

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

*2005 Annual Report*



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## **A. MERCURY IN BIRDS OF THE SAN FRANCISCO BAY-DELTA: TROPHIC PATHWAYS, BIOACCUMULATION AND ECOTOXICOLOGICAL RISK TO AVIAN REPRODUCTION**

**By Steven E. Schwarzbach, Thomas H. Suchanek, Gary H. Heinz, Joshua T. Ackerman, Collin A. Eagles-Smith, Terrence L. Adelsbach, John Y. Takekawa, A. Keith Miles, David J. Hoffman, Susan E. Wainwright-De La Cruz, Sarah E. Spring, Mark A. Ricca, and Thomas C. Maurer**

### **B. INTRODUCTION TO THE PROJECT**

**Background:** The Bay-Delta watershed has a legacy of mercury (Hg) contamination from both Hg mining and gold extraction. This Hg contamination threatens 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 cited the need for information on Hg effects in birds as a requirement for adaptive restoration of the Bay-Delta ecosystem and recognized the sensitivity of avian reproduction to methylmercury (MeHg) (Wiener et al. 2003). 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. Assessing the ecotoxicological risk of Hg is hampered by an inadequate understanding of MeHg exposure among different foraging guilds of birds, and the lack of integration between field and laboratory approaches.

**Project Goal:** Our goal is to integrate a field assessment of Hg exposure and effects with a laboratory assessment of the variation in sensitivity of avian embryos to MeHg to better define the risks of Hg to birds. Our field approach will evaluate the hazard of Hg to three foraging guilds of aquatic birds with different potential risks. 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 improve interpretation of our field data, provide vital data on the variation in Hg sensitivity among avian species, and establish and refine MeHg dose-response relationships and threshold concentrations associated with avian embryo toxicity.

**Objectives:** Our research program has 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.

Objective III: laboratory investigations on the differential sensitivity of avian taxa to Hg and determine No Observed Adverse Effects Level (NOAEL). concentrations in mallards through the use of controlled laboratory feeding experiments.

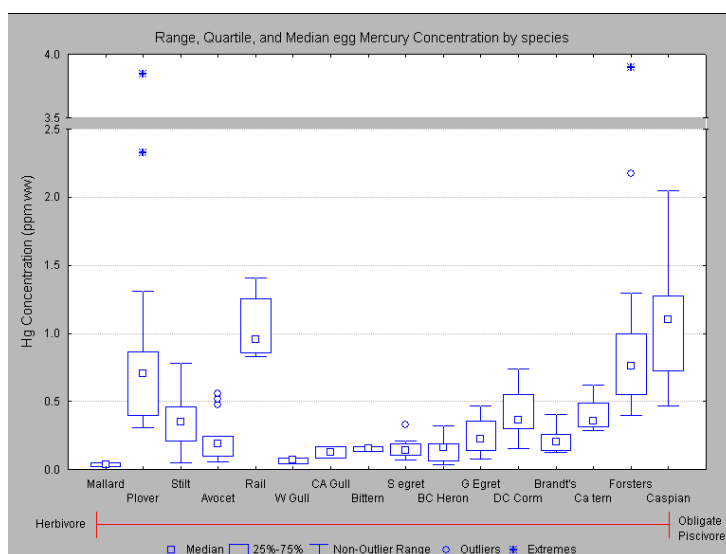


**The Guild Approach:** Estuarine waterbirds form distinct foraging guilds that are distinguished by their feeding method, diet preferences and habitat use. These guilds include (1) surface-feeding recurvirostrids (American avocet: *Recurvirostra americana*, and black-necked stilt: *Himantopus mexicanus*), (2) diving benthivores (surf scoter: *Melanitta perspicillata*), and (3) obligate piscivores (Caspian tern: *Sterna caspia*, and Forster's tern: *Sterna forsteri*). Each guild represents a unique foraging pathway within the Bay-Delta ecosystem for Hg bioaccumulation. The highest mean Hg concentrations in avian eggs are found in Caspian and Forster's terns, but surprisingly high concentrations are also found in stilts. Scoters wintering in the San Francisco Bay have highly elevated liver total Hg concentrations (see Figs. 1 and 8). Therefore, examining variation in major foraging guilds will provide a more comprehensive understanding of bioaccumulation processes related to avian use of habitats in all areas of the estuary.

