ERP Proposal Application Form

Section 1: Summary Information

1. Project title:	Water Quality Monitoring in the Cache Slough Complex
2. Applicant name:	University of California at Davis, Aquatic Toxicology Laboratory
3. Contact person:	Dr. Swee Teh
4. Address:	VM:APC, 1321 Haring Hall, University of California at Davis
5. City, State, Zip:	Davis, CA 95616
6. Telephone #:	(530) 754-8183
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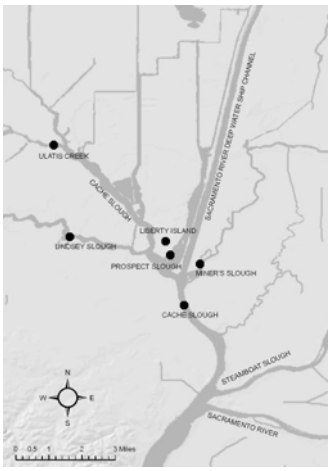
8. Email address:	sjteh@ucdavis.edu
9. Agency Type:	Federal Agency ☐ State Agency ☐ Local Agency ☐ Nonprofit Organization ☐ University (CSU/UC) ☒ Native American Indian Tribe ☐
10. Certified nonprofit organization:	Yes ☒ No ☐
11. New grantee:	Yes ☒ No ☐
12. Amount requested:	**$993,488** for the primary tier and $501,516 for a secondary optional tier
13. Total project cost:	$1,060,336 for the primary tier and $514,668 for a secondary optional tier
14. Topic Area(s):	(1) Ecosystem Water and Sediment Quality (2) Estuary Food Web Productivity
15. ERP Project type:	(1) Monitoring (2) Research
16. Ecosystem Element:	(1) Contaminants (2) Freshwater Fish Habitats, Delta Sloughs
17. Water Quality Constituent:	(1) Pesticides (2) Toxicity of Unknown Origin and Contaminants, Trace Metals, Persistent Organic Contaminants and Endocrine Disrupting Chemicals
18. At-Risk species benefited:	Sacramento splittail, Delta smelt, Chinook salmon and Longfin smelt
19. Project objectives:	The objective of the proposed work is to clarify both the sources and the ecological importance of contaminants in the Cache Slough Complex by evaluating potential impacts on resident species at six sites within the area. Several monitoring tools will be utilized including laboratory toxicity tests, *in situ* monitoring, bioassessment using artificial substrates, analytical chemistry, effect concentration testing and biomarker endpoints.
20. Time frame:	October 1, 2011 – September 30, 2013 for the primary tier. October 1, 2011 – September 30, 2014 if the secondary tier (additional monitoring) is included.

Section 2: Location Information

1. Township, Range, Section: and the 7.5 USGS Quad map name.	T6N,R3E,sec29: Liberty Island T4N,R3E,sec10: Rio Vista
2. Latitude, Longitude (in decimal degrees, Geographic, NAD83):	Cache Slough Integrator: 38.221, -121.673 Prospect Slough: 38.243, -121.679 Lindsey Slough: 38.251, -121.723 Ulatis Creek: 38.291, -121.730 Miner's Slough: 38.239, -121.666 Liberty Island: 38.249, -121.681
3. Location description:	The Cache Slough Complex is a region located in the north Delta where Cache Slough and the southern Yolo Bypass meet. All monitoring sites are located on various waterways within this complex, and will be sampled exclusively by boat. *In situ* exposure sites and artificial substrate samplers will be located as close to the monitoring sites as possible. Surface water samples will be collected mid-channel, while *in situ* testing and benthic macroinvertebrate monitoring will be conducted near shore. 
4. County(ies):	Sacramento County and Solano County.
5. Directions:	All sampling and *in situ* sites will be accessed by boat. The Rio Vista boat launch, located on Rivermile 13 of the Sacramento River, will be the starting point for each field event. To reach the Cache Slough Complex monitoring sites, travel upstream on the Sacramento River to reach the confluence of the Sacramento River and Cache Slough. Continue traveling beyond the confluence, up Cache Slough to access all sampling sites located in the various sloughs of the complex.
6. Ecological Management Region:	Sacramento-San Joaquin Delta and Yolo Basin
7. Ecological Management Zone(s):	Delta and Alluvial River Floodplain
8. Ecological Management Unit(s):	North Delta
9. Watershed Plan(s):	Not applicable
10. Project area:	The Cache Slough Complex encompasses approximately 45,000 acres of open water, marsh, floodplain, and riparian habitat.

11. Land use statement:	Agriculture is currently the dominant land use practice in the Cache Slough area. Urban and industrial use is also present, but limited. Land use practices are not expected to change over the next five years.
12. Project area ownership:	% Private_____ % State___100___ % Federal_____ *Enter ownership percentages by type of ownership*.
13. Project area with landowners support of proposal:	Not applicable

Section 3: Landowners, Access and Permits

1. Landowners Granting Access for Project: (Please attach provisional access agreement[s]) Not applicable	
2. Owner Interest: Not applicable	
3. Permits: Not applicable	
4. Lead CEQA agency: Not applicable	
5. Required mitigation:	Yes ☐ No ☒

Section 4: Project Objectives Outline

1. <u>List task information:</u>

The specific goals of this proposal address the ERP Strategic Goals and Objectives (Appendix D), ERP Stage 2 Conservation Strategy priority topic areas of **Goal 6: Water and Sediment Quality- Objective 1 and 2**. The proposal primary ERP topic areas are 1) **Ecosystem Water and Sediment Quality** and 2) **At-Risk Species Assessment.** This proposal is a **research monitoring** project type and the primary ecosystem element is **Stressors.**

Toxic contaminants may be one of several factors acting to lower pelagic productivity in the Delta. The goal of the proposed work is to assess the role of chemical contaminants in the observed decline of pelagic species in the Cache Slough area of the Bay-Delta estuary, an important nursery for pelagic species that has been targeted for habitat restoration, but where toxicity has been observed on multiple occasions in recent years (California DWR 2007, Werner *et al.* 2010). The proposed project would bring the ERP closer to accomplishing the objective of eliminating toxic impacts in the Bay-Delta estuary by quantifying the distribution of toxicity in the Cache Slough area and by suggesting candidate activities and substances that appear to be the sources of the toxicity. This project would include estimation both of direct effects of contaminants on fish health and of indirect impacts on fish populations caused by water quality conditions toxic to species at lower trophic levels by utilizing a comprehensive toxicity monitoring approach.

Our proposed project is intended to conduct research that tests hypotheses identified in the Delta Regional Ecosystem Restoration Implementation Plan (DRERIP) Evaluation Summary Report with regards to water quality. Laboratory toxicity tests will evaluate the hypothesis that test organisms exposed to ambient samples within the Cache Slough Complex will exhibit reduced performance relative to laboratory controls and that test organism performance differs between ambient samples. *In situ* exposures will test the hypothesis that test organism performance differs between ambient samples. Comparison of effect concentration data to detected chemical concentrations will test the hypothesis that chemical analysis has revealed contaminants sufficient to account for the severity of toxicity present in the ambient sample. Artificial substrate samplers will test the hypothesis that macroinvertebrate communities colonizing the artificial substrates during the same sampling event differ between sampling locations. This comprehensive approach will aid the ERP's vision for the strategic objective of reducing toxicant loads in the Bay-Delta watershed. The results of this project can also be used to support existing programs for controlling agricultural and urban point and non-point substances.

2. Additional objectives:

The results of our study can also be used to determine what impact, if any, concurrent habitat restoration has on water quality, as our proposed testing sites are adjacent to sites slated for restoration.

3. Source(s) of above information:

California DWR. 2007. Delta Risk Management Strategy – Phase 2: Evaluation of Risk Reduction Strategies.

Werner, I., L.A. Deanovic, C. Reece, D. Markiewicz, M. Stillway, J. Khampanh. 2010. Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008 – 2010. Final Report to the Interagency Ecological Program, California Department of Water Resources.

Section 5: Conflict of Interest

To assist ERP staff in managing potential conflicts of interest as part of the review and selection process, we are requesting applicants to provide information on who will directly benefit if your proposal is funded. Please provide the names of individuals who fall in the following categories:

- Persons listed in the proposal, who wrote the proposal, will be performing the tasks listed in the proposal, or who will benefit financially if the proposal is funded; and/or
- Subcontractors listed in the proposal, who will perform tasks listed in the proposal, or will benefit financially if the proposal is funded.

