Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Signature Page

Each applicant submitting a proposal to the CALFED Bay-Delta Program Ecosystem Restoration Program must submit a signed Signature Page.

Failure to sign and submit this form will result in the application not being considered for funding.

The individual signing below declares the following:

- the truthfulness of all representations in this proposal;
- the individual signing the form is authorized to submit the application on behalf of the applicant (if applicant is an entity or organization; and
- the applicant has read and understood the conflict of interest and confidentiality discussion in the PSP Section 2.4 and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in this PSP.

Proposal Title:

Mercury in San Francisco Bay-Delta Birds: Trophic Pathways, Bioaccumulation and Ecotoxicological Risk to Avian Reproduction

_____
Authorized Signature

_____
Printed Name

____U.S. FISH and WILDLIFE SERVICE_____
Organization

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form I - Project Information

All applicants must complete this form for their proposals. *Failure to answer these questions will result in the application not being considered for funding.*

1. Proposal Title:

Mercury in San Francisco Bay-Delta Birds: Trophic Pathways, Bioaccumulation and Ecotoxicological Risk to Avian Reproduction

2. Proposal Applicants:

Thomas H. Suchanek, U.S. Fish and Wildlife Service
Steven E. Schwarzbach, U.S. Geological Survey
Gary H. Heinz, U.S. Geological Survey

3. Corresponding Contact Person:

Thomas H. Suchanek
U.S. Fish and Wildlife Service
Division of Environmental Contaminants
2800 Cottage Way
Sacramento, CA 95825
916-414-6599
Tom_Suchanek@fws.gov

4. Project Keywords:

At-risk Species
Bioaccumulation
Contaminants
Environmental Risk Assessment
Heavy Metals (mercury, PBDEs, PCBs, selenium)
Trophic Dynamics and Food Webs
Wildlife Ecology

5. Type of project:

RESEARCH.

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

NO.

7. If yes, is there an existing specific restoration plan for this site?

NO.

8. Topic Area

ECOSYSTEM WATER AND SEDIMENT QUALITY.

9. Type of applicant

FEDERAL AGENCY.

10. Location – GIS coordinates (centrum)

Latitude: 37.8999 N
Longitude: 122.3636 W
Brooks Island, Contra Costa County, CA
Datum: (leave blank)

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

This project will be conducted within the San Francisco Bay-Delta Estuary from San Pablo Bay and Suisun Bay in the north to south San Francisco Bay south of the San Mateo Bridge and extending further south to the lower Guadalupe River. The project will extend into the lower portions of the Sacramento-San Joaquin Delta and Suisun Bay. Habitats that will be studied include open waters of the Bay and Delta and the surrounding shallow bay margins as well as salt ponds and other diked wetlands that serve as suitable habitat for species from the guilds proposed for study. Some representative sites and coordinates are presented below (coordinates are presented in decimal degrees (NAD 27)).

Napa-Sonoma Marshes Wildlife Area Pond 3: 38.1290 N; 122.2967 W
Hayward Regional Shoreline: 37.6335 N; 122.1452 W
Baumberg Pond 10: 37.6080 N; 122.1301 W
Don Edwards National Wildlife Refuge Pond A7: 37.4435 N; 122.0089 W
Don Edwards National Widlife Refuge Environmental Education Center: 37.4569 N; 121.9690 W
Charleston Slough (Mountain View): 37.4376 N; 122.0960 W

11. Location – Ecozone

Code 15: Landscape

12. Location – Counties

Napa, Solano, Santa Clara, Alameda, San Francisco, Contra Costa, Marin, Sonoma, Lassen, Modoc, Shasta

13. Location – City. Does your project fall within a city jurisdiction?

NO

14. If yes, please list the city:

15. Location – Tribal Lands. Does your project fall on or adjacent to tribal lands?

NO

16. Location – Congressional District.

5th

17. Location – California State Senate District & California Assembly District

California State Senate District Number: 5
California Assembly District Number: 9

18. How many years of funding are you requesting?

THREE.

19. Requested Funds:

a. Are your overhead rates different depending on whether funds are state or federal?

NO

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

c. If no, list single overhead rate and total requested funds.

Overhead rates for USFWS depend on whether the funds are to be used within the USFWS or passed through to subcontractors. The "internal" USFWS overhead rate is 26.5%. The overhead rate for funds passed on to subcontractors is 4.5%. The budget has been calculated to account for the portions of the various tasks and subtasks that will be performed by USFWS versus other subcontractors, including USGS.

d. Do you have cost share partners already identified?

YES

If yes, list partners and amount contributed by each.

USGS (Western Ecological Research Center- WERC; Patuxent Wildlife Research Center- PWRC): The USGS normally requires 35.65% overhead, but as a sub-contractor to the USFWS is only requiring 15% overhead, saving 20.65% overhead costs equivalent, for this project, equivalent to ca. $505,925. WERC will also be cost-sharing a portion of the work from ongoing coastal ecosystem research programs including a "Place-based Program" on salt pond science support, wetland restoration monitoring studies, and diving benthivore foraging ecology and contaminant research, equivalent to ca. $403,755. PWRC is providing cost-share for the salaries of Dr. Heinz (GS-14) and Dr. Hoffman (GS-15) on the egg injection component of this study, equivalent to $302,912 for the three-year period of the project. In addition, Drs. Hoffman and Melancon of PWRC will conduct biochemical assays associated with oxidative stress and P450 enzyme induction, respectively, for which they will not charge salary. They are leaders in their respective fields of expertise and their partnership is a significant addition to this project. They will conduct assays, assist in interpretation of results, report writing, and publication of study findings. Partnership with PWRC staff researchers is funded by PWRC directly and is estimated at $15,000 each for Drs. Hoffman and Melancon for the biomarker work. Over the 3 year life of the project this partnership component has an estimated additional value of $90,000.

Bird tissues and eggs are not at this time a regular part of the RMP contaminant monitoring program. SFEI, however, is very interested in the bird results of this investigation and we intend to link with results of the RMP as well. The value of their program to our project is difficult to quantify but we will work in close cooperation with the institute and share results.

e. Do you have potential cost share partners?

YES

If yes, list partners and amount contributed by each.

No amounts have been promised to date. Potential cost share partners we have collaborated with in the past, and entities with whom we will seek partnerships if this project is funded include: (1) The Regional Monitoring Program for Trace Substances, implemented by the San Francisco Estuary Institute (SFEI), (2) San Francisco Bay Regional Water Quality Control Board, (3) U.S. EPA Region IX, (4) California State Water Resources Control Board, and (5) CalEPA

f. Are you specifically seeking non-federal cost share funds through this solicitation?

NO.

If yes, list total non-federal funds requested.

g. If the total non-federal cost share funds requested above does not match the total state funds requested in 19a, please explain the difference.

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

NO.

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

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

YES.

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

Below are listed grants obtained by the three lead Principal Investigators of this proposal (Heinz, Schwarzbach, Suchanek).

Heinz, G.H. and D.J. Hoffman (1999-2003: CALFED Grant # ERP-99-B06) Assessment of ecological and human health impacts of mercury in the Bay-Delta Watershed*: Task 3B: Laboratory assessment of the hazards of mercury to reproduction in aquatic birds.*

Schwarzbach, S. and T. Adelsbach (1999-2003: CALFED Grant # ERP-99-B06). Assessment of ecological and human health impacts of mercury in the Bay-Delta watershed. *Task 3A: Field assessment of avian mercury exposure in the Bay-Delta ecosystem.*

Suchanek, T.H. and D.G. Slotton (1997-2000: CALFED Grant # ERP-97-C05). The effects of wetland restoration on the production of methyl mercury in the San Francisco Bay-Delta System.

Suchanek, T.H., D.G. Slotton and D.C. Nelson (1999-2003: CALFED Grant # ERP-99-B06) Assessment of ecological and human health impacts of mercury in the Bay-Delta Watershed*: Task 5A - Source Bioavailability and Mine Remediation Feasibility.*

Reid, F., M. Bias, J. Takekawa (2001-2003: CALFED Grant # 2001-E212) Ecological Monitoring of Tolay Creek and Cullinan Ranch Tidal Wetland Restoration Projects in North San Francisco Bay.

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

NO

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

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

NO.

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

NO.

If yes, identify project number(s), title, and funding source.

25. Please list suggested reviewers for your proposal. (optional)

Name	Organization	Phone	Email
Charles Henny	USGS-Corvallis, OR	541-757-4840	charlesjhenny@usgs.gov
Daniel Anderson	U.C. Davis, Davis, CA	530-752-2108	dwanderson@ucdavis.edu
Harry Ohlendorf	CH_2M-Hill	916-920-0212	hohlendo@ch2m.com
Donald Axelrad	Fla. Dept. Envt'l. Protection	850-245-8306	don.axelrad@dep.state.fl.us
Bart Hoskins	U.S.E.P.A.	617-918-8375	hoskins.bart@epa.gov
David Evers	Biodiversity Research Inst.	207-781-3324	david.evers@briloon.org
Sudeep Chandra	Univ. of Wisconsin	530-583-3279	schandra@ucdavis.edu

26. Comments.

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form II - Executive Summary

All applicants must complete this form for their proposals. ***Failure to answer these questions will result in the application not being considered for funding.***

Proposal Title:

Please provide a brief but complete (about 300 words) summary description of the proposed project; its geographic location, project type, project objective, approach to implement the proposal, hypotheses and uncertainties, expected outcome and relationship to CALFED ERP and/or CVPIA goals.

1. Executive Summary

The CALFED Mercury Strategy Document recognizes reproductive success of birds as a sensitive and important endpoint relative to mercury (Hg) contamination. However, methylmercury (methyl-Hg) bioaccumulation in birds has been difficult to predict from concentrations in water and sediment at specific sites, because avian species from different foraging guilds use diverse habitats and consume distinctly different prey items. Each guild represents a unique foodweb for Hg bioaccumulation. We propose a research and monitoring program encompassing three objectives (1) field studies of avian dietary Hg exposure and bioaccumulation in three major foraging guilds (surface feeders, piscivores, benthivores); (2) field studies on reproductive success including use of advanced telemetry techniques (to study habitat use, movements, survival and to locate individuals breeding outside the study area), as well as biomarker and histopathological analysis (to assess contaminant impacts to individuals and populations); and (3) laboratory studies to examine interspecific mercury sensitivity in avian eggs and to estimate No Observed Adverse Effects Levels (NOAEL) for methyl-Hg in mallards, a standard used for comparing relative sensitivities across multiple species. We will also evaluate the potential influence of other contaminants of concern including selenium (Se), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) that co-occur with Hg. The proposed project will be conducted within the San Francisco Bay-Delta Estuary from San Pablo Bay and Suisun Bay in the north to the lower Guadalupe River in south San Francisco Bay, and the lower Sacramento-San Joaquin Delta. Habitats prioritized for study include open waters of the Bay-Delta, surrounding shallow waters, and salt ponds and other diked or natural wetlands surrounding the Bay-Delta that serve as suitable habitat for species from the guilds proposed for study. The goal is to integrate field and laboratory studies to evaluate differences in Hg exposure and subsequent effects to birds, thus providing a scientific foundation to assist resource managers and environmental regulators in prioritizing Hg control and remediation strategies, as well as guide on-going and future restoration projects.

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form III - Environmental Compliance Checklist

All applicants must complete this form for their proposals. *Failure to answer these questions will result in the application not being considered for funding.*

Successful applicants are responsible for complying with all applicable laws and regulations for their projects, including the National Environmental Policy Act (NEPA) and the California Environmental Quality Act (CEQA).

Any necessary NEPA or CEQA documents for an approved project must tier from the CALFED Programmatic Record of Decision and Programmatic EIS/EIR to avoid or minimize the projects adverse environmental impacts. Applicants are encouraged to review the Programmatic EIS/EIR and incorporate the applicable mitigation strategies from Appendix A of the Programmatic Record of Decision in developing their projects and the NEPA/CEQA documents for their projects.

1. **CEQA or NEPA Compliance**
   a. Will this project require compliance with CEQA? **NO**
   b. Will this project require compliance with NEPA? **NO**

   If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

   Compliance is not required because the USFWS will operate under a Categorical Exclusion for this research project under the authorities listed below (see #3 below).

2. **If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies). Please write out all words in the agency title other than United States (use the abbreviation US) or California (use the abbreviation CA). If not applicable, put None.**

   CEQA Lead Agency: U.S. Fish and Wildife Service
   NEPA Lead Agency (or co-lead:) U.S. Fish and Wildlife Service
   NEPA Co-Lead Agency (if applicable):

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

   CEQA

   - [x] Categorical Exemption
   - [ ] Negative Declaration or Mitigated Negative Declaration
   - [ ] EIR
   - [ ] none

NEPA

- [x] Categorical Exclusion
- [ ] Environmental Assessment/FONSI
- [ ] EIS
- [ ] none

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.

CEQA Categorical Exemption
Title 14. California Code of Regulations
Chapter 3. Guidelines for Implementation of the California Environmental Quality Act
Article 19. Categorical Exemptions
Section 15306. Information Collection

Class 6 consists of basic data collection, research, experimental management, and resource evaluation activities which do not result in a serious or major disturbance to an environmental resource. These may be strictly for information gathering purposes, or as part of a study leading to an action which a public agency has not yet approved, adopted, or funded.

NEPA Categorical Exclusion
516 Departmental Manual, Section 6, Appendix 1

1.4.B. Resource Management. Prior to carrying out these actions the Sevice should coordinate with affected Federal agencies, State, Tribal and local governments.
(1) Research, inventory, and information collection activities directly related to the conservation of fish and wildlife resources which involve negligible animal mortality or habitat destruction, no introduction of contaminants, or no introduction of organisms not indigenous to the affected ecosystem.

Note: Authority cited: Sections 21083 and 21087, Public Resources Code; Reference: Section 21084, Public Resources Code.

CEQA/NEPA Process

a. Is the CEQA/NEPA process complete? YES
b. If the CEQA/NEPA process is not complete, please describe the dates for completing draft and/or final CEQA/NEPA documents.
c. If the CEQA/NEPA document has been completed, please list document name(s):

4. **Environmental Permitting and Approvals**

Successful applicants must tier their project's permitting from the CALFED Record of Decision and attachments providing programmatic guidance on complying with the state and federal endangered species acts, the Coastal Zone Management Act, and sections 404 and 401 of the Clean Water Act.

The CALFED Program will provide assistance with project permitting through its newly established permit clearing house.

Please indicate what permits or other approvals may be required for the activities contained in your proposal and also which have already been obtained. Please check all that apply. 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 – **REQUIRED – Our current permits expire 3/04, and we will renew.**

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

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land. **– None required at this time**
Agency Name:

Permission to access state land. **– REQUIRED – In process of obtaining permission.**
Agency Name: **California Department of Fish and Game**

Permission to access federal land. **– REQUIRED – In process of obtaining permission.**
Agency Name: **USFWS - Don Edwards National Widlife Refuge**

Permission to access private land. - **None required at this time**
Landowner Name:

Comments. If you have comments on any of the above questions, please enter the question number followed by a specific comment.

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form IV - Land Use Checklist

All applicants must complete this form for their proposals. Failure to answer these questions will result in the application not being considered for funding.

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

NO

2. If you answered yes to #1, please answer the following questions:

a. How many acres will be acquired?

b. Will existing water rights be acquired?

c. Are any changes to water rights or delivery of water proposed?

d. If yes, please describe proposed changes.

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

YES

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

NO

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

RESEARCH ONLY

5. If you answered yes to #3, please answer the following questions:

a. How many acres of land will be subject to a land use change under the proposal?

b. Describe what changes will occur on the land involved in the proposal.

c. List current and proposed land use, zoning and general plan designations of the area subject to a land use change under the proposal.

d. **Is the land currently under a Williamson Act contract?** (For multiple sites, answer Yes if true for any parcel, and provide an explanation in the Comments box below)

e. **Is the land mapped as Prime Farmland, Farmland of Statewide Importance, Unique Farmland or Farmland of Local Importance under the California Department of Conservation's Farmland Mapping and Monitoring Program?** For more information, contact the California Department of Conservation, Division of Land Resource Protection, Farmland Mapping and Monitoring Program (http://www.consrv.ca.gov/dlrp/FMMP/index.htm). (For multiple sites, answer Yes if true for any parcel, and provide an explanation in the Comments box below)

f. If yes, please list classification:

g. Describe what entity or organization will manage the property and provide operations and maintenance services.

6. Comments.

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form V - Conflict of Interest Checklist

All applicants must complete this form for their proposals. *Failure to answer these questions will result in the application not being considered for funding.*

You may update your information at any time. The [update proposal] button is located at the bottom of this form.

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):

Thomas H. Suchanek, U.S. Fish and Wildlife Service, ECD, Sacramento, CA
Steven E. Schwarzbach, U.S. Geological Survey, BRD, Sacramento, CA
Gary H. Heinz, U.S. Geological Survey, PWRC, Laurel, MD

Collaborators who helped write proposal:
Terrence L. Adelsbach USFWS, ECD, Sacramento, CA
Collin A. Eagles-Smith, USFWS, ECD, Sacramento, CA
John Y. Takekawa, USGS, WERC, Vallejo, CA
Susan E. Wainwright-De La Cruz, USGS, WERC, Vallejo, CA
A. Keith Miles, USGS, WERC, Davis, CA
D.J. Hoffman, USGS, PWRC, Laurel, MD

Additional personnel who will be performing tasks.
Nicole Athearn
Deborah Jaouen
Carolyn Marn
William Perry
Liza Ryan
Julie Yee

Subcontractor(s):

Are specific subcontractors identified in this proposal?

YES.

If yes, please list the name(s) and organization(s):

(For details, see Form VII – Budget Justification)
1) U.S. Geological Survey
2) Ecoscan Telemetry, Watsonville, CA
3) Argos Space Agency, Largo, MD
4) Point Reyes Bird Observatory, Stinson Beach, CA
5) Trace Element Research Laboratory (TERL), Texas A&M Univ, College Station, TX
6) San Francisco Bay Bird Observatory (SFBBO), Alviso, CA
7) Battelle Marine Sciences Laboratory, Sequim, WA
8) California Department of Fish & Game, Sacramento, CA
9) University of Waterloo, Ontario, Canada
10) Patuxent Wildlife Research Center (PWRC), Laurel, MD
11) Christopher Babcock, D.Q. University, Davis, CA
12) Department of Toxic Substances Control (HML), Berkeley, CA

Helped with proposal development

Are there persons who helped with proposal development?

YES.

If yes, please list the name(s) and organization(s):

None other than those USFWS and USGS agency personnel listed as collaborators on the cover page of the proposal text.

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form VI: Budget Summary

YEAR 1

Year 1													
Objective	**Task**	**Subtask**	**Direct Labor Hours**	**Salary (per year)**	**Benefits (per year)**	**Travel**	**Supplies and Expendables**	**Equipment**	**Services or Consultants**	**Other direct costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
I. Trophic Pathways and Species patterns	**Tern Guild**												
	Task Total		3,840	$210,060	$5,091	$4,025	$20,300	$0	$133,557	$0	$373,033	$66,824	$439,857
	Recurvirostrid Guild												
	Task Total		5,849	$106,478	$22,303	$7,037	$18,766	$0	$136,227	$0	$290,811	$37,010	$327,821
	Diving Duck Guild												
	Task Total		5,529	$100,718	$21,323	$7,612	$31,266	$0	$80,737	$0	$241,656	$37,169	$278,825
	Hg QA/QC		0	$0	$0	$0	$0	$0	$19,865	$0	$19,865	$894	$20,759
Objective Total			15,218	417,256	48,717	18,674	70,332	0	370,386	0	925,365	141,897	1,067,262
II. Reproduction and Toxic Effects	Tern Guild												
	Task Total		2,246	$182,488	*	*	*	$0	$201,336	$0	$383,824	$57,419	$441,243
	Recurvirostrid Guild												
	Task Total		2,976	$62,134	$16,563	$2,300	$9,000	$0	$85,860	$0	$175,857	$22,021	$197,878
	Diving Duck Guild												
	Task Total		113	$2,778	$972	$1,725	$29,000	$0	$81,465	$0	$115,940	$18,145	$134,085
	Hg QA/AC		0	$0	$0	$0	$0	$0	$10,125	$0	$10,125	$456	$10,581
Objective Total			5,335	$247,400	$17,535	$4,025	$38,000	$0	$378,786	$0	$685,746	$98,041	$783,787
III. Embryo Sensitivity	Tern Guild (+ cormorants)												
	Task Total		1,645	$39,721	$10,239	$867	$9,334	$0	$10,165	$0	$70,326	$13,902	$84,228
	Recurvirostrid Guild												
	Task Total		1,699	$34,916	$11,600	$1,405	$9,584	$0	$10,165	$0	$67,670	$12,817	$80,487
	Diving Duck Guild												
	Task Total		1,699	$34,916	$11,600	$1,405	$9,584	$0	$10,165	$0	$67,670	$12,817	$80,487
	Hg QA/QC		0	$0	$0	$0	$0	$0	$2,610	$0	$2,610	$117	$2,727
Objective Total			5,043	$109,553	$33,439	$3,677	$28,502	$0	$33,105	$0	$208,276	$39,653	$247,929
IV. Data Handling, Mapping, & QC			458	$12,500	$4,375	$0	$0	$0	$0	$0	$16,875	$3,405	$20,280
Year 1 Totals			26,054	$786,709	$104,066	$26,376	$136,834	$0	$782,277	$0	$1,836,262	$282,995	$2,119,257

Form VI: Budget Summary
YEAR 2

Year 2													
Objective	**Task**	**Subtask**	**Direct Labor Hours**	**Salary (per year)**	**Benefits (per year)**	**Travel**	**Supplies and Expendables**	**Equipment**	**Services or Consultants**	**Other direct costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
I. Trophic Pathways and Species patterns	Tern Guild												
	Task Total		3,840	$219,316	$5,091	$4,525	$17,167	$0	$84,607	$4,250	$334,956	$67,669	$402,625
	Recurvirostrid Guild												
	Task Total		5,849	$106,478	$22,303	$9,037	$15,633	$0	$106,763	$0	$260,214	$35,456	$295,670
	Diving Duck Guild												
	Task Total		5,529	$100,718	$21,323	$9,112	$31,266	$0	$61,365	$0	$223,784	$36,600	$260,384
	Hg QA/QC		0	$0	$0	$0	$0	$0	$9,530	$0	$9,530	$429	$9,959
Objective Total			15,218	$426,512	$48,717	$22,674	$64,066	$0	$262,265	$4,250	$828,484	$140,153	$968,637
II. Reproduction and Toxic Effects	Tern Guild												
	Task Total		2,246	$191,753	*	*	*	$0	$170,276	$4,250	$366,279	$59,603	$425,882
	Recurvirostrid Guild												
	Task Total		2,976	$62,134	$16,563	$3,800	$9,000	$0	$73,564	$0	$165,061	$21,770	$186,831
	Diving Duck Guild												
	Task Total		137	$3,334	$1,167	$2,225	$29,000	$0	$76,825	$0	$112,551	$18,189	$130,740
	Hg QA/QC		0	$0	$0	$0	$0	$0	$5,445	$0	$5,445	$245	$5,690
Objective Total			5,359	$257,221	$17,730	$6,025	$38,000	$0	$326,110	$4,250	$649,336	$99,807	$749,143
III. Embryo Sensitivity	Tern Guild (+ cormorants)												
	Task Total		1,631	$41,399	$10,648	$1,467	$4,666	$0	$9,998	$0	$68,178	$13,837	$82,015
	Recurvirostrid Guild												
	Task Total		1,698	$36,153	$12,009	$2,005	$4,916	$0	$9,998	$0	$65,081	$12,634	$77,715
	Diving Duck Guild												
	Task Total		1,698	$36,155	$12,009	$2,005	$4,916	$0	$9,998	$0	$65,083	$12,634	$77,717
	Statistical Modelling		173	$5,000	$1,750	$0	$0	$0	$0	$0	$6,750	$1,362	$8,112
	Hg QA/QC		0	$0	$0	$0	$0	$0	$750	$0	$750	$34	$784
Objective Total			5,200	$118,707	$36,416	$5,477	$14,498	$0	$30,744	$0	$205,842	$40,501	$246,343
IV. Data Handling, Mapping, & QC			458	$12,500	$4,375	$0	$0	$0	$0	$4,375	$16,875	$3,405	$20,280
Year 2 Totals			$26,235	$814,940	$107,238	$34,176	$116,564	$0	$619,119	$12,875	$1,700,537	$283,865	$1,984,402

Form VI: Budget Summary
YEAR 3

Year 3 Objective	Task	Subtask	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies and Expendables	Equipment	Services or Consultants	Other direct costs	Total Direct Costs	Indirect Costs	Total Cost
I. Trophic Pathways and Species patterns	Tern Guild												
	Task Total		2,308	$160,227	$2,153	$1,650	$6,500	$0	$2,560	$4,250	$177,340	$45,418	$222,758
	Recurvirostrid Guild												
	Task Total		2,863	$52,628	$13,401	$2,000	$0	$0	$10,000	$0	$78,029	$14,175	$92,204
	Diving Duck Guild												
	Task Total		2,863	$52,628	$13,401	$2,000	$0	$0	$0	$0	$68,029	$13,725	$81,754
Objective Total			8,034	$265,483	$28,955	$5,650	$6,500	$0	$12,560	$4,250	$323,398	$73,317	$396,715
II. Reproduction and Toxic Effects	Tern Guild												
	Task Total		2,246	$200,456	*	*	*	$0	$20,000	$4,250	$224,706	$55,147	$279,853
	Recurvirostrid Guild												
	Task Total		2,976	$62,134	$16,563	$3,800	$9,000	$0	$20,000	$0	$111,497	$19,360	$130,857
	Diving Duck Guild												
	Task Total		593	$11,418	$2,441	$2,225	$44,000	$0	$48,000	$0	$108,084	$21,806	$129,890
Objective Total			5,815	$274,008	$19,004	$6,025	$53,000	$0	$88,000	$4,250	$444,287	$96,313	$540,600
III. Embryo Sensitivity	Tern Guild (+ cormorants)												
	Task Total		1,524	$33,558	$11,074	$1,734	$7,667	$0	$20,572	$0	$74,605	$12,787	$87,392
	Recurvirostrid Guild												
	Task Total		1,524	$33,558	$11,074	$1,734	$7,667	$0	$20,572	$0	$74,605	$12,787	$87,392
	Diving Duck Guild												
	Task Total		1,524	$33,558	$11,074	$1,734	$7,667	$0	$20,572	$0	$74,605	$12,787	$87,392
	Statistical Modelling		173	$5,000	$1,750	$0	$0	$0	$0	$0	$6,750	$1,362	$8,112
	Hg QA/QC		0	$0	$0	$0	$0	$0	$5,235	$0	$5,235	$236	$5,471
Objective Total			4,745	$105,674	$34,972	$5,202	$23,001	$0	$66,951	$0	$235,800	$39,958	$275,758
IV. Data Handling, Mapping, and QC			458	$12,500	$4,375	$0	$0	$0	$0	$0	$16,875	$3,405	$20,280
Year 3 Totals			19,052	$657,665	$87,306	$16,877	$82,501	$0	$167,511	$8,500	$1,020,360	$212,992	$1,233,352
Entire Project Totals			71,341	$2,259,314	$298,610	$77,429	$335,899	$0	$1,568,907	$21,375	$4,557,159	$779,853	$5,337,012

Project 3 Year Total = $5,281,042

Hg QA/QC Costs = $55,970

3 Year Total with QA/QC = $5,337,012

Ecosystem Restoration Program - 2002 Proposal Solicitation Package (PSP): Form VII - Budget Justification

All applicants must complete this form for their proposals. ***Failure to answer these questions will result in the application not being considered for funding.***

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

For all labor, yearly estimated hours are given for Years 1/2/3 as xx/xx/xx.
tbn = "to be named" personnel.

USFWS:
Objective I (Trophic Pathways): Suchanek (GS-13) direct charge time only (see below) = 60/60/35; Adelsbach (GS-11) = 850/850/680; Eagles-Smith (GS-9) = 700/700/563; tbn biologist #1 (GS-9) = 456/456/295; tbn biologist #2 (GS-7) = 309/309/180; tbn biologist #3 (GS-7) = 250/250/100
Objective II (Reproduction/Toxic Effects): Suchanek (GS-13) direct charge time only (see below) = 30/30/30; Adelsbach (GS-11) = 850/850/850; Eagles-Smith (GS-9) = 675/675/675; tbn biologist #1 (GS-9) = 300/300/300; tbn biologist #2 (GS-7) = 247/247/247; tbn biologist #3 (GS-7) = 144/144/144
Objective III (Embryo Sensitivity): Adelsbach (GS-11) = 57/57/0; Eagles-Smith (GS-9) = 50/50/0
Objective IV (Data Handling, Mapping, and QC): Adelsbach (GS-11) =100/100/100; tbn (GS-9) = 100/100/100.

