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JUL 22 1997

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento CA 95814

Research Proposal Entitled
"Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary"
RFP: 1997 Category III Ecosystem Restoration Projects and Programs
Principal Investigator - William A. Bennett

Dear Colleague:

It is our pleasure to present for your consideration the referenced proposal in response to **the CALFED Bay-Delta Program RFP.**

Please call on the principal investigator for scientific information. Administrative questions may be directed to me or my assistant, René Domino, at the above address and phone number. We request that correspondence pertaining to this proposal and a subsequent award be sent to the Office of Research and to the principal investigator.

Sincerely,


Sandra M. Dowdy
Contracts and Grants Analyst

Enclosure

cc: W. A. Bennett

DWR MAIL ROOM

97 JUL 24 PM 2:23

Proposal to: CALFED Bay Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, California 95814

Title of Project: Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary

Total Amount Requested: 437,326

Requested Start Date: 01/01/98

Proposed Duration of Project: 2 Years

Applicant Information:

The Regents of the University of California
Office of the Vice Chancellor for Research
410 Mrak Hall
University of California
Davis, California 95616.8671

Principal Investigator Information:

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Type of Organization and Status: Non-profit Public Institution of Higher Education

Tax Identification Number: 94.6036494.W

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Administrative/Contractual/Financial Contact:

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Participants/Collaborators in Implementation: Interagency Ecological Program

RFP Project Group: (3) Other Services

Approvals

William A. Bennett 7/11/97
Principal Investigator Date

James S. Ulegg 21 July '97
Department Chair Date

Sandra M. Dowdy
Official Signing for Organization Date
Sandra M. Dowdy
Contracts and Grants Analyst

**Role of Contaminants in the Decline of Delta Smelt
in the Sacramento-San Joaquin Estuary**

Principal Investigators:

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Collaborator:

Interagency Ecological Program

RFP Project Group:

Other Services

Period of Performance: 1/1/98-12/31/99

Amount Requested: \$437,326.00

EXECUTIVE SUMMARY

**Role of Contaminants in the Decline of Delta Smelt
in the Sacramento-San Joaquin Estuary****William A. Bennett Ph.D., Swee J. Teh Ph.D.¹, Susan L. Anderson Ph.D.**

Bodega Marine Laboratory, University of California, Davis

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The delta smelt (*Hypomesus transpacificus*) is currently listed as a threatened species under the Federal and State Endangered Species Acts. Despite intensive monitoring of their distribution and abundance, little is known about the factors regulating the population. Unlike many other resident populations, delta smelt abundance has remained low during recent years despite apparently favorable habitat conditions (high freshwater outflow). Although water diversions, exotic species, habitat loss and degradation are thought to affect delta smelt, there is increasing concern that contaminants from commercial, domestic, and agricultural sources may also be important. This suspicion was supported in 1996, when a strong larval delta smelt year-class apparently suffered high mortality during the juvenile stage, and bioassays indicated Sacramento River water was toxic to fish. Therefore, to facilitate restoration there is an urgent need to quantify the importance of contaminant exposure, in relation to other factors regulating the delta smelt population.

We propose to evaluate the relative importance of contaminant effects in comparison to other potential sources of mortality for the delta smelt population. To do this we will quantify the overall health, condition, and growth rate of delta smelt collected from various habitats encompassed by the Interagency Ecological Program (IEP) monitoring surveys over last 6 years (1992-1997). These extend from the lower Sacramento and San Joaquin Rivers, to San Pablo Bay and Napa River (i.e. the known range of the species). Our interdisciplinary investigation of these samples will employ evaluations of: (1) *histopathology biomarkers* of exposure and organ/tissue condition, and (2) *biomarkers of DNA damage*, with (3) *otolith growth rate analyses* of individual smelt. Integration of these state-of-the-art techniques for individual smelt will quantify contaminant effects on individuals that can be related to consequences for the delta smelt population.

Two years of support are requested. *In year one*, our initial objective will be to examine the year-class failure in 1996, using samples archived by DFG surveys (Sweetnam), and IEP Entrapment Zone Studies (Bennett). We will (1) *determine whether sublethal tissue or genetic alterations due to contaminant exposure affected growth rate*. Regulation of growth rate during early life is often a key factor affecting year-class success in aquatic populations, because it can lengthen the period of susceptibility to predation and/or eventually decrease fecundity. We will next (2) *coordinate field sampling at key locations/times* with DFG/USGS/USBR to obtain specimens for evaluations requiring special fixatives for specific biomarkers and link interpretations with water quality monitoring programs. (3) *Laboratory exposures with commonly detected chemicals, and growth experiments* will provide standards for genetic and histological biomarkers, and otolith growth rates. These investigations will

strengthen interpretations from biomarker evaluations of the archived samples. *In year two*, we will examine (1) *whether condition and growth rate varies among years (1992-1998)* that had different outflow, habitat (food) conditions, and loadings of contaminants, as well as (2) *continue with sampling, exposure, and growth studies*. The majority of basic research on the effectiveness of the various techniques and their application to delta smelt has been completed or is currently in progress.

Data for individuals will be translated to the population level by *integrating within* and among life stages, habitats, and years, and comparisons with information on other factors to develop survival estimates for use in population modeling. Therefore, this study will provide a *comprehensive investigation of the role of contaminants on the delta smelt population* by integration of results from several levels of biological organization, space and time. Even with the potential absence of contaminant effects, such investigations will provide the first quantification of population stressors which will considerably increase understanding of the factors regulating the delta smelt population.

