MONITORING RESPONSES OF THE DELTA SMELT POPULATION TO MULTIPLE RESTORATION ACTIONS IN THE SAN FRANCISCO ESTUARY

A. **Project Description: Project Goals and Scope of Work.**

1. Problem, Goals and Objectives:

A primary goal of the CALFED Ecosystem Restoration Program (ERP) is to recover at-risk native species, particularly the delta smelt (*Hypomesus transpacificus)*, currently listed as threatened under the Federal and State Endangered Species Acts (CALFED 2000). A variety of ecosystem restoration actions have been implemented to "improve and increase aquatic habitat and ecological functions" (CALFED 2000) in the hope of restoring the delta smelt population. However, a coherent plan to investigate the relative importance of various mechanisms influencing the population or the effectiveness of the restoration initiatives does not exist.

Local monitoring for fish occurs at a few of the restoration sites, but there is no way to track the extent to which delta smelt use restored sites and whether restoration benefits those individuals or the population. Similarly, extensive monitoring of most life stages of delta smelt by the Interagency Ecological Program (IEP), which produces the official measures of delta smelt abundance, can detect trends in the population in space and time, but cannot reveal causes of spatial or temporal variability in delta smelt (Bennett 2005). Both types of monitoring are vital components for assessing the potential benefits of restoration actions; however, both must be explicitly linked with quantitative measures of the mechanisms by which changes in habitat influence the population.

Missing from the current mix of monitoring programs is any systematic effort to link across scales of biological organization. Fish respond to their environment as individuals; what we see as a population response is actually the cumulative outcome of many individual responses. Restoration or other management actions affect local conditions, which in turn affect individual fish through changes in growth rate, fecundity, or mortality risk. Therefore to understand the population-level consequences of restoration, it is necessary to look beyond mere numbers of fish, and to measure variables that provide information about these vital rates.

Our primary goal is to implement a state-of-the-art monitoring program to link key vital parameters for delta smelt collected by existing monitoring programs with survival to adulthood. The unit of interest in this monitoring program is the individual fish. Advances in understanding and measurement techniques allow us now to investigate how individuals vary in their current and historic state, as determined by vital parameters such as growth rate and feeding success. These vital parameters can then be traced to likely times and regions of influence, either positive or negative, on the smelt. When these parameters have been measured on individuals collected throughout the year and over the entire range of the species, influences contributing to year-class success can be determined and traced to their source. This will provide the most novel and technologically advanced monitoring, allowing us to assess the value of habitat restoration by providing mechanistic linkages between local habitat improvements and the abundance patterns as determined by ongoing IEP surveys. Delta smelt are a threatened fish, therefore, our plan is extremely cost-effective and environmentally friendly because we are proposing to extract the greatest possible amount of information from fish that will be killed and eventually discarded in the course of monitoring population abundance.

Out of the 165 actions listed by the ERP (http://calwater.ca.gov/Solicitation/ERP_PSP_Tools.), at least 15 propose benefits to delta smelt. Of these, actions at 9 sites (Figure 1) claim to be improving habitat for delta smelt by:

- creating shallow-water habitat, or restoration of natural flow regimes by levee breaches or flooding of islands
- improvements in water quality through reclamation of salt ponds or reductions in toxic chemicals in runoff.

Although these restoration actions are well intended and involve significant investment and effort, rarely are the proposed benefits to the smelt population specifically defined. Most of actions are small in scope relative to the entire delta smelt habitat, and are occurring simultaneously without any pre-project data, thereby preventing a linkage of these actions to the smelt population. Thus potential benefits will be difficult to decipher from the variety of overlapping influences on the population.

To understand how restoration actions influence delta smelt, we must first define the mechanisms through which population-level benefits might arise. Improvements in habitat quantity or quality resulting from restoration are likely to influence five factors that can be measured for delta smelt and used to assess population-level benefits. *Our objectives are to make concurrent, linked measurements of the following population variables to help understand how environment, restoration, and management activities affect the fish:*

1. Growth efficiency and body condition
2. Impairments from exposure to toxic chemicals
3. Survival to the adult stage.
4. Spawning success
5. Food composition and abundance

We previously developed the methodology for measuring the first four of these parameters for delta smelt (reviewed in Bennett 2005); the fifth is a standard technique. Our approach is novel in combining various sources of information about the state of individual fish. For example, we will combine information from fish otoliths with that from histopathology of tissues to distinguish among potential mechanisms influencing fish condition and growth (reviewed in Bennett 2005). This information, together with direct information on feeding, will give us a complete picture of the nutritional status and health of each fish. In addition, we have recently developed the technology to identify micro-chemical signatures in delta smelt otoliths, which provide a chemical history of the fish's historical movements. With this technique we can now trace the natal origins, as well as track the occurrences of these fish throughout their life history to four regions within the estuary that encompass the delta smelt habitat (Hobbs et al. 2004a). Thus, we can reliably measure changes in vital rates and then link them to a specific region in which they occurred.

Restoration actions at the 9 sites targeting delta smelt fall neatly within the regions identified using the chemical signatures in otoliths (Figure 1). They include:

- North Delta - Sacramento River region
- Central Delta - San Joaquin River region
- Suisun Marsh region
- Napa River region

Our primary objective, therefore, is to collaborate with the system-wide monitoring by the IEP and with local monitoring efforts at the restoration sites to collect and archive delta smelt for analysis using the above approach. Our second objective is to apply the same methods to fish collected during water export operations at the South Delta facilities. This is important because potential benefits from restoration may help to compensate for, or could be offset by, the large numbers of young delta smelt lost during water export operations (Bennett 2005).
We propose this monitoring program as Phase 1 of a multi-phase project, with the intention of further refining our methodology, increasing the numbers of samples, and encouraging involvement by all relevant restoration projects as they come on-line in subsequent phases.

Our proposed monitoring also satisfies various criteria deemed as priority needs by the CALFED ERP as outlined in the PSP. Our program can track trends, evaluate implementation, assess effectiveness, and provide measures for model validation of restoration actions. It provides a multi-institutional initiative, involving scientists at the University of California, San Francisco State University, and the IEP, as well as potential contributions from environmental groups and municipalities involved with local restoration. The partnerships established with this program also provide a highly interdisciplinary approach that may serve as a backbone onto which additional projects funded from diverse sources may be linked. Moreover, with this program we would have the ability to evaluate the performance of delta smelt at a variety of scales, and produce results that would be integrally linked with the IEP and other monitoring programs, and facilitate the work of the interagency Data Assessment Team (DAT) in refining the Delta Smelt Risk Assessment Matrix (DSRAM).

Background on the life cycle

The following discussion is based mainly on the recently published review paper on the ecology of the delta smelt population (Bennett 2005). Key features are outlined in our conceptual model (Figure 1) that depicts the spatiotemporal extent of the population, our monitoring emphasis, and general approach for measuring delta smelt vital parameters. Nine restoration actions (solid circles) and the natal regions (open ovals) within the delta smelt habitat (yellow shading) are linked to a life-cycle diagram showing the temporal sequence of various processes that influence survival at the different life-stages sampled by the IEP monitoring surveys (Figure 1). Delta smelt are primarily an annual species with a small number of individuals living and potentially spawning at two years of age. Our monitoring will concentrate on four key periods of the life cycle, each of which is surveyed by the IEP. All IEP monitoring programs discussed here sample throughout the range of the concurrent life stage of delta smelt, and all take ancillary measurements including temperature, salinity, and length of the fish.

Spawning season: Adult delta smelt spawn in freshwater in spring when water temperatures lie within about 15 to 20ºC. Adult fish are monitored by the spring Kodiak trawl survey from March to May, and fish are identified to reproductive state. Spawning distribution, inferred from monitoring of fish during the transition from ripe to spawned condition, varies with hydrological conditions. In dry years delta smelt spawn primarily in the North Delta region, while in wet years spawning is more evenly distributed among regions, including the Napa River. Pesticides that enter the habitat with freshwater run-off from agricultural fields in late winter may impair egg or sperm development in some regions (Thompson 2000, Bennett 2005). Restoration actions may improve spawning success in different regions by creating shallow-water habitat or by improving water quality. However, if restored habitats are dominated by exotic fishes such as inland silversides, improvements in spawning could be offset by increased predation on delta smelt larvae (Bennett and Moyle 1996, Bennett 2005).

Delta smelt spawn adhesive eggs, but little is known of the spawning habitat other than they probably utilize shallow-water or shoreline areas, as does their closest relative the surf smelt (*Hypomesus pretiosus*), a marine species that sometimes frequents the estuary. Only one egg has been found in the field (K. Fleming, DFG, Pers. Comm.).

Post-larval stage: Delta smelt hatch out as yolk-sac larvae and grow and develop on endogenous energy supplies until they begin to feed at about 5 mm total length (TL). At about 15-20mm TL delta smelt are considered post-larvae: they have finished developing a functional swim bladder and fin-folds. This life-stage is monitored by the 20mm survey from April to June. The initial distribution of post-larvae is generally similar to that of adults during the spawning season, but the smelt move seaward so that they are in the Low-Salinity Zone (LSZ) by July (Bennett et al. 2002). As in many fishes, survival through this stage is influenced by several factors (Figure 1). Feeding success and exposure to toxic pesticides may be especially important, either directly causing mortality or, more likely, by impairing growth and reducing survival. Rapid growth during early life history is an essential feature of recruitment success in fishes because losses to predation tend to be highest on the smallest fish (Houde 1987, 1989).

Feeding success at first feeding and later may be particularly poor for delta smelt because the composition of their zooplankton prey has been changed dramatically by the introduction of several exotic species over the last 2-3 decades (Kimmerer et al. 1994, Kimmerer and Orsi 1996, Orsi and Ohtsuka 1999, Nobriga 2002). Biomass of calanoid copepods, which are the principal prey of delta smelt (Nobriga 1998, 2002, Lott 1998), has been lower in spring in and near the LSZ, including the western Delta, since 1987. Total copepod biomass has been supplemented since 1993 by the introduced cyclopoid copepod *Limnoithona tetraspina*, but this copepod is apparently too small to be readily consumed by delta smelt (Lott 1998, Bouley 2004).

Pesticides are known to occur in the regions occupied by larval and post-larval delta smelt (Kuivila and Foe 1995, Crepeau and Kuivila. 2000, Moon et al. 2000, Thompson et al. 2000, Bennett 1996, 2005). We previously detected growth impairments in delta smelt post-larvae due to poor feeding and toxic exposure (summarized in Bennett 2005). Entrainment in the freshwater export facilities also causes considerable mortality during this period. Currently, however, this mortality is estimated only for fish larger than 20mm, and the extent to which entrained fish reflect only those spawned in the south Delta (lower portion of our Central Delta region), or arrive there from other areas of the Delta, is a topic of considerable uncertainty. Restoration actions that improve flow regimes and water quality, or create habitat, may enhance preferred prey species and feeding success, and reduce impairment from exposure to toxic chemicals. However, such benefits may be offset by increases in fish entrainment. Restoration actions that create or improve habitat may enhance juvenile survival if the changes do not favor exotic fishes disproportionately (Grimaldo et al. 2004, Bennett 2005).