Primary Contact for Proposal: Swee Teh
Primary Investigator: Swee Teh
Co-Primary Investigator: Richard Breuer (DWR) and Kean Goh (DPR)
Supporting Staff: Linda Deanovic
Subcontractor: None

Provide the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

Last Name	First Name	Organization	Role
Markiewicz	Dan	UCD	Writer
Stillway	Marie	UCD	Writer
Reece	Charissa	UCD	Writer

Section 6: Project Tasks and Results Outline

1. Detailed Project Description

The San Francisco Estuary (SFE) is the largest estuary on the West Coast, comprised of a complex ecosystem formed by the convergence of freshwater from the Sacramento-San Joaquin Rivers (SSJR) and the Pacific Ocean. The estuary has been markedly altered by human activities over the last 150 years, which has caused dramatic declines in the abundance of many species of fish and other organisms across the food web. These declines have been attributed to habitat degradation and loss, food limitation, and to the toxicity of legacy and contemporary environmental contaminants. Declines in both estuarine zooplankton and native fish suggest a trophic linkage between the species. Furthermore, the conceptual model of the Pelagic Organism Decline (POD) proposes that bottom-up interactions from nutrient loading and contaminant inputs may be contributing to the profound decline in fisheries (POD Report 2007).

The goals of the proposed work are to 1) assess the role of chemical contaminants in the observed decline of pelagic species in the Cache Slough Complex, including impacts to lower trophic levels; 2) identify when possible the class of chemical(s) causing any toxicity observed; and 3) apply the results of this study towards the reduction of contaminant

loads and concentrations of toxic contaminants in the Bay-Delta, with the ultimate goal of these waters being free from ecologically damaging concentrations of toxic substances.

The Cache Slough is a freshwater tidal marsh in the northern reaches of the upper SFE (Moyle 2008) that drains into the Moore Tract and Yolo Bypass, and receives tidal fluctuation from the Sacramento River (Lund *et al.,* 2008). The slough is considered a productive site due to its relatively high primary productivity with abundant zooplankton and larval fish (Lund *et al,* 2008). The slough also supports spawning and nursery habitat, notably Liberty Island, for native fish such as Delta smelt, Sacramento splittail and Chinook salmon (Moyle *et al* 2004, Moyle 2008, Sommer *et al*. 2001).The Cache Slough area has been targeted for habitat restoration efforts aimed at reestablishing healthy populations of native fish species (California DWR 2007). However, a likely obstacle to the restoration of fish populations is the impacts of water-borne contaminants. A four-year toxicity monitoring study by the UC Davis Aquatic Toxicology Laboratory (ATL), supported by the Department of Water Resources (DWR) and Interagency Ecological Program, revealed toxicity to *H. azteca* in water samples collected downstream from the confluence of Cache Slough and Ulatis Creek on multiple occasions (Werner *et al.* 2010). **The objective of the proposed work is to clarify both the sources and the ecological importance of contaminants in the Cache Slough area by evaluating potential impacts on resident species at a variety of sites throughout the area.** Restoration efforts can only succeed if levels of contaminants in the Cache Slough Complex are low enough to allow the existence of robust aquatic communities capable of supporting sustainable populations at every trophic level.

We propose a research plan that combines comprehensive contaminant and toxicity monitoring of ambient water from the important larval smelt and splittail habitats of Cache Slough Complex, habitats associated with good primary production, through the use of (i) field and laboratory bioassays of water column toxicity to phytoplankton, zooplankton, amphipods, and native fish of California, (ii) comprehensive chemical analysis of toxic samples, (iii) effect concentration testing to examine the sensitivity of test organisms to detected contaminants, (iv) bioassessment of benthic macroinvertebrates colonizing artificial substrates, and (v) investigation of biomarkers characteristic of exposure to water from various sampling locations, water quality conditions and toxic events. Six (6) sites will be monitored throughout the Cache Slough area from February 2012 to January 2013. A second year of monitoring is optional which would extend the monitoring period to January 2014.

Test species used in bioassays will include phytoplankton (the diatom *Thallasiosira pseudonana*), zooplankton (the calanoid copepods *Eurytemora affinis* or *Pseudodiaptomas forbesi*), amphipods (the epibenthic organism *Hyalella azteca*) and fish (the Sacramento splittail, *Pogonichthys macrolepidotus*). Laboratory toxicity bioassays will test the hypothesis that test organisms exposed to ambient samples show reduced performance relative to laboratory controls and that test organism performance differs between ambient samples. After laboratory exposure to ambient samples and control water, larval splittail will be raised in control water for an additional month to assess biomarker responses. Field toxicity tests will involve deployment of *in situ* devices submerged in the waterway. These *in situ* exposures will involve *H. azteca* and Sacramento splittail, but not the plankton species, which are too small to be feasibly included in such testing. Field bioassays will test the hypothesis that test organism performance differs between ambient samples.

In the event that ambient water is found to be acutely toxic in laboratory testing, a comprehensive chemical analysis will be performed by the Co-Principal Investigator, Dr. Kean Goh (Department of Pesticide Regulation, in-kind). Chemical analysis will include pyrethroid, organophosphate, carbamate, and metal scans to ensure that a full spectrum of chemical classes is identified. These particular scans were chosen in part due to the northern Delta waterways' 303(d) listing for chlorpyrifos, DDT, diazinon, group A pesticides, selenium, mercury, PCBs and unknown toxicity. Chlorpyrifos is one of the most heavily used agricultural insecticides, and has recently been shown to be present at toxic concentrations in Ulatis Creek and agricultural drains (California Regional Water Quality Control Board Agricultural Waiver Program, 2007). Diazinon, cyfluthrin, bifenthrin and permethrin were detected in 2007, in water column samples from various sites in the Delta (Werner *et al.*, 2008).

Results of chemical analyses of samples exhibiting acute toxicity will be compared to existing published effect concentration values for sensitive resident species, such as those found in the US Environmental Protection Agency (EPA) Ecotox Database, to determine if elevated contaminant concentrations are likely to have caused toxicity to a specific test species. In cases of significant analytical detections for which published effect concentration data are not available, effect concentration tests will be performed with the affected species to determine the number of toxic units present in the acutely toxic sample and to expand the knowledge of the sensitivities of species resident in the Delta. Comparison of effect concentration data to detected chemical concentrations will test the hypothesis that chemical analysis has revealed contaminants sufficient to account for the severity of toxicity present in the ambient sample.

Bioassessments will involve the deployment of Hester-Dendy artificial substrates in order to avoid the confounding effects of between-site differences in substrate and to highlight differences in macroinvertebrate community diversity and

abundance associated with differences in water quality. Hester-Dendy plates will be deployed during two (2) five-week-long colonization events, once in April 2012 and again in October 2012. The timing of the colonization events was chosen to coincide with established index periods for the examination of benthic macroinvertebrate (BMI) communities, and the October event is expected to capture the effects of contamination by first flush runoff. Five (5) replicate plates will be deployed at each site. Subsamples of macroinvertebrates colonizing the artificial substrates will be counted and identified to family. This level of taxonomic resolution allows rapid processing of samples, requires only a moderate level of taxonomic expertise, and can provide an assessment of biological condition nearly as precise as identification to genus (Pond *et al.,* 2008). Macroinvertebrate community structure will be quantified using an array of metrics including the recently developed multimetric Central Valley IBI (Rehn *et al.,* 2008). Due to the use of artificial substrates, these measures of community integrity will not be comparable to measurements involving sampling of natural instream habitats. We will test the hypothesis that macroinvertebrate communities colonizing the artificial substrates during the same sampling event differ between sampling location.

Field and laboratory bioassay data, chemistry results, effect concentration values, and bioassessment data will be synthesized to evaluate the limitations that water-borne contaminants may place on the health of pelagic populations in the Cache Slough area. Additionally, the California Department of Pesticide Regulation's Pesticide Use Reporting database, as well as U.S. Geological Survey's National Land Cover database, will be consulted in order to examine associations between toxic events, land use practices, and pesticide application, with the ultimate goal of identifying potential sources of toxic contaminants. This comprehensive approach will aid the ERP's vision for the strategic objective of reducing toxicant loads in the Bay-Delta watershed. The results of this project can also be used to support existing programs for controlling agricultural and urban point and non-point substances, such as identifying contaminants for future development of TMDLs and meeting Basin Plan objectives.

Results and evaluations from this collaborative effort will be made available either as an electronic archive on the Web to CALFED or as a series of publications in peer-reviewed scientific journals (*e.g.,* CALFED Science Journal: *San Francisco Estuary and Watershed Science*). For quality assurance and quality control (QA/QC), this study will follow the detailed methodology of the SWAMP Quality Assurance Project Plan. This will allow for comparisons between this study's laboratory results and other related monitoring studies in the CALFED region.

Data collected from this monitoring study will provide information that can be used to address the following questions: 1) Are contaminant effects in the Cache Slough Complex becoming more or less prevalent? 2) Is water quality adequate for the recovery of at-risk fish species?

