ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

Project Information

1. **Proposal Title:**

   ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

2. **Proposal applicants:**

   Swee Teh, University of California, Davis
   Guohua Zhang, UCD
   Ted Sommer, DWR
   Randy Baxter, CDFG
   Silas Hung, UCD
   Peter Moyle, UCD
   Paul Lutes, UCD

3. **Corresponding Contact Person:**

   Swee Teh
   Aquatic Toxicology Program
   One Shields Avenue, 1321 Haring Hall, VM:APCB University of California-Davis
   530 754-8183
   sjteh@ucdavis.edu

4. **Project Keywords:**

   At-risk species, fish
   Fish Health Biology
   Heavy Metals (mercury, selenium, etc.)

5. **Type of project:**

   Research

6. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

7. **Topic Area**:

   At-Risk Species Assessments

8. **Type of applicant:**

University

9. **Location - GIS coordinates:**

Latitude: 38.417

Longitude: -121.467

Datum:

Describe project location using information such as water bodies, river miles, road intersections, landmarks, and size in acres.

This work will be conducted in conjunction with Mr. Ted Sommer (DWR), Mr. Randy Baxter (DFG), Dr. Peter Moyle (CALFED), and Dr. Silas Hung (CALFED 99-N07). Therefore, we will work under their Endangered Species Act take permits. The field sampling locations selected will be within Sutter and Yolo Bypass, and the vicinity of the Sacramento- San Joaquin rivers adjacent to the Bypass, as well as Suisun Marsh, the Cosumnes River flood plain, and areas of the Delta adjacent to the Cosomnes and Mokelumne rivers (e.g., Snodgrass Slough).

10. **Location - Ecozone:**

3.4 Colusa to Verona, 3.5 Verona to Sacramento, 8.4 Sutter Bypass, 10.1 Cache Creek, 10.2 Putah Creek, 1.1 North Delta, 11.1 Cosumnes River, 11.2 Mokelumne River, 2.1 Suisun Bay & Marsh

11. **Location - County:**

Yolo

12. **Location - City:**

Does your project fall within a city jurisdiction?

No

13. **Location - Tribal Lands:**

Does your project fall on or adjacent to tribal lands?

No

14. **Location - Congressional District:**

3rd district

15. **Location:**

California State Senate District Number: ?

California Assembly District Number: 8

16. **How many years of funding are you requesting?**

17. **Requested Funds:**
    a) Are your overhead rates different depending on whether funds are state or federal?

    Yes

    If yes, list the different overhead rates and total requested funds:

State Overhead Rate:		10%
Total State Funds:	$ 859,496.00	
Federal Overhead Rate:	48.5%	
Total Federal Funds:	$1,160,319.60	

    b) Do you have cost share partners already identified?

    No

    c) Do you have potential cost share partners?

    No

    d) Are you specifically seeking non-federal cost share funds through this solicitation?

    No

    If the total non-federal cost share funds requested above does not match the total state funds requested in 17a, please explain the difference:

18. **Is this proposal for next-phase funding of an ongoing project funded by CALFED?**

No

Have you previously received funding from CALFED for other projects not listed above?

Yes

If yes, identify project number(s), title(s) and CALFED program.

97-B81650	**Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary**	**CALFED**
99-N07	**(Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (Pogonichthys macrolepidotus): A Biomarker Approach**	**CALFED**

19. **Is this proposal for next-phase funding of an ongoing project funded by CVPIA?**

No

Have you previously received funding from CVPIA for other projects not listed above?

No

20. **Is this proposal for next-phase funding of an ongoing project funded by an entity other than CALFED or CVPIA?**

No

Please list suggested reviewers for your proposal. (optional)

Dr. Sam Luoma USGS (650) 329-4481 snluoma@usgs.gov

Dr. Mike Johnson UCD (530) 752-8837 mbjohnson@ucdavis.edu

Dr. Chris Foe SWRCB (916) 255-3113 foec@rb5s.swrcb.ca.gov

Dr. Mike Anderson CalEPA (916) 255-6625 Manders7@dtsc.ca.gov

21. **Comments:**

If the above reviewers are not availablle, we would like to have: Dr. Marshall Adams, Oak Ridge National Laboratory, phone: (865) 576-736, SMA@ORNL.GOV Dr. Bruce Thompson, SFEI, Phone:(510)231-5613, brucet@sfei.org Dr. Mark Myers, NOAA-NWFSC, phone:(206)860-3329 mark.s.myers@noaa.gov Dr. Thomas Braunbeck(University of Heidelberg)(braunbeck@urz.uni-heidelberg.de) phone: 0049-(0)6221-545668

Environmental Compliance Checklist

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

1. **CEQA or NEPA Compliance**
   a) Will this project require compliance with CEQA?

      No
   b) Will this project require compliance with NEPA?

      No
   c) If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

      University of California-Davis will ensure that all their employee complying to the laws and regulations.

2. **If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies).** *If not applicable, put "None".*

   CEQA Lead Agency:
   NEPA Lead Agency (or co-lead:)
   NEPA Co-Lead Agency (if applicable):

3. **Please check which type of CEQA/NEPA documentation is anticipated.**

   CEQA
   -Categorical Exemption
   -Negative Declaration or Mitigated Negative Declaration
   -EIR
   Xnone

   NEPA
   -Categorical Exclusion
   -Environmental Assessment/FONSI
   -EIS
   Xnone

   If you anticipate relying on either the Categorical Exemption or Categorical Exclusion for this project, please specifically identify the exemption and/or exclusion that you believe covers this project.

4. **CEQA/NEPA Process**
   a) Is the CEQA/NEPA process complete?

      Not Applicable

   b) If the CEQA/NEPA document has been completed, please list document name(s):

5. **Environmental Permitting and Approvals** (*If a permit is not required, leave both Required? and Obtained? check boxes blank.*)

LOCAL PERMITS AND APPROVALS

Conditional use permit

Variance

Subdivision Map Act

Grading Permit

General Plan Amendment

Specific Plan Approval

Rezone

Williamson Act Contract Cancellation

Other

STATE PERMITS AND APPROVALS

Scientific Collecting Permit Obtained

CESA Compliance: 2081

CESA Compliance: NCCP

1601/03

CWA 401 certification

Coastal Development Permit

Reclamation Board Approval

Notification of DPC or BCDC

Other

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation

ESA Compliance Section 10 Permit

Rivers and Harbors Act

CWA 404

Other Obtained

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land.
Agency Name:

Permission to access state land.
Agency Name:

Permission to access federal land.
Agency Name:

Permission to access private land.
Landowner Name:

6. **Comments.**

This proposal's State (CDFG) and Federal (USFWS) fish collecting permits are already obtained by Mr. Sommer (DWR), Mr. Baxter (DFG), and Dr. Moyle.

Land Use Checklist

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

1. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

2. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

   No

3. **Do the actions in the proposal involve physical changes in the land use?**

   No

   If you answered no to #3, explain what type of actions are involved in the proposal (i.e., research only, planning only).

   research only

4. **Comments.**

Conflict of Interest Checklist

<u>ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT</u>

Please list below the full names and organizations of all individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal or who will benefit financially if the proposal is funded.
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.
- Individuals not listed in the proposal who helped with proposal development, for example by reviewing drafts, or by providing critical suggestions or ideas contained within the proposal.

The information provided on this form will be used to select appropriate and unbiased reviewers for your proposal.

Applicant(s):

Swee Teh, University of California, Davis
Guohua Zhang, UCD
Ted Sommer, DWR
Randy Baxter, CDFG
Silas Hung, UCD
Peter Moyle, UCD
Paul Lutes, UCD

Subcontractor(s):

Are specific subcontractors identified in this proposal? No

Helped with proposal development:

Are there persons who helped with proposal development?

No

Comments:

Budget Summary

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

Please provide a detailed budget for each year of requested funds, indicating on the form whether the indirect costs are based on the Federal overhead rate, State overhead rate, or are independent of fund source.

State Funds

Year 1												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** *(per year)*	**Benefits** *(per year)*	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Field Survey	3477	80674.9	18521.9	500.00	63600	0	0	0	163296.8	16329.68	179626.48
2	Laboratory Experiment	1160	26907.5	6177.6	0	36600	0	0	0	69685.1	6968.51	76653.61
3	project management	504	11688.6	2683.5	500.00	0	0	0	0	14872.1	1487.21	16359.31
		5141	119271.00	27383.00	1000.00	100200.00	0.00	0.00	0.00	247854.00	24785.40	272639.40

Year 2												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** *(per year)*	**Benefits** *(per year)*	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Field Survey	3477	84708.65	19448.00	525	66780	0	0	0	171461.65	17146.17	188607.82
2	Laboratory Experiment	1160	28252.88	6486.48	0	38430	0	0	0	73169.36	7316.94	80486.30
3	Program management	504	12273.03	2817.68	525	0	0	0	0	15615.71	1561.57	17177.28
		5141	125234.56	28752.16	1050.00	105210.00	0.00	0.00	0.00	260246.72	26024.68	286271.40

Year 3												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** *(per year)*	**Benefits** *(per year)*	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Field Survey	3477	88944.08	20420.39	551.25	70119.00	0	0	0	180034.72	18003.47	198038.19
2	Laboratory Experiment	1160	29665.52	6810.80	0	40351.50	0	0	0	76827.82	7682.78	84510.60
3	Project Management	504	12886.68	2958.56	551.25	0	0	0	0	16396.49	1639.65	18036.14
		5141	131496.28	30189.75	1102.50	110470.50	0.00	0.00	0.00	273259.03	27325.90	300584.93

Grand Total=859495.73

Comments.

Budget Justification

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

Direct Labor Hours. Provide estimated hours proposed for each individual.

Dr. Swee Teh (3 years)= 1512 direct hours; Dr. Guoha Zhang (3 years) = 6048 direct hours; SRA IV (3 years) = 3024 direct hours;Lab Assistant (3 years) = 3024 direct hours; Student assistant (3 year) = 1512 direct hours; Dr. Silas Hung (3 year) = 302 direct hours (no cost); Dr. Peter Moyle (3 year) = 302 direct hours (no cost); Dr. Paul Lutes (3 years) = 302 direct hours (no cost).

Salary. Provide estimated rate of compensation proposed for each individual.

Dr. Swee Teh ($41/hr); Dr. Guoha Zhang ($27.80/hr); SRA IV ($39.66/hr); Lab Assistant ($24.59/hr);

Benefits. Provide the overall benefit rate applicable to each category of employee proposed in the project.

Dr. Swee Teh (17%); Dr. Guoha Zhang (23%); SRA IV (23%); Lab Assistant (23%)

Travel. Provide purpose and estimate costs for all non-local travel.

$1,000.00 for attending meetings outside of Davis to present posters or oral presentation of results.

Supplies & Expendables. Indicate separately the amounts proposed for office, laboratory, computing, and field supplies.

1. Office supplies including telephones, fax, printing,and mailing; surcharge fees for Departmental equipment usage = $2,000.00 per year; 2. Computer upgrades and softwares = $3,000.00 per year; 3. laboratory supplies including chemical reagents for exposing fish and making of fish diets; Laboratory glassware; histological slides and slide boxes, polishing diamond paste and sand papers for otolith preparation, Surcharge fees from CABA at UCD for tanks, water and rooms, surcharges fees for processing tissue samples = $19,000 per year; 4. chemical wastes removal by EH&S = $5,000 per year; 5. publications and poster preparations cost = $1,000.00; 6. LA-ICP-MS analysis at $30.00 per sample 6A (Task 1). Field study: 1620 samples (3 fish species X 30 fish X 6 sites X 3 samples (liver, otolith, and fin ray) $30 X 1620 = 48,600.00 6B (Task 2). Laboratory study: 720 samples (8 concentrations x 3 tissue samples X 30 fish) $30 X 720 = $21,600

Services or Consultants. Identify the specific tasks for which these services would be used. Estimate amount of time required and the hourly or daily rate.

Not applicable

Equipment. Identify non-expendable personal property having a useful life of more than one (1) year and an acquisition cost of more than $5,000 per unit. If fabrication of equipment is proposed, list parts and materials required for each, and show costs separately from the other items.

not applicable

Project Management. Describe the specific costs associated with insuring accomplishment of a specific project, such as inspection of work in progress, validation of costs, report preparation, giving presentatons, reponse to project specific questions and necessary costs directly associated with specific project oversight.

Dr. Swee Teh will be the Principal Investigator. He will work with Dr. Guahua Zhang in coordinating field sampling with Mr. Ted Sommer, Mr. Randy Baxter, Dr. Peter Moyle, and Dr. Silas Hung. Dr. Teh will work with Drs. Zhang, Lutes, and Hung to experimental design tanks and raise fish for laboratory study. 10% of Drs. Teh and Zhang times will be involved in preparation of animal protocols, experimental design,statitical analysis, surpervising technicians and students, submitting quarterly and final report to CALFED, poster presentations and publication of results in peer-reviews scientific journals.

Other Direct Costs. Provide any other direct costs not already covered.

not applicable

Indirect Costs. Explain what is encompassed in the overhead rate (indirect costs). Overhead should include costs associated with general office requirements such as rent, phones, furniture, general office staff, etc., generally distributed by a predetermined percentage (or surcharge) of specific costs.

University charges 10% State and 48.5% Federal overhead for all research perform within the university. Investigators are requires to pay for their own Office supplies (e.g., phone, fax, paper, etc...).

Executive Summary

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

The research proposal objectives are to: 1) determine health indices of bluegill, longfin smelt and splittail from three habitats and life styles 2) determine migratory patterns of splittail and longfin smelt 3) investigate metallic contaminant effects on bluegill, longfin smelt, splittail and sturgeon 4) assist in testing effectiveness of floodplain restoration activities. We predict that by using otoliths and fin rays for age identifications and LA-ICP-MS to determine chemical fingerprinting in growth increments will enable us to reconstruct the migratory pattern of the fish. Hence, allow us to track fish movement based on chemical environments and provide data to their whereabouts from early to adult life stages or in between spawning. Laboratory exposure provides specific bioindicators of metal exposure help to evaluate and validate field exposure. An integrated health response with respect to metallic contaminants exposure will be derived by incorporating liver and gonads metal concentrations, otolith and fin rays elemental fingerprinting, histopathology, and conditional index into multivariate analyses, will provide site-specific health index of each fish species. Integration of these results with ongoing efforts by CALFED-funded IEP studies to monitor effort of improving Bypass floodplains and to survey fish populations will enable us to evaluate the health of floodplain habitats. Geographic locations selected will be Sutter and Yolo Bypass, vicinity of Sacramento-San Joaquin Rivers adjacent to the Bypass, Suisun Marsh, Cosumnes River floodplain, and areas of the Delta adjacent to Cosumnes and Mokelumne rivers. This project fits well with the long-term goal of CALFED Science Program of building a body of knowledge to continually improve the effectiveness of restoration actions, goal section-3406(b)(1) of CVPIA to identify adverse environmental effects of central valley project, address priorities issues developed in MR-5&-6, SR-7, SJ-3&-5, DR-2,-4&-6, BR-5, and actions and Goals-1,-4, and-6 of ERP.

Proposal

University of California, Davis

ASSESSING HEALTH INDICES AND MIGRATORY PATTTERNS OF FISH TO SUPPORT RESTORATION EFFORT

Swee Teh, University of California, Davis
Guohua Zhang, UCD
Ted Sommer, DWR
Randy Baxter, CDFG
Silas Hung, UCD
Peter Moyle, UCD
Paul Lutes, UCD

TITLE: ASSESSING HEALTH INDICES AND MIGRATORY PATTERNS OF FISH TO SUPPORT RESTORATION EFFORT

A. Project Description:
1. Problem

Fish of the Sacramento and San Joaquin Estuary are potentially exposed to a large variety of contaminants whose combined effect(s) on reproductive and developmental parameters are largely unknown. State and Federal biologists have difficulty in assessing the reproductive and developmental parameters in endangered and threatened fish species because of the lack of information on the habitats and spawning behavior of these fishes. Hence, little has been done examining morphological abnormalities in local fish exposed to environmental contaminants in field study. Reproductive and developmental parameters are among the most important sentinel endpoints for assessing exposure to contaminants. It is clear that contaminants that adversely affect reproductive and developmental parameters ultimately affect populations as well. In many locations where fish populations are in severe decline or individuals are experiencing decreased growth, survival and reproduction, contaminants (including endocrine-modulating compounds) are known to be present; however, little research has been done to identify the role of contaminants and endocrine-modulating compounds. It is important to realize that fish are exposed to a bewildering array of natural stressors and low levels of complex toxicants throughout their life history. It is known that fish accumulate pollutants in body tissues as they grow, so the deleterious effects may become apparent only when concentrations in tissues reach a threshold level after several months or years. Alternately, a toxicant present at low levels may be lethal only to very early life history stages. A decline in population due to contaminants may therefore become apparent only several years after a pollution incident when low numbers of a particular year-class is recorded. It is usually difficult to relate the health effects to the time of contaminant exposure without tracing back through life history, i.e., where the fish were roaming several months and years ago.

Inundation of the Yolo Bypass floodplain has been found to be an important seasonal spawning and rearing habitat for several federally listed and sport fish. (Sommer *et al*., 1997). As a result, the CALFED Estuarine Restoration Program includes Yolo Bypass as a key area for habitat management and restoration. Ranges of actions are being considered including improved floodplain-river connectivity and managed inundation of floodplain habitat in dry years. The hypothesis that managed flooding can be used to support native fish production was recently tested using a model floodplain by Sommer and associates (in preparation), who found that Sacramento splittail (*Pogonichthys macrolepidotus*) will spawn in a dry year if appropriate spawning condition is available. From the risk assessment standpoint, it is critical to investigate the health of the aquatic organisms using the bypass and similar habitats because there is a possibility that the spawning ground may contain unforeseeable problems such as the influx of metals and pesticides from nearby channels or the recycling of contaminants already present in the floodplain.

Therefore, from an adaptive management standpoint of evaluating the benefits of restoration efforts, assessing the health of fish and relating the deleterious effects to long-term contaminant exposure to populations should not be neglected.

The specific goals of the present proposal are:
1) to assess the health indices in three different habitats' fish species [bluegill (freshwater), splittail (freshwater/estuary), and longfin smelt (estuary/marine)] collected from various locations in the Sacramento/San Joaquin Rivers, Bay-Delta regions, and Sutter and Yolo Bypass floodplains
2) to develop otolith and fin ray trace elemental fingerprinting using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) technique
3) to determine the deleterious health effects of metallic contaminants under laboratory and field study
4) to integrate otolith and fin ray chemical profiles to regional differences in chemical environment and metal concentrations in liver and gonads to the health of fish at different habitats,
5) to validate effects of metal pollutants on fish health as a function of habitats and fish's life style
6) to determine the migratory patterns of splittail and longfin smelt

The objectives of this proposal are to demonstrate the use of health indices on three different habitats fish species to assist in testing the effectiveness of Sutter and Yolo Bypass floodplain restoration activities, to determine migratory pattern of splittail and longfin smelt, and to investigate the metallic contaminant effects on fish health. An integrated health response of fish with respect to metallic contaminants exposure at each collection site will be derived by incorporating liver and gonadal metal concentrations, otolith and fin rays elemental fingerprintings, histopathology, and conditional index into multivariate analyses. This will provide us with site-specific health index of each fish species. Integration of these results with ongoing efforts by CALFED-funded IEP studies [Sommer (DWR), Baxter (DFG), and Moyle (UCD)] to monitor effort of improving Bypass floodplains and to survey fish populations will enable us to evaluate the health of floodplain habitats.

The hypotheses to be tested in this study are:

H1: The inundation of Yolo Bypass floodplain has no short- or long-term health effects on fishes caused by uptake of contaminants.

H2: There are no sites differences in fish health indices between fish using the Yolo Bypass and fish in the nearby Sacramento-San Joaquin River or Bay-Delta.

H3: There is no difference in otolith and fin rays elemental fingerprinting among fish from freshwater, freshwater/estuary, and estuary/marine.

H4: Uptakes of metal and age lines in otoliths are not affected by temperature changes; therefore, the deleterious health effect on fish is not related to temperature changes.

2. Justification

The use of fish health and migratory pattern in coordinated environmental monitoring programs can be a valuable, cost-effective approach for assessing the exposure and effects of environmental contaminants and can help in evaluating restoration efforts. Preliminary laboratory studies of splittail (CALFED project# 99-N07) have provided strong evidence that selenium induced teratogenesis (loss of tail, kyphosis, lordosis, and scoliosis) in splittail embryos during musculoskeletal development (Teh *et al.,* 2001). Morphological abnormalities (kyphosis, lordosis, and scoliosis) were also seen in splittail larvae that have survived the 96 hr diazinon (1.25 ppm) acute toxicity testing. Chronic selenium-dietary exposure of juvenile splittail revealed significantly slower growth and increased incidences of intersex fish in higher dose groups (Figure 1). Field investigations shown that apparently healthy adult splittail collected at several locations in the Sacramento-San Joaquin Rivers were found to bioaccumulate selenium to concentrations of as high as 22 $\mu g.g^{-1}$ in liver and 59 $\mu g.g^{-1}$ in gonads. Histological analysis revealed primordial germ cell necroses and inflammations in the gonads of some fish. Results from the combined laboratory and field studies indicated that bioaccumulation of high levels of selenium seems to have little or no direct toxic effects on adult fish but have profound effects on reproductive success and development of the embryo. These results suggest that pollutant effects on fish health need to be evaluated in field monitoring programs. In response to the priority issue MR-5 and specific ERP goals 1, 4, and 6, such evaluations would help to ensure that restoration is not threatened by degraded environmental water quality.

One of our objectives in TASK 1 of this study is to assess the health indices in three fish species (bluegill, longfin smelt, and splittail) collected from various locations in the Sacramento and San Joaquin Rivers, Bay-Delta regions, and floodplains. Conditional factors (length and weight), metal concentrations (bioaccumulation) in liver and gonads, histopathology of gill, liver, kidney, and gonads will be determined in each fish. The purpose is to define the health differences in fish from three different habitats (freshwater, Freshwater/estuary, and estuary/marine). Although the principal objective of this study does not focus on organic endocrine disrupting chemicals, use of histopathologic biomakers, a component of the health indices, is the most direct method of determining the irreversible effects of endocrine disrupting compounds. Other than seeing direct damage such as atresia of oocytes, necrosis of spermatogonia, and intersex in male and female fish, it is often difficult to prove to what extent the damage affects such functions as hormonal and enzyme imbalances in response to endocrine-modulating compounds. Liver and blood samples will be archived for future choriogenin and vitellogenin determination if intersex are identified in fish.

Otoliths (earstones) have been used by State and Federal fish biologist as indicators of metabolically inert timekeeper (fish aging) and environmental recorder. Because of their metabolically inert nature, otoliths combine time-keeping properties with storage of geochemical information. They are therefore ideal material to track the movements of individual fish exposure to different environments (Campana et al, 1994; 1995). Measuring concentrations of various elements in tissues has widespread utility. The successful reconstructions of the fish's life history such as temperature history (Patternson *et al.,* 1993), detection of anadromy (Kalish, 1990; Secor, 1992; Limbury, 1995), determination of migration pathways (Thresher *et al.,* 1994; Thorrold *et al.,* 1997) and stock identification (Edmonds *et al.,* 1989) have been reported using isotope analysis. Some trace elements such as Lanthanum (La), Samarium (Sm), Cerium (Ce), Barium (Ba), Mercury (Hg) and Manganese (Mn) have shown the consonance between the increased concentrations in the otolith and elevated concentrations in fresh water after exposure (Ennevor and Beames 1993; Geffen *et al.,* 1998). Single-element additions of Strontium (Sr), Cadmium (Cd) and Ba to salt water also resulted in significant incorporation into the otolith (Campana 1999). Zhang (2000) reported that concentrations of lead (Pb) in the pectoral fin rays of Chinese sturgeon were several hundred to even thousand times higher than in otoliths of other fish species from similar locations, indicating that fin rays could also absorb harmful heavy metals. Hence, the addition of fin rays as a substitute for otoliths proved feasible especially, when otoliths are not present, e.g., in sturgeon. Therefore, we will include fin rays in addition to otoliths for aging and elemental fingerprinting to ensure that pollutant exposure was not missed.

Our preliminary study using otoliths and fin rays in laboratory and field splittail revealed significant findings. Table 3 and Figure 2 revealed significant elevated Se concentrations, i.e., Se/Ca ratio was 1-2 order of magnitude higher in the highest Se concentration group than in the control group after a 9-month Se dietary exposure. Figure 3A showed profile of ^{88}Sr: ^{44}Ca and ^{138}Ba: ^{44}Ca ratios from the center to the edge of otolith of a wild splittail collected from Sherman Island. This profile provides evidence that the splittail are migrating from low-Sr, high-Ba environment (freshwater) to high-Sr, low-Ba environment (estuary). Additional analysis revealed elevated ^{88}Cr:^{44}Ca, ^{202}Hg:^{44}Ca and ^{208}Pb: ^{44}Ca ratios indicating higher levels of Cr, Hg, and Pb in the estuary (Figure 3B). These results revealed the possibility of using otolith and fin rays as indicators of past metal exposure. Therefore, the second objective of the TASK 1 study is to develop a chemical indicator of past chemical exposure using otolith and fin rays. We postulate that bluegill (freshwater), longfin smelt (estuary/marine), and Sacramento splittail (freshwater/estuary) would have distinctive patterns of chemical indicators because of their differences in food intake and metal concentrations in freshwater, estuarine, and marine habitats. When otolith and fin rays growth increments (age lines) can be determined, chemical profiles within each increment will be analyzed using LA-ICP-MS for elemental composition. We predict that otolith and fin rays not only can help us to determine fish age, but also can be used to reconstruct migratory patterns and past metal exposures of fish.

Trace metals are of biological interest because of their role as micronutrients and toxicants. Trace metals enter the San Francisco estuary from a wide variety of urban and agricultural runoff, industrial effluents, old mines, and from weathering of soils and rocks within the watershed; thus metal bioaccumulation patterns are complicated. Report of CVRWQB, 1998 has shown that there can be high regional differences in the chemical environment within the Sacramento/San Joaquin Delta with concentration differences of 27-300X between the lowest and highest sites; temporal differences at the same site vary only 2-15X, much lower than regional differences (Table 1). Brown and Luoma (1995) was the first to demonstrate that Asian clam is an effective biosentinel for studying processes controlling trace element contamination and bioavailability as well as environmental differences in trace metal exposure. The metallic contaminants effects on reproduction in *Potamocorbula amurensis* and *Macoma balthica* have been studied (Brown and Luoma, 1998; Hornberger *et al.,* 1999). Concentrations in clam tissues indicated some distinct metal contamination gradients through the North Bay (Brown and Luoma, 1998). Table 2 showed the significances differing body burdens of metallic contaminants in clams collected from four USGS stations for the past ten years (Ms. Cindy Brown, USGS). Because splittail and white sturgeon feed extensively on benthic invertebrates (including clams), it is believed that these fish will bioaccumulate trace metal in tissues to levels that may affect their health (growth and reproduction). These differences in metallic contaminant concentrations among various sites in the Sacramento-San Joaquin Rivers and Delta provide a sound foundation for using otolith and fin rays as indicators of past chemical exposure in the fish.

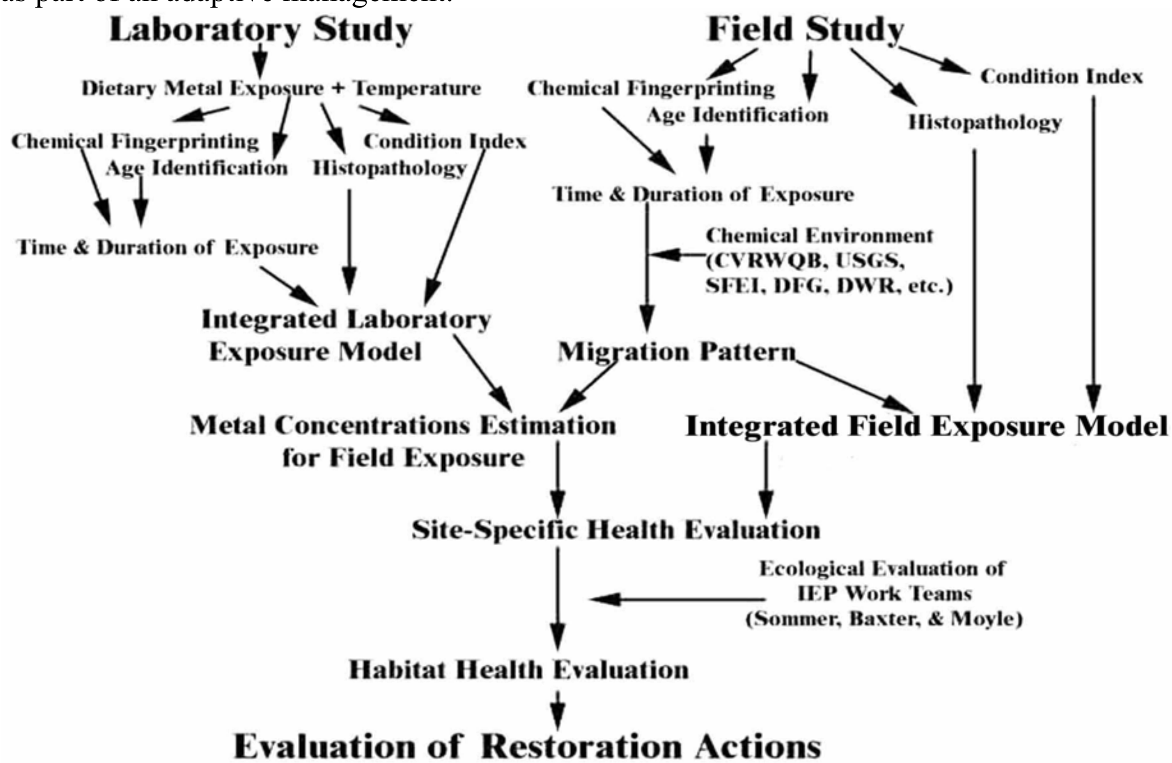
We therefore propose in TASK 2 to perform laboratory exposure of fish to different concentrations of metals, under different temperature conditions. In the laboratory, we can develop a detailed record of the environment to which the fish was exposed and then examine the element composition of otoliths and fin rays using LA-ICP-MS. In addition, the synergistic effects of various metals will be investigated using health indices approach as described in TASK 1. Our justification of using laboratory Purified Casein diet instead of natural food (e.g., Asian clam) for laboratory study is the presence of unknown contaminants in the clams. The justifications of using white sturgeon (*Acipenser transmontanus)* in laboratory study are: 1) as a surrogate species for the threatened green sturgeon (*A. medirostris)* to study the effect of metallic contaminants, 2) to explore the possibility of using fin rays as future elemental fingerprinting for green and white sturgeon. However, green sturgeon will be used if it is available at UCD. Finally, we propose to study the effects of temperature on metal exposures is because temperature affects the bioaccumulation of metals through regulating the metabolism of fish (Radtke *et al*., 1990; Campana, 1999), therefore, it may have significant effects on accumulation of metals in otoliths and fin rays especially when contaminant levels are very low.

It is anticipated that laboratory evaluations in TASK 2 will provide information on temperature and contaminant effects on the health of splittail and sturgeon. TASK 2 will help to establish a linear relationship between dietary concentrations to accumulation of metals in liver, otolith and fin rays (see flow chart below). When age lines of the fish can be determined, elemental fingerprinting will provide chemical profiles linking time to metal exposures and health effects. The data collected will be used to compare and evaluate the findings of our field study (TASK 1).

2A: Conceptual model

There are growing interests in using biomarkers to indicate environmental health and safety (Luoma and Fisher, 1997). These biomarkers include soft tissues, hard tissues and chemical indicators (Radtke, 1989; Campana, 1999). Our conceptual model is outline below. We predict that by using otoliths and fin rays for age identifications and LA-ICP-

MS to determine chemical profiles or fingerprintings in growth increments will enable us to reconstruct the migratory pattern of the fish. Furthermore, by restructuring the migratory pattern of fish, we may be able to track the fish (Splittail) movement around the San Francisco estuary based on food and water chemical environment (CVRWQB, 1998; Brown and Luoma 1998) and provides data to their whereabouts from early to adult life stages or in between spawning. Laboratory exposure model provides specific bioindicators of metal exposure will help to evaluate and validate field exposure model. An integrated health response of fish with respect to metallic contaminants exposure at each collection site will be derived by incorporating liver and gonadal metal concentrations, otolith and fin rays elemental fingerprintings, histopathology, and conditional index into multivariate analyses. This will provide us with site-specific health index of each fish species. Integration of these results with ongoing efforts by CALFED-funded IEP studies [Sommer (DWR), Baxter (DFG), and Moyle (UCD)] to monitor effort of improving Bypass floodplains and to survey fish populations will enable us to evaluate the health of floodplain habitats. Such abilities are essential to evaluate restoration efforts as part of an adaptive management.



2B. Scope of Work

2B1. Geographic Boundaries

This work will be conducted in conjunction with Mr. Ted Sommer (DWR), Mr. Randy Baxter (DFG), Dr. Peter Moyle (CALFED), and Dr. Silas Hung (CALFED 99-N07). Therefore, we will work under their Endangered Species Act take permits. The field sampling locations selected will be within Sutter and Yolo Bypass, and the vicinity of the Sacramento- San Joaquin rivers adjacent to the Bypass, as well as Suisun Marsh, the Cosumnes River flood plain, and areas of the Delta adjacent to the Cosomnes and Mokelumne rivers (e.g., Snodgrass Slough).

2B2. Selection of fish species

1. Field study: Three fish species, Bluegill, *Lepomis macrochirus* (freshwater), longfin smelt, *Spirinchus thaleichthys* (estuary/marine), and Sacramento splittail, *Pogonichthys macrolepidotus* (fresh/estuary) will be selected for this study. The reasons for selection of such species are based on: 1) bluegill is a introduced resident fish that lives its whole life within an area (stationary fish) while Sacramento Splittail and longfin smelt are native fish that live in freshwater/estuary and estuary/marine habitats during different periods of life (migratory fish). 2) By comparing fishes among the freshwater, estuary/marine, and freshwater/estuary habitats, the success of identifying pollutant effects and tracing the migratory pattern of the fish is higher because there is a unique chemical pattern in the otolith and fin rays of freshwater/estuary (splittail) that is not found in either freshwater (bluegill) or estuary/marine fish (longfin smelt).

2. Laboratory Study: splittail and White sturgeon, *Acipenser transmontanus* (as surrogates for green sturgeon, *Acipenser medirostris*) and were selected because of their availability at Center of Aquaculture and Aquatic Biology at UCD for experimental study. Although not territorial, the majority of their lives (except for annual spawning migrations in freshwater) are spent in intimate contact with contaminated sediments and waters of the Estuary. Because splittail and sturgeon feed extensively on Asian clam (Ms. Robin Steward, USGS), the laboratory exposures will base on metal concentrations determined by Ms. Cindy Brown (USGS) in Asian clams (Table 2).

2B3. Condition Index, Histopathology, and chemical indicators

1. Condition Index: Gross measurements and weights will be used to determine condition index (CI), gonadosomatic (GSI) and hepatosomatic (HSI) indices in fish. CI is a measure of "plumpness" and defined as (body weight in grams)(100) / length in cm3. GSI is the gonad to body weight ratio and HSI the liver to body weight ratio. All three indices are broad measures of general health. Changes in CI specifically reflect alterations in growth and nutritional status, while fluctuations in GSI are associated with sexual maturity and reproductive status. Differences in HSI may reflect sex, sexual maturity, or general health and nutrition. Contaminant-induced alterations of somatic and/or gonadal growth will therefore be reflected by changes in these indices. Adverse effects may be detected by analysis of liver and gonads. Gonadosomatic (100 X gonad weight /body weight) and hepatosomatic (100 X liver weight/body weight) indices have proven to be sensitive and simple indicators of responses when compared in fishes from contaminated versus reference sites. One example has been the findings of decreased hepatosomatic (HSI) and gonadosomatic indices (GSI) in fishes living in waters receiving effluents from pulp and paper mills (Jobling, *et al.,* 1996).

2. Histopathology: Histopathology has been recommended as a physiological approach to pollution investigation (Sindermann, 1985). Histopathology has been used with both fish and aquatic invertebrates for the assessment of contaminant-mediated adverse effects (Adams *et al.,* 1989 & 1999; Teh *et al* 1997; 1999). Histologic lesions can be examined in a tissue specific manner, allowing identification of target organs for specific xenobiotic

compounds (Wester and Canton, 1986). Histologic damage in reproductive organs can be directly linked to population level effects, and detection of lesions in early life stages (embryos and larvae) of fish and other aquatic organisms is considered one of the most sensitive means of assessing adverse effects induced by xenobiotic compounds (Weis and Weis, 1987; Teh *et al.,* 1999). Histopathologic analysis is particularly relevant to field investigations (Meyers *et al.,* 1994), allowing rapid detection of in vivo toxicity, thereby helping to prioritize sites for more detailed analysis. Direct histological damage of the liver and gonads has been documented in several studies involving heavy metals, pesticides, and pulp and paper mills effluents (Singh *et al.,* 1994; Teh *et al.,* 1997; 1999). Histopathology is the most direct method of determining the irreversible effects of endocrine disrupting compounds. Because other than seeing direct damage such as atresia of oocytes, necrosis of spermatogonia, and intersex in male and female fish, it is often difficult to prove to what extent the damage affects such functions as hormonal and enzyme imbalances in response to endocrine-modulatin compounds. Therefore, HSI and GSI when coupled to histopathologic analysis of the liver and gonads, effects of endocrine-modulating compounds may be detected and quantified.

<u>3. Chemical Indicators</u>: We will use LA-ICP-MS to probe the otoliths and fin rays of fish. This technique combines low detect limits (0.1-0.01mg.g^{-1}), wide dynamic range (ng.g^{-1} to %) with spatial resolution of < 10 µm (Wang *et al.,* 1994). There are consistent differences in the composition of the otoliths for fish inhabiting different areas, especially Sr which is proven elemental indicators of anadromy between freshwater and saltwater environments (Kalish, 1990; Secor *et al.,* 1995; Tzeng *et al.,* 1997; Tsukamoto *et al.,* 1998). After the introduction of LA-ICP-MS, more elements and isotopes have been proven capable of being indicators of spatial differences of fish. For example, Barium (Ba) is also an indicator of anadromy (Thorrold *et al.,* 1997; Zhang, 2000). We will use at least 28 elements published in the literature as our target elements, then select elements that can indicate elemental differences of fish from different locations in the Sacramento-San Joaquin estuary and floodplains. Subsequently, according to these patterns, we will determine where the fish have been and the types of pollutant the fish have been exposed to by checking elemental trajectories in fish otoliths.

3. Approach.

An integrative approach utilizing health indices, otolith and fin ray elemental composition analysis (fingerprinting), and liver and gonadal metal bioaccumulation analysis was selected for this study because of the need to resolve confounding factors (natural and anthropogenic) common to all complex ecosystems. Histopathologic biomarkers can be highly sensitive indicators of individual health, and help identify target organs and biological effects. Liver and gonadal metal bioaccumulation analysis helps identify contaminant type and concentration. Otolith and fin rays analyses provide age identifications and elemental fingerprinting of past metal exposure, which can help in reconstructing migratory patterns of fish. Finally, an integrated health response of fish with respects to metallic contaminants exposure at each collection site will be derived by incorporating liver and gonadal metal concentrations, otolith and fin rays elemental fingerprintings, histopathology, and condition indices into a multivariate discriminant analysis. This will provide us with site-specific health index of each fish species. Results of this study, in conjunction with studies proposed by IEP work team (Mr. Randy Baxter and Mr. Ted Sommer), CALFED funded project# 99N07 and ongoing studies of Dr. Moyle), will be used to evaluate the health of fish in different water bodies that cross multiple regions can help in develop a fish model for future contaminant monitoring study. In addition, this data may provide important information in assessing effectiveness of shallow water habitat on ecologically important aquatic organisms.

TASK 1: **Assessing health indices and migratory patterns of fish in the Sacramento-San Joaquin Estuary and restoration floodplains**

We propose to determine the health indices of one fresh (bluegill), one estuary/marine (longfin smelt), and one fresh/estuary resident fish (Sacramento splittail). 20-40 adult fish of each species will be collected depend on availability. Target areas of fish collection will be closely related to works conducted by Mr. Ted Sommer, Mr. Randy Baxter, Dr. Peter Moyle and from our ongoing CALFED#99-N07 project. Gross examination of individual fish will be scored and recorded in a Logbook. Observations will be recorded for general conditions, such as presence or absence of fin disease, body and/or mouth sores, and internal and external parasites. Fish will be weighed and measured for a condition index. Liver and gonads will be weighed to determine liver and gonadal somatic indices. Gill, liver, kidney, and gonad will be fixed in 10% buffered formalin for immunohistochemical, and histopathological analyses. Subsamples of liver and gonad will be frozen in liquid nitrogen for metals (ICP-MS) analysis. Otolith and fin rays will be collected for age determination and subjected to LA-ICP-MS elemental fingerprinting.

1. Preparation of liver and gonad for ICP-MS: Liver and gonad will be frozen in liquid nitrogen temperature and stored at -80 ^{0}C freezers for later analysis. Upon analysis, tissues will be vacuum dried, weighed and digested in nitric acid following the methods of EPA 1991. The target elements will be analyzed using ICP-MS (See below).

2. Preparation of Otolith and fin ray for LA-ICP-MS: Otoliths and fin rays will be removed from each fish, clean of adhering tissue, and rinsed in distilled water. Tissues will be embedded on a 2.5 cm X 2.5 cm clean plastic microscope cover slip and grounded to the middle plane with wet and dry silicon carbide paper (600-1200 grit). Tissues will then be polished with 30-mm and 1-mm diamond pastes, ultrasonically cleaned in double-deionized water for 5 min, triple rinsed with double-deionized water and air-dry under the clean hood. After drying, tissues will be sealed in paper bags for subsequent LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) assay.

3. ICP-MS and LA-ICP-MS Elemental Analysis: Measurements of otolith and fin ray will be carried out on a Merchantek UV-266-nm Nd:YAG laser sampler and a Finnigan Mat ELEMENT ICP-MS and measurements of liver and gonad will be by Finnigan Mat ELEMENT ICP-MS located in the Beijing Research institute of Uranium Geology. At least 28 previously reported elements including Ag, As, Cd, Cr, Co, Cu, Hg, Ni, Pb and Se will be adopted in our analysis. Calcium will be used as an internal standard and the concentrations of other elements are estimated against the Ca concentration. The

detection limits of the ICP-MS will be calculated using the gas blanks. The core and edge of each hard tissue will be ablated in order to get the elemental data on the natal and captured locations. Transects of each hard tissue also will be ablated to provide the profile of chemical composition change from the time of hatch to the time of sampling.

TASK 2: **Laboratory investigation of metal and temperature stressors effects on white sturgeon and splittail health indices and using otolith and fin rays elemental composition as an indicator of past metals exposure**

We propose to determine the changes of metal concentrations in the tissues of white sturgeon and splittail exposed to different levels of heavy metals and under different temperature regimes. Temperature experiment will be conducted to assess how temperature affects growth hence the concentrations of heavy metals uptake in the tissues. The results will help to test the effects of temperature on growth increments and hence provide critical information on seasonal (temperature) differences in metal uptake in field study. Prior to the start of the experiments, white sturgeon and splittail will be weighed to determine average body weight (BW) in grams. Samples of fish tissues and diet will be taken to verify contaminant levels in diet as well as determine baseline levels in fish. The pattern of metal accumulation in the fish otolith and fin rays will be compared with liver and gonad concentrations at individual fish health level in order to evaluate the metals exposure effects.

1. Metal Stressor Experiments (**Year 1 & 2**): Six metals, selenium (Se), Nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), and silver (Ag) will be used. Dietary exposures were based on metal concentrations in clam tissues (Table 2). Each experiment will last four months with 24 treatments and two replication tanks per treatment. The system consists of 48 circular fiberglass tanks (90 L) and tanks are supplied with aerated 18±1 ^{0}C well water at 1.5 L/min. Initially, 960 juvenile fish (**Year 1**: splittail; **Year 2** Sturgeon) will be transferred from the large tanks and distributed randomly among the 48 tanks at 20 fish/tank. Fish will be exposed to concentrations of metals in the diet as outlined in table 4.

2. Temperature Stressor Experiment (**Year 3**): The system consists of 10 circular fiberglass tanks (90 L) and tanks are supplied with aerated well water at 1.5 L/min. 400 fish will be transferred from the large tanks and distributed randomly among the 10 tanks at 40 fish/tank. Each experiment will last four months with five temperature regimes (10±1 ^{0}C, 14±1, 18±1 ^{0}C, 22±1 ^{0}C, and 26±1 ^{0}C) and each treatment was replicated in 2 tanks. One of the above metal exposure groups with the least fish health effects will be selected for this experiment. The fish will be fed a ration of 5% BW per day with food being divided into two feedings (morning, afternoon). Daily monitoring will include recording water temperature, mortality, as well as flushing individual tanks. Water quality (O_2, ammonia, nitrate, nitrite, pH) will be assessed on a weekly basis. Fish will be weighed every two weeks to adjust ration size.

3. Health indices and LA-ICPMS analyses: See TASK 1.

<u>Integrate Data Analysis of TASK 1 and 2</u>: Statistical analyses will be carried out using parametric and non-parametric multivariate approaches. In order to conform to the statistical assumptions, all elemental data will be initially examined for normality and homogeneity of variance. Elemental differences between locations in field and between treatments in laboratory experiments will be compared by ANOVA. Regression analysis will be used in determining the relationships between exposure concentrations in diet or temperature regime and concentrations in tissues. These laboratory results will be applied in estimating metal concentrations in the field. Comparisons of differences in trajectories across the hard tissues will be made with repeated measures ANOVA. Linear discriminant function analysis (LDFA) and artificial neural networks (ANN) will be used to determine classification accuracy to the fishing location. Most of samples from each location will be used to train the neural network and the remaining samples as a test data set. We will try to track the past migratory history of fish by using these two models. Integrating chemical data, histopathological and condition indices, the canonical correlation analysis (CCA) will be employed to determine which indices are strong factors in affecting site-specific health. Site relationship will be assessed with principle components analysis (PCA) to determine if the sites can be separated into groups on the basis of chemical composition, histopathology and condition indices.

TASK 3: Program Management

Dr. Swee Teh will be the Principal Investigator. He will work with Dr. Guahua Zhang in coordinating field sampling with Mr. Ted Sommer, Mr. Randy Baxter, Dr. Peter Moyle, and Dr. Silas Hung. Dr. Teh will work with Drs. Zhang, Lutes, and Hung to experimental design tanks and raise fish for laboratory study. Standard operating procedures (SOP), QA/QC, and animal uses protocols will be developed before initiation of experiments. The investigators, as university and State employee, are committed to generate data and publications that are accessible to all public. All data analyses and report writing will be in Excel and word format as instructed by CALFED. Data will be stored and backup in three locations: in two computers, zip disks, and CDRoms. Programmatic quarterly and final report in the format described in Attachment F will be submitted to CALFED. CALFED manager will be notified if there are any changes in plan.

4. Feasibility

<u>1. Field Study:</u> Fish samples and Endangered Species Act take permit needed for this study are those already obtained by IEP and CALFED funded works (Mr. Randy Baxter, Mr. Ted Sommer, and Dr. Peter Moyle) and our ongoing CALFED 99-N07 works. As such, we have minimized sampling costs and fish take. Therefore, chances of successful field collecting are high.

2. <u>Laboratory Study</u>: We will use white sturgeon and splittail that are available at UCD. The proposed research will be done at three locations on the campus of UC Davis: the Aquatic Toxicology Program (ATP) laboratory, the Center of Aquaculture and Aquatic Biology Center (CABA), and the Animal Science Nutrition Laboratory (ASNL). Sturgeon and Splittail will be maintained at the CABA facility, which is equipped with a

1500 sq. ft. metal Quonset hut and a 800 sq. ft. laboratory. The Quonset hut is equipped with a flow-through freshwater system, supplying 19 ± 1 ^{0}C well water at a rate of up to 400 L/min to 72 small (90 L) and six large (700 L) fiberglass tanks. Adjacent to the Quonset is an outdoor flow-through system with 16 large (700 L) fiberglass tanks. The 800 sq. ft. laboratory is equipped with electronic balances, refrigerators, freezers, and additional instruments and supplies needed to maintain fish and prepare samples. CABA laboratories, tanks, and animals are monitored on a daily basis by CABA personnel and 24 hrs a day via remote sensing.

All sturgeon and splittail diet formulations (standard and test diets with contaminants) will be prepared in the ASNL laboratory equipped with several feed mixers, a California Laboratory Pellet Mill (and associated steam generator), a high pressure liquid chromatography (HPLC) machine, a gas liquid chromatography (GLC) machine, a spectrophotometer, and an atomic absorption machine. Biochemical, histochemical, and histological studies will be performed in the ATP and CNL laboratories. These laboratories are equipped with one freeze-dryer, a dehydration processor, three microtomes, three high resolution light microscopes with photography attachments, a Bio-Rad 2-D gel electrophoresis systems, and 24-bit digitizer CCD camera, three –20^{0}C freezers, and one –80^{0}C freezers. Based on the available of fish and laboratory for the propose study, we are confident that the tasks proposed in our study can be fulfilled.

