, essentially food limitation (Sommer at al 2007).

Seasonal blooms of the toxic colonial cyanobacteria *Microcystis aeruginosa* may have played a role in the decline in pelagic fish through changes in water quality and trophic interactions (POD Management Team 2007). *Microcystis* could be having an impact on the pelagic foodweb through its toxicity (essentially a water quality effect) or because it may be an inferior food for zooplankton (resulting in food limitation), though actual impacts are unknown. *Microcystis* likely reduces phytoplankton food quality and thereby habitat quality for preferred pelagic fish prey such as *Pseudidiaptomus forbesi*.

Elsewhere, *Microcystis* blooms have caused zooplankton community shifts to smaller and more tolerant species in eutrophic lakes, rivers, and upper estuaries globally (Chorus and Bartram 1999, Paerl 1988). Impacts to zooplankton are species-specific and due to a combination of toxicity, nutritional deficiency, and consumption-resistant morphology. The cumulative response of the zooplankton community depends on the dynamics of each system. A large body of research indicates that trophic impacts due to interference with grazing by herbivorous zooplankton can be substantial (Christoffersen 1996, Chorus and Bartram 1999, Zurawell et al 2005). Thus, there is good reason to believe that *Microcystis* blooms in the upper SFE could exacerbate food limitation and declines in the abundance of zooplankton that are prey to pelagic fish.

Microcystis blooms have been a regular feature of the Delta for almost a decade, yet there is virtually no information on their impacts on the pelagic foodweb. Blooms of *Microcystis* were initially observed in localized surface scums in the late 1990's , though the species was recorded previously in non-bloom forming quantities (Lehman et al 2008, Bay Delta Tributaries Project). In the last 9 years, annual blooms have extended over wide regions of the Delta, in salinities of 0.1-18, beginning in June and reaching their peak between September and October (Lehman et al 2008). The geographic range of the blooms has been expanding, implying permanent establishment in the SFE (POD Management Team 2007). Although *Microcystis* was previously most abundant in low-flow waters of the Central Delta, the most recent monitoring from 2007 suggests that its abundance in Antioch has become comparable to that in the Central Delta (POD Management Team 2007, personal observation). *Microcystis* now occurs seasonally from Suisun Bay to the freshwater habitat of rivers feeding the Delta, with greatest abundance in the Central-South Delta and more recently at the confluence of the Sacramento and San Joaquin Rivers (Lehman 2005, POD Management Team 2007).

Here I propose to investigate the contribution of *Microcystis* to the decline in pelagic organisms through a careful study of food limitation, habitat quality, and trophic interactions with zooplankton. This work will build on my current graduate research investigating the toxic and nutritional impacts of Microcystis on copepods.

I will quantify and model routes of exposure of SFE zooplankton to *Microcystis* using a combination of field and laboratory experiments, apply novel molecular methods for measuring in-situ ingestion rates, and collect information on how *Microcystis* changes species-specific survival and consequently the community composition of SFE zooplankton. The decline of the pelagic ecosystem to historically low levels places *Microcystis* as a potentially significant strain on the planktonic foodweb, and the extent of this strain is ripe for investigation.

Background

Cyanobacterial blooms are a key issue in aquatic management for a variety of reasons that stem from toxin exposure to the foodweb and changes in the trophic transfer of carbon (Chorus and Bartram, 1999, Hansson et al, 2007). Blooms of toxic cyanobacteria now globally threaten water quality, human uses such as water consumption and fishing, and fundamental ecological functions (Anderson and Garrison 1997, Paerl 1988, Stahl-Delblanco et al. 2003, Graneli and Turner 2006). *Microcystis aeruginosa*, one of the most common species of freshwater cyanobacteria worldwide, forms colonies, and is dominant in many eutrophic and hyper-eutrophic waters including upper reaches of estuaries (Paerl 1988, Christoffersen 1996). In recent years there has been an increase in the abundance and distribution of toxic *Microcystis* blooms in the landward portion of the San Francisco Estuary (SFE), with unknown impacts to the foodweb (Lehman et al 2008).

Microcystis blooms occur under environmental conditions that include high nutrients, low dissolved carbon, stratified water column, and warm water temperatures. Given the current state of the SFE and the life history of *Microcystis*, these blooms are likely a permanent seasonal trait (POD Management Team 2007). Monitoring and predicting the impacts of such blooms is of fundamental interest to aquatic research and management, and its potential impact on food limitation in the SFE cannot be ignored (Ouelette and Wilham 2003, Chorus and Bartram 1999).

Microcystis is known to shift zooplankton community composition towards smaller species, or those able to graze on it directly (Lampert 1987, Chorus and Bartam 1999, Nadini 2003, Graneli and Turner 2006). However, the majority of the information pertaining to *Microcystis* is based on laboratory studies using *Daphnia* spp. as the test organism, while only a small fraction is based on copepods (reviewed in Wilson et al 2006). Copepods are the dominant zooplankton in the SFE so information on copepod – *Microcystis* interactions will benefit management of the SFE as well as other estuaries (POD Management Team 2007, Wilson et al 2006). Mechanistic evidence from laboratory studies shows that *Microcystis* typically suppresses feeding in large zooplankton grazers because of its colonial morphology, toxic properties, and nutritional deficiency (Wilson et al 2006, Lampert 1987, Christoffersen 1996, DeMott and Moxter 1991). However, the role of *Microcystis* on trophic shifts in zooplankton communities is not clear because it is difficult to observe in the field and species-specific differences cause contradictory effects (Kumar 2003, Ghaouani et al 2003, Kirk and Gilbert 1992).