2. Background and Conceptual Models

In the last several years, abundance indices of numerous pelagic fish species residing in the Sacramento-San Joaquin Delta of California have shown marked declines and record lows for the endemic Delta smelt, age-0 striped bass, longfin smelt, and threadfin shad (Stevens and Miller, 1983; Stevens *et al*., 1985; Moyle *et al*., 1992; Moyle and Williams, 1990). While several of these species have shown evidence of long-term declines, there appears to have been a precipitous "step-change" to very low abundance during the period 2002-2004 (Sommer *et al*. 2007). Toxic contaminants are one of several factors acting individually or in concert to lower pelagic productivity. Contaminant toxicity has been documented in shellfish, fish, mammal and bird species from the Bay-Delta, with the most serious contaminant problems in the Bay-Delta and its main stem rivers and tributaries coming from mine drainage, agricultural drainage and urban runoff (Vol. 1 ERPP, 2000).

Three fundamental elements have been recommended by the US EPA as a comprehensive approach to contaminants control (US EPA, 1991) and form the foundation of this proposal. These three elements of biomonitoring include toxicity testing, bioassessment and analytical chemistry. In addition, *in situ* monitoring provides an additional contribution to evaluating contaminants by providing the mechanism to perform "real time" toxicity testing.

Pelagic fish may be affected by contaminants directly, and contaminants may also reduce the health of populations of other organisms in the food web on which pelagic fish depend. In our study design, we propose to examine the toxicity of water-borne contaminants to resident organisms in three of the lower trophic levels. We have chosen species that are easily obtainable, and are tractable for laboratory culture and toxicity testing in the laboratory/field. This proposed study will include larval stages of the endemic Sacramento splittail, a *species of special concern* in the Delta. The calanoid copepods *P. forbesi* and *E. affinis* will be used because they are abundant in the upper SFE and are an important food source to key fish species (IEP 1987, Meng and Orsi 1991) including the endangered Delta smelt (Lott 1998, Nobriga 2002). These copepods also demonstrate considerable variability in spatial and seasonal abundance in certain habitats of the upper SFE (Obrebski *et al* 1992) such as Cache Slough. The amphipod *H. azteca* has been used extensively in our

previous pelagic organism decline studies (Werner *et al*, 2010) and is very sensitive to current use pyrethroid insecticides. The traditional US EPA surrogate species Selenastrum capricornutum is not salt tolerant enough to be used in toxicity tests of Bay-Delta water samples. Consequently, we will use a euryhaline diatom (*T. pseudonana)* as a better representation of important phytoplankton species in the SF Delta.

The conceptual model (Figure 1) outlines our approach to evaluating contaminants in the Cache Slough Complex. The specific site locations for water sample collections are illustrated in Figure 2.

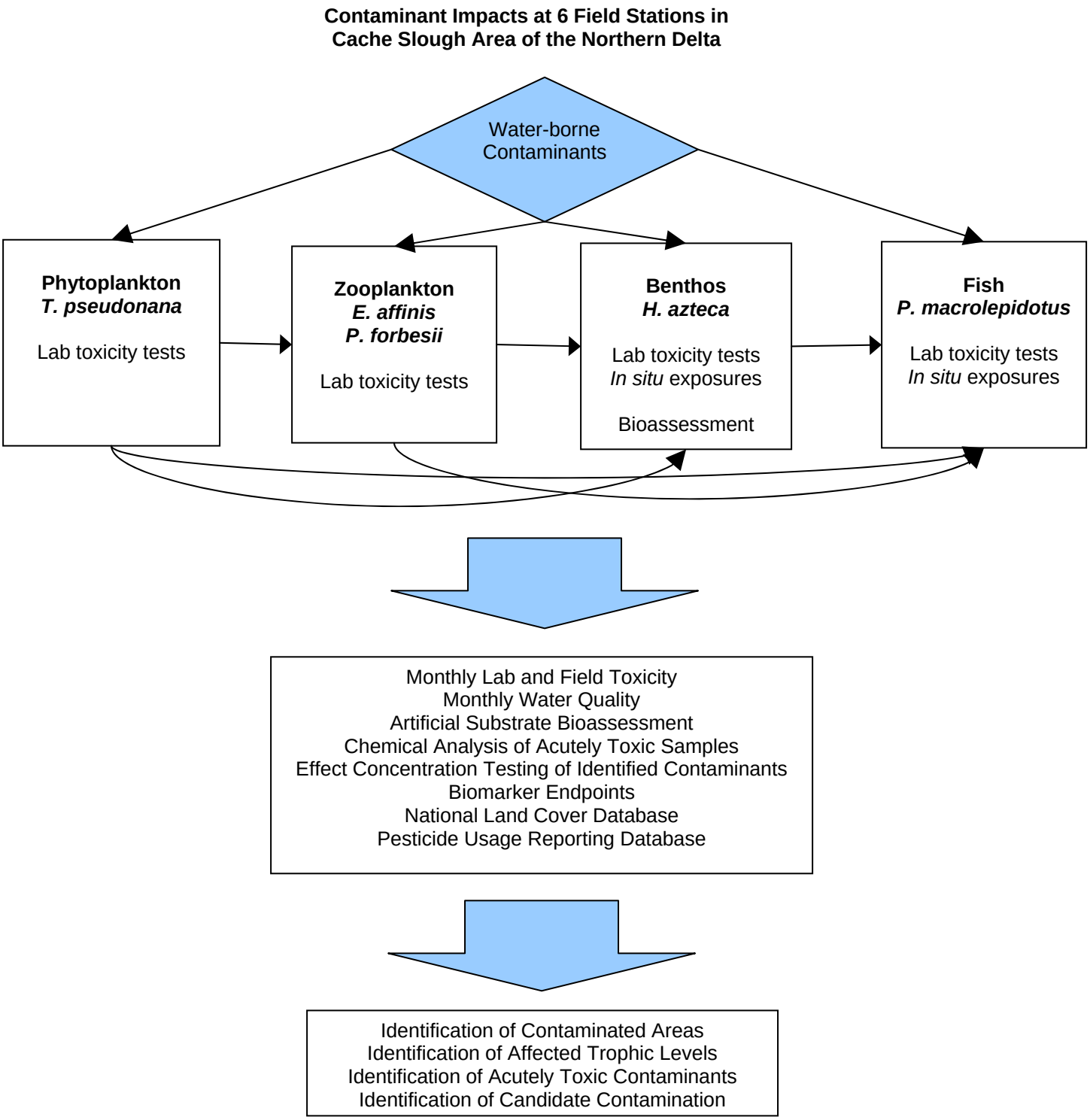


Figure 1. Conceptual model of the potential direct and food-web-mediated effects of contaminants on pelagic organisms in the Cache Slough area, including proposed measurements of contaminant effects, names of resident species to be used in toxicity bioassays, multiple lines of evidence to be collected, and final conclusions of the proposed project.

