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Proposal No.	Region

(Pages A13-A18)

Section 1: Summary Information

1. Project title:	Health of Threatened Fish: Role of Contaminants, Disease, and Nutrition
2. Applicant name:	University of California at Davis
3. Contact person:	Dr. Swee Teh
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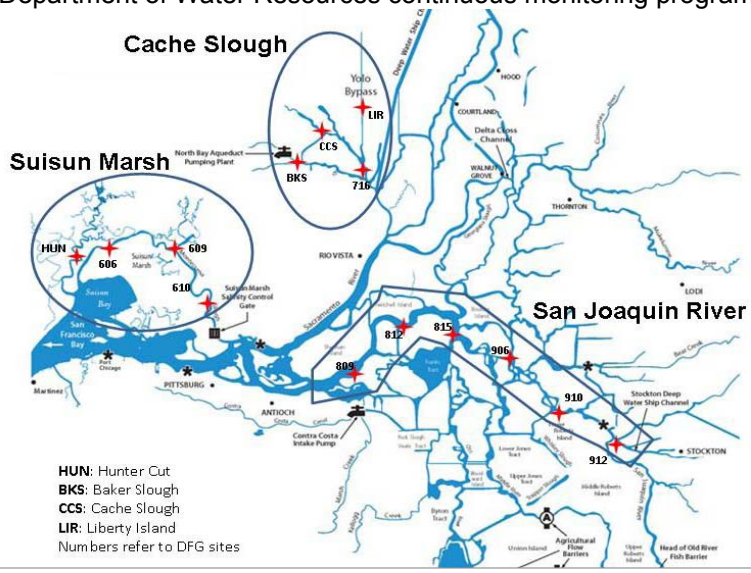
9. Agency Type:	Federal Agency ☐ State Agency ☐ Local Agency ☐ Nonprofit Organization ☐ University (CSU/UC) ☒ Native American Indian Tribe ☐
10. Certified nonprofit organization:	Yes ☐ No ☒
11. New grantee:	Yes ☒ No ☐
12. Amount requested:	$**953,448**
13. Total project cost:	$**953,448** + in-kind services from DFG and USBR
14. Topic Area(s):	Primary: At-Risk Species Assessment Secondary: Ecosystem Water and Sediment Quality, Estuary Food Web Productivity
15. ERP Project type:	Primary: Research Secondary: Monitoring, Pilot/Demonstration
16. Ecosystem Element:	Primary: Bay-Delta Aquatic Food Web Secondary: Contaminants,
17. Water Quality Constituent:	Primary: Toxicity of Unknown Origin and Contaminants Secondary: Nutrients and Oxygen Depleting Substances
18. At-Risk species benefited:	Threadfin shad (*Dorosoma petenense*), striped bass (*Morone saxatilis*), Sacramento splittail (*Pogonichthys macrolepidotus*).
19. Project objectives:	Determine the biological effects of contaminants, pathogens/diseases, and nutritional status in threatened pelagic fishes and establish baseline health
20. Time frame:	2 years (July 1st, 2011 - June 30th, 2013)

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** Liberty Island – DR2068: 38.329, -121.693 Cache Slough: 38.295, -121.742 Barker Slough Pumping Plant: 38.276, -121.797 Cache Slough N. of Cable ferry: 38.241, -121.686 **Suisun Marsh** Hunter Cut at Montezuma Slough: 38.156, -122.053 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 N. of Jersey Point: 38.053, -121.689 San Joaquin River W. of Oulton Point: 38.090, -121.641 San Joaquin River at Medford Island: 38.052, -121.509 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 either part of the Department of Fish and Games routine monitoring or at locations within the Department of Water Resources continuous monitoring program. 
4. County(ies):	Sacramento County, 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]) Not Applicable	
2. Owner Interest: Not Applicable	
3. Permits: Not Applicable	Not Applicable
4. Lead CEQA agency:	Not Applicable
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, 2 and 3

Goal 2: Ecological Processes; Objective 1 and 2

Goal 4: Habitats; Objective 1 and 5

Goal 6: Water and Sediment Quality; Objective 1, 2 and 3

The proposal primary ERP topic areas are 1) **At-Risk Species Assessment** and 2) **Ecosystem Water and Sediment Quality.** This proposal is a **research monitoring** project type and the primary ecosystem element is **Stressors.**

The goals of this study are the following:

1. Evaluate the potential correlations between the different measurements of biological effects and physicochemical parameters
2. Determine the effect of fish nutritional status on susceptibility to pathogens/disease and contaminants as determined by biomarker responses
3. Analyze the fish health profile by integrating the outcome of the biological effects
4. Describe the current fish health status and use as baseline information

2. Additional objectives:

Objective 1 (Additional): Determine the potential differences in the severity of biological effects between the species caught from the less productive (San Joaquin River) and more productive habitats (Suisun Marsh and Cache Slough)

Objective 2 (Additional): Determine if the species collected from the productive habitats are more nutritionally adequate than the species from the less productive habitat

Objective 3 (Additional): Develop specific cell lines for the isolation and culture of viral agents from the threatened species

Objective 4 (Additional): Determine if the fish health status is a good indicator of the habitat health

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: Dolores V. Baxa (UCD), Randall D. Baxter (DFG), Ping-Shi Wu (Lehigh University, PA), Peter Moyle (UCD), Ronald P. Hedrick (UCD)
Supporting Staff: Shawn Acuna, Ida Flores, Sarah Lesmeister, Tomofumi Kurobe, Ching Teh
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.

Last Name	First Name	Organization	Role
Feyrer	Fred	US Bureau of Reclamation	Feyrer is PI of IEP funded project: "*Life history and biology of splittail"* with Teh as Co-PI. Feyrer will actively cooperate by providing adult splittail, threadfin shad, and striped bass

Section 6: Project Tasks and Results Outline

Developing a Baseline Health Status for Threatened Fish in Critical Habitats of the San Francisco Estuary: An Ecological Approach of Evaluating the Effects of Contaminants, Disease, and Fish Nutrition

1. Detailed Project Description

The decline of fish populations in the San Francisco Estuary (SFE) has raised serious concerns regarding the overall degradation of this unique ecosystem. A core issue is the unprecedented decrease in the abundance of pelagic organisms including four pelagic fishes collectively referred to as the "Pelagic Organism Decline", POD (POD Report 2007, Sommer et al. 2007). Food quality and quantity, habitat availability, and water quality are among the key elements that have been explored to some extent in relation to the fish declines (POD Report 2007). Currently lacking among the scientific studies is baseline information on the health of native or introduced fish including POD species. A concerted effort to establish a baseline fish health data, as we propose in this study, is critically relevant as multiple stressors are present in the Delta that have disrupted and will continue to render debilitating effects to the estuary and its fishery resources.

The purpose of this proposed study is to evaluate the impacts of two key stressors, contaminants and disease, including environmental factors, and the underlying role of nutritional adequacy on fish health. The cumulative effects of these factors will be used as criteria to establish the general health status of two pelagic species, striped bass (*Morone saxatilis*) and threadfin shad (*Dorosoma petenense*) currently in population decline, and the endemic Sacramento splittail (*Pogonichthys macrolepidotus*) as a species of special concern and as an indicator species for the SFE. The sedentary tule perch (*Hysterocarpus traskii*) will be included as a reference control to determine the site specificity of biological effects in the three species with migratory behavior. Targeting both geographic and temporal distribution, juvenile to adult stages of the four species will be collected from sites in Cache Slough, Suisun Marsh, and San Joaquin River. These habitats were selected because Cache Slough and Suisun Marsh have high primary productivity that support nursery rearing and spawning of these species and other fishes while San Joaquin River have less productive habitats due, in part, to contaminant loading and decreased plankton production. Based on the habitat features, potential gradients of the stressors and fish nutritional status may be found in the candidate sites chosen for this study. It is important to note that the temporal and spatial distribution of contaminants in the Delta remains poorly understood (Anderson et al. 2007).

In this study, we define fish health as a state characterized by a set of biological criteria including 1) fish condition factor and organo-somatic indices, 2) biomarker responses, 3) pathogens/diseases, and 4) fish nutritional status. These biological effects will be analyzed for temporal and spatial trends of health and condition and used as baseline information in the two POD species, the endemic Sacramento splittail, and the tule perch as reference control. Although the current condition is not realistic baseline information as multiple stressors have been present in the SFE for decades, baseline health may provide a valuable reference on potential trends in improvement or degradation of fish health. Unique to the POD phenomenon are pressing issues such as geographic, seasonal, and temporal variability of fish populations and contaminant inputs, difficult evaluation of responses in a complex water body, and lack of a reference condition, all of

which warrants a rapid and progressive mitigating approach (Anderson et al. 2007). In the proposed study, our unifying goal is to establish an innovative conceptual framework that proposes relationships among stressor effects, ecosystem function, and the general health of fish currently at risk of population decline. **Although it is impossible to determine the precise factors contributing to the health of a free-ranging species caught at a certain site, the use of an integrated approach incorporating fish condition indices in concert with assessments of biological effects and in comparison to the health of a sedentary reference control fish can provide good indicators of the general health of the species.**

Establishing baseline health information is requisite to modeling or assessing changes in the health of other threatened species and changes to the health of the ecosystem itself. Our study will provide the essential ground work that ties the impacts of exposure to contaminant stressors and microbial diseases known to be present in the aquatic environment as combined with potential effects of nutrition. Furthermore, integrating fish health data with ongoing long-term Delta water quality, hydrology, and ecosystem monitoring studies by the State and Federal agencies 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 important fish species. Our results may serve as a framework to link several ecological and management factors as charged to the POD committee to uncover sustainable solutions to address current challenges associated with fish declines in the Delta. Our collaborative aim is to contribute to management and restoration efforts in the Delta by investigating comprehensive fish health in these sites through assessments that integrate potential effects of key stressors. An ecosystem perspective on fish health is relevant to understanding the types of management approaches that need to be implemented in the SFE. By understanding the incremental and cumulative effects of multiple stressors, baseline information on fish health may provide critical insights into system-wide patterns of normal prevalence and distribution (spatial and temporal) of different life stages, and their ability to sustain growth and reproduction following exposures to environmental stressors. It is essential to collect baseline information prior to major structural changes in water delivery, such as the proposed construction of the peripheral canal, the “Two Gates” fish protection demonstration project, and other water management operations so that the potential impacts of such changes can be properly assessed in the future.

Because exposure to contaminants has been one of the most important stressors in the Delta for decades, the presence of pathogens/disease in wild populations is one of the ultimate health indicators representing the cumulative effects of multiple stressors and other environmental variables (Hedrick 1998). The nutritional status of the fish can affect the impacts of contaminants and disease. Fish nutrition, in part, may reflect shifts in food quality and availability, which has been identified as an important factor in fish declines in the estuary (POD Report 2007). This investigation will attempt to understand these relationships from a perspective utilizing an integrated approach of measuring the effects of environmental stressors and other emerging factors that could profoundly alter the Delta and its fishery resources. We propose to answer key questions such as:

- Do fish exhibit reduced nutritional status when exposed to contaminants and/or disease?
- Are nutritionally inadequate fish capable of sustaining growth and reproduction?
- What are the fundamental and significant health problems confronting these fish in these habitats, and which risks to health warrant intervention?

To address these questions, the specific objectives of this proposal are to:
Objective 1: Evaluate the potential correlations between the different measurements of biological effects and physicochemical parameters
Objective 2: Determine the effect of fish nutritional status on susceptibility to pathogens/disease and contaminants as determined by biomarker responses
Objective 3: Analyze the fish health profile by integrating the outcome of the biological effects
Objective 4: Describe the current fish health status and use as baseline information
Our research will be divided into two major tasks with several subtasks. These tasks and associated hypotheses are:

Task 1 – Project Management
Task 2 – Fish Health Evaluation
Task 2–1. Fish sampling
Task 2–2. Fish condition and biomarkers study
Task 2–3. Pathogens/disease
Task 2–4. Proximate analysis to determine fish nutritional status
Task 2–5. Integration and data analysis

H_1: The effects of multiple stressors (contaminants, environmental factors, disease) vary among fish species and their life stages across habitats and seasons
H_2: Multiple stressors negatively affect the fundamental fish functions (growth and reproductive capacity)
H_3: Fish nutritional status is negatively altered after exposure to multiple stressors
H_4: Multiple stressors act in concert and cause biological effects that negatively impact overall fish health

PREVIOUS RESEARCH

In an effort to address the potential causes of the POD, research investigations that ensued focused on broad components including bottom-up (food), top-down (predation), and habitat factors (physical and chemical) (Sommer et al. 2007). To date however, no single ultimate cause of the POD has been identified indicating that a combination of multiple stressors are the likely cause of the population collapse (Sommer et al. 2007, MacNally et al. 2010, Thompson et al. 2010). Among the key stressors associated with POD include altered hydrology of habitats (water export pumping for domestic and agriculture use), habitat changes affecting reproduction and recruitment, invasion of exotic species including toxic algae and toxicant loading, climate, and food web changes resulting in species composition shifts and predation (Linville et al. 2002, Lehman et al. 2005, Bennett 2005, Sommer et al. 2007, Davis et al. 2008, Jassby 2008). The SFE Delta is a highly complex and dynamic ecosystem that each of the stressors can potentially influence and modify other factors rendering prior efforts of correlative data analyses from the past 30 years unsuccessful at identifying the specific cause(s) of the POD (Bennett and Moyle 1996, Sommer et al. 2007, MacNally et al. 2010, Thompson et al. 2010, Glibert 2010).

CRITICAL UNKNOWNS

Our study addresses a critical need for information on the impacts of recurring stressors in the Delta notably contaminants, disease, environmental stress, and the underlying role of nutrition on fish health. To the best of our knowledge, the comprehensive effects of these key stressors remain undetermined to date among fish in the SFE particularly in light of their implications to declining fishery resources. Stressors and their impacts are altered over time and remain a compelling threat to the general health of fish and other organisms in the estuarine ecosystem. The Delta Independent Science Board recently underscored the role of stressors in the Delta based on the current state of knowledge: "*Not one stressor, or group of stressors in the California Delta is the most important, the stressors are interactive and complex"* http://www.deltacouncil.ca.gov/delta_science_program/publications/sci_news_0211_stressors.html. Early warning indicators of exposures to contaminants have been rarely examined in the Delta. We will address this information gap by identifying biomarkers that can be used to assess the sub-lethal effects of chemical contaminants particularly to growth and reproduction of at risk fish species. The declining environment requires a massive task of characterizing the adverse effects of multiple stressors particularly to threatened species to determine whether chronic dysfunctional reproductive capacity in surviving fish may ultimately result in decreased fecundity and fertility, and potentially affect population level. Emerging challenges from incremental and cumulative effects of the key stressors that we propose to investigate warrant further understanding on their impacts to fundamental fish functions, consequences to general health status, and implications to population recruitment.

2. Background and Conceptual Models

Several pelagic species showed substantial declines from 2002 to 2004 in the upper SFE. Included in this trend were record low numbers of delta smelt, young of the year striped bass, and close to historic minimum for threadfin shad and longfin smelt (Sommer et al. 2007). Splittail share similar decreasing trends and habits as that of delta smelt and longfin smelt. Also in decline during this time period were calanoid copepods that are important food sources to pelagic species (POD Report 2007). Significant threats to these species are changes in water management and hydrologic infrastructures (diversions) that reduce spawning and rearing areas and other low-salinity habitats in Suisun Bay (Meng and Moyle 1995). In general however, low recruitment of pelagic fishes has been linked to poor survival and growth of larval and juvenile fishes (Bennett and Moyle 1996) that prey primarily on zooplankton (Moyle 2002, Kimmerer 2006). Over time, species invasions triggered the severe competition for food resources resulting in persistently low food supply and zooplankton taxonomic shifts (Winder and Jassby 2010). Consequently, the limited availability of food resources for estuarine fish impacted the abundance indices of pelagic species (Sommer et al. 2007).

By utilizing field-based fish health investigations, our proposed study aims to establish the potential causal relationships among key stressors (contaminants, disease), environmental factors (dissolved oxygen, turbidity, temperature, salinity), nutritional status, and a panel of fish health responses. This comprehensive approach as demonstrated in our conceptual model **(Fig. 1)** will provide information on the relative importance of the cumulative impacts of multiple stressors in at-risk species and other aquatic organisms in the SFE Delta. This approach may also benefit other aquatic ecosystems with declining fisheries resources. In view of the critical interaction of multiple stressors and the host-pathogen-environment continuum, fish health monitoring has been the basis of a unique and unprecedented strategy of establishing a healthy ecosystem in the Great Lakes http://www.glfc.org/research/reports/stephen_fish_health.pdf. Evaluating the impacts of toxic substances and pathogens/disease on threatened fish in the SFE-Delta is warranted as the general health status of fish is more likely impaired in polluted waters (Moller 1985).

CONCEPTUAL MODEL

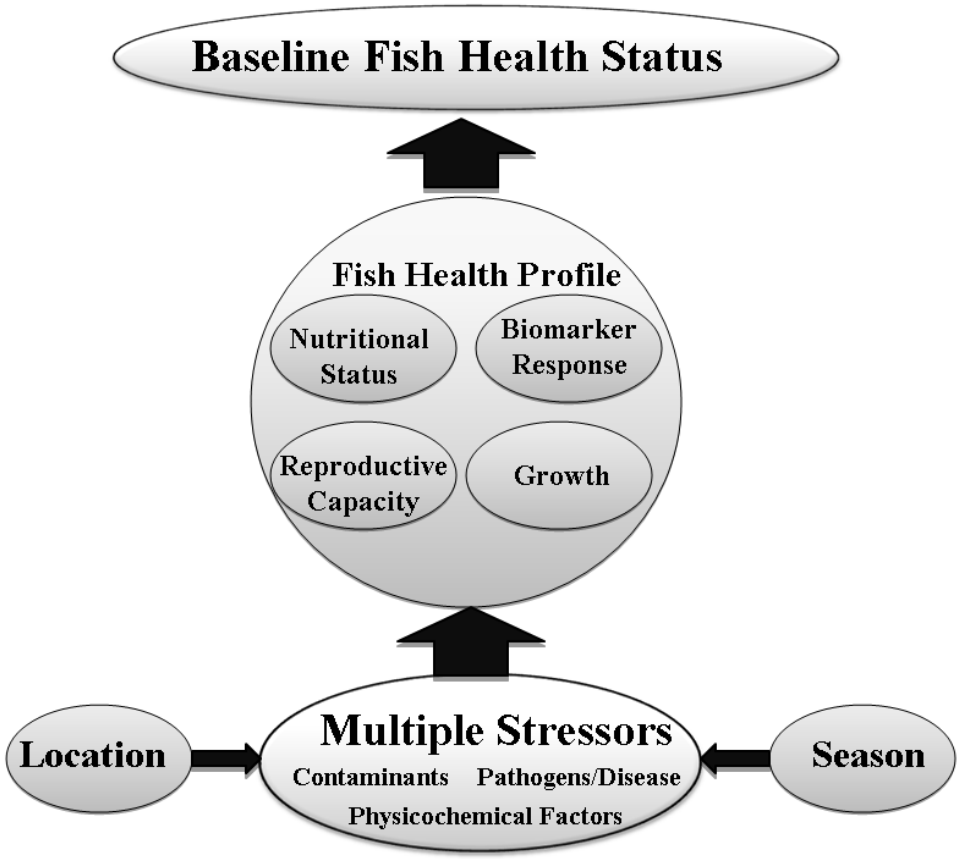


Figure 1. Conceptual model for establishing the baseline health of threatened fish in the San Francisco Estuary. Multiple stressors such as contaminants, pathogens/disease, and physicochemical factors that vary temporally and spatially act in concert to determine the general fish health profile. Key stressors, sub-lethal contaminant exposures (biomarker responses), and nutritional status interact and impact the fish fundamental functions of reproduction and growth. Baseline health indicates the current condition as a result of the incremental and cumulative impacts of multiple stressors.

Rationale for species

Investigating biological effects in striped bass and threadfin shad is warranted in view of their placement in the POD and as an endemic species of special concern (Sacramento splittail). Although declining in abundance, threadfin shad and striped bass are broadly distributed within the Delta and Suisun Marsh for needed specimens. Splittail is also broadly distributed at least seasonally and remains sufficiently abundant. Further, these fish will provide context to the nutritional aspect of the study in relation to their feeding ecology as they utilize the benthic (splittail), pelagic (threadfin shad), as well as top consumers (striped bass) in the estuarine food web. As these species are migratory, the sedentary tule perch will be used as reference control to verify the temporal and spatial differences in biological effects in the target species due to exposures to toxicants and stressors at different habitats during movements across the SFE. Measurements of biological effects due to stressors present in occupied habitats can be estimated directly in the tule perch. Moreover, tule perch feed facultatively in the water column like threadfin shad or on the bottom or another substrate similar to striped bass and splittail (Moyle 2002). Benthic and pelagic food resources may be exposed to different toxicants and stressors that may be integrated by tule perch and to some degree striped bass, but not by threadfin shad and splittail.

Description and distribution of species

Threadfin shad (TFS) is a pelagic clupeid introduced to the SFE as an alternative prey species for sport fish (e.g. striped bass) and other declining species such as the Delta smelt (Kimsey 1954). The TFS can tolerate highly altered

eutrophic waters, optimum growth occurs at 22–24°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).

Sacramento splittail is a native endemic cyprinid and a benthic estuarine forager in the SFE (Moyle 2002). Due to fluctuating abundances and changes in habitat availability, splittail was listed in 1999 under the Endangered Species Act of 1973 and delisted in 2003 despite the uncertainty in population recovery (Moyle 2002). As a key predator in the benthic food web and because of the strong relationship between their population size and foraging habitat, splittail was designated a species of special concern and as an indicator species for the SFE by the USFWS and CDFG (Baerwald et al. 2007). Splittail spawn in shallow water habitats, such as in Cache Slough from February to July (Sommer et al. 1997), and feed on zooplankton (Moyle 2002, Baerwald et al. 2007). Juvenile stages migrate to estuarine habitats such as Suisun Marsh to grow into adults in two to four years. Splittail adapt well in estuarine waters (10-18 ppt) and are found in the Delta, Suisun Bay, Suisun Marsh, and other parts of the SFE and tributaries (Moyle 2002).

Striped bass is an estuarine piscivorous perciform introduced to the SFE as a sport fish in the late 1800s. Striped bass are found in freshwater portions of the Delta year-round with certain populations migrating to Suisun, San Francisco Bay, and the Pacific Ocean. The fish migrate to freshwater to spawn beginning in May and peaks in June and July (Moyle 2002). The larvae and juveniles forage on zooplankton in productive habitats such as Suisun Marsh. While striped bass populations historically increased to levels supporting commercial and sport fisheries (Skinner 1962), significant declines since 2000 placed them in the POD (Armor et al. 2005).

The tule perch is a non-migratory member of the surfperch family, native to rivers and estuaries of central California, the sole member of its genus and the only freshwater member of its family (see Moyle 2002). The Sacramento-San Joaquin subspecies occurs in large tributaries to the SFE. They are broadly distributed in the Sacramento Valley and estuarine tributaries, the Delta, Suisun Marsh, and estuarine reaches of Petaluma River. Tule perch are viviparous, mating occurs in July–September, and females give birth to precocious young in May–June; brood size depends on food abundance (e.g. benthic and pelagic invertebrates).

Contaminants, Environmental Factors, and Fish Health

Fish are robust indicators of the ecological conditions in aquatic habitats. As such, fish health monitoring is an important and ecologically relevant approach of determining the effect of stressors to fundamental fish functions such as reproduction, growth, and recruitment. Reproductive and developmental parameters are among the most important sentinel endpoints for assessing exposure to contaminants. While thousands of chemical contaminants are continuously being introduced into the SFE (Kuivila and Hladik 2008, Werner et al. 2008, Johnson et al. 2010), few studies to date have focused on determining the biological effects of contaminants particularly among fish experiencing population declines in the Delta. The survival and growth of delta smelt and striped bass were altered following exposure to delta water (Werner et al. 2000). Exposure to Hood water containing wastewater effluent or runoff from Sacramento urban areas suggested negative impacts to growth and development of larval delta smelt (Connon et al. 2010)

The presence of contaminants is highly likely in locations where fish populations are in severe decline or when individual fishes are experiencing decreased growth, survival, and reproduction (Arcand-Hoy and Benson 1998, Kidd et al. 2007). Because these biological effects are likely indicators of endocrine disrupting chemicals (EDCs), exposure and effects of EDCs in threatened pelagic species in the upper SFE are critically warranted. Agricultural drainage, wastewater treatment plants and urban runoff are a significant input of contaminants to the SFE (San Francisco Estuary Institute 2004, Leatherbarrow and McKee 2004). Contaminants such as pesticides and selenium are dominant in agricultural runoffs while urban discharges are mostly contaminated with PCBs and dioxins. As these observations demonstrate,

there is a need to better understand the role of contaminant affecting fish health in the SFE. Delta smelt and other aquatic species of the Delta SFE may also be exposed to a suite of emerging contaminants that includes pharmaceutical agents and personal care products, many of these emerging contaminants are known to affect reproduction via toxicity to the endocrine system (Daughton and Ternes 1999, Kolpin et al. 2002).

Increased concentrations of mercury and the emerging contaminant flame retardants polybrominated diphenylethers (PDBEs) affecting human and wildlife health were found to be prevalent among fish in the San Francisco Bay (Greenfield et al. 2004, Davis and Greenfield 2004) but remains to be determined in the Sacramento-San Joaquin River Delta. Data synthesis using chemical and toxicity analyses of water in the SFE Delta demonstrated that exposure to contaminants is generally detrimental to pelagic organisms (Johnson et al. 2010). One significant conclusion drawn from the report on contaminant effects noted: ***"while future research can address our understanding of the relative sensitivity of POD species to various contaminants in the system, it cannot recreate history".*** This underscores the importance of establishing adequate background data, such as fish health status, which can provide critical baseline information to understand key relationships among factors surrounding the decrease or increase in populations of aquatic organisms in the SFE. From a management standpoint of adapting relevant management strategies and restoration efforts within the Delta region, assessing the health of at-risk and sport fish by determining deleterious effects from long-term exposures of populations to chemical contaminants should be a priority.

Establishing causal or correlative relationships among environmental contaminants and fish health is a notoriously difficult task to tackle due to the complex interaction of ecologic factors such as temperature, food quality and quantity, and habitat availability (Ham et al. 1997). For example, extreme water quality (e.g. temperature, DO, pH) and pollutants can cause stress in fish leading to disease (Wood 1979). Exposure to contaminants can influence host susceptibility and pathogen infectivity (Riley et al. 2008). Host nutritional status affects pathogen-host interaction while infection aggravates nutritional deficiencies that in turn exacerbate infections (Solomon and Scott 1994). The contribution of a specific stressor can be difficult to quantify when a suite of stressors affects a population. In general however, contaminants target the fish immune function, which can enhance disease susceptibility (Casillas et al. 1993, Sanders et al. 1996). Interactions between pathogens, pollutants and other environmental stressors have been rarely examined but the synergistic interactions of these risk factors have been observed (Arkoosh et al. 1998, Clifford et al. 2005). Studies that establish correlations between specific chemical pollutants and disease impacts on wild fish populations are to date, lacking. Climate change is one of the key environmental stressors to fish and other food web organisms globally and in the SFE (Lehman 2004). The role of climate shifts in the declining state of the Delta ecosystem and its fishery populations is an enormous task that remains to be determined. In our proposed study, we will determine the potential relationships between temperature shifts and the different indices of fish health across collection sites and times.

Pathogens, Disease, and Fish Health

The role of disease in the variability of fish populations in the SFE has received less attention than many other potential causes. Microbial pathogens are natural components of the aquatic environment therefore fish populations are subjected to continuous or repeated exposure to microbial infections. Pathogens and hosts evolve in response to each other and to ecosystem conditions consequently, pathogens may occur in the absence of disease (Riley et al. 2008). Although pathogens co-evolve naturally with native fish, pathogens are also key agents of diseases (Austin and Austin 1999) that may cause morbidities and mortalities in sub-clinically infected hosts under extreme environmental factors (Foott et al. 2003, Stocking and Bartholomew 2005). The importance of microbial disease in wild fish populations has been the primary impetus for the National Wild Fish Health Survey initiated in 1997 by the U.S. Fish and Wildlife Service (http://www.fws.gov/wildfishsurvey). One of the agents of concern to wild fish is viral hemorrhagic septicemia virus (VHSV) historically affecting trout culture in Europe. VHSV was identified as the cause of massive die offs among wild fish in the Great Lakes region (Elsayed et al. 2006, Gagne et al. 2007, Lumsden et al. 2007). The spread of this virus among native freshwater and marine fish is one example of the profound effects of infectious diseases on wild fish populations.

In a stressed ecosystem such as the SFE, invasive species harboring exotic pathogens can affect the abundance

of the host population (Lafferty et al. 2004). For example, severe intestinal infections due to a myxozoan parasite were demonstrated in the invasive yellowfin goby (YFG), *Acanthogobius flavimanus*, during a monitoring program by Dr. Peter Moyle's group in Suisun Marsh in 2005 (Baxa, Stover, Clifford, Kurobe, Teh, Moyle, and Hedrick; manuscript in progress). The myxosporean was detected from YFG immediately after collection from Suisun marsh and from gobies that were moribund or that succumbed to infection after 2 weeks in captivity. Our concern about this parasite is due, in part, from wide variations of annual catch per unit effort in YFG that were abundant from 1992 to 2001 and declined thereafter (Meng et al. 1994, Matern et al. 2002). Invasion ecology suggests that disease can either reduce (if it affects the invader) or increase (if initiated by a relatively immune invader) the impact of introduced species (Simberloff and Gibbons 2004). The basic myxozoan life cycle alternately infects fish and oligochaete worms and when these hosts die, they liberate spores into the water column which are infectious to the opposite host (i.e. spores from fish infects worms and vice versa). Carriers of latent infections may transmit the parasite to indigenous species in the marsh. Species specificity of the goby parasite is unknown, but it can likely infect native fish species. Exotic species in the SFE may alter host population density by carrying pathogens deemed more pathogenic to naïve hosts (Riley et al. 2008).