Juvenile stage: The juvenile stage is monitored primarily by the summer tow-net survey from June to August, and the September fall mid-water trawl survey. Our previous work showed that a recruitment bottleneck may occur in late summer as juveniles transition into the adult stage. A stock-recruit model (Figure 2) indicated that survival during this transition may be density dependent in some years. Approximately 60% of juveniles examined in our previous study had growth impairments due to poor feeding success at this life-stage (Bennett 2005). Food abundance, competition, and habitat volume are commonly associated with density-dependent survival (Houde 1987, 1989, Cowan et al. 2000, Rose et al. 2001) and a similar relationship exists for juvenile striped bass (Kimmerer et al. 2000), but the factors contributing to this pattern of density dependence or to poor-feeding success in late summer are currently unknown (Bennett 2005).

Adult stage: Delta smelt adults are monitored by the fall mid-water trawl survey from October to December. During this period we will primarily focus our efforts on understanding the natal origins and occurrences of adults to define the relative contribution made by the various sub-regions to annual year-class success.

2. Justification:

As a “big-R” species, delta smelt have been a priority of CALFED for about a decade. The need to implement a cohesive scientific program to guide recovery efforts has been identified in the CALFED Ecosystem Restoration Program Strategic Plan, Stage 1 Implementation Plan, the Comprehensive Monitoring and Research Program (CMARP), and by a variety of CALFED scientific review panels (EWA Review Panel summary 2003), workshop proceedings (Brown and Kimmerer 2001, Kimmerer and Brown 2003), as well as the IEP Delta Smelt Research Strategy. Clearly, this need is even more compelling if we are to understand influences from a variety of significant restoration actions proposing to provide benefits for delta smelt.

Restoration actions must be evaluated at the population level. Although each restoration action involves considerable effort and capital investment, each is small in scope relative to the distribution of the delta smelt population. Thus, there is no assurance that a higher occurrence of delta smelt at restored sites has any influence on abundance or overall spawning success of the population. Moreover, there is no way to determine if potential benefits to delta smelt compensate for, or merely contribute to, fish lost to the water export facilities. Therefore, a program to assess benefits from restoring habitat needs to link local monitoring efforts at the restoration sites to population-level monitoring by the IEP, as well as fish taken by the water export facilities.

The goal of our work is to provide that linkage by implementing a monitoring program that measures several vital parameters for delta smelt reflecting individual responses to habitat quality. Because we also now have the ability to trace where these responses occurred during a fish's life, we can measure how changes in habitat quality may benefit delta smelt in different sub-regions. Our approach provides powerful integration across scales of biological organization, as well as in time and space, by extracting various types of information from large samples of individuals. Furthermore, we reiterate that this sampling will occur at little further cost in mortality to the delta smelt population, since most of our smelt will come from existing monitoring programs.

The primary strength of our interdisciplinary approach is its ability to integrate across a variety of scales to assess the spatiotemporal patterns by which various factors influence the delta smelt population. The conceptual pathways through which restoration actions may benefit the delta smelt population are shown in Figure 3. Restoration actions may benefit delta smelt at regional and population levels through changes in the spawning and feeding environment, water quality, and the fraction of fish entrained by the water export facilities. These actions have effects on the immediate environment of delta smelt, which in turn evoke responses of individuals that combine to determine a population response. The individual responses can then be measured, providing a way to trace effects back to their origins.

An alternative view of the measurements being taken (Figure 4) illustrates how different classes of measurements provide information on different vital rates. Individual variability due partly to spatial and temporal variability in the environment, together with risk of entrainment in export facilities, results in high or low survival for different subgroups of the population. The studies of otolith micro-chemistry on adult fish will provide information on the origins of the survivors, and will enable us to interpret the individual-based information measured on the younger fish. This powerful approach will enable us to determine what factors are most important in determining survival, and how those factors may interact.

By combining knowledge of the key predictors of high survival (e.g., good feeding success) with measurements of these predictors on fish collected near restoration sites, we will be able to at least begin to assess the efficacy of various classes of restoration actions in benefiting delta smelt.

Since this proposal was first submitted, concern over the decline in abundance of delta smelt and three other pelagic species has led to a radical shift in the emphasis and activities of IEP, and CALFED is being called upon to play a greater role. Indeed, a decline in abundance of key species following substantial expenditures for restoration in the Delta is seen by some as an embarrassment, and by many as a cause for concern about restoration actions and priorities. Although we do not know the cause of the decline of delta smelt, this project is perfectly poised to show whether reduced feeding rate or toxic impairment are likely contributors to the decline. Furthermore, the general approach, which maximizes information gained from each fish caught, should serve as a model for studies of the other species that have declined, and for studies of causes of biological change more generally. This is particularly true since the PI's are involved in related studies (see below) that will mutually benefit from this proposed study, with the result being a maximum of knowledge gained about this important fish.

Response to Reviewer Comments

The principal outcome of the review was a recommendation to cut funding for this proposal by about one half, and to eliminate the histopathology element. We have discussed these comments with agency scientists working on the Pelagic Organism Decline (POD), and have reached a somewhat different conclusion about how to accommodate the budget reduction that would also maximize the benefit to the POD process, which was developed after this proposal was reviewed.

The POD work plan defines some of the key contenders for causes of the decline in key fish species, including delta smelt. One potential causal pathway is through the food supply, and another is through anthropogenic or naturally-produced toxins, the latter from the toxic alga *Microcystis*. The best way to distinguish between effects of toxins and effects of food on delta smelt is through simultaneous measurements of several attributes of each individual fish, specifically including estimates of feeding success, feeding rate, growth rate, and toxic impairment. The best way to estimate toxic impairment while simultaneously getting information on feeding success in large samples of fish is through histopathology. Moreover, as previously proposed, bioassays are the best way to improve the resolution and certainty in our histological diagnosis of fish condition. To further support the POD process we will archive samples for future use with other biomarker techniques. Also, in the event that only small numbers of delta smelt are collected by the monitoring programs, as in 2005, we will include samples of longfin smelt (*Spirinchus thaleichthys*), a species in decline and of special concern to the POD process, in our evaluations of individual fish condition.

We therefore propose to reduce our effort by reducing personnel costs, examining 2 years (rather than 3 years) of field-caught fish, eliminating the search for delta smelt eggs, as well as other costs such as equipment that is no longer needed. This way we can keep all of the elements of our integrated project intact, and ensure the maximum information gain from each fish and each field sample.

3. Previously Funded Monitoring:

Our previous work focused on developing the techniques and overall approach, cooperating with the IEP sampling programs to collect specimens. Relatively speaking it was limited in scope as a monitoring program. We are now proposing to implement the approach as a program specifically linking various restoration actions to establish a mechanism for assessing their relative benefits to the population.

4. Approach and Scope of Work:

We propose a 3-year program to link individual variability in fish condition to population-level responses by focusing on vital parameters most likely to influence year-class strength. We approach this problem from two directions: we will examine key determinants of survival at the individual level, specifically those that influence individual growth rate, focusing on larvae and juveniles originating in each of the four regions. We will then determine the age, growth rate, and geographic origin of surviving fish to determine where most of the survivors come from, looking back in time to determine which sets of conditions and responses most closely predict success. Effects of export pumping occur at the sub-population level (i.e., we assume the probability that a fish will be killed at the pumps depends only its position, not its condition), and these will also be factored into the retrospective analysis. This will enable us to determine the relative importance of export losses in each of the four regions, in comparison to other sources of mortality.

We propose to use delta smelt collected by the IEP monitoring surveys, as well as from restoration projects as they initiate local monitoring for fish. For these fish we will then examine a suite of biological responses using histopathology of fish tissues, as well as growth and assessment of natal origin using otoliths. These measurements we will then be used as input to size-structured and individually-based population models (see Related Projects).

Our previous work (Bennett et al. 1995, Bennett et al. 2002, Bennett 2005) provides a solid foundation for the proposed monitoring program. We have carefully examined the ecology and potentially critical points in the life cycle (Figures 1,2), readily distinguished the effects on fish condition of poor feeding success from those of exposure to toxic chemicals (Figures 5), and then assessed potential consequences for growth rate and mortality ((Figures 6, Bennett 2005). In addition, we will be able to link the indicators of feeding success with information on actual diet and composition of the ambient food supply. Finally, by combining this approach with otolith microchemistry we can trace back through time and identify the region in which each individual was spawned and reared until it was caught in field sampling (Figure 7). This novel approach will also provide the information necessary to track the potential benefits from ecosystem restoration at the sub-regional and population levels.

The relationship among the studies, the measurements made in each, and the individual and population responses to the environment are shown in Figure 4. Again, the objective is to capitalize on the fact that measurements identifying population responses can only be made on individuals at different life stages. When this information is then summarized over regions and the entire habitat it can provide the most effective avenue for assessing the relevance of habitat restoration at a variety of scales, especially at the population level. In other words, we will proceed from what we can reliably measure to what matters for the population (and the species), as well as the concerns of management.

We propose to include an integrated suite of monitoring studies into the IEP surveys that estimate delta smelt abundance (Figures 3,4). Feeding studies will characterize the ambient food supply where fish are caught, and their gut contents will provide a link between local food production and its benefit to individuals. Histopathology studies will then provide us with crucial information on the extent to which growth is influenced by the feeding environment or by exposure to toxic chemicals in the ambient waters. Otolith studies will then measure the actual rate of growth over time periods of days to weeks. Otolith microchemistry will be used to identify the regions where individuals were born and lived until capture. All of this information will then be synthesized with estimates of abundance, as well as the fraction of fish entrained at the water export facilities, both based on data collected by the IEP. This will provide a comprehensive understanding of the regional contribution to annual population abundance, the mechanisms underlying regional performance, as well as the natal regions where fish are most susceptible to entrainment in the export facilities. This is the most technologically feasible way to understand the potential value of local habitat restoration actions for the delta smelt population.

We request three years of funding to implement this interdisciplinary monitoring program that will be composed of four integrated components (Tasks):

Task 1 – Fish sampling, growth and natal history (Bennett, Hobbs)
Task 2 - Measures of fish condition using bioassays and histopathology biomarkers (Teh)
Task 3 – Estimates of food availability and consumption (Kimmerer, Bennett).

Task 1: Fish Sampling, growth and natal history (Bennett, Hobbs).

Fish Sampling. Field specimens will be collected in cooperation with the ongoing IEP monitoring surveys and from efforts at the restoration sites as they get underway. With the IEP, delta smelt will be obtained from four surveys targeting different life stages:

- Spring Kodiak Trawl spawning survey (SKT)
- Spring post-larval survey (20mm)
- Summer juvenile Tow-Net Survey (TNS)
- Fall Mid-water Trawl survey (MWT)

In each survey sampling occurs approximately bi-weekly over a 2-4 month period, except that the MWT survey is monthly. Each survey encompasses nearly the entire distribution of delta smelt at the targeted life stage. Maps showing the distribution of sampling stations for each survey are available at the website for the Central Valley, Bay-Delta Branch, California

Department of Fish and Game (http://www.delta.dfg.ca.gov/). Our objective will be to collect approximately 100 specimens from each survey, or about 500 fish each year, by accompanying IEP personnel in the field. Where possible, samples will be weighted towards monitoring stations near the restoration sites, although strong tidal dispersion in the pelagic habitat of delta smelt implies that the regional scaling may be the most appropriate spatial scale for investigation.