Dr. William A. Bennett (PI) has ten years of research experience on the ecology of Bay/Delta fish populations, during which he has been a frequent collaborator with IEP member agencies. *Dr. Swee J. Teh (Co-PI)* manages one of the nation's most highly reputable environmental toxicology and histopathology laboratories. Our previous collaboration identified the importance of rice pesticides in regulating the survival of larval striped bass. *Dr. Susan L. Anderson (Co-PI)* is one of the foremost researchers on the effects and consequences of environmental toxicants on the genetic condition of aquatic organisms, and has also worked on Bay/Delta contaminant issues for over a decade. Integration of our collective expertise constitutes an innovative interdisciplinary strategy for expediently addressing the delta smelt population problem. We do not foresee any third party impacts.

This project is supported by IEP. It was developed and will continue in close coordination with ongoing IEP programs. Extensive discussions and reviews of this project were provided by the IEP Contaminant Effects and Resident Fishes Work Teams, as well as Scientific Advisory Group. These teams will also provide peer review. Collaborations will continue with DFG delta smelt monitoring and DWR otolith work (Sweetnam, Grimaldo), IEP funded delta smelt culturing projects (Dorshov, UCD), and various water quality monitoring programs (e.g. SWRCB, USGS, SFED). In addition, this project will be integrated with the Bay/Delta entrapment zone (EZ) studies, conducted by W. Kimmerer (SFSU), W. Bennett (BML) and J. Burau (USGS) with IEP support (about \$120K a year for past 4 years). The EZ study has been quantifying the influence of hydrodynamics on biological constituents (phytoplankton, zooplankton, fish), which reside in the delta smelt nursery habitat.

Restoration of the delta smelt population is uncertain without quantifying the effects of selected stressors on the population. Monitoring of abundance alone is insufficient to fulfill current restoration goals. Therefore, our proposed investigation to determine whether toxicants affect condition and growth efficiency of delta smelt will make unique contributions towards the CALFED goal to restore the population. Integration of this study with existing monitoring programs and research efforts will dramatically improve the cost-effectiveness and impact of the project.

Budget Costs: Year One: \$224,704; Year Two: \$212,622

PROJECT DESCRIPTION

Project Description & Approach

Currently, little is known about the factors regulating the threatened delta smelt (*Hypomesus transpacificus*) population of the Sacramento-San Joaquin estuary. Exposure to toxic substances is now considered a potential factor in the decline of the population, yet no efforts have been made to distinguish the effects of contaminants from other potential sources of mortality. Our goal is to quantify the overall health, condition, and growth rate of delta smelt through several life-stages (collected from various habitats and years in the estuary) to identify whether contaminants influence delta smelt year-class success. This interdisciplinary investigation will adopt an innovative strategy, integrating evaluations of histopathology biomarkers of exposure and organ/tissue condition, and biomarkers of DNA damage, with growth rate analyses of individual smelt.

Archived samples, field collections, and limited laboratory investigations will be used to evaluate the health and growth rate of delta smelt under selected conditions. Specifically, we will use samples archived by the Interagency Ecological Program (IEP) monitoring surveys (1992-1997) and our own collections (1994-1996) to: (1) determine whether variation in biomarker expression occurs among sampling locations and times, (2) determine whether body condition and DNA damage are related to habitat quality and/or contaminant exposure, (3) evaluate stage-specific and interannual variation in these responses, and (4) identify the potential relationship between biomarkers of exposure and condition with growth rates of specimens among sampling locations and times.

Field collections at key locations and times each year will be used to further investigate the responses described above as well as to: (1) evaluate the potential relationship between biomarker responses and water quality evaluations from ongoing monitoring programs, and (2) examine biomarker responses which require frozen tissue, blood samples, and other technique-specific fixatives.

Laboratory investigations will be conducted to: (1) determine whether impaired body condition and DNA damage are induced in embryos and larvae following exposure to ambient waters and single toxicants, and to (2) to validate counts of otolith-rings from field-caught specimens with those from known-age larvae.

Such information will be quantified and integrated for specimens collected at different life stages (embryos, larvae, juveniles, adults). This will distinguish the relative and/or interactive importance of toxic exposure from other potential sources of mortality (e.g. food limitation, and parasites/disease; DWR/USBR 1993). For example, a larval smelt with an impaired liver due to exposure to pesticides may also be growing slowly compared with normal individuals, reducing its probability of surviving. Such information will be compiled among individuals from different habitats (e.g. salinity, slough, channel), locations (e.g. Delta, Napa River), and year-types (wet vs. dry). Comparisons among these spacio-temporal scales and hydrodynamic conditions will facilitate evaluation of the relevance of condition and growth rate for regulating recruitment and/or reproductive success. Moreover, this information will be compared with that being compiled for other factors (e.g., predation, Bennett 1995; entrainment, DWR/USBR 1993) to more accurately describe potential effects of contaminant

exposure on the delta smelt population. The results of our evaluations will be suitable for translation into parameters for future use in individual based (stage-structured) population models (e.g. Crowder et al. 1992).

Geographic Boundaries and Priority Habitats

The boundaries of this project include the known range of delta smelt historically sampled by IEP monitoring programs, which encompass the lower Sacramento and San Joaquin Rivers, Delta, Suisun Bay, Suisun Marsh, San Pablo Bay, and Napa River. Within this geographic range CALFED priority habitats from which specimens will be evaluated include, (1) tidal perennial aquatic habitat, (2) midchannel islands and shoals habitat, and (3) North Delta agricultural wetlands.