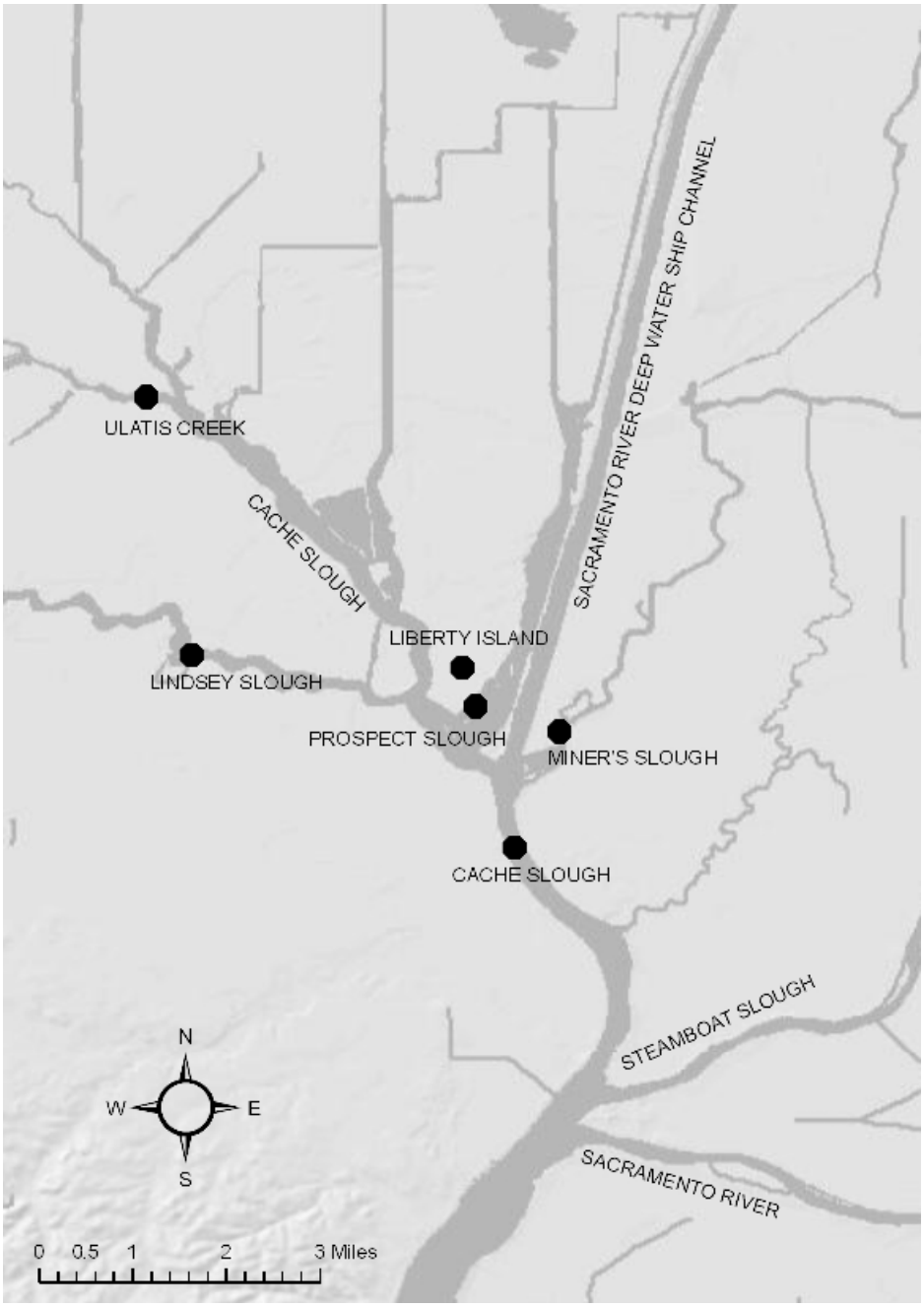


Figure 2. Proposed sampling locations in the Cache Slough Complex

3. Approach and Scope of Work

Our monitoring work will be conducted in collaboration with DWR, the California Department of Pesticide Regulation (DPR) and the UC Davis Department of Wildlife, Fish, and Conservation Biology. DWR is providing in kind services that support field work during the monitoring period, including personnel for site reconnaissance and *in situ* deployment up to 14 days per monitoring year. In addition, DWR will occasionally provide a boat in instances when the UC Davis boats are reserved. DPR is providing all analytical services associated with this project, including the analyses of multiple pesticide classes and metals. The UC Davis Department of Wildlife, Fish, and Conservation Biology will provide a boat operator and boat for up to 40 days per monitoring year.

3.1 Water Sample Collection

This project will consist of rain events and pre-determined sampling events at six locations in the Cache Slough Complex. Rain event samples will be collected following the first flush rain event as well as three precipitation based events within the dormant spray season. Sites selected for this project will include 1) Cache Slough downstream of Miner's Slough, 2) Miner's Slough, 3) Prospect Slough, 4) Ulatis Creek, 5) Lindsey Slough, and 6) Liberty Island. Site water will be collected by boat as mid-channel sub-surface grabs every month during the monitoring portion of this project. In addition to sample collection, field data, GPS coordinates and water quality data will be recorded at each site. Ambient samples collected in the field will be delivered to the UCD ATL for laboratory toxicity testing.

3.2 Laboratory Toxicity Testing

Toxicity testing will be conducted at UCD ATL and *in situ* at sample collection sites. Quality assurance measures will be included in this project to ensure the reliability of data generated in this project. Reference Toxicant tests will be conducted monthly to determine whether test species are responding typically during this study. Precision of monitoring endpoints will be determined through field duplicate samples and contamination will be evaluated through bottle blank samples. Species and methods to be utilized are outlined below.

3.2.1 *H. azteca*

Acute 96-hour water column toxicity testing for *H. azteca* will be based on sediment protocols outlined in Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (USEPA, 2000), and UCD Granite Canyon's Standard Operating Procedures Manual (Granite Canyon, 2007).

Organisms will be obtained from Aquatic Research Organisms (Hampton, NH). Prior to toxicity test initiation, animals will be placed into an environmental chamber maintained at 23 ± 2 ºC as specified in US EPA (2000), and acclimated to laboratory conditions for 48-hours.

Tests will consist of five replicate 250 ml glass beakers, each containing one square inch piece of Nitex® screen for artificial substrate, 100 ml of sample and 10 organisms. Tests will be initiated with 7-14 day-old organisms. Eighty percent of the test solution will be renewed at 48-hours, at which time debris and dead animals will be removed. Organisms will be fed 1 ml of food mixture per beaker at test initiation and after water renewal on day 2. Test vessels will be incubated in a temperature-controlled environmental chamber or water bath maintained at 23 ± 2 ºC with a 16-hour light: 8-hour dark photoperiod. Mortality will be measured daily.

3.2.2 Sacramento splittail

Acute 96-hour toxicity testing for Sacramento splittail will be based on protocols outlined in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (US EPA, 2002).

We have experience in inducing wild-capture and laboratory-raised adult Sacramento splittail to spawn in our laboratory (CALFED# 99-N07). We will perform induced spawning of adult Sacramento splittail and culture the larval splittail for this study. Prior to toxicity test initiation, animals will be placed in a temperature-controlled water bath and acclimated to laboratory conditions until their use in a test. Organisms will be tested at temperatures within their physiological tolerance and as close to the average field temperature for each sampling event.

The Sacramento splittail acute sublethal toxicity tests have been performed in our lab (Teh *et al,* 2004 and Teh *et al,* 2005) and will be used with modification. Tests will consist of four replicate 600 ml glass beakers each containing 250 ml of sample and ten organisms. Tests will be initiated with 21-day old splittail larvae. Eighty percent of the test solution will be renewed daily, at which time debris and dead fish will be removed from the test chamber. Fish will not be fed for the duration of the exposure. Tests will be conducted within the fish's physiological tolerance and as close to the average field temperature as possible, under a 16-hour light: 8-hour dark photoperiod in a temperature-controlled water bath. Mortality will be assessed daily. At test termination, the surviving fish will be raised in clean water for an additional month. Splittail will be sacrificed; gross examination for deformity and disease, measurements and weights will be used to determine growth and condition index. Cytochrome P450s, choriogenin, and vitellogenin will be analyzed to determine biomarker of exposure, and histopathology will be analyzed to determine biomarker of effects of contaminants.

3.2.3 Diatom

Toxicity testing for the estuarine or marine diatom *T. psdeuonana* will follow procedures described in the Guide for Conducting Static 96-hr Toxicity Test with Microalgae (ASTM E 1218-97a), and procedures outlined in Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (USEPA, 2002).

Cultures originally obtained from the University of Texas at Austin, the Culture Collection of Algae (UTEX) will be maintained at UCD ATL. Axenic algal cells will be placed in growth media for 4-7 days before test initiation to ensure the cells are in exponential growth.

The *T. pseudonana* 96-hour chronic tests will consist of four replicate 250 ml Erlenmeyer glass flasks with 100 ml of sample and 1 ml of 1.0 x 10^6 cell/ml *T. pseudonana*. Test vessels will be incubated in a temperature-controlled environmental chamber maintained at 21 ± 2 ºC under cool white fluorescent light with a 14-hour light: 10-hour dark photoperiod. Flasks will be in random placement kept in a mechanical shaker in continuous orbital motion at 100 oscillations per minute, and will be randomized twice daily. Cell growth will be measured at test termination.

3.2.4 Copepods

Except for two recent *Microcystis* studies (Ger *et al.* 2009 and 2010), the effects of contaminants on *P. forbesi* and *E. affinis* have rarely been examined. Therefore, toxicity testing conditions in this study will closely follow the US EPA standard toxicity testing procedures (US EPA, 2002) and culture techniques and conditions of copepod cultures that we developed in our laboratory (Teh *et al* 2008).

Batch cultures of *E. affinis* and *P. forbesi* have been successfully propagated for the past two years under controlled conditions in our laboratory and will be used for this study. Prior to toxicity testing, organisms will be acclimated to laboratory conditions until their use in a test. Only one calanoid copepod species, either *E. affinis* or *P. forbesi,* will be tested during each event. Copepod selection will be based on seasonal abundance trends and test methods will follow those used in previous studies conducted at our laboratory.

