ERP Proposal Application Form

For DFG use only	
Proposal No.	Region

Section 1: Summary Information

1. Project title:	DNA Barcoding and Quantitative PCR for zooplankton assessment
2. Applicant name:	University of California at Davis
3. Contact person:	Dr. Swee Teh
4. Address:	VM:APCB, 1321 Haring Hall, University of California, Davis
5. City, State, Zip:	Davis, CA 95616
6. Telephone #:	(530) 754-8183
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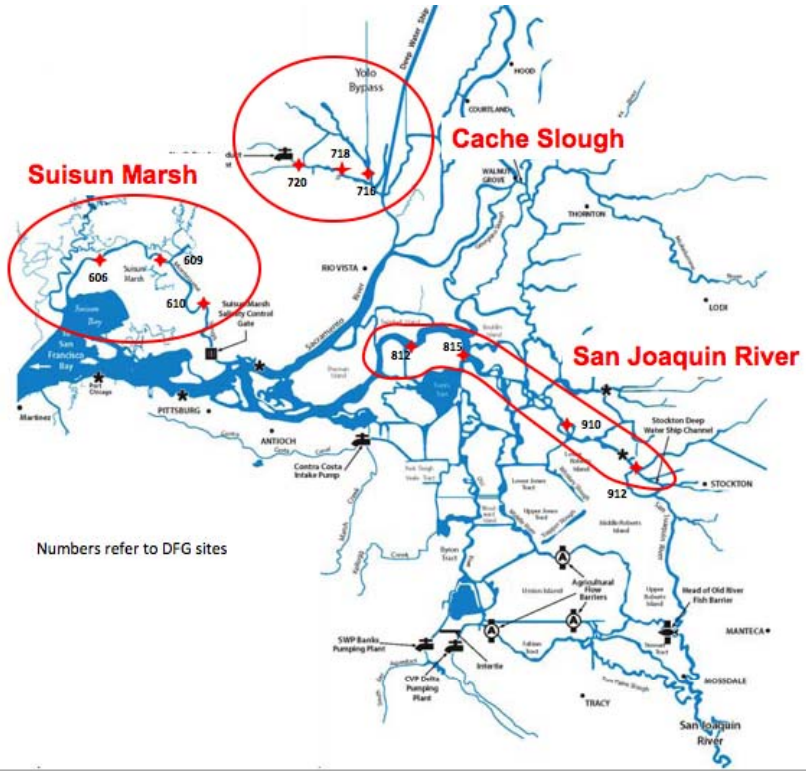
8. Email address:	sjteh@ucdavis.edu
9. Agency Type:	Federal Agency ☐ State Agency ☐ Local Agency ☐ Nonprofit Organization ☐ University (CSU/UC) ☒ Native American Indian Tribe ☐
10. Certified nonprofit organization:	Yes ☐ No ☒
11. New grantee:	Yes ☒ No ☐
12. Amount requested:	$868,417
13. Total project cost:	$868,417 + in-kind services (DFG)
14. Topic Area(s):	Primary: Estuary Food Web Productivity Secondary: At-Risk Species Assessment
15. ERP Project type:	Research, Monitoring, Pilot/Demonstration
16. Ecosystem Element:	Primary: Bay-Delta Aquatic Food Web
17. Water Quality Constituent:	Primary: Toxicity of Unknown Origin and Contaminants Secondary: Nutrients and Oxygen Depleting Substances
18. At-Risk species benefited:	Delta smelt (*Hypomesus transpacificus*), threadfin shad (*Dorosoma petenense*)
19. Project objectives:	Develop molecular-based techniques, DNA barcoding and qPCR, for qualitative and quantitative analysis of the micro and meso zooplankton community of the San Francisco Bay Delta Estuary
20. Time frame:	3 years (July 1st, 2011, June 30th, 2014)

Section 2: Location Information

1. Township, Range, Section: and the 7.5 USGS Quad map name.	**Please see 3 Location Description**
2. Latitude, Longitude (in decimal degrees, Geographic, NAD83):	**Cache Slough** Lindsey Slough: 38.258, -121.725 Barker Slough Pumping Plant: 38.261, -121.774 Cache Slough N. of Cable ferry: 38.241, -121.686 **Suisun Marsh** Montezuma Slough off Joice Slough: 38.169, -122.026 Montezuma Slough at Nurse Slough: 38.167, -121.938 Montezuma Slough at Rd. from Birds Landing: 38.119, -121.889 **Lower San Joaquin River** San Joaquin River mouth of Little Potato Slough: 38.080, -121.570 San Joaquin River W. of Oulton Point: 38.090, -121.641 San Joaquin River between Hog and Turner Cut: 38.002, -121.449 San Joaquin River at the mouth of Calaveras River: 37.983, -121.364
3. Location description:	The Cache Slough complex is a region located in the north Delta where Cache Slough and the southern Yolo Bypass meet. Suisun Marsh is a tidal wetland located in the southern portion of Solano County, including Montezuma Slough and Suisun Slough. The lower San Joaquin River is the second largest river in California and includes areas downstream of the City of Stockton to Jersey Point. All monitoring sites are located on various waterways within this complex, and will be sampled exclusively by boat. All sites are part of the Department of Fish and Games routine monitoring stations. 
4. County(ies):	Sacramento, Contra Costa, San Joaquin, and Solano County.
5. Directions:	All sampling will be accessed by boat. The Rio Vista boat launch, located in the City of Rio Vista, will be the starting point for Cache Slough. To reach Cache Slough travel upstream and enter the confluence of Cache Slough and the Sacramento River. Lloyd's Holiday Harbor, located in the City of Antioch,

	will be the starting point for Suisun Marsh. To reach Suisun Marsh travel downstream and enter the eastern confluence of Montezuma Slough and the Sacramento River. The Brannon island boat launch, located in the Brannon Island State Recreational Area, will be the starting point for the lower San Joaquin River. To reach the San Joaquin travel south along Three Mile Slough and enter the confluence of Three Mile Slough and the San Joaquin River.
6. Ecological Management Region:	Delta Region
7. Ecological Management Zone(s):	1.1,1.2,1.3,1.4 (Sacramento San Joaquin Delta) 11.1,11.2,11.3 (East Delta Tributaries) 2.1,2.2,2.3,2.4,2.5 (Suisun Marsh and North San Francisco Bay)
8. Ecological Management Unit(s):	North Delta, East Delta, Central/West Delta, Suisun Marshlands and Bay
9. Watershed Plan(s):	Not Applicable.
10. Project area:	The Cache Slough, Suisun Marsh and the lower San Joaquin River encompass approximately 450,000 acres of open water, marsh, floodplain, and riparian and urban habitat.
11. Land use statement:	Agriculture is currently the dominant land use practice in the Cache Slough and Suisun Marsh areas. Urban and industrial use is also present, but limited. The San Joaquin River area consists of a significant mixture of Urban, industrial and Agricultural land use. 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]) N/A	
2. Owner Interest: N/A	
3. Permits: N/A	
4. Lead CEQA agency:	N/A
5. Required mitigation:	Yes ☐ No ☒

Section 4: Project Objectives Outline

1. List task information:

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 1: Endangered and Other At-risk Species and Native Biotic Communities – Objective 1 and 3

Goal 2: Ecological Processes – Objective 1 and 2

Goal 4: Habitats – Objective 1 and 5

The goals of this study are the following:

- Establish a new zooplankton identification system using DNA barcoding for qualitative analysis of the micro and meso zooplankton community across the San Francisco Estuary (SFE)
- Develop qPCR assays to quantify the abundance of key zooplankton species including detecting all their life stages or forms
- Validate the molecular tools (DNA barcoding and qPCR assays) by comparing with traditional morphological techniques
- Compare the composition and abundance of zooplankton species in the gut contents of larval delta smelt and threadfin shad and from surface waters of the habitats from which the fish will be collected

2. Additional objectives:

The transfer of information generated from the study is an essential part of the project. Following validation of the DNA barcodes and specific qPCR in year 2 and 3 of the project, the application of the molecular tools will be demonstrated to ongoing plankton monitoring programs such as the Bay Delta Monitoring Program, which encompasses both phytoplankton and zooplankton monitoring in the SFE, and other relevant users such as the Interagency Ecological Programs (IEP) Pelagic Organism Decline Management Team including research laboratories contracted by CA DFG for morphological analyses of zooplankton. Information transfer will be additionally facilitated by conducting workshops to demonstrate the application of the molecular technologies to environmental managers, local agencies, and relevant research projects with phytoplankton and zooplankton study components.