### C. PROJECT TIMETABLE AND PROGRESS

CBDA approved the project for funding in December 2003 with planning and fieldwork in 2004 with cost-share support. By September 2005, we successfully completed two winter collections for scoters and our first year of breeding fieldwork. We have already produced several products including a scientific journal article, 3 general articles, and 11 presentations. We are now conducting wintering work and are preparing for the next breeding season which will run through September 2006. Our target completion date for the final field season is September 2007. We anticipate that the final report will be submitted in 2008.

We are currently on schedule to complete all project objectives. However, we are modifying a few tasks on the basis of results from initial fieldwork. Objective I: We found sample sizes and access insufficient for telemetry on two guilds in the North Bay (Task I.1, I.2). Instead, we will increase numbers in other study areas. Also, we were issued a restricted federal permit on Caspian terns (see Results), precluding telemetry. Thus, we increased efforts on Forster's terns. Objective II: We found sample sizes insufficient for full breeding studies in the North Bay (Task II.1, II.2). Instead, we increased breeding samples in other areas so that we will obtain suitable data in two field seasons. The restricted federal permit precluded Caspian tern breeding studies, so we focused more on Forster's terns and recurvirostrids. Objective III: The timing has changed because we had poor hatching success of recurvirostrid eggs for the egg injection study (likely due to rough courier shipment). Thus, we will collect new eggs in 2006 and hand deliver them to the incubation facility.



**Figure 1. Total Hg concentrations in waterbird eggs from the San Francisco Bay Estuary.**

## D. PROJECT HIGHLIGHTS AND RESULTS

Our initial fieldwork was completed in September 2005, and we are in the process of submitting samples for analysis or receiving a few initial results. Below, we discuss our preliminary results and the early study implications.

### 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 are identifying species differences in trophic pathways of MeHg exposure in the three guilds of aquatic birds.*

**Task I.1-** *Identify trophic pathways of MeHg exposure in surface feeding recurvirostrids (avocets and stilts) through the use of diet analysis, stable isotopes and identification of foraging sites by telemetry.*

We captured 95 avocet and 46 stilt adults from 4 March to 27 May 2005. Ninety-four of the 95 avocets and 30 of the 46 stilts were captured in the South Bay, due to a limited number of breeding recurvirostrids in the North Bay (Fig. 2). From each bird, we collected blood and feathers for Hg and stable isotope analyses. We marked 1 male avocet in the North Bay and 48 female avocets in the South Bay. We radio-marked 16 stilts (4 male and 12 female) in the North Bay and 28 stilts (15 male and 13 female) in the South Bay. Birds were radio-tracked daily from truck-mounted telemetry systems and weekly by aircraft. In total, we collected 1,569 avocet and 1,925 stilt telemetry locations.

We estimated fixed Kernel home ranges and 50% utilization distributions (hereafter core areas) during the pre-breeding time period (e.g. Fig. 3). We then randomly selected data points within each bird's core area to sample for their invertebrate prey. We used a combination of sweep nets and light traps at 3-4 randomly selected locations per bird to sample invertebrate prey. We also collected invertebrate density samples using a standardized sweep in each of the locations where diet samples were collected. Common species in our samples included Corixidae (water boatmen), *Artemia* (brine shrimp), *Mysis* (shrimp) and occasionally *Corophium* (amphipod).

We collected and salvaged pre-breeding avocets (39) and stilts (24), from 3 March through 4 April 2005. Twenty-seven of the 39 avocets and 9



Figure 2. Map of San Francisco Bay Estuary indicating the main study sites.