3.3 *In situ* Monitoring

In situ toxicity tests will be initiated concurrently with sample collection. *In situ* cages will be deployed by boat at near-shore locations adjacent to the mid-channel sampling sites used for toxicity testing. Exact locations for *in situ* deployment will be selected to minimize the likelihood that cages will be vandalized or removed. Five replicate cages for two species, *H. azteca* and Sacramento splittail, will be launched at each site to ensure adequate replication should vandalism occur. Water quality measurements including turbidity, pH, dissolved oxygen, electrical conductivity, specific conductance, and temperature will be measured at the time of deployment and exposure termination. Survival of organisms will be measured at test termination, following a 96-hour exposure. Splittail will be preserved for biomarker analysis to determine the sublethal effects of exposure. One set of cages will be deployed as a duplicate every other sampling event to assess the precision of the toxicity and biomarker endpoints.

3.4 Chemical Analysis

If acute toxicity is exhibited in laboratory tests, an analytical sample collected during the sampling event and immediately preserved with methylene chloride, will be sent to the laboratory of Dr. Kean Goh (Co-PI) of DPR in Sacramento, CA. DPR will conduct a comprehensive analysis to determine if contaminants contributed to the observed toxicity. Results of chemical analyses of samples exhibiting acute toxicity will be compared to existing published effect concentration values for the affected species, such as those found in the US EPA Ecotox Database, to determine if elevated contaminant concentrations are likely to have caused toxicity to a specific test species.

3.5 Effect Concentration Tests

In instances where there is a lack of supporting published effect concentration data for a particular species where acute mortality was observed in the initial laboratory toxicity test, we will conduct LC_{50} determination testing on the species of interest. Using our best professional judgment and based on chemical analysis results, we will test with the chemical most likely to have caused toxicity in order to determine resident species sensitivity to the chemical in question. Effect concentration tests will follow the species-specific protocols outlined above. These tests will generate LC_{50} effect concentration data.

3.6 Toxicity and Pesticide Use Patterns

We will complement the chemical analysis of toxic samples with an analysis of spatial and temporal associations between pesticide use and toxicity. Monthly toxicity testing results and monthly agricultural pesticide use data at the township resolution (2.6 km^2) from the Pesticide Usage Reporting database will be used for this analysis. Monthly pesticide use will be quantified in a buffer upstream of each study site using ArcGIS. We hypothesize that during months with significant rainfall and therefore runoff, agricultural pesticide use on this scale will be positively correlated with the degree of toxicity observed, except where unrecorded urban contaminants result in additional toxicity not corresponding to the agricultural pesticide applications recorded in the PUR database.

3.7 Water Quality

Various water quality parameters other than contaminants can affect toxicity test results. Thus, UCD ATL and our collaborator DWR will monitor several factors that might confound test results to aid in toxicity data interpretation. Water quality parameters of temperature, EC, pH, DO, ammonia-nitrogen, hardness, alkalinity and turbidity will be measured on all samples.

3.8 Artificial Substrate Methods

Five Hester-Dendy samplers will be deployed at each monitoring site during April 2012 and October 2013. Samplers will remain in the field for five weeks to allow benthic macroinvertebrates to colonize the plates of the samplers. Samplers will be carefully placed in five-gallon buckets filled with site water and brought back to the lab for preservation and taxonomic identification. Organisms will be gently removed from the plates, condensed into a smaller volume and preserved with ethanol. Organisms will be identified to the family level to determine species diversity and abundance. Five to ten percent of all samples will be sent to a second qualified laboratory to assess accuracy of our taxonomic identification and enumeration.

3.9 Biomarkers Approach

Surviving Sacramento splittail will be sacrificed at the end of laboratory and *in situ* toxicity tests and preserved for biomarker analysis of sub-lethal contaminants effects (sections 3.9.1 – 3.9.2).

3.9.1 Growth indices

The condition indices have been used extensively as indicators of the well-being of individual organisms in fish health and population assessments (Schmitt and Dethloff, 2000). Gross measurements and weights will be used to determine condition index (CI). The CI is a measure of "plumpness" and is defined as (body weight in grams) x (100)/length in cm^3. Changes in CI specifically reflect alterations in growth and nutritional status, but are also associated with sexual maturity and reproductive status. Contaminant-induced alterations of somatic growth will therefore be reflected by changes in CI.

3.9.2 Histopathology

Histopathology markers are good indicators of environmental stress as they provide visible biological endpoints and measurable responses to sub-cellular mechanisms that can integrate exposure over time (Myers and Fournie, 2002), Stentiford *et al,* 2003). As such, contaminant-mediated adverse effects in fish and aquatic invertebrates have been mostly evaluated using histopathology (Adams *et al*, 1989, 1999; Teh *et al*, 1997, 1999; Myers and Fournie, 2002). Histopathological biomarkers will be used in this study as sentinel endpoints to assess exposures of fish to contaminants in the SFE. Histologic damage in early life stages (embryos and larvae) of fish and other aquatic organisms is considered one of the most sensitive means of assessing adverse effects induced by xenobiotic compounds (Weis and Weis, 1987; Teh *et al,* 1999). Histopathologic analysis is relevant to field investigations because it provides rapid detection of *in vivo* toxicity thereby prioritizing sites for more detailed analysis (Myers and Fournie, 2002). Direct histological damage of the liver and gonads has been documented in several organisms exposed to heavy metals, pesticides, and industrial effluents (Singh *et al*, 1994; Teh *et al*, 2005). Histopathology is the most direct method of determining the irreversible effects of endocrine disrupting compounds (EDCs). Direct impairments in response to EDCs such as the presence of oocytes (intersex) and necrosis of spermatogonia in male fish is easier to detect than the extent of the damage. EDC impacts can be detected and quantified using combined HSI and GSI with histopathology of the liver and gonads. Histopathology combined with vitellogenin and choriogenin analyses is more effective in screening the presence of EDCs in the aquatic ecosystem.

Gill, gonads, kidney, and liver of each fish will be fixed in neutral buffered formalin for 24 hours, changed to 70% ethanol, and processed according to standard histology techniques (Humason, 1979). Lesions will be qualitatively scored as previously described based on severity (Teh *et al*, 2005). 0=normal or no lesion; 10=mild or less than 10% of the organ is affected, 20=moderate or 10-50% of the liver is affected, and 30=severe or > 50% of the liver is affected.

Choriogenin (CHG) and vitellogenin (VTG)

During reproduction in fishes, light and temperature act as cues to the brain to release hormones causing the ovary to produce estradiol (E2), the steroid with the most robust estrogen properties. E2 travels in the blood stream to the liver and induces production of VTG, a yolk precursor. This material is manufactured by liver cells and then released to tissues and blood spaces where it is transported back to the ovary to form oocyte yolks. Chemical and immunochemical detection methods are specific for VTG. A second hepatocyte derived product under endocrine (estrogen) control has been recently isolated, characterized, and used for antibody production in specific teleost fishes. This product called choriogenin (CHG) or zona radiata protein (ZRP) is used in eggshell (chorion) formation. CHG or ZRP when present in male fish are good biomarkers of exposure to endocrine-modulating compounds (EDCs) since the male would not normally produce ZRP or VTG. Levels of ZRP and VTG levels will be measured in plasma and liver by using a homologous VTG or ZRP antibody kit (Biosense, Norway) in a sandwich ELISA. Monoclonal antibodies to VTG and ZRP are commercially available (Biosense, Norway).

Cytochrome P450s

These enzymes are involved in the biotransformation of organic chemicals such as coplanar PCBs (Safe 1994), PAHs and some pesticides (Munkitterick *et al*, 1995). Induction of CYP1A1 by xenobiotic compounds has been well documented (Huggett *et al*, 1992). A fluorometric method that measures activity of the enzyme ethoxyresorufin O-deethylase (EROD) will be used to quantify CYP1A1 (Munkitterick *et al*, 1995). P450 induction is therefore a good indicator of exposure to organic contaminants.

3.10 Data Collection

UCD ATL generates records for sample receipt and storage, analyses and reporting. All raw toxicity test and water quality data will be recorded in non-erasable ink on standardized printed data sheets. The raw data will be entered into spreadsheets and manipulated with statistical programs, then photocopied and used when performing data interpretations. All data will be submitted to the DFG as part of the corresponding project reports in pre-formatted Excel spreadsheets that will include data from laboratory toxicity testing, *in situ* testing, water chemistry and BMI community diversity and abundance. All spreadsheets and statistical analyses will be proofread and checked for errors. All data will be filed and stored on site in a secure cabinet for seven years.

3.11 Statistical Analysis

We will evaluate laboratory toxicity test and biomarkers results by comparing performance in controls to performance in ambient samples using SWAMP standard statistical protocols (pairwise heteroschedastic t-tests and/or EPA TST) and by comparing performance among ambient samples using Tukey's multiple comparison procedure or pair-wise Wilcoxon Rank-Sum tests. Presence of toxicity will be evaluated as a statistically significant reduction in test organism performance or condition in ambient water compared to the control water. *In situ* toxicity data will be evaluated by comparing test organism performance among study sites using Tukey's multiple comparison procedure or pairwise Wilcoxon Rank-Sum tests.