3. Source(s) of above information:

Not applicable

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:	Randall D. Baxter, Dolores V. Baxa, Tomofumi Kurobe
Supporting Staff:	
Subcontractor:	

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

None

Section 6: Project Tasks and Results Outline

Assessment of zooplankton species composition and abundance in the San Francisco Estuary using DNA barcoding and quantitative PCR

1. Detailed Project Description

Since 2000, populations of pelagic organisms including California native fishes have declined substantially leading to the phenomenon known as the "Pelagic Organism Decline (POD)" (Sommer et al. 2007). The four species of the POD are the delta smelt *Hypomesus transpacificus*, longfin smelt *Spirinchus thaleichthys*, striped bass *Morone saxatilis*, and threadfin shad *Dorosoma petenense* (Feyrer et al. 2007, Sommer et al. 2007). In an effort to determine the potential mechanisms of the decline, some research has focused on broad components of stressors from which the limited availability of food resources, particularly zooplankton, was identified as a major factor affecting the abundance of POD species (Sommer et al. 2007).

In the Sacramento-San Joaquin Delta, several resident fish species including California native fishes thrive on zooplankton during their larval and juvenile stages. The zooplankton community of the upper San Francisco Estuary (SFE) has undergone long-term changes in composition and biomass due to the potential impacts from anthropogenic inputs including chemical contaminants, pesticide loading, ammonia, and changes in the algal community such as reduced algal biomass and regime shifts in algal species to less nutritious algae (Richards et al. 2004, Kusler 2009, Sarma et al. 2003, Ger et al. 2010). Reduced algal biomass has been linked to the introduction of the invasive Asian clam *Potamocorbula amurensis* (Sommer et al. 2007, Winder and Jassby 2010). The long-term and recent collapse of key pelagic fish species have been mainly attributed to major shifts in zooplankton composition, which consequently affected the quantity and quality of carbon available to planktivorous fish (Bennett and Moyle 1996).

The zooplankton composition of the SFE has been traditionally analyzed by morphological identification and counting using light microscopy to determine the species composition, abundance, and seasonal and spatial dynamics. Morphological classification of planktonic organisms is historically a difficult task because of the lack of distinguishing features particularly in small nondescript cryptic organisms (Nobriga 2002). The lack of distinct morphological attributes in certain zooplankton species in the SFE may result in incorrect classification rendering gaps on their ecological functions or contributions in the SFE food web.

The purpose of this study is to analyze and update the biodiversity of the micro and meso zooplankton community in the SFE using a new molecular-based approach known as DNA barcoding. DNA barcoding is a method for taxonomic identification which relies on the use of two or three standardized DNA regions that is specific for each species (Blaxter 2004)). With a sufficient DNA database, DNA barcoding provides an accurate and rapid identification of known species and the discovery of new cryptic organisms as the method does not require morphological identification. In addition, the establishment of a genetic database will enable us to develop other molecular analytical tools such as quantitative PCR (qPCR). In this proposed study, we will utilize DNA barcoding for general qualitative assessment of zooplankton composition present in the SFE and then apply qPCR to quantify the abundance of key zooplankton species found in certain habitats.

We propose to address the following questions:

- How diverse is the zooplankton species composition in the SFE?
- Are there cryptic organisms that cannot be identified by traditional microscopy?
- Are the molecular tools (DNA barcoding and qPCR) useful for monitoring the zooplankton community of the SFE?

- Which zooplankton species are present in the fish gut especially at larval stage, and are they considered good quality food?

In order to address these research questions, our specific objectives are to:

- Establish a new zooplankton identification system using DNA barcoding for qualitative analysis of the micro and meso zooplankton community across the SFE
- Develop qPCR assays to quantify the abundance of key zooplankton species including detecting all their life stages or forms
- Validate the molecular tools (DNA barcoding and qPCR assays) by comparing with traditional morphological techniques
- Compare the composition and abundance of key zooplankton species in the gut contents of larval delta smelt and threadfin shad and from surface waters of the habitats from which the fish will be collected

Our research is divided into two tasks with sub tasks and hypotheses

Task 1. Project management, transfer of information, outreach application

Task 2. Collaborative field studies – to collect zooplankton samples for assessment of species diversity of zooplankton across habitats and seasons by morphological analysis and DNA sequencing

Task 2–1. Morphological analysis and establishment of genetic database

Task 2–2. Develop the DNA barcode identification system for qualitative analysis and qPCR for quantitative characterization of the zooplankton community structure

Task 2–3. Validate the molecular tools by analysis (molecular methods vs. morphology) of zooplankton composition and abundance in critical habitats of the SFE that differ in productivity – (e.g. Cache Slough and Suisun Marsh with high productivity; San Joaquin River with low productivity)

Task 2–4. Apply the molecular tools for assessment of zooplankton composition and abundance in the habitat and in gut contents of planktivorous larval fishes

H_1: DNA barcoding is a reliable species identification tool for assessment of zooplankton diversity and abundance in the SFE

H_2: DNA barcoding identifies cryptic species of zooplankton present in the SFE that are not identified by morphological analysis

H_3: A tiered approach using molecular (DNA barcoding and qPCR) and morphological analysis is an effective tool for monitoring the composition and abundance of the SFE zooplankton community

H_4: The composition and abundance of zooplankton in the gut contents of pelagic fish at larval stage are affected by changes in time and habitat

H_5: DNA barcoding and qPCR analysis will address the interaction between the zooplankton composition in the habitats and the gut contents of larval fish

PREVIOUS RESEARCH

Zooplankton composition and abundance in the SFE has been intensively monitored by the California Department of Fish and Game (CA DFG) over the past 37 years (http://www.dfg.ca.gov/delta/data/20mm/CPUE_zoomap.asp). Zooplankton dynamics in Suisun Bay and the Sacramento–San Joaquin Delta in relation to environmental changes over the duration of the monitoring project has been recently described (Winder and Jassby 2010). To date, approximately 23 species of zooplankton have been identified by morphological analysis (Table 1), following annual surveys that mostly focused on major zooplankton species of interest in the SFE including *Sinocalanus doerrii, Eurytemora affinis, Acartia* spp*.,*

Pseudodiaptomus forbesi, and *Acartiella sinensis* (http://www.dfg.ca.gov/delta/data/20mm/CPUE_zoomap.asp). *E. affinis* was abundant in the mid 1970s and was the primary food source of pelagic fish species in the SFE. However *E. affinis* populations started to decline since 1975 with the most significant decrease during the summer and fall of the late-1980s, subsequent to the introductions of the overbite clam, *P. amurensis*, and the introduced calanoid copepod *P. forbesi* (Hennessy 2010). *P. forbesi* has been dominant in low and high salinity zones in the SFE since its introduction, and is mostly abundant in the summer (Hennessy 2010). *P. forbesi* has replaced *E. affinis* as an important food resource for the upper SFE for POD and other fish species in the summer and fall months (Baxter 2008).

Although reduced primary (phytoplankton) and secondary (zooplankton) production were associated with long-term declines of diverse fish populations in the upper SFE (Cloern 2007), an equivalent decrease in zooplankton carbon was not observed immediately following the decline of pelagic fish in 2002 (Sommer et al. 2007, Thomson et al. 2010). This suggests that altered prey composition due to zooplankton taxonomic shifts enhanced food limitations to estuarine fish (Winder and Jassby 2010). The dominance of small-sized cyclopoids (i.e. *Limnoithona*) reduced prey quality, foraging efficiency, nutritional value of zooplankton for fish, and the selective feeding mode of fish (Bouley and Kimmerer 2006). As such, decreased food quantity and quality has been linked to reduced growth and survival of key pelagic fishes in Suisun Bay and the Sacramento-San Joaquin Delta (Sommer et al 2007).

DNA barcoding is a new concept of identifying organisms based on species-specific DNA regions (Blaxter 2004). Because of the few number of well-trained taxonomists in aquatic organisms especially at lower trophic levels, publications on development of DNA barcoding system for phyto and zooplankton are limited. A representative study on zooplankton identified 507 individuals representing 61 species of Cladocera and 21 Copepod species in freshwater zooplankton assemblages from Mexico and Guatemala by DNA barcoding (Elias-Gutierrez et al. 2008). Their study showed that the number and composition of individuals identified by morphological methods was significantly underestimated compared to DNA barcoding. An on-going oceanographic research study focusing on taxonomic classification of krill (euphausiids) and crustaceans (crustacea) by DNA barcoding approach (Bucklin et al. 2007) is a part of a comprehensive biodiversity survey on “Census of Marine Life” (http://www.coml.org/). This study focuses on the assessment of the biodiversity and discovery of new organisms in extreme environments (i.e. deep sea), and it does not include conservation efforts of local ecology.