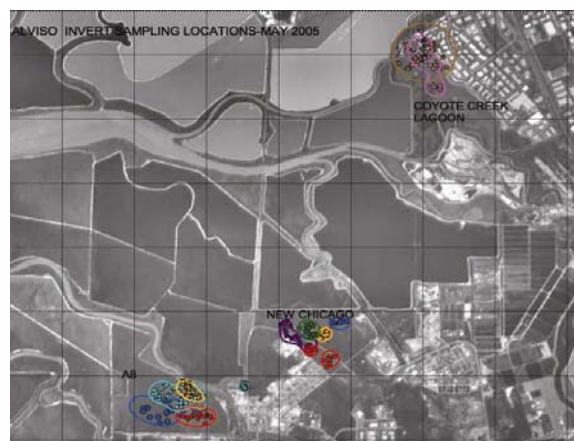


Figure 3. Telemetry locations and core areas of avocet and stilt home ranges in the South Bay.

of the 24 stilts were collected in the South Bay. During the breeding period, we collected 11 avocets and 17 stilts on their nests at 9-12 days incubation. We also collected their full clutches for quantifying maternal contaminant transfer and intra-clutch Hg variability. From each collected bird we sampled whole blood, blood plasma, red blood cells (RBC), muscle, liver, kidney and feathers for contaminant and stable isotope analyses (SIA), and gastrointestinal (GI) tracts for diet analysis. Analyses per tissue include the following: whole blood (THg, MeHg, SIA), plasma (THg, MeHg, SIA), RBC (SIA), muscle (THg, SIA), liver (THg, MeHg, Se, OCs), kidney (THg, MeHg), feathers (THg, SIA). Carcasses are also being archived for body condition proximate analysis pending additional funding. Tissues were flash frozen on dry ice upon collection and subsequently stored at -20°C. We are currently processing samples for laboratory analyses.

We analyzed GI tracts from 30 collected birds after they were observed actively foraging. Two-thirds of the GI tracts contained prey, and the most abundant prey item was Corixidae, followed by *Corophium*, gastropods, bivalves, Chironomidae, tiger beetles, and polychaetes.

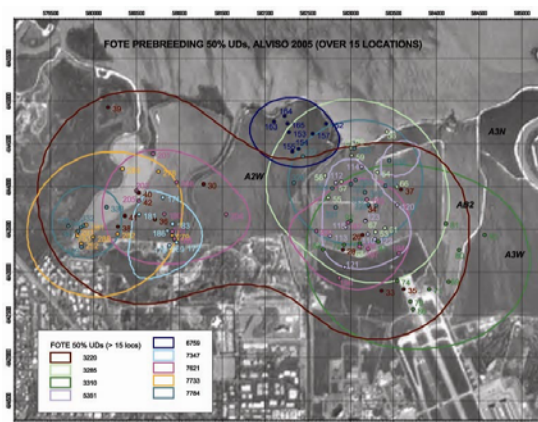
**Task I.2 - Identify trophic pathways of MeHg exposure in obligate fish-eating birds (terns) through the use of diet analysis, stable isotopes and identification of foraging sites by telemetry.**

We captured and radio-marked 50 Forster's tern adults from 12 April to 25 May 2005. We radio-marked 12 terns (7 male and 5 female) in the North Bay and 38 terns (19 male and 18 female) in the South Bay. From each tern, we collected blood and feathers for Hg and SI analyses and sexed them using DNA. Terns were radio-tracked daily from truck-mounted telemetry systems and weekly by aircraft. In total, we collected 1,398 Forster's tern telemetry locations. We estimated fixed Kernel home ranges and 50% core areas during the pre-breeding period (Fig. 4) and randomly selected data points within each bird's core area to sample for their fish prey.

We collected fish from 76 locations in 12 sites between 25 March and 16 June 2005 in the North Bay and 60 locations in 15 sites between 3 May and 16 July 2005 in the South Bay. Fish collected include topsmelt, jacksmelt, shiner perch, inland silverside, longjaw mudsucker, yellowfin goby, bay goby, staghorn sculpin, Pacific sardine, northern anchovy, Pacific herring, striped bass, Sacramento splittail, speckled sandab, and starry flounder. Each species of fish will be divided into appropriate size classes and analyzed for THg and SI.

We collected 20 pre-breeding Forster's terns, 10 each from the North and South Bay regions, and 28 (16 South Bay, 12 North Bay) breeding Forster's terns on their nests at 9-12 days incubation. We also collected their full clutches for quantifying maternal contaminant transfer and intra-clutch Hg variability. In addition to the tissues and analyses identified in Task I.1 we also sampled liver, kidney, and brain for analysis of oxidative stress enzymes (e.g. P450) and histopathology. The GI tracts of each bird were removed, fixed in 10% buffered formalin, and preserved in 70% ethanol. Diet items will be removed, identified to the lowest possible taxa, measured, dried, and weighed.