Evaluation of bioassessment data will also include examination of differences between sites during the same sampling event, and will likewise be tested using Tukey's multiple comparison procedure or pair-wise Wilcoxon Rank-Sum tests. Parameters to be evaluated will include absolute and proportional abundances of individual taxa as well as a suite of potentially informative metrics including the Central Valley IBI, a scale involving the metrics of collector richness, predator richness, % EPT taxa, % clinger taxa, and the Shannon diversity index.

Associations between toxicity and pesticide use will be examined using ANCOVA, with upstream pesticide use as a predictor variable, month as a covariate, and toxicity endpoints as response variables.

3.12 Equipment and Facilities

UCD ATL is a 3200 ft² facility containing five rooms and is fully equipped to conduct various toxicant exposure and surface water monitoring studies using the US EPA freshwater and non-promulgated resident toxicity testing species. The laboratory contains 17 environmentally-controlled chambers, and five environmentally-controlled water baths with programmable lighting and temperature regimes.

4. Deliverables

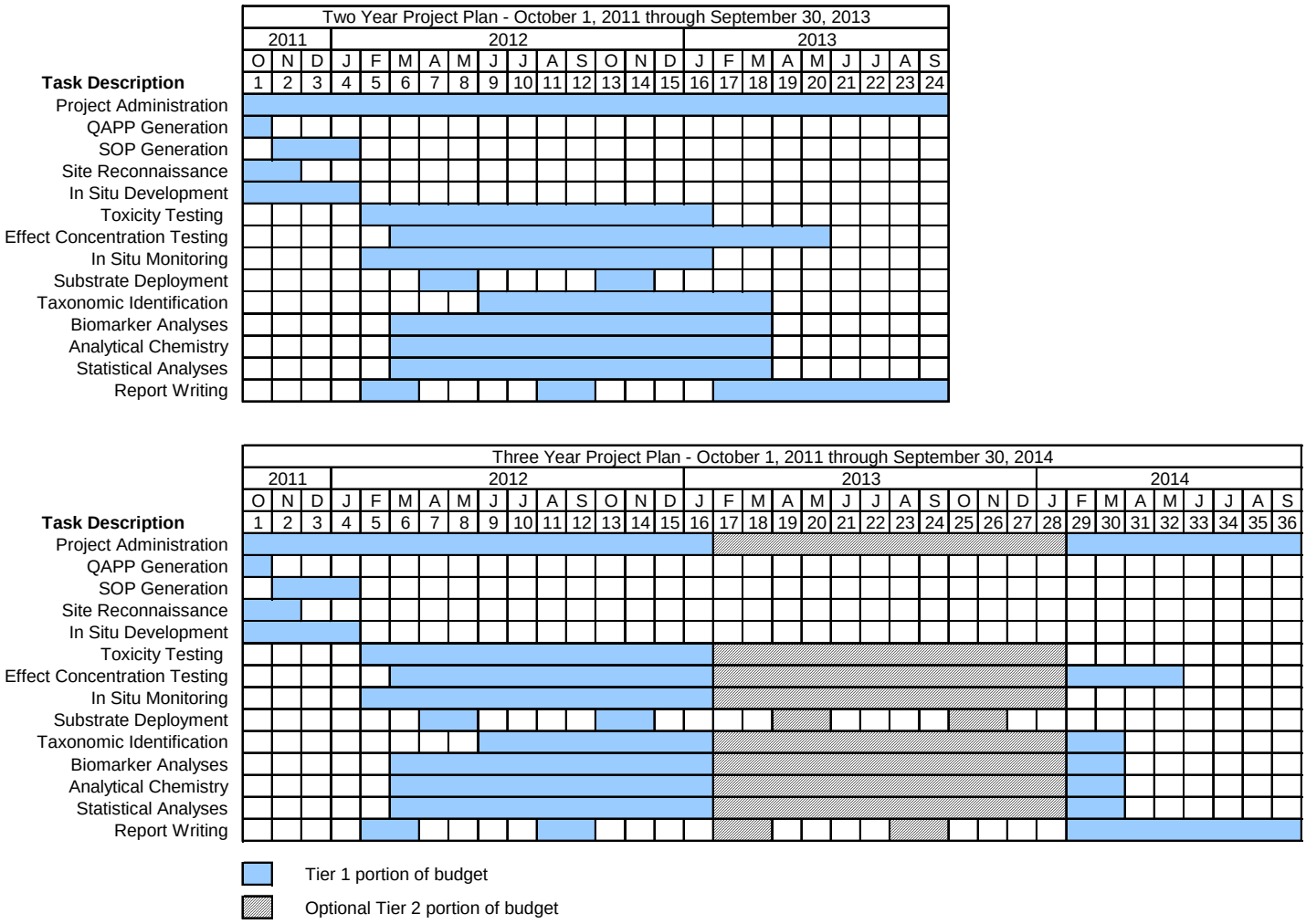
Project Deliverables will include: (i) annual presentations at the Bay-Delta Science Conference; (ii) two to four semiannual progress reports summarizing monitoring results; (iii) a compact disc containing all GPS coordinates related to *in situ* deployment, artificial substrate basket deployment and water sample collections; and (iii) a final report. The final report will provide a comprehensive overview of all data analyzed over the course of the project including a quality assurance summary, toxicity testing results, *in situ* monitoring results, family diversity and abundance of artificial substrate deployments, biomarker and histopathology results, and effect concentration data.

5. Feasibility

The proposed study is feasible due to a combination of 1) research experience, 2) minimal contingencies for project completion, and 3) the availability of research facilities. UC Davis has two (2) boats readily available for water sampling and *in situ* cage deployments; therefore, successful field investigation is very likely. Two (2) trained field assistants in our laboratory have been trained and certified with Mr. Teejay Orear (Graduate student of Dr. Peter Moyle) in operating their boat (UCD 21' Workskiff-135 HP outboard). In addition, in-kind service from Mr. Rich Breuer (Co-PI) from DWR will provide a boat when a UCD boat is not available. Personnel at UC Davis ATL have extensive experience in ambient water collections and are will versed in sample handling protocols and holding time requirements.

The timeline for this project (Table 1) was specifically designed to provide ample time before and after the monitoring portion of the project to allow for potential delays. The three (3) months preceding the monitoring period will allow for the generation of the Quality Assurance Project Plan; Standard Operating Procedure development; site reconnaissance and *in situ* cage building and testing. The nine (9) months following the monitoring period will allow for the finalization of effect concentration testing, completing statistical analyses and writing the final report. The constraints that can influence the schedule generally will depend on the turn around time for chemical analyses and taxonomic identification.

Table 1. Timeline for proposed monitoring project with Tier 1 and 2 Funding Options.



Project management decisions will be discussed at weekly laboratory meetings to ensure that all staff understand any recent changes in the project methods or schedule. In addition, meeting minutes will be emailed to any staff members that were unable to attend.

6. Relevance to the CALFED ERP

Our project is intended to conduct research that tests hypothesis identified in the DRERIP evaluation of the BDCP conservation measures and National Research Council OCAP biological opinion review and to address the uncertainties found. "Other stressor" conservation measures related to water quality were evaluated by DRERIP, and included measures to reduce the loads of: 1) endocrine disruption compounds (OCSM2); 2) pesticides and herbicides (OCSM4); and 3) toxic contaminants in storm water and urban runoff (OCSM5). This evaluation found that in general, reducing the amounts of chemicals in Delta waterways is expected to be beneficial for the covered fish species, even if the specific benefits are difficult to quantify (DRERIP, 2009).

This proposed project also directly addresses potential factors affecting productivity, specifically contaminants, in the Delta by utilizing species that are more ecologically relevant than standard surrogate toxicity testing species. This project incorporates the US EPA's three elements to biomonitoring to understanding contaminants by applying toxicity tests, bioassessment (artificial substrate studies) and analytical chemistry to sites within the Cache Slough Complex.

With the increase in use of pyrethroid pesticides in agricultural areas, and with urban area uses of pyrethroids making up nearly half of the total amount of pyrethroids used in the Central Valley (Werner and Oram, 2008), it is important to use a species in toxicity testing that is sensitive to this class of pesticides. Several authors have demonstrated *H. azteca's* sensitivity to pesticides (Werner, *et al* 2007; Ankley *et al*, 1995; Amweg *et al*, 2006; Burkepile *et al*, 2008; Deanovic *et al*, in progress), making it an ideal testing species for our region of interest. Moreover, our proposed site selections include a

variety of land use practices which directly addresses the needs identified in the PSP. Our use of water column toxicity testing with this species can be applied to OSCM4 and OSCM5 of the DRERIP Evaluation Summary Report (ESR), allowing us to detect concentrations of these pesticides at ecologically relevant amounts.