Expected Benefits

Our project will quantify the relative importance of selected stressors on a priority species, the delta smelt. In particular, we will distinguish the significance of contaminant exposure (*water quality stressor*) in comparison to other stressors (e.g. food limitation, parasitism/disease) that can result from changes in the quantity and quality of nursery habitat due to interannual differences in hydrography (*water management stressor*). This will constitute the first direct evaluation of stressor effects on the delta smelt population. Results will be useful for modeling delta smelt population dynamics and the degree to which further controls on contaminant discharges are required. Therefore, our project will benefit the delta smelt population by contributing crucial information that can facilitate restoration of this depressed population. In addition, this work will also contribute to a better understanding of Bay-Delta water quality. The integration of our collective expertise constitutes an innovative approach that will expediently address issues in support of the CALFED mission to sustain the biological resources of the Bay-Delta region.

Background and Biological/Technical Justification

The delta smelt is endemic to the Sacramento-San Joaquin estuary, and currently listed as a threatened species under the Federal and State Endangered Species Acts. It has relatively low fecundity and it is an annual species, such that the majority of adults die soon after spawning each year. While this life history strategy presumably contributes to the large interannual fluctuations in population abundance observed in monitoring data since 1959, the abundance of delta smelt has remained extremely low since 1982, except during infrequent "wet" years (Mole et al. 1992). Several factors have been suggested to explain the decline in delta smelt abundance, including changes in the quantity and quality of suitable nursery habitat (Herbold 1994), entrainment in water diversions (Moyle et al. 1992), and intraguild predation by exotic inland silversides (Bennett 1995, Bennett and Mole 1996). The effects of these factors are thought to be exacerbated in years of low freshwater outflow.

Toxic substances, however, can also affect the delta smelt population, and are suspected as important in recent years for several reasons (Bennett 1996, Fox and Miller 1996). *First*, delta smelt are an annual species such that contaminant effects could directly affect population dynamics relative to other species with many overlapping generations. *Second*, delta smelt are

known to spawn and occur during their more vulnerable early life stages where elevated concentrations of contaminants have been detected over time. *Third*, delta smelt abundance has been declining in even-numbered years (e.g. 1996) relative to odd-numbered years (e.g. 1993). Contaminant effects have been suggested as a potential cause of this phenomenon (B. Herbold USEPA, personal communication). This pattern was supported in 1996 when monitoring indicated successful larval recruitment, yet high mortality during the juvenile stage produced one of the lowest adult abundance indices on record, even though it was a wet year with optimal rearing habitat. *Fourth*, bioassay results from exposures with Sacramento River water in 1996 produced mortality to larval fathead minnows (Fox and Miller 1996) and mysid shrimps (Thompson 1996). These results are striking because the toxicity was found in the delta smelt spawning habitat during the spawning and rearing season, and in comparison to fathead minnows, most fishes (and presumably delta smelt) appear to be more sensitive to the types of chemicals detected. However, while such monitoring provides invaluable information on interannual year-class success and water quality, it alone is insufficient for substantiating potential relationships between contaminants, or other factors, and delta smelt abundance (Bennett 1996).

To assess the role of contaminants, the biomarker approach coupled with exposure assessment is the best strategy currently available. Chemical analysis is limited by the fact that bioavailability of toxicants and the effects of complex mixtures are difficult to predict from chemistry alone. Toxicity tests are limited by the fact that only lethal and simple sublethal effects are predicted and only select species are studied. Biomarkers are defined as biochemical, physiological and histological markers of anthropogenic stress (Hinton et al 1992; Huggett et al. 1992). These complement toxicity testing and chemical analysis because latent and long-term sublethal effects can be assessed and because effects on target organisms are assessed directly.

The potential value of the biomarker approach in evaluations of the delta smelt population decline are particularly compelling. *First*, this species is extremely fragile and would not be amenable to long term or routine toxicity testing. *Second*, archived samples exist that may contain information relevant to the population declines, and third, latent and sublethal effects can be assessed. Currently, there is no other approach available to accurately assess the effects of such stressors (Adams et al 1989; Hinton et al 1992; Huggett et al. 1992; The et al 1997). Our previous investigations using the histopathology biomarkers approach with larval striped bass (*Morone saxatilis*, which occur at similar times and locations with delta smelt, and also exhibit poor year-class success in low outflow years) indicated many larvae had been exposed to toxic compounds, potentially leading to slower growth and mortality (Bennett et al. 1995). These results suggest that the use of histopathology biomarkers will identify whether toxic compounds are contributing to mortality of delta smelt.

Genetic biomarkers may also reveal sublethal effects of contaminants on delta smelt. Genetic biomarkers are the biomarker responses which have been most firmly linked to detrimental effects on organisms and populations. Anderson and Wild (1994) and Anderson (1990) have conducted and reviewed investigations linking genotoxic responses to reproductive impairment in fish, polychaete worms, sea urchins, and other organisms. The mechanistic basis of these linkages are that chromosomal damage reduces fertility and induces embryo

abnormality. It is also well known that DNA damage can result in cancer in aquatic organisms. Genotoxic effects of pesticides are widely known. These include damages resulting in mutations, chromosomal breakage, cell death and cancer. DNA damages have been characterized in a range of model systems using endpoints such as sister chromatid exchange, chromosomal aberrations and gene mutations (Sternberg, 1979; Tezuka et al., 1980, Chen et al, 1981).