The first analyses from the lab have been total Hg (THg) concentrations in Forster's tern



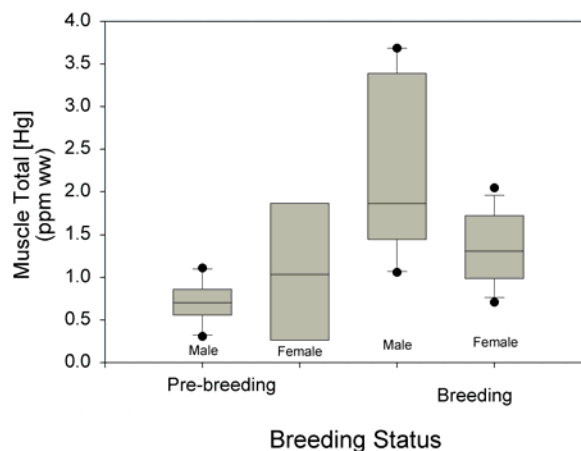
**Figure 4. Telemetry locations and core areas of Forster's tern home ranges in South Bay.**



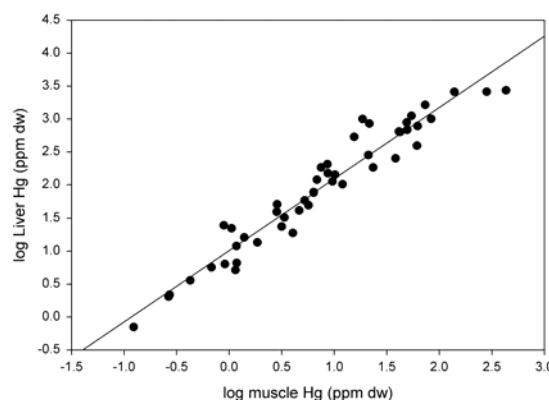
muscle. Mean THg concentrations in all collected adult Forster's tern muscle was 1.35 ppm wet weight (N=42). During the pre-breeding season, we found no difference in Hg between sexes (ANOVA:  $F_{1,18}=0.05$ ,  $P=0.83$ ; Fig. 5) or regions (ANOVA:  $F_{1,18}=0.01$ ,  $P=0.94$ ). However, during the breeding season, we found significant differences in Hg between sexes (ANOVA:  $F_{1,22}=7.33$ ,  $P=0.01$ ; Fig. 5) but not regions (ANOVA:  $F_{1,22}=0.90$ ,  $P=0.35$ ). Moreover, THg concentrations were significantly greater during the breeding season than during the pre-breeding season (ANOVA:  $F_{1,41}=22.87$ ,  $P=0.01$ ; Fig. 5). We also found that THg concentrations in liver were highly correlated to THg concentrations in muscle ( $R^2=0.94$ ,  $P=0.001$ ,  $N=47$ ; Fig. 6), indicating that both tissues are reflective of current body burden and dietary exposures.

Our preliminary data indicate that THg concentrations in tern muscle increase from the pre-breeding to breeding season, more so in males than females, suggesting that Hg bioaccumulation occurs rapidly in the San Francisco Bay Estuary (Fig. 5). The fact that male muscle THg concentration increases significantly more than female muscle THg over a 6 week time period indicates that females may be able to deposit Hg in eggs (males increased 1.51 ppm, whereas females increased only 0.32 ppm). Our results also indicate that as expected, Caspian tern muscle Hg is higher than that in Forster's terns (Fig. 7). This is likely because Caspian terns generally forage on larger fish than Forster's terns.