Our use of Sacramento splittail in both lab and *in situ* toxicity tests will evaluate the type, extent and severity of exposures to different contaminants based on their biomarker responses. Acute mortality expressed in toxicity tests will evaluate the presence of contaminants at high concentrations. Cytochrome P450s, choriogenin and vitellogenin will be analyzed in surviving fish at test termination to determine organic contaminants and endocrine disrupting chemicals exposure, and histopathology will be analyzed to determine effects of exposure to contaminants at sublethal levels. This aspect of the study directly addresses OCSM 2, 4 and 5 of the DRERIP ESP.

Chemical analyses applied to samples in which acute mortality is observed in toxicity tests will play an important role in supporting existing programs for controlling agricultural and urban point and non-point substances, such as identifying contaminants for future development of TMDLs, meeting Basin Plan objectives, and ultimately reducing the loads and concentrations of toxic contaminants in the Bay-Delta watershed to non-toxic levels.

Additional questions that will be addressed in this study include:

1. Is the community structure in Cache Slough Complex affected by contaminants?
2. What is the source of contamination, both geographically and chemically?
3. What is the extent, frequency and magnitude of toxicity in the Cache Slough Complex?
4. What are the spatial and temporal patterns associated with toxicity in samples collected from the major tributaries to Cache Slough?
5. If restoration efforts are adjacent to one of the site locations, is the community structure different at sites outside of the restoration effort?

While the use of diatoms, copepods and benthic macroinvertebrates and splittail in our study cannot necessarily provide direct links to the conservation measures evaluated in the water quality stressors component of the DRERIP ESP, they represent different trophic levels found in the Delta and are an important indicator of ecosystem health. The species used in this study play an important role in answering the questions of contaminant impact on community structures, spatial and temporal patterns in toxicity, as well as restoration effects on community structures.

This proposed project will incorporate the collaborative efforts of DWR, DPR and the UC Davis Department of Wildlife, Fish and Conservation Biology, thereby supporting priority two of the PSP.

The waterways of the Cache Slough Complex currently provide some of the best spawning and nursery areas for Sacramento splittail and the threatened Delta smelt. The future of the Delta, including water usage, has always been one of California's most pressing and complex issues and the role of contaminants has been a major component in determining ecosystem health. Our proposed study will complement larger CALFED goals by providing data that can be used to address the overall objectives in sustaining Bay-Delta ecosystem and pelagic organism health. UCD ATL has extensive experience in evaluating Delta ecosystem health, having worked with CALFED agencies such as DWR and the State Water Quality Control Board. We have recently completed a four-year POD toxicity monitoring study which was supported by both DWR and the Interagency Ecological Program, in which the role of contaminants was examined on a number of Bay-Delta sites. Our proposed study will link back to the POD project by taking the next logical step and focusing on evaluating the health of sites upstream and closer to potential sources of contamination.

7. Expected quantitative results (project summary):

The quantitative results associated with this project are not listed in Appendix E of the ERP. Our research focuses on the effects of contaminants in relation to aquatic ecosystems. Quantitative results related to this project will be water quality measurements, survival measurements, analytical chemistry measurements and biomarker endpoint measurements. All measurements will apply to the six (6) site locations designated in Section 2.3 of this proposal.

Table 2. Summary of intended quantitative results

Measurement Parameter	Number of Sites	Number of Measurements **per Monitoring Year**	Total Measurements **per Monitoring Year**
Water Quality Measurements			
Turbidity	6	40	240
Specific Conductance	6	40	240
Electrical Conductivity	6	40	240
pH	6	40	240
Dissolved Oxygen	6	40	240
Temperature	6	40	240
Total Ammonia/Unionized Ammonia	6	12	72
Toxicity Testing Measurements			
T. pseudonana Growth	6	12	72
E. affinis or *P. forbesii* Survival	6	12	72
H. azteca Survival	6	12	72
P. macrolepidotus Survival	6	12	72
LC50 Determination	Based on toxicity		Maximum of 4
In Situ Exposure Measurements			
H. azteca Survival	6	12	72
P. macrolepidotus Survival	6	12	72
Artificial Substrate Deployment			
Family Diversity	6	2	12
Family Abundance	6	2	12
Analytical Chemistry			
Metals	Based on toxicity		Maximum of 12
Pyrethroid Pesticides			Maximum of 12
Organophosphorous Pesticides			Maximum of 12
Carbamate Pesticides			Maximum of 12
Biomarker Measurements			
P. macrolepidotus Growth Indices	6	24	144
P. macrolepidotus Histopathology	6	24	144
P. macrolepidotus GHG	6	24	144
P. macrolepidotus VTG	6	24	144
P. macrolepidotus Cytochrome P450	6	24	144

8. Other products and results:

No additional products or results are expected from this proposed project.

9. Qualifications

Dr. Swee Teh is the Principal Investigator of the proposed project, and is responsible for the oversight of all activities outlined in the project description. Linda Deanovic is the project manager and will be responsible for managing, planning, and evaluating all environmental monitoring and analyses conducted by the Aquatic Toxicology Laboratory. Rich Breuer (Co-Principal Investigator, Chief, Environmental Water Quality and Estuarine Studies, DWR) is responsible for site reconnaissance within the proposed study area. Dr. Kean Goh (Co-Principal Investigator, Environmental Program Manager, DPR) is responsible for conducting or contracting the necessary chemical analyses of water samples. Dan Markiewicz (Statistician) will conduct all statistical analyses. All toxicity testing will occur at the Aquatic Toxicity Laboratory, UC Davis.

Dr. Swee Teh (Principal Investigator) is the interim Director of the ATL and is a research toxicologist and pathologist under the Department of Anatomy, Physiology and Cell Biology at UC Davis. Dr. Teh has conducted ecotoxicological research at UC Davis for 24 years, and has produced over 50 publications in this field. He has extensive experience in the area of biomarker approaches and aquatic biomonitoring and assessment, using both laboratory and *in situ* techniques.

Linda Deanovic, (Project Manager) is a Staff Research Associate IV at UC Davis for 24 years, and is responsible for the management and supervision of all employees at the ATL. Linda manages all ATL projects including ambient water toxicity testing, sediment testing, *in situ* analyses, indigenous species method development, Toxicity Identification Evaluations, bioassessment procedures and endocrine disruption studies. Most recently, with funding from the Interagency Ecological Program, Linda headed a project that assessed the effects of contaminants on pelagic species in the delta that are under decline (Pelagic Organism Decline). This project included toxicity testing and Toxicity Identification Evaluations (when triggered) using *H. azteca*, as well as *in situ* tests with native fish and invertebrate species, species sensitivity studies and the use of molecular field biomarkers for species of concern (developed in a previous study by the ATL). Linda and the ATL have been heavily involved in the development, validation, and implementation of acute and chronic bioassay methods and continue to work towards enhancing current laboratory protocols. Recently, Linda and her staff developed two new water column toxicity tests, one using Delta smelt and another using *H. azteca*.

Rich Breuer (Co-Principal Investigator) is the Chief of DWR's Environmental Water Quality and Estuarine Studies branch. With over 20 years of experience in the field, he has worked as a UC farm advisor, a licensed agricultural consultant and research scientist in the field of aquatic toxicology. Rich is, therefore, very familiar with the study area. As Chief, he currently manages numerous monitoring programs in the Sacramento San Joaquin Delta, as well as the DWR component of the Interagency Ecological Program. Rich also participates in SWRCB hearings regarding water rights decisions, provides scientific input to the Bay Delta Conservation Plan process to develop long-range biological protection goals for the Delta and its watersheds, and is a core member of the Pelagic Organism Decline management team. As Co-Principal Investigator under the proposed project, Rich's primary role will be to conduct site reconnaissance within the Cache Slough Complex.

Dr. Kean Goh (Co-Principal Investigator) is manager to the Environmental Program under DPR. In his current position, Dr. Goh oversees environmental monitoring of pesticides and research studies on pesticide fate and transport. He is responsible for the development of sampling and analytical methods, as well as the modeling, analysis, and mitigation of pesticide contamination in surface waters (as well as soil, and vegetation). He has produced over 30 peer-reviewed publications and book chapters on pesticides fate, monitoring, mitigation and analytical methods. As such, Dr. Goh will be responsible for conducting and/or contracting analytical services for the proposed project.