Actual sample sizes and their geographic distribution will be proportional to sampling success, which is influenced by delta smelt abundance and distribution, therefore, strongly influenced by climatic conditions. Overall, delta smelt cannot tolerate salinities above 19 psu or temperatures above 25^{o}C; over 90% of the fish occur below salinities of 9 psu and temperatures below 22^{o}C (Bennett 2004). Thus, in normal to wet conditions, delta smelt are more evenly distributed throughout their range, occurring as far west as the Napa river, whereas in dryer years their distribution is centered in the North Delta and lower Sacramento river (Table 1). Distribution also changes with life stage, with older life stages generally being more widely dispersed. Thus, we anticipate that the approximate proportions of samples from each region and life stage, and hence the potential contribution of each region, will fluctuate depending on climate, as outlined in Table 1. Fish will also be obtained from local sampling at restoration sites as those local monitoring efforts get underway.

Given the current low numbers of delta smelt sampled in the recent surveys in 2005-2006, we will augment our samples as necessary with longfin smelt in coordination with the POD management team.

During the TNS, MWT, and SKT surveys delta smelt are first identified and measured by IEP personnel. We will then weigh and decapitate them. Heads will be placed in 70% ethanol (ETOH) for later examination of otoliths, and bodies will be fixed in a 10% buffered formaldehyde solution for histopathological evaluations and gut contents. Additional samples may be preserved using alternative fixation techniques, kept cool on ice, or frozen in liquid nitrogen. Water samples will also be taken during fish sampling to validate micro-chemical signatures found in the otoliths, and refine our current spatial resolution. In the 20mm survey, fish are often too small to accurately identify or decapitate onboard, so samples will be split between containers with ETOH and formaldehyde solution, or an additional net sample will be taken at each site. We will also obtain specimens in cooperation with salvage operations at the State and Federal Water Project (Dept. of Water Resources, U.S. Bureau of Reclamation). This overall sampling strategy is cost-effective, extending the value of existing resources and minimizing the sacrifice of additional fish.

Fish Growth and Natal History - Delta smelt growth will be evaluated by measuring incremental change in larval and juvenile otoliths (Secor et al. 1991). In our previous work we tailored this methodology for delta smelt, successfully validating the daily periodicity of otolith formation between known-age and field-caught specimens and developed models for back-calculating the size of the fish at ages prior to capture (Hobbs et al. 2004b). Verification of ring-counting by 2 independent readers indicates our methods are accurate to within 4-5 days for juvenile specimens. We also successfully evaluated over 300 juvenile otoliths from the field. In brief, otoliths are removed by micro-surgical technique, coded, secured to a glass slide, polished, and analyzed using light microscopy aided by computerized image analysis. Digitized photographs of each otolith are archived for future use. Adult otoliths, however, are difficult to evaluate, because somatic growth and otolith deposition rate drops off markedly in the adult stage, therefore we are currently evaluating use of otolith weights and morphometrics to establish alternative ways to reliably age older individuals.

Chemical elements incorporated into otoliths as they form provide us with a powerful tool for identifying natal habitats and migration routes for delta smelt (Campana 1999). These elements include trace levels of rare earth and heavy metals dissolved in the waters surrounding the fish. Spatial differences in the concentrations of trace elements and heavy metals, and isotopic ratios of various elements have been well documented in the San Francisco Estuary (Ingram and Weber 1999, Hobbs et al. 2004a). Unique combinations of these signals can be recorded as otoliths form in different regions of the estuary. We have recently developed state-of-the-art technology to measure micro-chemical differences in otoliths for delta smelt (Figure 7, Hobbs et al. 2004a). Trace levels of elements within the otoliths are measured using Laser Ablation techniques in which a laser is focused on a minute portion of the otolith (5 μm diameter) to vaporize it. The vapor is transported into a mass spectroscopy instrument, and the concentrations of elements are measured. Measurements taken at the core of the otolith provide the micro-chemical signature of the natal habitat, whereas transects across the otolith can provide daily resolution of habitat use at the regional scale. Water samples will also be taken to further characterize and refine differences in elemental chemistry among regions.

Task 2: Ambient Bioassays and Histopathology (Teh)

Histopathology of fish organs and tissues provides us with an effective screening tool for assessing mechanisms influencing delta smelt condition or health (Hinton et al 1992, Teh et al 1997). We have successfully used this technique to distinguish the influences of poor feeding success from exposure to toxic chemicals in larval striped bass (Bennett et al. 1995) as well as delta smelt (Figures 5,6 , reviewed in Bennett 2004). A full assessment of potential chemical exposure is difficult and very expensive and therefore beyond the scope of monitoring.

Assessment of livers and gonads of bioassay survivors and field specimens by histopathology is well documented as a technique for determining characteristic signatures of alteration due to poor feeding and sub-lethal toxicity (Hinton et al., 1992, McCarthy and Shugart, 1990, Bennett et al. 1995, Teh et al., 1997). The primary advantage of this approach for our monitoring program is that it can distinguish the effects of food shortages from exposure to sub-lethal concentrations of contaminants on growth (abnormalities in the liver) and reproduction (abnormalities in the gonads) (Bennett et al. 1995).

We will employ both quantitative and qualitative histopathological techniques on about 500 specimens each year using methods described in Bennett et al. 1995; Teh et al 1997). Histology will be performed using whole larval specimens, because they can be embedded and sectioned (Bennett et al. 1995), and for key organs and tissues of older smelt, focusing initially on the liver and gonads where poor feeding and toxic chemicals produce effects (Teh et al. 2004b). Essentially, poor feeding depletes liver hepatocytes of energy reserves (glycogen), whereas exposure to toxic chemicals typically produces a variety of other alterations, including cancerous lesions. Formaldehyde-fixed tissues will be embedded in paraffin and sectioned at 3-5 microns thickness. Tissue sections will be mounted on glass slides and stained with hematoxylin and eosin (HE). Direct histological damage such as 1) liver dysfunction, 2) intersex, testicular necrosis and atrophy in male fish, and 3) ovarian atresia and germ cell necrosis in female will be assessed. All relevant tissues will be analyzed and qualitatively scored based on severity of gylcogen depletion or other cellular abnormalities (e.g., 0= normal; 10= mild or less than 10% of the organ is affected; 20= moderate or 10-50%; and 30= severe or > 50%). Further clarification of liver and gonad diagnoses will be made on subsets of specimens using electron microscopy to determine the nature and extent of cellular and organelle alterations (Bennett et al. 1995). In addition, a small number of field-caught fish will be frozen in liquid nitrogen for further analyses of stress proteins (hsp70), fatty acid composition, and glycogen level.

<u>Bioassays</u> will be used to evaluate long-term influences of sub-lethal effects. In particular, we will use them to gauge the *temporal responses* of fish organs and tissues to poor feeding or exposure to toxic chemicals. *This will provide us with standards to sharpen our histopathological diagnosis of field-caught specimens and allow us to more accurately align information with changes in growth and fish location through time*. Thus, our approach differs from traditional bioassays in that we are primarily interested in the temporal response of effects (i.e. how long does it take for a liver tumor to develop?) to a wide variety of chemicals, primarily pesticides, occurring in the delta smelt habitat. Fortunately, the majority of suspected chemicals tend to produce similar alterations in liver cells (hepatocytes) at the level we are using histopathology as a screening tool (Hinton 1994, Bennett et al. 1995, Teh et al. 1997, Schwaiger et al. 1997, Au 2004). Therefore, the actual chemical(s) used in these bioassays is of a lower concern than understanding the potential consequences of chemical concentration and timing to our 5 point grading system used on the field-caught specimens. Moreover, it is important to reemphasize that the objectives of our investigation are staged: first, we are concerned with if there is any compelling evidence of impact on the fish from chemicals; second, if so, then we will be concerned with what chemicals, or mix of chemicals, might be producing the impacts.

For the bioassays, larval and post-larval delta smelt will be obtained through the delta smelt culturing project (Baskerville-Bridges UCD, *personal communication*) and brought to the Aquatic Toxicology Program laboratories at University of California-Davis (UCD). Fish will be raised in flow-through systems at the Center for Aquatic Biology and Aquaculture, UCD. Water temperature will be maintained at $19 \pm 2\ ^{0}C$ with flow rate at 2 L/min.

Three types of bioassays will be performed: (1) fish will be kept for 4 weeks in different treatments with alternating schedules of feeding or food-deprivation lasting for 1 week. (2) fish will be exposed to environmentally relevant concentrations of commonly used insecticides and/or herbicides, including pyrethroid pesticides (esfenvalerate and permethrin) for 96 hours in a static beaker system according to the method of Teh et al. (2004a, b). Surviving fish will then be divided into fed or starved feeding treatments for 1 week followed by a normal feeding regime for 4 weeks. (3) Fish will be fed or starved for 1 week then divided into pesticide exposure treatments for 96 hr, followed by normal feeding regime for 4 weeks. In all bioassays, fish will be sampled at 0, 1, 2, 3, and 4 weeks for cumulative mortality, morphological anomalies, glycogen and lipid, stress proteins, histopathology, and growth determinations. Water quality parameters will also be recorded.

Task 3: Food availability and Feeding (Kimmerer)

Gut content analysis is the more traditional of our studies, but it provides information on the actual feeding of the delta smelt that cannot be determined in any other way. The time scale of gut content analysis is the shortest of any of our studies, on the order of hours, responding sharply to the ambient food environment. It is also necessary to describe the ambient food environment, which will enable us to estimate food selectivity (which we have found is difficult to determine in the laboratory on these fish), and also allow us to extrapolate feeding conditions to places and times where smelt are not observed.

Smelt for feeding studies will be collected as described under Task 1. Guts will be carefully dissected from fish to be used for histopathological analysis. All prey items from each gut will be dissected out, identified to the lowest taxonomic resolution, and measured if possible. Most of the prey will probably be copepods, which are usually relatively identifiable because their hard parts resist digestion, and because the species diversity in the LSZ is low. We believe that species-specific differences in prey are very important to feeding success, and therefore will be at great pains to identify as many prey items to species as possible.

Larvae that have been sectioned for histopathology cannot be dissected, so we will use the embedded sections to reconstruct gut contents. Total gut volume will be determined by image analysis of each slide followed by reconstruction using simple geometric shapes to represent each section. Some calibration will be necessary to identify prey by this method. We will feed common prey types, one species at a time, to suitable larval fish in the laboratory, section the fish as described above, and search for diagnostic features of the prey and hard parts that can be used to determine prey size (see Kimmerer 1984 for a similar approach).

Plankton samples will be taken concurrently with the samples for delta smelt. The 20mm survey already takes plankton samples using a 10-cm Clarke-Bumpus net attached to the larger net frame. Plankton counting and analyses will be done in collaboration with the IEP/POD . Arrangements will be made to take plankton samples in conjunction with the other surveys from which gut contents will be analyzed. All of these data will be placed in context using data from the long-term IEP zooplankton monitoring program (1972 – present; e.g., Orsi and Mecum 1986, Kimmerer and Orsi 1996, Kimmerer 2004).

Relationship to other studies

Several POD efforts and three current studies link closely with the work described here. Together these studies will provide a substantial gain in our understanding of the dynamics and feeding success of delta smelt. We anticipate considerable interaction with the current and proposed POD studies, and expect that our projects will contribute substantially to that process. Specifically, we plan to link our efforts with several specific POD efforts:

1) Gut content and fish condition studies (R. Gartz, DFG): The fundamental value of our work is that disparate types of information will be obtained from the same individuals. Thus gut contents on fish analyzed for otolith growth and histological condition is key. Work by the POD/IEP on gut and condition studies may broaden the implications of our studies to a larger sample size and potentially other species of concern. Every effort will be made to insure that our efforts complement the POD efforts in this area.

2) Invertebrate and fish toxicity testing (I. Werner, UCD). Our work will be coordinated with the UCD/POD efforts to determine toxicity of ambient water samples to fish and invertebrates.

3) Fecundity (K. Sousa, M. Gingrich, DFG). With pilot funds from a UCD Wildlife Health Center and DFG sponsored Resource Assessment Program, we obtained fecundity estimates for delta smelt sampled by the Spring Kodiak Trawl Survey in 2003-2004. This information has been provided to DFG and POD. We will collaborate with DFG to insure the continuation of such studies to broaden our collective understanding of reproductive success.

4) Ongoing and special fish sampling (R. Baxter). Our work relies on the successful execution of the ongoing sampling surveys, so every effort will be taken to fine-tune our sample handling protocols so that they do not interfere with these vital sampling efforts. Special sampling cruises offered by IEP/POD will greatly facilitate this effort by allowing us to implement modifications to existing protocols, and troubleshoot new methods (e.g. fish weight), to maximize the information gain from the samples.

Three current studies that will also be integrated with our proposed work include:

“Feeding Success of Delta Smelt” (Kimmerer and Bennett, IEP funding, work in progress): We are examining the population dynamics of two copepod species that appear to be important food resources for delta smelt, and along with the timing and degree of food limitation in delta smelt. Total copepod biomass has declined since the late 1980’s, and we are trying to understand how the seasonal pattern of these copepods interacts with the feeding requirements of delta smelt. This is the topic of an ongoing Master’s student project at SFSU, and it will provide a perfect springboard for the feeding studies to be conducted in this proposed project.

“Modeling the delta smelt population” (Kimmerer, Bennett, K. Rose, LSU, and S. Monismith, Stanford; anticipated start date April 2006). We will develop a series of models including matrix models, an individual-based model, and particle-tracking models to extend our field- and lab-based understanding of the biology of this fish. The individual-based model in particular will benefit greatly from the individual-based information developed in this project.

“Foodweb support for the delta smelt population” (Kimmerer and colleagues at SFSU; anticipated start date in early 2006). Our preliminary results suggest that delta smelt are frequently food limited. We will investigate the extent to which food limitation could be eased through management actions in the Delta, including nutrient management and flow conditions.

5. Feasibility:

Our research plan is feasible; each of the collaborators is an expert in their discipline of proposed work, and all have a successful background in interdisciplinary collaborative projects. The methods to be applied are already in use, or have been used in the past, and will be refined during the course of the project. A letter of support from the IEP also acknowledges collaboration with the IEP fish monitoring surveys (Appendix B). Feasibility will be further enhanced because we will be capitalizing on the use of fish that will be killed as a result of abundance monitoring, and thus will be considered as "take" under the IEP Endangered Species Act collection agreement with U.S. FWS. Bennett and students hold current State collecting permits and have been working with U.S. FWS to obtain a Federal permit for archiving delta smelt specimens over the last year. Currently, all specimens are housed at UCD under a Federal permit issued to Dr. P.B. Moyle.

6. Expected Outcomes and Products:

We anticipate the following products from the proposed work:
1.- Periodic oral presentations and peer review at IEP Estuarine Ecology Team and CALFED workshops. In particular, we will brief the POD management team at least two times per year, or more as dictated by the findings. We also regularly participate in the Environmental Water Account workshop each of the last 5 years focusing entirely or mainly on delta smelt, and delta smelt research receives close attention at the annual scientific review panel meeting convened by the CALFED Lead Scientist.
2.- Annual reports to CALFED/POD summarizing progress.
3.- Final report to CALFED summarizing results.
4.- Several publications in peer-reviewed journals with IEP personnel as co-authors where appropriate.

7. Data Handling, Storage, and Dissemination:

Data developed during this project will be stored in databases on computer disks. Digital photographs of each otolith evaluated will be stored on CD-ROM. A database of all delta smelt specimens in our collection is being maintained in EXCEL or ACCESS. This contains relevant information on collection date and location, as well as types of evaluations performed on individual specimens. In addition, data will be made available to the POD every six months for use in their analyses, and relevant data on individual condition, and zooplankton feeding will be placed in the IEP database after publications are submitted.

8. Public Involvement and Outreach:

In addition to regular communications with IEP, CALFED, and stakeholders, we will explore the use of science teachers or members of the lay public to help with local monitoring at restoration sites and the pilot egg survey.

9. Work Schedule:

Our proposed work schedule is outlined in Figure 8. (*Task 1*) Bennett will begin project planning in early 2006. We will then convene a project meeting to organize the work-plan and discuss project products. Field sampling will then begin in coordination with POD/IEP efforts, first with the IEP SKT in early spring. Otolith analyses will begin soon after the first samples are collected and continue throughout the year. Sampling will continue into June each year with the 20mm survey, and then in August with the TNS and September with the MWT. Sampling for adults will then occur in November or December with the MWT each year. (*Task 2*) Teh's research team will begin with histopathological analyses soon after field sampling begins and continue throughout the year. The bioassays will be conducted in late spring in year one and two concurrent with the production schedule of delta smelt in the culturing facility. (*Task 3*) Kimmerer's team will begin gut analyses and plankton sampling in early April with the 20mm survey and this will continue throughout the duration of the project. *(Task 4)* Bennett will convene at least 2 group meetings each year (probably in February and September) with all researchers associated with the project, as well as agency collaborators, to review progress, data synthesis, and discuss publications in preparation. Bennett will address the POD management team whenever necessary. Bennett and Kimmerer will present findings at CALFED EWA workshops, and annual reports will be presented to CALFED and IEP each July. All collaborators will provide input for the final report to be completed within 3-months after the project deadline.

Performance Measures

A variety of performance measures will be used to evaluate the progress and effectiveness of this project. Several criteria, typically associated with a basic scientific research project, will include, fulfilling the objectives of the work tasks and timeline as described above, completion of semi-annual progress reports, presentation of research findings at various professional conferences, and submission of manuscripts to peer-review scientific journals. In addition, the effectiveness of this project also will be measured through (i) coordination and cooperation with IEP/POD and CALFED related studies, (ii) communication of progress and results at regular meetings of various IEP project work teams, the IEP/POD management team, various CALFED workshops and programmatic reviews, as well as (iii) semi-annual deliverables of data developed by this project to the POD management team. The overall measure of this project's success will be the degree to which the information gained through accomplishing the objectives and tasks help to promote a better understanding of delta smelt, the factors limiting the population, and metrics for assessing restoration effectiveness.

B. Applicability to CALFED Bay-Delta Program ERP Goals, the ERP Draft Stage 1 Implementation Plan, and CVPIA Priorities.

1. ERP and CVPIA Priorities:

Our proposed work plan is central to several CALFED ERP Strategic Goals and priorities. The first Goal of the ERP is to "Achieve recovery of at-risk native species dependent on the Delta and Suisun Bay….." The Draft Stage 1 Implementation Plan states "The recovery of at-risk species is at the heart of the ERP." Delta smelt is at the heart of the at-risk species: it is clearly both the most at-risk native species in this region, and the most dependent on the Delta and Suisun Bay. To achieve recovery requires an understanding of how different actions contribute to recovery; thus, the degree of specificity incorporated in our proposed project is essential for determining how and where this recovery may be achieved, and for assessing progress.

Relevant priorities of the CVPIA include: "to protect, restore, and enhance fish, wildlife, and associated habitats in the Central Valley and Trinity River basins of California; to address impacts of the Central Valley Project on fish, wildlife and associated habitats;…to contribute to the State of California's interim and long-term efforts to protect the San Francisco Bay/Sacramento-San Joaquin Delta Estuary." All of these point to the need for attention to delta smelt.

Although the Environmental Water Account (EWA) is not part of ERP, there is a growing linkage among ERP, EWA, and CVPIA, which are becoming viewed as a comprehensive package designed to reduce conflicts over effects of water projects on key fish species. The EWA is increasingly focused on delta smelt, which appear to be more vulnerable to effects of export pumping than any of the other species of concern. An improvement in our understanding of vulnerabilities of delta smelt from different regions to export pumping should help sharpen the focus of EWA to those times of greatest effectiveness.

2. Relationship to Other Ecosystem Restoration Actions, Monitoring Programs, or System-wide Ecosystem Benefits:

Our proposed monitoring program will be integrally linked with the IEP and CALFED Science programs efforts to restore delta smelt. The overall approach and design of our program may be transferred and tailored for other aquatic species in the Sacramento-San Joaquin Watersheds and San Francisco Estuary.

The principal changes in the context for this study since it was originally proposed are that the two delta smelt-related proposals are being funded by the CBDA Science Program (described above), and that the Pelagic Organism Decline (POD) has gained prominence as a major problem requiring immediate attention. Current information about feeding, growth, and condition of delta smelt is limited to a small number of samples and a limited scope for interpretation. This information is essential if we are to develop a complete picture of the biology of delta smelt that can help determine which of the several possible causes of the recent decline is most important for this key species.

3. Additional Information for Proposals containing Land Acquisition:

N/A

C. Qualifications

Organizational structure. Bennett will serve as project leader and coordinate with the supervisors and boat captains of the IEP sampling surveys, and personnel at the fish salvage facilities. Bennett and Kimmerer will regularly discuss project findings and obtain feedback from the IEP Estuarine Ecology Team and CALFED ERP and Science programs. Collaborator responsibilities will closely follow their respective task descriptions and work schedule.

Dr. William A. Bennett received B.S. and Master's degrees in population biology from the University of Massachusetts at Boston, and Ph.D. in ecology from the University of California at Davis (UCD). Dr. Bennett has been a Postdoctoral Researcher at the Bodega Marine Laboratory and is currently an Associate Research Ecologist with the Watershed Science Center, John Muir Institute of the Environment, and faculty member of the Graduate Group in Ecology at UCD. Bennett has worked over 15 years on the ecology of fishes in the San Francisco Estuary, including survival of larval and adult striped bass, exotic inland silversides, the vertical migration behavior of larval fishes. Since arriving at UCD he has worked closely with the Interagency Ecological Program (IEP). He is an active member of IEP's Estuarine Ecology Team, Entrapment Zone Study Team, and Contaminant Effects Team. Recently, Dr. Bennett authored the CALFED white paper on delta smelt, recently published in San Francisco Estuary and Watershed Science. He was also the co-technical program chair for the first CALFED Science Conference.

Dr. Wim J. Kimmerer received his B.S. degree in chemistry from Purdue University and his Ph.D. in biological oceanography from the University of Hawaii. After positions at the Hawaii Institute of Marine Biology, University of Melbourne, and BioSystems Analysis Inc., an environmental consulting firm, he became a Senior Research Scientist at the Romberg Tiburon Center, San Francisco State University. Dr. Kimmerer's expertise is in marine and aquatic ecosystems, including physical, chemical, and biological oceanography, ecology of estuaries and lagoons, fisheries management, simulation modeling, and statistical analysis of data. His current research interests include estuarine ecology, zooplankton ecology, population dynamics of fish such as salmon and striped bass, and the effect of anthropogenic influences such as freshwater flow on estuarine and marine systems. Dr. Kimmerer has written over 80 papers and technical reports on these and related topics, including the CALFED White Paper on Open Water Processes. He has been closely involved with the Interagency Ecological Program, acting as chair of the Estuarine Ecology Team and the Entrapment Zone study team. He was a member of the CALFED Ecosystem Restoration Program Core Team, developing a strategic plan for the program, and is now a member of the Independent Science Board.
Note regarding conflicts: Kimmerer has a potential conflict of interest in that he is an advisor to the CALFED Lead Scientist on the Environmental Water Account, and a former member of the CALFED ERP Science Board. The Science Board position has been determined by CALFED's

attorney not to constitute a conflict provided the member does not actively participate in development of the Implementation Plan, or in the evaluation of proposals. Kimmerer has not been involved in these activities.

Dr. Swee J. Teh, is a research toxicology and pathology faculty-member at UC Davis, Dept. of Anatomy, Physiology and Cell Biology and has over 15 years of extensive field and laboratory research experience in ecotoxicology and biomarker studies. His research interests are in 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 publications, and travels nationally and internationally presenting talks and workshops in this area.

Dr. James A. Hobbs received a B.S. degree in Marine Biology from Sonoma State University, and recently completed a PhD. in Ecology from the University of California, Davis. Dr. Hobbs's dissertation research focused on development of otolith microstructure and microchemistry techniques to understand the population biology and ecology of delta smelt. He has been working with Dr. Bennett on San Francisco Bay Estuary issues for 9 years, and has been a frequent participant in the Estuary Ecology Team and Resident Fishes Team. Dr. Hobbs has presented his research findings at numerous local, national and international conferences. Dr. Hobbs has a publication in the Journal of Marine and Freshwater Research outlining the state of the art use of strontium isotopes in delta smelt otoliths to identify the natal origin of delta smelt, and served as the chair of a poster session showcasing the use of otoliths in fish ecology and restoration issues in the San Francisco Estuary at the "State of the Estuary Conference" and "CALFED Science Conference 2004. His current research interest focuses on the application of otolith based research studies to understand the population ecology of estuarine species and how key processes in the demographics are associated with habitats and restoration activities.

D. Cost

1. Budget

The budget is attached below.

2. Cost sharing

No cost-sharing is required.

3. Long-term funding strategy

Request funds for subsequent phases of the project from CALFED and other funding sources.

E. Compliance with Standard Terms and Conditions

The University of California, Davis takes exception to the following proposed “standard” clauses:

Exhibit A – Scope of Work Section III, Project Officials (add Administrative Contact)
Exhibit B – Attachment 3 – State Travel & Per Diem Expenses Guidelines (Delete)
Exhibit C – General Terms and Conditions for ERP Grants (Replace with GIA 101)
Exhibit D – Special Terms and Conditions for ERP Grants (Replace with UC IP Clause)

Please note with the exception of Exhibit A the above has previously been negotiated with CALFED/GCAPS on behalf of the University of California and agreeable language has been included in the following current ERP agreements with UC Davis (ERP-02D-P31, ERP-02D-P32, ERP-02D-P33, ERP-02D-P35, and ERP-02D-P51).

Exhibit A – Scope of Work, Section III, Project Officials. We request that a third individual be added as the administrative contact and will act on behalf of the Grantee in lieu of the Project Director.

F. Literature Cited

Au, D.W.T. 2004. The application of histo-cytopathological biomarkers in Marine pollution monitoring: a review. Marine Pollution Bulletin 48: 817-834.

Bennett, W.A. 1996. Framework for evaluating pesticide effects on fish populations. IEP Newsletter 8:7-12 (Spring 1996).

Bennett, W.A. 2005. Critical assessment of the delta smelt population in the San Francisco Estuary, California. San Francisco Estuary and Watershed Science. 3 (2) [CALFED online serial] 71 pages.

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.

Bennett, W.A., D.J. Ostrach, and D.E. Hinton. 1995. Condition of larval striped bass in a drought- stricken estuary: evaluating pelagic food web limitation. Ecological Applications 5: 680-692.

Bennett, W.A., W.J. Kimmerer, and J.R. Burau. 2002. Plasticity in vertical migration by native and exotic estuarine fishes in a dynamic low salinity zone. Limnology and Oceanography 47: 1496-1507.

Bouley, P.B. 2004. The feeding ecology of a highly abundant copepod, *Limnoithona tetraspina* (Oithonidae), in the San Francisco Estuary : Patterns of predation on natural prey assemblages.

MA Thesis, San Francisco State University.

Brown, R. and W.J. Kimmerer. 2001. Delta Smelt and CALFED's Environmental Water Account. http://www.science.calwater.ca.gov/pdf/2001_Delta_Smelt_Workshop.pdf

CALFED 2000. Ecosystem Restoration Program Plan Strategic Plan for Ecosystem Restoration. http://calwater.ca.gov/programs/ecosystemrestoration/ecosystemvol3restorationplan.shtml

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188: 263-297.

Cowan, J.H. K.A. Rose, and D.R. DeVries. 2000. Is density dependent growth in young-of-the year fishes a question of critical weight? Reviews in Fish Biology and Fisheries. 10:61-89.

Crepeau, K.L. and K.M. Kuivila. 2000. Factors controlling rice pesticide concentrations in the Colusa Basin Drain and the Sacramento River, California, 1990-1993, J. Environmental Quality, vol. 29(3): 926-935.

EWA Review Panel Summary. 2003. Review of the 2002-2003 Environmental Water Account (EWA) http://www.science.calwater.ca.gov/pdf/EWA_Panel_Report_2003.pdf

Grimaldo, L., R. E. Miller, C. M. Peregrin, and Z. P. Hymanson. 2004. Spatial and temporal distribution of native and alien larval fish assemblages in the southern Sacramento-San Joaquin Delta. In, F. Feyrer, L. Brown, J. Orsi, and R. Brown, eds. Early life history of fishes in the San Francisco Estuary and watershed. American Fisheries Society Symposium. Bethesda, Maryland.

Hinton, D.E., 1994. Cells, cellular responses, and their markers on chronic toxicity of fishes. In Malins, D.C., Ostrander, G.K. (Eds), Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives, Lewis Publishers, Boca Raton, pp. 207-239.

Hinton, D.E., et al. 1992. Histopathological Biomarkers. In: Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. R.J. Huggett, R.A. Kimerle, P.M. Mehrle, H.L. Bergman (eds.) Lewis Publishers, Inc., Boca Raton, FL.

Hobbs, J.A., J. Burton-Hobbs, and W.A. Bennett. 2005a. Retrospective determination of natal habitats for an estuarine fish using otolith strontium isotope ratios. Marine and Freshwater Research 56:1-6.

Hobbs, J.A., J. E. Burton, W.A. Bennett, and B. Baskerville-Bridges 2005b. Age and growth validation of delta smelt (*Hypomesus transpacificus*) in the San Francisco Estuary. U.S. Fishery Bulletin. (*Submitted, in Review*).

Houde, E. 1987. Fish early life history dynamics and recruitment variability. American Fisheries Society Symposium 2:17-29.

Houde, E. D. 1989. Subtleties and episodes in the early life of fishes. Journal of Fish Biology 35:29-38.

Ingram, B.L., P. K. Weber. 1999. Salmon origin in California's Sacramento-San Joaquin river system as determined by otolith strontium isotopic composition. Geology 27: 851-854.

Kimmerer, W. J. 2004. Open water processes of the San Francisco Estuary: >From physical forcing to biological responses. San Francisco Estuary and Watershed Science [online serial]. Vol. 2, Issue 1 (February 2004), Article 1.

Kimmerer, W. J., and J. J. Orsi. 1996. Causes of long-term declines in zooplankton in the San Francisco Bay estuary since 1987. Pages 403-424 *in* J. T. Hollibaugh, editor. San Francisco Bay: The Ecosystem. AAAS, San Francisco.

Kimmerer, W.J. and R. Brown. 2003. CALFED Bay-Delta Program Environmental WaterAccount Summary of the Annual Delta Smelt Technical Workshop, Santa Cruz, CA, August 18-19, 2003. http://www.science.calwater.ca.gov/workshop/water_ops_symposium.shtml

Kimmerer, W. J., E. Gartside, and J. J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San-Francisco Bay. Marine Ecology-Progress Series 113:81-93.

Kimmerer, W.J., J.H. Cowan Jr., L.W. Miller, and K.A. Rose. 2000.Analysis of an estuarine striped bass population: Influence of density-dependent mortality between metamorphosis and recruitment. Can. J.Fish. Aquat. Sci. 57: 478-486

Kuivila, K. M., and C. G. Foe. 1995. Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco estuary, California. Environmental Toxicology and Chemistry **14**:1141-1150.

Lott, J. 1998. Feeding habitats of juvenile and adult delta smelt from the Sacramento-San Joaquin estuary. IEP Newsletter 11:14-19.

McCarthy, J.F. and Shugart L.R. 1990. Biological Markers of Environmental Contamination. In: Biomarkers of Environmental Contamination. Lewis Publishers, CRC Press, Inc., Boca Raton, Florida, pp. 3-14.

Moon, G.E., K.M. Kuivila, C. Ruhl, and D.H. Schoelhamer. 2000. Exposure of Delta Smelt to dissolved pesticides in 1998 and 1999, IEP Newsletter, 13 (4): 27-33.

Nobriga, M. 1998. Evidence of food limitation in larval delta smelt. IEP Newsletter 11: 20-23.

Nobriga, M. 2002. Feeding ecology of larval delta smelt: a multi-year assessment of diet composition and feeding incidence in relation to environmental and ontogenetic influences. California Fish and Game. 88: 149-164.

Orsi, J., and W. Mecum. 1986. Zooplankton distribution and abundance in the Sacramento-San Joaquin Delta in relation to certain environmental factors. Estuaries **9**:326-339.

Orsi, J. J., and S. Ohtsuka. 1999. Introduction of the Asian copepods *Acartiella sinensis, Tortanus dextrilobatus* (Copepoda: Calanoida), and *Limnoithona tetraspina* (Copepoda: Cyclopoida) to the San Francisco Estuary, California, USA. Plankton Biology and Ecology 46:128-131.

Rose, K. A., J. H. Cowan Jr., K. O. Winemiller, R. A. Myers, and R. Hilborn. 2001. Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish and Fisheries 2: 293-327.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W., and Triebskorn, R. 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. Journal of Aquatic Ecosystem Stress and Recovery 6: 75-86.

Secor, D., J.M. Dean, E.H. Laban. 1991. Manual for Otolith Removal and Preparation for Microstructural Examination. Handbook for evaluation of fish otoliths. Belle Baruch Institute for Marine Biology and Coastal Research.

Teh, S.J., Adams, S.M. and Hinton, D.E. 1997. Histopathologic biomarkers of anthropogenic stress in resident redbreast sunfish (*Lepomis auritus*) and largemouth bass (*Micropterus salmoides Lac p de*) from contaminant impacted sites. Aquatic Toxicology. 37:51-70

Teh, S.J., Deng, X., Deng, D.F., Teh, F.C., Hung, S.S.O., Fan, W-M.T, Liu, J. and R.M Higashi 2004a. Chronic Effects of Dietary Selenium on Juvenile Sacramento Splittail (*Pogonichthys Macrolepidotus*). Environ. Sci. Technol.; 38(22) pp 6085 - 6093.

Teh, S.J., Zhang, G.H., Kimball, T, and F.C. Teh, 2004b. Lethal And Sublethal Effects Of Esfenvalerate And Diazinon On Sacramento Splittail Larvae. In F. Feyrer, L. Brown, J. Orsi, and R. Brown, eds. Early life history of fishes in the San Francisco Estuary and watershed. American Fisheries Society Symposium. Bethesda, Maryland.

Thompson, B. 2000. An overview of contaminant-related issues identified by monitoring in San Francisco Bay. Environmental Monitoring and Assessment 64:409-419.

H. Nonprofit Verification.

See Attachment A.

Table 1. Rough approximation of anticipated delta smelt sampling success (% fish) in years with high versus low freshwater outflow among regions of the San Francisco Estuary.

	Region							
	North Delta		Central Delta		Suisun Marsh		Napa River	
Monitoring Survey			Freshwater		Outflow			
	High	Low	High	Low	High	Low	High	Low
Spring Kodiak	30	80	30	10	25	10	15	0
20mm	25	80	25	10	25	10	25	0

Tow Net (TNS)	25	70	25	25	25	15	5	0
Mid-water trawl (MWT)	40	80	20	20	40	20	0	0

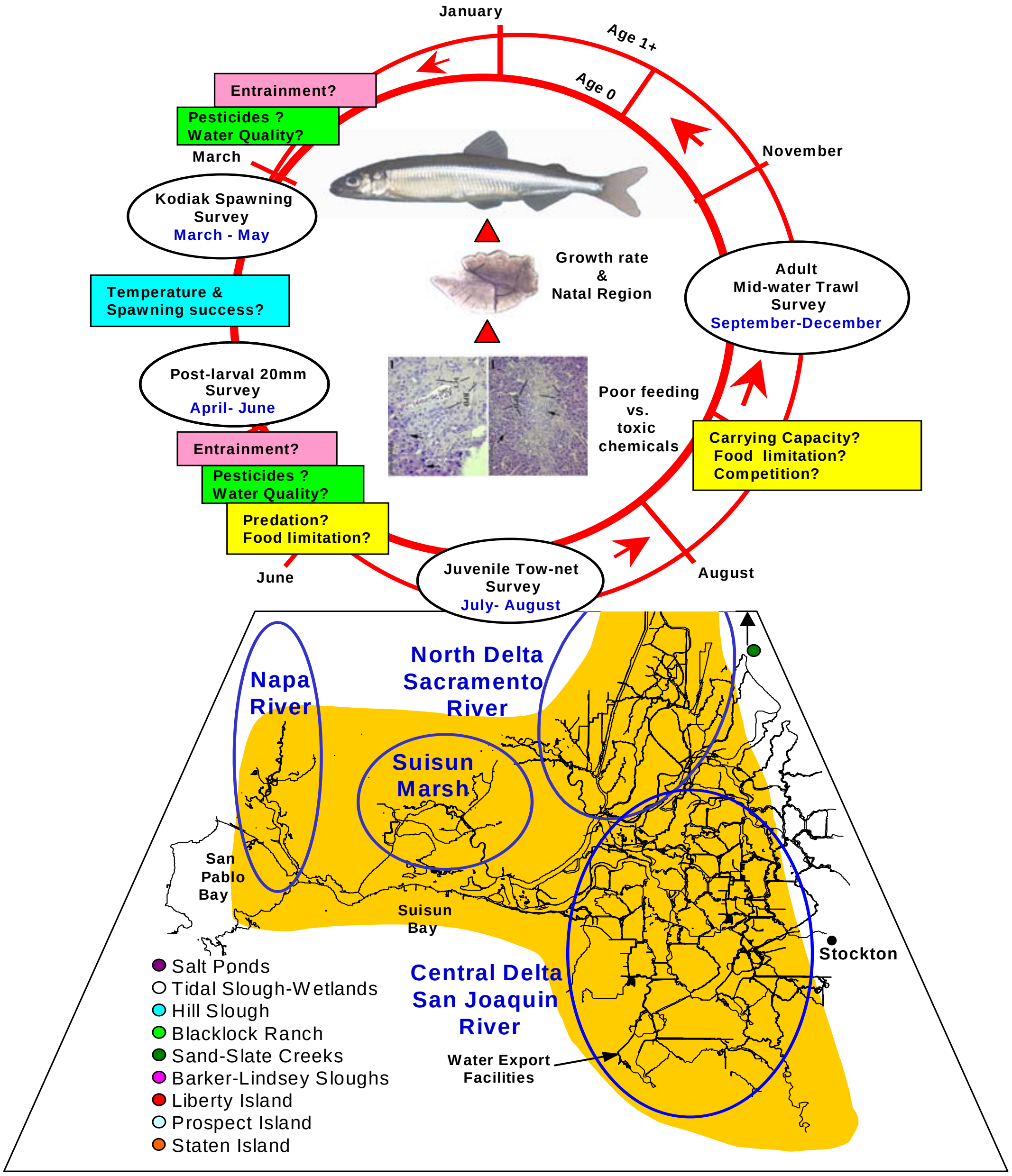


Figure 1. Delta smelt in space and time. A conceptual life cycle model shows key life stages to be monitored, the timing of factors influencing survival, and indivudally-based approach for measuring vital parameters from liver hepatocytes and otoliths for delta smelt. Also shown are the locations of nine restoration sites (colored circles) that propose benefits for delta smelt, as well as the natal regions (open ovals) identified by elemental signatures of otoliths within the range of the population (yellow shading) in the San Francisco Estuary.

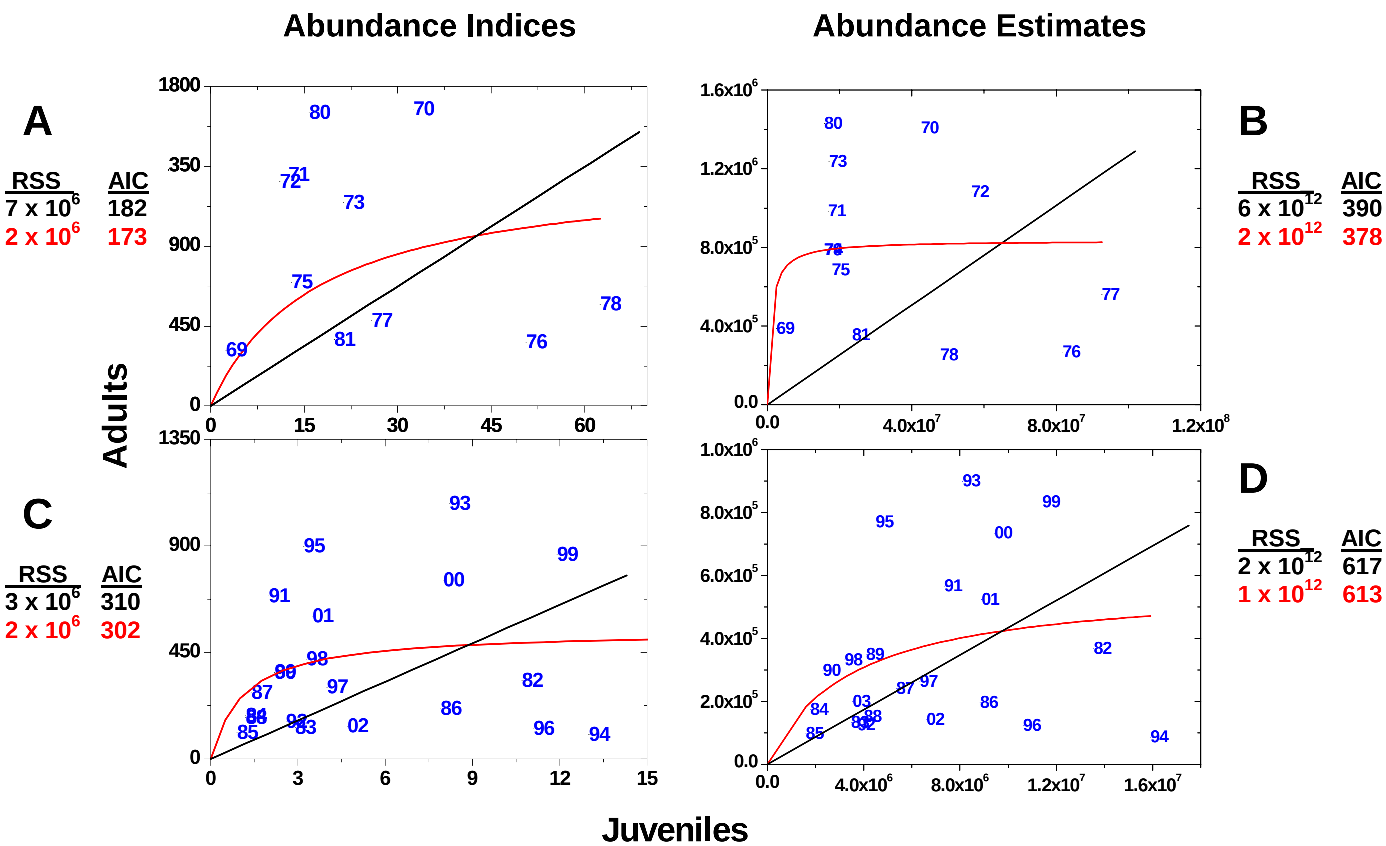


Figure 2. Stock-recruit relationships comparing fits of Beverton-Holt (density dependence) and linear (density independence) models using abundance indices and abundance estimates for the juvenile and adult stages. The period before the population decline (1967-1982) is shown (A, B) with similar fits for the post-decline period (C, D). Model fits are compared using the residual sum of squares (RSS) and Akaike's Information Criterion (AIC) where a lower values indicate the Beverton-Holt model provides a better fit to the data points than a linear model. Data points are shown as years (blue). (From, Bennett 2005.)

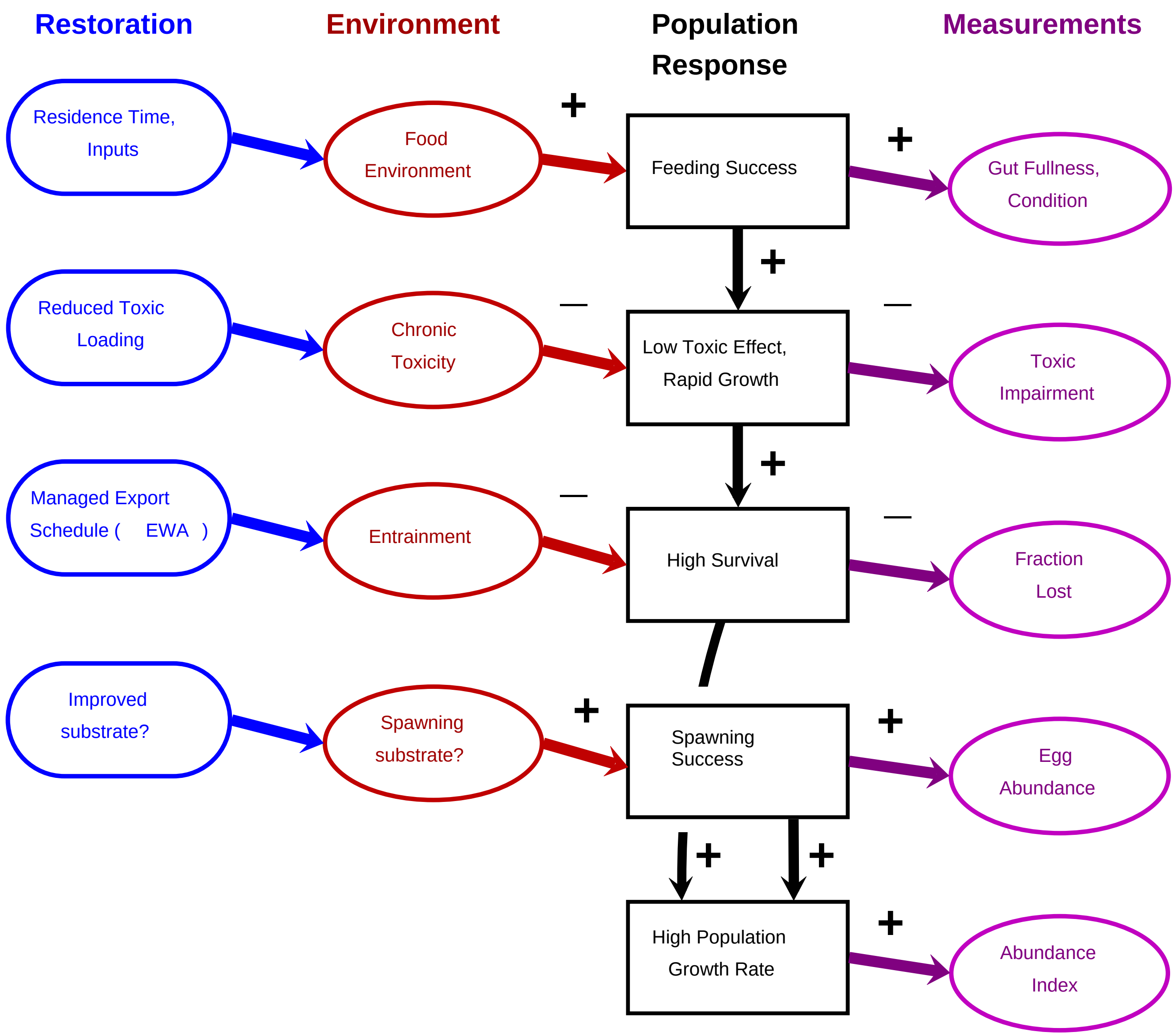


Figure 3. Conceptual model showing the pathways of potential effects resulting from restoration on the delta smelt population, and the measurements needed to monitor them in the San Francisco Estuary. Restoration actions that improve flow regimes or key nutrient inputs may influence the local production of food, improving feeding success, gut fullness, and liver condition promoting rapid growth rates for individuals. Reductions of toxic pesticide inputs would further improve growth leading to higher survival rates. Management of freshwater exports (e.g. use of the Environmental Water Account, EWA) would reduce the fraction of fish lost further increasing survival. Habitat improvements providing more spawning substrate may increase spawning success and egg abundance leading to higher growth rates for the population among years.

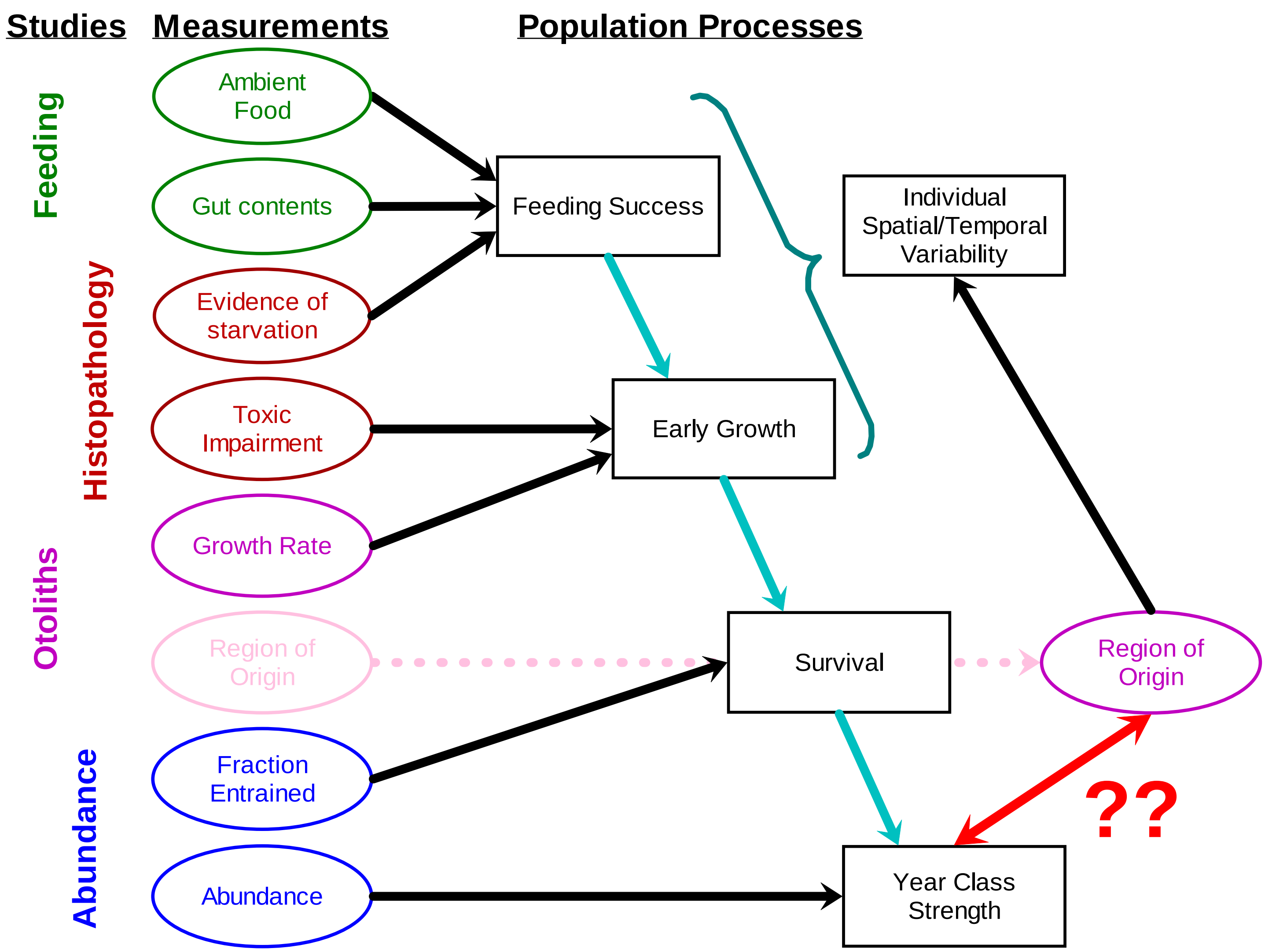
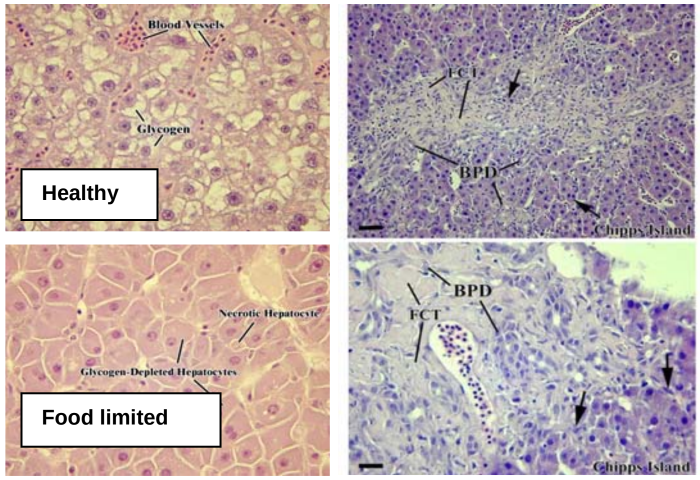


Figure 4. Logic of the monitoring design. Measurements to be made in this program and the IEP monitoring programs are listed in ovals on the left with the general category of studies, which map to the project tasks, in matching color at the far left. Black arrows indicate the population processes (in black) at which each measurement is directed. Most of the measurements focus on early life history. The feeding studies and one part of the histopathology study will determine feeding success; together with the remaining histopathology and otolith aging we will have a good idea of feeding, toxic compromise, and growth of each individual fish. Individual variability within and between regions where fish are collected will be determined. IEP monitoring efforts, listed in blue, determine abundance and the fraction of some life stages entrained at the south Delta export facilities. All of this information will be interpreted in terms of geography using the otolith microchemistry, used to place the individual data and entrainment data in a broader context. The principal question to be answered by this entire suite of techniques is how variability at the individual level, differing among regions, combined with differential exposure to entrainment in export pumps, translates into year-class strength and, conversely, to what extent year-class strength is determined by the observed regional pattern of variability.



Glycogen in liver (energy reserve)

Liver cancer from pesticide exposure

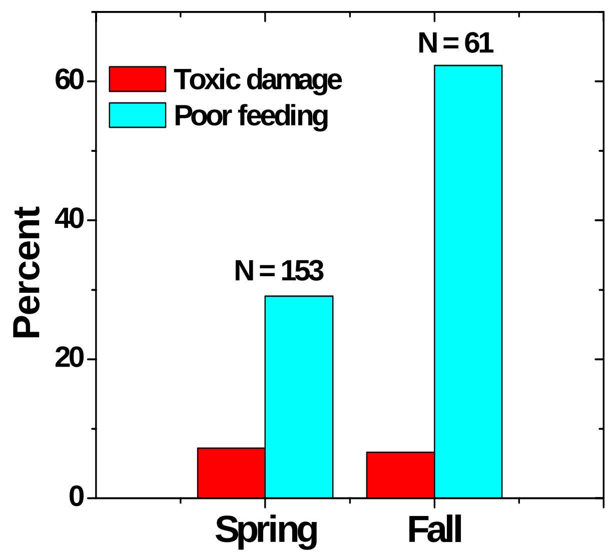


Figure 5, Histopathology of juvenile delta smelt livers. Photographs show differences between a healthy (glycogen enriched; light areas in hepatocytes) liver versus a food deprived liver (few light areas in hepatocytes), and livers damaged by exposure to pesticides. Also shown are results from field specimens indicating percent of livers damaged by pesticides versus poor nutrition from 1999-2000 (Form, Bennett 2005).

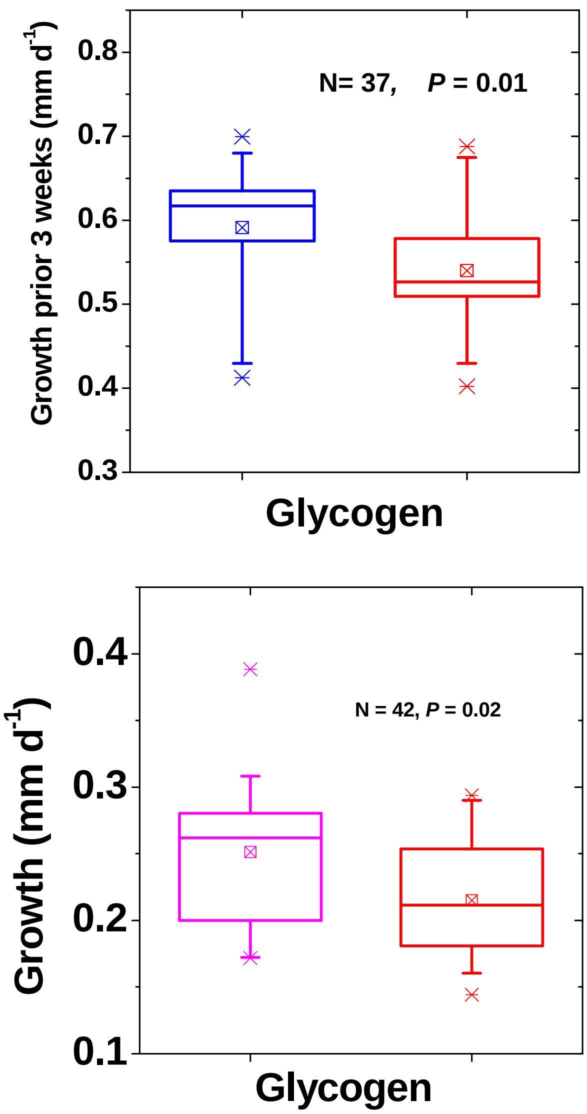


Figure 6. Relative growth during the 3 weeks prior to capture for healthy and undernourished juvenile delta smelt that had begun to feed exogenously when *E. affinis* declined in abundance during spring 1999 (upper panel). Similar relationship for juveniles in late summer (lower panel). Diagnosis of feeding condition was by histopathology of liver cells (From, Bennett 2005).

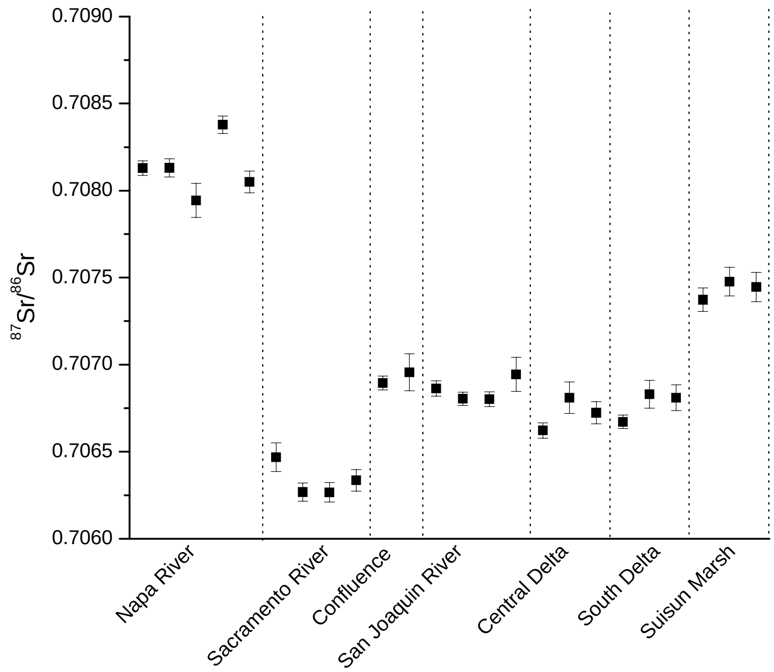


Figure 7. Differences in strontium isotope ratios ($^{87}Sr/^{86}Sr$) for individuals (2σ) collected at potential natal areas within the San Francisco Estuary (From, Hobbs et al. 2005a).

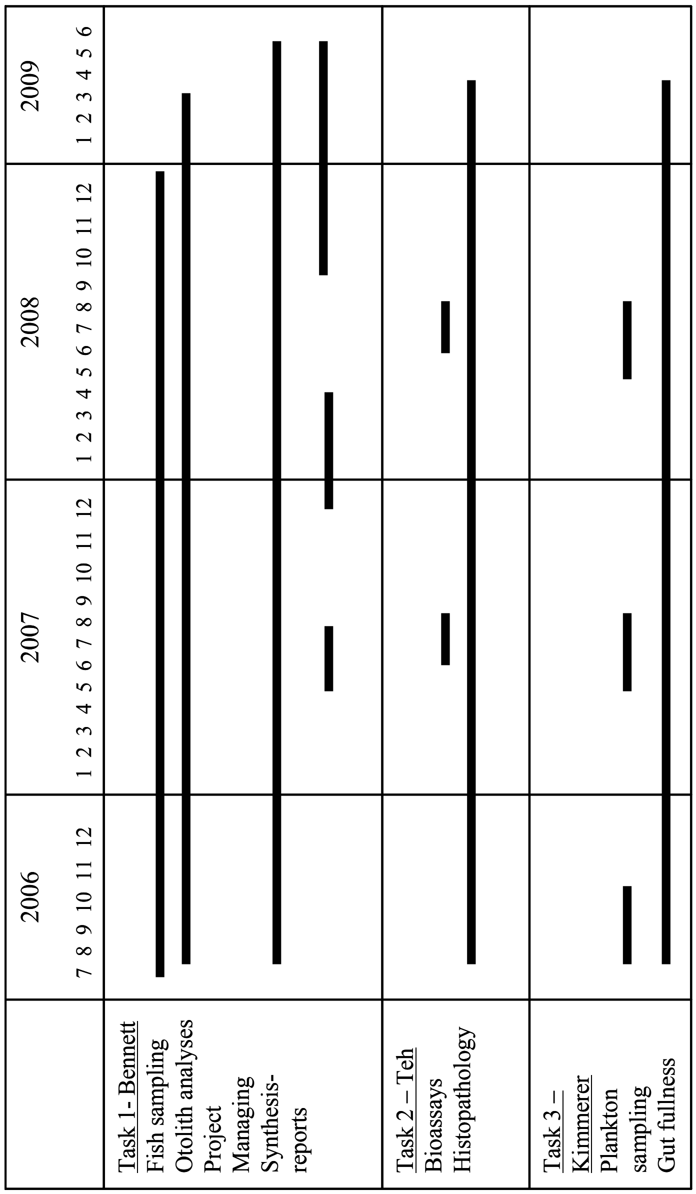


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Figure 8. Work Schedule. Thick lines show the monthly course of work for each Task.

BUDGET: CALFED 2004 PSP, BENNETT(PI)

	YEAR 1 (2006)			YEAR 2 (2007)			YEAR 3 (2008)		
A. PERSONNEL UCD	**SALARY**	**BENEFITS**	**TOTALS**	**SALARY**	**BENEFITS**	**TOTALS**	**SALARY**	**BENEFITS**	**TOTALS**
JMIE									
1. Bennett ,Assistant RS III/ Associate (75%)	$50,850	$13,221		$53,393	$13,882		$56,062	$14,576	
3. Jr. Specialist I (100%)	31,044	$6,209		$32,596	$6,519		$34,226	$6,845	
4. Jr. Specialist I (50%)	15,522	$3,104		$16,298	$3,260		$17,113	$3,423	
5. Cummings, Analyist (12.5%)	$6,345	$1,396		$6,662	$1,466		$6,995	$1,539	
TOTAL SALARY			$103,761			$108,949			$114,397
TOTAL BENEFITS			$23,930			$25,127			$26,383
SUBTOTAL PERSONNEL			**$127,691**			**$134,076**			**$140,779**
APCB									
Teh, Associate RB II (25%)	$21,250	$5,950		$22,313	$6,248		$23,428	$6,560	
Histopathologist (75%)	$63,750	$22,313		$66,938	$23,428		$70,284	$24,600	
Lab Assistant IV (75%)	$28,755	$8,627		$30,193	$9,058		$31,702	$9,511	
Undergraduates (25%)	$5,000	$150		$5,250	$158		$5,513	$165	
TOTAL SALARY (TEH)			$118,755			$124,693			$130,927
TOTAL BENEFITS(TEH)			$36,889			$38,891			$40,835
SUBTOTAL PERSONNEL(TEH)			**$155,644**			**$163,584**			**$171,763**
TOTAL SALARIES-BENEFITS			**$283,335**			**$297,659**			**$312,542**
B. SUPPLIES & EXPENSES									
JMIE, Bennett		$8,000				$5,000			$5,000
APCB, Teh		$20,000				$20,000			$20,000
TOTAL SUPPLIES-EXPENSES			**$28,000**			**$25,000**			**$25,000**
C. TRAVEL									
JMIE, Bennett		$7,000				$7,000			$7,000
APCB, Teh		$5,000				$5,000			$3,000
TOTAL TRAVEL			**$12,000**			**$12,000**			**$10,000**
D. UCD TOTAL DIRECT COSTS			**$323,335**			**$334,659**			**$347,542**
-		-			-			-	
E. UCD INDIRECT COSTS (25%)			$80,834			$83,665			$86,886
UC OVERHEAD ON SUBCONTRACT (1ST $25K)			$6,250						
TOTAL UCD INDIRECT COSTS			**$87,084**			**$83,665**			**$86,886**
E. SUBCONTRACT SFSU									

Kimmerer (2mo, 1mo,1mo)	$15,600	$7,488	$8,190	$3,931	$8,190	$3,931
Research Assistant II (4mo.,3mo.,2mo)	$13,000	$6,240	$10,238	$4,914	$6,825	$3,276
Student Assistant 1 ($14.00 per hour)	$19,600	$294	$20,580	$309	$5,880	$88
TOTAL SALARY		$48,200		$39,008		$20,895
TOTAL BENEFITS		$14,022		$9,154		$7,295
SUBTOTAL PERSONNEL		**$62,222**		**$48,162**		**$28,190**
SFSU, Kimmerer Travel		$1,000		$2,800		$4,000
SFSU, Kimmerer supplies		$3,000		$2,000		$500
Other Expensis (Student Fee, Boat time,Pub costs)		$4,000		$4,200		$500
TOTAL DIRECT COSTS		**$70,222**		**$57,161**		**$32,690**
Indirect Costs@50%		**$33,111**		**$26,481**		**$16,345**
TOTAL SFSU Subcontract		**$103,333**		**$83,642**		**$49,035**
F. TOTAL COSTS		**$513,752**		**$501,966**		**$483,463**
TOTAL AWARD (3-YEARS)						**$1,499,181**