CRITICAL UNKNOWNS

The species diversity of the zooplankton community in the SFE may not be completely characterized due to the limited capability of morphological analysis by light microscopy to identify the potential presence of other zooplankton species in the estuarine environment. Zooplankton monitoring in the SFE has mostly focused on ecologically relevant species such as the calanoid copepods that can be easily identified morphologically (Hennessy 2010). However, microzooplankton and other nondescript zooplankton are likely an integral part of plankton assemblages in the SFE, but which are difficult or impossible to identify by existing traditional morphological methods. While unrecognized or unidentified, these zooplankton species are potentially important food sources to other aquatic organisms in addition to larval and juvenile fishes in the SFE. **Importantly, molecular-based methods are currently lacking and are critically needed to provide a specific, rapid, and inexpensive tool to monitor the identification and quantification of zooplankton species in the SFE.** As competition for food resources is a critical factor affecting fish abundance in the SFE (Kimmerer et al. 1994, Sommer et al. 2007, Winder and Jassby 2010), gut content analysis is one method of determining the potential connections between food quality and availability and fish fitness in certain habitats. In larval fishes in particular, little is known about their stomach contents especially on ingested microzooplankton because their small and damaged or digested appendages are difficult or impossible to identify by morphological methods (Nobriga 2002). DNA barcoding and qPCR analyses of fish contents can specifically and rapidly identify and quantify ingested zooplankton species. Our

proposed research aims to address these information gaps and the relevance of zooplankton diversity assessment by molecular approach.

Table 1. Zooplankton species in the San Francisco Estuary as summarized from descriptions in Hennessy (2010) and Winder and Jassby (2010)

Zooplankton taxon	Species (or genus)	Reference
Copepoda	*Acartia* spp.	Hennessy 2010
	Acartiella sinensis	Hennessy 2010
	Eurytemora affinis	Hennessy 2010
	Limnoithona sinensis	Hennessy 2010
	Limnoithona tetraspina	Hennessy 2010
	Pseudodiaptomus forbesi	Hennessy 2010
	Sinocalanus doerrii	Hennessy 2010
	Tortanus dextrilobatus	Hennessy 2010
Cladocera	*Bosmina* sp.	Hennessy 2010
	Daphnia sp.	Hennessy 2010
	Diaphanosoma sp.	Hennessy 2010
Rotifera	*Asplanchna* spp.	Winder and Jassby 2010
	Keratella spp.	Winder and Jassby 2010
	Synchaeta bicornis	Hennessy 2010
	Trichocerca spp.	Winder and Jassby 2010
	Polyarthra spp.	Winder and Jassby 2010
Mysids	*Acanthomysis aspera*	Winder and Jassby 2010
	Acanthomysis hwanhaiensis	Winder and Jassby 2010
	Alienacanthomysis macropsis	Hennessy 2010
	Deltamysis holmquitae	Winder and Jassby 2010
	Hyperacanthomysis longirostris	Hennessy 2010
	Neomysis mercedis	Hennessy 2010
	Neomysis kadiakensis	Hennessy 2010

2. Background and Conceptual Models

Zooplankton and stressors

Zooplankton communities are an important food source of larval and juvenile fishes in the SFE. As such, characterizing the changes in zooplankton composition and abundance and their interaction with fish is a paramount step to advance our understanding of the relationship between zooplankton productivity and fish recruitment in the SFE. Our conceptual model as shown in **Fig. 1** demonstrates the various factors affecting the zooplankton community in the water column subsequently affecting the zooplankton composition in fish guts.

Zooplankton availability and quality are key factors correlated with fish recruitment in the SFE. The occurrence of fish declines overlapped with the decreased production of phytoplankton and native zooplankton in the last decade (Jassby et al. 2002, Sobczak et al. 2002, Kimmerer 2004, Bouley and Kimmerer 2006, Cloern 2007). Species composition, trends in abundance, and potential causes in the decline of certain zooplankton species were described in previous studies (Winder and Jassby 2010, Glibert 2010, Lehman 2000; Kimmerer 2005). An overall decreased production observed at multiple trophic levels indicated the reduced capacity of the Delta to support fisheries in recent decades (Baxter et al. 2008, Cloern 2007).

Aquatic contaminants can further complicate the potential effects of grazing rates, reproduction, sex ratios, and zooplankton survival (Medina et al. 2004, Sibley et al. 2004, Bengtsson et al. 2004, Ramirez-Perez et al. 2004). Contaminants can decrease zooplankton diversity (Richards et al. 2004) suggesting direct toxicity to zooplankton may be an important factor in population declines in the SFE (Kuivila and Foe 1995).

Species invasions have contributed largely to the long-term changes in zooplankton community composition and biomass in the SFE (Winder and Jassby 2010). The introduction of the filter-feeding clam *P. amurensis* and *Microcystis* in recurring seasonal blooms exacerbated the zooplankton declines (Carlton et al. 1990, Alpine and Cloern 1992, Ger et al. 2009). The local zooplankton assemblage, particularly copepods and mysids, is now displaced with introduced species throughout the Sacramento–San Joaquin Delta and Suisun following prolonged climate anomalies, changes in water quality and flow that enhanced the establishment of non-indigenous species. Due to food limitation and predation by *P. amurensis*, mysid populations and the calanoid copepod *E. affinis* decreased and these zooplankton are now replaced by smaller and less nutritious copepods, the *Limnoithona* spp. (Glibert 2010) and the cyanobacterium *Microcystis aeruginosa* (Lehman et al. 2000, 2005). *Microcystis* blooms due mainly to *M. aeruginosa* produce the hepatotoxin, microcystin, causing adverse effects to pelagic species in the Delta through direct toxicity or indirect impacts to the food web (Lehman et al. 2008 and 2010, Baxa et al. 2010). Recurring blooms and the shift from nutritious diatoms to small-sized flagellates and cyanobacteria are the main factors that limited the availability of food resources to primary consumers in the upper SFE (Lehman 2000; Kimmerer 2005). Multiple species invasions further triggered the long-term and severe competition for shared food resources, paving the way for massive declines in zooplankton populations in the SFE (Sommer et al. 2007).

Gut content analysis

The feeding ecology of organisms has been traditionally determined using gut content analysis. Larval delta smelt at first-feeding stage thrive on unicellular algae and rotifers under cultured conditions (Baskerville-Bridges et al. 2002). In contrast, delta smelt larvae raised in the field in 1992-1994 actively prey on cyclopoids and on the calanoid copepods *E. affinis* and *P. forbesi* at different developmental stages (Nobriga 2002). Importantly, analysis of their gut contents showed a high percentage of unidentified zooplankton due to enzymatic digestion in the stomach and because early copepod stages are morphologically similar among different species (Nobriga 2002). The stomach contents of postlarval (15 mm) and adult delta smelt demonstrated high proportions of calanoid copepods (*E. affinis* and *P. forbesi*) (Moyle and Herbold 1992). These zooplankton species were observed in the stomach contents of delta smelt sampled in 1972-1974 (*E. affinis*) and in 1988 (*P. forbesi*). Consistently present in the stomach of the delta smelt were the mysid (*Neomysis mercedis*). Unlike delta smelt, little is known about the gut contents of threadfin shad and other pelagic fishes in the SFE. The DNA barcoding and specific qPCR assays that we will develop in this study will provide the necessary tools for specific identification and quantification of ingested zooplankton particles among planktivorous fish species.

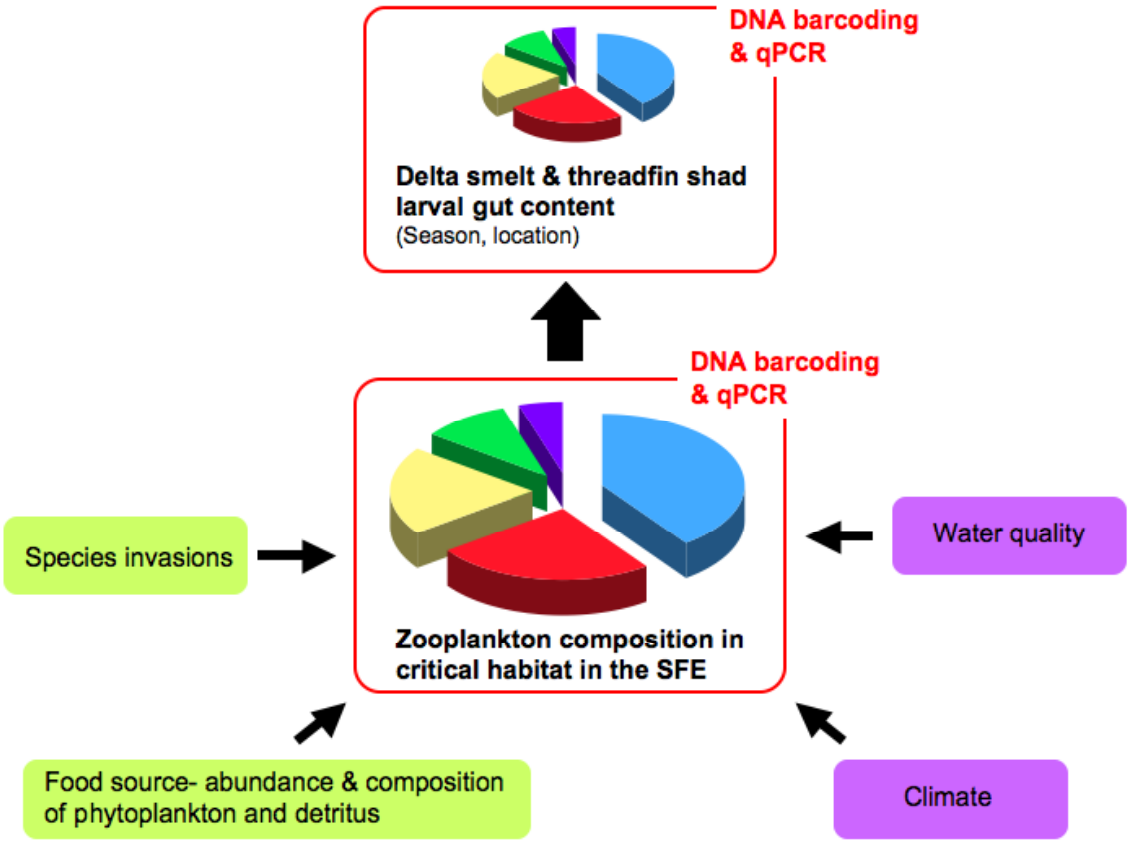


Fig. 1. Conceptual model: Zooplankton composition and abundance in the San Francisco Estuary may be altered through species invasions, environmental factors, and stressors. Planktivorous fishes that depend on zooplankton for food in their habitat may reflect the zooplankton composition present in their gut contents. Key biological stressors are indicated in green and physicochemical factors in purple. The zooplankton composition and abundance in the environment and in the gut will be analyzed by DNA barcoding and qPCR as highlighted in this figure.