A key component of our project will be to integrate the biomarker approach with assessment of the growth rates of individual specimens. Larval growth rate has been repeatedly shown to be important for eventual survival (Houde 1987; Bennett and Rogers-Bennett 1997), and our previous work (Bennett et al. 1995) has identified sublethal alterations in larval striped bass livers that may depress larval growth rates. Contaminant effects on growth can affect both the abundance and fecundity of the spawning adults. However, the mechanisms underlying growth rate regulation have rarely been quantified for teleosts. Our proposal to directly compare body condition and growth rate is a novel approach that can provide strong evidence concerning the consequences of potential tissue and/or genetic alterations for young smelt and population recruitment success, and for comparisons with other sources of mortality. Given the potential for exposure to various contaminants, and the paucity of information on the relative importance of stressors, our proposed work will facilitate effective management of this threatened species.

Proposed Scope of Work

We propose a 2-year investigation during which 3 specific approaches (histopathology biomarkers, genetic biomarkers, growth rate analyses) will be pursued and integrated for individual delta smelt larvae, juveniles and adults.

Archived specimens of larval, juvenile, and adult delta smelt will be used for much of the study. Sources of specimens include: DFG (Sweetnam) from 1992-1997, and Bennett's 1994-1996 collections from the IEP Entrapment Zone studies. Use of the historical collection facilitates an experimental design that compares the condition of young smelt between two "wet" years (1993, 1995) and two "dry" years (1992, 1994), and whether smelt condition differs among habitat areas (Suisun Bay, Delta, Napa River). We will proceed first with the extensive collection of samples available from 1996, a year in which optimal rearing conditions prevailed yet eventual year-class success was poor.

After surgical removal of otoliths, a suite of selected histopathologic and genetic biomarkers will be used to assess the potential responses of delta smelt to contaminant stressors (Sternberg, 1979; Tezuka et al., 1980, Chen et al, 1981, The et al 1997). *Histopathological evaluations* will be made using whole larval specimens, because they can be embedded and sectioned (Bennett et al. 1995), and for key organs/tissues of older smelt, focusing initially on the liver and reproductive system where many contaminant effects manifest. We will employ both quantitative and qualitative histopathological techniques (McCarthy and Shugart, 1990; Bennett et al. 1995; The et al 1997) to distinguish contaminant effects from other factors (e.g. poor nutrition). All relevant tissues will be scored and subsequently analyzed using methods described in Bennett et al. (1995) and The et al. (1997). Subsets of newly collected specimens will be scheduled for electron microscopic studies to

determine the nature and extent of cellular and organelle alterations. Quantitative histopathology provides the percentage of functional/healthy tissue (e.g. the percentage of liver actually occupied by the functional or viable hepatocytes) and percentage of tissue occupied by potential contaminant-related lesions, and therefore the degree of damage to the tissue/organ examined. Qualitative histopathological techniques detect whether apparent histologic lesions are related to environmental contaminant exposure and their potential physiologic effect, and thus, in field-caught specimens they are the only clear link between exposure to contaminants and tissue/organ alterations currently available. Histological processing of 5 larvae from 1994, and 2 juveniles from 1995 indicates that the majority of archived samples are suitable for such evaluations.

Genetic biomarkers of DNA damage are similar for a variety of organisms exposed to various contaminants (Sternberg, 1979; Tezuka et al., 1980, Chen et al, 1981; Anderson and Wild 1994). Anaphase aberration analyses will be conducted on larvae of delta smelt using the general methods of Hose (1985). Recent investigations in our laboratory have discerned that this technique is applicable to top smelt embryos and we found decreased mitotic index at the lowest dose tested (10 ppb) with the dithiocarbamate pesticide Ziram. DNA strand break analyses on adult delta smelt will be attempted using the Comet assay (Fairbairn et al, 1995). In this method, cells with damaged DNA appear like a comet with a long tail. Initially, assays will be conducted on fresh blood cells using methods of Pandrangi et al. (1995). Blood samples will be maintained on ice and returned to the laboratory for electrophoretic analyses. This work will be performed on specimens collected during 1999 (Year 2) to determine whether this assay is more cost effective than the anaphase aberration analyses (Year 1).

We will estimate growth rate by incremental change in the otoliths from larvae and juveniles using conventional methodology (Secor et al. 1991). Otoliths will be removed by microsurgical technique, coded, secured to a glass slide, polished, and analyzed using light microscopy aided by computerized image analysis. Our preliminary evaluations (Grimaldo, DWR; Sweetnam DFG) and prior experiences (Bennett and Rogers-Bennett 1997) indicate smelt are amenable to this analysis. Currently, we have evaluated 12 field-caught and 3 laboratory-grown (known-age) specimens which exhibit clear daily growth rings that are verifiable in shape and ring-count with known-age controls. Numerous larval specimens were archived this spring (1997) from UCD culturing projects to be used for further validation of growth rings.

Laboratory exposures of embryos and yolk sac larvae will also be used to further validate histopathological and genetic biomarker findings. These will be conducted first using individual toxicants and then ambient waters. These studies will be conducted to evaluate the range of biomarker responses (histological, and genetic). Initial laboratory exposures will provide us with detailed information regarding contaminant responses to specific chemicals and will help refine assay protocols, assess biomarker sensitivity and specificity, and define target organs and biological effect in delta smelt. Data will include responses at the genetic, molecular, cellular, tissue, and organismal level. We realize that laboratory models are simplistic and only remotely resemble "real world" situations, however, to begin to unravel complex interactions involved with exposure to contaminant mixtures, we must start with simple schemes. In addition, by assessing multiple biomarkers (in animals exposed to single contaminants) we will begin to

identify patterns of biomarker expression. These patterns will allow for the development of biomarker profiles which can be matched to specific contaminants, contaminant concentrations, exposure times and conditions. Preliminary laboratory exposures of embryos and larvae to diazinon have been performed and samples archived for biomarker analyses. Preliminary histopathological evaluations of embryos indicate that hatching time was delayed, and lesions were observed, including necrosis in the brain, eye, cardiac and skeletal muscle.