5. Performance Measure

There are no universally accepted set procedures for handling tissues samples for histopathologic and chemical biomarker assays. Therefore, we will be developing a QA/QC work plans for tissue samples collection to minimizing sample alteration and errors in data interpretation. QA/QC protocols will also be in place to standardize proper sample collection, storage, and processing. Animal care and maintenance, including monitoring of animal health and water quality, will be overseen by licensed veterinarian and assisted by full-time technical staff of Animal Facility Program at UCD.

The investigators have good records of accomplishment of tasks proposed in their work. They have been participating in services such as providing seminar and presentation to academic and international meeting. We will collect information or reports published by DFG, DWR, SWRCB, SFEI, USGS, and CALFED funded investigators on water quality and contaminants data to further justify our findings. We anticipate a minimum of 6 peer-reviewed papers will be published from the work of this study.

6. Data Handling and Storage

Standard operating procedures (SOP) and animal uses protocols will be developed prior to the initiation of study. The investigators, as university and State employee, are committed to generate data and publications that are accessible to all public. All data analyses and report writing will be in Excel and word format as instructed by CALFED. Data will be stored and backup in three locations: in two computers, Zip disks, and

CDRoms. Quarterly and final report will be submitted to CALFED. CALFED manager will be notified if there are any changes in plan.

7. Expected products/Outcomes

We believe that the use of fish health and migratory pattern in coordinated environmental monitoring programs can be a valuable, cost-effective approach for assessing the exposure and effects of environmental contaminants and help in evaluating restoration efforts. The assessing and evaluating the health indices of fish in the Yolo and Sutter floodplain will provide additional information as to the beneficial effort of floodplain restoration. Furthermore, by restructuring the migratory pattern of fish, we may be able to track fish movement around the Sacramento and San-Joaquin estuary and provide data to their whereabouts from early to adult life stage or in between spawning. This data will be valuable asset to State and Federal fish biologist of ecological interest. If successful, the results obtained from the proposed studies will substantially enhance current understanding of splittail migratory patterns. Furthermore, assessing the health indices of fish from three habitats and life style, will allow rapid detection of in vivo toxicity, thereby helping to prioritize sites for more detailed contaminant analysis.

We expect that the laboratory exposures will provide us with detailed information regarding contaminant responses to metals and will help refine assay protocols and define target organs and biological effect in splittail and white sturgeon species. Together, laboratory exposures and field surveys are expected to generate a wealth of new information regarding the response of fish to metallic contaminants. More importantly, we expect the study to validate an approach utilizing otolith and fin rays composition elemental analyses to develop specific chemical profiles, thereby linking contaminant exposure to adverse effects. As more data are developed and added to the diagnostic suite, response profiles will become even more unique, strengthening the link between specific contaminants and lesions in target species.

In summary, we believe that the study approach has broad application to a wide variety of impacted aquatic environments and that response profiles generated by this study could be used to evaluate contaminant-mediated effects in other aquatic fish and ecosystem. Although data generated by this study will reflect adverse effects in individual fish, we also expect to be able to correlate patterns of otolith and fin ray elemental profiles to the migratory pattern of splittail. An integrated health response of fish with respect to metallic contaminants exposure at each collection site will be derived by incorporating liver and gonadal metal concentrations, otolith and fin rays elemental fingerprintings, histopathology, and conditional index into multivariate analyses. This will provide us with site-specific health index of each fish species. Integration of these results with ongoing efforts by CALFED-funded IEP studies [Sommer (DWR), Baxter (DFG), and Moyle (UCD)] to monitor effort of improving Bypass floodplains and to survey fish populations will enable us to evaluate the health of floodplain habitats. Results of this study could also help guide management decisions with respect to determining acceptable contaminant levels in the environment, migratory patterns of at-risk fish species, and used to evaluate progress of restoration efforts. For mutual benefit, we will incorporate our findings into the collaborators' analysis packages to optimize information on contaminant effects on fish at shallow water habitats

8. Work Schedule: A three-year funding beginning on July 2002 is requested for this study. Task 1 and 2 can be separated if funding is limited.

TASK 1 (3 YRS): Drs. Teh and Zhang with the helps of technicians and students will work closely with Mr. Sommer, Mr. Baxter, & Dr. Moyle to collect field samples and process fish for condition index, histopathology, and chemical indicators.

TASK 2 (3 YRS): Drs Teh and Zhang with the helps of technicians and students will work closely with Dr. Hung and Lutes for laboratory exposure setup and culturing of splittail and white sturgeon (W**e will use green sturgeon if it is available for the study**). Dr. Zhang will be responsible for the experiment design and data collection. He will be assisted by technicians and student assistants.

TASK 3 (3 YRS): Drs Teh and Zhang will be responsible for animal protocols, OA/OC, quarterly and final reports, poster presentation, and published data in scientific journals.

STUDY	2002 JJASONDJFMAM	2003 JJASONDJFMAM	2004 JJASONDJFMAM
TASK 1	XXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXX
TASK 2	XXXXXX	XXXXXXXX	XXXXXXXX
TASK 3	X X XXXX	X X X XXXX	X X XXXXXXX

B. Applicability to CALFED ERP and Science Program Goals and Implementation Plan and CCPIA Priorities.

B1. ERP, Science Program and CVPIA Priorities.

Since three species of fish (freshwater, estuary/marine, and freshwater/estuary) at the Sacramento-San Joaquin regions and Bay-Delta regions were used and the results will provide direct evidence of pollutant effects in at-risk and native fish species. This proposal address priorities issues developed in MR -5 & -6, SR-7, SJ-3 & -5, DR-2, -4 & -6, BR-5, and actions and Goals-1, -4, and-6 of ERP.

The use of fish health and migratory pattern in coordinated environmental monitoring programs can be a valuable, cost-effective approach for assessing the exposure and effects of environmental contaminant and the outcome will help in evaluating restoration effort. Therefore, this research proposal fit well with the long-term goal of CALFED Science Program of building a body of knowledge that will continually improve the effectiveness of restoration actions.

One of the focuses of this proposal is to investigate the pollutant effects on endangered and threatened fish species in Bypass floodplain. This fit well with the goal section 3406 (b) (1) of the CVPIA to identify adverse environmental effects of the central valley project.

B2. Relationship to other Ecosystem Restoration Projects.

The results obtained from the proposed studies will substantially enhance current understanding of splittail migratory patterns and the beneficially of floodplain habitat. Assessing the health indices of fish from three different habitats and life style, will allow rapid detection of in vivo toxicity, thereby helping to prioritize sites for more detailed contaminant analysis. All are valuable information to currently or future funded ecosystem restoration projects. As stated earlier, healthier fish means healthier ecosystem, which implies that, the restoration project work.

B3. Requests for next-phase funding. No.

B4. Previous Recipients of CALFED Program or CVPIA funding.

CALFED 99-N07 (Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (*Pogonichthys macrolepidotus)*: A Biomarker Approach: Hung, Teh, and Davis. 65% complete. We have completed two seasons of field sampling and three laboratory studies. Two papers have been submitted for publication. In the final year of this project, we will focus on analyzing field samples for organochlorines and heavy metals and compares the chemical data to the biochemical and histopathological indicators. Currently, we are working on the dietary exposure of juvenile splittail to various concentrations of Selenium. Otolith and fin rays collected from this project will be subjected to elemental analysis to minimize sampling cost, if current proposal is funded.

CAlFED-B81650. (Role of contaminants in the decline of Delta Smelt in the Sacramento-San Joaquin Estuary). Bennett, Teh, and Anderson. 90% complete. This project has developed tools for quantifying the potential effects of poor food supply and contaminant exposure on the growth and survival of individual delta smelt collected in the IEP abundance surveys. Final report to be submitted to CALFED in October 2001.

B5. System-Wide Ecosystem Benefits.

Several CALFED and CVPIA funded projects will provide valuable information on water, tissues and sediments contaminant analyses on websites (e.g. IEP, USGS and SFEI). The proposed field survey work will closely coordinated with their actions. This study will help to establish causative links between contaminant exposure and biological effect in the Sacramento-San Joaquin regions, Bay-Delta regions, and floodplains and will pioneer an approach that can generally be applied to other aquatic ecosystems.

C. Qualifications:

1. The lead investigator, **Dr. Swee J. Teh**, is a research toxicology and pathology faculty at UC Davis, Dept. of Anatomy, Physiology and Cell Biology and has over 14 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.

2. Dr. Guohua Zhang is a postdoctoral researcher currently working in Dr. Swee Teh's laboratory and has 12 years of extensive field and laboratory research experience in fish ecology and biomarker studies. His research experience is focused on the impacts of human activities and natural processes on the habitats and biota of rivers and lakes in China, aquatic ecosystem conservation and restoration strategies, and conservation biology of endemic fishes. Most recently, His research interests are the uses of the hard tissues in stock discrimination and toxicological studies.

PUBLICATIONS: Selected from 15 peer-reviewed publications:

Zhang, G., J. Chang and G. Shu, 2000. Application of factor-criteria reconstruction analysis in the reproduction research of grass carp, black carp, silver carp and bighead in the Yangtze River. International Journal of General Systems, 29(3): 419-428

Zhang, G., S. Dan, Z. Miao and S. Deng, 1999. Otolith morphology of six cyprinid species in species and stock discrimination. Acta Hydrobiologica Sinica, 23: 683-688.

Zhang, G., Z. Deng, Y. Xu, Z. Miao, J. Chang and T. Song, 1998. Survey on the number and catches of weirs in Honghu Lake. Journal of Lake Sciences, 10(3): 68-74.

Zhang,G., W. Cao and Y. Chen, 1997. Effects of fish stocking on lake ecosystems in China. Acta Hydrobiologica Sinica, 21(3): 271-280.

Zhang,G., 2000. Otolith morphology and elemental composition with the application in stock discrimination of fish. Doctoral dissertation, Institute of Hydrobiology, Chinese Academy of Science, China.

3. Mr. Ted R. Sommer

EDUCATION: 1980 BA Aquatic Biology, UC Santa Barbara; 1983 MS Ecology, UC Davis. Presently completing PhD at UC Davis.

EXPERIENCE:1991-present Environmental Specialist, Depart. of Water Resources; 1985-1990; Senior Scientist, Western Biotechnology, Australia.

Certified Fisheries Scientist: American Fisheries Society 1994.

PUBLICATIONS: Selected from 20 peer-reviewed publications

Sommer, T., R. Baxter, and B. Herbold. 1997. The resilience of splittail in the Sacramento-San Joaquin Estuary. Transactions of the American Fisheries Society 126:961-976.

Sommer, T. R., W. C. Harrell, M. Nobriga, R. Brown, P.B. Moyle, W. J. Kimmerer and L. Schemel. 2001. California's Yolo Bypass: evidence that flood control can be compatible with fish, wetlands, wildlife and agriculture. Fisheries 26(8):6-16.

Sommer, T. R., M. L. Nobriga, W. C. Harrell, W. Batham, and W. J. Kimmerer. 2001. Floodplain rearing of juvenile chinook salmon: evidence of enhanced growth and survival. Canadian Journal of Fisheries and Aquatic Sciences 58(2):325-333

4. Mr. Randall D. Baxter, Senior Biologist Supervisor, California Department of Fish and Game, currently supervising the long-term fish monitoring studies conducted by the Department as part of the Interagency Ecological Program. Previous work includes eight years of study on the biology and ecology of Sacramento splittail and six years of study directed at a wide variety of freshwater, estuarine and marine fishes as part of the Delta Outflow San Francisco Bay Study.

5. **Dr. Silas S.O. Hung**, Professor of Animal Science Department, UCD has 24 years of extensive experiences in nutritional biochemistry and physiology. He has worked on nutrition, biochemistry, and culturing of many species of fish including rainbow trout, American eel, white sturgeon, Chinese sturgeon, triploid grass carp, common carp (growth hormone transgenic and non-transgenic), Atlantic salmon, hybrid tilapia, and Sacramento splittail. He has published more 70 papers in these areas. He is considered the world leading expert in the nutrition and feeding of sturgeon. Since 1983, he has published more than 40 papers in the nutrition and feeding of white sturgeon including one review paper and two book chapters. Dr. Hung has established an extensive network and collaborative research with scientists from other countries such Canada, China, Japan, Korea Norway, Sweden, and Taiwan.

6. **Dr. Peter B. Moyle** is a Professor of Department of Wildlife, Fish, and Conservation Biology. His research interests are in conservation of aquatic species, habitats, and ecosystems, including salmon; ecology of fishes of Sacramento-San Joaquin estuary; ecology of California stream fishes; impact of introduced aquatic organisms; use of flood plains by fish. Dr. Moyle has author or co-author of over 170 publications (mostly on fish in California), including five books/monographs. For a more complete lists, see http//wfcb.ucdavis.edu/www/Petermoyle/default.htm.

7. **Dr. Paul Lutes** is an Aquatic Animal Research Facilities manager; graduate studies in Physiological Ecology/fish reproduction; currently managing a two unit aquatic animal research facility focusing on aquatic toxicology, fish reproductive physiology, physiological ecology of fishes, fish behavior and neurobiology, fish nutrition, aquatic infectious diseases; supported recent/current facilities research activities including micro/macro-nutrients requirements in white sturgeon, whirling disease in salmonids, green sturgeon spawning and early rearing, surface water environmental monitoring, impact of agricultural water diversions on local fish species, herpes virus infection in white sturgeon and koi carp, selenium toxicity in white sturgeon juveniles, and olfaction in coho salmon; publications pertaining to final oocyte maturation in white sturgeon and white sturgeon nutrition; managed facilities include approximately 100,000 gallons total holding capacity in various tanks (5 to 7,000 gallon individual capacity), 3 wells totaling 2,000 gallons per minute in capacity, and water heating/chilling/ filtration/recirculation systems.

D. Cost: D1. Budget: See Web forms**.**

No budgets are required for Mr. Sommer (DWR), Mr. Baxter (DFG), and Drs. Hung, Lutes, and Moyle (UCD) as their salaries were covered by other projects and University.

D2: Cost-Sharing: No cost-sharing.

E. Local Involvement

This is a research project where 75% of the work will be conducted within a research university at UCD. As such local, environmental, landowner, conservancies groups were not aware of this project. This project, in conjunction with ongoing IEP work team of biomonitoring efforts of fish population by Mr. Randy Baxter (DFG) and Yolo Bypass floodplain by Mr. Ted Sommer (DWR), and multi-regional fish monitoring efforts by Dr Moyle, to assess health indices and migratory patterns of three fish species. The project can be a valuable, cost-effective approach for assessing the exposure and effects of environmental contaminant and help in evaluating restoration effort. We will be working in close collaboration with DFG's fish monitoring survey, IEP funded Floodplains projects, and various water quality monitoring programs (USGS, SWRCB, DFG, and SFEI).

Manuscripts from the proposed research will be submitted to high quality scientific journals for peer-reviews and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of paper at an international and national meeting. We do not foresee any third party impacts to result from this study.

F. Compliance with Standard Terms and Conditions

The project applicants are public university employee as such will comply with the standard State and Federal contract terms.

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Figure 1.Ovatestis and moderate primodial germ cell necroses in testis of 1-year-old splittail dietary exposed to 26.04 $\mu g.g^{-1}$ of selenium for 5 months

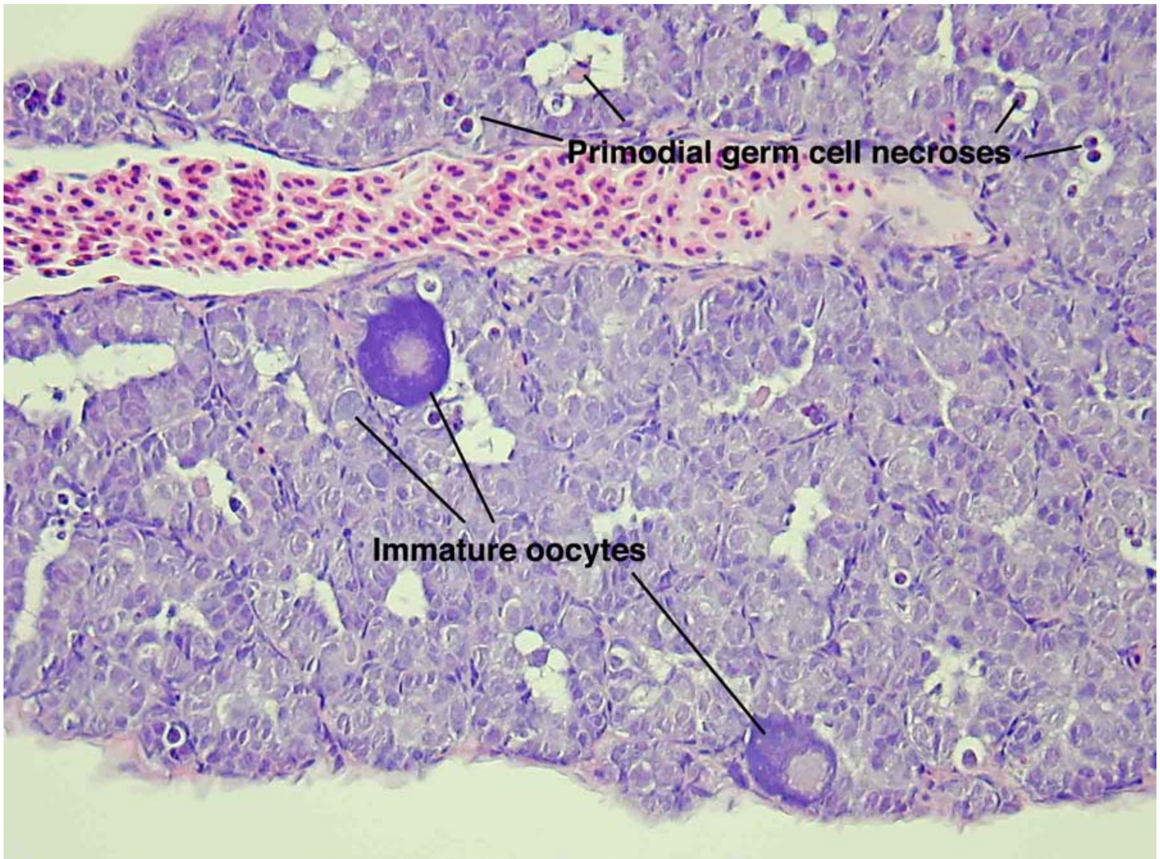

Figure 2. **Otolith of 16 month-old splittail glued on a plastic coverslip and processed for LA-ICP-MS analysis**

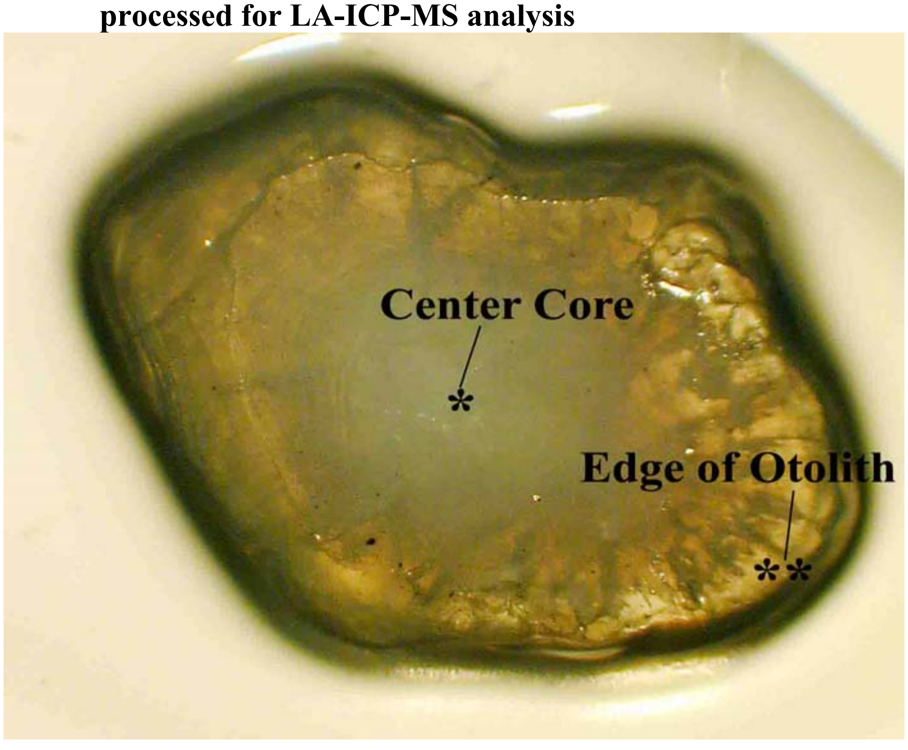

Table 1. Metal concentration differences in the Sacramento/San Joaquin Delta

	Highest concentration (ppb)	Site	Lowest concentration (ppb)	Site	Times of difference
Copper	140.5	Cache Creek	1.15	American River	122.2
Zinc	333	Yolo Bypass	4.6	Shasta Dam	72.4
Chromiun	303	Cache Creek	1.28	American River	236.7
Lead	33.3	Cache Creek	0.44	American River	75.7
Cadmium	0.47	Putah Creek	0.017	American River	27.6
Nickel	652	Cache Creek	2.2	American River	296.4

Table 2. Means metal body burden ($\mu g.g^{-1}$) in Asian clams from 1990-1999 (U.S.G.S. Ms. Cindy Brown)

	Near Chipps Island		Near Roe Island		Carquinez St off of Martinez harbor		San Pablo Bay	
Station	N	Mean (S.D.)	N	Mean (S.D.)	N	Mean (S.D.)	N	Mean (S.D.)
Ag	52	0.80 (0.38)	62	1.25 (1.22)	99	1.48 (1.05)	71	0.71 (0.48)
Cd	59	5.74 (1.34)	66	4.15 (1.21)	103	2.71 (0.65)	98	1.84 (0.58)
Cr	59	20.74 (14.46)	66	13.13 (9.55)	104	6.49 (6.63)	100	3.08 (1.59)
Cu	59	25.93 (6.11)	66	22.27 (5.83)	104	23.18 (5.05)	100	21.59 (5.77)
Ni	59	12.06 (4.56)	66	10.43 (4.50)	104	7.73 (3.11)	99	4.94 (1.61)
V	59	5.59 (9.22)	66	5.20 (4.74)	104	4.48 (4.94)	100	2.21 (1.30)
Zn	59	106.37 (18.47)	66	102.11 (22.13)	104	84.42 (13.38)	100	75.18(12.24)

Table 3. $^{82}Se/^{44}Ca$ ratio in Otolith before and after Se exposure (Mean ± SD)

Tissue Samples	Center core of otolith*	Edge of Otolith** 0.37 ppm of Se in PCdiet	57.62 ppm Se in PCdiets
Otolith	4.34E-07±3.39E-07	2.90E-07±1.17E-08	1.48E-05±1.27E-05
Fin ray	Not determined	2.04E-07±2.18E-07	5.21E-06±1.62E-06

* Center core of otolith is between 0-7 months of age, which is before se exposure.
** Edge of otolith is at approximately 16 months of age, 9 months after se exposure.

Figiure 3A. Profiles of ^{88}Sr: ^{44}Ca and ^{138}Ba: ^{44}Ca ratios from center core (1) to edge of otolith (10) in a splittail collected from Sherman Island indicating the evidence of splittail migrate from low-Sr, high-Ba environment (freshwater) to high-Sr, low-Ba environment (estuary). ^{138}Ba: ^{44}Ca ratio is magnified by 25X.

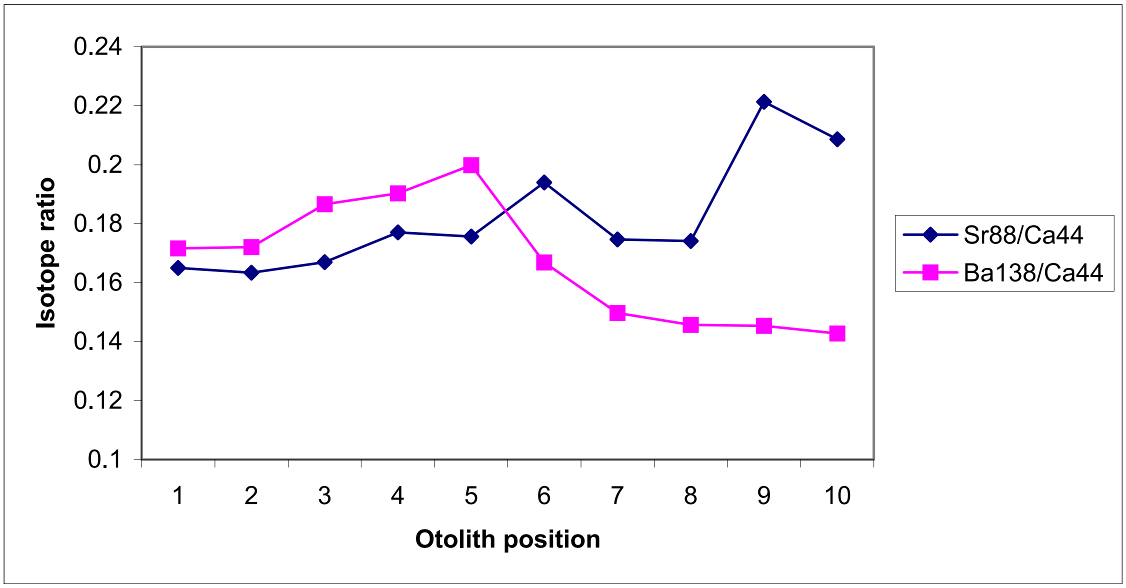

Figure 3B. Profile of six isotope ratios from the center to the edge of otolith of a splittail collected at the Sherman Island. The elevated ^{88}Cr: ^{44}Ca, ^{202}Hg: ^{44}Ca and ^{208}Pb: ^{44}Ca ratios are consistent with the changes of ^{88}Sr: ^{44}Ca and ^{138}Ba: ^{44}Ca ratios (Figure 3A), indicating the difference of chemical environments between freshwater and estuary. The ratios are magnified as follows: ^{65}Cu: ^{44}Ca (10X), ^{202}Se: ^{44}Ca (20X), ^{208}Ag: ^{44}Ca (100X), ^{202}Hg: ^{44}Ca (20X) and ^{208}Pb: ^{44}Ca (100X).

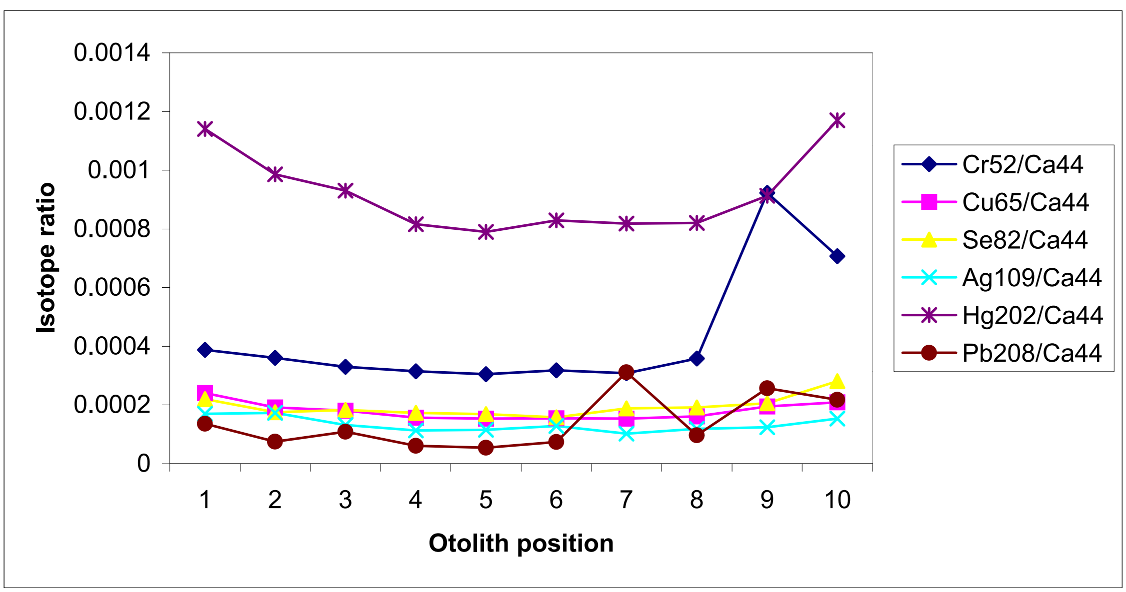

Table 4. Concentrations of metal (µg.g^{-1}) in Diet for Exposure Experiment

Group	Tanks	Cd	Cu	Ni
1	2	**50**	**1**	**1**
2	2	**20**	**10**	**10**
3	2	**5**	**50**	**20**
4	2	**1**	**100**	**50**
5	2	**1**	**100**	**1**
6	2	**5**	**50**	**10**
7	2	**20**	**10**	**20**
8	2	**50**	**1**	**50**
9	2	**1**	**1**	**50**
10	2	**5**	**10**	**20**
11	2	**20**	**50**	**10**
12	2	**50**	**100**	**1**

Group	Tanks	Cr	Se	Ag
1	2	**200**	**1**	**0.5**
2	2	**50**	**5**	**1**
3	2	**10**	**15**	**5**
4	2	**1**	**30**	**10**
5	2	**1**	**30**	**0.5**
6	2	**10**	**15**	**1**
7	2	**50**	**5**	**5**
8	2	**200**	**1**	**10**
9	2	**1**	**1**	**10**
10	2	**10**	**5**	**5**
11	2	**50**	**15**	**1**
12	2	**200**	**30**	**0.5**