DNA barcoding and quantitative PCR

DNA barcoding is a taxonomic identification method which relies on the use of two or three standardized DNA regions also known as “genetic markers” or “barcode”, which is specific for each species (Hebert et al. 2003). Because of its rapid and practical application, extensive research on taxonomic classification of various types of organisms have been conducted in mammals, birds, amphibians, and fish (Murphy et al. 2001, Khan et al. 2010, SanMauro et al. 2005, Kochzius et al. 2010). Although this technique requires the initial collaboration of taxonomists and molecular biologists to establish a comprehensive database linking genetic information to morphological features of organisms, specimens can be analyzed only by molecular technique (PCR and sequencing) once the method has been established. Currently, the Basic Local Alignment Search Tool (BLAST) at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Barcode of Life Data (BOLD) Systems, (http://www.barcodinglife.org) at the Consortium for the Barcode of Life are web sites available for species identification based on similarity comparisons of DNA barcodes. BOLD is specifically designed for DNA barcoding so that the database provides DNA sequence, primer sets used for PCR, specimen image and taxonomic information. Because the barcodes are unique for each species, DNA barcoding offers an accurate identification of known species, and the discovery of new ones, using specific gene tags or barcodes. This technique is relatively simple, applicable to all life stages of a species, can be performed using only parts of an organism, is culture-independent, and objective (Frezal et al. 2008). DNA barcoding provides an enormous access to systematics without the need for specialized training (Stoeckle 2003). Genetic analysis by DNA barcodes will clearly improve classifications of zooplankton

in the SFE by critical examination of precise morphological traits commonly used in taxonomy.

Development of the zooplankton database will enable us to further develop molecular tools such as qPCR, which can be used to estimate the abundance of certain zooplankton species. qPCR is a PCR-based molecular technique that can amplify the target sequence in a DNA sample and simultaneously quantify the copy number of the target gene. In our previous study, we have developed qPCR assays that were used to assess the abundance and proportion of toxin-producing *Microcystis aeruginosa* present among cyanobacterial assemblages in the SFE (Baxa et al. 2010). Using the same approach for developing specific qPCR assays for key zooplankton species, primer and probe sequences will be designed to the unique region of the target species. Once the qPCR assays are developed, we will be able to develop high-throughput identification and monitoring tools for zooplankton composition in water columns and stomach contents of fish. **Using qPCR, over 50 field or gut samples of zooplankton composition for ten different species can be analyzed in a week. The method will provide new insights into understanding the biodiversity and ecology of the zooplankton community in the SFE.**

Physical setting

We will conduct zooplankton and pelagic fish sampling in collaboration with the CA DFG in Cache Slough, Suisun Marsh and San Joaquin River. Productivity and species richness of those three sites are described in "3. APPROACH AND SCOPE OF WORK-*Rationale for collection sites*". Cache Slough is a freshwater tidal marsh in the northern reaches of the SFE (Moyle 2008) that drains into Moore Tract and Yolo Bypass with tidal fluctuations from the Sacramento River (Lund et al. 2007). Cache Slough includes high biodiversity of aquatic organisms and waterfowl, and sensitive habitats such as remnant riparian and vernal pools, providing over 32,900 acres of habitat for 80 listed species, including delta smelt. Suisun Marsh is located on the northern shore of Suisun Bay and is the largest fresh/brackish marsh in California. It contains 1,000 acres of intertidal and roughly 11,100 acres of subtidal aquatic, supporting a diverse assemblage of invertebrate, fish, bird, and mammal species (Engle et al. 2010). Salinity levels fluctuate seasonally between 0 and 16 ppt, and water temperatures between 5 and 25°C. San Joaquin River is predominantly an open river channel draining to Suisun Bay and is the second-longest river in California (530 km long). The average unimpaired runoff of the main stem of the river is about 2.22 km^3 per year. The San Joaquin and its eight major tributaries drain about 83,000 km^2 of California's San Joaquin Valley.

3. Approach and Scope of Work

Project plan and timeline

Year 1 will entail the development of barcodes by using zooplankton samples from the upper SFE Delta obtained from collaborations with ongoing monitoring projects of the CA DFG. Sequencing of genomic DNA from zooplankton samples will be the basis of a genetic database established during year 1. The quantification of dominant zooplankton species will follow by developing specific qPCR assays based on the obtained barcodes and established database in year 1. Years 2 and 3 will cover the validation and application phase of the barcodes and qPCR assays by assessment of the zooplankton composition and abundance of habitats that differ in productivity including gut content analysis of key larval species present in the habitats.

Task 1. Project management and coordination of information transfer

Dr. Swee Teh is the Principal Investigator (PI) and will be responsible for details of contract management and execution,

as well as ensuring coordination among tasks. The investigators will be in charge of their respective tasks in the study. Drs. Teh and Co-PI Mr. Randall Baxter from the CA DFG will direct fish and zooplankton sampling (Task 2-1) with the help of 2 graduate students and one technician. Co-PIs Drs. Dolores Baxa and Tomofumi Kurobe will lead the DNA sequencing (Task 2-1), and development of the molecular techniques (Task 2-2, -3 and -4). Drs. Teh and Baxa will manage the coordination of field studies in collaboration with on-going monitoring program at the CA DFG.

The investigators will share the responsibility of certain aspects of project management including allocation of resources, management of project staff, acquisition of supplies relevant to their tasks, and development of appropriate protocols for the various laboratory assays and field studies. The investigators will oversee the timely completion of their task and to ensure their goals and results are accomplished and reported in a timely manner and integrated as component of a manuscript for submission to a peer reviewed journal.

The transfer of information generated from the study is an essential part of the project. Following validation of the DNA barcodes and specific qPCR in year 2 and 3 of the project, the application of the molecular tools will be demonstrated to ongoing plankton monitoring programs such as the Bay Delta Monitoring Program, which encompasses both phytoplankton and zooplankton monitoring in the SFE, and other relevant users such as the IEP Pelagic Organism Decline Management Team including research laboratories contracted by CA DFG for morphological analyses of zooplankton. Information transfer will be additionally facilitated by conducting workshops to demonstrate the application of the molecular technologies to environmental managers, local agencies, and relevant research projects with phytoplankton and zooplankton study components.

Task 2. Collaborative field studies

This study component will be conducted in coordination with on-going zooplankton monitoring program of the California Environmental Monitoring Program by DFG to obtain zooplankton from water samples and to determine the temporal and spatial trends of zooplankton dynamics across gradients of physicochemical factors.

Rationale for collection sites

Zooplankton and fish samples will be obtained from the routine zooplankton monitoring survey of the CA DFG http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm. From the CA DFG sampling sites, we will select a total of 10 stations that will most closely represent the three habitats that differ in primary productivity- Cache Slough, Suisun Marsh and the San Joaquin River (**Fig. 2**).

Cache Slough is considered a productive site due to relatively high primary productivity with abundant zooplankton and larval fish (Lund et al. 2007). It 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). The slough has few invasive species, abundant in phytoplankton and zooplankton, and less flooding (Lund et al. 2007). **Suisun Marsh** contains critical habitats for nursery rearing of Sacramento splittail, striped bass, and Chinook salmon (Hobbs et al. 2006, Lund et al. 2007). Zooplankton and pelagic fish monitoring program has been extensively conducted to understand the ecology of the marsh (Engle et al. 2010). **The San Joaquin River** is considered less productive due to the reduced abundance of phytoplankton to support higher trophic levels and few viable habitats for spawning or larval rearing (Lund et al. 2007). The river is highly altered due to pollution and presence of invasive species (Baxter et al. 2008). These sites were chosen because gradients in biological effects due to contaminants, disease, and nutrition will likely be observed in these habitats that vary in productivity to support zooplankton community and spawning and rearing of fish species.