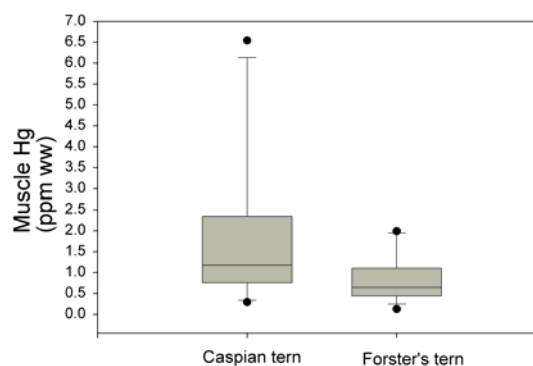
We were unable to reach agreement under our federal permit to capture and mark Caspian terns during the pre-breeding time period. After repeated requests, we were finally granted permission to capture terns during late August when chicks were >7 days old. At that time, we captured 16 adult Caspian terns, collected blood and feathers, and radio-marked 3 birds to conduct a pilot study in preparation for 2006. We were not allowed to collect Caspian terns in



**Figure 5.** Total Hg concentrations in pre-breeding Caspian tern and Forster's tern muscle.



**Figure 6.** Relationship between total Hg concentrations in Forster's tern liver and muscle.



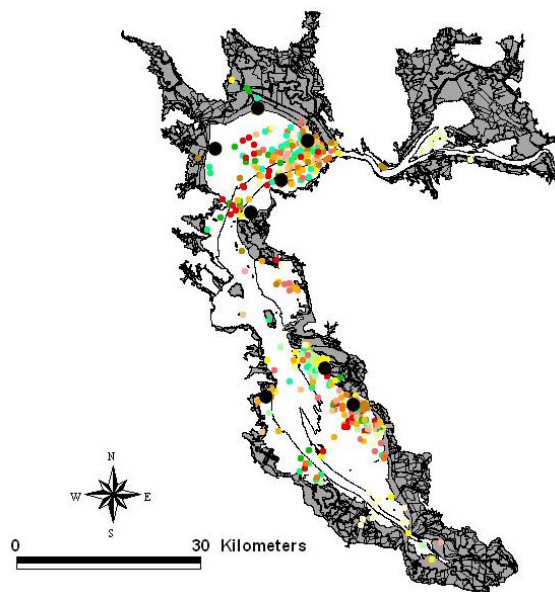
**Figure 7.** Total Hg concentrations in Forster's tern muscle during the pre-breeding and breeding seasons for each sex.



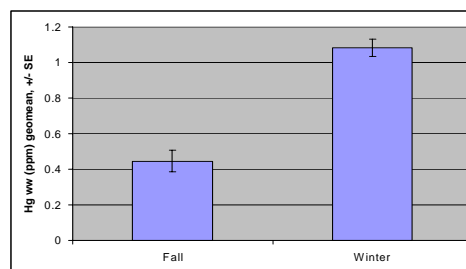
the South Bay, and were only permitted 10 pre-breeding collections in the North Bay. We adjusted our efforts to focus on Forster's terns.

**Task I.3 - Identify trophic pathways of MeHg exposure in benthivores (surf scoters) through the analysis of invertebrate prey, stable isotopes, and identification of foraging sites by telemetry.**

We used net guns to capture surf scoters in San Pablo Bay and the Central Bay (near San Leandro) in winter of 2004 and 2005 in coordination with USGS coastal ecosystem studies. During the wintering period, we captured 175 scoters and marked 160 with either very high frequency (VHF, N=149) or satellite platform terminal transmitters (PTT, N=11). We collected blood and feathers from each bird for Hg and SI analyses. VHF-marked scoters were tracked daily from 3 November to 15 April using truck-mounted null-peak telemetry systems and by aircraft once every two weeks. We obtained 3,152 telemetry locations on scoters, including nearly 1,500 foraging locations determined by transmitter attenuation during dives. These data were used to calculate both early (Nov to mid-Jan) and late (mid-Jan to March) fixed Kernel home ranges for each bird. Invertebrate prey items were collected from randomly chosen locations in areas where several foraging core areas overlapped (Fig. 8).