Fish health surveys were initiated in the SFE in 2005 in striped bass, threadfin shad, and other species by the CA Department of Fish and Game (CDFG) and the US Fish and Wildlife Service (USFWS) (Fish Health Survey 2005). Fish were presumed healthy as determined by length-weight data, and that striped bass with parasites were deemed healthier than fish without parasites. As the biological significance of these findings was unclear (Johnson et al. 2010), the USFWS conducted another survey in 2006 that focused on threadfin shad and longfin smelt (Foott et al. 2006; Fish Health Survey 2006). As abnormalities were not observed from among few samples (N=13) examined by histology, another survey was conducted in 2007 using large sample sizes (Fish Health Survey 2007). Although significant health problems were found in threadfin shad and longfin smelt in 2007, it is important to note that the fish were not evaluated for contaminants or nutrition-related deficiencies. Importantly, specific cell lines were not used in cell cultures in attempts of isolating viral agents. It is possible that the lack of specific cell lines may have affected the isolation and detection of viral agents if present.

Infectious diseases have been reported from key species in the SFE. For example, *Mycobacterium*, a bacterial pathogen causing serious diseases in mammals, humans, and almost all species of fish, was first reported in the SFE from striped bass (Hedrick et al. 1987) and in Delta smelt (Bruno et al. 1998). In yet another study, groups of Delta smelt caught from the Sacramento San Joaquin River showed the presence of *Mycobacterium* and the disease (mycobacteriosis) during broodstock development and swimming performance trials of progenies in the laboratory (Antonio-Baxa et al. 2000). These studies suggest that *Mycobacterium* may be present in a latent state in wild populations in the SFE and infections can progress from asymptomatic to clinical under stressful conditions of intensive culture and climate shifts.

Histopathological analyses of native Sacramento splittail from the Delta in 2001–2002 indicated significant toxic effects in the liver (Greenfield et al. 2008). Among the POD species, contaminant-related health problems have been mostly observed from striped bass as their spawning grounds are close to discharge point sources that are more likely affecting the survival of eggs and larvae (POD Report 2007). Liver lesions caused mortalities in striped bass in the Sacramento River (Bennett et al. 1995), and cestodes were found in stomachs and mesenteries (Arnold and Yue 1997). Viral infections were not observed from striped bass examined in 2006 (POD Report 2007).

Fish Nutrition and Health

Growth and reproduction in fish are maintained by receiving nutrients and energy through normal feeding. Food quality and quantity, water quality, contaminants, and disease can affect energy storage and allocation in fish. Stressed fish use more energy to survive hence less energy is available for growth and reproduction. Fish exposed to stressors, such as sturgeons fed with selenium-spiked diets, have lower energy storage, reduced growth, and poor reproduction (Tashjian et al. 2006). Salmonids infected with parasitic nematodes have low lipid contents and altered fatty acid composition compared to non-infected fish (Schaulfer et al. 2008). Severe decline in Chinook salmon population was

associated with an epizootic disease outbreak, but dietary deficiency exacerbated their vulnerability to disease (Benjamin and Bence 2003, Holey et al. 1998). As these examples demonstrate, most of the stored energy may have been utilized to cope with the effects of the stressors (i.e. infection and contaminant).

Lipids are one of the most important nutritional factors affecting the fitness of aquatic organisms by supplying energy and essential compounds for general metabolic functioning, somatic growth and reproduction (Müller-Navarra et al. 2000), and enhanced immunocompetency (Kiron et al. 1995). Lipid and fatty acids are more compact energy reserves than glycogen and are the major energy storage forms. Essential lipids support the physiological development and health of aquatic organisms therefore strengthens the nutritional status of aquatic food webs (Arts and Kohler 2009). Total lipid content in fish has also been linked to climate-induced community changes (Litzow et al. 2006). Several fatty acids play pivotal roles in the health of fish and other aquatic organisms (Arts and Kohler 2009). Lipids and fatty acids are important in fish reproductive competence such as egg quality, spawning, hatching rate, and larval survival (Rainuzzo et al. 1997, Sargent et al. 2002) including growth, behavior, vision, osmoregularity, membrane fluidity (thermal adaptation), and immune response (Arts and Kohler 2009). Further, total lipid energy can predict features of population dynamics. For example, total egg production is directly correlated to total lipid energy in cod suggesting lipid as a good indicator of successful recruitment in other trophic levels (Marshall et al. 1999).

The study of fatty acids in aquatic food webs has focused mostly on biomarker use in trophic transfer studies (e.g. Napolitano 1999, Dalsgaard et al. 2003, Iverson et al. 2004). In the SFE, trophic upgrade has been demonstrated in key zooplankton prey, the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*, due to the presence of essential fatty acids (Mueller-Solger et al. 2006). As such, the availability and abundance of these zooplankton species as good quality food sources can greatly affect the recruitment of pelagic populations (Mueller-Solger et al. 2002, POD Report 2007).

In aquaculture by contrast, fatty acids research is usually centered on the importance of only two or three fatty acids as essential dietary nutrients (Parrish 2009). Under starvation, fish will initially use saturated fatty acids for energy before utilizing other fatty acids. Changes in essential fatty acid contents may take a few weeks to few months depending on the age and growth rate of the species. Variable fecundity, fertility, and embryonic hatchability were shown in rohu (*Labeo rohita*) broodstock due to feeding on different dietary protein (Khan et al. 2005). Because the nutritional status of adult fish reflects the health of their progenies (Sargent et al. 1999), predicting the impacts of fatty acids and lipid storage from prey to consumers such as fish are relevant measures of nutritional adequacy (Adams 1998). In this context, total lipid analysis will be employed in the current study to evaluate the nutritional competency of the species.

A large body of evidence exists in teleosts demonstrating that dietary intake is largely reflected in tissue lipids (Arts and Kohler 2009). Because the dietary availability of even only two fatty acids may correlate with total lipid content (Parrish 2009), total lipids and the major energy storage forms are relevant measures of nutritional adequacy in fish that can, in part, predict their health status. In addition to total lipid analysis, nutritional composition and energy storage will be analyzed to determine the relevance of nutritional adequacy in the general health measures of the species.

3. Approach and Scope of Work

The field study component of this proposal will be conducted with on-going monitoring projects at UC Davis with Co-PI Dr. Peter Moyle, and with Co-PI Mr. Randal Baxter at the California Department of Fish and Game. We will also collaborate with Mr. Fred Feyrer at the US Bureau of Reclamation who will provide some of the fish samples from their IEP funded study on “Life History and Biology of Splittail”. To minimize resources for manpower and field sampling, we will collect fish samples under their Endangered Species Act take permits. Mr. Feyrer has agreed to coordinate and provide us with samples of splittail, striped bass, and threadfin shad from their IEP funded study. Graduate students from Dr. Teh’s laboratory have been trained and certified under the staff of Dr. Peter Moyle in operating their boat (UCD 21’ Workskiff-135 HP outboard) and in fish sampling in Suisun Marsh and Cache Slough. The laboratory components will be conducted in the Aquatic Toxicology Program lab and Fish Disease lab at UC Davis.

This interdisciplinary Fish Health monitoring program will utilize a suite of biological effect measurements based

on the following criteria: 1) Detecting differences in physiological and morphological health of fish based on condition factor and organo-somatic indices, 2) Integrating biomarkers capable of selectively recognizing specific types of contaminants (e.g. P450 induction from PCB exposure, vitellogenin or choriogenin induction in males from endocrine disruptor exposure) and biomarkers specific for both exposure and deleterious effects (e.g. endocrine disruption and histopathology), 3) Utilizing the presence of pathogens/disease as a significant health indicator due to the cumulative effects of multiple stressors and other environmental variables to wild fish populations, and, 4) Proximate analysis which determine the nutritional status of fish at different trophic levels: splittail (benthivore), threadfin shad (planktivore and filter feeder) and striped bass (top consumer).

TASK 1 − PROJECT MANAGEMENT AND INFORMATION TRANSFER

Dr. Swee Teh is the Principal Investigator (PI) and will be responsible for details of contract management and execution. Drs. Teh, Moyle, and Baxter will direct fish sampling (Task 2-1) with the help of 2 graduate students and one technician. Drs. Baxa and Hedrick will lead the pathogen and disease studies (Task 2-3). Drs. Teh and Baxa will manage the condition factor, organo-somatic indices and biomarker studies (Task 2-2), and nutrition studies (Task 2-4). Dr. Wu will lead the data integration task (Task 2-5). The investigators will share the responsibility of project management (Task 1) including allocation of resources, management of project staff, and development of appropriate protocols for the various laboratory assays and field studies. 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, and integrate reports and other outreach materials.

Information transfer is an essential part of the project, and fostering data synthesis is critical to project management. Year 2 of the project will be dedicated to dissemination of baseline fish health status for example to IEP POD Management Team, Bay-Delta Science Meetings, and workshops to ensure the information is transferred to local agencies, and environmental managers.

TASK 2 − FISH HEALTH EVALUATIONS

Task 2–1. Field sampling and environmental parameters (Teh, Moyle, graduate students, and student assistants)

This study element will be conducted to provide an environmental context for the different measurements of biological effects described in Tasks 2.2 – 2.4, and to determine if temporal and spatial trends of biological effects are related to gradients of physicochemical stressors. At each sampling site, the tidal stage, channel depth, temperature (°C), salinity (ppt), specific conductance (μS), dissolved oxygen, and water transparency (Secchi depth in cm) will be recorded. Water flow including other water quality data for each site will be obtained from CA DWR (http://cdec.water.ca.gov/queryTools.html) and other web sites.

Fish will be sampled from candidate sites in Suisun Marsh (HUN, 606, 609, 610), Cache Slough (BKS, CCS, LIR/Yolo Bypass, 716), and San Joaquin River (809, 812, 815, 906, 910, 912) **(Fig. 2).** Fish sampling will be conducted once a month for 4 months between the summer and fall for 2 years. These months were chosen to encompass the presence of juvenile stages for all the species. One or two trawls will be conducted at each site to collect a total of 10–15 fish/species/site depending on fish availability.

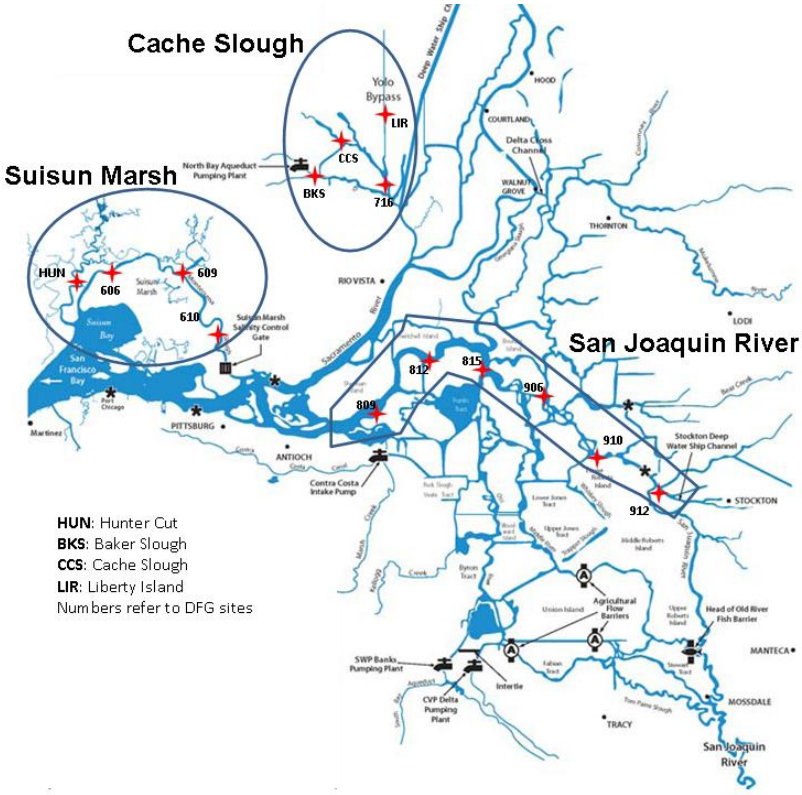


Figure 2. Map of the San Francisco Estuary indicating the sites (+) for collection of fish in Suisun Marsh, Cache Slough, and San Joaquin River.

Description and justification of collection sites – **Cache Slough** is a freshwater tidal slough in the northern reaches of the Delta (Moyle 2008) that drains to Moore Tract and Yolo Bypass and receives some Sacramento River water through tidal fluctuations (Lund et al. 2008). Cache Slough is considered a productive site due to relatively high residence times, abundant phytoplankton and zooplankton, less flooding, and few invasive species (Lund et al. 2008). Cache Slough provides spawning and nursery habitats, 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). Salinity fluctuates seasonally from 0 to 0.75 ppt and temperature from about 6 to 23°C. Cache Slough receives approximately 10 mgd of secondarily-treated sewage from Easterly Wastewater Treatment Plant (city of Vacaville) via Alamo Creek (http://www.ci.vacaville.ca.us/departments/public_works/wastewater_treatment.php). This discharge will tend to remain within Cache Slough and the region due to limited additional runoff entering the sloughs except when Yolo Bypass is in full flood. A gradient of this discharge may be observed in the sampling sites CCS and 716 **(Fig. 2)** due to tidal mixing and some regional runoff. **Suisun Marsh** is a fresh/brackish tidal marsh in the north of Suisun Bay and heavily diked to support freshwater habitat for waterfowl (Matern et al. 2002, Moyle 2008). The undiked portions of the marsh are critical habitats for nursery rearing of Sacramento splittail, striped bass, and Chinook salmon (Lund et al. 2008). Salinity levels fluctuate seasonally between 0 and 16 ppt, and between 5–25°C water temperatures. The Fairfield-Suisun Sewer District discharges tertiary treated municipal, commercial and industrial sewage at a rate of 16.1 mgd (2000-2002) into Boynton Slough, a northern tributary to Suisun Slough in the city of Fairfield (http://www.epa.gov/npdescan/CA0038024FP.pdf). Discharge from this facility will dilute on its way to the sampling site located in lower Suisun Slough **(Fig 2. "HUN"),** but should not be detectable in Montezuma Slough **(Fig. 2, 606, 609, 610)**. **The San Joaquin River** is predominantly an open river channel and considered less productive due to the reduced residence times associated with water transfer through the Delta (past lower stations **812, 815, 906** in **Fig. 2**) which in turn reduces the abundance of phytoplankton to support higher trophic levels and few viable habitats for spawning or larval rearing (Lund et al 2008); residence time and productivity is higher at stations 910 and 912. The river is highly altered due to pollution and presence of invasive

species. Seasonally, salinity in the sampling region ranges from 0 to 2ppt and temperature from 5 to 26-27°C. The Stockton Municipal Utilities Department operates a sewage treatment plant that discharges tertiary treated water from municipal, commercial industrial sources (ca 78 mgd) into the San Joaquin River just upstream from the Stockton Deepwater Port and only several miles above station 912 **(Fig. 2),** thus a gradient will be established. These sites were chosen because gradients in biological effects due to the stressors will likely be observed in these habitats that vary in productivity to support spawning and rearing of fish species including San Joaquin River spawning grounds of striped bass downstream of the sewage discharge point (POD Report 2007).

Fish sampling – prior to field sampling, we will consult with field managers at California Department of Fish and Game, Department of Water Resources, US Fish and Wildlife, and the US Bureau of Reclamation to obtain recommendations and to refine selections of sampling stations if needed to prevent accidental take of endangered delta smelt, and to maximize sampling success. All species will be collected with a four-seam otter trawl with a 1.5 X 4.3 m opening, 5.3 m length, and mesh sizes of 35 mm stretch in the body and 6 mm stretch in the cod end. The trawl is towed at approximately 4 km/hr for 5 minutes in the small sloughs and 10 minutes in the larger sloughs (to compensate for small catches) and river channels. Sampling is augmented with 30-m beach seines having a stretched mesh size of 6 mm. Beach seining has proven to be an effective means of sampling fish with minimal stress to the fish**.** Although the species are expected to have a widespread distribution, shoaling areas (<12-15 feet deep) will be sampled in San Joaquin River to maximize the chances of catching all species. Following capture, fish will be kept alive in separate holding tanks until necropsy. Time of holding will be less than 6 hours after capture.

Necropsy and processing of fish – fish will be individually netted, euthanized with an overdose of tricaine methanesulfonate (MS-222, Sigma). Individual weights and lengths of each fish species will be measured and recorded for condition factor and organo-somatic indices. Groups of 10-12 fish will be processed separately for measurements of biomarkers/histopathology, disease, and nutrition.

Task 2–2. Analyses of fish condition and biomarker responses – (Teh, Baxa, graduate students, histopathologist, and technician)

Condition factor and organo-somatic indices– these indices will be examined to provide a general estimate of the fish condition as determined by the relationship of length and weight and morphological conditions. Results will be integrated with biomarkers, pathogen/disease, and nutrition as the cumulative basis of fish health.

The well-being of fish and the population in general can be determined by analyses of condition factor and organo-somatic indices (Schmitt and Dethloff 2000) in addition to morphological and physiological methods (Goede 1989, Schmitt et al. 1999). Gross measurements and weights will be used to determine condition index (CI), gonadosomatic (GSI) and hepatosomatic (HSI) indices in fish. CI is a measure of "plumpness" and defined as (body weight in grams) x (100) / length in cm^3. GSI is the gonad to body weight ratio and HSI is the liver to body weight ratio. All three indices are broad measures of general health. Changes in CI are specific indicators of altered growth and nutritional status while fluctuations in GSI are associated with sexual maturity and reproductive status. Differences in HSI may indicate gender, sexual maturity, or general health and nutrition. Changes in these indices will therefore indicate contaminant-induced alterations of somatic and/or gonadal growth. Adverse effects may be detected by histopathological analysis of liver and gonads. Gonadosomatic (100 x gonad weight/body weight) and hepatosomatic (100 x liver weight/body weight) indices are sensitive and simple indicators of responses when comparing fishes from contaminated versus reference sites. For juvenile fish, the CI and HSI indices will be more relevant measures than GSI.

Biomarker studies – biomarkers are techniques that provide information on contaminant exposures and adverse biological effects. Because they can link exposures and effects, biomarkers are relevant tools for evaluating field exposed fish, laboratory toxicity, and in situ studies (Anderson et al. 2007). To determine the role of contaminants in fish health as established in the current study, a panel of biomarkers will be used based on the types of contaminants and chemicals

present in the Delta (Anderson et al. 2007, Johnson et al. 2010). The species will be evaluated on the type, extent, and severity of potential exposures to different contaminants based on their responses to the biomarkers. Biomarkers data will be integrated within the framework and goals of our investigation to adhere to precautionary measures established for POD-related studies (Anderson et al. 2007).

Histopathology – histopathology markers are good indicators of environmental stress as they provide visible biological endpoints and measurable responses to subcellular 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 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 disruption chemicals (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 a good indicator of exposure to organic contaminants.**

Metallothionein (MT) – this is a group of low molecular weight proteins rich in cysteine that regulates toxic and physiological metals (Hamer 1986, Olsson et al. 1987, Rosesijadi 1992). Elevated metals in aquatic systems can increase MT levels in aquatic species (Benson and Birge 1985, Olsson et al. 1987). MT has been successfully applied as a marker for exposure of bivalves and fish to metals in field and laboratory conditions (Viarengo et al. 1988, Couillard et al. 1993, Roesijadi 1994, Chan 1995). MT can also be induced by certain organic chemicals and stress factors such as injury, infections, or prolonged periods of starvation (Berger et al. 1995). Fish liver will be homogenized to obtain supernatant (Berntssen et al. 2004), and MT will be determined by a differential pulse polarographic method (Olafson and

Sim 1979, Olsson et al. 1987). Rabbit liver MT will be used as a standard, and the total protein content will be analyzed (Bradford 1976).

Immunohistochemistry (IHC) – antibodies to VTG, ZRP, P4501A1, HSP, and MT specific to striped bass, Salmon, largemouth bass, carp, and minnow (Biosense, Norway) will be used. Unlike biochemical assays, IHC can be used to assess biochemical expression (Teh and Hinton 1993) as it allows the precise localization of enzyme activity in specific organs and cells. In addition, a sandwich ELISA can be used to verify IHC results using a homologous antibody kit (Biosense, Norway).

IHC to detect microcystin and *Microcystis* DNA detection – due to recurring impacts of *Microcystis* in the estuary, localization of microcystin (MC, hepatotoxin produced from *Microcystis*) will be conducted on fish tissues using IHC. A monoclonal antibody to microcystin-LR (MC10E7, Alexis Corporation, Switzerland) will be used, which recognize all 4-Arg MCs. The hybridized antibodies will be visualized with an HRP reaction system. In addition, DIG-labeled probes specific to local *Microcystis* strains in the estuary (Baxa et al. 2010) will be used to localize *Microcystis* DNA in fish tissues. Fish samples with suspect *Microcystis*/MCs in tissues as indicated by histopathology will be verified using these techniques.

Task 2-3. Evaluate the presence of pathogens/disease (Hedrick, Baxa, and SRAIII) – By expanding prior studies, we have initiated the development of specific cell lines for virus isolation from threadfin shad, Sacramento splittail, and striped bass.

Pathogen and disease examination – procedures are described in detail in AFS-FHS (2005). Briefly fish will be euthanized with MS-222 and then kept on ice until used for pathogen isolation using aseptic conditions in the laboratory. Samples for bacterial and viral tissue culture should not be frozen but may be stored on ice for up to 24 hours or stored in buffered antibiotic and antifungal solutions or both.

External signs of infection (e.g. fin erosion, skin ulcer, eye disorder, hemorrhage, parasites, tumors, skeletal deformities, and lesions) and internal signs (e.g. liver and kidney coloration and nodules, and amount of mesentery fat) will be recorded for each fish during necropsy. Organ abnormalities (e.g. liver: normal, fatty, nodules, discoloration) will be examined and qualitatively scored based on severity. For example: 0=normal or no signs; 10=mild or few small focal areas, 20=moderate or diffuse but less than half of the body is affected, and 30=severe or greater than half of the body is affected. The presence of helminthes or other parasites in the coelomic cavity will also be scored accordingly: 0= absence of helminthes and other parasites, 20=moderate number of helminthes or parasites (N=1-20), 30=severe presence of helminthes or parasites (N=>20).

Isolation and identification of bacteria – conventional diagnostic procedures will be used as outlined in AFS-FHS (2005) in conjunction with the Analytical Profile Index System (API 20E, BioMerieux, France) for non-fastidious Gram-negative organisms. Using aseptic techniques, a portion of the kidney and spleen from each fish will be directly inoculated onto a general media for isolation of non-fastidious bacterial organisms and specific media for isolation of certain fastidious bacteria.

Rationale for developing specific cell lines for virus isolation – the replication of viruses in cells is generally species origin-dependent. For this reason, the successful isolation of potential viral agents requires the establishment of specific cell lines from the host species. The ability to culture cells in appropriate cell lines has been the basis for successful viral isolation and propagation of many viral agents (Wolf and Quimby 1976). Cell culture is central to establishing the etiology of many fish viruses as important disease agents (Hetrick and Hedrick 1993, Fryer and Lannan 1994). **We have developed cell lines from threadfin shad, splittail, including the Delta smelt using different organs and tissues of the targeted species. Striped bass cell line will be forthcoming.**

Cell culture – the kidney, spleen as well as other potential virus target organs will be used for virus isolation by cell culture following procedures described in AFS-FHS (2005).

Serology and molecular biology for viral and bacterial identification – viral agents and bacteria will be confirmed by serological procedures such as serum agglutination to specific antibodies of specific pathogens, fluorescent antibody test

(FAT), enzyme-linked immunosorbent assays (ELISA), serum neutralization tests, and PCR (AFS-FHS 2005). Identities of bacterial isolates will be verifed using 16S rRNA gene sequencing and viral isolates by nucleotide sequence analysis of selected genes (Cunningham 2002). Sequence alignments will be analyzed using Clustal W software (Thompson et al. 1994).

Identification of parasites – parasites will be initially assessed in histological sections. If significant parasite infections are present and they are not identified with currently published criteria, molecular techniques will be employed to describe the new agents. This will include the design of primers and probes based on the small subunit ribosomal DNA (ssu-rDNA) sequence of the parasite to detect specific infections by PCR. Following isolation/detection in media, cell culture, and PCR, the presence/absence of bacteria, viruses or parasites will be recorded and qualitatively scored for each fish. 0=absence of pathogens; 10=mild focal or patchy pathogen presence as detected in one organ; 20=moderate pathogen presence as detected in more than two organs; and 30=severe pathogen presence as detected from more than 3 organs.

Task 2-4. Conduct proximate analysis of major storage forms of energy to determine fish nutritional status (Teh, Baxa, graduate students, and technician)

Glycogen, lipid, and protein are the major forms of energy storage and will be measured to evaluate the nutritional status of fish in this study. Glycogen is the primary short-term storage energy in animals in the form of glucose and mainly synthesized and stored in the liver and muscle. Fish exposed to a short feeding duration or acute stress will first deplete glycogen from the liver and then muscle. Protein can be used as an energy source if the body lipid level goes lower than the storage threshold. Protein plays a pivotal role in biosynthetic activities in embryogenesis (Metcoff 1986).

Proximate analysis – for each of the four species, whole fish will be frozen in liquid nitrogen and transported to the laboratory at UC Davis and stored at -80°C until used for analysis. The liver and muscle tissues will be removed for fatty acid analysis and the remaining whole fish will be used for proximate composition (dry matter, lipid, and protein). The number of fish for proximate analysis will depend on fish size, with a minimum of 5 g dry sample needed for proximate composition and glycogen analysis.

Lipid extraction and analyses of fatty acids, glycogen, and protein composition – whole fish will be dried to a constant mass (ca. <5% change) at 100°C and then processed for lipids, protein, and ash using standard AOAC methods (1995). Total lipid will be analyzed by Soxhlet extraction method and protein will be estimated using the Kjeldahl method. Ash will be determined by drying samples in a muffle furnace at 600°C. Glycogen will be measured according to the method of Murat and Serfaty (1974). Fatty acid composition will be analyzed after lipids are extracted from liver or muscle (Folch et al. 1957). Methylation of fatty acids and gas-liquid chromatography determination of fatty acids will follow previous methods (Xu et al. 1993).

Task 2-5. Data integration and analyses (Wu, Teh, Baxter, Feyrer (collaborator), and graduate students) – Dr. Wu will direct the integration and analyses of data from the different measurements of biological effects

Data Preparation:

Results from Tasks 2.2–2.4 evaluated on cascading levels of biological organization (Adams 2001, Adams and Greeley 2000) will be analyzed. Biochemical, physiological, and histopathological data will be evaluated for the effect on sub-organism level. Health indicators at the individual level will include 1) condition factor and organo-somatic indices (CI, GSI, and HSI, 2) pathology and diseases, and 3) nutrition. Existing monitoring data from an IEP funded project of Mr. Randy Baxter (DFG) and Mr. Fred Feyrer (USBR) will be incorporated, size frequency distribution, sex ratio, and growth indicators will be analyzed at the population level. Qualitative observations including histopathological lesions, external and internal signs, and pathogens/disease will be systematically scored and presented as sum scores for each fish to allow statistical comparisons (HAI; Adams et al. 1993).

Statistical Analysis:

1. **Within biological level analysis** – Multivariate N-way Analysis of Variance/Covariance (MANOVA/MANCOVA) will be

conducted to evaluate the significant difference of health indices or measurements to study the effects of location, species, season (sampling time), and their interaction effects for each species separately. The use of MANCOVA helps with compensating the environmental factors (e.g. temperature, speed of flow, etc.) of interest in the evaluation. The test of homoscedasticity could base on Bartlett's test and variants of Levene's test. The significant multivariate analysis will then be followed by univariate ANOVA or ANCOVA and various multiple comparison procedure (e.g. variants of Tukey's Honestly Significant Difference procedure). Appropriate scaling will need to be considered, if the homogeneity of variances is absent. For the cumulative and incremental effect of health information, MANOVA or MANCOVA with different measurements across years/months as a prelude suffices to elucidate its significance. In parallel, nonparametric alternatives for MANOVA or MANCOVA will also be conducted for completeness.

2. Multilevel modeling/Integration of health information – the integration of multilevel information for statistical modeling is still an ongoing subject in statistical community. The integration of health information across various biological levels for health assessment in this project will be processes in the following steps for each of the species.
Screening nuisance measurements – using the labeling from index of biotic integrity (IBI) as the response and all the measurements as covariates in multinomial discrete choice model or generalized linear mixed effect model helps to filter out nuisance measurements. Canonical discriminant with variable selection can be adopted as well.
Implementation of aquatic ecosystem health index (AEHI, Yeom and Adams 2007) – fish health status will be assessed through empirical distribution of star-plot area. Based on the preserved measurements from previous step, AEHI will be conducted for annealing the status assignment. Note that the evolution of overall fish health status can be seen from the comparison of the empirical distributions of AEHI for different years. (Actually, the distribution of AEHI could also be used as baseline medium for assessment.) Thorough refinement of AEHI will be investigated by Dr. Wu regarding the configuration of index and the corresponding allocation of health status. As the prototypic AEHI implicitly assumes orthogonality among various levels of indices and focuses only on four out of six pairwise composite effects, an amendment could yield improvement on health status assessment.

3. Baseline health assessment profiling – to profile health assessment mechanism, Classification and Regression Tree approach (CART, Breiman et al. 1984) will be fitted for each species (could also for each year if the empirical distribution of AEHI differs significantly) to discover the inter-correlation among information measurements (nodes in a tree) as being parent-descendant composited towards successful classification of health status (healthy, partially impaired, impaired). The comparison of those species-specific trees can help to differentiate the inherent health mechanisms among species. On the other hand, the comparison of CART trees across temporal units for each species suffices to delineate the evolutionary mechanism of fish health, furnishing the baseline of health assessment profile. The change on the importance of each node (health measurement) of the health decision trees can provide the foundation for understanding the change of fish health status. **The use of CART tree does not only provide an easier summary of health information, but also the template of profiling fish health for the long term evaluation.**

4. Computation platform – all of the analyses mentioned above will be mainly based on SAS (SAS Inc. 1985) whenever applicable; secondary facilities will include both R and Matlab (R Development Core Team 2010, http://www.R-project.org, http://www.mathworks.com/)

4. Deliverables

Schedule of Deliverables (beginning from project start date)

Deliverable	Description	Completion (month)
Project summary, beginning	One-page summary for public at beginning of project	1
Semi-annual report	Report of findings for each task, delivered twice/year	6, 12, 18, 24
Final report	Report of project completion discussing research findings	24
Project summary, completion	One-page summary for public upon project completion	24
Project closure summary report	Summary of project and findings	24
Presentation at CALFED Science Conference	Presentation of current results	24
Other presentations at request of CALFED Science Program	Regional presentation of findings, once or twice/year	12, 24
National or international presentations	Presentation of findings, once a year	24
Draft scientific paper	Development of cell lines for specific isolation of virus from threatened fishes in the San Francisco Estuary	24
Draft scientific paper	Characterizing pathogens and diseases in threadfin shad, splittail, and striped bass: implications to Pelagic Organism Decline in the San Francisco Bay Delta Estuary	24
Draft scientific paper	Nutritional status of threadfin shad, striped bass and Sacramento splittail: Impacts to growth and reproduction	24
Draft scientific paper	Establishing health profile of threatened fish in the SFE Delta using an aquatic ecosystem health index: Analysis of biological effects due to multiple stressors – disease, contaminants (biomarkers), and nutritional status	24
Draft scientific paper	Development of baseline health information for threatened fish in the San Francisco Bay Delta Estuary	24

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. Dr. Moyle is a professor in the Department of Wildlife Fish and Conservation Biology, and has devoted a 30-year study of Suisun Marsh and its fishes. Dr. Hedrick is a fish disease specialist with 31 years of experience with an active research laboratory at UC Davis focused on pathogens of fish and shellfish. 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. Wu is an assistant professor of Mathematics at Lehigh University with a broad expertise in Nonparametric Curve fitting, Kernel Density Estimation, Functional Data Analysis, Bioinformatics, and Statistical Genetics. His current research on object data in complex format will facilitate the establishment of baseline fish health in this project.

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 Dr. Peter Moyle's monitoring program and from the IEP funded monitoring program of Mr. Fred Feyrer and Mr. Randy Baxter (DFG) to minimize sampling costs and fish take in this regard. 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 collections to minimize shipping time and to ensure fish are handled properly for biological effect measurements.

3) Measurements of biological effects will be conducted at the Aquatic Toxicology Program (ATP) laboratory and at the Fish Disease laboratory. ATP is fully equipped with facilities, instruments, and protocols for toxicology and nutrition studies. The Fish Disease laboratory is well established for disease diagnoses and research for controlled studies involving bacteria, viruses, and parasites. Importantly, the Fish Disease lab has developed a vast collection of unique cell lines for specific isolation and propagation of viral agents from fresh and seawater fishes

6. Relevance to the CALFED ERP

Relevance to this PSP – this research program directly addresses two priority topic areas of the ERP: 1) Topic 2 – "Tidal marsh restoration efforts in the Delta and Suisun Marsh to determine pelagic fish production" and "Potential factors affecting productivity such as contaminants" and, 2) "CALFED Multi-Species Conservation Strategy to achieve recovery of at-risk native species, and protect/restore functional habitat types in the Bay-Delta Estuary". This interdisciplinary project will incorporate the collaborative efforts of the California Department of Fish and Game, US Bureau of Reclamation, Lehigh University in Bethlehem, Pennsylvania, UC Davis Department of Wildlife, Fish and Conservation Biology, UC Davis Department of Medicine and Epidemiology (Fish Disease Laboratory), and UC Davis Department of Anatomy, Physiology, and Cell biology at the School of Veterinary Medicine, thereby supporting the focused priorities of the PSP.

The biological effects of key environmental stressors (contaminants, disease, and environmental factors) and fish nutrition will be characterized in at-risk species (threadfin shad, striped bass, Sacramento splittail) across habitats (Suisun Marsh, Cache Slough, and San Joaquin River) identified as priority sites for restoration projects in the SFE Delta. Because of the nature of the goals and the experimental design, this study entails extensive collaborations from multiple disciplines, and integration and synthesis of previous and current information. Given the limited financial and manpower resources and research facilities of most agencies, our findings will benefit resource managers including environmental regulators within and beyond the Bay-Delta Estuary to prioritize environmental and management efforts. Studies addressing the incremental and cumulative effects of multiple environmental stressors on the health and fitness of at risk fish populations are lacking. Although the cause of fish declines and other pervasive ecosystem problems in the Delta cannot be attributed to a single or combination of stressors, the Delta Independent Science Board emphasized the magnitude and complexity of interactive stressors that continue to threaten the Delta (http://www.deltacouncil.ca.gov/delta_science_program/publications/sci_news_0211_stressors.html). By focusing on environmental stressors that contribute most to the health and declines of fishery resources, our results may offer new insights on fish health that will substantially support management and restoration efforts and over all scientific evaluation of fish health status in the Bay Delta Estuary.

This study will advance our current understanding of contaminant exposure and effects by incorporating disease and nutrition to model the components that can be used to assess overall fish health. Assessing the health indices of fish will allow rapid detection of in vivo toxicity, thereby helping to prioritize sites for more detailed contaminant analysis. All are valuable information to current or future ecosystem restoration projects. Healthier fish means healthier ecosystem, suggesting that the restoration efforts are working. The study will evaluate the utility of biological effects (used singly or in combination) in assessing xenobiotic impacts on fish populations and communities to generate data that will form the basis of a model system defining transport, fate, and impact of contaminants, the underlying role of disease, and nutritional adequacy as health measures for aquatic organisms. Results of this study will have practical application as field data correlating biological effect measurements with contaminant loading may help guide management decisions in determining acceptable contaminant levels in the environment and description of normative presence of microorganisms as integral components of an ecosystem. More importantly, incorporating disease will not only aim to identify the presence of specific etiological agents but also as part of an integrated effort to document the biological effects of contaminants. Many of the biological effect measurements that we propose are proven biomonitoring tools that can be used to monitor xenobiotic exposure and effects in aquatic organisms from a variety of contaminated aquatic habitats, and help evaluate progress of remediation efforts. This aspect of the study directly addresses "other stressor" conservation measures of the DRERIP PSP.

Relevance to CALFED Issues Outside this PSP – while this study is important for understanding the biological effects of key stressors affecting threatened pelagic fishes in the SFE, other watersheds or ecosystems faced with similar challenges from stressors and degradation of fishery resources can also benefit from the results. Baseline fish health information can be merged with other relevant data management systems to complement larger CALFED goals and efforts by addressing the incremental and cumulative impacts of multiple stressors that affect the current or future decline or increase in populations of aquatic organisms.

7. Expected quantitative results (project summary):

This is a research and monitoring project, as such quantitative measures are not applicable. The project addresses:

- Comprehensive measurements of biological effects due to key stressors that affect the overall fish health. These measurements include 1) Condition factor and organo-somatic indices, 2) Biomarker responses including biochemical, histopathological, and physiological endpoints as indicators of contaminant exposure and effects, 3) Isolation and identification of pathogens/diseases using specific cell lines for virus isolation, and 4) Proximate analyses to determine fish nutritional status
- Measurements of fundamental fish functions notably growth and reproductive capacity and potential correlations to the cumulative effects of key stressors
- Statistical analysis using Classification and Regression Tree approach (CART) trees of biological effects data that will provide the foundation for understanding the summary of health information and the template of profiling fish health for long term evaluation
- Baseline fish health and potential use for predicting trends and patterns of fish health over time that may be altered due to emerging risk factors and other multiple stressors affecting the Delta and its fishery resources

8. Other products and results:

- Fish health monitoring to complement other restoration projects or programs within the Bay-Delta system that aims to regulate and manage the effects of anthropogenic activities associated with contaminant introductions and the additive impacts of infectious diseases and fish nutritional status

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. Dr. Teh and Dr. Baxa will direct Task 2.2 (Condition factor/indices and Biomarkers), and Task 2.4 (Nutrition).

2. Dr. Ronald P. Hedrick is a Professor in the School of Veterinary Medicine at UCD with 31 years of experience in fish health. His principal area of teaching and research over the past 27 years at the University has focused on infectious diseases of fish and shellfish. Current research programs deal with diseases due to viruses, myxozoans and microsporidians including well known and more recently emerging pathogens. Nearly all of the studies conducted (over 230 publications in peer-reviewed scientific journals) have been on diseases directly relevant to the health of California's aquatic resources. Dr. Hedrick's program stresses the use of controlled laboratory studies as directly relevant to ongoing disease issues in wild or captive populations of fish. These approaches have often clarified the role of biological factors that are difficult or impossible to evaluate in more complex field situations. Importantly, the insights obtained from these controlled laboratory studies have had direct impacts on large scale management approaches to improving the health of wild fish populations which are directly relevant to problems currently experienced by several fish species in the Delta and

San Francisco Estuary. Professor Hedrick and Dr. Baxa will work closely to provide the laboratory and technical staffing expertise for Task 2-3.

3. Dr. Peter Moyle has been working on the ecology and conservation of California's freshwater and sea-run fishes since 1969, resulting in the book, *Inland Fishes of California* (2002) and many other publications. He is author or co-author of five other books as well, including *Fish: an enthusiast's guide* (1996). He has been involved in the study of fishes of the San Francisco Estuary since the 1970s, including a 30 yr study of Suisun Marsh and its fishes. He has been part of a true interdisciplinary team that has created two influential reports on the future of the Delta, published by the Public Policy Institute of California in 2007 and 2008. In 2007, he received two national awards: the Outstanding Achievement Award of the Association of Fisheries Research Biologists and the Award of Excellence of the American Fisheries Society. He is a professor in the Department of Wildlife Fish and Conservation Biology and Associate Director of the Center for Watershed Sciences, UC Davis.

4. 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. Dr. Baxa will be directly involved in almost all aspects of the project importantly the fish disease component (Task 2–3).

5. Dr. Ping-Shi Wu is an assistant professor of Mathematics at Lehigh University with an extensive training in Statistics and a broad expertise in Nonparametric Curve fitting, Kernel Density Estimation, Functional Data Analysis, Bioinformatics, and Statistical Genetics. He has several peer-reviewed publications, and active participation in national and international meetings. His recent work proposed an innovative way to bridge multivariate data analysis with functional data analytic approach with excellent results in dealing with high dimension and low sample size data. In applications of cancer prognosis and screening of endocrine disrupting activity, the embedding substantially helped to achieve effective classification. Dr. Wu is currently devoted to research on analyzing object data, data in complex format, which will help to consolidate the establishment of baseline fish health in this proposal. Dr. Wu will direct the statistical analysis and interpretation of data (Task 2-5) in this proposed study.

6. Mr. Randall D. Baxter is a Senior Biologist Supervisor (Fisheries) with the California Department of Fish and Game and has over 22 years of experience sampling fishes and invertebrates in the San Francisco Estuary. He currently 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 and coauthored publications focused on splittail and longfin smelt. He is currently 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.

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Section 7: Project Budget

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

Title: Developing a Baseline Health Status for Threatened Fish in Critical Habitats of the San Francisco Estuary: An Ecological Approach of Evaluating the Effects of Contaminants, Disease, and Fish Nutrition			
CATEGORIES		**YEAR ONE**	**2011-2012**
A. PERSONNEL (Allows 3% COLA)	#of hour	hourly rate	Total
Swee J. Teh, PI (25% effort)	480	48	22,815
Dolores Baxa, Co-I (50% effort)	960	33	31,850
Ronald Hedrick Co-I (5% effort)	96	0	0
Peter Moyle Co-I (5% effort)	96	0	0
Sarah Lesmeister (50% effort)	960	19	17,833
Ida Flores (50% effort)	960	26	25,230
Histopathologist (40% effort)	768	57	44,120
Staff Research Associate III (50% effort)	960	22	21,432
Ching Teh, LA III (50% effort)	960	21	20,448
Three student assistants	1,500	10	15,000
SUBTOTAL PERSONNEL COSTS			198,728
B. FRINGE BENEFITS (Based on UCD Proposed Blended Rates)		**Benefits**	
Swee J. Teh, PI		44%	10,107
Dolores Baxa, Co-I		44%	12,867
Ronald Hedrick Co-I		0%	0
Peter Moyle Co-I		0%	0
Sarah Lesmeister		3%	481
Ida Flores		3%	681
Histopathologist		44%	19,545
Staff Research Associate III		44%	9,494
Ching Teh, LA III (50% effort)		44%	9,058
Student Assistants		5%	810
TOTAL FRINGE BENEFITS		29%	63,045
TOTAL PERSONNEL COSTS			**261,773**
C. TRAVEL			
PIs and staffs attend Scientific Meeting and field sampling			5,000
TOTAL TRAVEL COSTS			5,000
D. EQUIPMENT: None Requested			
TOTAL EQUIPMENT COSTS			0
E. SUPPLIES			
Histology and electron microscopy			8,000
Enzymes, microbiology media, virology-cell culture, antibodies			10,000
Nutrition (proximate and fatty acid analysis)			15,000
Glassware, reagents, and equipment lease			5,000
Biohazard disposal			2,000
Publication costs			300
TOTAL SUPPLY COSTS			**40,300**
F. CONTRACT/SERVICE AGREEMENT			
Ping-Shi Wu (Biostatistician)			15,000
TOTAL CONTRACTUAL COSTS (not exempt from F&A)			15,000
G. OTHER EXPENSES (Allows 10% annual increase per UCOP)			
Graduate Student Fee Remission: Resident + NonResident GSR			43,559
TOTAL OTHER COSTS (exempt from F&A)			43,559
H. TOTAL DIRECT COSTS (sum of a through g)			365,632
I. Indirect Costs/Charges			
Modified Total Direct (less fees/tuition)			322,073
25% of base			80,518
J. TOTAL PROJECT COSTS (sum of h & I)			**446,150**

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CATEGORIES		2012-2013	YEAR TWO
A. PERSONNEL (Allows 3% COLA)	#of hour	hourly rate	Total
Swee J. Teh, PI (25% effort)	480	49	23,499
Dolores Baxa, Co-I (50% effort)	960	34	32,806
Ronald Hedrick Co-I (5% effort)	96	0	0
Peter Moyle Co-I (5% effort)	96	0	0
Sarah Lesmeister (50% effort)	960	19	18,368
Ida Flores (50% effort)	960	27	25,987
Histopathologist (40% effort)	768	59	45,444
Staff Research Associate III (50% effort)	960	23	22,075
Ching Teh, LA III (50% effort)	960	22	21,061
Three student assistants	1,500	10	15,450
SUBTOTAL PERSONNEL COSTS			204,690
B. FRINGE BENEFITS (Based on UCD Proposed Blended Rates)		Benefits	
Swee J. Teh, PI		48%	11,350
Dolores Baxa, Co-I		48%	14,533
Ronald Hedrick Co-I		0%	0
Peter Moyle Co-I		0%	0
Sarah Lesmeister		3%	496
Ida Flores		3%	702
Histopathologist		48%	21,949
Staff Research Associate III		48%	10,662
Ching Teh, LA III (50% effort)		48%	10,172
Student Assistants		5%	834
TOTAL FRINGE BENEFITS			70,699
TOTAL PERSONNEL COSTS			**275,389**
C. TRAVEL			
PIs and staffs attend Scientific Meeting and field sampling			5,000
TOTAL TRAVEL COSTS			5,000
D. EQUIPMENT: None Requested			
TOTAL EQUIPMENT COSTS			0
E. SUPPLIES			
Histology and electron microscopy			8,000
Enzymes, microbiology media, virology-cell culture, antibodies			10,000
Nutrition (proximate and fatty acid analysis)			15,000
Glassware, reagents, and equipment lease			5,000
Biohazard disposal			2,000
Publication costs			300
TOTAL SUPPLY COSTS			40,300
F. CONTRACT/SERVICE AGREEMENT			
Ping-Shi Wu (Biostatistician)			15,000
TOTAL CONTRACTUAL COSTS (not exempt from F&A)			15,000
G. OTHER EXPENSES (Allows 10% annual increase per UCOP)			
Graduate Student Fee Remission: Resident + NonResident GSR			47,915
TOTAL OTHER COSTS (exempt from F&A)			47,915
H. TOTAL DIRECT COSTS (sum of a through g)			383,604
I. Indirect Costs/Charges			
Modified Total Direct (less fees/tuition)			335,689
25% of base			83,922
J. TOTAL PROJECT COSTS (sum of h & I)			**467,526**

	Year One	Year Two	Total 2-yr
Total project Cost	365,632	383,604	782,883
25% indirect Cost	80,518	83,922	170,565
Grand Total	446,150	467,526	953,448

Grand Total for Two years = $953,448

2. Budget justification:

Personnel

Swee J. Teh, PhD (25% time) will be responsible for the coordination and overall supervision of the project (Task 1. project management). He will be responsible for reporting and assisting in the experimental design and analysis of studies conducted. Drs. Teh and Moyle with the help of 2 graduate students (50% time), one laboratory assistant (50% time), and 3 student assistants will direct fish sampling and data analyses (Task 1 and Task 2-1). Drs. Teh and Baxa (50% time) will coordinate efforts of 2 graduate students (50% time), 1 laboratory assistant (50% time), 1 histopathologist (35% time) and 3 student assistants, supervise and participate in Task 2-2 to evaluate condition factor, organo-somatic indices, and biochemical and histopathology biomarkers among species collected at different sites and seasons, and in task 2-4 to conduct proximate analysis of major storage forms of energy to determine fish nutritional status. In addition, Drs. Baxa and Hedrick with the help of a SRA (50% time) will have the principal responsibility for Task 2-3 to evaluate the presence of pathogens/disease of this study. Ping-Shi Wu, PhD, a biostatistician will be responsible for data integration and analyses for performance measure (Task 2-5).
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 of threadfin shad, Sacramento splittail, striped bass, and tule perch 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: Histopathology preparations, i.e., glass slides, paraffin, glycomethacrylate, antibodies, immunohistochemical and histological reagents, and electron microscopy ($8,000), DNA extraction, PCR & in-situ hybridization, isolation and identification of microbial pathogens, cell cultures, genetic sequencing of pathogens ($10,000), proximate composition analysis and fatty acid analyses of fish tissues ($150 per sample for 100 samples = $15,000), Enzyme, chemicals and molecular reagents, buffer and standard solutions for pH, salinity, ELISA bioassay, glasswares; and dissecting microscope and compound microscopes lease, ($5,000), biohazard disposal ($2,000), and publication costs ($300).

Year 2: Histopathology preparations, i.e., glass slides, paraffin, glycomethacrylate, antibodies, immunohistochemical and histological reagents, and electron microscopy ($8,000), DNA extraction, PCR & in-situ hybridization, isolation and identification of microbial pathogens, cell cultures, genetic sequencing of pathogens ($10,000), proximate composition analysis and fatty acid analyses of fish tissues ($150 per sample for 100 samples = $15,000), Enzyme, chemicals and molecular reagents, buffer and standard solutions for pH, salinity, ELISA bioassay, glasswares; and dissecting microscope and compound microscopes lease, ($5,000), biohazard disposal ($2,000), and publication costs ($300).

Other Expenses:

2 Graduate student fees: year 1 ($43,559), Year 2 ($47,915).

3. Administrative overhead:

Indirect Costs:
The current indirect cost rate for VM:APC and University is 25%

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· 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.			