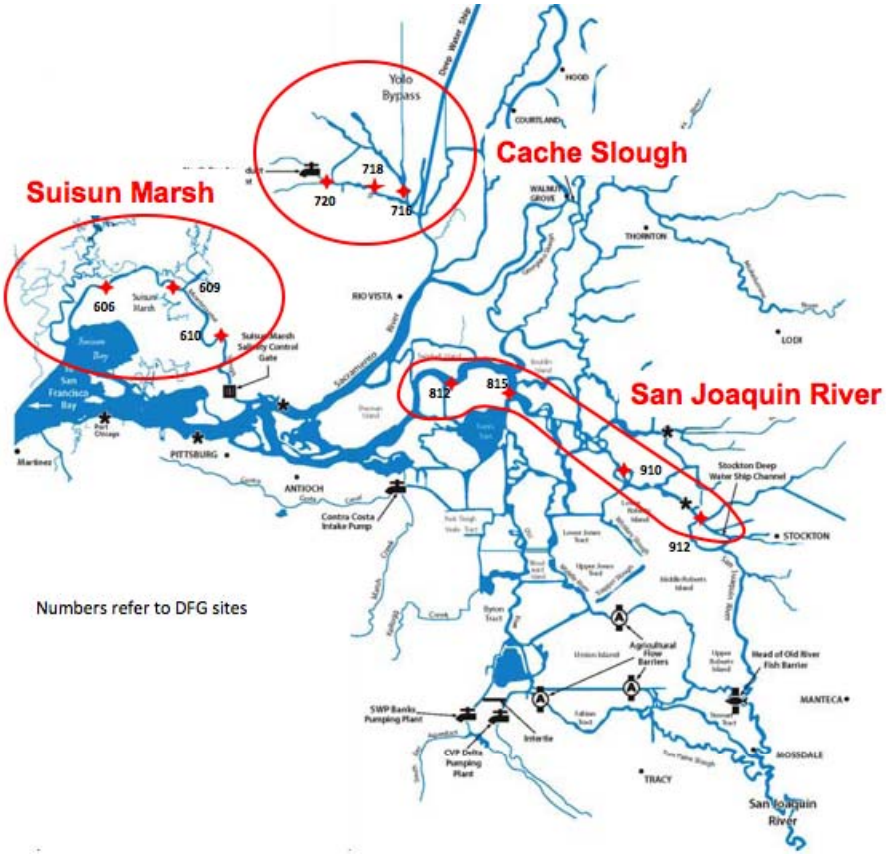


Figure 2. Sampling sites for zooplankton (for DNA barcoding) and the pelagic fish species- delta smelt and threadfin shad (for gut content study). The samples will be collected from a total of 10 candidate stations representing Suisun Marsh, Cache Slough, and San Joaquin River (indicated by red stars). These sampling stations are corresponding to CA DFG sampling locations.

Zooplankton sampling method

Zooplankton will be provided by Co-PI Mr. Randall Baxter, Department of Fish and Game, from the routine Zooplankton Survey. A total of 10 sites that correspond with fish sampling stations in Cache Slough, Suisun Marsh, and San Joaquin River will be sampled (**Fig. 2**). Zooplankton samples will be collected as detailed by CA DFG (Hennessy 2010). At each sampling site, tidal stage, depth, temperature (°C), salinity (ppt), specific conductance (μS), dissolved oxygen, and water transparency (Secchi depth in cm) will be recorded. Zooplankton samples from archived samples and from collections in previous and current years will be subjected to DNA barcoding, validation of molecular tools, and assessments of potential relationships between zooplankton composition in habitats and abundance in stomach contents of planktivorous larval fishes (Task 2).

Rationale and description of pelagic fish species for gut content analyses

This proposed study will include larval stage of the endemic delta smelt and the threadfin shad. The pelagic species were chosen because of their placement in the POD. These species are planktivorous during the larval stage of development, whose prey is selected depending on the age (and size) and by the availability of zooplankton.

Threadfin shad (TFS) is a pelagic clupeid introduced to the SFE in 1953 as an alternative prey species for sport fish (e.g.

striped bass) in response to declines in the populations of prey species (Kimsey 1954). Possessing a robust physiology, the TFS can tolerate highly altered eutrophic watersheds with optimum growth at 22–24^{o}C, and spawns between April and August (Moyle 2002). Planktivorous TFS feed on zooplankton, phytoplankton and detritus in the water column. Most TFS do not live longer than 2 years and is the only pelagic species found exclusively in the freshwater portions of the Bay-Delta ecosystem year-round (Johnson et al. 2010). Larval stages are found in shallow water habitats such as Cache Slough while juveniles move to open-waters such as the Sacramento Ship Channel and the San Joaquin River (Moyle 2002). Considered an important prey species in the upper SFE, TFS abundance can affect the abundance of other predator species such as the striped bass (Armor et al. 2005).

Delta smelt is a member of *Osmeridae* which is endemic in the SFE (Moyle 2002). Delta smelt has been listed as a threatened species since 1993 under the Endangered Species Act (Bennett 2005) as a result of the POD (Bennett 2005). They are known for their distinctive cucumber aroma and feed on copepods in the pelagic zone of the SFE. Delta smelt are primarily an annual fish, spawning only once before dying (Moyle 2002). During the fall, delta smelt migrate upstream to Suisun Bay and the confluence of Sacramento and San Joaquin River. Delta smelt spawn between March and June (Moyle and Herbold 1992, Bennett 2005) in shallow freshwater habitat such as Cache Slough. Larval delta smelt are scattered throughout the watershed of the Sacramento and the San Joaquin Rivers and are more abundant in less altered habitats of Cache Slough and Liberty Island. Delta smelt remain in low salinity zones until they become juveniles, then they slowly move to more saline waters of Suisun Marsh (Bennett 2005).

Fish sampling method

Fish will be provided by Co-PI Mr. Randall Baxter, Department of Fish and Game, from the routine 20mm Trawl Survey. A total of 10 sites that correspond with zooplankton sampling stations in Cache Slough, Suisun Marsh, and San Joaquin River will be sampled (**Fig. 2**). Fish samples will be collected as detailed by CA DFG (http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm). A total of 5–10 fish per species from both archival samples from previous and current collection times will be sub-sampled from each station sample. The sub-samples will be analyzed for gut content as described in Task 2-4. At each sampling site, tidal stage, depth, temperature (°C), salinity (ppt), specific conductance (μS), dissolved oxygen, and water transparency (Secchi depth in cm) will be recorded.

Zooplankton and fish sampling schedule

Zooplankton and fish will be collected following the CA DFG sampling schedule. In year 1, only zooplankton samples will be collected from the DFG designated sampling stations every other month during the entire sampling period (i.e. March, May and July). These samples will be used for morphological analysis and establishment of genetic database (Task 2-1) and for the development of the DNA barcode identification system and quantitative PCR (Task 2-2).

In year 2 and 3, fish and zooplankton sampling will occur simultaneously every month between March and June from all the sampling stations (**Fig. 2**). These months were chosen to encompass the presence of juvenile stages of all the fish species. The collected juvenile fish and zooplankton samples will be used for validation and application of the barcodes and qPCR assays by assessment of zooplankton composition and abundance of habitats that differ in productivity including gut content analysis of key larval species present in the habitats (Task 2-3, 2-4).

Task 2–1. **Morphological analysis and establishment of genetic database**

In year 1, zooplankton samples will be initially examined by morphological analysis at the CA DFG. Morphologically identified zooplankton species will be subjected to DNA sequencing at UC Davis to obtain standardized DNA regions (DNA barcodes). Pictures of each zooplankton showing essential appendages and other features for morphological identification will be taken under a microscope. Up to 50 species from each representative habitat and sampling time will be identified, and subjected to DNA barcoding. Zooplankton which cannot be identified morphologically will also be processed. In year 2, the samples will be split into half for morphological taxonomic identification and the remaining half for quantification by molecular methods to validate the qPCR assays (Task 2-3). In year 3, zooplankton samples collected from the water column and from fish gut contents will be analyzed by qPCR at UC Davis (Task 2-4).

PCR amplification and gene sequencing

DNA barcoding relies on the use of more than one standardized DNA regions. Two of the most commonly used DNA regions, mitochondrial cytochrome c oxidase I gene (mtCOI) and 18S ribosomal DNA (rDNA) (Blaxter 2004) will be amplified by PCR using genomic DNA extracted from morphologically analyzed individual zooplankton from Task 2-1 as follows: genomic DNA will be extracted using a standard phenol-chloroform procedure (Sambrook and Russel 2001), followed by PCR with High Fidelity Taq polymerase (Invitrogen). Available primers for zooplankton mtCOI gene will be used for PCR (Elias-Gutierrez et al. 2008, Bucklin et al. 2007). Degenerate primers for the other target (18S rDNA) will be designed based on the sequence from other representative zooplankton species available in GenBank Database. Cycling conditions for target genes, especially for annealing temperature, will be optimized for each primer set. Amplified DNA fragments at expected size will be extracted from the gel and submitted to Davis Sequencing Service to determine the DNA sequences. Sequencing reactions will be performed from both 5' and 3' ends to obtain full-length and high quality barcode sequences.