Possible role of *Microcystis* in the food limitation of pelagic fish

Increasing blooms of the cyanobacteria *Microcystis aeruginosa* in the freshwater and low salinity zones of the SFE come at a time of historically low abundances of both calanoid copepods and nutritious phytoplankton, raising concern for further depletion of high quality fish food (Mueller-Solger et al 2002, Lehman et al 2005, POD Management Team 2007, Sommer et al 2007). To our knowledge, there is no

information regarding the mechanisms by which *Microcystis* changes zooplankton community structure or the routes of exposure in the SFE, but it has the potential to reduce habitat quality for copepods.

High abundances of consumption-resistant algae such as *Microcystis* may impact the foodweb in three ways, 1) Reduce the quality of total phytoplankton biomass; 2) increase foodchain length by shifting zooplankton grazing to microbial sources, reducing overall zooplankton productivity; 3) change the zooplankton community composition by favoring smaller, non-herbivorous copepods such as cyclopoids, rotifers, and protozoans (Kirk and Gilbert 1992, Smith and Gilbert 1995, Ghadouani 2003, DeMott and Tessier 2002). The trophic response of the pelagic foodweb to increased cyanobacterial primary production is governed by the identity of zooplankton and their feeding ecology (Stibor et al 2004, Lampert 1987). It is necessary to quantify in-situ grazing rates and feeding ecology of zooplankton to understand the dynamics of *Microcystis* impacts to the foodweb.

Why copepods? Implications of the Delta's shifting copepod community

Copepods dominate metazoan/mesozooplankton in the Delta and are the main food source for small pelagic fish (Mueller Solger et al 2006, Kimmerer 2004). Calanoid copepods that can consume phytoplankton (*E. affinis* and *P. forbesi*) form shorter and more productive food chains that fuel the pelagic ecosystem. A decline in such copepods may signal alternative trophic pathways, in part due to *Microcystis* blooms, shifting the zooplankton towards non-herbivorous species. Long term monitoring indicates significant shifts in the copepod community (POD Management Team 2007), including a more variable abundance in all species, a decrease in the once common calanoid *E. affinis*, its replacement with *P. forbesi* as in the diets of fish, and increased abundances of non-herbivorous copepods including cyclopoids and *Acartiella*. The SFE's previously abundant calanoids *P. forbesi* and *E. affinis* have been in decline, with potentially severe consequences on pelagic fish (Sommer at al 2007).

Pre-1980's monitoring shows that *E. affinis* was dominant year round, and other copepods were not so abundant. Peaks of *E. affinis* abundance are now limited to spring. *P. forbesi* takes over in early summer, and shares habitat with the predatory *Acartiella sinensis* and the small cyclopoid *Limnoithona* (Kimmerer 2004, Bouley and Kimmerer 2006). These changes, the results of introductions from East Asian estuaries, have effectively reshaped the zooplankton community, with likely changes in the trophic structure of the foodweb.

Copepod feeding ecology, food limitation, and *Microcystis*

Copepods feed selectively on a wide variety of food particles ranging from the smallest phytoplankton to protozoans, detritus, and larger prey including other copepods (Nejstgaard et al 2008, DeMott and Felix 1991, Kumar 2003, Koski et al 1999). Mechanisms behind food selection are species-specific and include biochemical and mechanical cues as well as the mode of feeding, i.e. raptorial versus suspension feeding (Bouley and Kimmerer 2006, Gasparani and Castelt 1997). The SFE has always had relatively low rates of primary production and phytoplankton abundance, probably because of the high concentration of suspended sediments (Jassby et al 2002, Kimmerer 2004). Together, these attributes are known to select for omnivorous zooplankton that have selective ability to capture prey (Gasparini et al 1999). Omnivorous copepod feed on both microbial and autotrophic food chains, though species-specific adaptation to a certain diet is important (DeMott 1995, Kumar 2003, Panosso et al 2003).

Suspension-feeding calanoid copepods such as *E. affinis* can select particles following predictions of an optimal diet model, i.e. to maximize energy input per unit of expenditure (DeMott and Moxter 1991, Gasparani and Castelt 1997). Calanoid copepods have been shown to successfully select against *Microcystis* (DeMott and Moxter 1991, Koski et al 1999). The strength of selection depends on the feeding adaptation of species, abundance of nutritious food particles, and suspended sediment (DeMott

and Moxter 1991, Gasparini and Castel 1997). As long as adequate nutritious particles are available, selective feeding is an adaptation to avoid harmful or non-nutritious particles. Strong feeding selection is an advantage to copepods, as they are able to switch between different food sources to maximize benefit from a variable resource. Overall, copepod response to changes in food quality, such as an increase in *Microcystis*, will be a function of the species-specific adaptations for ingesting phytoplankton versus protozoans (DeMott and Moxter 1991, Kumar 2003)