**Figure 8. San Francisco Estuary telemetry locations (colored circles) and prey samples (black circles) of**



**Figure 9. Total Hg in livers of surf scoters in North and Central Bay, early and late winter 2004.**

We collected 159 foraging scoters in the winter in three time periods (early: Nov-Dec, mid: Jan-Feb, and late: Mar-Apr) and three sub-bays (Suisun, San Pablo, and Central Bays). We processed birds and archived liver, kidney, breast muscle, whole blood, plasma, and feathers for THg, MeHg, Se, and SI analyses, and we removed GI tracts. At each scoter collection site, we used an Eckman dredge and collected eight benthic grab samples to determine prey composition and for THg, MeHg, Se, and SI analyses.

Scoter tissues and prey items are currently being analyzed for THg and MeHg. Preliminary analyses indicated an increase in liver THg between early (Fall) and late winter (Fig. 9;  $t_{38} = -3.62$ ,  $P = 0.01$ ), with greater differences in the North Bay ( $t_{14} = 2.33$ ,  $P = 0.01$ ). Our results suggest that despite efforts to regulate contaminant sources, migratory birds such as scoters continue to increase their THg loads significantly while wintering in San Francisco Bay.

**Objective II. Field Studies of Hg Effects on Bird Reproduction**

*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 contaminants of concern, to reproductive failure.*

**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.*

We are presenting Tasks II.1 and II.2 together for easier interpretation.

Nesting Studies --- We monitored recurvirostrid and tern nests weekly at several sites in the North and South Bay (Table 1). Overall, nesting densities were very low in the North Bay, and those birds that did nest in the North Bay had poor nesting success due to depredation. Therefore, we concentrated most of our efforts in the South Bay and, during the next field season (2006), we will concentrate all our nest monitoring efforts in the South Bay. In total, we monitored 419 avocet, 168 stilt, and 581 Forster's tern nests; we were not permitted to monitor Caspian tern nests.

Among recurvirostrids, nest success was highest for avocets nesting on islands in Alviso salt pond A16 (86%) where terrestrial predators had limited access. Conversely, nest success was much lower in Alviso salt pond A8 (35%) where California gulls depredated many nests. Stilts, on the other hand, experienced moderate nest success within Alviso's New Chicago Marsh (48%) where emergent vegetation protected nests from aerial predators but not terrestrial predators (Fig. 10). Forster's terns experienced higher nest success than recurvirostrids at each site (range: 57%-94%) due to their habit of nesting on islands with limited access by terrestrial predators and nesting colonially which can deter aerial predators. The number of eggs hatching in a successful nest (hereafter hatching success) ranged between 81-85% for avocets, 88% for stilts, and 78-83% for Forster's terns.



**Figure 10.** *Hatching stilt nest in New Chicago Marsh.*

**Table 1.** *Total number of nests found and nest success by site and species.*

Site	Avocet		Stilt		Forster's Tern	
	Nests Monitored	Nest Success	Nests Monitored	Nest Success	Nests Monitored	Nest Success
<b>North Bay</b>						
Rush Creek	2	---	24	3%	0	---
Pond 4	13	5%	0	---	0	---
Figeras Tract	0	---	11	67%	0	---
Pond 2	0	---	0	---	136	57%
Eden Landing	24	33%	32	44%	21	62%
<b>South Bay</b>						
A16	164	86%	3	---	168	94%
A8	188	35%	0	---	115	73%
New Chicago	28	33%	98	48%	17	63%
A1	11	60%	0	---	124	94%
<b>Total</b>	<b>430</b>		<b>168</b>		<b>581</b>	

We collected eggs from avocet, stilt, and Forster's tern nests. These collections included both failed to hatch and random, viable eggs. Collected eggs will be analyzed for Hg, Se, SI, and OCs, and comparisons will be made between viable eggs and failed to hatch eggs. We also collected one egg randomly from several clutches at 9-12 days in incubation, and then the remaining eggs were followed for hatching success. Hg levels in the collected eggs will act as a surrogate for Hg levels in the remaining eggs; we will examine whether Hg levels in eggs influences hatching success in the wild, to complement our lab egg injection studies. We also collected entire clutches to examine intra-clutch variation in Hg levels among eggs to make sure collected eggs are appropriate surrogate eggs. Hg analyses are currently being conducted and results are not yet available.