Task 2-2. **Development of DNA barcode identification system and quantitative PCR**

Development of DNA barcode identification system

The DNA sequences obtained from Task 2-1 will be deposited in the GenBank database at the National Center for Biotechnology Information (NCBI) as well as the Barcode of Life Database (BOLD) established by the Consortium for the Barcode of Life (Ratnasingham and Hebert, 2007). Taxonomic and source information (e.g. taxonomic classification, location, year, and photo) will be deposited in the databases along with DNA sequences. For organisms that are difficult or impossible to identify morphologically, only DNA sequences will be determined and added to the database to facilitate their identification by DNA sequence similarity search.

Development of qPCR assays

qPCR assays will be developed for key zooplankton species that are ecologically relevant in the SFE. A small portion of the standardized DNA region (< 100 bp), which is well conserved among populations of the species but distinct from other closely related species, will be utilized as a target. Nuclear gene (18S rDNA) is a preferred target for quantification than mitochondrial gene (mtCOI) as the number of mitochondria in a cell varies widely by organism and tissue type. Primers and probes for qPCR will be designed by Primer Express software ver. 3.0 according to the manufacturer's instruction (Applied Biosystems). Probe-based method (TaqMan analysis, Applied Biosystems) will be utilized to maximize amplification efficiency and specificity. Reactions will be run by StepOne Real-Time PCR System from Applied Biosystems. Standard curve will be generated using serial dilutions of synthesized single-stranded DNA with known copy number calculated based on Avogadro's number. Specificity and sensitivity of the assays will be evaluated by spiking gDNA extracted from target and closely related species.

Difficulties are sometimes encountered in the interpretation of qPCR results because the method can only enumerate the copy number of genes that does not reflect the number of individual organisms. In Task 2-2, a new approach expressing qPCR results by biomass will be attempted. Laboratory cultures of *E. affinis* at the Aquatic Toxicology Program at UC Davis will be subjected to qPCR analysis. The ratio of the zooplankton wet weights and gene copy numbers as obtained by qPCR will be calculated to provide a biomass estimate. This approach will be validated by statistical analysis using Analysis of Variance (ANOVA) to develop a formula for converting qPCR results into wet weight.

Task 2–3. **Validation of molecular tools by analysis of zooplankton composition and abundance in critical habitats in the SFE**

Habitats that show gradients in productivity will be examined for species composition and abundance of key zooplankton species using the molecular methods and traditional morphological analysis. Zooplankton samples obtained from a total of 10 sampling stations (Fig. 2) will be sampled as described above, see *Zooplankton sampling method* (Task 2).

Zooplankton samples will be thoroughly mixed in the preservative solution and split into two subsamples. One half of the sample will be used for morphological identification at CA DFG and the other half will be analyzed at UC Davis by qPCR as described in Task 2-2. The zooplankton composition and the number of zooplankton species along with their percentage in total sample will be determined by morphological identification. Biomass of the targeted copepod species in the sample will be analyzed by qPCR and expressed as percentage in total biomass. Correlation (e.g. linear regression) of the two data sets from traditional counting and biomass from qPCR will be analyzed statistically using ANOVA.

Task 2–4. **Application of the molecular tools for assessing potential relationships between zooplankton composition in habitats and abundance in gut contents of planktivorous larval fishes**

Gut contents of delta smelt and threadfin shad at larval stage will be analyzed by qPCR as follows: wet weight of pooled zooplankton from the surface water of each sampling station (**Fig. 2**) will be measured and recorded, followed by homogenization and genomic extraction according to the method as described above (Task 2-1). Diversity and quantity of zooplankton in each sample will be analyzed by qPCR developed in Task 2-2 and 2-3. Subsequently, pool of gut contents of larval fishes from each sampling station (N=5–10) will be weighed and processed in same manner. qPCR will be performed for zooplankton species found in surface waters. Obtained qPCR results will be converted into wet weight based on the equation obtained from Task 2-2, and the results will be expressed as the composition of zooplankton biomass.

Species richness will be examined by the Shannon-Weaver diversity index (*H'*), which is frequently used for assessment of biodiversity in habitats based on the number of species or genera and their relative abundance in a sample (Shannon and Weaver 1949).

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i)$$

The proportion of species *i* relative to the total number of species (p_i) will be calculated, and then multiplied by the natural logarithm of this proportion (ln p_i). The resulting product will be summed across total number of species in a community *S*, and multiplied by -1. We will examine differences in biodiversity richness of the three designated locations by ANOVA. In addition, total and relative biomass of zooplankton species within and between sites will be analyzed by ANOVA as well.

4. Deliverables

Research findings and progress from these tasks will be disseminated through reports, presentations at national and local meetings, and in articles submitted to both the IEP Newsletter and peer-reviewed scientific publications (Table 2). In addition, data collected from the zooplankton survey will be integrated into the current zooplankton monitoring program of the CA DFG to supplement the identification of micro zooplankton species that are difficult or impossible to identify. Furthermore, the zooplankton DNA database will be integrated with ongoing long-term Delta water quality, hydrology, and ecosystem monitoring studies by the State and Federal agencies that may potentially provide essential information on the biological effects of environmental variables such as temperature changes and salinity intrusion, or combination of these multiple stressors and their effect on the composition and abundance of zooplankton species in the San Francisco Bay Estuary.

Table 2. Deliverables for each task. All investigators will be involved in each task of Project management (Task 1) and Collaborative field work (Task 2)

Task	Description	Deliverables
1	Project management	• Semi-annual reports • Final reports • Project summaries for public (beginning/completion) • Project closure report • Presentations at CALFED Science conferences
2-1	Morphological analysis and establishment of genetic database	• Presentations at regional and national/international conferences • Draft scientific paper: Biodiversity of zooplankton species in SFE • Release zooplankton database
2-2	Development of DNA barcode identification system and quantitative PCR	• Presentations at regional and national/international conferences • Draft scientific paper: Development of qPCR assays for key zooplankton species
2-3	Validation of molecular tools by analysis of zooplankton composition and abundance	• Presentations at regional and national/international conferences • Draft scientific paper: Estimating the abundance of key zooplankton species across critical habitats in SFE
2-4	Evaluating zooplankton composition in fish guts and habitats by qPCR	• Presentations at regional and national/international conferences • Draft scientific paper: Correlating zooplankton composition in habitats and gut contents of planktivorous fish

5. Feasibility

The proposed study is feasible due to a combination of 1) research experience, 2) few contingencies for project completion, and 3) availability of research facilities.

1) Dr. Teh is a comparative toxicologist/pathologist with over 20 years of extensive field and laboratory research experience in carcinogenesis, ecotoxicology, endocrine disruption, and biomarker studies. Mr. Randall Baxter from the CA DFG has over 22 years of experience sampling fishes and invertebrates in the San Francisco Estuary and currently directing the two long-term fish monitoring surveys, Summer Townet and Fall Midwater Trawl, in the SFE Delta. Dr. Baxa is a research scientist with a broad background and training in infectious diseases of fish for the last 20 years. Her

research is currently focused on the key interplay between infectious diseases and toxicants. Dr. Kurobe is a well experienced molecular biologist with a background in Fish Pathology, Aquatic Bioscience and Aquatic Toxicology, and has a broad knowledge in molecular techniques described in this proposal, such as DNA barcoding and qPCR.

2) Our proposed study is not dependent on the outcomes of other investigations. At-risk and sport fish samples and Endangered Species Act take permit needed for this study are those already obtained by Mr. Randall Baxter's (DFG) IEP funded monitoring program UC Davis has two boats readily available for fish sampling therefore successful field sampling is very likely. We will apply for our own collectors' permit to cover any permit expiration between the sampling groups. Two trained field assistants from Dr. Teh's laboratory will assist in fish and zooplankton collections to minimize shipping time and to ensure fish are handled properly for biological effect measurements.

3) We have access to instruments and facilities needed to conduct the proposed research. Dr. Swee Teh's laboratory (Aquatic Toxicology Program) at UC Davis has the necessary equipments and facilities for conducting the DNA barcoding experiment and culture of *E. affinis* which is critical in the development of new enumeration approach for the qPCR data (Task 2-2). For running the DNA sequencing reactions and qPCR, DNA samples will be submitted to on-campus facilities, Davis Sequencing Service (http://www.davissequencing.com/) and Real-Time PCR Research and Diagnostics Core Facility (http://www.vetmed.ucdavis.edu/vme/taqmanservice/).

The ultimate goal of this project is the development of the DNA barcoding system for zooplankton identification in the SFE. The system relies on a database linking genetic information to morphological features of organisms as described in the background narrative. Because zooplankton identification is challenging, only a few publications characterizing these planktonic organisms are available. Collaborating with the CA DFG will greatly facilitate the development of the molecular methods by utilizing their zooplankton data that they generated from monitoring the zooplankton community of the SFE over the last 37 years. This research project will develop a comprehensive DNA database of zooplankton in the SFE relatively easily that can be used as a basis for zooplankton assessment for research studies and for routine monitoring of natural habitats.