USGS:
Objective I (Trophic Pathways):
Jaouen (GS-6/2) = 1023/1023/1023; Miles (13/6) = 129/129/129; Ryan (student) 898/898/898; Takekawa (GS-13/3) = 73/73/73; Wainwright-De La Cruz (GS-9/4) = 490/490/490; other tbn technical support = 10280/10280/3440
Objective II (Reproduction/Toxic Effects): Athearn (GS-9/2) = 470/470/470; Marn (GS-11/4) = 779/779/779; Takekawa (GS-13/3) = 44/44/44; Wainwright-De La Cruz (GS-9/4) = 221/221/221; other tbn technical support = 1600/1600/1600
Objective III (Embryo Sensitivity):Athearn (GS-9/2) = 235/235/235; Takekawa (GS-13/3) = 15/15/15; Wainwright-De La Cruz (GS-9/4) = 98/98/98; Yee (GS-12/2) = 0/173/173; Heinz (GS-14) = 1240/1240/1240; Hoffman (GS-15) = 728/728/728; tbn (GS-9) = 2080/2080/2080; tbn (GS-7) = 2080/2080/2080.
Objective IV (Data Handling, Mapping, and QC): Athearn (GS-9/2) = 235/235/235; Perry (GS-12/7) = 223/223/223.

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

USFWS:
All USFWS salaries are based on a BioDay Rate of $650 for an 8-hr day ($81.25/hr), regardless of the employee's rank. This rate applies to all biologists' staff time, but not supervisory staff time, which is covered internally by the BioDay rate applied to all biologists working on the project. Therefore, most of the coordination and management time for Suchanek is covered internally under the BioDay Rate structure. In addition, Suchanek will participate occasionally in field and/or lab work, during which time he will charge biologist time (see breakdown above for personnel hours).

USGS (WERC):
Ahearn: GS-9/2 - $21.29/hr
Jaouen: GS-6/2 - $14.66/hr
Marn: GS-11/4 - $25.66/hr
Miles: GS-13/6 - $38.79/hr
Perry: GS-12/7 - $33.61/hr
Takekawa: GS-13/3 - $37.91/hr
Wainwright-De La Cruz: GS-9/4 - $22.67/hr
Yee: GS-12/2 - $28.89/hr
Other technical support: $18/hr

USGS (PWRC):
Heinz: GS-14/8 - $48.38/hr
Hoffman: GS-15/3 - $49.22/hr
tbn #1: GS-9/1 - $19.25/hr
tbn #2: GS-7/1 - $15.74/hr

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

USFWS: All benefits are covered under the BioDay Rate structure, so no additional costs are listed in the budget for USFWS associated benefits.

USGS: The following benefit rates apply:
35%: Athearn, Jaouen, Marn, Miles, Perry, Takekawa, Wainwright-De La Cruz, Yee
33%: Heinz, Hoffman, tbn #1, tbn #2
30%: Ryan
17%: Technical support (students)

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

USFWS: All field travel is covered under the BioDay Rate structure, so no additional costs are listed in the budget for USFWS associated field travel. Some additional travel costs, however, are listed under 'Other Direct Costs' for travel to meetings. Yearly costs are estimated at $7,500 for the last two years of the project.

USGS (WERC): All non-local travel will be to professional meetings to present project results. Yearly costs are estimated at $7,500 for the last two years of the project.

USGS (PWRC):
Travel to CALFED and other scientific meetings for two scientists: one GS-9, one GS-7 for Years1/2/3 = $2,600/$4,400/$5,200

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

USFWS: All supplies and expendables are covered under the BioDay Rate structure, so no additional costs are listed in the budget for USFWS associated supplies and expendables.

USGS (WERC):
Office supplies: $14,750 over three yrs
Computing: $5,000 over three yrs
Laboratory: $0
Field Supplies: $255,850 over 3 yrs (breakdown given below)

Field Supplies

	year 1	year 2	year 3	
4, ATS 8-band receivers @ 3.2K/ea (1 yr cost)	6400	0	0	
Equipment for benthic collections (Davis)	2850	2850	2850	
80 ducks @ $300/VHF radio (50 VHF in 3rd year)	24000	24000	15000	
70 adult + 40 chick recurves @ $150/radio	16500	16500	4400	
70 adult + 40 chick terns @ $150/radio	16500	16500	4400	
2.9K/PTT 10ducks	29000	29000	29000	
Telemetry system on 2 trucks @ 1.5K ea	3000	0	0	
Trapping supplies	2000	2000	0	
Surgery supplies	0	0	0	
Diet bird collection supplies	800	800	0	
Benthic collection supplies (Davis)	2500	2500	2500	
Total Per Year	103550	94150	58150	
Grand Total				255850

USGS (PWRC):
For Years 1/2/3 –
Office: $300/$300/300
Laboratory: $7,900/$3,200/$5,000
Computing: $500/$300/$200
Field: $2,300/$2,200/$10,000

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.*

1) Ecoscan Telemetry, Watsonville, CA
   - Robert Van Wagenen (pilot) aerial telemetry - to search for radio-marked birds
   - 384 hrs X $160/hr for 3 yrs

2) Argos Space Agency, Largo, MD
   - provide ARGOS satellite data services in cooperation with NOAA
   - variable time required: cost based on transmitters in use
   - $1,000 per satellite transmitter

3) Christopher Babcock, Deganawida-Quetzecoatl University, Davis, CA
   - surf scoter nest searching in Canada
   - 4 to 5 nest searches - $889/day X 45 days/yr for 3 yrs
   - includes charter flights, searchers' time, travel, supplies

4) Point Reyes Bird Observatory, Stinson Beach, CA
   - Nils Warnock – recurvirostrid nest searching, nest and chick fate, telemetry
   - 1,040 hrs/yr X $28.85/hr = $30,004/yr X 3 yrs

5) Trace Element Research Laboratory (TERL), Texas A&M Univ, College Station, TX
   - total Hg analyses: $54/sample X 4,020 samples for 3 yrs
   - Selenium analyses: $67/sample X 1,465 samples for 3 yrs

6) San Francisco Bay Bird Observatory (SFBBO), Alviso, CA
   - Cheryl Strong - field assistance for Objective II (tern tasks)
   - 693 hrs/yr X $28.85/hr for 3 yrs

7) Battelle Marine Sciences Laboratory, Sequim, WA
   - methyl-Hg analyses: $116/sample X 2106 samples for 3 yr total

8) California Department of Fish & Game (CDFG), Sacramento, CA
   -REQUIRED TO HAVE IN BUDGET-
   - 5% QA/QC Hg analyses:
   - total Hg analyses: $125/sample X 207 samples for 3 yr total
   - methyl Hg analyses: $245/sample X 113 samples for 3 yr total

9) CDFG: Marine Wildlife Veternary Care and Research Center, Santa Cruz, CA
   - Dr. Jim Hill - bird histopathology analyses –
   - $250/sample X 160 samples (total for 2 yrs)

10) University of Waterloo, Ontario, Canada
   - stable isotope analyses, including sample preparation
   - Carbon and Nitrogen: $26/sample X 4,180 samples for 3 yrs total
   - Sulfur: $36/sample X 3,500 samples for 3 yrs total

11) Patuxent Wildlife Research Center (PWRC), Laurel, MD
- biomarker analyses (years 1 & 2 only)
- oxidative stress (indicator of Hg contamination) $250/sample X 160 samples
- P450 analyses (indicator of PCB contamination) $60/sample X 160 samples

12) Department of Toxic Substances Control (HML), Berkeley, CA
- PCB/PBDE analyses
- $550/sample X 380 samples

13) USGS (PWRC) Student assistants @ $12.50/hr = $6,000/$10,375/$11,625

14) USGS (PWRC) Instrument maintenance - $900/$1,475/$1,307

15) USGS (PWRC) Egg collections by field cooperators - @ $40/hr = $7,600/$7,600/$5,000

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.*

NONE.

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 presentations, response to project specific questions and necessary costs directly associated with specific project oversight.*

Costs for USFWS management of this project by Suchanek and associated budget personnel in the Sacramento Fish and Wildlife Office will be covered internally by the USFWS BioDay Rate charges for all biologists funded on this project. Additional project management for USFWS on field activities will be conducted by Adelsbach and is covered within his salaried hours.

Costs for coordination with USFWS and USGS management of this project by Schwarzbach will be covered internally by the USGS Biological Research Division, Sacramento, CA. Additional project management for USGS on field activities will be conducted by Takekawa and Athearn and is covered within their salaried hours.

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

NONE.

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. *[CORRECTION: If overhead costs are different for State and Federal funds, note the different overhead rates and corresponding total requested funds on Form I - Project Information, Question 17a. On Form VI - Budget Summary, fill out one detailed budget for each year of requested funds, indicating on the form whether you are presenting the indirect costs based on the Federal overhead rate or State overhead rate. Our assumption is that line items other than indirect costs will remain the same whether funds come from State or Federal sources. If this assumption is not true for your budget, provide an explanation on the Budget Justification form.]* Agencies should include any internal costs associated with the management of project funds.

USFWS: A rate of 26.5% is applied to all internal USFWS costs. This indirect cost rate includes administration, clerical services, computer services, technical assistance, support facilities including furniture, phones etc., database management, and training.
A rate of 4.5% is applied to all pass-through sub-contracts, including, in this case, USGS.

USGS: For this project, the normal USGS indirect cost rate of 35.65% does not apply. As a sub-contractor to the USFWS, the USGS (both WERC and PWRC) is using an indirect cost rate of 15%, which covers: administration, clerical services, computer services, technical assistance, database management, training and support facilities, including furniture, phones, etc.

Joint Agency Proposal

submitted by:

U.S. Fish & Wildlife Service
U.S. Geological Survey

Thomas H. Suchanek, U.S. Fish & Wildlife Service, ECD, Sacramento, CA
Stephen E. Schwarzbach, U.S. Geological Survey, BRD, Sacramento, CA
Gary H. Heinz, U.S. Geological Survey, PWRC, Laurel, MD

in collaboration with:

Terrence L. Adelsbach USFWS, ECD, Sacramento, CA
Collin A. Eagles-Smith, USFWS, ECD, Sacramento, CA
John Y. Takekawa, USGS, WERC, Vallejo, CA
Susan E. Wainwright-De La Cruz, USGS, WERC, Vallejo, CA
A. Keith Miles, USGS, WERC, Davis, CA
D.J. Hoffman, USGS, PWRC, Laurel, MD

Mercury in San Francisco Bay-Delta Birds: Trophic Pathways, Bioaccumulation and Ecotoxicological Risk to Avian Reproduction

August 1, 2003

Note: This proposal was originally submitted as two separate proposals (USFWS – field studies; USGS – lab studies), but members of the previous review panel requested that we join the two efforts into one integrated package and to expand some of the priority issues. This has been done with a revised and expanded budget. In so doing, it has been difficult to provide a succinct proposal for such a large and diverse amount of work to be done. The basics of the approaches are given in the body of the text. Additional details, for those with further interest, are provided in a series of Tables and Appendices. Hopefully these extra materials will provide additional input to questions that might naturally arise from reading an abbreviated proposal.

A. Project Description

1. Problem

The Bay-Delta watershed has a legacy of mercury (Hg) contamination from both Hg mining and gold extraction. This Hg contamination is significant enough to threaten both human health and ecosystem function. Hg bioavailability within subregions of the watershed and even the watershed as a whole, ultimately may be increased by certain restoration approaches. Therefore, Hg complicates the analysis of CALFED restoration alternatives. Reduction and/or control of Hg within the watershed needs to be guided by appropriate human and ecotoxicological endpoints as well as an understanding of the factors affecting Hg bioaccumulation. The Review Panel that drafted the Mercury Strategy Document (Wiener et al. 2003 – pg. 25, lines 10-12) cited the need for information on Hg effects in birds as a requirement for adaptive restoration of the Bay-Delta ecosystem. In addition, the Review Panel recognized the sensitivity of avian reproduction to methylmercury(methyl-Hg; pgs. 24-26). Reproductive success in birds is believed to be more sensitive to methyl-Hgthan adult or juvenile survival (Finley and Stendell, 1978; Heinz, 1979; Scheuhammer, 1991; Tejning, 1967; Wiener et al., 2003) and consequently should be a point of focus for any biological work done in the Bay-Delta ecosystem. The usefulness of using avian reproduction as a sensitive endpoint has been demonstrated in other regions of the country where there is significant Hg contamination of aquatic ecosystems. For example, adverse effects of Hg in juvenile egrets in Florida, (Bouton et al., 1999) and impaired reproduction in common loons in New England and Wisconsin have both been linked to Hg contamination of the aquatic environment (Evers et al., 2000). Assessing the ecotoxicological risk of Hg is hampered by an inadequate understanding of methyl-Hg exposure among different foraging guilds of birds (Lovvorn and Gillingham 1996a, 1996b), the migratory patterns of some species, the potential for variation in the sensitivity to methyl-Hg by species and life stage, the paucity of avian feeding studies that assess methyl-Hg effects and the complications inherent in using only a field approach to assessing toxicological impacts from methyl-Hg.

Project Goal: Our goal is to use an integrated field and laboratory approach to evaluate the risks of Hg exposure to birds. This information is necessary to devise and prioritize Hg control strategies protective of Bay-Delta avian species. Effective control strategies are imperative in light of planned wetland restoration projects that have the potential to increase methyl-Hg availability and toxicity to wildlife. Specifically we propose to integrate a field assessment of exposure and effects with a laboratory assessment of the variation in sensitivity of avian embryos to methyl-Hg. Our field approach will evaluate the relative hazard of Hg to three foraging guilds of aquatic birds in conjunction with the ongoing assessment of Hg sources, loadings and bioavailability conducted by the CALFED Hg Project. We will determine which, if any, of these species are experiencing adverse effects in the field that may be linked with Hg exposure. We will also evaluate the potential influence of other contaminants of concern (COCs), primarily selenium (Se), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether (PBDE), which co-occur with Hg in some areas of the Bay-Delta. Our complementary laboratory approach will greatly improve interpretation of our field data, provide vital data on the variation in Hg sensitivity among avian species, and establish and refine methyl-Hg dose-response relationships and threshold concentrations associated with avian embryo toxicity.

We propose a research program with three objectives focused on representative species of aquatic birds from three distinct foraging guilds known to be at risk from Hg contamination.

Objective I: field studies of avian dietary Hg exposure and bioaccumulation in each foraging guild. Objective II: field studies of the effects of Hg bioaccumulation on reproduction and other parameters. Objective III: laboratory investigations on the differential sensitivity of avian taxa (from the guilds represented above) to Hg and determine No Observed Adverse Effects Level (NOAEL) concentrations in mallards through the use of controlled laboratory feeding experiments. Data obtained from each objective will be used to quantify Hg exposure and effects risks to aquatic birds, especially in relation to potential restoration projects that could increase methyl-Hg in regions of the Bay-Delta ecosystem.

OBJECTIVES:

Objective I. Field Studies of Avian Dietary Hg Exposure and Bioaccumulation
Using diet analysis, stable isotope techniques, Hg analysis of prey, and radio telemetry, we will identify species and geographic differences in trophic pathways over a range of exposures to methyl-Hg in three guilds of aquatic birds (littoral benthic-feeding shorebirds, obligate piscivores and diving benthivores) that forage in epi-benthic, surface-water, and benthic foraging habitats, respectively.

Task I.1*- Identify trophic pathways and geographic patterns of methyl-Hg exposure in surface-feeding recurvirostrids (avocets and stilts) through the use of diet analysis, stable isotopes and identification of foraging sites by telemetry.* ***Task I.2*** *- Identify trophic pathways and geographic patterns of methyl-Hg exposure in obligate fish-eating birds (terns) through the use of diet analysis, stable isotopes and identification of foraging sites by telemetry.* ***Task I.3*** *- Identify trophic pathways and geographic patterns of methyl-Hg exposure in benthivores (diving ducks) through the analysis of invertebrate prey, stable isotopes and identification of foraging sites by telemetry.*

Objective II. Field Studies of Hg Effects on Birds
Conduct field assessments of Hg effects on aquatic birds by quantifying reproductive success in three foraging guilds over a range of Hg exposures and evaluating the relative contribution of Hg, and other COCs, to any identified adverse effects to avian embryos and chicks in the field.

Task II.1 *- Conduct field studies of reproductive success in recurvirostrids over a range of Hg environments to evaluate the fate of eggs and chicks.* ***Task II.2*** *- Conduct field studies of reproductive success in terns over a range of Hg environments to evaluate the fate of eggs and chicks, and to identify potential effects on growth and biochemical functions.* ***Task II.3*** *- Evaluate the reproductive success, adult body condition and successful migration of diving benthivores that over-winter in the estuary using satellite telemetry and stable isotopes.*

Objective III. Laboratory Studies of Hg Effects on Birds
Establish and refine dose-response relationships and threshold concentrations of methyl-Hg associated with embryo toxicity in selected avian taxa.

Task III.1 *- Use a laboratory approach (egg injection techniques) to determine and quantify variability in the sensitivity of selected avian species to methyl-Hg in the egg.* ***Task III.2*** *- Use a laboratory approach (egg injection techniques) to explore toxic interactions of combinations of methyl-Hg plus selenium (in the form of selenomethionine) in the avian egg.*[1] ***Task III.3*** *- Conduct a controlled feeding study using mallard ducks (Anas platyrhynchos) designed to establish a true NOAEL (No Observed Adverse Effects Level)* to which the results from egg injection studies (above) may be calibrated, and produce statistical models to compare the sensitivity of wild avian species (1) when their eggs are injected with methyl-Hg, versus (2) when the methyl-Hg is maternally deposited in the eggs.

2. Justification
Existing regulations and guidelines to protect humans and wildlife from Hg poisoning strongly suggest that avian reproduction is more sensitive to methyl-Hg than is human health. Hg concentrations in fish proposed for human health protection are based upon rates of human consumption and using EPA's current methodology the wet weight Hg concentration proposed is 0.3 ppm. The chronic effects threshold for fish is believed to be between 0.68 and 5.0 ppm (Niimi and Kissoon 1994, Hammerschmidt *et al.* 2002). Data from field studies suggest that fish-eating birds, on the other hand, may very well be at risk from fish Hg concentrations below 0.3 ppm but this is a matter of ongoing research (Evers et al. 2001). Data from controlled laboratory studies have shown that as little as 0.5 ppm mercury as methyl-Hg on a dry-weight basis in the diet of mallards (which is equivalent to about 0.1 ppm Hg on a wet-weight basis) causes a reduction in reproductive success (Heinz 1979). Further, egg injection studies have demonstrated that mallards are not the most sensitive avian species, which means that some wild species may have even lower dietary thresholds for harm (Heinz 2002). In the survey of Hg in avian eggs of the Bay and Delta previously funded by CALFED, we found three species had overall and/or site means above the lowest concentrations found to be toxic in mallards at 0.5 ppm fresh wet weight (fww) (Fig. 1). These species were the Caspian tern (*Sterna caspia*), which had site means ranging from 0.7 to 1.2 ppm, the Forster's tern (*Sterna forsteri*), which had site means between 0.5 and 1.63 ppm, and the California clapper rail (*Rallus longirostris obsoletus*), which had a mean of 0.82 ppm. Two other species, the snowy plover (*Charadrius alexandrinus*) and the black-necked stilt (*Himantopus mexicanus*), had a site mean concentration just below 0.5 ppm but had some eggs between the 0.5 and 0.8 ppm thresholds. In addition, overwintering migratory avian species in the Bay-Delta far outnumber locally breeding species, and many, such as diving ducks (Hoffman *et al.* 1998), accumulate significant concentrations of Hg that may cause reproductive harm. Because the Bay-Delta ecosystem is the most important estuary for wintering birds on the Pacific coast (Bellrose 1980, Page and Shuford 2000), Hg contamination in this region has the potential to influence several North American avian populations. Thus, the effects of Hg on reproduction of both SFB local and migratory species warrants study.). Because avian reproduction is one of the most

[1]This was an additional task recommended by the previous review panel.

sensitive indicators of Hg effects in the Bay-Delta, it has been recommended for consideration in any CALFED adaptive restoration plan (Weiner *et al.* 2003). However, many questions about the variability of Hg concentrations and effects across avian guilds remain unanswered. Our proposed study is designed to identify both the causes of inconsistent Hg concentrations seen across Bay-Delta avian guilds and the potential reproductive problems posed at these concentrations. Our combined field and laboratory approach will provide comprehensive information on differences in Hg exposure pathways and sensitivities among species.

***The Guild Approach*:**

Estuarine waterbirds form distinct foraging guilds that are distinguished by their feeding method, diet preferences and habitat use (Takekawa *et al.* 2001). These guilds include (1) surface feeding and littoral zone probing recurvirostrids (American avocet: *Recurvirostra americana*, and black-necked stilt: *Himantopus mexicanus*), (2) diving benthivores (surf scoter: *Melanitta perspicillata*), and (3) obligate piscivores (Caspian tern: *Sterna caspia*, and Forster's tern: *Sterna forsteri*). Each of these guilds represents a unique component of the foodweb and foraging pathway within the Bay-Delta ecosystem for Hg bioaccumulation. Piscivorous birds traditionally represent the species with the greatest bioaccumulation potential in aquatic systems. In the Bay-Delta ecosystem the highest mean concentrations of Hg in avian eggs are found in Caspian terns and Forster's terns (0.9 and 0.8 ppm (fww), respectively : Figs. 1 and 2). An individual Forster's tern egg collected in the South Bay had the highest Hg concentration in a single egg of any bird species yet sampled among 321 eggs from 15 species at a fresh wet weight concentration of 3.3 ppm (Schwarzbach and Adelsbach 2002). Some non-piscivorous birds in SFB (e.g. - California clapper rails, snowy plovers, black-necked stilts, American avocets, surf scoter and greater scaup (*Aythya marila*)) also exhibit elevated Hg concentrations in their eggs and livers (Ohlendorf *et al*. 1986b, 1991, Schwarzbach and Adelsbach 2002), with surprisingly large differences in contaminant concentrations of black-necked stilts and American avocets from the same area, reflecting the importance of their foraging differences (Fig. 3). Diving benthivores, such as surf scoters that winter in the estuary, have some of the highest Hg concentrations reported for adult birds in the ecosystem (Ohlendorf et al. 1986, Hothem et al. 1998). Therefore, examining variation in contaminant uptake by the major foraging guilds will provide a more comprehensive understanding of bioaccumulation related to avian use of habitats in the estuary.

Justification for Objective I: Field Studies of Avian Dietary Hg Exposure and Bioaccumulation

Methyl-Hg is one of the rare compounds which not only bioaccumulates, but also magnifies across trophic levels. Bioaccumulation factors in aquatic systems commonly exceed 10^6 (USEPA 1997). Most Hg transferred across trophic levels is methyl-Hg, the more bioaccumulative and toxic form. Methyl-Hg can occur in high enough concentrations in the environment to be toxic to humans, who receive most of their Hg exposure through consumption of fish. Avian reproduction is so sensitive to Hg because most of the Hg in the avian egg is methyl-Hg and the developing avian embryo is exposed to this methyl-Hg unprotected by any maternal metabolism after oviposition. Aquatic ecosystems tend to have higher rates of bioaccumulation and biomagnification than do terrestrial ecosystems (USEPA 1997). Thus, aquatic birds are uniquely vulnerable to methyl-Hg. As the factors that control methyl-Hg formation, transport and loading within the Bay-Delta ecosystem are

evaluated by other CALFED projects, it is important to include an avian bioaccumulation component as this endpoint is almost certainly going to be a key driver of Hg control strategies.

Findings of elevated egg Hg in the Bay-Delta survey by Schwarzbach and Adelsbach (2002) was not restricted to piscivorous birds. Elevated Hg concentrations were found in several non-piscivorous birds nesting near salt ponds or in tidal marshes: California clapper rail (0.83 ppm at Wildcat Marsh), black-necked stilts (0.45 ppm at salt ponds near Moffett), American avocets (0.31 ppm at Pond A16) and snowy plovers (0.45 ppm at Pond A22). These concentrations all exceeded those found in the exclusively piscivorous Brandt's cormorants (*Phalacrocorax penicillatus*) (0.19 ppm) nesting at Alcatraz Island. In addition, Hg concentrations in Bay-Delta benthivores' livers meet or exceed those that cause reproductive damage in other waterbirds (Barr 1986). Estuarine birds occupy distinct foraging niches and Hg bioaccumulation in avian species has been shown to vary by two orders of magnitude in avian eggs within the Bay-Delta ecosystem. Thus, we propose a guild approach to assess multiple dietary pathways to determine which foodwebs, from which habitat types, present the greatest Hg bioaccumulation hazard. Objective I seeks to document the pathways of exposure to key bird guilds, and quantify Hg biomagnification through the food web in order to develop an understanding of factors affecting the variability in degree of exposure within and between the various guilds.

Quantifying contaminant trophic pathways and bioaccumulation requires a thorough understanding of dietary energy sources, and the percentage each source contributes to the diet. We will use several methods, including stable isotope analysis (SIA) to perform this task[2]. SIA has become an increasingly popular tool for quantifying trophic interactions and organic matter/energy flow in food webs, as well as tracing migratory origins/breading grounds of insects, fish, and birds Hobson *et al.* 1997, Hobson *et al.* 2000). In addition, SIA has been successfully coupled with studies of persistent contaminants (e.g. organochlorines, Hg, etc.) as a means of quantifying trophic transfer efficiencies and biomagnification rates (Fig. 4C) (Mazak *et al.* 1997, Atwell *et al.* 1998). Although the application of SIA to the characterization of food webs is most amenable to systems of relatively low "complexity", our initial data indicate that there are distinct spatial differences in isotope signatures between habitats within the Bay-Estuary. Such divergence between sites is highly agreeable to tracing energy sources and contaminant exposure with SIA. Appendix A provides further details on benefits and limitations of using the SIA.

Recurvirostrids (American avocet and black-necked stilt): American avocets and black-necked stilts are surface feeders that forage in the water column or on sediment surfaces mainly for invertebrate prey (Robinson *et al.* 1997 and 1999). While both species are found in wetlands and salt ponds, they may exploit somewhat different microhabitats while foraging. Evidence suggests they feed in slightly different water depths (Hamilton 1975), and that stilts use vegetated areas in marshes and salt ponds, whereas avocets prefer to forage in more open areas (Rintoul *et al*. 2002). Major prey items for both species in salt ponds or saline inland wetlands include brine shrimp (*Artemia salina*), brine flies (*Ephydra spp*.), and terrestrial insects (Robinson *et al*. 1997, 1999). Other food items in brackish environments include vegetation and seeds, amphipods, isopods, small polychaetes and fish (Hamilton 1975, Robinson *et al.* 1997, 1999). Recurvirostrids have been shown to be quite vulnerable to contaminants, particularly Se (Ohlendorf *et al.* 1989, Williams *et al*. 1989). Mean methyl-Hg concentrations in black-necked stilt eggs at the Baumberg and Moffit salt

[2] See Objective I Approach for details on how these methods will be employed.

ponds were just under the 0.5 ppm (wet weight) threshold for embryotoxicity for bird eggs, while mean values in avocets from nearby sites were generally lower (Schwarzbach and Adelsbach 2002). The telemetry and foraging ecology work we propose will help clarify whether these differences represent site-specific contamination or differences in microhabitat use and diet between species.

Terns (Caspian tern and Forster's tern): Caspian and Forster's terns are colonial ground nesting birds. Among the 15 avian species assessed in the Bay-Delta ecosystem, the highest egg Hg concentrations have been documented within these two species (Schwarzbach and Adelsbach 2002). While cormorants can take larger prey from greater depths, they have only about one third to one half of the Hg found in tern eggs in the Bay-Delta ecosystem (Schwarzbach and Adelsbach 2002). Within the Bay-Delta ecosystem a large determinant of Hg exposure is where an organism feeds as well as what it feeds on. There are large differences between species feeding solely or partially on fish and much of this variation in exposure is likely attributed to differences in forage species and microhabitats being used for foraging. Hg exposure even amongst strict piscivores is heavily mediated by foodweb and ecosystem processes (Fig. 6). Previous work funded by CALFED with injections of methyl-Hg into double-crested (*Phalacrocorax auritus*) cormorant eggs has suggested they are less sensitive than mallards (Heinz 2002), but comparable embryo sensitivity data is not available for terns. Field and laboratory data are needed in terns to evaluate whether elevated Hg in tern eggs produces harm and what food web links are responsible for the findings of elevated egg Hg.