**Recurvirostrid Chick Studies** --- We captured 74 avocet and 33 stilt chicks within a few days of hatching and attached radio transmitters containing thermistor switches (Fig. 11). We weighed and measured chicks and collected downy feathers from the rump and mantle for Hg analysis. We located chicks daily from the time of radio attachment until their fate (depredated, dead, or fledged) was determined and we collected 471 avocet and 289 stilt chick locations. If the thermistor switch indicated the chick had died, we immediately used hand-held Yagi antenna systems and receivers to enter the marsh and find the transmitter, chick, and identify the cause of mortality. Locations and fates of chicks are displayed for the South Bay (Fig. 12).

Survival estimates to 21 days after hatching were 13.9% ( $\pm 4.2\%$  SE) for avocet chicks and 31.7% ( $\pm 10.1\%$  SE) for stilt chicks (Fig. 13). The odds of mortality were 2.52 (1.45-4.39) times greater for avocet chicks than for stilt chicks (Cox's Proportional Hazards Model:  $N=107$ ,  $X^2=10.67$ ,  $P=0.001$ ). For both species, the likelihood of mortality decreased with residual chick mass (Cox's Proportional Hazards Model:  $N=107$ ,  $X^2=6.18$ ,  $P=0.01$ ); chicks were 1.16 (1.03-1.31) times more likely to survive with each 1-g increase in residual body mass at hatching. Julian hatching date did not influence the likelihood of survival for either species (Cox's Proportional Hazards Model:  $N=107$ ,  $X^2=1.81$ ,  $P=0.18$ ).

We are currently in the process of analyzing chick feathers for Hg levels and, in the future, we will relate individual survival rates to their Hg levels. Other than contaminants, the main cause of avocet chick mortality was due to predators; in fact, 15 radio transmitters were found within the A6



Figure 11. Radio-marked avocet chick.



Figure 12. Telemetry locations and fates of avocet chicks in the South Bay.

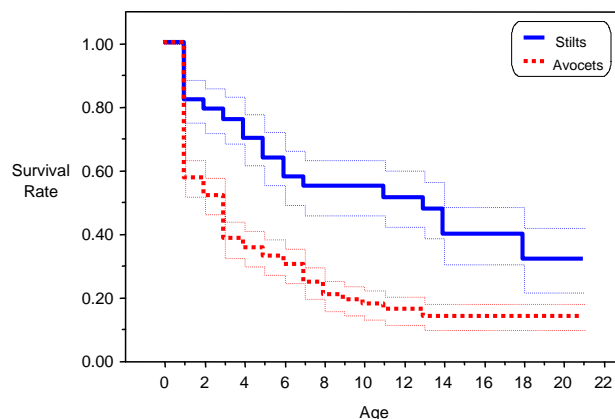
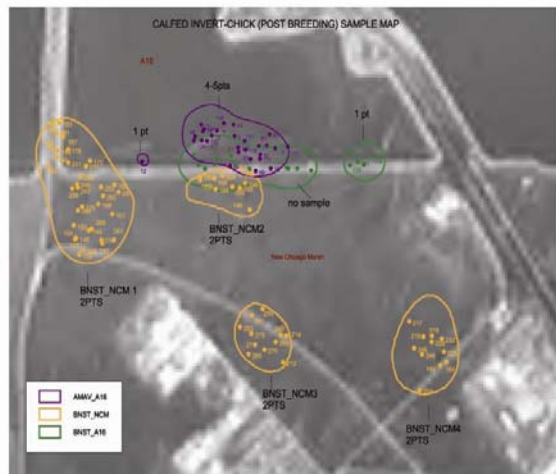


Figure 13. Survival rates of avocet and stilt chicks through 21 days post hatch estimated using telemetry.

California gull colony (currently estimated at >17,000 gulls; C. Strong, unpublished data) indicating that depredated chicks had been carried there by gulls. In contrast, no stilt chicks were depredated by gulls. Because most chicks were depredated within 7 days of hatching, we were not able to map individual home ranges of chicks as we had done for adults. Instead, we examined population ranges for chicks within each pond and used population core areas for sampling invertebrate prey of chicks. Similar to adult telemetry, we randomly selected individual tracking data points within the population core area to sample invertebrates (e.g. Fig.