We will also conduct field-collections at key locations/times throughout the study in collaboration with DFG (Sweetnam). New sampling will enable use of a variety of fixatives and preservation methods that will broaden the range of histopathological and genetic techniques applied to the specimens. Bennett has conducted limited field-sampling in collaboration with USGS water quality studies (Kuivila) in Suisun Bay during spring 1997.

Phasing of work and reports on findings will proceed as follows:

- Year One-**
- 1.- Coordinate and catalog archived specimens.
 - 2.- Begin histopathology, genetic, otolith analyses on 1996 samples.
 - 3.- Sampling for 1998 specimens, and coordinate with water quality sampling
 - 4.- Initiate laboratory experiments
 - 5.- Submit progress report on 1996 samples to CALFED and IEP Newsletter.
- Year Two-**
- 1.- Evaluate archived samples using histopathology, genetics, otolith analyses.
 - 2.- Continue laboratory experiments.
 - 3.- Sampling for 1999, and coordination with water quality sampling.
 - 5.- Evaluate 1998, 1999 samples.
 - 6.- Integrate all results into final report to CALFED.

Data Evaluation

Data analyses will evaluate and integrate, genetic, histopathological and growth rate indices for specimens among habitats and years. This will include: *(1) decide on and evaluate summary statistics for exposure biomarker data* from the archived samples, comparing among locations/habitats and years; *(2) compare exposure biomarker data from the archived samples with those obtained from laboratory exposures, and existing water quality information;* *(3) develop appropriate statistical analyses based on data structure* using various clustering, ordination, and other variance analyses, to integrate indices (genetic, histopathologic, growth) among life-stages, and space/time locations; *(4) relate evaluations of contaminant effects with other factors*, translating this information into stage-specific duration and mortality estimates for projection of delta smelt population dynamics.

Peer review of the data evaluation process will constitute periodic presentations of progress at regular meetings of the IEP Estuarine Ecology (EET), Resident Fishes (RFT) and Contaminant Effects (CET) Project Work Teams, as well as presentations at local and national meetings of various professional societies.

Implementability

We do not foresee any substantive barriers to implementing this project. All specimen collections will occur with IEP under their permit guidelines.

COSTS AND SCHEDULE TO IMPLEMENT PROJECT

Budget Costs

Two years of support are requested. Budget costs are broken down by Principal Investigator and year (Table 1), because the division of tasks is fairly distinct among investigators. The phasing/scheduling of specific tasks is shown in Table 2.

Dr. William A. Bennett will serve as Principal Investigator. Direct salary costs for Bennett represent a 50% time commitment to the project. Specific tasks (Table 2) include overall organization of the project and coordination with IEP agencies, cataloging of archived specimens, overall study design, otolith analyses, field sampling, laboratory exposure/growth work, data analyses, presentations, and report writing. A Post Graduate Researcher (James Hobbs) will commit 50% time to assisting Dr. Bennett with these tasks, several of which will need to occur simultaneously. Miscellaneous Costs include supply funds for otolith analyses, field sampling, laboratory growth, computer software, and general laboratory/office operations related to the project. In addition, these costs include travel funds for research, project meetings, and presentations at the annual IEP conference, and a national conference of a professional society.

Dr. Swee Teh (Co-Principal Investigator) will be responsible for all histopathological analyses, laboratory exposures. Direct Salary Costs reflect a 50% time commitment of Dr. Teh on these and his primary task of evaluating and interpreting the large number of histopathological preparations (several hundred slides). Dr. Teh also requires two assistants, a 50% time commitment of a Laboratory Assistant II (Ching Teh) for histological processing, as well as a 20% time commitment of a Staff Research associate III, (Tammy Harrington) who specializes in electron microscopy. Miscellaneous Costs consist of supply funds for histology, immunochemistry, electron microscope rental, laboratory exposures, and general laboratory/office operation. In addition, these costs include expenses for travel to project meetings, and presentations at one national conference of a professional society.

Dr. Susan Anderson (Co-Principal Investigator) will be responsible for all genetic analyses, assist with laboratory exposures, field collections, and interpretation of these results. Direct Salary Costs for Dr. Anderson include her 25% time commitment to the project, as well as a 50% time commitment of a Post Graduate Researcher I (Jana Machula), for assistance in the genetic processing and analysis. Miscellaneous Costs include supply funds for the genetic analyses, laboratory/office operation. In addition, funds are included for travel to project meetings, field sampling, and one national conference of a professional society.

Our project is supported by IEP, and will collaborate with IEP programs. In particular, sample analyses will be integrated with the IEP funded Bay/Delta entrapment zone (EZ) studies on the influence of hydrodynamics on the feeding success of fishes in the delta smelt rearing habitat. IEP will be allowing us to accompany regular sampling surveys for special collections (Sweetnam, DFG) and will contribute a 20% time commitment of L. Grimaldo (DWR) for integration his preliminary otolith evaluations with our larger effort.

We request CALFED funds because this project is intended to guide the efforts of IEP and CALFED to restore the delta smelt population. To effectively accomplish this goal the project requires a large scope which is beyond IEP expertise and funding for directed studies.

Funding of individual components (investigators), or for only a single year of work will dramatically reduce the effectiveness and efficiency of supplying information on the importance of stressors to the restoration process. Potentially, CALFED funding could be incremental after year one, although this would interrupt the efficiency of the work plan.