Caspian terns are the largest tern species and forage on the open bay, salt ponds, as well as in freshwater ponds and rivers. Caspian terns nesting on salt pond levees near the Napa river for example forage in the open water of Suisun Bay, the Napa River, San Pablo Bay as well as in the North Bay salt ponds. Forster's terns are smaller but more numerous with more breeding colonies. Forster's terns nest on salt pond levees near the Napa River and have nested at 28 sites in the South Bay since 1992 (Goals Project 1999). Our initial analysis identifies significantly different $\delta^{13}C$ and $\delta^{15}N$ values for identical fish species in salt pond versus open bay habitat (Fig. 4A, 4B, 4D). The potential for Hg exposure is also greatest in the salt pond environment (Fig. 4C) suggesting that birds foraging in these areas are likely at higher risk for reproductive impairment than those individuals feeding in the open bay. The distinct differences in isotope signatures of prey items from salt pond versus open bay allows for a robust quantification of the proportion of an individuals diet which is derived from each habitat. Our initial analysis of Forster's tern eggs (Fig. 5) also indicates that spatial sub-habitats within the Bay possess distinctive isotopic signatures which allow for a site specific evaluation of dietary exposure and risk of reproductive impairment.

Diving benthivores (surf scoter): Diving waterfowl are bay and sea duck species from the tribes Aythyini, Mergini and Oxyurini that forage for prey in open bay, salt pond, and slough habitats. The primary winter prey of diving ducks is benthic macro-invertebrates, but several species also feed on plant materials, fish roe, and other items. The San Francisco Bay-Delta estuary is the most important diving duck wintering site in the lower Pacific Flyway, and frequently harbors well over 300,000 individuals (Trost 1998, 2000; U.S. Fish and Wildlife Service 2001). The most abundant species on the open Bay include surf scoter, greater and lesser scaup (*Aythya marila* and *A. affinis*), canvasback (*A. valisineria)*, ruddy ducks (*Oxyura jamaicensis*), and bufflehead (*Bucephala albeola*). North American populations of several of these species are currently in decline,

particularly surf scoter, scaup, and canvasback (Hodges 1996, Savard *et al.* 1998, Austin *et al.* 2000, Sea Duck Joint Venture SDJV Management Board 2001).

Surf scoters are one of the most numerous benthic-foraging ducks in the Bay-Delta ecosystem. As much as 78% of the lower Pacific Flyway wintering population is found in the estuary (Accurso 1992; Trost 1998, 2000; U.S. Fish and Wildlife Service 2001). Scoters accumulate some of the highest concentrations of Hg (12.5 ppm dw in liver) and Se (119 ppm dw in liver) of any avian species in SFB (Ohlendorf *et al.* 1986, White *et al.* 1989, Ohlendorf *et al.* 1991, Hoffman *et al* 1998). Such concentrations are beyond those associated with adverse effects to reproduction in dabbling ducks (*Anas spp*) (Ohlendorf *et al*. 1989, Skorupa and Ohlendorf 1991, Heinz and Hoffman 1998). In addition to reproductive effects, hepatic Hg concentrations have been associated with decreased body, liver, pancreas, and heart weights (Hoffman *et al.* 1998, Takekawa *et al*. 2002), and oxidative stress was correlated with Se and Hg concentrations in SFB surf scoter and scaup (Hoffman *et al.* 1998).

Justification for Objective II. Field Studies of Hg Effects on Birds

It is very likely that avian reproduction is a more sensitive toxicological endpoint for Hg in the Bay-Delta ecosystem than human health and that Hg concentrations are high enough to impair avian reproduction. To date, however, reproductive studies in the field to assess the impacts of methyl-Hg in birds in the Bay-Delta ecosystem have only been done with California clapper rails - a species with elevated Hg and depressed egg hatchability (Schwarzbach *et al.* unpublished data). Other studies have been limited to the assessment of Hg concentrations in eggs without the assessment of impacts to embryos or chicks.

To conduct meaningful studies of Hg contamination in the field, we need to select species most conducive for nest study as well as Hg bioaccumulation. We need a gradient of Hg concentrations and we need to be able to follow the fate of eggs and chicks after hatch, while assessing the degree of Hg bioaccumulation associated with this fate. Hg bioaccumulation is, on average, greater in piscivorous species, but significant Hg bioaccumulation has also been found in other guilds in the Bay-Delta ecosystem (California clapper rails, recurvirostrids and diving ducks) leading to new hypotheses about trophic transfer of methyl-Hg related to sediment dynamics within the wetland margins. This evidence makes these studies especially relevant to management decisions on potential restoration projects because the planning process needs to incorporate toxicological considerations for both human and ecosystem health.

Important factors that make a species more amenable to field investigation of reproductive success include the ability to make multiple visits to the nest with a minimum of disturbance to the incubating birds and with a minimum of cost and hazard to the investigator. Two guilds of birds with species nesting in the Bay-Delta ecosystem lend themselves to this approach - recurvirostrids and terns. In addition, species from a third guild, the diving ducks, have very high Hg concentrations in their livers accumulated during their winter stay in the Bay-Delta system. The lack of cross-seasonal studies of methyl-Hg reproductive effects has been cited by several sources (Henny *et al.* 1995, Savard *et al*. 1998, Miles 2000, Luoma and Presser 2000, Wiener *et al.* 2003). Satellite telemetry technology now makes it possible to overcome this key data gap as they can be followed to their breeding grounds (http://www.werc.usgs.gov/scoter). Our work will pioneer efforts to link winter Hg accumulation of a migratory species with potential reproductive effects on distant breeding grounds. Since migratory wintering birds comprise the majority of birds using the

Bay-Delta ecosystem, this cross-seasonal work is imperative for understanding the ramifications of Hg accumulation in migratory birds in the Bay-Delta region that breed elsewhere.

Justification for Objective III: **Laboratory Studies of Hg Effects on Birds**
Given the complexity of field studies, including the presence of many other environmental stressors, complementary controlled laboratory studies are needed to refine our ability to attribute impaired reproduction to a specific contaminant such as Hg. The June 26, 2003 draft of the "CALFED Mercury Strategy Document" (Wiener *et al.* 2003) recognized the need for a combined field and laboratory approach to assess the hazards of methyl-Hg to avian reproduction (page 25, lines 30-31). In predicting the adverse effects of Hg on birds in the field, avian researchers typically rely upon a few benchmark laboratory studies that have established threshold concentrations in bird eggs associated with impaired hatchability and altered behavior of hatched chicks likely to result in reduced juvenile survival. Laboratory feeding studies with methyl-Hg that have demonstrated reduced hatchability of avian eggs include Fimreite (1971) at egg Hg concentrations of 0.5 to 1.5 ppm (fww) in ring-necked pheasants (*Phasianus colchicus*) and Heinz (1979) which found that effects upon hatchability were associated with average egg concentrations of 0.8 ppm (fww) in mallard ducks. These studies were not designed to establish NOAELS (No Observed Adverse Effect Levels) or LOAELS (Lowest Observed Adverse Effects Levels) for ecological risk assessments, but instead provided opportunistic observations of effects at the relatively few number of concentrations tested. Another key uncertainty for evaluating the importance of Hg in limiting avian reproduction in the Bay-Delta region is the relative applicability of pheasant and mallard Hg toxicity thresholds to estuarine birds indigenous to the region such as terns, herons egrets, diving benthivores, recurvirostrids and cormorants. This uncertainty is related to two factors: 1) the uncertainty of extrapolating from one species to another and 2) the uncertainty of extrapolating from laboratory to field settings where birds must cope with the multiple stresses of survival in the wild. What is needed is a quantitative knowledge of inter-species differences in the response to methyl-Hg in the egg and a definitive assessment of the NOAEL and LOAEL in a key bird species by which the relative sensitivity of the embryos of other wild species to methyl-Hg may be calibrated.

3. Approach

Objective I: Field Studies of Avian Dietary Pathways of Hg Bioaccumulation

Invertebrate Prey Sampling: We will sample north and south Bay avian prey base using transects along habitat gradients. Three transects will be established in San Pablo Bay and 3 in south San Francisco Bay. Transects will initiate at the same shallow, subtidal depth, proceed approximately straight line, and end at the center of a salt pond. Start and end points will be GPS coordinates. Habitat gradients will be sampled along each transect: 3 subtidal, 3 intertidal, 3 marsh, 3 slough, and 3 salt ponds stations will be established at similar tidal depth or vegetative characteristic (preferably cordgrass [*Spartina* spp.] or pickleweed [*Salicornia*] spp. marsh); stations will be a minimum of 50 m apart and identified as GPS coordinates. To determine diversity and biomass, 3 random Eckman core samples will be taken at each station. Station samples will be composited as one sample per each habitat gradient per each transect, and statistical means established for each

San Pablo or South Bay gradient. The transects will be sampled in concert with migratory bird studies in year 1 and 2. A total of 180 benthic samples will be collected. In addition, 3 pelagic sweeps of a large mesh (.05 mm) planktonic tow will be conducted per each station in the shallow subtidal, and salt ponds, and composited as one sample per gradient per transect (72 samples). Sample processing (N = 252) will be conducted in years 1, 2, and 3.

Fish Prey Sampling: Because both tern species are obligate piscivores, invertebrate sampling for this guild serves mainly to establish and isotopic baseline for each foraging area and identify primary trophic pathways in the secondary nodes within the food web. In order to characterize tern dietary exposure, we will be conducting fish sampling in concert with the invertebrate collections. Salt ponds and intertidal zones will be sampled with a 60m bag seine, each sweep being replicated three times, following standard methods identified in Murphy and Willis (1996). At least 5 individuals from each size class of each species will be collected for contaminant and isotope analysis. In addition, all fish captured will be identified to species, weighed, and measured. Monthly sampling to characterize species-specific relative abundance presence/absence will be conducted during the avian breeding season. Open water salt pond habitat will be sampled via gill nets. One hour setting time will be rigorously followed to ensure adequately comparable CPUE values, and prevent extensive indirect mortality of non-target species. Open water Bay habitat will be sampled with standardized surface and mid-water trawls. Each tow will be replicated three times for each transect. To the extent possible, fish sampling will be coordinated with ongoing monitoring programs by California Department of Fish and Game, Department of Water Resources, and University of California, Davis. In order to account for the transient nature of certain fish species with the SF Bay Estuary, we will collect fish for contaminant and isotope analyses early and late in the breeding season. This will allow us to more confidently identify any temporal diet shifts in terns that result from changes in individual prey availability.

Avian Foraging Characterization: Additional subtidal and intertidal sites in San Pablo and South San Francisco Bay will be sampled depending on the avian foraging characteristics observed in the habitats defined above. For example, if the 3 subtidal gradients sampled in San Pablo Bay occur outside of observed avian foraging areas, 3 sites within avian foraging areas will be sampled for comparison. However, in order to define avian preference relative to prey availability, additional samples will be necessary. Stations established for foraging characterization will be sampled as defined above. Additional samples (N = 108) will be collected for diversity and biomass.

Prey Chemistry and Pathology: Caloric value, nutrient chemistry (e.g., N, P, Ca), stable isotope signature, pathology (parasites, abnormalities), and Hg concentrations will be determined to define dietary parameters that affect avian predators. We will collect in mass and analyze the most commonly consumed prey species from each gradient using a modified otter trawl, bottom seine, or suction dredge in the aquatic habitat, and baited pit traps or hand dredges in the marsh environment When possible, we will target up to 3 of the most common phyla (e.g., mollusk, crustacean, polychaete, insect) consumed by each avian species and up to 2 species from each phylum from each gradient. One composited sample from each of the 5 gradients per each taxon (6 possible samples) per each of 6 transect per year 1 and 2. Each of these samples will be subdivided for the analyses of the 6 determinations. (Total number of samples = 360).

Primary and Secondary Contaminants: The primary contaminant being investigated in this project is Hg. During the first year, a representative suite of tissue samples will be analyzed for total recoverable Hg, with a subset of each type of matrix analyzed for methyl-Hg in order to establish a typical ratio of methyl:total Hg in specific organs, tissues and prey items. Once the methyl:total Hg ratio (and its variability) is determined for most matrices, future Hg analyses will focus primarily on total Hg, but with more additional verification for invertebrate prey because their methyl:total Hg ratios are known to be more variable. Hg will be analyzed on 6 avian tissue or organ types (blood, feathers, muscle, liver, kidney and brain) and in prey of these avian species. Blood and feathers will also be evaluated for possible use as non-lethal indicators of Hg exposure and risk. Analyses of prey from foraging sites of targeted avian species will provide spatial and temporal analyses of environmental loads and distribution, and verification of exposure at those foraging sites relative to concentrations determined in avian predators.

Secondary analytes include, but will not be limited to, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and selenium (Se). These secondary analytes have been documented at high levels either in representative prey species or within individual adults or their eggs or both within the geographic scope of this proposed project. These secondary analytes are of toxicological significance to the species being proposed for study as they are readily bioaccumulated and may have effects on reproduction and individual survival.

***Telemetry: Adult Recurvirostrids, Terns, and Diving Ducks*:** Captured birds will be marked with USFWS leg bands, as well as color bands, to facilitate identification of individuals throughout the remainder of the project. Further details of our capture methods can be found in Appendix B. For a subset of these captured birds, very high frequency (VHF) radio transmitters will be applied and used to determine local and regional movements, home ranges, and foraging locations of species from each guild. This will allow us to establish links between foraging areas, Hg in prey at these locations, and Hg accumulation in adult birds. Ultimately, these data will help elucidate the differences in Hg concentrations we document among avian species in the Bay-Delta region. Telemetry methods are well established for waterbirds from all guilds we propose to study (Warnock and Takekawa 1996, Kenow *et al.* 1997, Takekawa *et al.* 2002, Hickey *et al.* unpub. data). This tool is an effective means of determining site fidelity and core use areas for individual birds (Worton 1987, Kenward 2001) (Fig. 7). In the case of diving ducks, specific foraging locations can be determined by listening for signal attenuation when a bird is diving (Custer and Custer 1996, Takekawa *et al.* unpublished data).

Satellite telemetry is a highly effective tool for linking wintering and breeding grounds of migratory birds (Takekawa et al. 2000), and will help us to determine the cross-seasonal effects of Hg on breeding surf scoters. In this method, small satellite platform terminal transmitters (PTTs) attached to a bird transmit position data to polar orbiting satellites, which are in turn sent to us electronically. Satellite data will help us find and collect eggs from nesting surf scoters in their northern breeding grounds (see Objective II) (Fig. 8).

***Dietary Studies - Recurvirostrids, Terns, and Diving Ducks*:** Core component 5 of the CALFED Hg Strategy Document (Wiener et al. 2003) involves an assessment of ecological risk to protect fish and wildlife from adverse effects of methyl-Hg exposure. One of the objectives stated in this

component is to identify habitats, areas, and trophic pathways associated with elevated, potentially harmful methyl-Hg exposure. To that end, we propose to utilize stable isotope technology as a complementary means for identifying pathways of methyl-Hg exposure in at-risk avian species. SIA will not only provide a quantitative assessment of methyl-Hg trophic transfer, but will assist in identifying the amount Hg that is accumulated by avian predators in the Bay-Delta ecosystem versus Hg that is carried in by the birds from other locations. In addition, SIA will be useful in identifying sites of exposure in birds that forage over a large spatial range within the system.

We will use a combination of stable isotope analysis (SIA), and traditional diet approaches (gastrointestinal (GI) tract analysis, and direct feeding observations) to determine trophic pathways and quantify exposure sources of Hg and secondary contaminants in all three guilds. This information will be coupled with data gathered through telemetry (see previous section), allowing us to examine site-specific variability in diet, Hg exposure, and Hg biomagnification for all three guilds. Because avian predators are highly mobile, their diets (and thus contaminant exposures) may be temporally and spatially variable. Significant seasonal and annual variability has been documented in invertebrate prey at some foraging sites (Fig. 9), which can influence energy flow and Hg bioaccumulation. Thus, in sacrificed birds we will be analyzing stable isotopes in a suite of tissues with varying turnover times, representing diet over several temporal and spatial scales. [3]

Blood and liver will be used as short term diet indicators. While liver has perhaps the shortest turnover rate of all the tissues, blood has the benefit of allowing for repetitive sampling over time without requiring sacrifice of the animal. Controlled feeding trials have shown that isotope ½-lives range from 2.6 days in liver to 11.4 days in whole blood. When blood fractions are separated, Hobson and Clark (1993) identified that the isotopic ½-life in plasma was 2.9 days while that in the cellular fraction was 29.8 days. It is also desirable to gather data on longer-term diet sources, in order to differentiate the local isotopic signatures from those incorporated at other spatial locations. Thus we propose to include SIA of muscle tissue. Turnover rates of muscle tissue results in integration of diet over a span of several weeks to several months (Hobson and Clark 1992). In order to determine potential effects of maternal diet (thus contaminant exposure) on chicks, it is useful to examine dietary constituents during egg formation. Hobson (1995) and Hobson *et al.* (1997) examined C and N isotope fractionation and turnover during egg formation, and found that both yolk and albumin isotope signatures are directly related to maternal diet during egg formation. In circumstances where maternal nutrient reserves are abundant, endogenous protein and lipid reserves may be partitioned into the developing egg as well. This has potential implications for contaminant exposure for the Diving Duck Guild in particular, as they overwinter in the Bay-Delta region and breed in arctic freshwater systems. Evaluation of endogenous nutrients (and contaminants) allocated from Bay-Delta feeding grounds will be addressed in the Diving Duck Guild for Objective II. All eggs will have lipids extracted (Hobson et al., 1997) prior to $\delta^{13}C$ analysis to prevent interference from lipid variability in eggs. Feathers provide another tissue of value for isotopic analyses. Because feathers are biologically inert once grown (Mizutani *et al.*, 1992), they provide data on diet during the feather growth period. Thus for species which molt prior to entering the Bay-Delta system, feathers provide an excellent baseline indicator of isotope signature from the wintering grounds and migration route, and will allow us to identify isotope carryover relative to Bay-Delta signatures in other tissues. Furthermore, during the time period when chicks are being fed by adults, isotopic analysis of chick feathers will represent both chick

[3] Refer to sub-sections of each guild for details on sample sizes for each tissue.

and adult diets. Variation in basal $\delta^{15}N$ signatures between habitats can convolute interpretation and comparison of trophic levels occupied by individuals between habitats. We will determine $\delta^{15}N$ in obligate primary consumers, such as *Potamocorbula* from each habitat and calculate a baseline trophic position following Vander Zanden and Rasmussen (1999).

We will use GI tract examination as a direct means of diet analysis that will complement and validate SIA. While this method requires that we collect birds, we will coordinate our effort with other project tasks so that tissues from birds collected for GI tract analysis will also be used for contaminant and SIA.

We will collect adult surf scoters, terns, avocets, and stilts (60 scoter and 40 terns, avocets, and stilts per year) from sites identified as important foraging areas by radio-marked birds. Each bird will be observed to ensure it is feeding for at least 10-15 minutes before it is collected by air gun or shotgun with appropriate sized steel shot. The exact collection location will be recorded in UTMs with a GPS unit. To prevent digestion of prey items in the GI tract, we will process birds immediately after collection at a pre-determined on-site processing area. Using appropriate clean techniques to ensure proper handling of other tissues for contaminant analysis, we will excise and remove the entire upper GI tract, including esophagus, crop, proventriculus, and gizzard, and place it in 70% ethanol. Processing will involve rinsing esophagus and proventriculus contents in a 0.5 mm sieve, then sorting and identifying each organism to species. We will determine overall wet and dry biomass, wet and dry mass by species, as well as length and width of each organism. Gizzard contents will be sorted and identified to species and genus when possible. After processing, samples will be archived at the USGS Davis Field Station in 70% ethanol with 1g/l rose bengal.

Objective II: Field Studies of Hg Effects on Birds

RECURVIROSTRID GUILD:

Reproductive monitoring: We will monitor hatchability and fledging success of stilt and avocets using established protocols (Marn 2003). Nests of radio-marked and non-marked birds will be tagged, aged, and monitored weekly until hatching. Color-banded nestlings will be followed to determine fledging fate and compared to fates of radio-marked chicks (see post-hatch telemetry). We will collect information on number and stage of eggs or chicks, adult presence, and egg and cause-specific nest fates. One fresh egg will be collected from each of 10 randomly selected nests at each study site, and fail-to-hatch eggs will be salvaged opportunistically. Collected eggs will be assessed for viability and any late-stage embryos examined for gross abnormalities indicative of Hg or other contaminant toxicity (e.g., malposition, gross malformations of the eyes, brain, wings and legs). Eggs will be analyzed for Hg and other priority contaminants in the Bay. We will use egg Hg concentrations to estimate the effect of Hg on within clutch hatchability (Marn 2003, Hosmer and Lemeshow 1989). Stable isotopes in eggs will be used to determine Hg sources from parents.

Adult Sampling: Blood (plasma and cellular fraction), liver, muscle and feather from 40 each of foraging adult stilts and avocets collected for diet analysis in Objective I will be analyzed for stable isotopes (C, N, S), as well as total and methyl-Hg (blood and liver only). Gross anatomical necropsies will also be performed on all collected birds and tissue will be sampled and archived for histological analyses. This will provide evidence of the contaminant load they acquire during their

residency on the breeding grounds in the Bay-Delta system. Details on our protocols for necropsy are provided in Appendix C.

Nestling Sampling: At each study site we will euthanize 10 avocet and 10 stilt chicks at approximately 10 to 14 days post-hatch. Chick blood and tissues will be preserved for stable isotope, contaminant, and histopathology analyses. Methods for collection and preparation of individual bird samples will follow the methods described in Henny et al. (2002). On-site gross anatomical necropsies and histological analyses will be conducted by staff of the California Department of Fish and Game's Marine Wildlife Veterinary Care and Research Center in Santa Cruz, CA. Remaining tissues will be archived for potential histopathological analyses.

Post-hatch Telemetry and nestling developmental assessments: Post-hatch behavior and survival can be influenced by Hg-induced immune deficiencies and neurological damage (Henny et al. 2002). We will use radio telemetry on recurvirostrid chicks to measure behavior and survival (Marn 2003). Within 24 hours of hatch, stilt and avocet broods will be captured by hand and all chicks will be weighed, measured and marked with color-bands. One randomly chosen chick from each captured brood (40 in total) will be marked with a small transmitter (1.3-1.5 g, model BD-2, Holohil Systems Ltd., Woodlawn, ON) attached by a subcutaneous anchor (Newman et al. 1999) or subcutaneous suturing (Marn 2003). Marked chicks will be tracked as described for adults in Objective I for 4-5 weeks until they fledge (Gibson 1971). Home range, habitat use, foraging areas, survival rates and dispersal distances will be determined and compared to Hg concentrations in chick blood.

Mercury may also influence chick growth (Henny *et al*. 2002). Because avocet and stilt chicks are precocial and leave the nest area shortly after hatch, consistent recapturing to monitor growth is difficult. We monitor growth by weighing and measuring recaptured radio-marked and opportunistically recaptured banded chicks at least once before fledging. To minimize effects on survival we will attempt to recapture chicks >10 days old (Marn 2003).

TERN GUILD:

Because preliminary work from an earlier CALFED funded project (Schwarzbach and Adelsbach 2002) has revealed low reproductive success in two species of terns nesting in the Bay-Delta ecosystem, we will conduct an in-depth research and monitoring program aimed at identifying specific causes of reproductive impairment. Individual adults and nestlings will be collected from treatment and reference colonies. A thorough diagnostic veterinary exam, histopathological assessment, and comprehensive biomarker analysis will be conducted on each bird. Selected biomarkers are indicative of exposure to Hg and PCBs and will be used in conjunction with analytical chemistry results from each tissue matrix. This information will be used to assess the affects of contaminants on individual health, which can be linked to survival, and ultimately to population level parameters. We will also quantify overall reproductive success, including hatchability, nestling survival, fledging success, and nestling growth.

Reproductive Monitoring: Monitoring will evaluate (1) the size of each tern colony (numbers of nest and adults), (2) hatchability (the proportion of eggs incubated to full term that successfully hatch) and (3) fledgling success (average number of young fledged per breeding pair). Methodology will follow standardized observational and data collection protocols described in

Collis et al. (2002) and Roby et al. (2002, 2003). Use of these protocols will ensure that results are comparable with other reproductive monitoring studies being conducted at other colonies within the Pacific coast populations of Forster's and Caspian terns. A minimum of 50 nests will be marked and monitored at each breeding colony in each of the study regions. In years 1 and 2 one egg from ten randomly selected nests from each region will be collected and analyzed for contaminants and stable isotopes. Contaminant levels in these eggs will be compared to the fate of sibling eggs remaining in the nest and to the fate of eggs from the other marked nests (Blus 1982, 1984; Custer et al. 1990). Up to 10 fail-to-hatch eggs also will be collected from marked nests and analyzed for contaminants and stable isotopes. Contaminant concentrations will be compared between the potentially viable eggs and failed-to-hatch eggs.

***Adult Sampling*:** Ten adult terns of each species from each region (up to 30 adults each for Forster's tern and Caspian tern) will be euthanized and analyzed for gross anatomical necropsy, histological data, and analysis of contaminants and stable isotopes during two periods of the breeding cycle: (1) the pre-nesting phase when they arrive on the breeding grounds and (2) the post-nesting phase before individuals depart for fall migrations. Organs/tissues to be sampled include blood (plasma and cellular fraction), feathers, muscle, liver, brain, kidney and where possible, stomach contents. All samples will be analyzed for Hg and all except brain and kidney will be analyzed for stable isotopes. This will provide evidence of the contaminant load they acquire during their residency on the breeding grounds in the Bay-Delta system. Biomarker analysis will also be conducted on tissue samples from the late season collections.

***Nestling Sampling*:** During the nestling phase, at least 50 live tern chicks from each species from each region (total of 150 chicks each for Caspian and Forster's tern) will be marked with USFWS leg bands (denoting banding year, banding site, and individual) and color leg bands (or marking paint for very small nestlings). Chick banding will facilitate successful aging of the birds as well as ensure that siblings are not being sampled for biomarker assessment or chemical analysis.

At approximately 10 to 14 days post hatch, up to 10 chicks from each species in each of the three regions (total of 30 chicks each for Forster's tern and Caspian tern) will be euthanized. Methods for collection and preparation of individual bird samples will follow the methods described in Henny et al. (2002). A range of biochemical and histological biomarkers will be analyzed for contaminant effects and will include (1) oxidative stress biomarker for Hg exposure (Henny et al. (2002) and (2) P450 enzyme induction for PCB exposure (Custer et al. (2001). Biomarker analyses for Hg and PCB's are well established and provide useful information on the link between exposure and effects in individuals. Information on effects will be useful in determining the nature and extent of impacts from these contaminants on individual health and survival which can, in turn, affect population level processes. On-site gross anatomical necropsies and histological analyses will be conducted by staff of the California Department of Fish and Game's Marine Wildlife Veterinary Care and Research Center in Santa Cruz, CA. Biochemical analyses will be conducted by researchers at the Patuxent Wildlife Research Center (PWRC). Further details on protocols for biomarker analyses can be found in Appendix D (oxidative stress biomarker) and Appendix E (P450) and for histology in Appendix F.

***Nestling Developmental Assessment*:** Three visits will be conducted to each breeding colony in each of the regions outlined above during the post hatch phase of the breeding cycle. The first visit will be in the early hatching phase, one at approximately half way through the nestling phase, and a final visit near fledging. The nestling phase usually lasts from 35 to 45 days so visits would be approximately 10 days apart. At each visit physical measurements of 50-100 individual chicks of various ages will be obtained to develop time-specific growth curves for Caspian and Forster's terns (Palacios and Anderson – unpublished data). These data will be used to characterize pre-fledging development in order to assess possible trends in nestling development that may be correlated with Hg and/or PCB contamination.

***Post-hatch Telemetry*:** Post-hatch behavior and survival can be influenced by Hg-induced immune deficiencies and neurological damage (Henny et al. 2002). We will use radio telemetry on tern chicks to measure behavior and survival (Marn 2003). Up to 40 chicks will be caught within 10 days of hatching, banded, and marked with a small subcutaneous anchor (Newman et al. 1999) or glue-on transmitters (<1 g, model BD-2, Holohil Systems Ltd., Woodlawn, ONT). Chicks will be tracked as described above (Objective I: Approach - Telemetry**)**, and home range, habitat use, foraging areas, survival rates and dispersal distances will be determined and compared to Hg concentrations in chick blood.

DIVING DUCK GUILD:

***Cross-seasonal linkage and egg analyses* :**
Migratory birds in the Bay-Delta far outnumber breeding birds. The key to understanding how Hg may influence the millions of migratory shorebirds and waterfowl that winter in SFB is the determination of their reproductive success on distant breeding grounds. Diving ducks have a propensity to accumulate high contaminant loads while over-wintering in the Bay-Delta (Ohlendorf *et al*. 1989, Hoffman *et al.* 1998) and using nutrient reserves acquired during winter for egg production (Alisauskas and Ankney 1992) leaves them vulnerable to Hg-induced reproductive harm.

We will use satellite telemetry coupled with conventional telemetry to identify migratory stopover sites and breeding locations of surf scoter migrating from SFB. In spring of 2003, we conducted a successful pilot study to determine the migration route and breeding sites of 14 satellite-marked and 28 conventionally-marked SFB surf scoters (see http://www.werc.usgs.gov/scoter). During late winter, we will capture 10 female surf scoters and mark them with satellite transmitters (PTTs). We will fly telemetry transects at 16 km intervals in a 160 km radius of each PTT-marked bird to conventionally-marked birds and land to find and collect one egg and any feathers found in the nest as well as from nests of any other scoters found in nest searches. Eggs will be analyzed for methyl-Hg and other potential contaminants (identified above). We will compare stable isotope signatures in yolk and albumin to determine if contaminant sources to the egg are from endogenous female reserves (derived from Bay-Delta prey) or exogenous sources on the wintering ground (Alisauskas and Ankney 1992, Hobson *et al.* 1997, Hobson *et al.* 2000). Contaminants data from these eggs will be compared with winter foraging information. Isotope signatures will also be compared in feathers and eggs from marked and unmarked birds to determine if unmarked birds winter in Bay-Delta system, and thus if birds that winter together return to the same breeding areas.

Objective III: Laboratory Studies of Hg Effects on Birds

The June 26, 2003 draft of the CALFED Mercury Strategy Document (Wiener *et al.* 2003 – pg. 25, lines 30-31) recognized the need for a combined field and laboratory approach to assess the hazards of methyl-Hg to avian reproduction. Our laboratory research on the effects of methyl-Hg on reproductive success in birds is designed to complement our field research and to address important questions that may be difficult to address solely through fieldwork. Our laboratory approaches to measuring the sensitivity of bird reproduction to methyl-Hg are divided into three main parts:

Egg Injections with Hg: The Review Panel recognized the importance of egg injection studies and recommended that, "Dose-response relations and threshold concentrations for reproductive effects should be estimated with controlled laboratory experiments, such as egg-injection studies for birds (Heinz, 2002)..." In response to the Review Panel's recommendation, we will increase the number of species tested with egg injections and, with species that have already been tested, we will improve sample sizes. Our goal is to derive models for predicting the harm methyl-Hg poses to the reproductive success of aquatic birds within the San Francisco Bay-Delta ecosystem. The first step in deriving these models is to quantify the sensitivity of each species to graded doses of methyl-Hg injected into the egg. We know from the results of our injection studies conducted so far that there are substantial differences in sensitivity among species. For example, with some species that seem to be relatively resistant to methyl-Hg, such as the mallard, double-crested cormorant, and sandhill crane (*Grus canadensis*) our data show that it takes between 0.8 to 1.6 ppm injected Hg to begin causing mortality of embryos (Heinz 2002). By contrast, in very sensitive species, such as the white ibis (*Eudocimus albus*), clapper rail (*Rallus longirostris*), and snowy egret (*Egretta thula*) the toxic thresholds fall between 0.1 and 0.4 ppm injected Hg. The sensitivity of each species will be quantified as the LC50 (Lethal Concentration to 50% of the test animals) and the LOAEL (Lowest Observable Adverse Effect Level). The LC50 will be calculated as the concentration of Hg that has to be injected into the eggs of that species to kill 50% of the embryos. The LOAEL will be the lowest injected concentration of Hg that causes a statistically significant increase in embryo mortality above the mortality recorded for control eggs.

The second step in our modeling of species sensitivity will be to calculate how the toxicity of injected methyl-Hg compares to the toxicity of methyl-Hg deposited into eggs by the mother. For example, suppose injected Hg were found to be twice as toxic to embryos as naturally deposited Hg; in this hypothetical case one would then multiply the LOAEL determined by egg injections for each species by two to estimate the concentration of naturally deposited Hg that will begin to pose a hazard to the survival of avian embryos. Once we estimate the lowest concentration of methyl-Hg in the eggs of a wide variety of birds that is the threshold for reproductive impairment, we can then monitor Hg concentrations in the eggs of birds in the Bay-Delta area to determine if any contain hazardous levels of Hg. The same thresholds can serve to determine whether restoration of the Bay-Delta has succeeded in reducing Hg concentrations in bird eggs to safe levels. Our egg injection procedure will enable us to determine the sensitivity of many more species than could ever be studied in the wild. Among the species whose eggs are tested will be many from the Bay-Delta region (including species from each of the three feeding guilds [recurvirostrids, terns; and diving ducks] that are a focus for our field studies). In addition, some species not native to the Bay-Delta will be tested to broaden the range of species examined. With a greater the range of species tested

with statistically adequate sample sizes, our models for predicting harm to various kinds of fish-eating and other aquatic birds will improve. Knowing how variable a very wide range of species can be in their sensitivity to methyl-Hg will enable us to estimate safety margins needed to protect species that have not been tested, such as endangered species and species that breed in remote areas.

The third step in our laboratory-based modeling of the risk of methyl-Hg to the reproductive success of birds will be to translate harmful concentrations of Hg in eggs to harmful concentrations in the diet. Some of the data needed to make this connection will come from the field, where Hg in eggs can be correlated with Hg in the diet of that species, and other data will come from our controlled breeding study with mallards that will be described later. Once harmful concentrations of methyl-Hg in the diet for various species have been determined, these concentrations can be compared to how much Hg is in fish and other food items in the Bay-Delta and can be used to help set restoration goals.

Interactions Between Hg and Selenium: Selenium, which is known to interact with Hg in various ways (Cuvin-Aralar and Furness, 1991), is also elevated in places in the Bay-Delta (Miles and Ohlendorf, 1993; Lonzarich et al., 1992). The Review Panel suggested that work be done to explore the possible harmful interactions between Hg and selenium (page 26, lines 14-18 of the Mercury Strategy Document – Wiener *et al.* 2003). The way selenium may interact to alter Hg poisoning in birds is largely unexplored. In a laboratory study, a combination of methyl-Hg and selenomethionine fed to mallards had far worse embryotoxic effects than either the Hg or selenium alone (Heinz and Hoffman, 1998; Hoffman and Heinz, 1998). In the field, it would be impossible to find all the statistically needed combinations of Hg and selenium in wild populations of birds, and, even in the lab, feeding breeding birds several different levels of Hg and selenium, alone and in combination, would greatly exceed available pen space. However, virtually unlimited numbers of eggs are available for injecting from a game farm species such as the mallard, making comprehensive interaction studies possible and practical. In the laboratory we will inject combinations of methyl-Hg plus selenomethionine to assess toxic interactions.

Controlled Feeding Study: On page 26 of the CALFED Mercury Strategy document (Wiener *et al.* 2003) the Review Panel recommended that, "Dose-response relations and threshold concentrations for reproductive effects should be estimated with controlled laboratory experiments, such as egg-injection studies for birds (Heinz 2002) [we addressed egg injections above in study 1] or controlled dietary exposures (Heinz and Hoffman 2003)." Laboratory studies have shown that methyl-Hg in the diet of breeding birds can impair reproduction (Tejning, 1967; Fimreite, 1971; Heinz, 1979), but there are not enough data to predict the *degree* of harm as Hg concentrations in the diet, and consequently in eggs, increase. Our laboratory feeding study will provide the data to model the risk to reproduction associated with increasing dietary and egg concentrations of Hg. We will use mallards because they have been successfully used in past studies to assess Hg toxicity and the results from previous studies provide the soundest data that have been used for predicting threshold Hg concentrations that cause reproductive effects (Heinz, 1979; Heinz and Hoffman, 1998, 2003). Although egg injection studies have shown that the embryos of mallards may not be as sensitive to methyl-Hg as are the embryos of other species, it would be too costly and lengthy a process to establish breeding colonies of these wild birds in captivity. The mallard model will quantify the worsening harm to reproduction as dietary and egg concentrations of Hg increase. We

will use this mallard model, in conjunction with our findings from egg injections of mallard and wild bird eggs, to predict the degree of harm wild birds may experience as environmental contamination of Hg increases. These findings will not only allow those charged with restoration of the San Francisco Bay-Delta ecosystem to know when Hg concentrations in the diet or eggs of wild birds are harmful, but how much harm is being done. Further details on laboratory methods may be found in Appendix G.

4. Feasibility

Program Management: The investigators collaborating on this joint USFWS/USGS study have worked closely in the past on several other Hg projects and have developed an efficient protocol for developing and implementing large-scale field and laboratory studies. Dr. Suchanek (overall Project Manager) has considerable experience in coordinating and managing several successful large-scale ecosystem-level programs (see Qualifications). With the exception of bird transmitters (which are disposable and considered supplies), all major equipment items currently exist among the participating agencies. Our timelines have been developed with conservative safeguards for unexpected contingencies.

***Field Studies*:** Dr. Schwarzbach is an experienced avian toxicologist and program manager who will lead the USGS field component. Field studies of nesting birds are necessarily constrained by seasonality of bird nesting behavior and the logistical constraints of adequate lead time for obtaining or renewing permits, scouting nest locations, obtaining permission for access, etc. We are currently in the process of renewing all required collecting and access permits in anticipation of this project. Based upon our 2000/2001 survey of egg Hg concentrations in the Bay-Delta ecosystem in 15 species of birds at over 40 locations (Schwarzbach and Adelsbach), we know where elevated Hg concentrations are likely to be found in bird eggs and thus where to look for potential reproductive impairment. We have demonstrated the feasibility of satellite telemetry in tracking diving ducks to their breeding grounds in a 2003 pilot study (Takekawa, Wainwright-De La Cruz) and have previously conducted stable isotope work in food webs of the bay (Adelsbach, Miles, Suchanek and Eagles-Smith). We have expertise in conducting breeding bird nesting studies (Schwarzbach, Adelsbach, Takekawa, Wainwright-De La Cruz) and the collection of avian prey and stomach contents of birds for analysis of Hg concentrations in diet (Miles) as well as conducting ecological risk assessments for methyl-Hg (Schwarzbach).

***Laboratory Studies*:** Drs. Heinz and Hoffman are recognized leading authorities on the development of laboratory methods to evaluate toxic endpoints for birds and will lead the laboratory component at the Patuxent Wildlife Research Center (PWRC). Based on work we have already completed (Heinz), injecting eggs with methyl-Hg is a feasible way of measuring the toxicity of Hg to the embryos of many species of wild birds. We have shown that cooperators can collect fresh eggs from the field, ship them to Patuxent, and we can successfully inject and incubate them. The injection of various combinations of Hg and Se will follow the same general approach we have used so far with Hg alone. The captive feeding study should present no problems because the staff at the PWRC are very experienced in captive breeding studies with mallards, including several studies with Hg.

5. Performance Measures

Our work will contribute to the understanding of the scope, nature and magnitude of Hg risk to birds in this system and will be of sufficient scientific quality to be published in peer reviewed

journals as well as faster turn-around local management-oriented outlets (e.g. IEP Newsletter). The result should have direct management implications for deciding what Hg concentrations the Bay-Delta ecosystem can tolerate without impairing avian reproduction and where Hg remediation efforts should focus when the goal is not only the protection of human health, but ecosystem function as well. Thus, we hope to answer not only the "so what" question about Hg and methyl-Hg in the Bay-Delta ecosystem from the perspective of ecological health risks, but also provide the sound scientific basis to answer the "so what now" question.

High quality peer review is one of the best ways to ensure that the project successfully meets its objectives. Project performance can be evaluated by accomplishment of the following measures:

- Formalize agreements with collaborating partners
- Submit quarterly fiscal and programmatic reports
- Refine and approve annual sampling plans through peer review
- Sample all matrices successfully
- Meet chemical analyses data quality criteria
- Complete chemical analyses and QA/data reports within 5 months of sampling
- Complete peer-reviewed annual project findings and progress reports for CALFED
- Present findings at review meetings
- Produce peer-reviewed final report
- Present findings and raw data on the web
- Publish results in peer-reviewed journals

Success can be quantified by the timeliness, quantity, and quality of these products.

6. Data Handling and Storage

Field/Lab Data: Data handling and storage will follow Federal Geographic Data Committee (FGDC) metadata standards. Field data will be recorded on data sheets, notebooks, or personal digital assistants and entered into digital files stored on computer hard disks. All data will be compiled, QA/QC checked, and archived at research and/or agency facilities with mirrored drives, tape backup, and redundant copies at a different location. While individual researchers will retain files for analyses, the complete dataset will be housed on a data server at the USGS Western Ecological Research Center, Vallejo, CA. Lab data will be handled in a similar fashion, with the complete dataset housed at the Patuxent Wildlife Research Center, Laurel, MD.

GIS Data: Field data will be incorporated into a geographic information system (GIS) for spatial analyses of point, line, or polygon coverages. All field data will be collected with spatial references of latitude/longitude coordinates determined from digitized maps or Global Positioning System (GPS) units with Wide Area Augmentation System (WAAS) correction for <5 m accuracy if possible. Data will be projected into Universal Transverse Mercator (UTM), Zone 10, with NAD83 horizontal datum. Elevation data will be tied to the vertical datum of NGVD29 or NAVD88, and water depths and corresponding bathymetry will be adjusted to NGVD29.

Chemical Analyses: We will follow the guidelines specified under the existing "Quality Assurance Project Plan for the CALFED Project" (Puckett and van Buuren 2000), which was developed for the earlier CALFED Mercury Study, for all of our analytical work. We also anticipate following the guidelines of the "CALFED Mercury QA/QC Program" currently being developed for all CBDA Hg research.

7. Expected Products/Outcomes

Information developed in this project will be provided to the Regional Water Quality Control Boards in San Francisco Bay (Region 2) and the Central Valley (Region 5), and submitted to peer reviewed journals for publication. We will document our progress through quarterly reports and a final report to CALFED and provide a forum to share our data with all the other ongoing Hg studies in the Bay-Delta ecosystem as well as the larger scientific community. Final reports and data, once completed, will be made available through USFWS and USGS websites with links to the Delta Tributaries Mercury Council (DTMC) web site. We will present our results at CALFED Science Conferences, the State of the Estuary Conference and other professional society meetings. We will be collaborating with the ongoing Hg study by Marvin-DiPasquale *et al.* (2002) and if desirable, we would be willing to host a CALFED-sponsored Bay-Delta Mercury Workshop to provide a more unified understanding of Hg risk from a more holistic ecosystem perspective. Drs. Suchanek and Marvin-DiPasquale have already organized and co-chaired a similar and very successful effort in the past on "The Influence of Natural and Anthropogenic Processes on Mercury Cycling in Mine-Dominated Aquatic Ecosystems" at the American Geophysical Union (AGU) meeting in San Francisco in 2001.

8. Work Schedule

This project involves primarily two years of concentrated field work (especially during the spring/summer breeding seasons), three years of lab work, chemical analyses during the fall, some winter field work, and data analysis/evaluation during the winter, followed by a third year of reproductive monitoring, benthic sample processing, final data analysis, interpretation and report/publication writing. Graphical representations of the flow of tasks and sub-tasks associated with each guild (i.e., recurvirostrids, terns, diving ducks) as well as the laboratory egg injection studies and mallard feeding studies, are shown in Figs. 10, 11, 12 and 13.

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

1. CALFED ERP, Science Program and CVPIA Priorities.

ERP Goals: This project is directly applicable to 4 of 6 ERP Goals: (1) assist/recover at-risk species, (2) rehabilitate/support native aquatic communities, (4) protect/restore functional habitat for ecological and public values, and (6) improve/maintain water and sediment quality. By determining the environmental concentrations of Hg that negatively affect wildlife species, our data will provide guidelines with which the CBDA can develop restoration priorities in locations that have the potential to increase methyl-Hg exposure to wildlife (e.g., in wetland restoration sites), which is applicable to all four goals. Our data, in conjunction with data from the other ongoing Hg projects, can provide a holistic ecosystem perspective that is protective of our native wildlife species.

2. Relationship to Other Ecosystem Restoration Projects

We will seek to coordinate our work with existing CALFED-funded Hg projects (e.g. – Marvin-DiPasquale *et al.* 2002; Stephenson *et al.* 2002) as well as all other Hg studies in this region and related restoration projects (see examples in Figs. 14 and 15) and studies, including the proposed study on wetland restoration processes and Hg methylation in marsh habitats (Yee *et al.* 2003).

USGS is also a lead agency in monitoring the large North Bay and South Bay salt pond wetland restoration projects, and results acquired on Hg risks from this study will be incorporated in the scientific support framework for restoration decisions of the participating management agencies (e.g. - USFWS, CDFG, Coastal Conservancy, Corp of Engineers).

Specific studies with which we will coordinate most closely are:

Marvin-Dipasquale, M., R. Stewart, N.S. Fisher and R.P. Mason 2002. Evaluation of mercury transformations and trophic transfer in the San Francisco Bay/Delta: Identifying critical processes for the Ecosystem Restoration Program. CALFED-ERP funded grant.

Stephenson, M., K. Coale, G. Gill and M. Puckett 2002. Transport, Cycling, and Fate of Mercury and Monomethyl Mercury in the San Francisco Delta and Tributaries: An Integrated Mass Balance Assessment Approach. CALFED-ERP funded grant.

Davis, J.A., M. Stephenson, M. Mack and D.G. Slotton 2003. A Pilot Regional Monitoring Program for Mercury in Fish in the Bay-Delta Watershed. CALFED Proposal Submitted for Directed Action.– Aug 1, 2003

Yee, D., J. Collins, J. Davis, J. Evens, S.E. Schwarzbach, J. Takekawa, M. Marvin-DiPasquale and D. Krabbenhoft 2003. Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems. CALFED Proposal Submitted for Directed Action.– Aug 1, 2003

3. Next -Phase Funding - None requested; this is a new project.

4. Previous Recipients of CALFED or CVPIA Funding.

Suchanek was awarded two previous CALFED grants to study Hg in the Bay-Delta ecosystem/watershed: (1) Suchanek & Slotton (1998-2002): *The effects of wetland restoration on the production of methyl mercury in the San Francisco Bay-Delta System*, and (2) Suchanek, Slotton & Nelson (1999-2003): *Source Bioavailability and Mine Remediation Feasibility*, which was Task 5A of the earlier multi-institutional funded CALFED Mercury Project "*Assessment of ecological and human health impacts of mercury in the Bay-Delta Watershed*." In addition to agency reports, two preliminary in-progress communications have been produced from these studies to date: Suchanek *et al.* (1999a) and Slotton *et al.* (2001), but the bulk of the data are being prepared for 3 peer-reviewed publications from each grant. One additional paper is nearly ready for submission (Domagalski *et al.* – in prep). See Literature Cited list for details.

Schwarzbach and Adelsbach (under the auspices of USFWS) were awarded a CALFED grant under Task 3B of the CALFED Mercury Project (see above) – "Field Assessment of Avian mercury exposure in the Bay/Delta Ecosystem." The draft report is available on the web at Gary Gill's web site. This report is still awaiting comment from the peer review team before becoming final. This work will be submitted for publication in the journal Ecotoxicology this fall.

Heinz and Hoffman were awarded a CALFED grant under Task 3A of the CALFED Mercury Project (see above) to begin the study of the effects of methyl-Hg injected into the eggs of wild birds. In addition to agency reports, two publication products have been produced from these studies to date: (Heinz and Hoffman 2003b, 2003c). Additional data are being prepared for two peer-reviewed publications from this work. See Literature Cited list for details.

Takekawa was one of three investigators (including Reid and Bias) on a previous CALFED grant: Ecological Monitoring of Tolay Creek and Cullinan Ranch Tidal Wetland Restoration

Projects in North San Francisco Bay. This work is ongoing and has resulted in agency reports and two publications in preparation.

5. System-Wide Ecosystem Benefits.

The proposed project will gather information on priority pollutants occurring in the CALFED Solution Area (CSA), enabling us to predict patterns in accumulation and effects on avian species inhabiting the CSA. The process-oriented focus on bioaccumulation pathways among diverse avian guilds across a gradient of mercury concentrations in prey and other media will make the data generated from this study widely applicable to other regions of the CSA and to other species occurring in these areas. The field components will be combined with laboratory approaches to test and validate differential effects within and between species to provide a strong model by which to begin predicting Hg accumulation in higher trophic level avian species and the potential individual and population level effects that might result. The integration of field and laboratory techniques will make the link between bioaccumulation and effects in avian species increasingly strong.

Information gathered from this proposal can, in turn, be used to help guide restoration efforts in other related areas such as acquisition, restoration, and conservation of wildlife habitat within the CSA. As the CALFED Ecosystem Restoration Program is implemented there will be many projects targeted at identifying and remediating pollution sources within various regions. Data gathered by this project will be useful in identifying the effects of Hg sources in the CSA and establishing adequate protective levels for wildlife. Data generated from this project will also be helpful in understanding the role that other contaminants of concern have on sensitive avian species in the Sacramento-San Joaquin River Delta and, ultimately, how habitat restoration projects in these regions may affect these problems. This provides a strong ecosystem benefit by greatly improving the ability of ecosystem managers to make informed management decisions regarding restoration and management activities in the context of a Hg contaminated ecosystem.

6. Additional Information for Proposals Containing Land Acquisition. Not applicable.

C. QUALIFICATIONS:

(See Literature Cited section for full references of publications listed in this section.)

Thomas H. Suchanek-- Experience: Currently- Deputy Chief: Environmental Contaminants Division, USFWS, Sacramento. Previously - Research Ecologist @ UC Davis (1982-2001). Has expertise in and has led numerous extensive research programs involving a variety of environmental contaminants, including ecosystem impacts of oil (Exxon-Valdez oil spill), radionuclides in fishes (environmental effects of the Farallon Islands Nuclear Waste Dump Site), thermal pollution (Long Island Sound) and, most recently (1990-present), mercury contamination in CA -- Clear Lake (Sulphur Bank Mercury Mine), the Bay-Delta ecosystem and Cache Creek. Expertise in use of stable isotope techniques to track trophic pathways for nearly 20 yrs (see Suchanek *et al*.1985 and Bern *et al*. 1999 in Lit. Cite). Education: B.A. in Biology, University of Connecticut (1965); M.S. in Ecology & Evolution - SUNY @ Stony Brook, N.Y. (1972); Ph.D. in Zoology, University of Washington, Seattle (1979). Publications: Suchanek has over 130 publications, nearly 50 of which

are from peer reviewed journals or solicited works. See Literature Cited list (Suchanek) for relevant publications.

Steven E. Schwarzbach--Experience: Currently - research manager at USGS Biological Research Division. Previously - Chief: Environmental Contaminants Division, USFWS, Sacramento for thirteen years. Education: Ph.D. in Ecology, UC Davis (1989) on the effect of contaminants on avian reproduction. He has designed and directed numerous multidisciplinary field studies of environmental contaminant impacts to fish and wildlife in California including studies in the Klamath Basin, Sacramento Valley, Tulare Basin, San Luis Refuge Complex, Cache Creek and intertidal marshes of San Francisco Bay. Contaminant studies in which Dr. Schwarzbach has been involved have focused on Hg, selenium, organophosphate pesticides, aquatic herbicides, organochlorines, trifluoracetic acid, acid mine drainage, ammonia, and eutrophication effects upon water quality. His scientific interests have most recently been focused on Hg and Se in birds of the San Joaquin Valley and San Francisco Bay. He has directed field investigations on contaminant hazards to clapper rails in the South Bay in 1991 and 1992 and the North Bay in 1998 and 1999 and directed a bay-wide investigation of Hg bioaccumulation in birds of San Francisco estuary for CALFED and the Regional Water Quality Control Board. Relevant Publications include: Schwarzbach *et al.* 2001, Schwarzbach and Adelsbach 2002, Schwarzbach *et al.* (in prep.).

John Y. Takekawa--Experience: federal research biologist in California for 15 years; research specialty ecology of migratory waterbirds with technical specialty in application of radio telemetry; studies focused on the Pacific Rim, CA, and SFB; Goals Project Focus Team co-chair, BCDC Subtidal Habitats panel, NOAA Airport Runways panel, established the USGS SFB Estuary Field Station located on SPB in 1995. Education: Ph.D. 1987, Iowa State Univ., Ames, Iowa; Animal Ecology/Statistics minor, M.S. 1982, Univ. of Idaho, Moscow, ID;Wildlife Resources, B.S. 1979, Univ. of Wash., Seattle; Wildlife Science/Forestry. Selected Publications: Takekawa *et al.* 2001a, Takekawa *et al.* 2001b, Warnock and Takekawa 1995.

A. Keith Miles--Experience: federal research biologist, California 10 years, Arctic 10 years, Chesapeake Bay 6 years, Channel Islands 6 years; Chair Ecotoxicology, Graduate Group in Ecology, University of California, Davis (UCD); Contributor, Member, SFB Goals Project; Leader, USGS Davis Field Station, UCD. Research focus is the effects of contaminants on estuarine and marine habitats; emphasis is to determine consequences of accumulation of contaminants and discriminating effects caused by contaminants from naturally occurring changes in wildlife populations; effects of contaminants on the structure of invertebrate and vegetative assemblages and the potential for accumulation among specific prey guilds of migratory waterbirds and marine mammals; recent research to determine the effects of contaminants on fossorial animals in the Mojave Desert. Education: Ph.D. Wildlife Ecology/marine ecology, Oregon State University (OSU), June 1987, Corvallis, OR; M.S. Wildlife Biology, OSU, August 1976; B.S. Zoology, Howard University, June 1972, Washington, D.C. Selected Publications: Miles and Ohlendorf 1993, Miles and Tome 1997, Anthony *et al.* 1999, Miles *et al.* 2002.

Gary H. Heinz – Experience: research biologist in the contaminants program at the Patuxent Wildlife Research Center since 1969, with laboratory and field work on the effects of contaminants

on birds, including measurements on behavior, reproduction, survival, growth, accumulation and loss rates, and the interpretation of contaminant residues in tissues. He has studied heavy metals (especially mercury), selenium, organochlorine pesticides, cholinesterase inhibiting pesticides, PCBs, and oil. Education: B.A. - Biology, Lehigh University, 1965; M.S. - Wildlife Biology, Michigan State University, 1967; Ph. D. - Wildlife Biology, Michigan State University, 1969. Publications: more than 70 peer-reviewed articles and book chapters about environmental contaminants. He was a co-editor for the books *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* and *Environmental Contaminants and Terrestrial Vertebrates: Effects on Populations, Communities, and Ecosystems.* Working in collaboration with Dr. Hoffman, Dr. Heinz has published many articles on the toxicity of mercury and selenium to birds. Their data have been used to help establish threshold dietary and egg levels of mercury and selenium that harm avian reproduction; these thresholds have been applied by resource managers and regulatory agencies to protect wild birds. See Literature Cited list (Heinz) for relevant publications.

David J. Hoffman – Experience: research physiologist in the contaminants program at the Patuxent Wildlife Research Center since 1976, with laboratory and field research involving the effects of contaminants such as heavy metals, selenium, organochlorine and cholinesterase inhibiting pesticides, PCBs, and oil on birds and other wildlife. Dr. Hoffman is a recognized authority in the study of the teratogenic effects of contaminants on wildlife. In addition, he has extensive experience in the physiological effects of contaminants on wildlife, including the use of non-destructive biomarkers. Education: B.S. - Zoology, McGill University, 1966; Ph. D. - Developmental Zoology and Physiology, University of Maryland, 1971. Publications: over 100 peer-reviewed articles and book chapters dealing with environmental contaminants. He was the lead editor for the book *Handbook of Ecotoxicology*. See Literature Cited list (Hoffman) for relevant publications.

Susan Wainwright-De La Cruz – Experience: wildlife biologist with USGS WERC since 1998; over 10 years of wildlife research experience in San Francisco Bay focusing on contaminants in migratory and resident birds. Previous work includes assessing endocrine modulation in birds and fish using biomarker and hispathological techniques; using radio telemetry to identify foraging ranges and contaminant risks of aquatic birds; interpreting effects of trace element and organic contaminants on avian body condition and reproduction. Education: Ph.D. Candidate, Ecology (Ecotoxicology emphasis), Univ. of CA, Davis; M.S. 1998, Wildlife and Fisheries Sciences, Texas A&M Univ.; B.S. 1992, Biology, Univ. of CA, Davis. Selected Publications: Mora and Wainwright 1998, Wainwright *et al.* 2001, Takekawa *et al.* 2002.