The long-term decline in phytoplankton abundance in the Delta raises concern that there might not be enough high quality food for *P. forbesi* and *E. affinis*, which are the dominant prey species for larval fish (Mueller-Solger et al 2006, POD Management Team 2007, Sommer 2007). Both are suspension-feeding copepods that can survive and reproduce on a sole diet of nutritious phytoplankton, but probably feed opportunistically on protozoans and even detritus in the Delta (Bouley and Kimmerer 2006, Mueller Solger et al 2006 , Gasparini et al 1999). Feeding experiments show that *P. forbesi* selects high quality phytoplankton such as diatoms over microbial food (Bouley and Kimmerer 2006, Mueller Solger et al 2006). *E. affinis* selects smaller food particles under high suspended sediments, but ingests nutritious phytoplankton under less turbidity (Gasparini and Castel 1997). Additionally, laboratory tests indicate that *P. forbesi* has higher survival when fed *Microcystis* in a mixed diet with other phytoplankton, compared to *E. affinis* (Ger et al, in prep). *Microcystis* can exacerbate food limitation for copepods adapted to phytoplankton diets. My current doctoral work investigates the acute and chronic toxicity, as well as the dietary effects of *Microcystis* in a mixed diet on the copepods *P. forbesi* and *E. affinis* under controlled laboratory conditions. We found that survival in these copepods is a function of *Microcystis* ratio in the diet, higher mortality occurred for non-toxic *Microcystis*, and mortality ceased after 4-7 days for toxic *Microcystis*, provided in a mixture with high quality food. Results show that impacts of *Microcystis* on *P. forbesi* depend on available high quality phytoplankton food sources (Ger et al, in prep).

On the other hand, the dominant, small cyclopoid *Limnoithona tetraspina* shows a clear adaptation for microbial prey, especially heterotrophic ciliates. While there is probably some prey overlap between *L. tetraspina* with *P. forbesi* and *E. affinis*, the distinct diets and feeding adaptations of these copepods place them in somewhat separate foodchains (Bouley and Kimmerer 2006). All three copepods are likely limited by food supply (POD Management Team 2007). Blooms of *Microcystis* may shift copepod grazing to microbial food, and select for species such as *Limnoithona* or *Acartiella*. It has been suggested that *Limnoithona* is able to avoid predation by Delta smelt due to its non-motile behavour, and thus may be a high-cost food for pelagic fish (Bouley and Kimmerer 2006). *Microcystis* is unlikely to interfere with the feeding of such species.

qPCR as a new method for quantifying copepod feeding and trophic interactions

The emergence of ecosystem-based management, coupled with increased awareness of human impacts on ecosystems, has driven the need for organismal data that have greater resolution and accuracy (Hoffmann and Gaines 2008). Recent advances in DNA based biomarkers for the rapid and precise diagnosis of *Microcystis* abundance, toxicity, and species-specific foodweb interactions with zooplankton are a response to this need (Ouellette and Wilhelm 2003, Nejstgaard 2008). Given the complexity of multiple stressors and a constantly changing environment, managers will increasingly rely on DNA-based biomarkers to monitor trophic dynamics and assess ecosystem function (Hoffmann and Gaines 2008). The proposed project will apply this emerging method to track the species specific feeding ecology and response of the SFE's zooplankton community to *Microcystis*.

Recent improvements in molecular methods such as quantitative PCR provide rapid and sensitive diagnosis for the identification of copepod diets based on prey DNA sequences, including *Microcystis* (Nejstgaard et al 2008, Oberholster et al 2008). There are several ways to quantify copepod ingestion and

prey selection such as isotopic and microscopic in-vitro methods, and fluorescence based in situ estimates of gut contents. However each method has its limitations due to bottle effects, time constraints, biases due to differential pigment degradation, and limitation to pigmented prey. Advances in the use of DNA based biomarkers imply that prey DNA can be used as a quantitative measure of feeding selection and ingestion rates in copepods.

Nejstgaard et al. (2008) found that the 18s DNA target gene of the algal prey in a marine copepod can be used as a marker for algal prey. The relationship between PCR detection and prey gut content becomes increasingly quantitative when the ambient prey gene concentration is known. The breakdown of prey DNA is the main reason preventing this method of being fully quantitative and independent of calibrations. Still, because DNA is a chemically stable molecule in living cells, the breakdown of DNA in guts should be less than that of chemically unstable pigments, which are commonly used to quantify ingestion. Another study successfully used qPCR to amplify target *Microcystis* genes in the field from cladocerans (Oberholster et al 2008). That study used the *mcy* gene cluster, which encodes microcystin synthetase, as a molecular marker for ingestion by *Daphnia. magna*. Gut content analysis revealed that majority of the *Microcystis* cells were still intact, implying minimal loss of DNA. Using the *mcy* gene cluster also enables the detection and quantification of the microcystin production of the specific *Microcystis* strain ingested. This is the only method that can quantify in-situ ingestion of different *Microcystis* strains (Oberholster et al 2008).

Development of calibrated qPCR ingestion measurements such as the work proposed here will provide the most accurate measure of in-situ measurement of ingestion, without the costs and limitations of other methods (Nejstgaard et al 2008). Although only recently used to measure in-situ zooplankton ingestion and prey selection, PCR-based molecular tools for ecologists are rapidly evolving and new applications of such techniques will improve our knowledge of the trophic interactions between *Microcystis* and zooplankton. Developing a PCR-based method for quantifying ingestion will also benefit routine monitoring of zooplankton during *Microcystis* blooms to determine the extent and fate of *Microcystis* ingestion in zooplankton.