Schedule Milestones

Scheduling milestones are indicated in Table 2. First, we will catalog existing specimens collected during 1996 by geographical location and habitat. Habitat will be defined primarily by salinity and either slough or channel. Additional cataloging of 1992-1997 specimens assess sample sizes and spatial coverage to refine the study design. Our goal will be to identify the most appropriate sample years to include in a "wet" year vs. "dry" year comparison of specimen condition. Most of this important task will be completed by March 1998, at which time we will convene a project workshop including all investigators and collaborators to present and discuss a detailed work plan. This will include presentations of preliminary results, coordinating availability of cultured specimens for laboratory investigations, field sampling for spring 1998, and potential synoptic sampling with water quality monitoring programs.

Otolith, histological, and genetic analyses will proceed throughout the project. Otolith evaluations have already begun (in collaboration with DFG/DWR), and will continue, first on laboratory specimens archived in 1997, and the 1996 field specimens, and expanded in 1998-1999 to include selected specimens from other years. *In year one*, histopathological analyses will proceed on specimens from preliminary 1997 and 1998 laboratory exposures as well as 1996 field specimens. In year one, genetic analyses using the anaphase aberration technique will begin on embryo and larval specimens from 1997 laboratory exposures, and larval specimens from 1996 collections. This work will also include Comet analyses of blood samples from field specimens collected during spring 1998. By August 1998, preliminary results will be presented at regular meetings of IEP Project Work Teams (e.g. Contaminant Effects, Resident Fishes, Estuarine Ecology). By January 1999 we will submit a progress report to CALFED on the evaluations of 1996 specimens, laboratory exposures, and other project activities. In addition, results will be presented at the annual IEP conference in March 1999.

In year two, first, specimen cataloging of years 1992-1998, and study design will be refined, and another project workshop convened by March to discuss work plans and coordinate with culturing efforts, field sampling, and synoptic sampling with water quality monitoring programs. Histological and otolith evaluations will concentrate on selected specimens from 1992-1998 and laboratory exposures. Genetic analyses will proceed on these samples where appropriate, and with 1999 field specimens. By August 1999, work progress and results will be presented at regular meetings of IEP Project Work Teams. By December 1999, a final report will be presented to CALFED synthesizing all project findings including the implications of results for modeling and management of the delta smelt population.

Third Party Impacts

We do not foresee any third party impacts to result from this study.

Table 1. Budget Costs for Principal Investigators and Phase (year) for tasks outlined in text.

Project Phase and Task	Direct Labor Hours	Direct Salary and Benefits	Overhead to UCD (25.5%) Salary/Misc.	Miscellaneous Supplies/Travel	Total Costs
Year One: Tasks for Bennett (PI) Hobbs (Assistant) Bodega Marine Lab	2,080 hrs.	\$39,462	\$13,378	\$13,000	\$65,840
Year One: Tasks for Anderson (PI) Machula (Assistant) Bodega Marine Lab	1,560 hrs.	\$37,420	\$12,602	\$12,000	\$62,022
Year One: Tasks for Teh (PI) Teh, Harrington (Assistants) Dept. Anat., Phys., & Cell Biology	2,496 hrs.	\$60,665	\$19,677	\$16,500	\$96,842
Year Two: Tasks for Bennett (PI) Hobbs (Assistant) Bodega Marine Lab	2,080 hrs.	\$40,311	\$12,319	\$8,000	\$60,630
Year Two: Tasks for Anderson (PI) Machula (Assistant) Bodega Marine Lab	1,560 hrs.	\$38,141	\$12,275	\$10,000	\$60,416
Year Two: Tasks for Teh (PI) Teh, Harrington (Assistants) Dept. Anat., Phys., & Cell Biology	2,496 hrs	\$62,968	\$18,607	\$10,000	\$91,575

Table 1a: Budget Detail - Year I

A. Personnel

EMPLOYEE/ TITLE	SALARY	BENEFITS	TOTALS
William Bennett, PGR IV	18,356	4,589	22,945
James Hobbs, PGR I	14,057	2,460	16,517
Susan Anderson, Res. Biologist II	17,186	3,008	20,194
Jana Machula, PGR II	14,660	2,566	17,226
Swee Teh, SRA IV	26,282	6,308	32,590
Ching The, LA III	13,279	3,187	16,466
Tammy Harrington, SRA III	9,362	2,247	11,609
<i>Total Salaries and Benefits</i>	113,182	24,365	137,547

B. Supplies and Expenses

Bennett	10,000
Anderson	10,000
Swee Teh	15,000

Total Supplies and Expenses **35,000**

C. Travel

Bennett	3,000
Anderson	2,000
Swee Teh	1,500

Total Travel **6,500**

D. Total Direct Costs **179,047**

E. Indirect Costs @ 25.5% of MTDC* **45,657**

F. Total Costs **224,704**

Table 1b: Budget Detail - Year II

A. Personnel

EMPLOYEE/ TITLE	SALARY	BENEFITS	TOTALS
William Bennett, PGR IV	18,631	4,844	23,475
James Hobbs, PGR I	14,268	2,568	16,836
Susan Anderson, Res. Biologist II	17,443	3,140	20,583
Jana Machula, PGR II	14,880	2,678	17,558
Swee Teh, SRA IV	27,060	6,765	33,825
Ching The, LA III	13,675	3,419	17,094
Tammy Harrington, SRA III	9,639	2,410	12,049
<i>Total Salaries and Benefits</i>	115,596	25,824	141,420

B. Supplies and Expenses

Bennett		5,000	
Anderson		8,000	
Swee Teh		8,500	
<i>Total Supplies and Expenses</i>			21,500

C. Travel

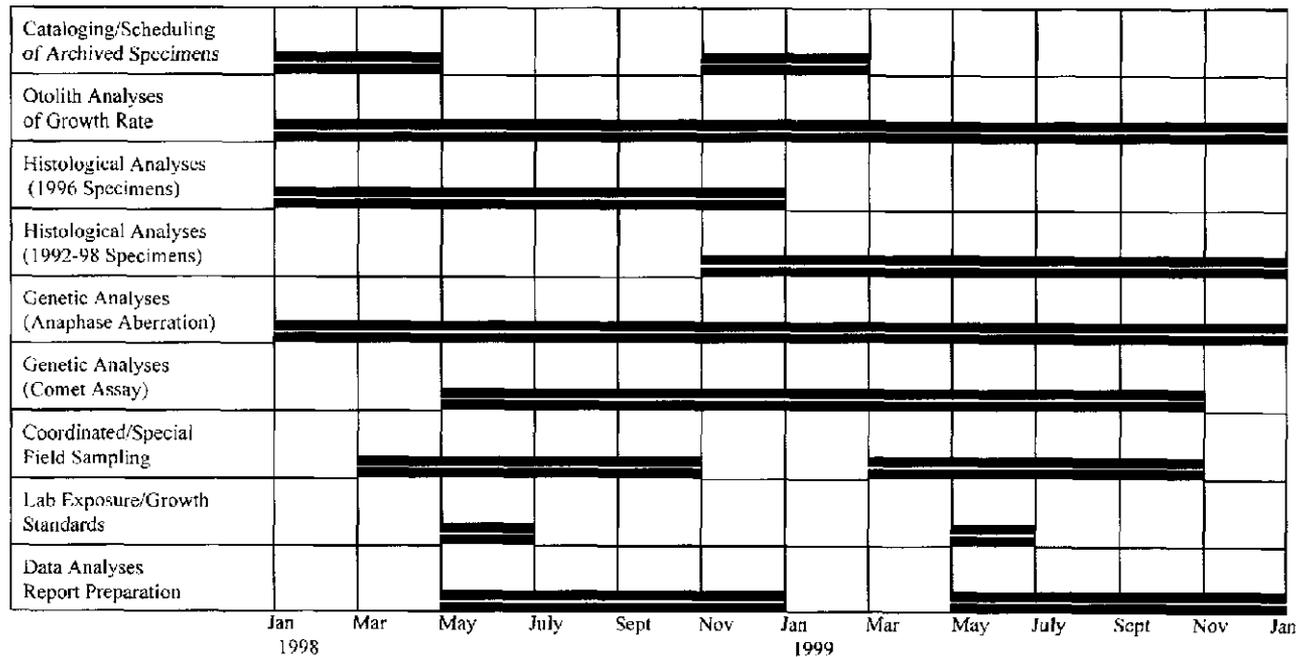
Bennett		3,000	
Anderson		2,000	
Swee Teh		1,500	
Total Travel			6,500

D. Total Direct Costs **169,420**

E. Indirect Costs @ 25.5% of MTDC* **43,202**

F. Total Costs **212,622**

Table 2. Schedule/phasing of work tasks.



1-000400

1-000400

APPLICANT QUALIFICATIONS

Dr. William A. Bennett (Ph.D. in ecology, UCD, 1993) will serve as Principal Investigator and main contact with CALFED for this project. Dr. Bennett will be responsible for coordinating project team meetings and field sampling with cooperating agencies, cataloging of archived samples and otolith evaluations with DFG/DWR, overall study design, data analysis, and ecological interpretation of results. Dr. Bennett has been a self-supported Postdoctoral Researcher at the Bodega Marine Laboratory (BML) since 1993, currently being promoted to Assistant Research Scientist, a position to be based at UCD and BML. Bennett has worked extensively on the ecology of Bay-Delta fishes, and since 1987 has been the author/Principal Investigator on various grants/contracts from (DWR/IEP, totaling over \$700K; USBR, totaling 80K). This work includes: identifying factors affecting the survival of larval striped bass, particularly rice-field pesticides, in collaboration with Dr. David Hinton's fish pathology laboratory at UCD (Bennett et al. 1995); identifying the effects of the exotic inland silverside on delta smelt (Bennett 1995); the interaction of larval fish behavior and hydrodynamics of the Bay-Delta entrapment zone (Bennett 1997); and, the effect of ocean conditions on the decline of the striped bass population (Bennett and Howard 1997). Dr. Bennett has also worked as an Environmental Specialist for the USEPA, completing a review of the potential effects of pesticides on Bay/Delta fish populations (Bennett 1996). He has been an active member of IEP's Estuarine Ecology Project Work Team (EET) since 1991, and a member of the Contaminant Effects Project Work Team. Dr. Bennett has also been an invited speaker at several workshops on Bay-Delta resources, universities, and national conferences.

Dr. Swee J. Teh (Ph. D. in comparative pathology, UCD, 1996) is a fish pathologist, managing the Aquatic Toxicology Laboratory at UCD for the past decade, a program headed by Dr. David Hinton. He will be primarily responsible for the histopathological assessment of delta smelt and the interpretation and integration of this data with Drs. Bennett and Anderson. He has been a Principal Investigator and/or managed grants from various Federal agencies, including USEPA (totaling over \$1 million), NCI (totaling over \$2.4 million), and USDE (\$275K). Dr. Teh has extensive experience with enzyme- and immunohistochemistry, light and electron microscopy, fish pathology, carcinogenesis, and morphometry. He is the primary or co-author on over a dozen referred publications related to fish histopathology and immunohistochemistry, including Teh and Hinton (1993), Teh et al. (1997), and Teh and Hinton, (*in press*). His spouse, Foo-Ching Teh is a histotechnician skilled at tissue processing and microtomy of glycomethacrylate (GMA)-embedded tissues. She also has significant experience with enzyme- and immunohistochemistry, and will be responsible for processing, sectioning, and staining of all fixed tissues for histopathologic assessment.

Dr. Susan Anderson (Ph.D., UCD, 1983) has over a decade of research experience in genetic ecotoxicology. She will be primarily responsible for the genetic evaluations of delta smelt and the interpretation and integration of results with Drs. Teh and Bennett. International recognition includes receipt of a Pew Scholarship in Conservation and the Environment (1992, among first 30 worldwide) for research examining effects of contaminants on reproduction and genetic diversity of aquatic organisms. Selected publications include: Anderson and Harrison (1990), Anderson et al. (1993), Anderson and Wild (1994), and Sadinski et al. (1995). In addition, she has taught courses and presented numerous invited lectures in Europe and the

United States. She has served as Principal Investigator on 8 grants related to genetic ecotoxicology which have totaled over \$2 million. In 1993, she organized the Napa Conference on Genetic and Molecular Ecotoxicology which was sponsored by NIH. The proceedings of this conference have been widely cited and are recognized as having established the contemporary definitions and practice within the field. Dr. Anderson has recently resigned as Group Leader for Ecological Research at the Lawrence Berkeley National Laboratory (LBNL) to accept a position at UC Davis, Bodega Marine Laboratory. She also has extensive experience in resource management issues within the San Francisco Bay Delta Ecosystem. In 1986-90 she was on the staff of the San Francisco Bay Regional Water Quality Control Board where she coauthored the 1987 revisions to the Water Quality Control Plan for San Francisco Bay ("Basin Plan") and established the first comprehensive toxicity biomonitoring program in the region. At LBNL, she served as PI on several Bay-related projects that resulted in: successful redesign of both urban stormwater and wastewater reclamation marshes, reevaluation of sediment quality criteria and toxicity testing procedures, and reconsideration of sediment quality remediation goals at the Mare Island and Alameda Naval facilities.

Our project is supported and will proceed in close cooperation with ongoing IEP programs. Extensive discussions and reviews of this proposal have been obtained from IEP's Contaminant Effects and Resident Fishes Project Work Teams, as well as IEP's Scientific Advisory Group, all of which support this proposal. We are working in cooperation with DFG (Sweetnam), who are providing the majority of delta smelt specimens archived from IEP monitoring surveys, and sampling for collections of fresh specimens. In addition, DWR will providing a partial time commitment of L. Grimaldo who has begun otolith evaluations, which we will expand with his collaboration. IEP funded culturing projects at UCD (Dorshov, Mager, Lindberg) have been providing embryo and larval specimens. Moreover, we will be coordinating efforts with various water quality monitoring programs in the Bay-Delta, including SWRCB (Foe), USGS (Kuivita), SFEI (Thompson).

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ATTACHMENT A

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ATTACHMENT B.

Letter of Support

**CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD —
CENTRAL VALLEY REGION**

3443 ROUTIER ROAD, SUITE A
SACRAMENTO, CA 95827-3098
PHONE: (916) 255-3000
FAX: (916) 255-3015



CALFED Bay-Delta Program
1416 Ninth Street, Suite 1155
Sacramento, California, 95814

**Letter of support for CALFED Category III proposal entitled “Role of
Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin
Estuary”**

The Interagency Ecological Program’s (IEP) Contaminant Effects Project Work Team (PWT) strongly recommends that the CALFED Category III program fund the proposal entitled “Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary” by William Bennett, Swee The, and Susan Anderson.

The Contaminant Effects PWT was formed a year and a half ago at the request of the IEP Directors with the mission to “acquire and disseminate information on the effects of contaminants on aquatic resources in the Central Valley and Estuary and to provide recommendations to Decision Makers aimed at minimizing contaminant related effects on populations of aquatic organisms”. The group is composed of about 30 aquatic toxicologists, chemists, population biologists and hydrologists from academia, state and federal agencies, and the private sector.

The PWT began by evaluating all instances where pollutants were suggested as possibly exerting population level impacts and concluded that there was insufficient evidence to ascertain whether impacts were occurring although a disturbingly large number of bioassays with surface water regularly tested toxic and concentrations of some chemicals appeared elevated in both river water and tissue samples. Therefore, the PWT decided to develop proposals in key areas where the problems appeared scientifically tractable and the results of large ecological significance. Proposals were written by small teams of researchers and were extensively reviewed by the entire group. In addition, they were submitted to the IEP’s Science Advisory Group for their comments and revised appropriately.

The result of this process was the Smelt proposal by Bennett *et al.* which the PWT believes is scientifically sound and critical for understanding and controlling the impact, if any, of contaminants on aquatic resources in the Estuary. Such knowledge is essential for fulfilling CALFED’s mandate to fully restore the ecological function of the Estuary. Therefore, the PWT strongly urge CAT III to fund the work.

If you have any questions please feel free to call me at (916)-255-3113.

Christopher Foe

Christopher Foe, Chair
Contaminants Effect PWT

NONDISCRIMINATION COMPLIANCE STATEMENT

COMPANY NAME	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
--------------	--

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

OFFICIAL'S NAME	Sandra M. Dowdy Contracts and Grants Analyst	
DATE EXECUTED	JUL 22 1997	EXECUTED IN THE COUNTY OF YOLO
PROSPECTIVE CONTRACTOR'S SIGNATURE	<i>Sandra M. Dowdy</i>	
PROSPECTIVE CONTRACTOR'S TITLE		
PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	