Terrence L. Adelsbach--Experience: Biologist, U.S. Fish and Wildlife Service, Environmental Contaminants Division, Biomonitoring and Investigations Branch, Sacramento CA. Jan 2000-Present. Independently plan, develop, and conduct field studies relating resource management, water quality and environmental protection issues to environmental contaminants. Primary field of study is in the effects of Hg and PCB's on aspects of avian ecology such as foraging and reproduction. Studies to date include assessment of Hg hazard to avian taxa breeding in the Sacramento-San Joaquin Delta and San Francisco Bay Estuary; assessment of tetrachloro dibenzo-p-dioxin toxic equivalents (TEQ's) concentrations and toxicological significance in eggs of three

tern species breeding in the San Francisco Bay Estuary; effects of foraging ecology on Hg exposure in two species of terns in south San Francisco Bay: a stable isotope approach; effects of Hg and TEQ's on hatchability and reproductive success in three species of terns in San Francisco Bay. Education: B.S. University of California, Davis (UCD), Wildlife and Fisheries Biology, June 1997; Ph.D. Student, UCD., Ecology Graduate Group, Ecotoxicology emphasis. Selected Publications: Schwarzbach and Adelsbach 2002, Schwarzbach and Adelsbach (in prep). See Literature Cited section for details.

Collin A. Eagles-Smith--Experience: Biologist with U.S. Fish and Wildlife Service, Environmental Contaminants Division since 2003; more than 5 years experience in food web ecology, specializing in ecological applications of stable isotopes, and mercury bioaccumulation in complex aquatic/marine ecosystems of California, including: Clear Lake, Cache Creek, Eagle Lake, and the San Francisco Bay and Delta. Education: B.S. 2000, Environmental and Resource Sciences, UC Davis; Ph.D. Candidate, Ecology (Ecotoxicology emphasis), UC Davis. Selected Publications: Suchanek *et al.* 2003; Eagles-Smith *et al.* unpublished data; McEachern *et al.* in press.

D. Cost

1. Budget: The budget and budget justification are provided in Forms VI and VII, respectively.
2. Cost-sharing: Cost-sharing information is provided in Form I.

E. Local Involvement

This project is a research and monitoring effort and will likely not have the public outreach issues with modification of the landscape associated with physical restoration. The information, however, should be of great interest to regional water quality control boards and other seeking to devise effective Hg control strategies and it is our intention to meet regularly with representatives of both the Central Valley Regional Water Quality Control Board (Chris Foe) and the San Francisco Bay Regional Water Quality Control Board representatives (Lynn Suer, Karen Taberski) that are actively engaged in developing Hg control targets and strategies. We will also work closely with local resource management entities such as the U.S. Fish and Wildlife Service Refuge System (Marge Kolar and Clyde Morris) and the California Department of Fish and Game Wildlife Management staff (Larry Wyckoff and Tom Hufman). Both agencies have large land holdings within and surrounding the CALFED Solution Area. Both agencies will also be undertaking large restoration projects relative to recent and past land acquisitions that involve Hg contamination issues. We plan to work closely with our partners in each agency to help guide and prioritize these restoration efforts. We will also likely be working with local agencies and private property owners to acquire access to key sites for the field objectives.

F. Compliance with Standard Terms and Conditions

In compliance with the federal Anti-Deficiency Act of 1998 (31 U.S.C. 1341), the Consolidated Appropriations Act of 2001 (Law # 106-554) and OMB 2002 Memorandum M-03-01, the USFWS

and the USGS must receive payment in advance for any funds originating from non-federal entities, before any work on this project could take place.

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TABLES

Table 1. - Detailed Budget for Year 1

Objective	Task	Subtask	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies and Expendables	Equipment	Services or Consultants	Other direct costs	Total Direct Costs	Indirect Costs	Total Cost
I. Trophic Pathways and Species patterns													
	Tern Guild												
		Diet and Stable Isotope Studies	1,649	$133,940	*	*	*	$0	$39,650	$0	$173,590	$37,278	$210,868
		Hg and Secondary Contaminants	576	$46,764	*	*	*	$0	$87,080	$0	$133,844	$16,311	$150,155
		Banding and Telemetry	1,615	$29,356	$5,091	$4,025	$20,300	$0	$6,827	$0	$65,599	$13,235	$78,834
	Task Total		**3,840**	**$210,060**	**$5,091**	**$4,025**	**$20,300**	**$0**	**$133,557**	**$0**	**$373,033**	**$66,824**	**$439,857**
	Recurvirostrid Guild												
		Diet and Stable Isotope Studies	3,526	$62,788	$13,751	$2,656	$3,133	$0	$41,400	$0	$123,728	$18,473	$142,201
		Hg and Secondary Contaminants	130	$3,472	$1,215	$356	$1,333	$0	$78,000	$0	$84,376	$4,796	$89,172
		Banding and Telemetry	2,193	$40,218	$7,337	$4,025	$14,300	$0	$16,827	$0	$82,707	$13,741	$96,448
	Task Total		**5,849**	**$106,478**	**$22,303**	**$7,037**	**$18,766**	**$0**	**$136,227**	**$0**	**$290,811**	**$37,010**	**$327,821**
	Diving Duck Guild												
		Diet and Stable Isotope Studies	3,526	$62,788	$13,750	$2,656	$2,133	$0	$14,660	$0	$95,987	$17,067	$113,054
		Hg and Secondary Contaminants	130	$3,472	$1,215	$356	$1,333	$0	$59,250	$0	$65,626	$3,953	$69,579
		Banding and Telemetry	1,873	$34,458	$6,358	$4,600	$27,800	0	$6,827	$0	$80,043	$16,149	$96,192
	Task Total		**5,529**	**$100,718**	**$21,323**	**$7,612**	**$31,266**	**$0**	**$80,737**	**$0**	**$241,656**	**$37,169**	**$278,825**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$19,865**	**$0**	**$19,865**	**$894**	**$20,759**
Objective Total			**15,218**	**417,256**	**48,717**	**18,674**	**70,332**	**0**	**370,386**	**0**	**925,365**	**141,897**	**1,067,262**
II. Reproduction and Toxic Effects													
	Tern Guild												
		Nest and Egg Studies	1,514	$123,013	*	*	*	$0	$55,800	$0	$178,813	$35,109	$213,922
		Chick post-hatch survival	732	$59,475	*	*	*	$0	$145,536	$0	$205,011	$22,310	$227,321
	Task Total		**2,246**	**$182,488**	*	*	*	**$0**	**$201,336**	**$0**	**$383,824**	**$57,419**	**$441,243**
	Recurvirostrid Guild												
		Nest and Egg Studies	1,697	$35,058	$9,160	$1,150	$3,000	$0	$43,620	$0	$91,988	$11,721	$103,709
		Post-hatch survival	1,279	$27,076	$7,403	$1,150	$6,000	$0	$42,240	$0	$83,869	$10,299	$94,168
	Task Total		**2,976**	**$62,134**	**$16,563**	**$2,300**	**$9,000**	**$0**	**$85,860**	**$0**	**$175,857**	**$22,021**	**$197,878**
	Diving Duck Guild												
		Nest and Egg studies	113	$2,778	$972	$1,725	$29,000	$0	$81,465	$0	$115,940	$18,145	$134,085
	Task Total		**113**	**$2,778**	**$972**	**$1,725**	**$29,000**	**$0**	**$81,465**	**$0**	**$115,940**	**$18,145**	**$134,085**
	Hg QA/AC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$10,125**	**$0**	**$10,125**	**$456**	**$10,581**
Objective Total			**5,335**	**$247,400**	**$17,535**	**$4,025**	**$38,000**	**$0**	**$378,786**	**$0**	**$685,746**	**$98,041**	**$783,787**
III. Embryo Sensitivity													
	Tern Guild (+ cormorants)												
		Egg injections (Hg)	1,525	$31,027	$10,239	$867	$9,334	$0	$10,165	$0	$61,632	$11,598	$73,230
		Egg collections and prep	107	$8,694	*	*	*	$0	$0	$0	$8,694	$2,304	$10,998
	Task Total		**1,645**	**$39,721**	**$10,239**	**$867**	**$9,334**	**$0**	**$10,165**	**$0**	**$70,326**	**$13,902**	**$84,228**
	Recurvirostrid Guild												
		Egg injections (Hg)	1,525	$31,027	$10,239	$867	$9,334	$0	$10,165	$0	$61,632	$11,598	$73,230
		Egg collections and prep	174	$3,889	$1,361	$538	$250	$0	$0	$0	$6,038	$1,218	$7,256
	Task Total		**1,699**	**$34,916**	**$11,600**	**$1,405**	**$9,584**	**$0**	**$10,165**	**$0**	**$67,670**	**$12,817**	**$80,487**
	Diving Duck Guild												
		Egg injections (Hg)	1,525	$31,027	$10,239	$867	$9,334	$0	$10,165	$0	$61,632	$11,598	$73,230
		Egg collections and prep	174	$3,889	$1,361	$538	$250	$0	$0	$0	$6,038	$1,218	$7,256
	Task Total		**1,699**	**$34,916**	**$11,600**	**$1,405**	**$9,584**	**$0**	**$10,165**	**$0**	**$67,670**	**$12,817**	**$80,487**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$2,610**	**$0**	**$2,610**	**$117**	**$2,727**
Objective Total			**5,043**	**$109,553**	**$33,439**	**$3,677**	**$28,502**	**$0**	**$33,105**	**$0**	**$208,276**	**$39,653**	**$247,929**
IV. Data Handling, Mapping, & QC			**458**	**$12,500**	**$4,375**	**$0**	**$0**	**$0**	**$0**	**$0**	**$16,875**	**$3,405**	**$20,280**
Year 1 Totals			**26,054**	**$786,709**	**$104,066**	**$26,376**	**$136,834**	**$0**	**$782,277**	**$0**	**$1,836,262**	**$282,995**	**$2,119,257**

Table 2 - Detailed Budget for Year 2

Year 2													
Objective	**Task**	**Subtask**	**Direct Labor Hours**	**Salary (per year)**	**Benefits (per year)**	**Travel**	**Supplies and Expendables**	**Equipment**	**Services or Consultants**	**Other direct costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
I. Trophic Pathways and Species patterns	Tern Guild												
		Diet and Stable Isotope Studies	1,649	$140,784	*	*	*	$0	$40,300	$2,125	$183,209	$39,684	$222,893
		Hg and Secondary Contaminants	576	$49,176	*	*	*	$0	$37,480	$2,125	$88,781	$15,281	$104,062
		Banding and Telemetry	1,615	$29,356	$5,091	$4,525	$17,167	$0	$6,827	$0	$62,966	$12,703	$75,669
	Task Total		**3,840**	**$219,316**	**$5,091**	**$4,525**	**$17,167**	**$0**	**$84,607**	**$4,250**	**$334,956**	**$67,669**	**$402,625**
	Recurvirostrid Guild												
		Diet and Stable Isotope Studies	3,526	$62,788	$13,751	$3,656	$3,133	$0	$41,400	$0	$124,728	$18,674	$143,402
		Hg and Secondary Contaminants	130	$3,472	$1,215	$856	$1,333	$0	$48,536	$0	$55,412	$3,571	$58,983
		Banding and Telemetry	2,193	$40,218	$7,337	$4,525	$11,167	$0	$16,827	$0	$80,074	$13,210	$93,284
	Task Total		**5,849**	**$106,478**	**$22,303**	**$9,037**	**$15,633**	**$0**	**$106,763**	**$0**	**$260,214**	**$35,456**	**$295,670**
	Diving Duck Guild												
		Diet and Stable Isotope Studies	3,526	$62,788	$13,750	$3,656	$2,133	$0	$14,660	$0	$96,987	$17,269	$114,256
		Hg and Secondary Contaminants	130	$3,472	$1,215	$856	$1,333	$0	$39,878	$0	$46,754	$3,182	$49,936
		Banding and Telemetry	1,873	$34,458	$6,358	$4,600	$27,800	0	$6,827	$0	$80,043	$16,149	$96,192
	Task Total		**5,529**	**$100,718**	**$21,323**	**$9,112**	**$31,266**	**$0**	**$61,365**	**$0**	**$223,784**	**$36,600**	**$260,384**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$9,530**	**$0**	**$9,530**	**$429**	**$9,959**
Objective Total			**15,218**	**$426,512**	**$48,717**	**$22,674**	**$64,066**	**$0**	**$262,265**	**$4,250**	**$828,484**	**$140,153**	**$968,637**
II. Reproduction and Toxic Effects	Tern Guild												
		Nest and Egg Studies	1,514	$129,258	*	*	*	$0	$52,320	$2,125	$183,703	$37,171	$220,874
		Chick post-hatch survival	732	$62,495	*	*	*	$0	$117,956	$2,125	$182,576	$22,432	$205,008
	Task Total		**2,246**	**$191,753**	*	*	*	**$0**	**$170,276**	**$4,250**	**$366,279**	**$59,603**	**$425,882**
	Recurvirostrid Guild												
		Nest and Egg Studies	1,697	$35,058	$9,160	$2,150	$3,000	$0	$37,472	$0	$86,840	$11,646	$98,486
		Post-hatch survival	1,279	$27,076	$7,403	$1,650	$6,000	$0	$36,092	$0	$78,221	$10,124	$88,345
	Task Total		**2,976**	**$62,134**	**$16,563**	**$3,800**	**$9,000**	**$0**	**$73,564**	**$0**	**$165,061**	**$21,770**	**$186,831**
	Diving Duck Guild												
		Nest and Egg studies	137	$3,334	$1,167	$2,225	$29,000	$0	$76,825	$0	$112,551	$18,189	$130,740
	Task Total		**137**	**$3,334**	**$1,167**	**$2,225**	**$29,000**	**$0**	**$76,825**	**$0**	**$112,551**	**$18,189**	**$130,740**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$5,445**	**$0**	**$5,445**	**$245**	**$5,690**
Objective Total			**5,359**	**$257,221**	**$17,730**	**$6,025**	**$38,000**	**$0**	**$326,110**	**$4,250**	**$649,336**	**$99,807**	**$749,143**
III. Embryo Sensitivity	Tern Guild (+ cormorants)												
		Egg injections (Hg)	762	$16,132	$5,324	$867	$3,666	$0	$7,634	$0	$33,623	$6,287	$39,910
		Hg/Se interactions	762	$16,132	$5,324	$600	$1,000	$0	$2,364	$0	$25,420	$5,128	$30,548
		Egg collections and prep	107	$9,135	*	*	*	$0	$0	$0	$9,135	$2,421	$11,556
	Task Total		**1,631**	**$41,399**	**$10,648**	**$1,467**	**$4,666**	**$0**	**$9,998**	**$0**	**$68,178**	**$13,837**	**$82,015**
	Recurvirostrid Guild												
		Egg injections (Hg)	762	$16,132	$5,324	$867	$3,666	$0	$7,634	$0	$33,623	$6,287	$39,910
		Hg/Se interactions	762	$16,132	$5,324	$600	$1,000	$0	$2,364	$0	$25,420	$5,128	$30,548
		Egg collections and prep	174	$3,889	$1,361	$538	$250	$0	$0	$0	$6,038	$1,218	$7,256
	Task Total		**1,698**	**$36,153**	**$12,009**	**$2,005**	**$4,916**	**$0**	**$9,998**	**$0**	**$65,081**	**$12,634**	**$77,715**
	Diving Duck Guild												
		Egg injections (Hg)	762	$16,133	$5,324	$867	$3,666	$0	$7,634	$0	$33,624	$6,288	$39,912
		Hg/Se interactions	762	$16,133	$5,324	$600	$1,000	$0	$2,364	$0	$25,421	$5,129	$30,550
		Egg collections and prep	174	$3,889	$1,361	$538	$250	$0	$0	$0	$6,038	$1,218	$7,256
	Task Total		**1,698**	**$36,155**	**$12,009**	**$2,005**	**$4,916**	**$0**	**$9,998**	**$0**	**$65,083**	**$12,634**	**$77,717**
	Statistical Modelling		**173**	**$5,000**	**$1,750**	**$0**	**$0**	**$0**	**$0**	**$0**	**$6,750**	**$1,362**	**$8,112**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$750**	**$0**	**$750**	**$34**	**$784**
Objective Total			**5,200**	**$118,707**	**$36,416**	**$5,477**	**$14,498**	**$0**	**$30,744**	**$0**	**$205,842**	**$40,501**	**$246,343**
IV. Data Handling, Mapping, & QC			**458**	**$12,500**	**$4,375**	**$0**	**$0**	**$0**	**$0**	**$4,375**	**$16,875**	**$3,405**	**$20,280**
Year 2 Totals			**$26,235**	**$814,940**	**$107,238**	**$34,176**	**$116,564**	**$0**	**$619,119**	**$12,875**	**$1,700,537**	**$283,865**	**$1,984,402**

Table 3 - Detailed Budget for Year 3

Year 3													
Objective	**Task**	**Subtask**	**Direct Labor Hours**	**Salary (per year)**	**Benefits (per year)**	**Travel**	**Supplies and Expendables**	**Equipment**	**Services or Consultants**	**Other direct costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
I. Trophic Pathways and Species patterns	Tern Guild												
		Diet and Stable Isotope Studies	1,225	$109,791	*	*	*	$0	$0	$2,125	$111,916	$29,658	$141,574
		Hg and Secondary Contaminants	428	$38,360	*	*	*	$0	$0	$2,125	$40,485	$10,729	$51,214
		Banding and Telemetry	655	$12,076	$2,153	$1,650	$6,500	$0	$2,560	$0	$24,939	$5,031	$29,970
	Task Total		**2,308**	**$160,227**	**$2,153**	**$1,650**	**$6,500**	**$0**	**$2,560**	**$4,250**	**$177,340**	**$45,418**	**$222,758**
	Recurvirostrid Guild												
		Diet and Stable Isotope Studies	2,620	$46,378	$11,214	$1,000	$0	$0	$0	$0	$58,592	$11,821	$70,413
		Hg and Secondary Contaminants	130	$3,472	$1,215	$500	$0	$0	$0	$0	$5,187	$1,046	$6,233
		Banding and Telemetry	113	$2,778	$972	$500	$0	$0	$10,000	$0	$14,250	$1,307	$15,557
	Task Total		**2,863**	**$52,628**	**$13,401**	**$2,000**	**$0**	**$0**	**$10,000**	**$0**	**$78,029**	**$14,175**	**$92,204**
	Diving Duck Guild												
		Diet and Stable Isotope Studies	2,620	$46,378	$11,214	$1,000	$0	$0	$0	$0	$58,592	$11,821	$70,413
		Hg and Secondary Contaminants	130	$3,472	$1,215	$500	$0	$0	$0	$0	$5,187	$1,046	$6,233
		Banding and Telemetry	113	$2,778	$972	$500	$0	0	$0	$0	$4,250	$857	$5,107
	Task Total		**2,863**	**$52,628**	**$13,401**	**$2,000**	**$0**	**$0**	**$0**	**$0**	**$68,029**	**$13,725**	**$81,754**
Objective Total			**8,034**	**$265,483**	**$28,955**	**$5,650**	**$6,500**	**$0**	**$12,560**	**$4,250**	**$323,398**	**$73,317**	**$396,715**
II. Reproduction and Toxic Effects	Tern Guild												
		Nest and Egg Studies	1,514	$135,125	*	*	*	$0	$10,000	$2,125	$147,250	$36,821	$184,071
		Chick post-hatch survival	732	$65,331	*	*	*	$0	$10,000	$2,125	$77,456	$18,326	$95,782
	Task Total		**2,246**	**$200,456**	*	*	*	**$0**	**$20,000**	**$4,250**	**$224,706**	**$55,147**	**$279,853**
	Recurvirostrid Guild												
		Nest and Egg Studies	1,697	$35,058	$9,160	$2,150	$3,000	$0	$10,000	$0	$59,368	$10,410	$69,778
		Post-hatch survival	1,279	$27,076	$7,403	$1,650	$6,000	$0	$10,000	$0	$52,129	$8,950	$61,079
	Task Total		**2,976**	**$62,134**	**$16,563**	**$3,800**	**$9,000**	**$0**	**$20,000**	**$0**	**$111,497**	**$19,360**	**$130,857**
	Diving Duck Guild												
		Nest and Egg studies	593	$11,418	$2,441	$2,225	$44,000	$0	$48,000	$0	$108,084	$21,806	$129,890
	Task Total		**593**	**$11,418**	**$2,441**	**$2,225**	**$44,000**	**$0**	**$48,000**	**$0**	**$108,084**	**$21,806**	**$129,890**
Objective Total			**5,815**	**$274,008**	**$19,004**	**$6,025**	**$53,000**	**$0**	**$88,000**	**$4,250**	**$444,287**	**$96,313**	**$540,600**
III. Embryo Sensitivity	Tern Guild (+ cormorants)												
		Hg/Se interactions	762	$16,779	$5,537	$867	$1,000	$0	$16,896	$0	$41,079	$6,023	$47,102
		Mallard Feeding Study	762	$16,779	$5,537	$867	$6,667	$0	$3,676	$0	$33,526	$6,764	$40,290
	Task Total		**1,524**	**$33,558**	**$11,074**	**$1,734**	**$7,667**	**$0**	**$20,572**	**$0**	**$74,605**	**$12,787**	**$87,392**
	Recurvirostrid Guild												
		Hg/Se interactions	762	$16,779	$5,537	$867	$1,000	$0	$16,896	$0	$41,079	$6,023	$47,102
		Mallard Feeding Study	762	$16,779	$5,537	$867	$6,667	$0	$3,676	$0	$33,526	$6,764	$40,290
	Task Total		**1,524**	**$33,558**	**$11,074**	**$1,734**	**$7,667**	**$0**	**$20,572**	**$0**	**$74,605**	**$12,787**	**$87,392**
	Diving Duck Guild												
		Hg/Se interactions	762	$16,779	$5,537	$867	$1,000	$0	$16,896	$0	$41,079	$6,023	$47,102
		Mallard Feeding Study	762	$16,779	$5,537	$867	$6,667	$0	$3,676	$0	$33,526	$6,764	$40,290
	Task Total		**1,524**	**$33,558**	**$11,074**	**$1,734**	**$7,667**	**$0**	**$20,572**	**$0**	**$74,605**	**$12,787**	**$87,392**
	Statistical Modelling		**173**	**$5,000**	**$1,750**	**$0**	**$0**	**$0**	**$0**	**$0**	**$6,750**	**$1,362**	**$8,112**
	Hg QA/QC		**0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$5,235**	**$0**	**$5,235**	**$236**	**$5,471**
Objective Total			**4,745**	**$105,674**	**$34,972**	**$5,202**	**$23,001**	**$0**	**$66,951**	**$0**	**$235,800**	**$39,958**	**$275,758**
IV. Data Handling, Mapping, and QC			**458**	**$12,500**	**$4,375**	**$0**	**$0**	**$0**	**$0**	$0	**$16,875**	**$3,405**	**$20,280**
Year 3 Totals			**19,052**	**$657,665**	**$87,306**	**$16,877**	**$82,501**	**$0**	**$167,511**	**$8,500**	**$1,020,360**	**$212,992**	**$1,233,352**
Entire Project Totals			**71,341**	**$2,259,314**	**$298,610**	**$77,429**	**$335,899**	**$0**	**$1,568,907**	**$21,375**	**$4,557,159**	**$779,853**	**$5,337,012**

Project 3 Year Total = $5,281,042

Hg QA/QC Costs = $55,970

3 Year Total with QA/QC = $5,337,012

Table 4: Hg concentrations (site means) in tern eggs from colonies with the San Francisco Bay region. Sample sizes in parentheses.

Mercury Concentrations in Tern Eggs

Region	Location	Least Tern	Forster's Tern	Caspian Tern
No. SF Bay	Napa Marsh		0.64b (6)	0.90b (5)
Central SF Bay	Alameda	0.3 (6)		0.72b (5)
	Hayward Shoreline		0.50b (5)	
So. SF Bay	Charleston Slough		0.59b (5)	
	Salt Pond A16		1.62a (5)	
	Salt Pond A7			1.18a (5)

FIGURES

Fig. 1 – Hg concentrations in eggs of 16 avian species breeding in the Bay-Delta ecosystem.

Fig. 2 – Hg concentrations in eggs from 16 avian species from the San Francisco Bay region by feeding group.

Fig. 3 – Range of Hg in eggs of aquatic birds from the San Francisco Bay region that consume primarily invertebrates. Values for black-necked stilts and American avocets are for randomly collected viable eggs. Values for snowy plover and California clapper rail for fail-to-hatch eggs only.

Figure 4. A. Analysis of Variance: effect of site (open bay vs. salt ponds) on ^{13}C. B. Analysis of Variance: effect of site (open bay vs. salt ponds) on ^{15}N. C. Log Hg vs ^{15}N. D. ^{13}C vs. ^{15}N scatterplot for open bay sites (blue dots) versus salt ponds (red squares).

Figure 5. Stable Isotopes in Forster's tern eggs from San Francisco Bay Region. The stippled zone indicates eggs from sites more influenced by freshwater food resources, whereas the non-stippled zone is from a region influenced more by marine food resources.

Fig. 6 – Hg concentration in eggs of facultative and obligate piscivorous birds from the San Francisco Bay region. The degree of piscivory increases from left to right along the abscissa.

Fig. 7 – Estimated home ranges in black-necked stilts in Napa-Sonoma marsh. VHF telemetry will help confirm site fidelity and foraging areas.

Fig. 8 – Migratory routes and ultimate breeding areas (Northwest Territories, Canada) of San Francisco Bay surf scoter satellite-marked in March 2003.

Fig. 9 – Seasonal change in invertebrate taxa abundance at three North Bay salt ponds.

Fig. 10 - Timeline for recurvirostrid guild studies during Year 1

Fig. 11 - Timeline for tern guild studies during Year 1.

Fig. 12 - Timeline for diving duck guild studies during Year 1.

Fig. 13 – Timeline for laboratory studies during Year 1.

Fig. 14 – Wetland Restoration and Enhancement Projects in the North Bay region.

Fig. 15 – Wetland Restoration and Enhancement Projects in the South Bay region.

Figure 1. Hg concentrations in eggs of 16 avian species breeding in the Bay-Delta ecosystem.

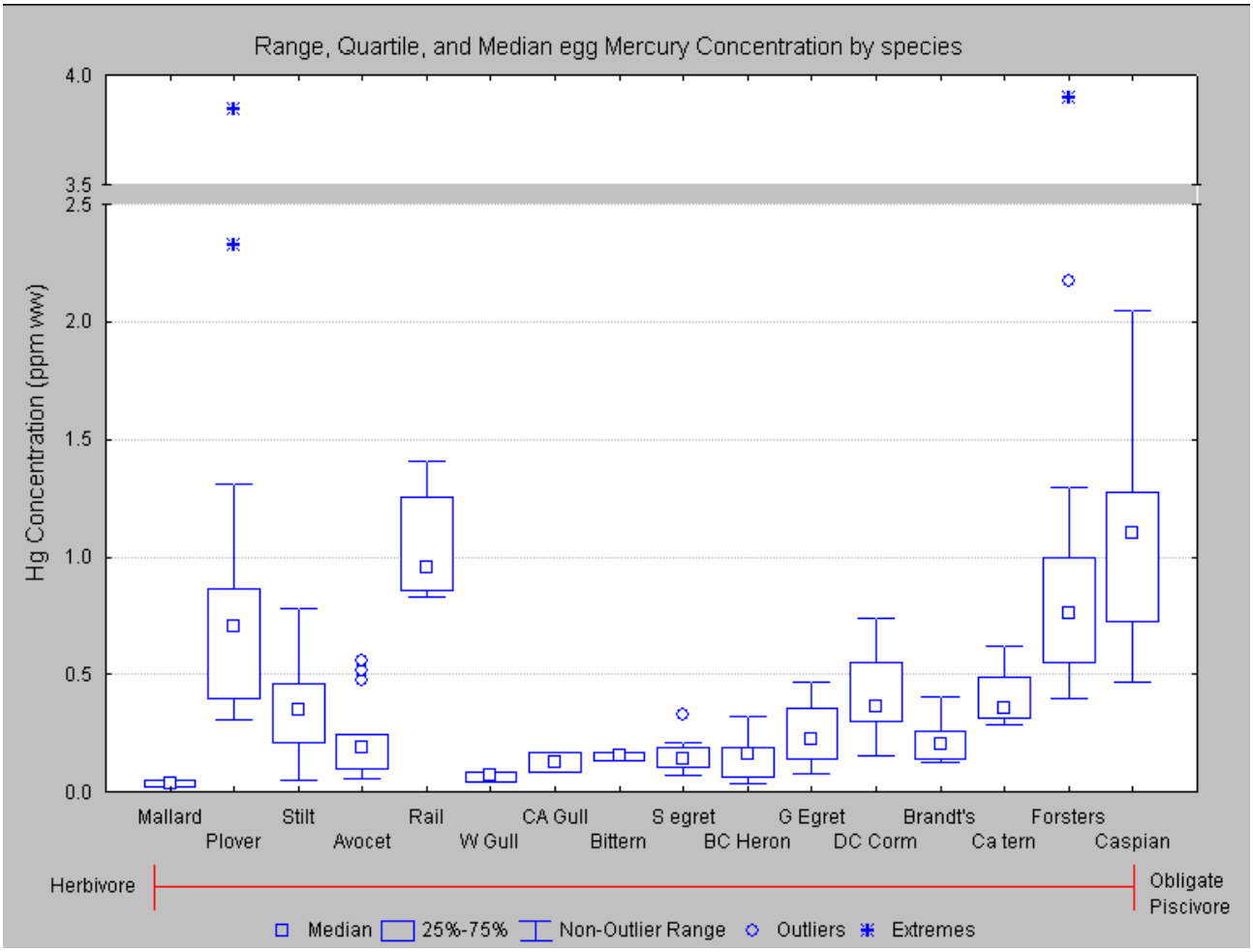


Figure 2. Hg concentrations in eggs from 16 avian from the San Francisco Bay region by feeding group.

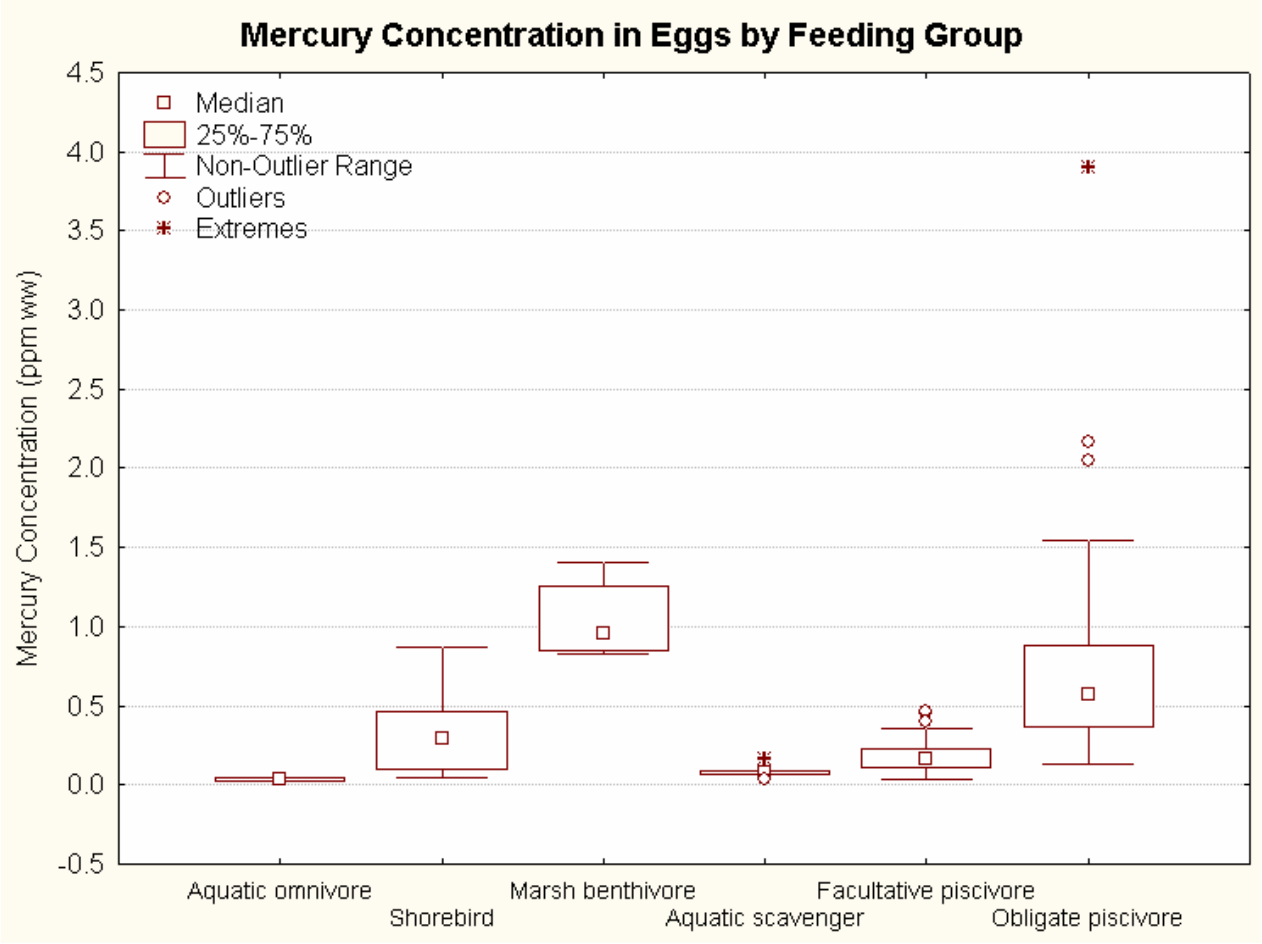
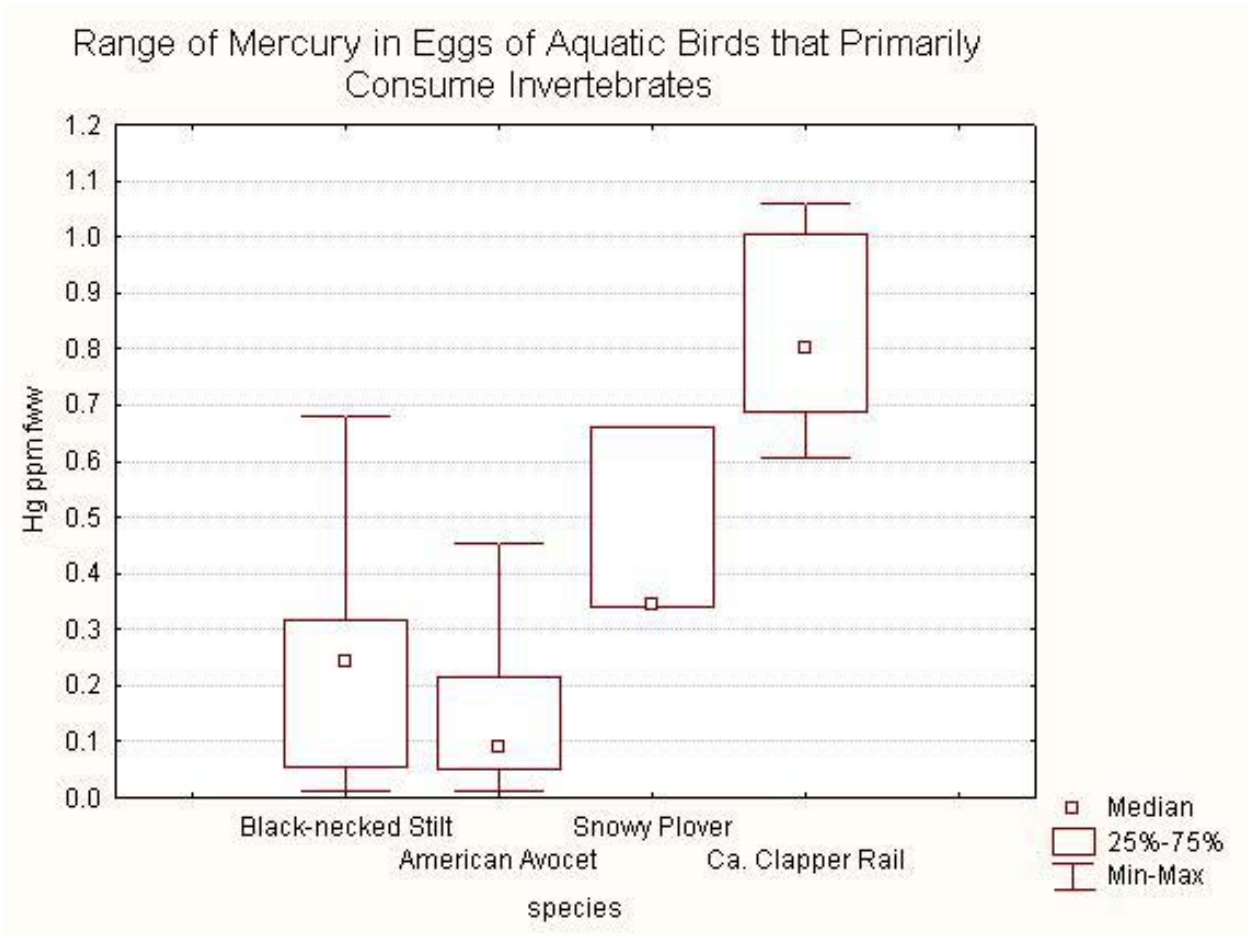


Figure 3. Range of Hg in eggs of aquatic birds from the San Francisco Bay region that consume primarily invertebrates. Values for black-necked stilts and American avocets are for randomly collected viable eggs. Values for snowy plover and California clapper rail are for fail-to-hatch eggs only.



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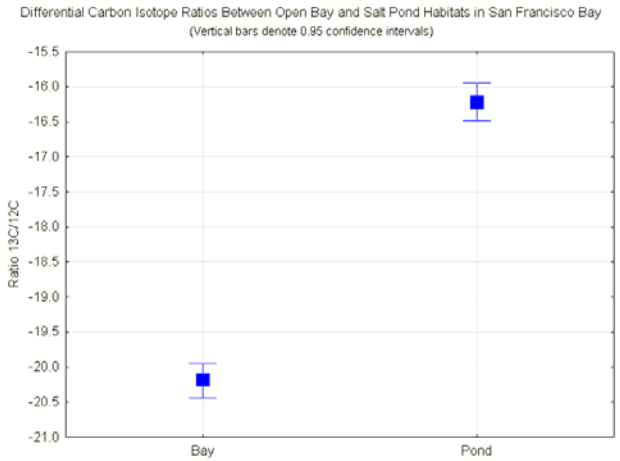
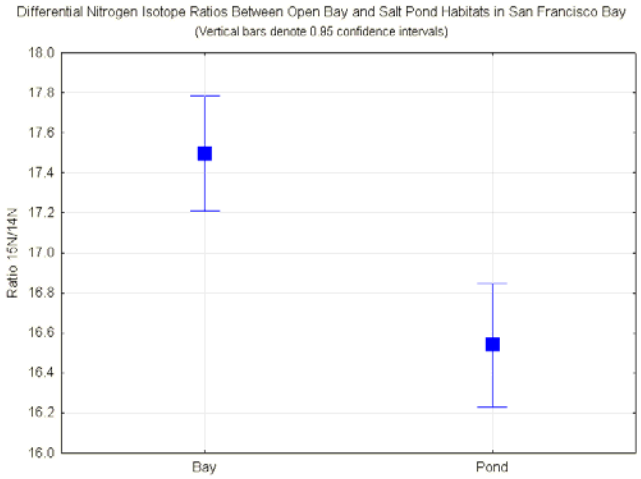
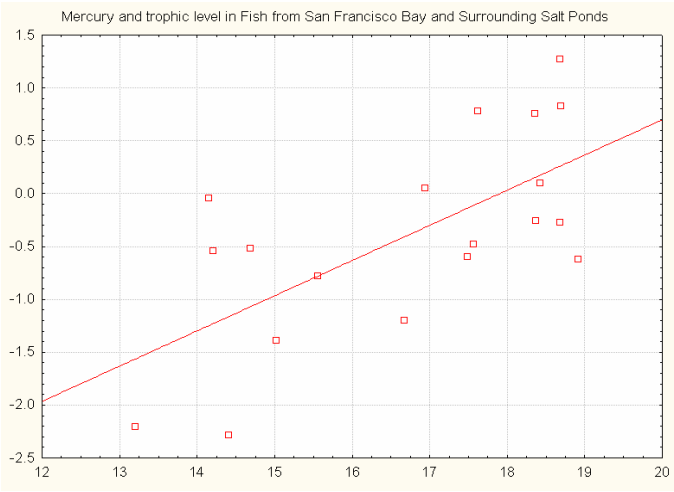
A.

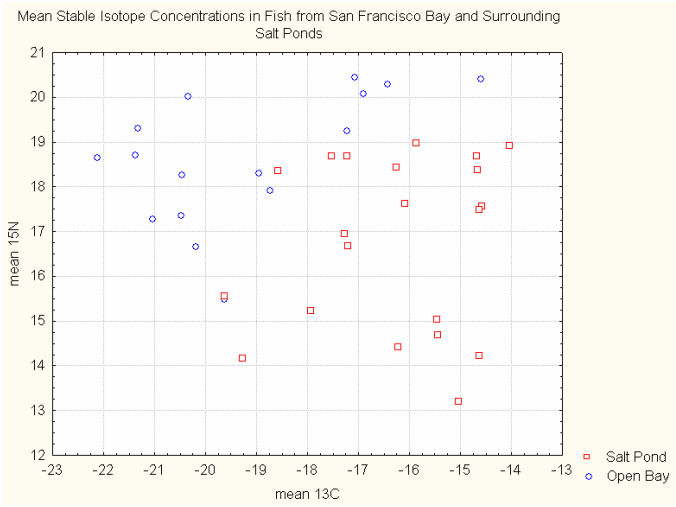


B.





D



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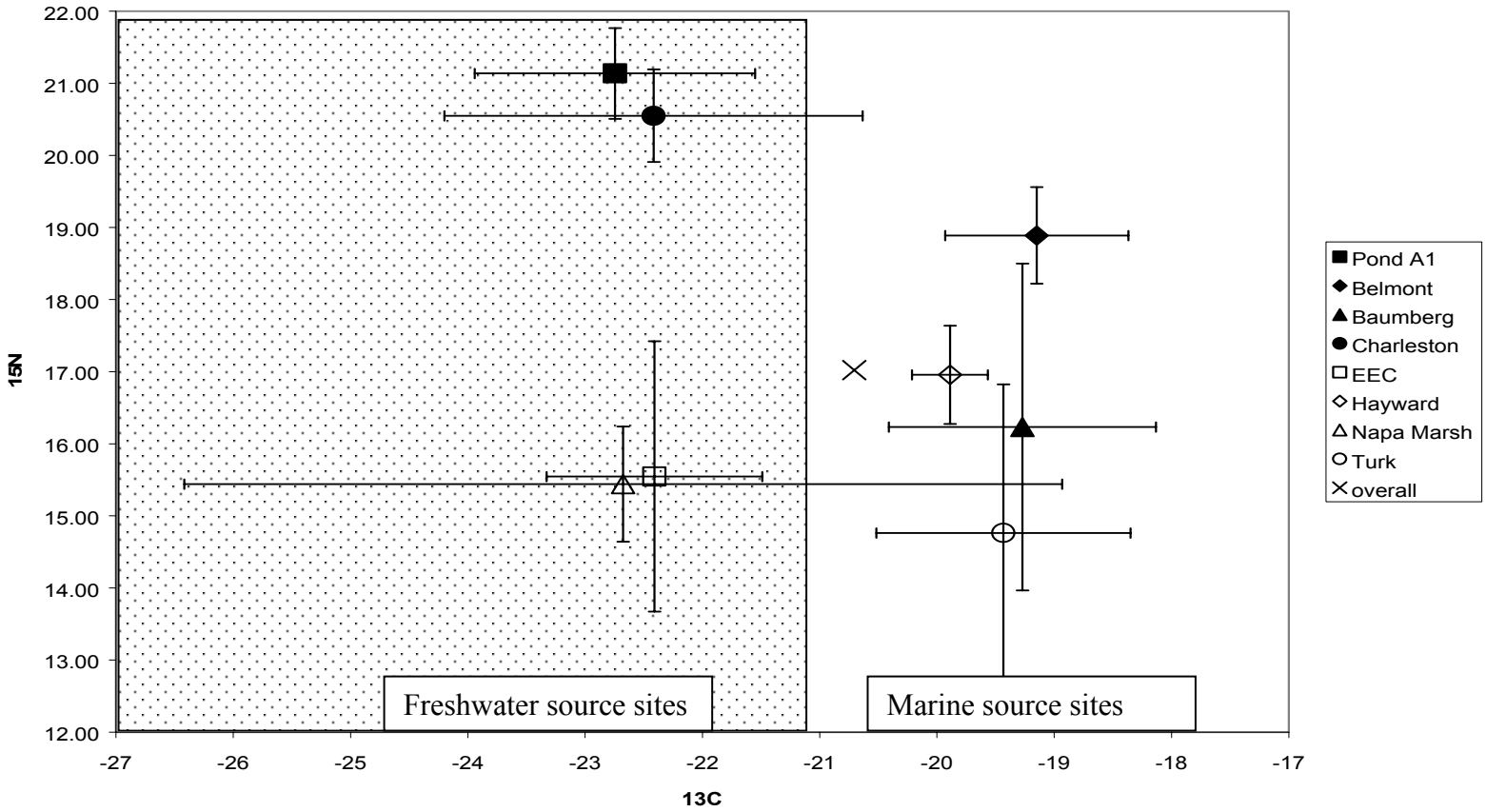

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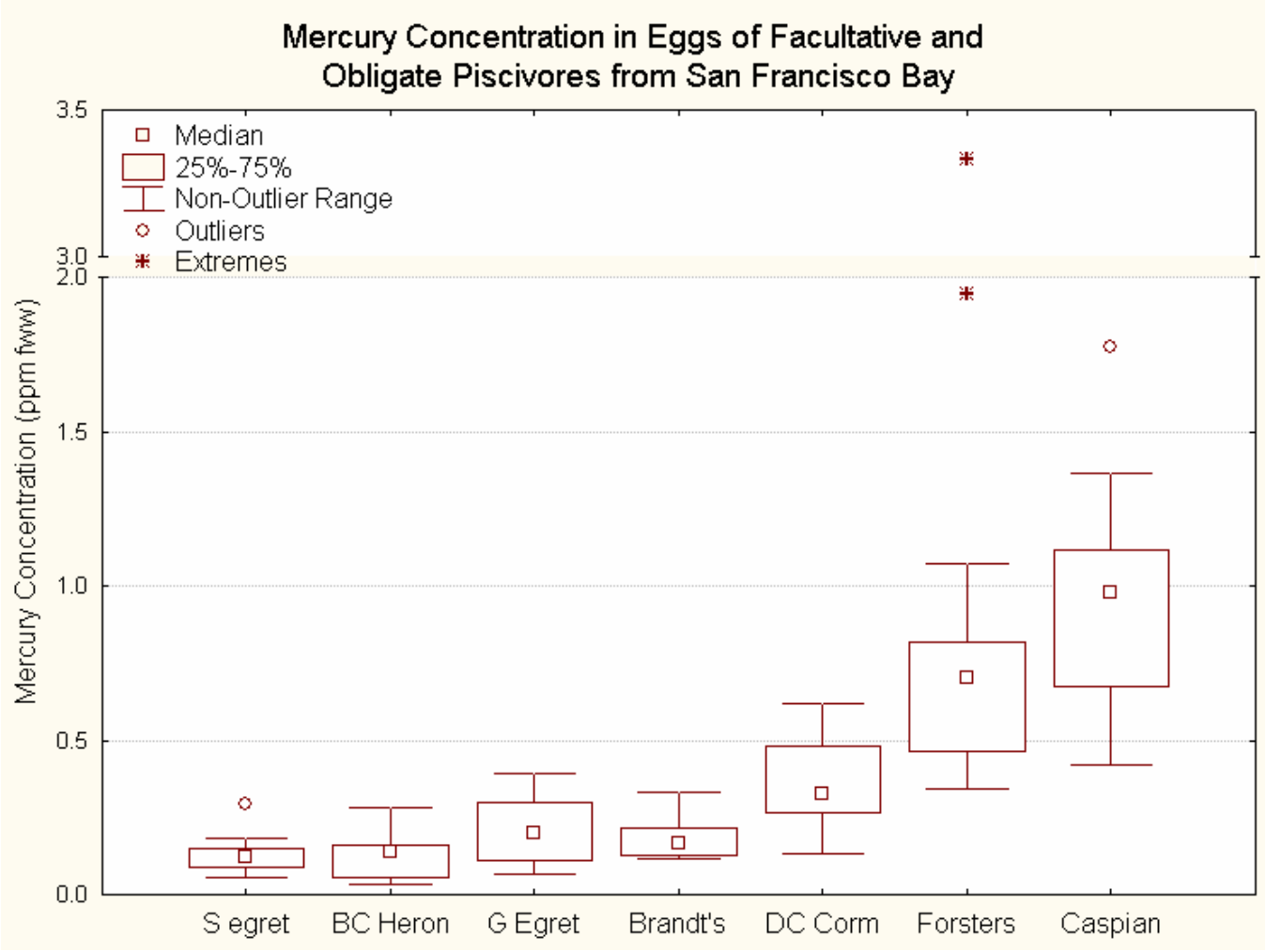

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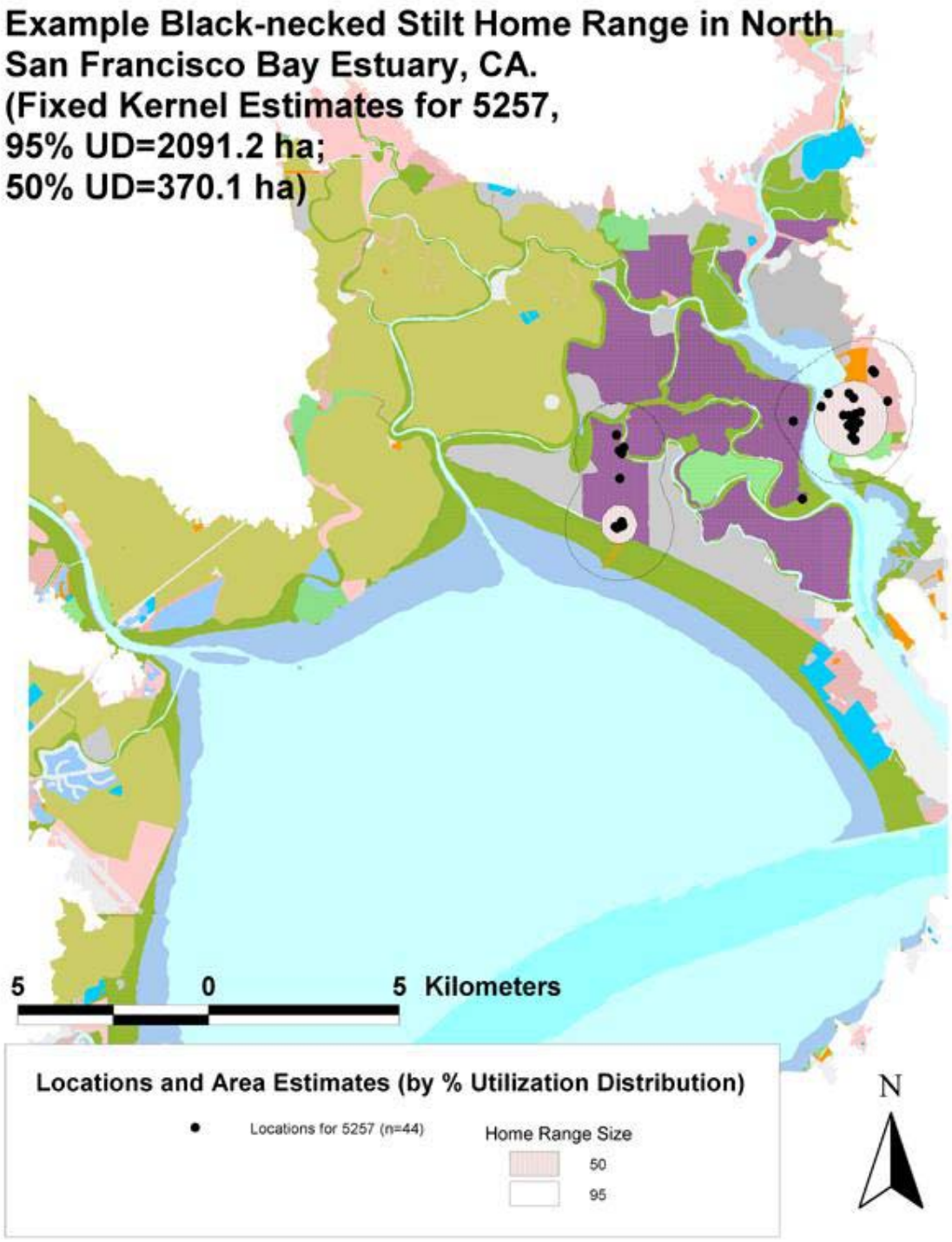


Figure 8. Migratory routes and ultimate breeding areas (Northwest Territories, Canada) of San Francisco Bay surf scoters that were marked with satellite transmitters in March 2003.

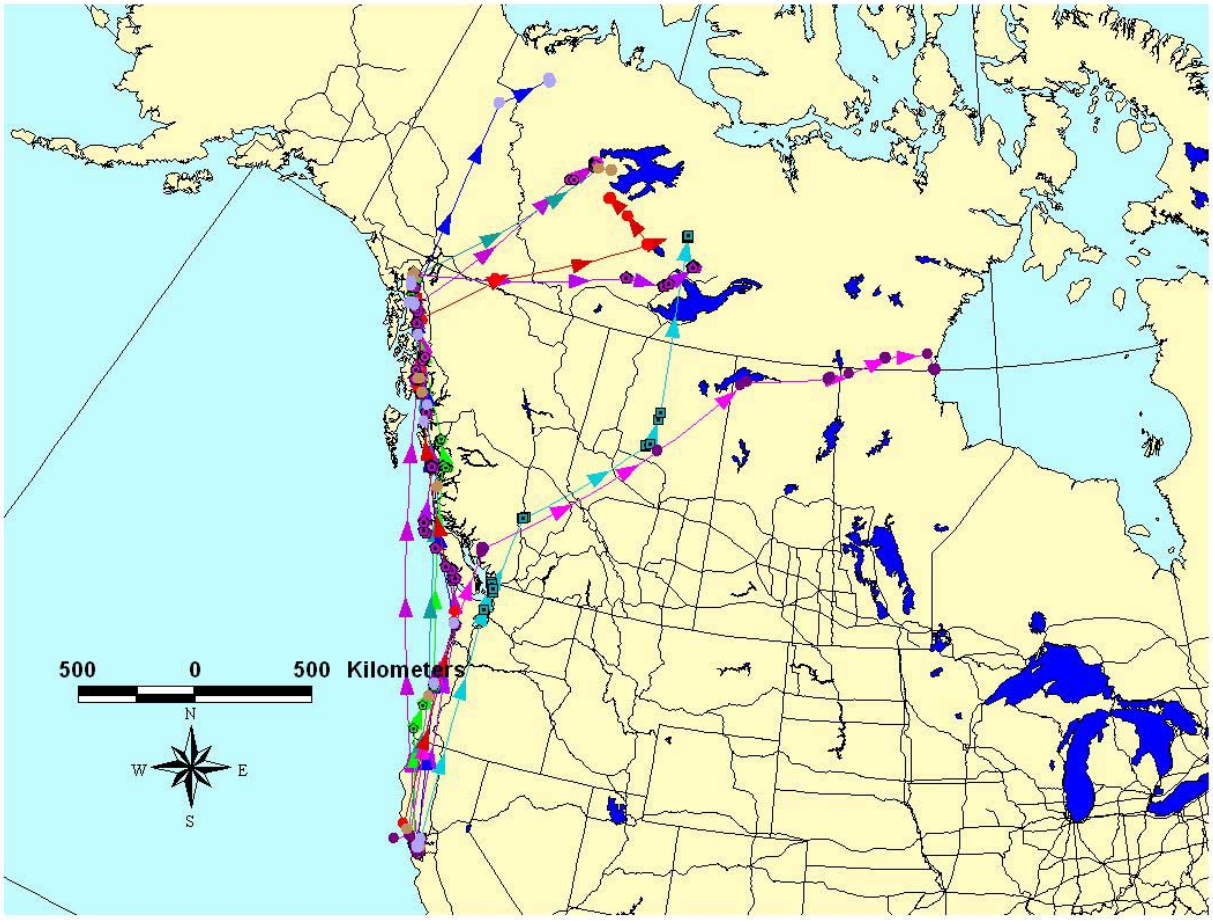
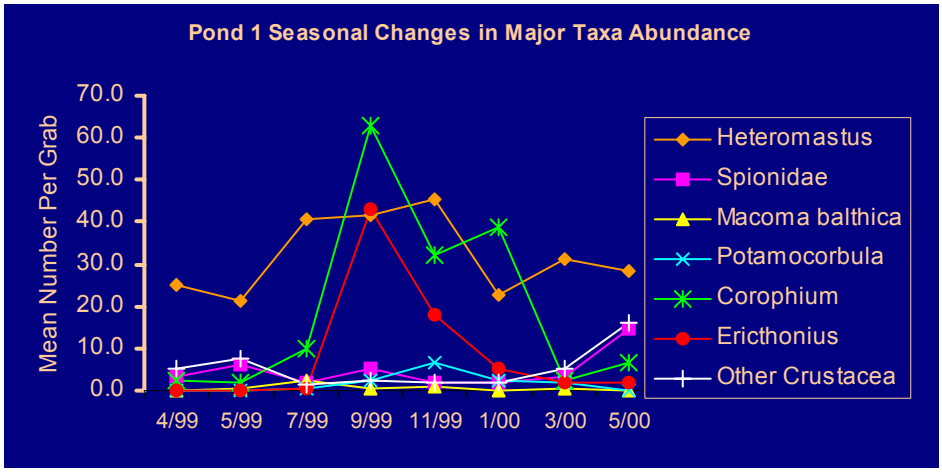
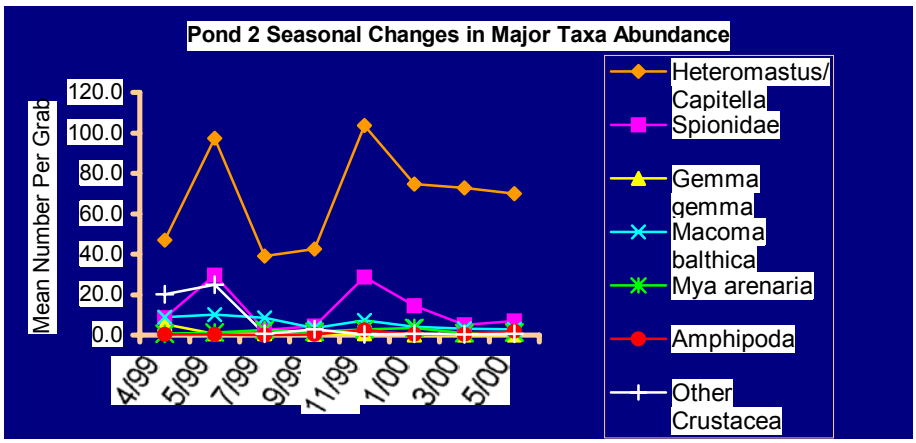


Figure 9. Seasonal change in invertebrate taxa abundance at three North Bay salt ponds.





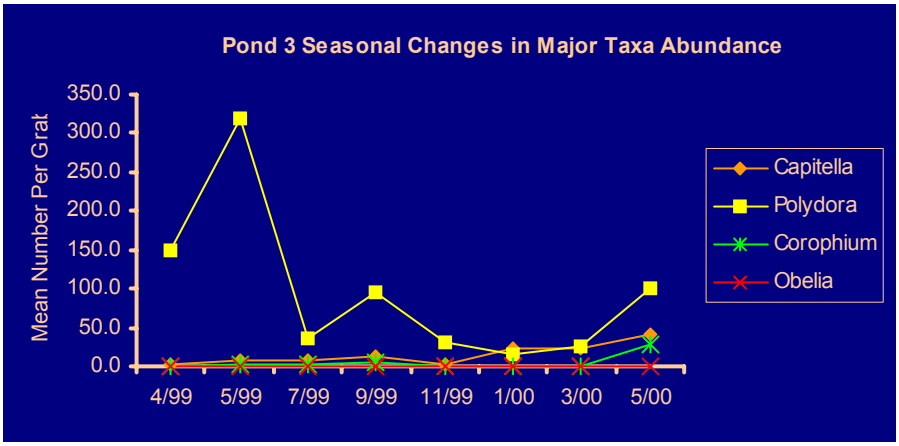


Figure 10. Timeline for recurvirostrid guild studies during Year 1.

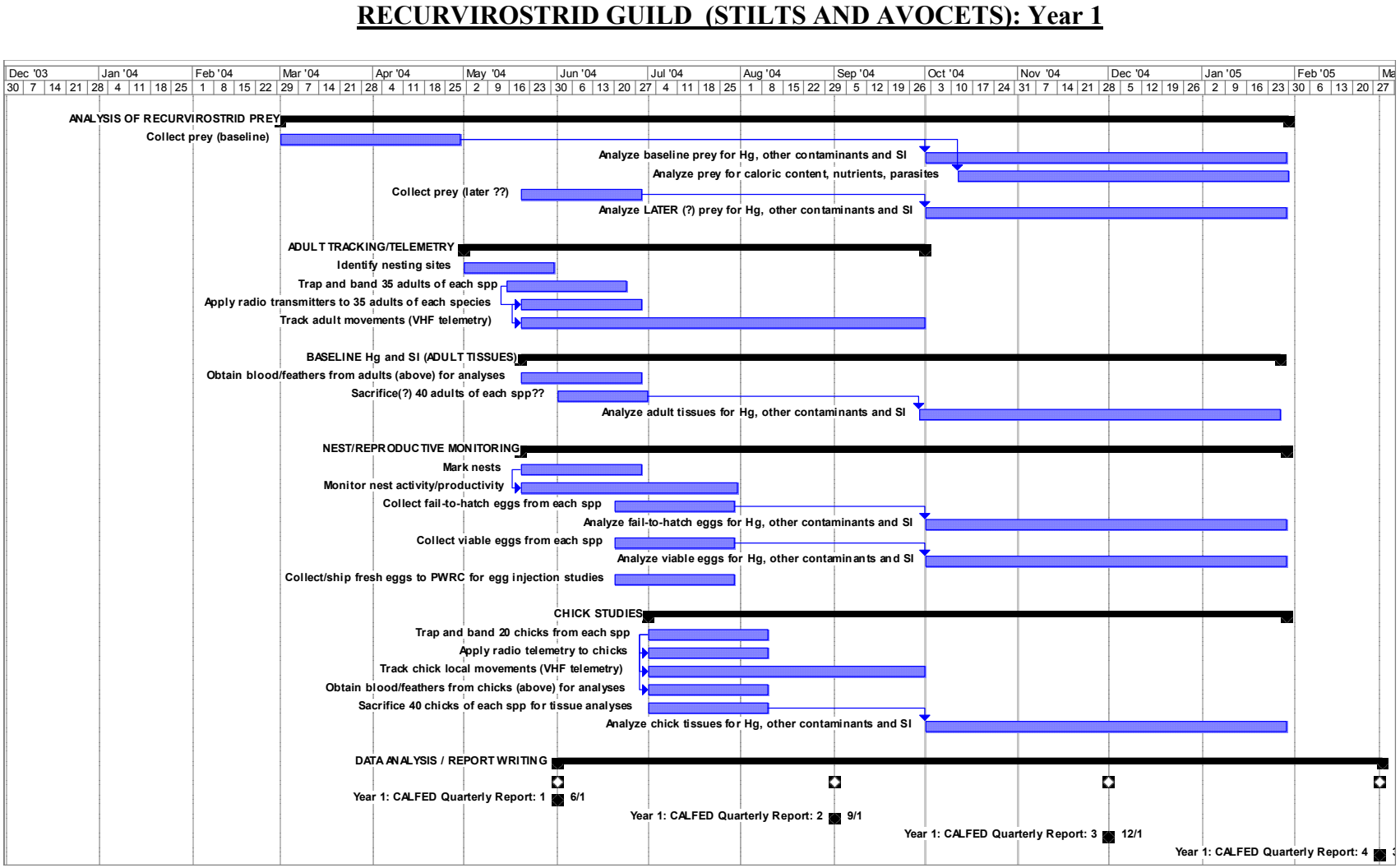


Figure 11. Timeline for tern guild studies during Year 1.

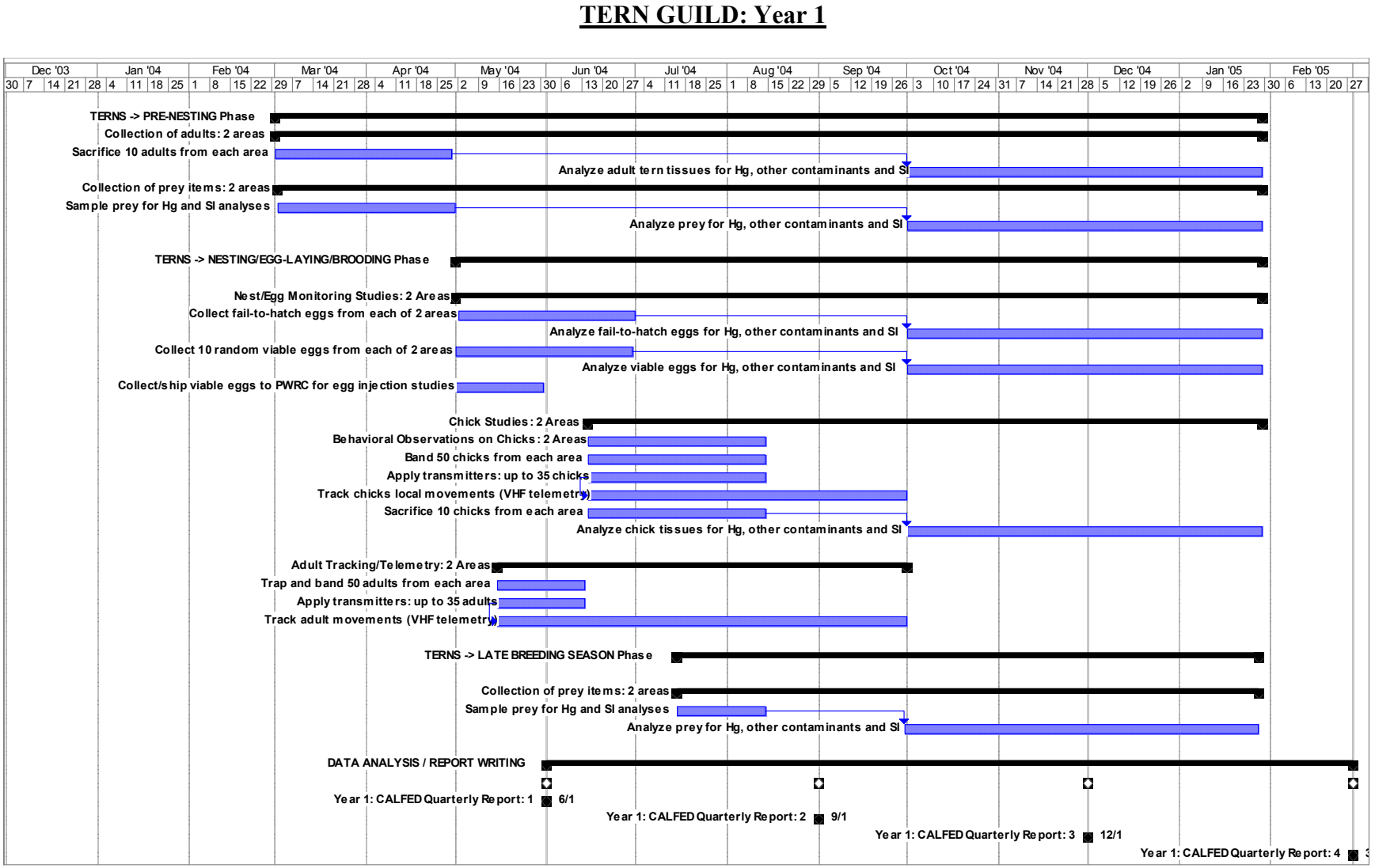


Figure 12. Timeline for diving duck guild studies during Year 1.

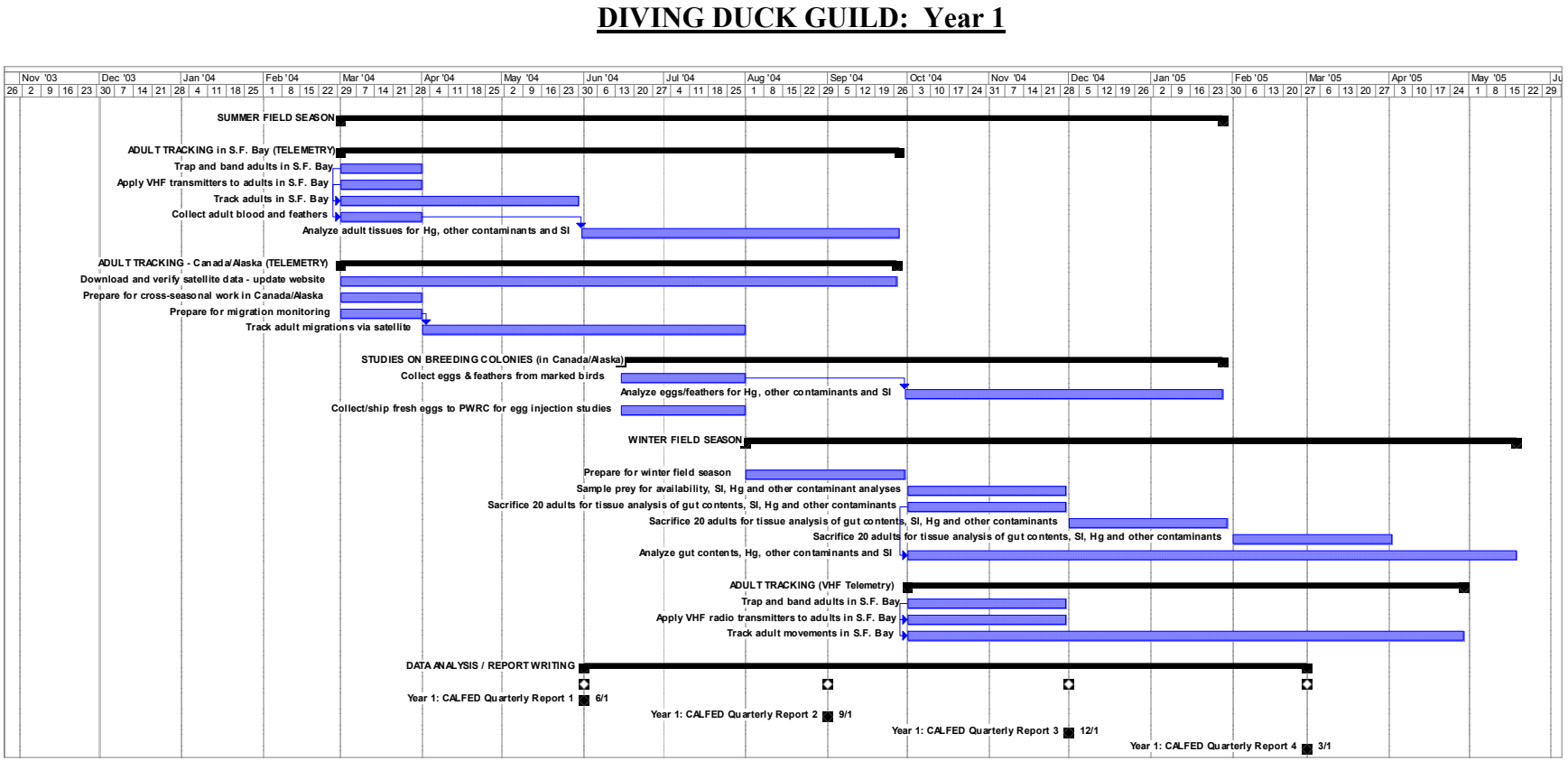


Figure 13. Timeline for laboratory studies during Year 1.

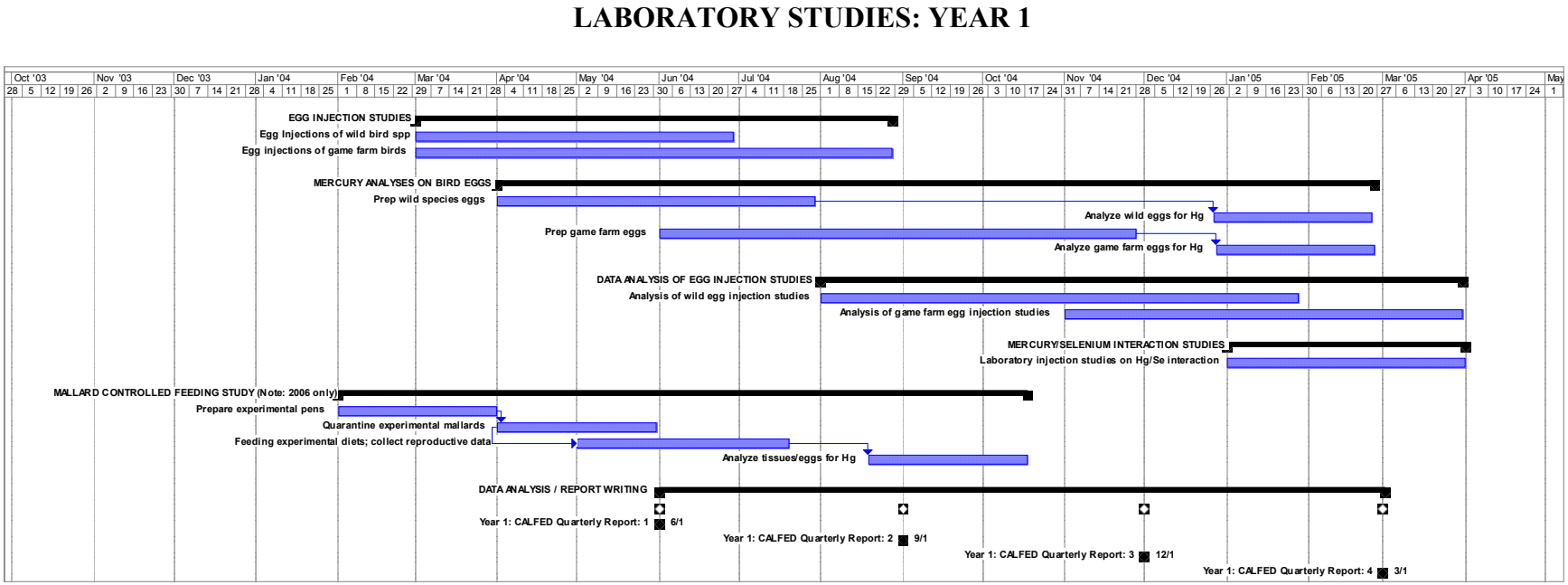


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Figure 14. Wetland Restoration and Enhancement Projects in the North Bay region.

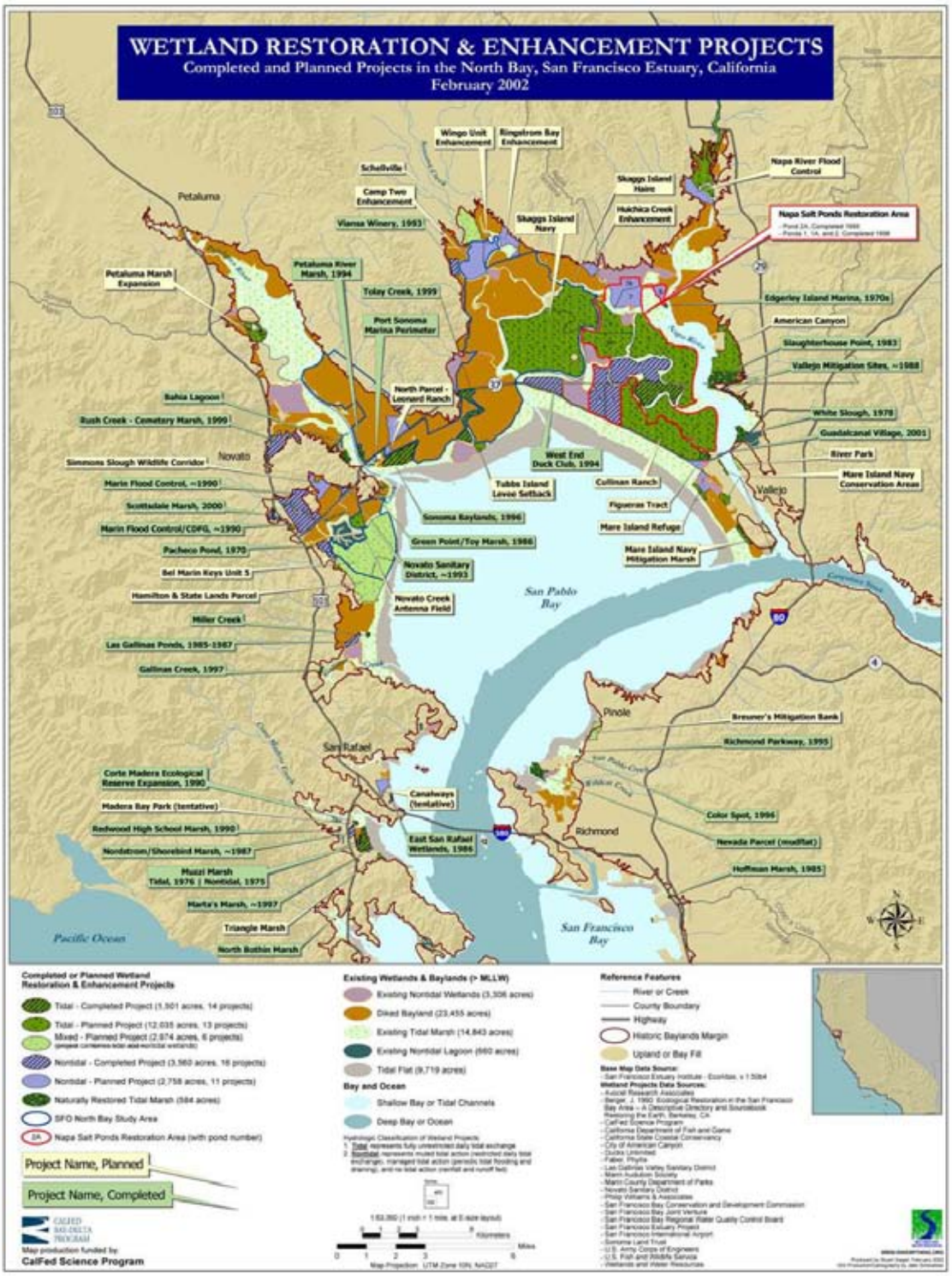

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Figure 15. Wetland Restoration and Enhancement Projects in the South Bay region.

APPENDICES:

Appendix A. Benefits and limitations of stable isotope analyses.

Appendix B. Capture methods for birds.

Appendix C. Protocols for necropsy

Appendix D. Protocols for Collection and Storage of Bird Plasma, Tissues, and Embryos for Oxidative Stress Analysis

Appendix E. Collection and Processing of Tissue Samples for Cytochrome P450 Related Assays.

Appendix F. Data collected for histological analyses

Appendix G. Rationale for Conducting Laboratory Studies and Details of Methods to be Used

APPENDIX A: BENEFITS AND LIMITATIONS OF STABLE ISOTOPE ANALYSES

In biological systems, enzymatic reactions often discriminate one isotope of the same element over another and result in the ratios of light to heavy isotopes changing (or fractionating) predictably from reactants to products. C ($\delta^{13}C$) and N ($\delta^{15}N$) isotopes are the two most commonly utilized in food web and energy flow investigations. Carbon isotope ratios are generally employed to trace the basal energy flow through food webs. Primary producer $\delta^{13}C$ values are primarily controlled by photosynthetic pathway (e.g. C_3 vs. C_4), and inorganic carbon source (e.g. atmospheric CO_2 vs. dissolved inorganic carbon), and are conserved (or change very little) from prey to predator. Thus a consumer's $\delta^{13}C$ signature reflects that of the basal primary producer(s) from which its energy is ultimately derived. As a result, one can trace the extent to which higher order consumers gain energy and nutrients from isotopically distinct habitats or specific trophic pathways in the food web. Nitrogen isotopes provide a valuable complement to carbon isotopes because they are relatively homogeneous among primary producers (within habitats), but increase predictably from prey to predator. As a result, once the basal $\delta^{15}N$ signature has been determined, the $\delta^{15}N$ value of a consumer acts as a quantitative proxy for trophic position. When examining feeding and/or movement between specific habitat types (marine vs. terrestrial or freshwater), S isotopes are particularly useful as they have unique marine signatures (relative to other habitats) and are passed conservatively through trophic interactions. In spatially and temporally complex systems such as the San Francisco Bay-Estuary the inclusion of these isotopes as an addition to C and N can help to both further elucidate trophic pathways, and identify the extent of marine, freshwater, and terrestrial feeding in highly mobile, avian predators.

SIA provides several advantages to traditional methods of dietary analysis. (1) SIA provides a time-integrated index of diet within a range of temporal scales from days to years, depending upon tissues analyzed and tissue turnover rates. One of the main drawbacks of traditional dietary methods is that they only provide a "point-in-time" estimate of diet, and thus are subject to bias sources such as collection time of day and rare opportunism. (2) Compared to a quantitative analysis of gut contents which often requires microscopic observation, measuring, and weighing, SIA is much less time consuming and less susceptible to individual investigator bias. (3) SIA provides quantitative estimates of diet sources that are actually assimilated, not just ingested. For example, often detrital or plant matter may be ingested while foraging on associated fauna and this substrate material may not provide much nutritional value to the consumer. Traditional methods of diet analysis cannot separate these factors and may grossly overestimate the importance of plant or detrital resources to the consumer. In addition, traditional dietary analyses often over estimate the importance of diet items containing "hard parts" which are not rapidly digested. Easily digested or "mushy" prey items can be overlooked as they may not be present in the stomach long, or quickly become unidentifiable. (4) SIA of easily available tissues (feathers, fur, scales, etc.), collection of which poses limited risk to the life of an organism, often precludes the need to sacrifice specimens, which is another drawback to gut content analysis.

Although SIA does confer certain unique advantages to ecologists performing dietary studies, the information garnered can be misleading in its own right and is most effectively exploited when coupled with the more traditional methods of gut content analysis and direct feeding observations.

Some of the disadvantages include: (1) the inability to separate the importance of diet items with similar isotopic signatures, (2) isotope signature carryover from other habitats, (3) uncertainty in the half-life of isotope signatures in specific tissues relative to tissue turnover time, (4) uncertainty in the fractionation factors of isotopes due to intra and inter-specific differences in metabolic rate, body condition, lipid content, and life stage, (5) inability to identify rare diet items that may contribute significantly to contaminant exposure. However, minimizing the influence of these disadvantages can be accomplished to a significant degree by validating SIA results with gut content analysis and feeding observations, as well as designing studies in ways which specifically address and (to the extent possible) avoid these potential problems.

APPENDIX B: CAPTURE METHODS FOR BIRDS

Our capture methods include proven techniques, such as rocket netting and bow net trapping, for Caspian and Forster's terns, black-necked stilts, and American avocets. Scoters will be captured with net guns and trapping techniques we developed in open water habitats (Takekawa *et al.,* unpublished data). Stilts, avocets, terns, and surf scoters (30 adults of each species per year, except up to 80 surf scoter for cross-seasonal work) will be marked prior to breeding with radio transmitters that emit unique frequencies and weigh <3% of the bird's body mass. Each bird will be weighed, measured, bled (shorebirds <0.5 ml, terns <1 ml, surf scoters 2 ml), banded, radio-marked, and released less than 1 km from the capture site. Stilts and avocets will be marked with unique plastic color bands, as well as 2.5 g (model PD-2sp, Holohil Systems Ltd, Woodlawn, ONT) transmitter glued to a metal leg band and placed on the upper left tibia (Plissner *et al.* 2000). Transmitter attachment styles for other species will include glue-on or leg band attachment of 1.4 g transmitters (model BD-2, Holohil Systems Ltd., Woodlawn, ONT) on terns (Morris and Burness 1992, Sirdevan and Quinn 1997), and implant transmitters (<25 g, model A2320, Advanced Telemetry Systems, Insanti, MN; Korschgen et al. 1996) for surf scoters (Korschgen *et al*. 1996). Ten adult female surf scoters will be marked with 20 g implant (Microwave Telemetry, Inc., Columbia, MD) PTTs during each year of our study.

APPENDIX C: PROTOCOLS FOR NECROPSY

Necropsy Record Details
(All of the following tissues will be fixed in 10 percent neutral buffered formalin)

Body condition (clinical finding only)
Musculoskeletal (Pectoral musculature)
Integument (portion of Ventral sternal tract, including feathers)
Oral cavity
Eyes (both)
Ears (taken only if a lesion is present)
Nares (taken only if a lesion is present)
Trachea
Syrinx
Air sacs (if a lesion is detected in a particular air sac will be collected, otherwise, the abdominal air sac will be collected)
Pleura
Lungs
Thyroid *
Thymus *
Peripheral nerve * (brachial plexus nerve)
Heart
Peritoneum
Kidney *
Ureter
Gonads
Adrenal
Liver *
Spleen *
Esophagus
Crop
Proventriculus (including stomach contents collected in a separate bag)
Gizzard (including stomach contents collected in a separate bag)
Intestine
Pancreas
Cloaca
Bursa *
Bone marrow (tibiotarsus)
Nervous system (brain) *
Pituitary
Spinal cord (cervical spine cord) *
* Tissues that will routinely be examined histologically from every bird; all of the other tissues will be collected for possible future examination, if necessary.