6. Relevance to the CALFED ERP

Our proposed study fulfills two of the priority topics of the CALFED Ecosystem Restoration Program on:

1) Intertidal restoration to estuarine productivity, provide spawning and rearing habitat for native fishes using the Delta, and which accommodate long-term habitat changes resulting from climate change.

2) Assessing flora and fauna response to restoration; determining changes in productivity, and monitoring hydrology and geomorphic changes in restored areas.

The DNA-based methods will provide a specific and rapid tool for routine monitoring of the composition and abundance of zooplankton across habitats in the SFE. Developing an improved monitoring system to investigate the species diversity and abundance of local zooplankton communities will provide a better understanding on interactions among habitat productivity, fish recruitment and connections to other environmental processes. The outcomes of this study will provide the necessary tools for identifying zooplankton species accurately and rapidly in open waters throughout the San Francisco Estuary and circumventing the limitations of traditional methods used in the current monitoring programs. Thus, this research study will crucially benefit restoration efforts for California native fishes by providing improved monitoring tools that generate rapid and specific information on the identity, quantity, and distribution of zooplankton across the Bay Delta Estuary as important food sources to many of its aquatic organisms.

Assessments of zooplankton biodiversity as we propose in this study can be used in future investigations that aim to restore estuarine productivity. Characterizing zooplankton diversity is central to understanding poorly resolved issues in the Delta such as 1) potential relationship and interaction of phytoplankton and zooplankton and ecosystem- scale responses to fish declines or extinctions, 2) ecological impacts by introduction of exotic species, 3) long-term or cyclic changes in population of pelagic organisms due to direct exposure to contaminants (e.g. ammonia, pesticides) or indirect toxicity to food sources, and 4) factors regulating plankton dynamics and productivity of the estuarine ecosystem.

7. Expected quantitative results (project summary):

This is a research and monitoring project, as such quantitative measures are not applicable. The project will produce following outcomes:

- Genetic database for qualitative (DNA barcodes) and quantitative (qPCR) characterization of macro and micro zooplankton species in the SFE
- Updating information on zooplankton diversity and abundance across habitats of the SFE
- Rapid and accurate identification of all life stages of zooplankton including eggs, larvae, and adults
- Potential discovery of new taxa that are difficult or impossible to identify by morphological analysis
- Molecular genetics of local zooplankton species as a basis for future monitoring of zooplankton
- The rapid and specific assessment of zooplankton composition and abundance by DNA barcoding and qPCR can be used as early warning tools to address the management of habitats at risk of zooplankton taxonomic shifts

Developing molecular-based techniques (DNA barcoding and qPCR) for zooplankton studies in the SFE is critically warranted to 1) Assess zooplankton composition and abundance rapidly and precisely, 2) Determine the relationship between zooplankton composition and fish fitness across critical habitats and, 3) Enhance the current zooplankton monitoring efforts by identifying and quantifying small nondescript zooplankton species that cannot be assessed by traditional morphological methods.

8. Other products and results:

- DNA barcodes from zooplankton in the SFE will be useful for analyzing the origin of invasive zooplankton species and their life cycles. For example, the presence of eight exotic zooplankton species, including *P. forbesi* and *L. tetraspina* known as good and poor diets, respectively for pelagic fish in the SFE are thought to be introduced from their natural habitat in Asia by ballast water from trade ships. DNA barcodes can help provide the scientific evidence to support the origin of these species.
- Zooplankton DNA barcodes in the SFE can be compared to DNA barcodes of zooplankton found in other locations to determine the geographic movements of exotic zooplankton species and their implications.
- DNA barcodes are useful for life cycle studies because gene sequences are unaltered in all developmental stages.

9. Qualifications

1. Dr. Swee J. Teh is the interim director of Aquatic Toxicology Laboratory at UCD, Department of Anatomy, Physiology and Cell Biology, and is the lead investigator of the proposed study. Dr. Teh has over 20 years of extensive field and laboratory research experience in ecotoxicology and biomarker studies. His research interests span the fields of developmental biology, nutrition, toxicology and pathology with special emphasis on adverse health, reproductive, and

embryonic developmental effects of environmental endocrine disruptors and contaminants in invertebrate, fish and shellfish populations. He has an extensive experience in submitting quarterly and annual reports to CALFED (now Delta Science) and has previously managed broad projects and contracts. Dr. Teh has over 50 peer-reviewed publications and has traveled nationally and internationally to present his work in conferences and workshops. Dr. Teh will be responsible for the overall direction of the project.

2. Mr. Randall D. Baxter is a Senior Biologist Supervisor (Fisheries) with the California DFG who has over 22 years of experience sampling fishes and invertebrates in the San Francisco Estuary. He supervises biologists conducting the two long-term fish monitoring surveys, Summer Townet and Fall Midwater Trawl. He's been involved with the development of sampling programs to assess the distribution and habitat use of several native fishes and has authored or coauthored publications focused on delta smelt, splittail and longfin smelt. He is a member of the Pelagic Organism Decline Management Team, which since 2005 has taken an interdisciplinary, multifaceted approach to investigating factors associated with the decline of four pelagic fishes in the San Francisco Estuary, and has coauthored a paper and a couple technical reports with that group.

3. Dr. Dolores V. Baxa is a research scientist at UCD and is the lead researcher at the Aquatic Toxicology Program on studies involving the key interplay between infectious diseases and toxicants and how they impact fish health. Dr. Baxa has a broad range of background and training in infectious diseases of fish for the last 20 years with over 30 peer-reviewed publications. She has maintained rigorous research projects in bacteriology, parasitology, and molecular biology that assess the transmission, interaction, and detection of disease agents in various fish and other secondary hosts in fresh and marine water environments. Her recent project involved the development of molecular techniques to evaluate the dynamics of toxic *Microcystis* in the San Francisco Estuary.

4. Dr. Tomofumi Kurobe is a post-doctoral fellow from Tokyo University of Marine Science and Technology in Japan and has expertise in Aquatic Science, Aquatic Toxicology, Fish Pathology and Molecular Biology. Currently, he is working in Dr. Teh's laboratory at the University of California, Davis and is involved in ecology and toxicology research to address various issues ongoing in California. Dr. Kurobe is a well experienced molecular biologist and has extensive laboratory research experience, especially in species identification based on standardized DNA sequence. He has published over 15 papers in phylogenetic classification of aquatic organisms, development of diagnostic assays for fish pathogens and initial characterization of emerging myxozoan parasites in aquaculture.

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Section 7: Project Budget

1. Detailed Project Budget *(Excel spreadsheets can be used)*

Title: Assessment of zooplankton species composition and abundance in the San Francisco Estuary using DNA barcoding and qPCR

CATEGORIES	2011-2012		YEAR ONE
A. PERSONNEL (Allows 3% COLA)	#of hour	hourly rate	Total
Swee J. Teh, PI (10% effort)	192	48	9,126
Dolores Baxa, Co-I (50% effort)	960	33	31,850
Tomo Kurobe (100% effort)	1,920	19	37,120
Graduate Student (50% effort)	960	19	17,833
Lab Assistant III (50% effort)	960	21	20,448
Three student assistants	1,500	10	15,000
SUBTOTAL PERSONNEL COSTS	6,492		131,377
B. FRINGE BENEFITS (Based on UCD Proposed Blended Rates)			
Swee J. Teh, PI		44%	4,043
Dolores Baxa, Co-I		44%	12,867
Tomo Kurobe		44%	14,996
Graduate Student (50% effort)		3%	481
Staff Research Associate III		44%	9,058
Student Assistants		5%	810
TOTAL FRINGE BENEFITS			42,257
TOTAL PERSONNEL COSTS			**173,634**
C. TRAVEL			
PIs and staffs attend Scientific Meeting and field sampling			5,000
TOTAL TRAVEL COSTS			5,000
D. EQUIPMENT			
TOTAL EQUIPMENT COSTS			0
E. SUPPLIES			
DNA barcoding system sequencing			30,000
Kits (PCR, gDNA extraction)			10,000
Glassware, reagents, and equipment lease			5,000
Biohazard disposal			2,000
Publication costs			0
TOTAL SUPPLY COSTS			47,000
F. CONTRACT/SERVICE AGREEMENT			
TOTAL CONTRACTUAL COSTS (not exempt from F&A)			0
G. OTHER EXPENSES (Allows 10% annual increase per UCOP)			
Graduate Student Fee Remission			14,229
TOTAL OTHER COSTS (exempt from F&A)			14,229
H. TOTAL DIRECT COSTS			
(sum of a through g)			239,862
I. Indirect Costs/Charges			
Modified Total Direct (less fees/tuition)			225,634
25% of base			56,408
J. TOTAL PROJECT COSTS			
(sum of h & I)			**296,271**

ERP Proposal Application Form

CATEGORIES	2012-2013		YEAR TWO
A. PERSONNEL (Allows 3% COLA)	#of hour	hourly rate	Total
Swee J. Teh, PI (10% effort)	192	49	9,400
Dolores Baxa, Co-I (50% effort)	960	34	32,806
Tomo Kurobe (100% effort)	1,920	20	38,234
Graduate Student (50% effort)	960	19	18,368
Lab Assistant III (50% effort)	960	22	21,061
Three student assistants	1,500	10	15,450
SUBTOTAL PERSONNEL COSTS	6,492		135,319
B. FRINGE BENEFITS (Based on UCD Proposed Blended Rates)			
Swee J. Teh, PI		48%	4,540
Dolores Baxa, Co-I		48%	14,533
Tomo Kurobe		48%	16,938
Graduate Student (50% effort)		3%	496
Staff Research Associate III		48%	10,172
Student Assistants		5%	834
TOTAL FRINGE BENEFITS			47,514
TOTAL PERSONNEL COSTS			**182,833**
C. TRAVEL			
PIs and staffs attend Scientific Meeting and field sampling			5,000
TOTAL TRAVEL COSTS			5,000
D. EQUIPMENT			
			0
TOTAL EQUIPMENT COSTS			0
E. SUPPLIES			
DNA barcoding system sequencing			6,000
Kits (PCR, gDNA extraction)			10,000
Glassware, reagents, and equipment lease			5,000
Biohazard disposal			2,000
Publication costs			500
TOTAL SUPPLY COSTS			23,500
F. CONTRACT/SERVICE AGREEMENT			
TOTAL CONTRACTUAL COSTS (not exempt from F&A)			0
G. OTHER EXPENSES (Allows 10% annual increase per UCOP)			
Graduate Student Fee Remission			
TOTAL OTHER COSTS (exempt from F&A)			15,651
H. TOTAL DIRECT COSTS			
(sum of a through g)			226,984
I. Indirect Costs/Charges			
Modified Total Direct (less fees/tuition)			211,333
25% of base			52,833
J. TOTAL PROJECT COSTS			
(sum of h & I)			**279,817**

ERP Proposal Application Form

CATEGORIES	2013-2014		YEAR THREE
A. PERSONNEL (Allows 3% COLA)	#of hour	hourly rate	Total
Swee J. Teh, PI (10% effort)	192	50	9,682
Dolores Baxa, Co-I (50% effort)	960	35	33,790
Tomo Kurobe (100% effort)	1,920	21	39,381
Graduate Student (50% effort)	960	20	18,919
Lab Assistant III (50% effort)	960	23	21,693
Three student assistants	1,500	11	15,914
SUBTOTAL PERSONNEL COSTS	6,492		139,379
B. FRINGE BENEFITS (Based on UCD Proposed Blended Rates)			
Swee J. Teh, PI		49%	4,783
Dolores Baxa, Co-I		49%	16,321
Tomo Kurobe		49%	19,021
Graduate Student (50% effort)		3%	511
Staff Research Associate III		49%	10,716
Student Assistants		5%	859
TOTAL FRINGE BENEFITS			52,211
TOTAL PERSONNEL COSTS			**191,590**
C. TRAVEL			
PIs and staffs attend Scientific Meeting and field sampling			5,000
TOTAL TRAVEL COSTS			5,000
D. EQUIPMENT			
None Requested			0
TOTAL EQUIPMENT COSTS			0
E. SUPPLIES			
DNA barcoding system sequencing			6,000
Kits (PCR, gDNA extraction)			10,000
Glassware, reagents, and equipment lease			5,000
Biohazard disposal			2,000
Publication costs			500
TOTAL SUPPLY COSTS			23,500
F. CONTRACT/SERVICE AGREEMENT			
TOTAL CONTRACTUAL COSTS (not exempt from F&A)			0
G. OTHER EXPENSES (Allows 10% annual increase per UCOP)			
Graduate Student Fee Remission			17,216
TOTAL OTHER COSTS (exempt from F&A)			17,216
H. TOTAL DIRECT COSTS			
(sum of a through g)			237,306
I. Indirect Costs/Charges			
Modified Total Direct (less fees/tuition)			220,090
25% of base			55,023
J. TOTAL PROJECT COSTS			
(sum of h & I)			**292,329**

	Year One	Year Two	Year Three	Total 3-yr
Total project Cost	239,862	226,984	237,306	704,153
25% indirect Cost	56,408	52,833	55,023	164,264
Grand Total	296,270	279,817	292,329	**868,417**

Grand Total for Three years = $868,417

2. Budget justification:

Personnel

Swee J. Teh, PhD (10% time) is the Principal Investigator (PI) and will be responsible for details of contract management and execution, as well as ensuring coordination among tasks. The investigators will be in charge of their respective tasks in the study. Drs. Teh and the collaborators at the California Department of Fish and Game will direct zooplankton and fish sampling (Task 2-1) with the help of one graduate student (50% time), one Lab Assistant (50%), and 1-2 student assistants. Drs. Baxa (50% time) and Kurobe (100% time) will lead the DNA sequencing analyses and development of the molecular techniques (Task 2-2 and 2-3) with the help of one graduate student and 2-3 student assistants. In addition, Drs. Teh and Baxa will manage the coordination of field studies in collaboration with on-going monitoring program at the California Department of Fish and Game; 1-2 student assistants and one graduate student will provide assistance on corresponding morphological analysis at CDFG. All investigators will share the responsibility of data integration and analysis (Task 2-5) and project management (Task 1) including allocation of resources, management of project staff, acquisition of supplies, and development of appropriate protocols for the various laboratory assays and field sampling. Importantly, all investigators will oversee the timely completion of the different tasks, establish collaborative activities to integrate study goals with other research and restoration projects or monitoring programs in the SFE, integrate reports and outreach materials, and manage other tasks as required. All investigators will be responsible for preparation of technical reports and manuscripts.

Fringe Benefits

Fringe Benefits have been calculated using actual benefit rates as indicated on the preceding budget page.

Travel

Travel funding is requested to support field sampling at: [UC Davis Fleet Svc Rental Fee, 7 passenger Van ($70/day) + Fleet Svc Mileage Fee: $0.30/mi for 100 mi/day ($30.00)] X 15 days = $100/day X 5 days/month for 4 months = $2,000, Boat and truck fuels ($1,000), vehicle liabilities and maintenance ($500), Boat safety training ($500), and the presentation of findings and the presentation of findings and developments at major national and scientific meetings ($1000).

Equipment

NA

Supplies

Year 1: Sequencing of standardized DNA regions of 2 target genes ($15/sequence X 2 sequence reaction X ~1000 = $30,000); Kits for molecular analysis such as PCR, genomic DNA extraction, gels ($10,000), chemicals and molecular reagents, buffer and standard solutions for pH, salinity, ELISA bioassay, glasswares; and dissecting microscope and compound microscopes lease,, pipette tips, microtubes, and misc disposal supplies ($5,000), and biohazard disposal ($2,000).

Year 2: Sequencing of standardized DNA regions of 2 target genes ($15/sequence X 2 sequence reaction X ~200 = $6,000); Kits for molecular analysis such as PCR, genomic DNA extraction, gels ($10,000), chemicals and molecular reagents, buffer and standard solutions for pH, salinity, ELISA bioassay, glasswares; and dissecting microscope and compound microscopes lease,, pipette tips, microtubes, and misc disposal supplies ($5,000), biohazard disposal ($2,000), and publication costs ($500).

Year 3: Sequencing of standardized DNA regions of 2 target genes ($15/sequence X 2 sequence reaction X ~200 = $6,000); Kits for molecular analysis such as PCR, genomic DNA extraction, gels ($10,000), chemicals

and molecular reagents, buffer and standard solutions for pH, salinity, ELISA bioassay, glasswares; and dissecting microscope and compound microscopes lease,, pipette tips, microtubes, and misc disposal supplies ($5,000), biohazard disposal ($2,000), and publication costs ($500)

Other Expenses:

Graduate student fees: year 1 ($14,229), Year 2 ($15,651), and Year 3 (17,216).

3. Administrative overhead:

Indirect Costs:
The current indirect cost rate for VM:APC at University of California Davis is 25%

· Class Waiver	No.: **03R-135**	Date Approved: **5/9/2003**	Sponsor Code:
Campus: **OP**	Reason: **C** [A=vital interest; C=sponsor policy]		
Sponsor Name: *CALIFORNIA STATE AGENCIES*			
Project Title: **CALIFORNIA STATE AGENCY AGREEMENTS****			
Waiver Rate: **25.00% MTDC***			
Notes: *UNLESS OTHERWISE SET FORTH IN STATUTE, REGULATION, OR PUBLISH POLICY THAT APPLIES TO ALL RECIPIENTS. C&G MEMO 03-02. SEE OTHER STATE CLASS WAIVERS FOR SPECIFIC PROGRAMS.			