The objective of the proposed project is to 1) develop a calibrated qPCR based method to quantify in-situ *Microcystis* ingestion using target zooplankton species, 2) apply this method in field mesocosm and laboratory feeding experiments to quantify in-situ ingestion of *Microcystis* and subsequent changes in zooplankton survival and community composition, and 3) compile results to determine impacts to zooplankton community composition. This will help to identify management alternatives specific to the Delta through a comparison to other affected estuaries around the world. The objectives will answer the following questions:

- How useful is qPCR as a monitoring tool for *Microcystis* ingestion?
- What are the trophic dynamics by which *Microcystis* changes the zooplankton community composition?
- Does *Microcystis* reduce the habitat quality of preferred fish prey such as *P. forbesi*?
- What zooplankton species benefit and suffer from increased *Microcystis*?
- Are there predictable changes in estuarine foodwebs due to *Microcystis* blooms?
- What is the contribution of *Microcystis* to the food limitation of pelagic fish?

Approach

We will test the hypothesis that *Microcystis* abundance causes predictable shifts in the SFE's zooplankton community with a series of laboratory experiments and field measurement of in-situ ingestion rates. DNA-based markers will be applied using quantitative PCR to estimate ingestion rates under laboratory conditions with the target copepod, ***P. forbesi***, and calibrated using established techniques such as radiolabeling. A combination of these techniques will be used to quantify *in-situ* ingestion rates of zooplankton in the field and in laboratory under controlled conditions. Our aim is to determine a potential relationship between *Microcystis* abundance and the change in zooplankton species composition based on species-specific ingestion and survival rates. Results can be used to model the zooplankton community response (survival, species composition) to various scenarios of *Microcystis* abundance (biomass, toxicity, duration) to understand the role that *Microcystis* plays in food limitation of pelagic fish in the SFE.

Culturing

A large quantity of ***P. forbesi*** will be used in the experiments. Small (4L beakers) batch cultures as well as a large mass culture (50 – 100 L) will be maintained to ensure enough test organisms. Cultures will be started from those existing at UC Davis, in collaboration with Dr Swee Teh's lab, and supplemented periodically with copepods from the field. The batch cultures will serve as backup stock to insure against possible loss or degradation of the large culture. Copepods will be fed a mixture of highly nutritious commercially available algal diet (Instant Algae, Reed Mariculture). Cultures will be kept at 24 ^{0}C and 5.0 salinity, and water will be changed regularly to minimize accumulation of ammonia and bacteria. Constant aeration will keep food particles suspended. Copepod density will be monitored regularly and cultures will be diluted as necessary to maintain copepod density below 300/L. Culturing medium and feeding will follow the current method I use (Ger et al, in prep). Only healthy, active late stage juveniles and adults will be chosen for ingestion experiments.

Microcystis cultures will be maintained in a separate room under light- and temperature-controlled conditions. Various strains of *Microcystis* will be obtained from the University of Texas Culture Collection (UTEX), including strains that do not produce microcystin (MC) toxins (UTEX 2386), which will be used as an additional control for *Microcystis* MC content during the ingestion study. Axenic batch cultures will be maintained in 500 ml glass flasks using autoclaved equipment and air filters to prevent contamination. A modified ASM-1 medium will be used under a low light intensity and photoperiod of 16L: 8D at 25^{o} C (Reynolds and Jaworski 1978). We will establish protocols to ensure that cultures of copepods and *Microcystis* remain clean. Specific areas and equipment will be designated for each culture to prevent cross-contamination.

Development of qPCR for in-situ ingestion rates

We will use commercially available primers for use in a rapid and specific PCR test to detect the presence of cyanobacteria, *Microcystis* (MC+ and MC- strains), and toxic *Microcystis* (MC+ only) based on the toxin genes microcystin peptide synthetase B (*mcy*B) and microcystin polyketide synthase D *(mcy*D) (Ouelette et al. 2006). The *mcy* gene cluster can be used as a molecular marker for detection and quantification of *Microcystis*, both in water samples as well as zooplankton guts (Oberholster et al 2006, Ouellette and Wilhelm 2003). Gene copy numbers of cyanobacteria - specific 16S rDNA, *Microcystis* - specific 16S rDNA, and the *mcy* gene cluster will be used to calculate abundance of cells carrying these genes. Fragments of the 16S rDNA gene regions will be amplified, the amplicons cloned, and the plasmids sequenced to design primers for use in a real-time quantitative PCR (qPCR) to estimate the abundance of toxic *Microcystis* in the sample based on the *mcy*D gene (Rinta-Kanto et al 2005, Ouellette and Wilhelm 2003). Probes will be designed following the specifications of Applied BioSciences (Foster

City, CA), and probe specificity will be tested on various *Microcystis* strains before the probes are used for field samples. We will collaborate with Dr Swee Teh and Dr Dolores Baxa from UC Davis to develop and apply this method for quantifying in-situ zooplankton ingestion of *Microcystis*. Dr. Baxa has been developing the use of qPCR and successfully applied it for quantifying gene copy numbers of field *Microcystis* samples. We will work together to further develop this method for use in ingestion studies. Initial development will take place in Dr. Teh's laboratory at UC Davis, though the PCR laboratory at the Romberg Tiburon Center will also be available.

Laboratory ingestion and calibration:

Copepods will be fed *Microcystis* and collected for qPCR analysis as described above. A factorial design of three diet concentrations for at least two different (MC+ and MC-) strains of *Microcystyis* (25%, 50% and 100% *Microcystis* diet by carbon, bringing the total food to 500 $\mu gC.L^{-1}.day^{-1}$ using Instant Algae) with three feeding periods (1, 2, and 6 hours) will be used to allow adequate time for acclimation of copepods to the diet. Copepods will be starved 2 hours prior to experiment to allow gut evacuation. There will be three replicates per treatment, and each replicate will consist of 15 copepods in a 1 L glass beaker. Controls will be fed with the maintenance diet and include a starved control to validate methods (0%, 25%, 50%, and 100% of 500 $\mu gC.L^{-1}.day^{-1}$ Instant Algae diet). From each replicate, two groups of 5 copepods will be randomly selected, rinsed rapidly with deionized water to ensure complete removal of *Microcystis* from the water and exterior of copepods, and placed in a 1.5 ml plastic vial for PCR analysis.

In a separate section of the laboratory authorized for the use of radioactive substances, radiolabeled ^{14}C *Microcystis* will be fed to copepods as an independent measure of ingestion. Rinsed copepods will be filtered or collected in scintillation vials (in groups of 20-30/replicate) and stored for analysis by liquid scintillation (Hargis 1977, Rohrlack 1999). 250 ml of exponentially growing *Microcystis* will be spiked with 1 ml of 22 $\mu Ci\ H^{14}CO_3$ solution, and incubated for 24 hours. This spiked culture will be then fed to the copepods following the same experimental design described for incubation for qPCR. We will calculate the copepod ingestion rate (I) based on the ratio of activity per cell of *Microcystis* and activity per copepod according to the equation below (modified from Hargis 1977). We will also confirm *Microcystis* ingestion using epifluorescence microscopy.

$$I\ [cells.(copepod.hour)^{-1}] = (^{14}C\ ml.\ ^{14}C\ copepod^{-1})\ \times (cells.ml^{-1}) \times (exposure\ hours)^{-1}$$

The relationship between the two ingestion rates (radiolabeled versus qPCR) will give a calibration curve that can be used to estimate ingestion rates from qPCR data taken on copepods collected in the field (Oberholster et al 2006, Nejstgaard et al 2008).

Field collection of zooplankton and Microcystis: in-situ ingestion

Both zooplankton and *Microcystis* will be collected from the same site, with measures taken to minimize the release of toxins from damaged *Microcystis* to zooplankton during the tow. *Microcystis* is generally patchy in its distribution, forming aggregated scums due to wind and wave action. We will take advantage of this patchy distribution and collect zooplankton where *Microcystis* is minimal, and within the same general site, and collect *Microcystis* where it is most dense.

Zooplankton will be collected from various sites in the SFE including Antioch and Suisun Bay, before, during, and after the *Microcystis* bloom to provide a range of conditions. Collection will be done from boat and shore, from the surface layer, using a standard 147μm mesh net equipped with a flow meter. We will fill several previously cleaned 20 L carboys with ambient surface water from the same site, and dilute concentrated zooplankton in the carboys. *Microcystis* will be collected and concentrated using a zooplankton net tow from the surface, and stored in additional carboys until arrival at the RTC for

analysis. Temperature, salinity, and standard water quality parameters will be recorded for each site. We will make sure that the zooplankton and *Microcystis* tows, as well as the water collected have similar physical properties including salinity.

Zooplankton in carboys will be brought to RTC within two hours of each collection and separated into species, rinsed with filtered surface water from the same site and immediately frozen in liquid nitrogen to prevent the loss of gut contents. Specific zooplankton included in analysis will be *P. forbesi*, E. *affinis*, *A. sinensis*, *L. tetraspina*, as well as other copepods and cladocera if time permits. For each species, 5 adults will be placed in a 1.5 ml vial, and frozen immediately upon separation for PCR analysis of target *Microcystis* genes. The *Microcystis* abundance (μg C/L), water quality (dissolved oxygen, temperature, salinity, pH, conductivity), total chlorophyll, and zooplankton species composition will be determined for each site and sampling event. Collections site will be selected based on *Microcystis* and zooplankton abundance, and will likely include Antioch, and possibly Mildred Island, locations of the most dense blooms.

Mesocosm experiments: survival, ingestion, change in species composition

Using the carboys from above, we will evaluate the impact of *Microcystis* abundance on in-situ grazing rates, survival, and change in species composition (abundance of species *x*/total species abundance) of zooplankton. To do this, we will set up 20 L mesocosms in a light and temperature controlled chamber, and vary initial *Microcystis* biomass. There will be three treatments (low, ambient, and high *Microcystis*), and two replicates. The whole procedure will be repeated at least once more to allow temporal replication. Each container will be sampled every 3 hours during the first 12 hours, and then once every 24 hours for three days.

This setup will be repeated in the spring, prior to bloom formation. As there will be no *Microcystis* naturally, we will add known amounts (based on summer abundances) of cultured *Microcystis* to run the experiments in the same design explained above. This will measure the potential impact of *Microcystis* on the spring zooplankton community.

In the laboratory, *Microcystis* will be rinsed in filtered surface water, filled into graduated cylinders, and allowed to separate from foreign particles by letting it remain in filtered surface water. By its positively buoyant nature, *Microcystis* will concentrate at the surface and stabilize its biovolume after 30 minutes, making it possible to modify initial abundances. Once it stabilizes, *Microcystis* biomass for the mesocosm experiment will be manipulated using the biovolume of colonies. The relationship between *Micocystis* biomass and biovolume will be quantified by using previously established conversions. Biomass per volume will be measured in μg C (with a GC).

At least three samples of zooplankton (5 animals each) will be taken for each replicate and feeding period, zooplankton samples frozen immediately to minimize loss of gut contents, and later isolated to species as described above to quantify *Microcystis* ingestion, change in abundance over time (survival), and change in overall zooplankton community. Experiments will be terminated after three days and samples will be stored at -80^0C till analysis. A continuously circulating system will be maintained by using available supplies such as a motorized paddle at the bottom of each mesocosm to minimize settling of sediments.

Data analysis and modeling

Results will be analyzed using standard statistical techniques such as ANOVA, with graphical analysis used to ensure data meet the criteria for each technique used. Calibration between different ingestion measurements will be analyzed using standard regression methods. Results from the above experiments will be combined to develop simple population dynamics models of zooplankton based on *Microcystis*

abundance, initial zooplankton composition, and ingestion. These models will be used to predict the impact of *Microcystis* on the pelagic foodweb of the SFE, focusing on zooplankton resources for fish for which diet and ingestion rate information are becoming available. Finally, the models will be combined with a literature review from comparable estuaries with *Microcystis* blooms to try to identify long-term solutions to manage *Microcystis* in a changing estuary.

Output/Anticipated Benefits and Products

This project will provide a quantitative diagnosis of the mechanisms by which *Microcystis* impacts the SFE's pelagic foodweb. It will show the degree of impact to the food quantity and quality for zooplankton, and provide predictions for the response of the foodweb to future *Microcystis* blooms. Studies that focus on the trophic interactions at the base of the foodweb are critical for management of higher trophic levels such as fish. This project benefits the CALFED priority areas as it explores how zooplankton and the primary food resource to larval pelagic fish respond to the expanding blooms of *Microcystis*.

Connectivity and variation in food quality among habitats of the SFE is now emerging as an important research area as more information becomes available. The POD Management team compiled evidence for bottom-up food limitation as an important factor for the decline in pelagic organisms, yet finer scale observations point to new processes that regulate the system's response to changes. It remains unclear why *P. forbesi* has declined in the Western Delta and Suisun Bay but not in the Southern Delta (POD Management Team 2007). Total phytoplankton biomass (chlorophyll) did not decline in these regions between 1995-2004, but *P. forbesi* abundance showed different trends. Such differences raise questions about phytoplankton food quality and the importance of source populations for key copepods such as *P. forbesi*.

Food quality for pelagic zooplankton is determined by the relative contribution of various phytoplankton and microbial species to the total particle pool available to primary grazers (POD Management Team 2007). Areas rich in high quality phytoplankton particles (such as the southern Delta) may create critical source populations of fish prey including *P. forbesi*. *Microcystis* can reduce the amount of high quality phytoplankton ingested by *P. forbesi*, and threatening habitat quality in its source populations. While delta smelt are probably not directly affected by *Microcystis* blooms, the source populations of its preferred prey may be threatened in the Delta. The proposed project will address how *Microcystis* affects the feeding ecology of and habitat quality of zooplankton, focusing on copepods, making it possible to predict impacts to *P. forbesi* and its source populations.

This research will benefit the first CALFED priority area: Trends and Patterns of Habitats, Populations and System Response to a Changing Environment, where the goal is to develop research that includes responses of primary production, habitat quality and connectivity, ecosystem function and ecosystem services. We propose to quantify mechanisms by which *Microcystis* blooms can change phytoplankton food and subsequent habitat quality, as well as the pelagic ecosystem function. In addition, results can be used to predict how *Microcystis* impacts spread within the SFE due to foodweb connectivity and leakage of effects. Specifically, we will help explain how *Microcystis* impacts in the freshwater Delta of the SFE can affect zooplankton abundances in the low salinity zone, which is the summer habitat for many fish species. In addition, the proposed project will benefit CALFED's second priority area of Aquatic Invasive Species, by predicting the foodweb response to *Microcystis*, one of the species of concern.

Once developed, the use of DNA based biomarkers to monitor in-situ zooplankton ingestion of *Microcystis* will provide a simple and highly effective indicator of the fate of this toxic cyanobacteria in the pelagic foodweb as well as provide an advanced method to monitor species specific exposure in the future. Benefits from the application of this method will extend beyond the SFE, as *Microcystis* blooms

are causing problems in many parts of California, including the Russian River and Klamath River reservoirs.

Effective management actions to improve pelagic ecosystem function require more detailed investigations into the causes and mechanisms of the declines (Sommer et al 2007). Overall, CALFED and especially the POD management team will benefit from the results and predictions of this project because it will clarify the trophic interactions and feeding ecology of highly significant zooplankton species as a function of *Microcystis* abundance and strain, explaining the potential variation in zooplankton due to *Microcystis*. Specific products will be available as follows.

Project Timeline

Year 1: January 2009 - January 2010

- Prepare laboratory supplies and start copepod and *Microcystis* cultures (January – March 09)
- Develop PCR detection of *Microcystis* in *P. forbesi*, calibrate with radiolabels, quantify ingestion rates (March 09 – July09)
- Determine in-situ ingestion in field and effects on community (July 09 – October 09)
- Develop PCR analysis of field ingestion (October 09 – January 10)

Year 2: January 2010 – January 2011

- Data analysis, report and manuscript preparation (January 10 – March 10)
- Determine ingestion under pre-bloom conditions (March 10 - June 10)
- PCR analysis to determine effects on pre-bloom community (June 10-August 10)
- Data analysis, report, and manuscript preparation (August 10 – January 11)

Potential peer-reviewed publications that will result from this project can include: 1) Development of qPCR method for in-situ *Microcystis* ingestion, 2) In-situ ingestion of *Microcystis* in field zooplankton using qPCR, 3) Role of *Microcystis* in the survival, ingestion, and zooplankton community shifts in the SFE.

Benefits to fellow: I am a graduate student at UC Davis, working on toxic and nutritional effects of *Microcystis* on copepods in the laboratory. Continuing to work on *Microcystis*-zooplankton interactions is a logical step, and extending research beyond the laboratory to the actual foodweb implications using novel methods is highly attractive way to get involved in developing my interests, knowledge base, and skills. I will gain valuable experience by designing and managing several experiments, working with and meeting new colleagues from various scientific backgrounds and agencies, and first-hand exposure to the management challenges facing one of the most heavily impacted estuaries in the world. This is a fascinating opportunity to learn new skills, be in a stimulating environment for research ideas, meet future collaborators, and to prepare for a career in coastal ecology and management. The proposed work will create at least three manuscripts to be published in peer-reviewed journals which will help build my career. Additionally, I will be receiving post doctoral training in laboratory management, teaching, writing, and interviewing skills from Dr. Kimmerer. Finally, this project will get me further involved with my interest to look at changes in coastal foodweb processes and ecosystem function due to human activity.

Benefits to research mentor: Dr.Wim Kimmerer has several ongoing projects dealing with food abundance for pelagic fish. Processes that affect zooplankton abundance and diversity directly relate to and enhance these projects. Understanding the effects of changes in growth rate and mortality processes is key to understanding changes in abundance. Although traditionally studies of these processes have

focused on food quantity and predation, there is growing interest in the effect of food quality and specifically biogenic toxins, which can influence zooplankton and fish through a variety of mechanisms.

Benefits to community mentor: Dr. Anke Mueller-Solger will be involved with the development and application of novel methods to quantify the fate and impact of *Microcystis* to the pelagic foodweb. As a senior environmental scientist at the Department of Water Resources, and the current chair of the POD Management Team, Dr. Mueller-Solger has extensive experience with trophic interactions and food limitation in the pelagic foodweb, a genuine interest in alternative trophic pathways at the base of the foodweb, and a desire to see new molecular methods such as DNA based biomarkers to quantify trophic interactions.

Benefits to CALFED: Improving water quality, ecosystem health and function, and reversing the decline in the pelagic foodweb of the SFE are central objectives of CALFED. The recent decline in pelagic fish and the listing of Delta smelt have put pressure to find causes of such trends, yet it has become clear that a more holistic understanding of the pelagic foodweb is necessary for reversing the decline in pelagic fish and their dominant food source. The increasingly frequent *Microcystis* blooms present a potential threat to CALFED's basic objectives of supporting water quality and ecosystem health. Please see above for the specific benefits to CALFED management objectives.

References cited

Anderson D. and Garrison D. 1997. Preface to the special issue for the Ecology and Oceanography of Harmful Algal Blooms. LIMNOLOGY AND OCEANOGRAPHY, 42(5) part 2.

Bay Delta Tributaries Project. Online Database available at http://bdat.ca.gov

Bouley, P. and W.J. Kimmerer. 2006. Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. MARINE ECOLOGY PROGRESS SERIES 324: 219-228.

Chorus I and Bartram J (Eds). 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management. SPON Press, on behalf of the World Health Organization.

Christoffersen K. 1996. Ecological implications of cyanobacterial toxins in aquatic food webs. PHYCOLOGIA, v. 35(supplement 6): 42-50.

DeMott WR, and Moxter F. 1991. Foraging on cyanobacteria by copepods: responses to chemical defenses and resource abundance? ECOLOGY, 75(5): 1820-1834

.

DeMott WR.1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. FRESHWATER BIOLOGY, 33 (2), 171–180

DeMott WR and Tessier A. 2002. Stoichiometric constraints vs. algal defenses: testing mechanisms of zooplankton food limitation. ECOLOGY, 83(12):3426-3433.

Ghaouani A, Pinel-Alloul B, Prepas EE. 2003. Effects of experimantall induced cyanobacterial blooms on crustacean zooplankton communities. FRESHWATER BIOLOGY, 48:363-381.

Gasparani S and Castelt J. 1997. Autotrophic and heterotrophic nanoplankton in the diet of the estuarine copepods *Eurytemora affinis* and *Acartia bifilosa*. JOURNAL OF PLANKTON RESEARCH 19(7):877-890.

Gasparini S, Castel J, Irigonen X .1999 Impact of suspended particulate matter on egg production of the estuarine copepod, *Eurytemora affinis.* JOURNAL OF MARINE SYSTEMS, 22(2-3):195-205

Ger KA, Teh SJ, Goldman CR. 2007 – SUBMITTED. Acute and Chronic effects of dissolved MC-LR on survival and population growth of dominant calanoid copepods of the San Francisco Estuary

Graneli, E; Turner, JT (Eds). 2006. Ecology of Harmful Algae, Ecological studies 189. Springer ISSN 0070-8356

Hansson L-A, Gustaffson S, Rengefors K, Bomark L. 2007. Cyanobacterial chemical warfare affects zooplankton community composition. FRESHWATER BIOLOGY 52:1290-1301.

Hargis JR. 1977. Comparison of techniques for the measurement of zooplankton filtration rates. LIMNOLOGY AND OCEANOGRAPHY, 22(5):942-945.

Hoffmann GE and Gaines SD. 2008. New Tools to Meet New Challenges: Emerging Technologies for Managing Marine Ecosystems for Resilience. BIOSCIENCE 58(1):43-52.

Jassby, AD, Cloern JE, Cole BE. 2002. Annual primary production: patterns and mechanisms of change in a nutrient rich tidal ecosystem. LIMNOLOGY AND OCEANOGRAPHY, 47(3):698-712

Kimmerer, W. 2004. Open Water Processes of the San Francisco Estuary: From Physical Forcing to Biological Responses. San Francisco Estuary and Watershed Science. Vol. 2, Issue 1 (February), Article http://repositories.cdlib.org/jmie/sfews/vol2/iss1/art1

Kirk K, Gilbert J. 1992. Variation in herbivore responses to chemical defenses zooplankton foraging on toxic cyanobacteria. ECOLOGY, v. 73 (6): 2208-2217.

Koski M, Engstrom, J, Viitasalo, M. 1999. Reproduction and survival of the calanoid copepod *Eurytemora affinis* fed with toxic and non-toxic cyanobacteria. MARINE ECOLOGY-PROGRESS SERIES, v186: 187-197

Kumar R. 2003. Effect of different food types on developmental rates and demographic parameters of *Phyllodiaptomus blanci* (Copepoda:Calanoida). ARCHIVES FUR HYDROBIOLOGIE v. 157(3):351-377.

Lampert W. 1987. Laboratory studies on zooplankton-cyanobacteria interactions. NEW ZEALAND JOURNAL OF MARINE AND FRESHWATER RESEARCH, 21: 483-490.

Lehman PW, Boyer G, Hall C, Waller S, Gehrts K. 2005. Distribution and toxicity of a new colonial *Microcystis aeruginosa* in the San Francisco Bay Estuary, California. HYDROBIOLOGIA, 541:87-99.

Lehman PW, Boyer G, Satchwell M, Waller S. 2008, The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcystins concentration in San Francisco Estuary. HYDROBIOLOGIA, 600:187-204

Muller-Solger, A, Jassby AD, Muller-Navarra D. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). LIMNOLOGY AND OCEANOGRAPHY, 47(5): 1468-1476.

Mueller-Solger AM, Hall CJ, Jassby AD, and Goldman CR. 2006. Food resources for zooplankton in the Sacramento-San Joaquin Delta. Final Report to the Calfed Ecosystem Restoration Program, May 2006.

Nadini S. 2003. Responses of rotifers and cladocerans to *Microcystis aeruginosa*: A demographic study. AQUATIC ECOLOGY 34:227-242.

Nejstgaard JC, Frischer ME, Simanelli P, Troedsson C, Brakel M, Adiyaman F, Sazhin AF, Artigas LF. 2008. Quantitative PCR to estimate copepod feeding. MARINE BIOLOGY, 153:565-577.

Ouellette JA and Wilhelm S. 2003. Toxic cyanobacteria: the evolving molecular toolbox. FRONTIERS IN ECOLOGY AND ENVIRONMENT, 1(7):359-366.

Oberholster PJ, Botha AM, Cloete TE. 2006. Use of molecular markers as indicators for winter zooplankton grazing on toxic benthic cyanobacteria in an urban Colarado Lake. HARMFUL ALGAE, 5:705-716.

Paerl HW. 1988. Nuicance phytoplankton blooms in coastal, estuarine, and inland waters. LIMNOLOGY AND OCEANOGRAPHY, 33(4,part2):823-847.

PanossoR, Carlsson P, Kozlowsky-Suzuki B, Azevedo SMFO, Graneli E.2003. Effect of grazing by a neotropical copepod Notodiaptomus, on a natural cyanobacterial assemblage of toxic and non-toxic cyanobacterial strains. JOURNAL OF PLANKTON RESEARCH, 25(9):1169-1175.

POD Management Team 2007. 2007. Pelagic Organism Decline Progress Report: Synthesis of Results. Baxter R, Rich Breuer R, Chotkowski M, Feyrer F, Gingras M, Herbold B,Mueller-Solger AM, Nobriga M, Sommer T, Souza K.

Reynolds, CS and Jaworski GHM. 1978. Enumeration of natural *Microcystis* Populations. BRITISH PHYCOLOGY JOURNAL, 13: 269-277.

Rohrlack T, Christoffersen K, Dittmass E, Nogueira I, Vasconcelos V, Borner, T. 2005. Ingestion of microcystins by Daphnia: Intestinal uptake and toxic effects. LIMNOLOGY AND OCEANOGRAPHY, 50(2):440-448.

Smith AD, Gilbert LG. 1995. Relative susceptibilities of rotifers and cladocerans to *Microcystis aeruginosa*. ARCHIV FUR HYDROBIOLOGIE, 132(3):309-336.

Stahl-Delbanco A. Hansson L, and Gyllstrom. 2003. Recruitment of resting stages may induce blooms of Microcystis at low N:P ratios. JOURNAL OF PLANKTON RESEARCH, 25(9):1099-1106.

Stibor H, Vadstein O, Diehl S, Hansen T, Hantzche F, Katechakis A, Lippert B, Loseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y. 2004. Copepods act as a switch between alternative trophic cascades in marine pelagic foodwebs. ECOLOGY LETTERS, 7:321-328.

Sommer T, Armor C, Baxter R, Breuer R, Brown L, Chotkowski M, Culberson S, Feyrer F, Gingras M, Herbold B, Kimmerer W, Mueller-Solger A, Nobriga M, Souza K. 2007. The collapse of pelagic fishes in the upper San Franciso Estuary. FISHERIES,32(6): 270-277.

Wilson, AE; Sarnelle, O; Tillmanns, AR. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. LIMNOLOGY AND OCEANOGRAPHY, 51(4): 1915-1924.

Zurawell RW, Chen H, Burke JM, Prepas EE. 2005. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, Part B, 8:1-37.