APPENDIX D: PROTOCOLS FOR COLLECTION AND STORAGE OF BIRD PLASMA, TISSUES, AND EMBRYOS FOR OXIDATIVE STRESS AND HISTOLOGICAL ANALYSIS

Blood Collection, Hematocrit, and Plasma Separation

1. Collect 3 to 5 ml of blood from the jugular vein, using lithium-heparinized bead monovette syringes.

2. Invert blood for 5 minutes on rocker prior to taking hematocrit using standardized hematocrit centrifuge and reader scale.

3. Remove some whole blood for residue chemistry, and leave 1 to 2 ml to centrifuge within same plastic syringe on clinical centrifuge for 10 minutes at setting of 2500 RPM. Gently pour or pipet off plasma and freeze in a cryovial in liquid nitrogen. Can be later shipped on dry ice.

Tissue Collection

1. For Oxidative Stress:

(a) right half of the brain into a cryotube into liquid nitrogen.
(b) one cryotube of liver (1-2 grams) off the tip of the larger lobe after a small piece off tip removed for histopath.
(c) posterior right kidney (or 1-2 grams) into the cryotube after a small piece off end removed for histopath.

2. For Histopath:

Place in a 20 ml plastic scintillation vial of 10 percent neutral buffered formalin:

(a) other half of the brain.
(b) small sliver of liver off the tip of the larger lobe.
(c) small piece of posterior right kidney.
(d) small cross section of back bone including mid thoracic spinal cord.
(e) spleen
(f) bursa

Unhatched Embryos

Place in 70 percent denatured ethanol with approximately 5 to 10 times the weight as volume of ethanol added.

Oxidative stress analysis

Many of the biochemical measurements chosen have been used to indicate mercury toxicity in birds, or are known to reflect organ damage and related physiological disturbances. Basic methods and assay conditions are described by Hoffman and Heinz (1998). Indicator assays of potential mercury-related effects follow: whole blood - hematocrit; blood plasma - total glutathione peroxidase (T-GSH-Px), selenium dependent glutathione peroxidase (S-GSH-Px), glutathione reductatase (GSSG - reductase), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholinesterase (ChE), uric acid (UA), creatinine (CRN), glucose (GLU), total plasma protein (TPP), albumin (ALB), cholesterol (CHL), triglycerides (TRG), calcium and inorganic phosphorus; liver - T-GSH-Px, S-GSH-Px, GSSG-reductase, glutathione-S-transferase (GSH-S-transferase), glucose-6-phosphate dehydrogenase (G-6-PDH), reduced glutathione (GSH), oxidized glutathione (GSSG), total sulfhydryl concentration (TSH), thiobarbituric acid reactive substances (TBARS), protein bound thiol (PBSH) and total thiol (TSH); kidney - GGT, UA, GLU, and enzymes related to glutathione metabolism, oxidative stress and TBARS per liver assays; brain - adenosine triphosphate (ATP) and cholinesterase (AChE), plus all liver variables.

Hoffman, D.J. and Heinz, G.H. (1998). Effects of mercury (Hg) and selenium (Se) on glutathione metabolism and oxidative stress in mallard ducks. *Environ. Toxicol. Chem.* **17**, 161-166.

APPENDIX E: COLLECTION OF TISSUE SAMPLES FOR CYTOCHROME P450 RELATED ASSAYS.

Liver tissue is removed and placed in a cryotube and immediately frozen in liquid nitrogen (dry ice is an option). If the gall bladder breaks, blot off any bile from the liver samples. A good sample size is 0.2 – 0.25 g per sample. (Two such samples in separate cryotubes allow me to have a sample in reserve in case anything goes wrong with the assay, but this isn't necessary.) Collect the liver samples from the same area of each liver. The liver samples must be maintained at these cold temperatures until assayed. We usually ship with generous amounts of dry ice. They will be stored in mechanical ultracold freezers [80^{o} C at the USGS Patuxent Wildlife Research Center until thawing for microsome preparation and assay for cytochrome P450 associated dealkylase activities (ethoxyresorufin-O-dealkylase(EROD) and benzyloxyresorufin-O-dealkylase(BROD)].

Two tail feathers are removed from the nestling and the base section is placed in a tube containing 10 percent buffered formalin of a depth to more than cover the feather sample. The frond part of the feather is available for heavy metal analysis. The tubes of feather bases in buffered formalin are kept at room temperature.

Laboratory Procedures

Following descriptions of microsome preparation and EROD and BROD assays are adapted from Melancon (1996) with the descriptions modified slightly from the ASTM publication to reflect recent modifications to the procedure, mainly pipetting procedures and the use of a different protein assay that does not require heating to a high temperature and which can be run in a microwell plate.

MICROSOME PREPARATION - Liver samples are thawed on crushed ice and homogenized in four mls ice-cold buffer (1.15 percent potassium chloride in 0.01 M sodium/potassium phosphate, pH 7.4) per g of tissue using a Polytron (at setting 5 for 20 sec) while maintaining the tube in a beaker of crushed ice. In order to maintain an adequate volume for the Polytron to function effectively and without excessive frothing, the minimum volume of buffer used is 1.0 ml even when the sample is less than 0.25 g. The supernatant from a 20 min, 11,000g centrifugation of the homogenate is centrifuged at 100,000 for 60 min to obtain the microsomal pellet. Because the field samples processed are generally 0.5 g or less they are generally resuspended in 2.0 ml per g of tissue weight of ice-cold buffer (0.05 M sodium/ potassium phosphate, 0.001 M disodium ethylenediamine tetraacetate, pH 7.6) using a motor driven stainless steel and teflon pestle in a glass homogenizing vessel. Smaller samples are resuspended in 4.0 ml or 10.0 ml buffer per g of tissue to ensure that there is adequate volume for effective resuspension and to reduce loss to vessel walls, etc.

MONOOXYGENASE ASSAY PROCEDURES - Because of the small volume (an effective working volume of approximately 0.26 ml) of the wells in a 96 microwell plate, the amount of microsomes needed for assay is also small. Use of such a fluorescence microwell plate scanner

began at PWRC in 1990 and became the only approach used for the resorufin-based monooxygenase assays in 1991. Currently, four resorufin generating dealkylases are assayed on this instrument. They are benzyloxyresorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD), methoxyresorufin-O-dealkylase (MROD), and pentoxyresorufin-O-dealkylase (PROD). EROD and BROD will be utilized in this study. Data are automatically placed into computer files which are transferred to a spreadsheet for necessary calculations.

The quantity of microsomes assayed is therefore based on the amount of liver used in their preparation and varies by assay, species and life stage. The quantity of microsomes selected for each assay is that which gives a linear response over the time of the assay, that is proportional to the amount of microsomes added and that falls within the range of the standard curve. In the case of highly induced samples it may be necessary to repeat the assay with less microsomes to have fluoresecence readings that are linear for enzyme activity and fall within the standard curve. Substrate concentrations are selected that give maximum velocity and are constant over the duration of the assay. Because of the high cost of NADPH the concentration of NADPH selected is that which gives a reading of at least 10 fluorescence units for uninduced samples or that for which doubling the NADPH concentration gives less than a 20 percent increase in monooxygenase activity.

As the assays are currently performed, the 96 microwell plate contains 24 samples in triplicate, a triplicate resorufin standard curve with 0 to 0.6 nmol per well and three wells with reference microsomes. Each well receives 150 ul of pH 7.4, 0.066M Tris buffer (TB) containing substrate, 50 ul of pH 7.4, 0.066M Tris buffer, and 50 ul of TB containing microsomes. This is preincubated in the dark at assay temperature (37^{o} C) for 10 minutes followed by addition of NADPH in 10 µl of TB and the plate is placed in the fluorescence microwell plate reader. An initial reading is taken followed by seven additional readings at 90 sec intervals (Approximately 75 sec are required to read the plate.). After checking fluorescence readings for proper instrument functioning and linearity with time, the fluorescence units are translated to nmoles of product by utilizing the resorufin standard curve. Protein concentrations are determined by the bicinchoninic acid method (Pierce) adapted to a microwell plate reader, and monooxygenase activity calculated as nmol or pmol product per min per mg microsomal protein. Assays utilize the amount of microsomes derived from 0.65 to 5.2 mg of liver per well, with 1.25 to 5.0 µM substrate and 0.125 or 0.25 mM NADPH.

The **10 percent buffered formalin solution** is made to neutral pH with phosphate buffer. Its possible to purchase this already made and even to buy sample containers prefilled with it. (At Fisher Scientific a case of 100 tubes lists for $60.00.)

The **immunohistochemical detection of CYP1A** in slides of fixed feather sections is done with an anti-avian CYP1A primary antibody that was generated in rabbit (Brown et al, 1996) and an anti-rabbit secondary antibody as developed at Patuxent.

References

Brown, R.L., Levi, P.E., Hodgson, E. and Melancon,M.J., 1996. Purification and immunological detection of $-napthoflavone- and phenobarbital-induced avian cytochrome P450 enzymes. Environ. Toxicol. Chem. 15, 2293-2298.

Melancon, M.J., "Development of Cytochromes P450 in Avian Species as a Biomarker for Environmental Contaminant Exposure and Effect: Procedures and Baseline Values", Environmental Toxicology and Risk Assessment: Biomarkers and Risk Assessment (5th. Volume), ASTM STP 1306 , David A. Bengston and Diane S. Henshel, Eds., American Society for Testing and Materials, Philadelphia, 1997.

APPENDIX F: DATA COLLECTED FOR HISTOLOGICAL EXAMINATIONS

Data Collected for Histological Examinations

Bursa
- Follicular diameter measurement; largest (um). BUFOLL
- Number of mitotic figures per field of view (400x). BUMIT
- Lymphoid depletion; 1 to 3, increasing severity. BULYMPH
- Wall thickness measurement (grid units). BUWALL

Liver
- Inflammation; 1-5, increasing severity. LVINFLAM
- Black pigment in Kupffer cells and clusters; 1-4 increasing severity. LVPIG
- Vascular change in hepatocytes; 1-5, increasing severity. LVVAC

Kidney
- Extramedullary hematopoeisis; 1-3, increasing severity. KDEMH
- Lymph nodules; 1-3, increasing severity. KDLYMPH
- Interstitial inflammation; 1-5, increasing severity. KDOTHER
- Pyelonephritis; 1-3 increasing lymphocytic infiltrate associated with ureter and collecting ducts. KDURETER
- Distention of Bowman's space; 1-3, increasing severity. KDENLG
- Vascular change proximal tubular epithelium; 1-5, decreasing uniformity cells and increasing vascular change of nuclei. KDVAC
- Vascular change and necrosis in distal tubular epithelium; 1-5, decreasing uniformity of cells and increasing vascular change out in nuclei. KDVAC2

Peripheral nerve
- Vascular change (size) and inflammation; 1 to 5, increasing severity. NVVAC

Spleen
- Number of a journal centers in the field of view (40x close). SPGERM
- Lymphoid depletion; 1 to 4, increasing. SPLYMPH

Thymus
- Lymphoid depletion of medullary area follicles, 1 to 4. THYLYMPH
- Measurement of smallest diameter (um). THYMSMAL
- Count of number of follicles per 100 grid units. THMDEE

Thyroid
- Colloid goiter or microfollicular change in glands; 1 to 5, 1= mild goiter, 2= normal, 3 to 5 decreasing follicular size and increasing cell height THYRCO

APPENDIX G: DETAILED RATIONALE AND PROTOCOLS FOR LABORATORY ANALYSES

A. Rationale for Laboratory Studies

Fish-eating birds sometimes contain high levels of Hg (Hg) (Hesse et al., 1975; Sepulveda et al. 1999) because the fish they eat readily accumulate Hg (Schreiber, 1983; Wiener and Stokes, 1990). In general, 95 to 99% of the Hg in fish is in the form of methyl-Hg (Wiener and Spry, 1996), and methyl-Hg is the most harmful form to birds (Heinz, 1996; Thompson, 1996; Wren et al., 1995). High dietary levels of Hg have been implicated in impaired reproduction in common loons (*Gavia immer)* in the Great lakes region (Barr, 1986). Survival of loon chicks from Wisconsin was lower in lakes where Hg in the blood of chicks was highest.

In controlled laboratory studies with black ducks (*Anas rubripes*), mallards (*Anas platyrhynchos*), chickens (*Gallus gallus*), and ring-necked pheasants (*Phasianus colchicus*) reproduction in birds has been shown to be very sensitive to methyl-Hg (Finley and Stendell, 1978; Heinz, 1979; Tejning, 1967; Fimreite, 1971), yet virtually nothing is known about the thresholds of Hg needed to harm reproduction in wild birds. To date, the levels of Hg permissible in the diet or eggs of wild birds have been based on these lab studies with mallards and other easily reared species. Until more data on the embryotoxicity of Hg to wild birds is gathered, the protection of reproduction in wild species will be mostly a matter of guess work.

Breeding a variety of fish-eating birds in captivity and feeding them various concentrations of methyl-Hg would be an excellent way of determining how much Hg it takes to cause reproductive harm. Unfortunately, no controlled laboratory studies have been done to examine the effects of Hg on reproductive success of fish-eating birds, and it would take many years to establish these breeding colonies and would cost many hundreds of thousands of dollars per species to complete the studies. Consequently, little of this work is likely to be done in the near future. We have chosen to pursue an alternative method, which is to bring fertile wild bird eggs into our lab, inject them with various doses of methyl-Hg, and compare the sensitivity of these wild species to the sensitivity of mallards, chickens, and ring-necked pheasants, for which both injection and feeding studies have already been done. The ranking of sensitivity to methyl-Hg turns out to be the same whether methyl-Hg is deposited by the female or injected into the egg: pheasants are the most sensitive of the three lab species, chickens are of intermediate sensitivity, and mallards are the least sensitive. Knowing that species seem to respond to injected methyl-Hg in the same order of sensitivity that they do to naturally incorporated Hg suggests that we will be able to rank the sensitivities of many wild species and estimate how much Hg in their diet and eggs is sufficient to cause reproductive impairment. Such data are currently not available by other means. To date, we have injected the eggs from a total of 18 species with methyl-Hg. Based on our research findings thus far, there are clear differences in the sensitivity of various species of birds to injected Hg (Heinz, 2002). Currently, when field studies reveal certain concentrations of Hg in the eggs of a wild species of bird, an assessment of potential hazard is based largely on a comparison of the Hg levels in the wild bird eggs with the Hg levels shown to be associated with reproductive impairment in published feeding studies with captive mallards (Heinz, 1979; Heinz and Hoffman, 1998; Hoffman and Heinz, 1998; Heinz and Hoffman, 2003) and, to a lesser extent, with results

from one laboratory study with ring-necked pheasants (Fimreite, 1971). The practice of using default values from mallard studies to protect wild bird eggs from Hg poisoning will now have to be re-evaluated in light of the results from egg injection studies.

Because both elevated Hg and selenium have been reported in the eggs and tissues of birds from the San Francisco Bay-Delta area, a logical extension of our Hg injection studies is to inject combinations of Hg and selenium into the same egg.

The controlled feeding study we have planned for mallards is justified because the four species studied so far in the lab (black duck, mallard, chicken, and pheasant) were only fed one or two different dietary concentrations of methyl-Hg. From so few treatments it is impossible to understand the dose -response relation between Hg and reproductive success. Data from these carefully controlled lab studies will always play some role in judging the harm of methyl-Hg to avian reproduction. Having data to quantify changes in reproductive success with increasing Hg concentrations in the diet, and subsequently in eggs, will be of great use when assessing harm of Hg to wild birds, especially because the wild birds themselves are not likely to be brought into captivity for controlled breeding studies. The findings from very rigorously controlled reproductive studies with captive mallards combined with the results of egg injection studies with both mallards and wild species offers the best chance laboratory research has of predicting harm from Hg to wild birds.

B. Details of methods to be Used for Laboratory Studies

Egg injection studies - We conducted many studies, using thousands of chicken and mallard eggs, to develop a standardized protocol that we then use for injecting the eggs of wild birds. We tested the toxicity of methyl-Hg chloride when it was dissolved in various solvents, including water, corn oil, propylene glycol, DMSO, acetone, ethyl alcohol, soybean oil, Crisco, canola oil, peanut oil, Vaseline, and olive oil. While various solvents had their advantages, we found that corn oil and propylene glycol were both good solvents, but for different ages of embryos. Corn oil induces low mortality in controls when eggs are injected at the stage of a 3-day-old chicken embryo, but is very toxic to eggs that have not undergone any incubation. By contrast, propylene glycol is not very toxic when injected prior to incubation of the eggs, but becomes toxic when injected at 3 days of age. Because we wanted to be able to could cull out infertile and early dead embryos prior to injecting the eggs, and this can be done by candling eggs at about 3 days of development, we decided to use corn oil, which is not very harmful to embryos by that time.

We also ran many tests to determine where to inject the doses of Hg. Because nearly all the Hg in a bird egg is found in the egg albumen, we decided not to inject the Hg into the egg yolk. That left two options. One was to inject the Hg directly into the albumen by drilling a hole through the shell into the wet contents. Unfortunately, we sometimes saw too much mortality of control eggs (those in which only the solvent was injected, without any methyl-Hg). The second option, the one we chose to use for the wild bird eggs, was to inject the dose of Hg into the air cell and let the solvent penetrate the inner shell membrane and carry the methyl-Hg into the albumen that way. In early studies with mallard eggs, we discovered that nearly all of the injected dose of methyl-Hg does penetrate into the albumen of the egg.

We studied the effects that the orientation of the eggs during incubation might have on mortality. We discovered that incubation of Hg-treated eggs on their ends, with the blunt end

(called the cap) of the egg pointing upward, resulted in much heavier mortality than with the egg sitting on its side in the incubator. Also, we read and discovered for ourselves that the eggs of wild birds tend to hatch better when incubated on their sides. Therefore, the eggs of all species were incubated on their sides.

Other variables we investigated with mallard and chicken eggs included the volume of solvent to inject, the type of disinfectant used to swab the eggshell prior to drilling the hole, the size of the hole drilled in the cap end of the egg through which the injection was made, the effect of sealing the hole with a hot glue gun, the temperature of the egg when injected, and the temperature of the solvent. We also mixed dyes into the solvents to be able to observe the distribution of solvents into the albumen of the egg.

Although there is still room for much more experimentation with different injection procedures, we are satisfied that the standardized protocol we use, and which is described below, gives good dose response curves and detects differences in species sensitivity to methyl-Hg.

Standardized Protocol

Eggs are collected in the field by cooperators who have the appropriate state and federal collecting permits. We have cooperators collect eggs from areas where Hg contamination is known to be low. We advise these cooperators to collect only fresh eggs, meaning those that have not undergone any incubation by the parents. All eggs are labeled as to the nest they came from. These eggs from the field are shipped back to our lab in foam-lined boxes by overnight delivery. Once the eggs come to our lab, we wash them in a dilute solution of Betadine to disinfect them and randomize them to the injection treatments they will later receive. We place one restriction on the randomization process, and that is to put eggs from the same nest in different treatments.

We then write a code number on each egg that identifies it to treatment. The eggs are placed on their sides in a Kuhl incubator (Kuhl Incubator Company, Flemington, NJ). We devised special trays that enable the eggs to turn about 180 degrees every hour. The eggs of many wild birds require this degree of turning. The temperature is set at 37.5EC for all species except chickens and pheasants, for which 37.6EC is recommended. The humidity inside the incubator is adjusted for each species so that the percentage weight loss of the eggs over the full course of incubation is about 14 to 16%, based on a sample of eggs we periodically weigh. Cracked and infertile eggs are eliminated, as are eggs that die prior to the time of injection. The eggs from different species are all injected at the same stage of embryo development. This stage was standardized as the development of a 3-day-old chicken embryo, which is equivalent to about a 4-day-old mallard embryo. Some species take less than 3 days and some take more than 3 days to reach the appearance of a 3-day-old chicken embryo. We know approximately when this stage will be reached by each species, based on the length of the incubation period compared to that of the chicken, but we confirm the stage by candling the eggs.

We inject eggs with a geometric progression of Hg doses. These doses are calculated to produce concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, or 6.4 ppm Hg on a wet-weight basis in the contents of the egg (minus the weight of the shell). With the game farm species, and with some of the wild bird eggs, we have enough eggs to also have a group that is not injected with corn oil. These uninjected eggs serve to demonstrate what effects the control solution of corn oil without methyl-Hg might have on embryo survival. The Hg is in the form of methyl-Hg chloride. Few species of wild bird eggs receive the entire series of treatments, all the way from 0 to 6.4 ppm

Hg, because we do not have large enough sample sizes from the wild birds, and past studies with related species generally gives us an appropriate range to work within. To prepare the solutions of Hg, we dissolve the methyl-Hg chloride into corn oil, starting with a solution that produces 6.4 ppm Hg when 1 microliter of corn oil is injected per gram of egg contents. Then we make serial dilutions to achieve the lower concentrations.

When the embryos within a set of wild bird eggs reach the embryological equivalent of a 3-day-old chicken embryo, we remove the eggs from the incubator, swab the cap end with alcohol, and drill a small hole through the cap end of the eggshell, below which the air cell lies. The air cell is a small pocket of air created in the cap (blunt) end of a bird's egg by a natural separation that exists between the outer and inner shell membranes. The inner shell membrane separates the air cell from the wet contents of the egg. Solvents such as corn oil, when injected into the air cell, penetrate the thin inner shell membrane and carry the methyl-Hg into the albumen of the egg. We use solutions of Hg in corn oil that are warmed to a temperature similar to that of the egg and inject 1 microliter of solution per gram of egg contents into the air cell of the egg, using the Hg treatment to which that set of eggs has been randomly assigned. We then seal the holes with a hot glue gun and keep each set of injected eggs in the vertical position for one-half hour to allow the corn oil to spread over the surface of the inner shell membrane. After one-half hour, the eggs are returned to their sides and are placed back in the incubator.

At about 3-day intervals we candle the eggs to check for dead embryos. Eggs containing dead embryos are opened to determine the stage of embryonic development at which death occurred and to examine the embryos for deformities. When embryos die before about one week of age, it is very hard to examine them for deformities because they are small and generally are decomposed if dead for a day or two. About two days prior to the anticipated hatching day, we transfer the eggs to hatching trays in a separate incubator, where the eggs are not turned every hour. The temperature in the hatching incubator is set at about 37.2EC and at a relative humidity of about 70 to 80%. Records are kept on which eggs hatch or fail to hatch. Unhatched eggs are opened and the embryos examined for deformities.

A sample of 5 or 6 eggs from each species (usually cracked or infertile eggs that we salvaged for this purpose) are saved for Hg analysis. Our objective in analyzing a sample of eggs from each species is to verify that Hg concentrations in eggs from the field are low.

With the eggs of wild species of birds, hatching success in artificial incubators, even for control eggs, may be much less than 100%. It is difficult to get the incubator conditions set to mimic how the parents incubate their eggs; normally there is no information on the incubation conditions the parents use. Sometimes there is very good embryo survival of wild bird eggs up close to the time of hatching and then some unexplained mortality will occur in the last couple of days. One way to overcome this unavoidable, late mortality that is common even for controls, is to calculate embryo survival up to some point close to hatching, but not all the way to hatching. For statistical comparisons, we use embryo survival through 90% of the incubation period.

We have used our standardized protocol with the eggs of 15 species of wild birds. One of our goals is to see just how different in sensitivity various kinds of birds can be. When we know the statistical distribution of sensitivities for many wild species, we may be better able to estimate how different an untested species could conceivably be, but this estimation will require a large data base of eggs from tested species.

In addition to the ready availability of eggs from chickens, mallards, and pheasants, these three lab species have one other advantage. For all three species, controlled laboratory studies have been done in which breeding adults have been fed methyl-Hg and the reproductive success of the Hg-treated birds has been compared to the success of controls. With all three species, the concentration of Hg deposited in the egg by the female that is associated with reproductive problems is known. Therefore, one can compare the concentration of naturally deposited methyl-Hg that results in embryonic mortality with the concentration that must be injected into eggs to cause the same degree of mortality. By comparing these two concentrations in the three lab species, we will arrive at an adjustment factor (from lab injected Hg to naturally deposited Hg). This adjustment factor will be used to estimate the concentrations of naturally occurring Hg in the eggs of wild birds that will cause reproductive impairment.

We use a Fishers exact probability test to determine the lowest injected concentration of Hg that causes a statistically significant ($P<0.05$) decrease in embryo survival through 90% of the incubation period compared to controls. LC50s are calculated using appropriate models for dose response relations.

The egg injection procedure is ideally suited to studying complex interactions between Hg and selenium. Using unlimited supplies of mallard or chicken eggs we will arrange many different combinations of Hg and selenium doses, covering combinations that have been reported in eggs from the Bay-Delta area. The studies will have a control group in which only clean corn oil is injected into the eggs, groups injected only with varying doses of Hg, groups injected only with varying doses of selenium, and groups injected with different combinations of Hg plus selenium.

In our injection studies, in addition to measuring the effects of injected Hg, or Hg plus selenium, on embryo survival, we measure other effects. In feeding studies with breeding adult birds, methyl-Hg deposited in eggs is known to cause teratogenic effects in avian embryos (Heinz and Hoffman, 1998). We have observed the same array of deformities in embryos from eggs injected with methyl-Hg. Selenium also causes deformities in embryos (Ohlendorf et al., 1986; Hoffman and Heinz, 1988) Consequently, all hatched chicks and dead embryos will be examined for deformities. Methyl-Hg also causes changes in physiological biomarkers such as glutathione peroxidase, glucose-6-phosphate dehydrogenase and glutathione-S-transferase, and promotes oxidative stress with changes in glutathione and thiol status as well as lipid peroxidation of various tissues (Hoffman and Heinz, 1998; Henny et al., 2002). These changes have been mainly characterized in adult and juvenile birds but remain to be examined in embryos and hatchlings. We will measure these biomarkers in samples of chicks hatching from eggs treated with different doses of Hg and assess various tissues such as liver, brain and kidney, as well as nondestructive sampling of plasma and blood.

Feeding study - Our experimental design for the laboratory feeding study with mallards will be based on what we have learned from many other captive breeding studies we have successfully carried out at the Patuxent Wildlife Research Center over a period of 30 years. Individual pairs of adult mallards will be randomly assigned to outdoor breeding pens. Hg treatments will be randomly assigned to pens. Based on past studies we have done with mallards, we know that a diet of 0.5 ppm Hg, as methyl-Hg, on a dry-weight basis produces a mean of about 0.8 ppm Hg in eggs on a wet-weight basis and a small decrease in reproductive success (Heinz, 1979). The value of 0.8 ppm Hg in eggs on a wet-weight basis represents something very close to a reproductive

effects LOAEL for mallards. This LOAEL has been confirmed in a recent captive breeding study with mallards (Heinz and Hoffman, 2003). In another study, a diet of 3 ppm Hg on a wet-weight basis resulted in about 6 to 7 Hg in eggs and a more pronounced reduction in reproductive success (Heinz 1976). In a study with black ducks, also fed a diet containing 3 ppm Hg, Hg residues in eggs and reproductive effects were similar to those with mallards (Finley and Stendell, 1978). In a third study with mallards, a diet of 10 ppm Hg, dry-weight, resulted in 16 ppm Hg, wet-weight, in eggs and only about a fourth as many eggs hatching (Heinz and Hoffman, 1998).

Based on the findings of these previous studies, we will try to bracket reproductive effects ranging from a no effect treatment to close to a 100% reduction in reproductive success. Such results are likely when several dietary treatments of Hg are spread out over a range starting at about 0.25 ppm Hg on a dry-weight basis and ending at 12 to 16 ppm Hg. Controls will be fed only the solvent in which the Hg is dissolved. Diets will start at least one month prior to the start of egg laying and will continue until a female has laid about 20 eggs. One or more eggs from each female will be saved for Hg analysis. Endpoints will include percent fertility of eggs, percent hatch of fertile eggs, percent survival of hatchlings, teratogenic effects, and physiological biomarker effects. Data will be analyzed by analysis of variance to determine the no-effect and lowest-effect dietary concentrations of Hg. The results also will be fit to a model to predict degree of reproductive effect versus dietary concentration of Hg and concentration in eggs.

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