Dan Markiewicz (Statistician) has conducted research and provided statistical services at the UC Davis ATL for over 8 years. With a strong scientific and statistical background, Dan currently focuses on the analysis and management of toxicity, water quality and bioassessment data at the ATL. He also produces technical reports and peer-reviewed publications, assures compliance with data analysis protocols, and performs aquatic water column and sediment toxicity bioassays. He has extensive experience with USEPA and SWAMP protocols for the analysis of toxicity data. He has conducted univariate and multivariate modeling and hypothesis testing including ANOVA, ANCOVA, MANOVA, multiple linear regression, Fisher's exact test, Student's t-test, Mann-Whitney U test, tests of normality and homogeneity of variance, and ordination methods include principle component analysis and non-metric multidimensional scaling. Dan will be responsible for the statistical analyses and management of data relevant to the proposed project.

All toxicity testing will occur at the ATL. The ATL is a State-certified laboratory with over 20 years of experience investigating surface water quality and aquatic ecosystem health in watersheds throughout California. The lab is part of the Department of Anatomy, Physiology and Cell Biology in the UC Davis School of Veterinary Medicine, and is involved in both research and teaching activities. A variety of experiments and projects occur at the lab, including aquatic organism toxicity tests, as defined by the United States Environmental Protection Agency (US EPA), using surrogate as well as resident species. The ATL has successfully developed and implemented water column toxicity tests for non-standard species of interest in the Sacramento-San Joaquin watersheds, including Delta smelt and *H. azteca*, and is active in the development of novel methods to enhance current laboratory protocols.

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Section 7: Project Budget

1. Detailed Project Budget:

Budget
Water Quality Monitoring in the Cache Slough Complex

STAFF LEVEL	Number of Hours	Hourly Rate	Year 1 Subtotal	Number of Hours	Hourly Rate	Year 2 Subtotal	**Tier 1 Funding Request**	Number of Hours	Hourly Rate	Year 3 Subtotal	**Tier 2 Funding Request**	**Line Item Total**
PERSONNEL (Allows 3% COLA)												
Swee J. Teh, PI (25% effort)	520	43.87	22,815	520	45.19	23,499	**46,314**	520	46.55	24,204	**24,204**	**70,518**
Linda Deanovic (75% effort)	1560	33.40	52,112	1560	34.41	53,675	**105,787**	1560	35.44	55,285	**55,285**	**161,072**
Staff Research Associate II (100% effort	2080	19.85	41,280	2080	20.44	42,518	**83,798**	2080	21.05	43,794	**43,794**	**127,592**
Graduate Student (50% effort)	1040	15.59	16,212	1040	16.06	16,698	**32,910**	1040	16.54	17,199	**17,199**	**50,109**
Post-doctoral Researcher (50% effort)	1057	17.85	18,870	1057	18.38	19,436	**38,306**	1057	18.93	20,019	**20,019**	**58,325**
Laboratory Assistant II (100% effort)	1446	20.61	29,808	1446	21.23	30,702	**60,510**	1446	21.86	31,623	**31,623**	**92,133**
Statistician (50% effort)	1040	21.44	22,296	1040	22.08	22,965	**45,261**	1040	22.74	23,654	**23,654**	**68,915**
Histotechnician (50% effort)	1040	19.66	20,448	1040	20.25	21,061	**41,509**	1040	20.86	21,693	**21,693**	**63,202**
Three student assistants	1765	8.50	15,000	1765	8.76	15,450	**30,450**	1765	9.02	15,914	**15,914**	**46,364**
Boat Captain (DWR In kind services)	100	59.00	5,900	120	61.95	7,434	**13,334**	40	65.05	2,602	**2,602**	**15,936**
Personnel Cost Subtotal			238,840			246,004	**484,844**			253,385	**253,385**	**738,229**
FRINGE BENEFITS												
Swee J. Teh, PI			6,160			7,097	**13,257**			7,431	**7,431**	**20,687**
Linda Deanovic			20,949			23,724	**44,673**			25,044	**25,044**	**69,717**
SRA II			16,595			18,793	**35,388**			19,839	**19,839**	**55,226**
Graduate Student			211			217	**428**			224	**224**	**651**
Post-doctoral Researcher			3,699			3,907	**7,605**			4,144	**4,144**	**11,749**
Laboratory Assistant II			11,983			13,570	**25,553**			14,325	**14,325**	**39,878**
Statistician			8,963			10,151	**19,114**			10,715	**10,715**	**29,829**
Histotechnician			8,220			9,309	**17,529**			9,827	**9,827**	**27,356**
Student Assistants			195			201	**396**			207	**207**	**603**
Boat Captain (DWR In kind services)			2,587			3,260	**5,847**			1,217	**1,217**	**7,064**
Fringe Benefits Subtotal			76,974			86,968	**163,942**			91,755	**91,755**	**255,697**
OPERATING EXPENSES												
Supplies and Materials			62,000			49,500	**111,500**			37,000	**37,000**	**148,500**
Printing and Duplicating (Publication Costs)			300			300	**600**			300	**300**	**900**
Graduate Student Fees			14,229			15,651	**29,880**			17,216	**17,216**	**47,096**
Travel			5,000			5,000	**10,000**			5,000	**5,000**	**15,000**
Boat Fuel and Maintenance (DWR In kind)			2,000			4,000	**6,000**			1,000	**1,000**	**7,000**
Analytical Chemistry (DPR In kind)			16,667			25,000	**41,667**			8,333	**8,333**	**50,000**
Operating Expenses Subtotal			81,529			70,451	151,980			59,516	59,516	**211,496**
DIRECT COST SUBTOTAL			397,343			403,423	800,766			404,656	404,656	**1,205,422**
ADMINISTRATIVE OVERHEAD												
Indirect Costs @ 25% (less fees/in kind)			95,778			96,943	192,722			96,860	96,860	**289,582**
GRAND TOTAL FOR FUNDING REQUEST			493,121			500,366	**993,488**			501,516	**501,516**	**1,495,004**
TOTAL IN KIND SERVICES FROM DPR			10,487			14,694	**25,181**			4,819	**4,819**	**30,000**
TOTAL IN KIND SERVICES FROM DWR			16,667			25,000	**41,667**			8,333	**8,333**	**50,000**

2. Budget justification:

Staff Level: Funding will support the salary and benefits for eleven (11) individuals. A three percent (3%) cost of living increase is budgeted for the second and third project years. Swee J. Teh, PhD (25% time) is the Principal Investigator (PI) and will be responsible for details of contract management and execution, as well as ensuring coordination among tasks. Linda Deanovic (75%) will be the onsite supervisor for the majority of laboratory and field work. The Staff Research Associate (SRA) II (100% time) will supervise laboratory and field activities during the weekends and participate in the execution of daily tasks including toxicity tests and field exposures. The graduate student (25%), Post-doctoral Researcher (25%), and student assistants will execute a number of toxicity tests and *in situ* exposures. The Laboratory Assistant (LA) II (100%) will be responsible for accompanying experienced staff on field events and will also participate in toxicity tests performed in the laboratory. The Statistician (50%) will be responsible for analyzing all laboratory and field data, organizing electronic files and participating in limited number of lab activities. The histotechnician (50%), graduate student (25%) and Post-doctoral Researcher (25%) will be responsible for all biomarker analyses. They will also be responsible for maintaining organism cultures. Three (3) Student Assistants will assist with day-to-day activities including

washing glassware, labeling glassware and measuring water quality parameters. Several reports will be generated as contract deliverables, which require the participation of the PI, Co-PI, SRAs, LA, Graduate Student, Post-doctoral Researcher and the Statistician. As an in kind service, DPR is providing one (1) individual to help in the field for a total of 26 days. A five percent (5%) cost of living increase is included for this individual. Fringe benefits rates and annual increases are defined by the University's composite fringe benefit rates for fiscal years 2011-2014.

Operating Expenses: Funding will support supplies and materials, printing and duplicating costs, graduate student fees, and travel. Supplies include histological and biochemical materials such as enzymes, antibodies, chemical reagents, glass slides, paraffin, glycomethacrylate, immunohistochemical and histological reagents. Toxicity testing materials include laboratory glassware, disposable items and hazardous waste costs. Field supplies include items used to construct *in situ* apparatus such as acrylic sheeting, PVC plumbing, wire mesh and general hardware. Water quality parameters will be measured using a pH meter and probe, a dissolved oxygen meter and probe, a conductivity meter and probe. Publication costs include posters and color plate cost. Graduate student fees will support the single graduate student's tuition costs. Travel will support costs associated with field work including vehicle rentals and fuel costs, and occasional trips to meetings. As an in kind service, DWR is providing a boat when the UCD boats are not available. DPR is providing the analytical chemistry associated with toxicity tests as an in kind service.

3. Administrative overhead:

The University of California has a long standing indirect cost waiver with California State agencies to maintain indirect costs at twenty-five percent (25%) (Waiver # 03R-135). These indirect costs support both the University as a whole and the Department of Anatomy, Physiology and Cell Biology in the School of Veterinary Medicine. More specifically, the funds support general clerical work, office support, accounting and payroll, Department level supervision, and general office supplies.