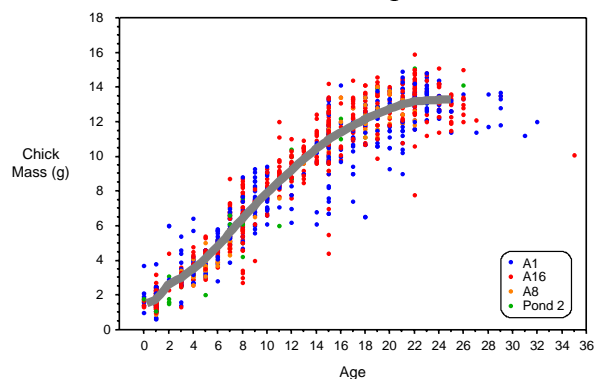
**Figure 14.** Population core areas of avocet and stilt chicks used to sample invertebrate prey in the South Bay.

an indicator of Hg accumulation after hatching. We are currently in the process of analyzing these feathers for Hg levels and we will relate individual growth rates to Hg levels in the future. In total, we captured 1,290 Forster's tern and 12 Caspian tern chicks (including recaptures). We used 4 indices of chick growth rate: 1) mass (Fig. 15), and 2) wing, 3) culmen, and 4) tarsus length. We also collected chicks just prior to fledging at  $24.2 \pm 4.2$  days old. We immediately processed chicks for future analyses of Hg in blood, feathers, muscle, liver, and kidney. Here we present only muscle data as the other Hg analyses are in progress.

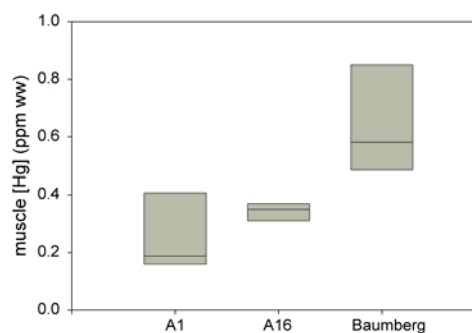
We found significant differences in chick muscle Hg levels among tern colonies (ANOVA:  $F_{2,10}=8.04$ ,  $P=0.01$ ; Fig. 16). We found no overall difference in growth rates among colonies (MANCOVA: colony:  $\lambda=0.92$ ,  $F_{9,253}=1.0$ ,  $P=0.40$ ; date:  $\lambda=0.94$ ,  $F_{3,104}=2.4$ ,  $P=0.07$ ; colony x date:  $\lambda=0.92$ ,  $F_{9,253}=1.1$ ,  $P=0.40$ ), despite significant differences in hatching dates among ponds (ANOVA:  $F_{3,1280}=509.0$ ,

14). We sampled chick invertebrate prey in a similar fashion to adult birds (see Task I.1).

**Tern Chick Studies** --- We used mark-recapture methodology to determine growth rates and survival of tern chicks in relation to Hg contamination levels at four Forster's tern and one Caspian tern colony. We captured and released Forester's tern chicks weekly at A16 (South Bay) and Pond 2 (North Bay), and every two weeks at A8 and A1 (both South Bay). We captured and released Caspian tern chicks during only 2 visits to the A7 (South Bay) tern colony due to permit restrictions. We individually marked, measured and weighed each chick, and collected downy feathers from the rump and mantle for Hg analysis to indicate egg Hg concentrations. We also collected breast feathers from recaptured chicks as



**Figure 15.** Forster's tern chick mass growth in relation to age (days) at each colony.



**Figure 16.** Total Hg levels in Forster's tern chick breast muscle from three different colonies in the San Francisco Bay.



$P < 0.001$ ; Fig. 17). However, at the individual level, wing growth rates of chicks declined with increasing concentrations of muscle Hg ( $R^2 = 0.71$ ,  $N = 6$ ,  $P = 0.12$ ; Fig. 18), but mass and culmen growth rates were unrelated to Hg levels (all  $P > 0.8$ ). These preliminary results underscore the importance of examining effects of Hg at the individual level. In the future, we will use our mark-recapture dataset of 680 tern chicks to relate individual growth rates to Hg concentrations in feathers. This larger dataset will help elucidate the relationship between growth and Hg levels.

**Task II.3** – