ERP Proposal Application Instructions

All of the fields in the application form are required for all projects, except where specifically noted. Any supplementary information must be included at the end of this application. For forms and examples, please see Appendix B.

To check a box, right click on the box and highlight "Properties." Click on the circle next to "Checked." Click "OK."

Section 1: Summary Information

1. Project title:	*What are the optimal environmental conditions for longfin smelt reproduction?*
2. Applicant name:	*Dr. James Hobbs*
3. Contact person:	*Dr. James Hobbs*
4. Address:	*Dept. of Wildlife,Fish and Conservation Biology, UC Davis* *One Shields Ave. .*
5. City, State, Zip:	*Davis Ca. 95616*
6. Telephone #:	*530-752-0205*
7. Fax #:	*530-752-9364*
8. Email address:	*jahobbs@ucdavis.edu*
9. Agency Type:	Federal Agency ☐ State Agency ☐ Local Agency ☐ Nonprofit Organization ☐ University (CSU/UC) ☒ Native American Indian Tribe ☐
10. Certified nonprofit Organization:	Yes ☐ No ☐ If yes, specify the nonprofit organization registration number: *See www.pd.dgs.ca.gov/smbus/nonprofit*
11. New grantee:	Yes ☒ No ☐
12. Amount requested:	*$604,962*
13. Total project cost:	*Sum of amount requested plus all cost share funds and services, from detailed budgetl.*
14. Topic Area(s):	*2) At-risk species assessment*
15. ERP Project type:	*1) Research; 2) Monitoring).*
16. Ecosystem Element:	*1) Delta sloughs; 2) Bay-Delta aquatic foodweb; Bay-Delta hydrodynamics; essential fish habitat).*
17. Water Quality Constituent:	*1) Salinity; 2) Turbidity, nutrients and oxygen depleting substances).*
18. At-Risk species benefited:	*Longfin Smelt*
19. Project objectives:	*Develop aquaculture techniques for longfin smelt*
20. Time frame:	*August 1, 2012-July 31, 2015*

Section 2: Location Information

1. Township, Range, Section: and the 7.5 USGS Quad map name.	*Bryon, California*
2. Latitude, Longitude (in decimal degrees, Geographic, NAD83):	*37.8260227971098 86,-121.596701145172 12*
3. Location description:	*Skinner Fish Facility, Fish Conservation and Culture Laboratory.*
4. County(ies):	*Contra Costa*
5. Directions:	*From I-5 take I-205 West to Grant Line Rd, then right on Bryon Rd.*
6. Ecological Management Region:	*Delta*
7. Ecological Management Zone(s):	*Sacramento-San Joaquin Delta*
8. Ecological Management Unit(s):	*South Delta*
9. Watershed Plan(s):	*BDCP, Delta Vision Plan, CALFED Ecosystem Restoration Program*
10. Project area:	*NA*
11. Land use statement:	*Describe current and anticipated future (next 5 years) land uses in the watershed.*
12. Project area ownership:	% Private_____ % State___100_____ % Federal_____ *Enter ownership percentages by type of ownership.*
13. Project area with landowners support of proposal:	*Project will be restricted to open access public waterways.*

Section 3: Landowners, Access and Permits

1. Landowners granting access for project: (Please attach landowner provisional access agreement[s]): *List and reference attached access agreements. Also map ownerships on attached project maps and diagrams. See sample forms in PSP Appendix B.*	
2. Owner Interest: *not applicable*	
3. Permits:	*DFG scientific collection permit, state and federal ESA take permits. I have the DFG scientific collecting permit and am appying for the take permits.*
4. Lead CEQA Agency:	*not applicable*
5. Required Mitigation:	Yes ☐ No ☒ *Is the work in the proposed project required as mitigation pursuant to CEQA or other authority? (See PSP Part III I. Environmental Compliance) Check and explain if yes.*

Section 4: Project Objectives

1. List task information:
Achieve recovery of at-risk native species dependent on the Delta and Suisun Bay as the first step toward establishing large, self-sustaining populations of these species; support similar recover of at-risk native species in San Francisco Bay and the watershed above the estuary; and minimize the need for future endangered specieslistings by reversing downward population trends of native species that are not listed.

Objective 1: Achieve, first, recovery and then large self-sustaining populations of the following at-risk native species dependent on the Delta, Suisun Bay, and Suisun Marsh: Central Valley winter-, spring- and fall/late fall-run chinook salmon ESUs, Central Valley steelhead ESU, delta smelt, longfin smelt, Sacramento splittail, and green sturgeon.

Objective 3: Enhance and/or conserve native biotic communities in the Bay-Delta estuary and its watershed, including the abundance and distribution of the following biotic assemblages and communities: native resident estuarine and freshwater fish assemblages, anadromous lampreys, and estuarine plankton assemblages.

This study will describe what habitats of the bay-delta system are critical for the recovery of delta smelt and longfin smelt, and will provide data for evaluating the restoration of habitats in the study areas.

2. Additional objectives:
Describe any additional objectives not described above.

3. Source(s) of above information: *List references*

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: Dr. James Hobbs
Primary Investigator Dr. James Hobbs
Co-Primary Investigator: Dr. Joan Lindberg and Dr. Swee Teh
Supporting Staff: Shawn Acuna, Galen Tigan
Subcontractor:

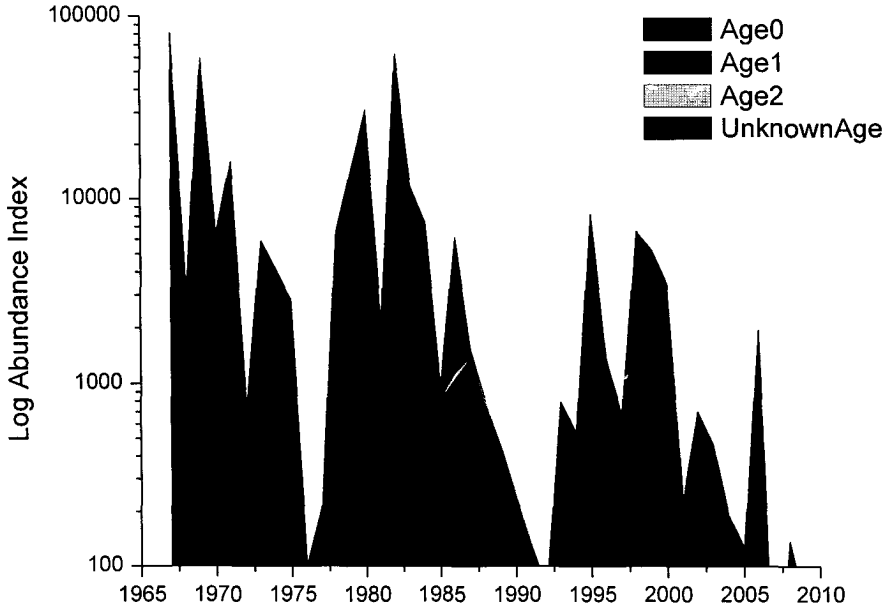
Provide the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

Last Name	First Name	Organization	Role
Baxter	Randy	DFG	Advisor
Sommer	Ted	DWR	Advisor

Section 6: Project Tasks and Results Outline

1. Detailed Project Description

The longfin smelt population, along with several other pelagic fishes in the San Francisco Bay-Delta Estuary has collapsed! (Figure 1.)



Currently, population indices hover around 5% of historic levels (http://www.delta.dfg.ca.gov/data/mwt/), with little sign of improvement with recent wet conditions (WY2006 & 2010). The drivers implicated for the decline (e.g. salvage, freshwater exports, food limitation, and poor water quality) remain unresolved regarding the decline of longfin smelt (**Baxter et al. 2008a**). Current research efforts supported by the Interagency Ecological Program and the National Academy of Science Review Panel seek to utilize individual-based modeling approaches to understand how different drivers affect the population dynamics of this threatened species. Unfortunately, very little information critical to the effective execution of such models exists for longfin smelt. For example, basic information regarding vital rates, (e.g. development times, growth rates and fecundity are wholly lacking for this species). Moreover, much of the reproductive and early life history associated with environmental tolerances is not known. Our onoing IEP funded studies suggest salinity tolerance of the early life stages may be much lower than anticipated based on salinities at which fish are found in the wild (**Hobbs et al 2010**).

The primary purpose of this project is to develop methods to successfully raise longfin smelt in a laboratory culture setting. Doing so would also provide a better understanding of the range of suitable environmental conditions for longfin smelt reproduction, development and growth. The focus of this study addresses multiple topic areas outlined in the 2010-11 Calfed Ecosystem Restoration Program Delta PSP, including the primary goal of the program; recover endangered or at-risk native species. Using captive breeding experiments we will gain a better understanding of the basic biology and ecology of longfin smelt. Currently we are conducting pilot culture experiments with longfin smelt funded by IEP, which is revealing several questions that need to be addressed regarding the proper environmental conditions necessary for larval rearing. Primarily,

(1), What salinity and temperature parameters are optimal for incubation of eggs that will maximize successful hatch?

(2), What age/stage are longfin smelt larvae competent for brackish water exposure?

Thus far we have been successful at collecting reproductive adults from the wild with the assistance of the California Department of Fish and Game and U.S. Fish and Wildlife Service monitoring programs. Our preliminary experiments suggest egg incubation and successful hatching can occur at salinities up to 4-ppt. Moreover we can successfully rear larvae for ~3 months at salinities less than 2-ppt. However it is not yet clear at what age or life-stage, larvae are competent for higher salinities. Meanwhile field surveys suggest longfin smelt less than 20-mm can occur at salinities as high as 10-ppt, but larvae found in high salinities may not survive as suggested by otolith strontium isotope geochemistry studies (**Baxter, *unpublished iep data*; Hobbs et al. 2010).** Thus retention of larval longfin smelt in the low salinity habitats of X2 may be the key driver of longfin smelt abundance trends.

The proposed study is feasible due to a combination of 1) our decade lon experience culturing delta smelt, 2) few contingencies for project completion (i.e. studies are already ongoing), and 3) availability of research facilities (Fish Culture and Conservation Laboratory at the Skinner Fish Facility).4.) Ongoing collaboration with state and federal agencies willing to procure adult smelt for our research purposes. The expertise of this research team spans the disciplines of fish ecology, biology and culturing, aquatic toxicology, otolith microchemistry, pathology, and nutrition. The combination of these tools at the individual level can provide critical vital rates data that can then inform ongoing individual based modeling efforts and provide the Ecosystem Restoration Program with sufficient biological and ecological information to develop effect management and restoration efforts specifically designed to benefit longfin smelt.

2. Background and Conceptual Models

Once one of the most abundance species in the San Francisco Bay-Delta, the longfin smelt population crashed in 2003 along with several other pelagic organisms (**Sommer et al 2007, Rosenfield and Baxter 2007**). The "Pelagic Organisms Decline" resulted in a wide ranging investigation of many old and few new factors thought to impact fish populations in the delta, including the most politically prominent cause, the loss of fish in large pumping facilities located in the South Delta (**Bennett and Moyle 1995**). During this time period the volume of water exported out of the delta increased by approximately 30%, when export rates increased during the winter months (**IEP_POD Report 2005**). However, other compelling changes to water quality also occurred, such as increased ammonia concentrations from waste water treatment on the Sacramento River, increased loadings of new forms of pesticides from adjacent agriculture fields, and massive blooms of toxic microcystis algae in the Delta. Concomitant with the increased freshwater exports, the volume of available habitat for pelagic organism was reduced along with an eastward shift in distribution towards the pumps (**Feyrer et al 2008, Nobriga et al 2008**). As a result of the recent collapse, a consortium of conservation and non-profit organizations led by the Bay Institute petitioned the State and Federal Government for listing the longfin smelt under the endangered species act (**The Bay Institute 2007**). Final decisions regarding the federal status are still ongoing.

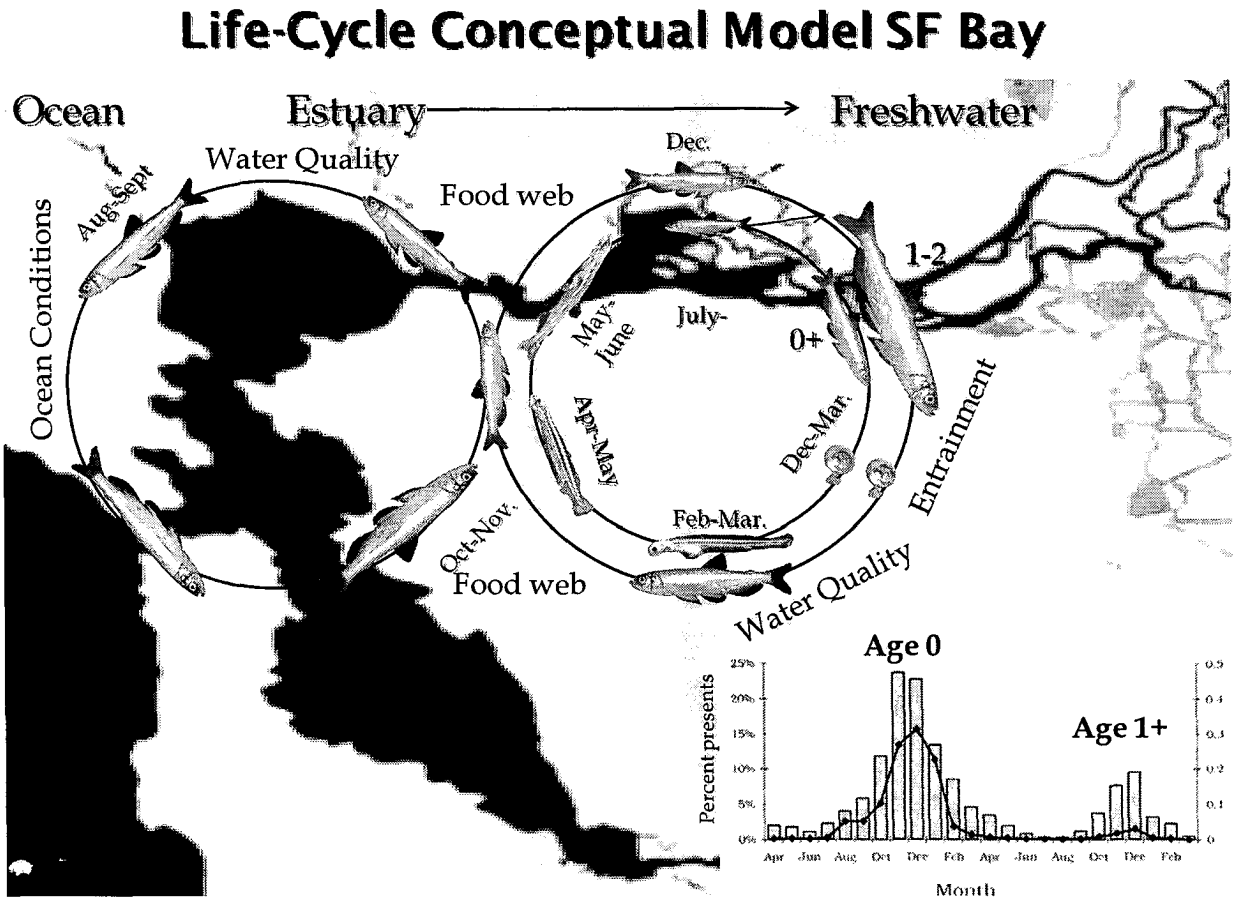
The longfin smelt is a small euryhaline fish that ranges from San Francisco Bay to the Frazer River in Canada. Several isolated populations are historically known to occur in Humboldt Bay, Klamath River Estuary, Columbia River Estuary, Lake Washington and the Frazer River Estuary, however records of current population status in other estuaries is virtually non-existent. Longfin smelt live 2-3 years of age and spawn primarily as 2 year olds in the tidal freshwaters of estuaries (**Moyle 2002**). Longfin smelt rear in brackish waters for approximately 6-9 months, with a contingent of the population migrating into nearshore ocean habitats and another that remains up estuary in low salinity waters of Suisun Bay (**Rosenfield and Baxter 2007**). The population structure is currently a question of considerable debate, with the question of population connectivity among the San Francisco Bay population with other adjacent populations at the heart of endangered species status. This issue is currently being addressed by our collaborators at the *center for genetic research at UC Davis*. In addition we are currently investigating the prevalence of ocean migrations in the population with otolith strontium isotope ratios.

Because longfin smelt utilize habitats other than the delta during its life-cycle, they are thought to be a good

indicator species for the greater ecosystem health of the estuary and near-shore environments. Moreover, integration of health status for this species among different habitats will provide novel insights regarding the different factors associated with the pelagic organism decline (POD) as all perturbations are operating primarily in the Delta. However, nearshore ocean productivity has also declined significantly over a similar time period as the POD. Longfin smelt condition and health status of segments of the population utilizing the Delta, estuary and marine habitats will provide managers with an indicator of the health of each habitat and allow us to pinpoint the geographic extent of various factors such as pesticide exposure, food limitation or disease.

CONCEPTUAL MODEL

The following conceptual model of the longfin smelt population biology and potential factors associated with their recent decline pertain to the objectives in this proposed study and is not intended to serve as a comprehensive review of potential drivers of the population. (Figure 2).



Life-Cycle of Longfin Smelt.

Longfin smelt utilize freshwater, low-salinity, brackish and nearshore ocean habitats throughout their 2-3 year life-cycle. Larvae occur in freshwater to brackish habitats, whereas juveniles and sub-adults can be found throughout SF Bay at salinities greater than 30-ppt. It appears that juvenile and adult longfin smelt reside in deep, cool and marine habitats in the fall (**Rosenfield and Baxter 2007**). There also appears to be a movement of fish to the ocean during the second summer of life, and large spawning run to the confluence of the Sacramento and San Joaquin River starting in December (**Rosenfield and Baxter 2007**). Spawning is thought to occur in freshwater, however recent preliminary results from egg incubation salinity studies by the Fish Conservation and Culture Laboratory, UC Davis, suggests larvae can successfully hatch at salinities up to 5-ppt and possibly higher. However we have yet to determine the appropriate age, or size at which fish can tolerate brackish conditions in the lab. Meanwhile otolith strontium isotope ratios of otolith cores from wild

fish may indicate successful spawning and hatching of longfin smelt eggs in higher salinities than previously recognized in laboratory experiments. However it remains unclear whether longfin smelt core chemistries are derived from maternal sources or environmental sources. Tasks 1 and 2 of this study would fill critical gaps in our knowledge of the stage durations, physiological tolerances of larvae to brackish water. Task 3 will help us determine whether the otolith core chemistry reflects a maternal contribution or environmental exposure.

Relationship between longfin smelt abundance-X2 position.

The abundance index of age-0 longfin smelt in the Fall Midwater Trawl declined significantly after the drought of 1987-1994 (**Rosenfield and Baxter 2007**). The abundance of age-0 longfin smelt is also strongly positively related to freshwater outflow as indexed by the position of X2 in the winter to spring period (**Kimmerer et al., 2002a,b**). This relationship changed after the 1987-1992 drought, such that juveniles produced significantly fewer adults after the drought. Moreover during the POD years the slope of this relationship declined again (**Fish et al., 2009**). (Figure 3). These observations suggest that significant population regulation takes place during the early life stages in spring and summer months when the longfin smelt are distributed primarily in upper estuary low-salinity and freshwater delta habitats. The reduction in longfin smelt abundance after 1987 has been attributed to the reduction in upper estuary productivity — which declined to very low levels by the mid-1990s (**Jassby et al., 1995, 2002**; **Kimmerer and Orsi 1996**; **Orsi and Mecum 1996**). The mechanism resulting in the recent decline in longfin smelt production, however remains unknown. However our recent evidence may suggest that exposure to high salinities during the early life stages may have detrimental effects on survival and may be a contributing factor to the relationship between longfin smelt abundance and flow.

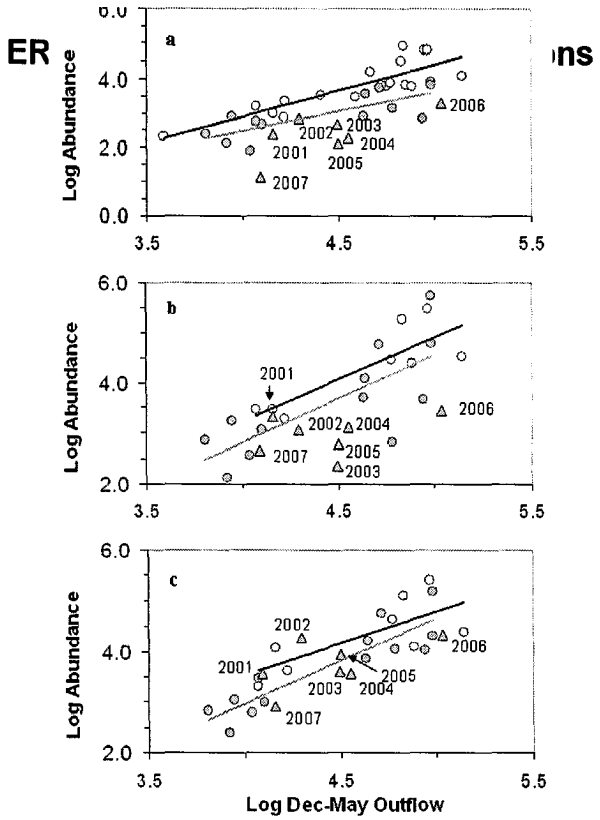


Figure 3. Longfin smelt annual abundance indices plotted on December through May average delta outflow for a) Fall Midwater Trawl (all ages); b) Bay Study Midwater Trawl Age 0; c) Bay Study Otter Trawl Age 0. Relationships depicted are pre-*Corbula amurensis* (1967-1987; open circles, black line) and post-*Corbula amurensis* (1988-2000; filled circles, grey line) and more recent years during the Pelagic Organism Decline (POD) (2001- 2007, grey triangles, no line).

Effects of Water Quality

The San Francisco Estuary is plagued with a history of heavy metal, pesticide-herbicide use and recently increased concentrations of ammonia in the rivers. Contaminants known from sediments in the Sacramento-San Joaquin Delta include Mercury, Selenium, legacy organochlorines (**Werner et al. 2008**), and pyrethroid insecticides (**Oros and Werner 2005**). Pyrethroid insecticides are highly toxic to aquatic organisms and are increasingly used in both agricultural and urban applications (**Oros and Werner 2005; Werner 2008**). A detailed time series of contaminant concentrations and distribution is not available for the San Francisco Estuary. However, certain contaminants have distinct spatial distributions, with heavy metals being more prevalent in Suisun and San Pablo Bay, while pesticides and herbicides are more prevalent in the delta (**Thompson et al. 2000. RMP 2008**). In addition the increase in ammonia concentrations recently is thought to impact the lower Sacramento River only (**IEP 2009**).

2. Approach and Scope of Work

We propose a 3-year study to develop aquaculture techniques for the threatened longfin smelt and examine the effects of salinity and temperature on development, survival, growth and condition of longfin smelt in culture. We will employ our interdisciplinary toolbox to develop aquaculture techniques for longfin smelt, advance our knowledge of longfin smelt biology, and begin to understand the mechanism controlling the population abundance. This study will expand upon our IEP funded project entitled "**How will longfin smelt respond to**

Fall X2 manipulations?: Experimentally determining early life-stage sensitivity to salinity", and provide the necessary research to complete the life-cycle of longfin smelt under culture condition.

The goals of this research will be to identify the range and scope of salinity and temperature requirements of early life stages of longfin smelt to advance the culture efforts. This work will also provide information regarding vital rates such as stage duration, growth and maternal contribution to offspring. This research will be divided into several tasks:

1) Laboratory culture development (Joan Lindberg FCCL Lab)
2) Biomarker evaluation of larvae exposed to different salinities and temperatures (Swee Teh, Aquatic Toxicology Lab)
3) Maternal contribution vs. environmental influence on otolith core geochemistry. (Jim Hobbs, Otolith Lab)
4) Data integration and project management. (Jim Hobbs)

This approach will provide key insights into longfin smelt biology and we fully anticipate beneficial products from this research, such as proper rearing condition for longfin smelt in culture, biomarkers of salinity and temperature stress and experiment determination of maternal contribution to offspring, via otoliths geochemistry studies.

Objectives and hypotheses- We propose to address a variety of key questions intended to develop culture techniques for longfin smelt and provide new tools to examine the biology and ecology which will guide management actions. Hypothesis testing in this regard may constitute parameter estimation of relative effects more than strict falsification of alternatives. These include but are not limited to

1. What are the optimal rearing conditions for egg, larvae and juvenile stages?
2. What are the stage durations, survival and growth rates of different life stages?
4. At what age/stage are longfin smelt competent for brackish water?
5. Does otolith core chemistry reflect maternal or environmental contribution?
6. Can we use otoliths chemistry information from field studies to inform culture methods.

Task 1. Laboratory Culture Development.

Task 1.1 Broodstock Collection. **Hobbs** (years 1-3)

We collect longfin sub-adults and adults for broodstock and spawning at two ages and at two times during this proposal. We propose collecting just prior to the spawning season (October-November) and during the spawning season (December - February) to determine if fish caught earlier may have better survival than fish in spawning condition. A confounding factor is water temperature in the wild. We have found that delta smelt survival is far better when the water temperatures have fallen to 13°C or below. We will coordinate with state and federal monitoring programs to obtain the wild longfin. Over the last couple of years we have been able to obtain surplus 2-year old longfin adult smelt from the USFWS monitoring program at Chipps Island. The wild smelt were collected in spawning condition in December in (2008-2009), and the FCCL successfully induced spawns from most of the captured fish. In the current proposal the collection effort will focus on obtaining 1-year old and 2-year old fish prior a couple of months prior to spawning as well as smelt in spawning condition. We will be coordinating with the CDFG and USFWS monitoring and sampling programs. In addition, we will fish our delta-smelt gear (100' lampara net deployed off bow of skiff) at select times and locations when surveys indicate longfin smelt are most abundant and during surveys by UC Davis in Suisun Marsh and South San Francisco Bay monitoring studies.

.

The wild-captive broodfish will be maintained in a re-circulating and biofiltered system which currently consists of two holding tanks (1000-L circular tanks), with planned expansion to 4-6 tanks total for task 3, Maternal contribution to offspring. Salinity of the recirc-system is held at the salinity in which the fish are captured, and will be increased (to 15-30PPT) should the smelt survive long past the spawning period.

We will feed mysid shrimp, amphipods, and brine shrimp nauplii, and or wild copepods, in an attempt to feed some of their preferred foods in the wild. Weaning to prepared feeds will be initiated after feeding is established in captivity. Tanks are siphoned or wiped down every other day, allowing inspection of fecal remains and live prey, indicating what the fish are eating and what they are avoiding.

Task 1.2 Salinity and temperature rearing conditions for longfin smelt. **Lindberg, FCCL**

Years 1-2 Experiments

Embryo incubation test:

In-vitro fertilization of longfin smelt will occur and embryos will be incubated in an up-welling column style incubator system, under three salinity conditions: 0, 2, and 4-ppt saltwater and three temperatures (10, 15 and 18 ^{o}C). Larvae hatch and dead are quantified and successful hatchlings are retained in 19-L black buckets. Years 1 and 2 will be dedicated to determining the optimal conditions for embryo incubation.

Larval rearing conditions:

Hatched larvae are stocked into tanks (70-130L black circular tanks on a re-circulating and bio-filtered system at 12-14 °C) at a density of about 40 larvae/liter. Larvae are maintained under green water conditions *(Nannochloropsis,* Reed Mariculture Inc., San Jose, CA), and fed rotifer and brine shrimp nauplii every two hours (**Bridges et al 2003,2005**, (the culture manual)). Larvae will be reared at 1-ppt and 4-ppt on separate recirculating banks of tanks. Sub-sampling of the larvae will be conducted every two weeks to document vital rates (growth, development) and provide materials for task 2, staging gill-chloride cell development and density; 10 fish will be preserved in formalin solution for each condition.

Year 3 Experiments:

(1). Effect of varying salinity exposure on larval smelt survival and gill-chloride cell development and density.

Larvae will be reared under oscillating salinity conditions, varying from 3-6ppt over a 72hour period (ca. 24 hours per salinity). Larvae will be sub-sampled as described above for vital rates, biomarker analysis, and they will be compared to the groups of fish reared under constant salinity conditions. These rearing conditions are meant to represent swings in salinity conditions (either tidal or anthropomorphic) that could be experienced by longfin eggs and embryos in the wild. Larvae reared under each of the 3 rearing conditions will be tested for their ability to transfer to higher salinities (2, 4, 6, 8, 10ppt) at several ages (10, 15, 20, 25, 30 dph) in short term exposures (20 larvae I 2-liter beakers with aeration for 24 hours).

(2). Effect of thermal conditions on larval smelt growth and survival.

We will rear fish in 20L tanks at three temperatures (12, 15 and 18 ^{o}C; 3 replicates each stocked at a density of 10/L) for a period of 30 days from a single salinity value based on results of 48 hour salinity trials. Fish from this experiment will be archived for biomarker evaluation (Task 2) oxygen isotope analysis (Task 3).

Based upon year 1 experimental results we will attempt to expand and extend salinity rearing trials to incorporate multiple salinities at several life-stages to determine the time at which longfin can transfer successfully to higher salinities. In addition we will expand the temperature experiments to incorporate salinity in a 3 x 3 experiment. As above, the behavior of fish will be monitored intermittingly and survival monitored daily.

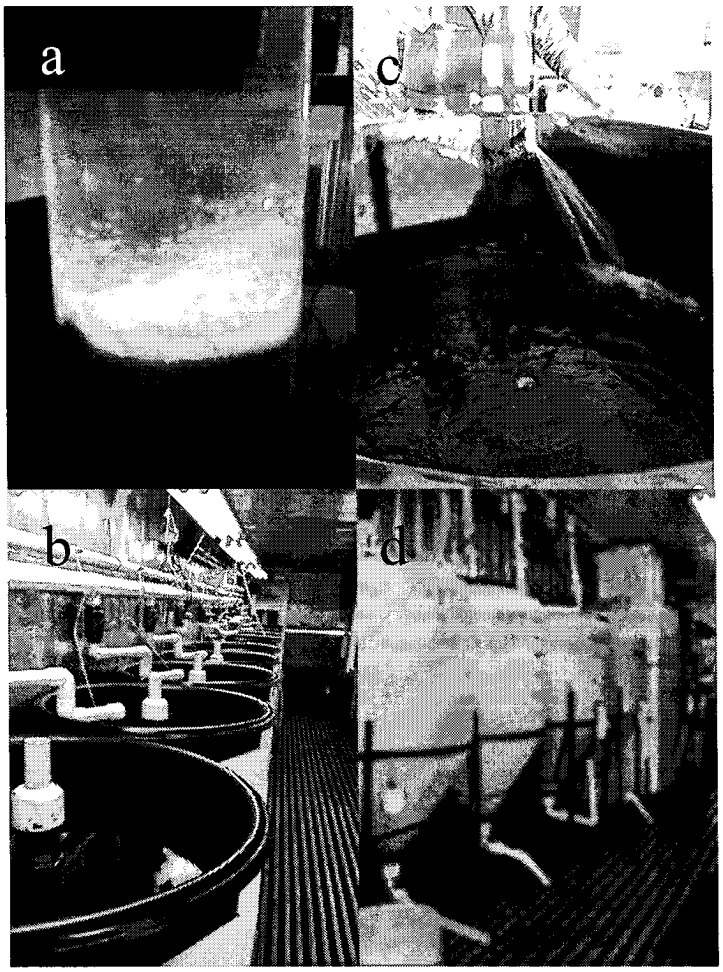


Fig 4: a) egg incubation b) 80 L larval rearing tanks c) adult broodstock tanks, d) live prey culture system.

Task 2. Biomarkers of salinity competence. Teh lab

Task 2.1 Biomarker studies

A suite of biomarkers was selected to provide evidence of: 1) competency for osmoregulating in saline conditions, 2) the nutritional status of adult LFS at various stages of ovarian maturation and development and 3) uptake of "doped" strontium isotope ratios into embryos (Task 3).

A. Histopathology – histopathology markers are good indicators of environmental stress (reviewed in **Myers and Fournie 2002**) as they provide visible biological endpoints and measurable responses to subcellular mechanisms that can integrate exposure over time (**Stentiford et al. 2003**). For this reason, 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 as to assess exposures of LFS to various salinity and temperature (see Task 1.2). Histologic damage in early life stages (embryos and larvae) of fish and other aquatic organisms is considered one of the most sensitive means of assessing adverse effects induced by xenobiotic compounds (**Weis and Weis 1987**, **Teh et al. 1999**).

Gill, gonads, and liver 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.

B. Proximate analysis of major storage forms of energy to determine fish nutritional status - 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 deplete glycogen from the liver and then muscle. Although the major biological functions of protein are to provide essential amino acid and nitrogen for normal functions in animal, protein can be also used as energy source if the body lipid level goes down lower than the threshold of storage. Protein plays a pivotal role in biosynthetic activities during early stages of embryogenesis (**Metcoff 1986**).

Proximate analysis – 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**).

Choriogenin and vitellogenin – during reproduction in fishes, light duration 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 vitellogenin (VTG), a yolk precursor. This material is manufactured by liver cells (hepatocytes) 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 or zona radiata protein (ZRP) is used in eggshell (chorion) formation. These products when present in male fish are good biomarkers of exposure to endocrine-modulating compounds 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).

Task 3. Maternal or Environmental Contribution to Offspring Otolith Chemistry. Hobbs

In our previous work with longfin smelt we were able to reconstruct the salinities of nursery habitats from otolith core strontium isotope ratios ^{87}Sr:^{86}Sr. (**Hobbs et al 2010**). This information can be used to inform us on proper salinities for rearing larvae in a laboratory setting. However, strontium isotope ratios ^{87}Sr:^{86}Sr in the natal otolith core of longfin smelt from the wild can reflect either the salinity at which the larvae are hatched or can be a proxy for the salinity at which the mother underwent oocyte maturation, as marine derived strontium can be passed along to offspring and longfin smelt are known to migrate to the ocean (e.g salmon).

In this task we will conduct experiments to determine the degree to which maternal and environmental conditions affect otolith core chemistry. This will be experimentally tested in two ways. Using a solution of strontium with a "doped" strontium isotope ratio ^{87}Sr:^{86}Sr we will:

(3.1) Inject the doped solution into the peritoneal cavity of ripe mothers and examine the otolith core of offspring for an altered isotope ratio.

Three groups of anesthetized fish, chosen based on external examination of reproductive stage, will be respectively injected with: 1) 1cc/ 500 g wet mass of an isotonic saline solution (~1% salinity as control), 2) 1cc/ 500 g wet mass of "doped" solution of strontium (concentration of the stable ^{86}Sr isotope is manipulated to create an artificial ratio that does not occur in nature) *In vitro* fertilization will be accomplished through manual expression of gametes in a subset of the fish from each treatment. Embryos will incubate in flow-through, column-style, incubators. Upon hatch, larvae will be moved to 70L black tanks and reared at the FCCL for about three months to an approximate length of 15-20 mm standard length. Up to 75 individuals (25 from each treatment) will be sent to the UC Davis Interdisciplinary Center for ICP-MS for strontium isotope analysis.

(3.2) Expose freshly expressed and fertilized eggs to the doped solution.

In one batch of 1000 eggs per treatment (3 replicate treatments) will be exposed to the "doped" strontium isotope solution prior to fertilization for 10 minutes. Fertilized eggs will be inclubated in flow through chambers as in task 1. In a second batch eggs will be fertilized prior to exposure for 10 minutes to the doped solitution and again incubated as in task 1. Upon hatch, larvae will be moved to 70L black tanks and reared at the FCCL for about three months to an approximate length of 15-20 mm standard length. Up to 75 individuals (25 from each treatment) will be sent to the UC Davis Interdisciplinary Center for ICP-MS for strontium isotope analysis.

4. Deliverables.

Deliverable for the project will include:
Oral presentations for the Delta Science Program Biennial Conference, which I have presented at in the past two meetings. To the American Fisheries Society, Cal-Nevada Chapter meetings and National meetings. To the Interagency Ecological Program annual meeting.

Annual progress reports, and two peer reviewed publications.

Databases of age and growth information will be provided to DFG and DWR, and other University collaborators and agency staff upon request.

Below is a schedule of work to be performed.

Proposed work will start in fall 2011 Task 1&2 would begin the following spring spawning period. Task 3: Otolith Age and microchemistry validation work at the Center for ICP-MS will take place in spring (March-April) and fall (October-November).

- Development of cultured fish for research, preserved developmental series of larvae and report on development of longfin smelt culture methodologies.
- Culture of larvae and juveniles reared from adults held at prolonged times in the lab prior to spawning to evaluate reliability of otolith core tracing salinity exposure of developing longfin smelt.
- Results will be presented at the at the Delta Science Conference or IEP workshop following termination of this 12-month study.
- Report at termination of project

With this study we hope to develop many of the specific methods for successful longfin culture, thereby elucidating physical parameters that enable or improve holding and rearing success. Comparisons can be drawn between the several life stages of delta smelt and longfin smelt and relative survival and/or growth for fish reared under one or more salinity conditions, as follows: holding of adult wild fish, fecundity (or egg-clutch) estimates, spawning and fertilization of eggs, and the rearing of larvae and juveniles. Species segregation, of the two smelts in question, and location in the natural habitat appears to depend on salinity, at least for several life stages, and manipulation of the position of X2 in the fall could affect these life stages significantly. This study will provide validation for otolith strontium isotope-salinity relationship developed in (May, Israel and Hobbs, Population Genetics and Otolith Geochemistry, 2008-137 "*IEP 2008 Work Plan to Evaluate the Decline of the Pelagic Species of the Upper San Francisco Estuary*"), as well as provide information for studies regarding the influence of Fall X2 and variable delta salinity management strategies.

7. Feasibility

Building on over a decade of experience in culturing delta smelt, our culture techniques will be adapted to accommodate the more euryhaline longfin smelt. Fecund longfin smelt adults will be collected by the Fish Conservation and Culture Laboratory staff, or retrieved from agency monitoring studies, per FCCL permit constraints. Adults are held in saline water (reflecting capture salinities). Fish are spawned through manual expression of gametes, fins clipped for later DNA analysis by the UC Davis Genomic Variation Lab, eggs incubated in column-style incubators, and larvae reared employing intensive-culture methods. The longfin smelt culture program will provide a supply of eggs and larvae for research use and will allow us to examine key aspects of their developmental biology, such as; determining incubation time for longfin smelt embryos in California. Monitor number of day's post-fertilization to hatch and record daily average temperature. Experiment addresses approximate duration of embryo vulnerability to disturbance, e.g., dredging or changing salinity.

We have a well established reputation and publication record regarding fish otolith geo-chemistry on delta smelt, longfin smelt and Sacramento splittail (Hobbs et al 2005, 2007a, 2007b, and Feyrer et al 2007b, c). The proposed research will take advantage of samples already collected in CDFG surveys, many of which have already been collected and are being prepared in addition to future samples so that ample sample sizes are available to address the proposed questions. The samples are currently housed and covered under State and Federal permits to UC Davis (Dr. Peter Moyle and the IEP take permit) and Hobbs permit for longfin smelt is currently under review.

6. Relevance to the CALFED ERP

This project is directly related to the CALFED goals to restore and protect native and threatened species (CALFED 2000). The proposed research also directly address topic 1 of the 2010 Delta Stewardship Council RFP, and could benefit ancillary studies investigating the use of strontium isotope ratios to address questions such as: How do native migratory fishes navigate through the San Francisco estuary? What factors affect their migratory behavior? What are the management implications? We are also addressing key questions regarding the physiological tolerances and adaptive traits of native fish species that determine their resilience to existing and emerging stressors?

This research is also relevant and extremely important for understanding the mechanisms associated with the Pelagic Organism Decline and is a key data need for the 2009 IEP study plan. My previous research on the delta smelt was funded through the CALFED Fellowship Program, and the IEP POD study. I am currently

developing otolith techniques for the longfin smelt under funding from the IEP POD 2009 Study Plan. This work is also relevant and crucial for the Bay Delta Conservation Plan, the OCAP Biological Opinion, and a key data need from the Blue Ribbon Task Force, Delta Vision Plan.

7. *Expected quantitative result (project summary):*

Information from this study will identify the critical environmental conditions for longfin smelt reproduction, produce a refuge population and aid in the recovery of these endangered species.

8. Other products and results:

9. Qualifications

James Hobbs, Ph.D., Assistant Research Scientist in the Department of Wildlife, Fish and Conservation Biology and an associate with the Interdisciplinary Center for Inductively Coupled Plasma Mass Spectrometry at UC Davis. Dr. Hobbs received his B.S. degree in Marine Biology from Sonoma State University, completed his PhD. in Ecology from the University of California, Davis and was a Sea Grant-CALFED Post-Doctoral Fellow at the University of California, Berkeley. His research focuses on development of otolith microstructure and microchemistry techniques to understand the population biology and ecology of commercially important and threatened species. Dr. Hobbs has published several articles in peer review literature regarding the application of laser ablation inductively coupled plasma mass spectrometry. He has received grants from the U.S. Forest Service to determine natal stream origins and migration history of Chinook and Coho salmon in the Klamath River; Army Corp of Engineers-Bonneville Power District to determine migration history and estuarine residency of spring Chinook salmon; Sonoma County Water Agency to determine estuarine residency in steelhead trout; Interagency Ecological Program to determine ocean residency in the threatened longfin smelt and natal origin of the endangered delta smelt

Dr. Swee J. Teh, Ph.D. is a research faculty in toxicology and pathology at UC Davis, Department of Anatomy, Physiology and Cell Biology and 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 40 peer-reviewed publications and has traveled nationally and internationally to present his work in conferences and workshops.

Dr. Joan Lindberg, Ph.D. Director, Fish Conservation and Culture Laboratory,
Biological and Agricultural Engineering Department, UC Davis

Initiated program to capture and culture delta smelt in 1992 for research purposes. In recent years the program has expanded and produces reliable supply of delta smelt for research, conducts research, and has developed a refugial population of delta smelt under genetic management. The refugial population constitutes a safeguard against extinction of this endangered species. The Fish Conservation and Culture Laboratory (FCCL) is located on State Water Project land near Byron, CA. Current research includes developmental biology, larval fish behavior, and adult reproductive biology and behavior of the delta smelt and development of culture techniques for the longfin smelt.

Select references: Lindberg JC, Baskerville-Bridges B, Van Eenennaam JP, and Doroshov SI. 2000. Update on delta smelt culture with an emphasis on larval feeding behavior. IEP Newsletter 13 (3): 45-49;

Baskerville-Bridges, B., J.C. Lindberg, and S.I. Doroshov. 2004. The effect of light intensity, alga concentration, and prey density on the feeding behavior of delta smelt larvae. *in* F. Feyrer, L. Brown, R. Brown, J. Orsi, editors. Early life history of Fishes in the San Fransisco Estuary and Watershed. *American Fisheries Society Symposium* 39:219-227;

Baskerville-Bridges, B., J.C. Lindberg, J. Van Eenennaam, and S.I. Doroshov, 2004. Culture of Delta Smelt (*Hypomesus transpacificus*) in Support of Environmental Studies and Restoration: 5-year summary 1998-2003. CALFED Bay-Delta Program Project #2000-B03, pp59;

Baskerville-Bridges, B., J.C. Lindberg, and S.I. Doroshov, 2005, Manual for the Intensive Culture of Delta Smelt *Hypomesus transpacificus*, pp62; Fisch, K., Rettinghouse, T., Ellison, L., Tigan, G., Lindberg, J., and B. May. 2009. Delta smelt refugial population development and genetic management - -2009 season summary. Interagency Ecological Program Newsletter. 22(3):

10. Literature Cited

Adams SM and 6 co-authors (1989). The use of bioindicators for assessing the effects of pollutant stress on fish. Marine Environmental Research 28: 459–464

Adams SM and 4 co-authors (1999). Ecological risk assessment in a large river-reservoir: Bioindicators of fish population health. Environmental Toxicology and Chemistry 18: 628–640

AOAC Association of Official and Analytical Chemists (1995). 16th ed. Official Methods of Analysis of AOAC, vol.1. Association of Official Analytical Chemists, Arlington, VA.

Barnett-Johnson, R., T.E. Pearson., F.C. Ramos., C.B. Grimes. and R.B. MacFarlane. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. Limnology and Oceanography. 53(4)1633-1642.

Baskerville-Bridges, B., J. Lindberg, and S.I. Doroshov. 2003. The effect of light intensity, alga concentration and prey density on the feeding behavior of delta smelt larvae. *in* F. Feyrer, L. Brown, R. Brown, J. Orsi, editors. Early life history of Fishes in the San Francisco Estuary and Watershed. American Fisheries Society, Santa Cruz, California.

Baskerville-Bridges, B., J. Lindberg, J. Van Eenennaam, and S. Doroshov. 2005. Manual for the Intensive Culture of Delta Smelt (Hypomesus transpacificus). Report to CALFED Bay-Delta Program; pp62.

Baxter, J.M., Breuer, R., Brown, L. Chotkowski, M. Feyrer, F. Gingras, M. Herbold, B. Mueller-Solger, A. Nobriga, M. Sommer, T. Souza, K. 2008a. Pelagic Organism Decline Progress Report: 2007 Synthesis of Results. http://www.science.calwater.ca.gov.

Baxter, J.M., Breuer, R., Brown, L. Chotkowski, M. Feyrer, F. Gingras, M. Herbold, B. Mueller-Solger, A. Nobriga, M. Sommer, T. Souza, K. 2008b. Interagency Ecological Program 2008 Work Plan to Evaluate the Decline of Pelagic Species in the Upper San Francisco Estuary, http://www.science.calwater.ca.gov

Bennett, W.A. and P.B. Mole. 1996. Where have all the fishes gone?: factors producing fish declines in the San Francisco Bay estuary. In, San Francisco Bay the urbanized ecosystem. J.T. Hollibaugh, editor. AAAS Symposium volume.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. 47:2219–2227.

Cope, J.M. and A.E. Punt. 2007. Admitting aging error when fitting growth curves: an example using the von Bertalanffy growth function with random effects. Canadian Journal of Fisheries and Aquatic Sciences 64: 205–218. doi:10.1139/F06-179.

Culleton, B.J., D.J. Kennett, T.L. Jones. 2009. Oxygen isotope seasonality in a temperate estuarine shell midden: a case study from CA-ALA-17 on the San Francisco Bay, California. Journal of Archaeological Science. 36: 1354-1363.

Devereux I. (1967) Temperature measurements from oxygen isotope ratios of fish otoliths. Science 155, 1684-1685.

Faure, G., and T.M. Mensing. 2005. Isotopes. Principles and Applications, 3rd Edition. Wiley and Sons. Hoboken New Jersey.

Feyrer, F., M. Nobriga, and T. Sommer. 2007a. Multi-decadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 64:723-734.

Feyrer, F.F., T Sommer and J.A. Hobbs. 2007b. Living in a dynamic environment: Variability of Age-0 Splittail in tributaries of San Francisco Bay. Transactions of the American Fisheries Society. 136(5) 1393-1405.

Feyrer, F.F., J.A. Hobbs, M.A. Baerwald, T Sommer, Q.Z. Yin, K Clark, B May and W.A Bennett. 2007c. Otolith microchemistry provides information complimentary to microsatellite DNA for a migratory fish. Transactions of the American Fisheries Society. 136(2) 469-476.

Fish, M., D. Contreras., V Afentoulis., J. Messineo., and K. Heib. Spring 2009. 2008 Fishes Annual Status and Trends Report for the San Francisco Estuary. IEP Newsletter, V22(2)17-36Spring.

Folch J, M Lees, GH Sloane- Stanley (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biology and Chemistry 226: 497–509

Gao, Y.W., Beamish, R.J., 1999. Isotopic composition of otoliths as a chemical tracer in population identification of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 56, 2052–2068.

Gauldie, R.W., C.E. Thacker, and N.R. Merrett. 1994. Oxygen and carbon isotope variation in the otoliths of Beryx splendens and Coryphaenoides profundicolus. Comparative Biochemisty and Physiology. 108A(2/3): 153-159.

Hobbs, J.A., L. Lewis, N. Ikemiyagi, T. Sommer and R. Baxter. 2010.. The use of otolith strontium isotopes to identify critical nursery habitat for a threatened estuarine fish. Environmental Biology of Fishes *89:557-569.*

Hobbs, J.A., W. A. Bennett, N Ikemiyagi, E Brown. Looking Back to Go Forward: The application of otolith growth and micro-chemistry to determine spatial-temporal variability in recruitment success for delta smelt. *Final Report to Calfed. Jan 2009.*

Hobbs, J.A., W. A. Bennett., J.E. Burton., and B. Baskerville-Bridges. 2007a. Modification of the biological intercept model to account for ontogenetic effects in laboratory-reared delta smelt (*Hypomesus transpacificus*). Fishery Bulletin. 105:30-28.

Hobbs, J.A. J.E. Burton, and W.A. Bennett. 2007b. Classification of larval and adult delta smelt to nursery areas using trace elemental fingerprinting. Transactions of the American Fisheries Society. 136(2) 518-527.

Hobbs, J.A., Q. Yin, J. Burton-Hobbs, and W.A. Bennett. 2005. Retrospective determination of natal habitats for an estuarine fish with otolith strontium isotope ratios. Journal of Freshwater and Marine Research. 56: 655-660.

Humason GL (1979). Animal tissue techniques. Freeman, San Francisco

IEP-POD Report 2005. Review Panel Report: San Francisco Estuary Sacramento-San Joaquin Delta Interagency Ecological Program on Pelagic Organism Decline. . Review Panel Members: M.D. Bertness, S.M. Bollens, J.H. Cowan, Jr., R.T. Kneib, P. MacCready, R.A. Moll, P.E. Smith, A.R. Solow, and R.B. Spies http://www.science.calwater.ca.gov/pdf/workshops/POD/IEP_POD_Panel_Review_Final_010606_v2.pdf

IEP POD 2009. Background/Summary of Ammonia Investigations in the Sacramento-San Joaquin Delta and Suisun Bay, Prepared by: A. Ballard, R. Breuer, F. Brewster, C. Dahm, C. Irvince, K. Larsen, A. Mueller-Solger. and A. Vargas.
http://www.science.calwater.ca.gov/pdf/workshops/workshop_ammonia_bckgrnd_paper_nh4-nh3_030209.pdf

Jassby, A. D., W. J. Kimmerer, S. G. Monismith, C. Armor, J. E. Cloern, T. M. Powell, J. R. Schubel, and T. J. Vendlinski. 1995. Isohaline position as a habitat indicator for estuarine populations. Ecological Applications 5(1):272-289.

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnology and Oceanography 47(3):698-712.

Kalish J. M. (1991) Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon ***(Arripis trutta). Mar. Biol.*** 110,
3747.

Kimmerer, W. J. and J. J. Orsi. 1996. Changes in the zooplankton of the San Francisco Bay Estuary since the introduction of the clam Potamocorbula amurensis. Pages 403-424. in J.T. Hollibaugh, editor. San Francisco Bay: the ecosystem. Pacific Division of the American Association for the Advancement of Science. San Francisco, California, USA.

Kimmerer, W. J. 2002a. Physical, biological, and management responses to variable freshwater flow into the San Francisco Estuary. Estuaries 25: 1275-1290.

Kimmerer, W. J. 2002b. Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages. Marine Ecology Progress Series 243:39-55.

Lindberg, J., B. Baskerville-Bridges and S. Doroshov. 2003. Two Reproductive Concerns Tested in Captive Delta Smelt, *Hypomesus transpacificus*, 2002: I. Effect of substrate and water velocity on spawning behavior, and II. Effect of temperature on embryo/larval survival. Report to the Interagency Ecological Program.

Lindberg JC, Baskerville-Bridges B, Van Eenennaam JP, Doroshov SI. 2000. Update on delta smelt culture with an emphasis on larval feeding behavior. *IEP Newsletter* 13 (1): 45-49.

Lindberg, J., R. Mager, B. Baskerville-Bridges and S. Doroshov . 1997. Status of delta smelt culture project. Interagency Ecological Program for the Sacramento-San Joaquin Estuary Newsletter, 10, (3): 31-32.

Malamud-Roam, F.; Phillis, C.; Ingram, B. L.; Schmitt, A. K.; Weber, P. K. 2008. Stable Oxygen isotopes in otoliths to reconstruct salmon and striped bass habitat use within the San Francisco Bay estuary AGU Fall Meeting Abstracts.

May, B, Israel, J. and J.A. Hobbs. 2008 Proposal to IEP POD. Population Genetics and Otolith Geochemistry Study in Support of IEP's Work Plan on Pelagic Organism Decline: Longfin Smelt.

Metcoff J (1986). Intracellular amino acid levels as predictors of protein synthesis. Journal of the American College of Nutrition 5: 107–120

Moyle, P.B. 2002. Inland Fishes of California, 2nd edition. University of California Press.

Murat JC, A Serfaty (1974). Simple enzymatic determination of polysaccharide (glycogen) content of animal

tissues. Clinical Chemistry 20: 1576–1577

Myers MS, JW Fournie (2002). Histopathological biomarkers as integrators of anthropogenic and environmental stressors. SM Adams (ed.), Bioindicators of Stress in Aquatic Organisms. Wiley and Sons, Boca Raton, FL, 221–287

Nobriga, Matthew L.; Ted R. Sommer; Frederick Feyrer; and Kevin Fleming. 2008. Long-Term Trends in Summertime Habitat Suitability for Delta Smelt (Hypomesus transpacificus). San Francisco Estuary and Watershed Science. Vol. 6, Issue 1 (February), Article 1.

Oros, D.R. and I. Werner. 2005. Pyrethroid Insecticides: an analysis of use patterns, distributions, potential toxicity and fate in the Sacramento-San Joaquin Delta and Central Valley: San Francisco Estuary Institute, Oakland, California.

Orsi, J. J., Mecum, W. L. 1996. Food limitation as the probable cause of a long-term decline in the abundance of Neomysis mercedis the opossum shrimp in the Sacramento-San Joaquin estuary. Pages 375-401 In Hollibaugh, JT (ed) San Francisco Bay: the ecosystem. American Association for the Advancement of Science. San Francisco, CA.

RMP 2009. 2008 RMP Annual Monitoring Results. A Report of the Regional Monitoring Program for Water Quality in the San Francisco Estuary, Mar 2010, Contr. No. 604:1-100pp.
Rosenfield, J.A., and R.D. Baxter. 2007. Population Dynamics and Distribution Patterns of Longfin Smelt in the San Francisco Estuary. Transactions of the American Fisheries Society. 136:1577-1592.

Sommer, T., C. Armor, R. Baxter, R. Breuer, L. Brown, M. Chotkowski, S. Culberson, F. Feyrer, M. Gingras, B. Herbold, W. Kimmerer, A. Mueller-Solger, M. Nobriga, and K. Souza. The collapse of pelagic fishes in the upper San Francisco Estuary el colapso de los peces pelagicos en la cabecera del estuario San Francisco. 2007. Fisheries 32(6): 270-277.

Stentiford GD, M Longshaw, BP Lyons, G Jones, M Green, SW Feist (2003). Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Research 55:137–159.
Teh SJ, SL Clark, C Brown, DE Hinton (1999). Enzymatic and histopathologic biomarkers as indicator of environmental contaminant exposure and effect in Asian clam (*Potamocorbula amurensis).* Biomarker 4: 497–509

Teh SJ, C Wong, V Furtula, FC Teh (2003). Lethal and sublethal toxicity of didecyldimethylammonium chloride (DDAC) in early life stages of white sturgeon (*Acipenser transmontanus*). Environmental Toxicology and Chemistry 22: 2152–2158

Teh SJ, DF Deng, I Werner, FC Teh, SS Hung (2005). Sublethal toxicity of orchard stormwater runoff in Sacramento splittail (*Pogonichthys macrolepidotus*) larvae. Marine Environmental Research 59: 203–216

The Bay Institute, Center for Biological Diversity, and N. R. D. Council. 2007. Petition to the State of California Fish and Game Commission and supporting information for the listing of the longfin smelt (Spirinchus thaleichthys) as an endangered species under the California endangered species act.
Thomspon, B., R Hoenicke, J.A. Davis, and A Gunther, 2000. An overview of contaminant-related issues identified by monitoring in San Francisco Bay. Environmental Monitoring and Assessment. 64:409-419.
Weis JS, P Weis (1987). Pollutants as developmental toxicants in aquatic organisms. Environmental Health Perspectives 71: 77–85

Werner I, Anderson S, Larsen K, and Oram J. 2008. Chemical stressors conceptual model. Sacramento (CA): Delta Regional Ecosystem Restoration Implementation Plan.

Xu RP, SSO Hung, B German (1993). White sturgeon tissue fatty acid composition are affected by dietary lipids. Journal of Nutrition 123: 1685–1692

Section 7: Project Budget

1. Detailed Project Budget

Instructions for Completing Total Project Budget

Each proposal must contain a detailed line item budget broken down into three categories: Personal Services, Operating Expense and Administrative Overhead. Additionally the budget must identify the amount being requested from DFG, the amount being provided by the applicant and the total cost for each line item. The amount requested from each source must be divisible by the listed unit cost. The total project budget must contain all project costs.

- Projects approved for funding will be required to submit invoices matching this budget format. Add or delete line items where not applicable.
- It is recommended you calculate, create and save your budget in *Microsoft Excel®* or similar spreadsheet program, as doing so will avoid costly and unfortunate budget errors; then export your budget to *Microsoft Word® or* compatible word processing program with the rest of your written proposal. If the proposal is funded, the information can be sent electronically to DFG staff without reformatting it. A fill and print budget template is provided in the ERP Proposal Application Form.
- It is recommended that the budget be in whole dollar amounts.

Personal Services Costs

All employee costs are required to complete the proposed project.

- List each personnel classification, their total hours, hourly pay rate, and the calculated total. **The calculated total must equal the line item calculation, including both the cost-share and requested amounts. (Do not include staff benefits in the hourly pay rate.)**
- A "Staff Benefit(s)" amount must be listed and calculated.
- Do not list subcontracts in this section. Subcontracts are listed as Operating Expenses.
- Do not list workers' compensation insurance in this section. Workers' compensation insurance is listed as an Operating Expense.

Operating Expenses

Include all materials, contractual services, equipment, and incidental costs.

Contractual Services are those necessary for the implementation of the proposal for which the applicant will subcontract. These services are undertaken by a provider external to the applicant's organization.

- List each subcontractor on a separate line. Provide names of subcontractor(s) if known.

Other Operating Expenses: Expenses related to the operation of the proposal.

- Provide as much cost detail as possible and practical. Use unit costs when applicable (per lb., per day, cubic yard, linear foot, etc.).
- Purchase of equipment with DFG funds is not normally allowed. See *Part II, #2 Project Budget*, for equipment definitions and restrictions.

Travel

Expenses must be consistent with state guidelines for reimbursed travel expenses. Per diem and mileage rates may not exceed State of California standards. State guidelines can be found at www.dpa.ca.gov/personnel-policies/travel/hr-staff.htm.

Streambed Alteration Permitting Fees

Fish and Game Code, Section 1600 et seq. authorizes the Department to recover the total costs it incurs to administer and enforce its Lake and Streambed Alteration Program by charging applicant fees for Lake and Streambed Alteration Agreements. The actual fees charged will depend on the total cost of the project. Before calculating the fee, be sure to read the definition of a project per the Lake and Streambed Alteration Program. The definitions, instructions and forms are available on the Lake and Streambed Alteration Agreements website at www.dfg.ca.gov/habcon/1600/forms.html.

Standard Agreement	
If project costs is:	**Permit fee will be:**
less than $5,000	$200
$5,000 to less than $10,000	$250
$10,000 to less than $25,000	$500
$25,000 to less than $100,000	$750
$100,000 to less than $200,000	$1,100
$200,000 to less than $350,000	$1,500
$350,000 to less than $500,000	$2,250
$500,000 or more	$4,000

Administrative Overhead

Administrative overhead should be applied only to projected administrative costs that cannot be recovered in other budget categories.

- Administrative overhead in excess of 10% must be justified on a separate attachment.

Budget

What are the Optimal Environmental Conditions for Longfin Smelt Reproduction?

PERSONAL SERVICES			
Staff Level 3yr total	**Number of Hours**	**Hourly Rate**	
Task 1			
Lindberg Staff	3360	$17.86	$60,024
Task 2			
Swee The	576	$48.97	$28,208
Shawn Acuna	1440	$19.92	$28,683
Graduate Student	2880	$19.14	$55,120
Student assistants	315	$9.81	$3,091
Task 3			
Jim Hobbs	1440	$35.79	$51,543
Jr. Specialist	1440	$18.43	$26,538
Subtotal			**$253,207**
Staff Benefits			
Task 1			
Lindberg Staff (40%)			$24,010
Task 2			
Swee The (40%)			$12,210
Shawn Acuna (40%)			$12,416
Graduate Student (40%)			$1,488
Student assistants (3%)			$93
Task 3			
Jim Hobbs (40%)			$20,617
Jr. Specialist (40%)			$10,615
Subtotal			**$81,450**
TOTAL PERSONAL SERVICES			**$334,657**
OPERATING EXPENSES			
Description			
Task 1			
New holding tanks, pumps, filters, power generator, each item <$5,000,two chillers @$2,500, prey culture equipment, artifically feed,			$41,736
Task 2			
Proximate and fatty acid analysis,Glassware, reagents, and histology, biohazard disposal, pub costs			$60,900
Task 3			
ICP-MS fees, travel, microscope and computer lease, supplies			$9,000
Subtotal			**$111,636**
Add/delete line items above for work to be performed by the contractor			
Total Operating Expenses			**$446,293**
Equipment			$0
Fee Remissions			$47,096
SUBTOTAL			
OVERHEAD @ 25% (Less Equipment, Fee Remissions)			$111,573
GRAND TOTAL			**$604,962**

2. Budget Justification

BUDGET JUSTIFICATION

Personnel

Dr. Joan Lindberg, PhD (5% time) **with** the assistant of Lab Assistant Specialists (100 %time or 7 months) will be responsible for culture experiments

James A. Hobbs, PhD (25% time) will be responsible for the coordination, overall supervision, broodstock collections and reporting of the UCD project. He will be responsible for the supervision of a Junior Specialist (25% time), conducting otolith microstructure and microchemistry to estimate the growth rate, and environmental history in Task 3. Dr. Hobbs and the Junior Specialist will participate in field sampling of fish and data analyses.

Swee J. Teh, PhD (10% time) will be responsible for the coordination and overall supervision of the UCD project. He will be responsible for reporting and assisting in the experimental design and analysis of studies conducted in Task 2. Dr. Teh will coordinate efforts of graduate student (50% time) and lab assistant III (25%), supervise and participate in field sampling of fish and standard operating protocols, and data analyses as well as the laboratory care, exposure, and maintenance of fish.

All investigators will be responsible for preparation of technical reports and manuscripts.

Fringe Benefits

Fringe Benefits have been calculated using estimated benefit rates (40%).

Travel

HOBBS:- Travel funding is requested to support field sampling of broodstock: Private vehicle use: $0.50/mi for travel to field sites to collect water samples and fish (Hobbs and Jr. Specialist), year for a total of $500, Per Diem is $35 per day, and the presentation of findings and developments at IEP workshops, informal meetings and the California Estuarine Research Society meetings.

TEH: - presentation of findings and developments at IEP workshops, informal meetings and the California Estuarine Research Society meetings ($500).

LINDBERG: presentation of findings and developments at IEP workshops, informal meetings and the California Estuarine Research Society meetings ($500).

Equipment

NA

Supplies

HOBBS: Includes service fees for laser ablation ICPMS @ the Interdisciplinary Center for ICPMS-UCD @$100.hr; 20hrs = $2,000 microscope rentals, otolith preparation slides, including, microscope slides, slide boxes, lapidary films, polishing alumina, section blades, $500. All costs are per year basis

TEH: Glassware, tanks, chemicals, and reagents for preparation of reconstituted water and fish exposure ($3000), proximate composition analysis and fatty acid analysis for fish tissue for field and laboratory experiment: $100/samples for 150 samples ($15,000) biohazard disposal and fish facility room user fees ($1000). And $300 for publication costs. All Costs on a per year basis.

LINDBERG: New holding tanks for longfin smelt adult holding studies, pumps filters power generator chillers in year 1 $25,236. Feed costs, disinfection and aquaria consumables $5000 per year.

Other Expenses:

TEH: Graduate student fees: 3 years ($47,906).

3. Administrative Overhead

Indirect Costs:

The current indirect cost rate is 25%

For DFG use only	
Proposal No.	Region

(Pages A13-A18)

Section 1: Summary Information

1. Project title:	

ERP Proposal Application Form

2. Applicant name:	
3. Contact person:	
4. Address:	
5. City, State, Zip:	
6. Telephone #:	
7. Fax #:	
8. Email address:	
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:	
13. Total project cost:	
14. Topic Area(s):	
15. ERP Project type:	
16. Ecosystem Element:	
17. Water Quality Constituent:	
18. At-Risk species benefited:	
19. Project objectives:	
20. Time frame:	

Section 2: Location Information

1. Township, Range, Section: and the 7.5 USGS Quad map name.	
2. Latitude, Longitude (in decimal degrees, Geographic, NAD83):	
3. Location description:	
4. County(ies):	
5. Directions:	
6. Ecological Management Region:	
7. Ecological Management Zone(s):	
8. Ecological Management Unit(s):	
9. Watershed Plan(s):	
10. Project area:	
11. Land use statement:	
12. Project area ownership:	% Private_____ % State_____ % Federal_____ *Enter ownership percentages by type of ownership.*
13. Project area with landowners support of proposal:	

Section 3: Landowners, Access and Permits

1. Landowners Granting Access for Project: (Please attach provisional access agreement[s])	
2. Owner Interest:	
3. Permits:	
4. Lead CEQA agency:	
5. Required mitigation:	Yes ☐ No ☐

Section 4: Project Objectives Outline

1. **List task information:**

2. **Additional objectives:**

3. **Source(s) of above information:**

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:
Primary Investigator:
Co-Primary Investigator:
Supporting Staff:
Subcontractor:

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

Last Name	First Name	Organization	Role

Section 6: Project Tasks and Results Outline

1. Detailed Project Description

2. Background and Conceptual Models

3. Approach and Scope of Work

4. Deliverables

5. Feasibility

6. Relevance to the CALFED ERP

7. Expected quantitative results (project summary):

8. Other products and results:

9. Qualifications

10. Literature Cited

Section 7: Project Budget

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

Budget			
Project Title			
			Totals
PERSONAL SERVICES			
Staff Level	**Number of Hours**	**Hourly Rate**	
Subtotal			
Staff Benefits @ %			
TOTAL PERSONAL SERVICES			
OPERATING EXPENSES			
Description			
Subcontractor Costs			
Materials			
Photographic Supplies			
Printing and Duplicating			
Office Supplies			
General Expense			
Travel and Per Diem			
Training			
Add/delete line items above for work to be performed by the contractor			
Total Operating Expenses			
EQUIPMENT			
SUBTOTAL			
OVERHEAD @ % (Less Equipment)			
GRAND TOTAL			

2. **Budget justification:**

3. **Administrative overhead:**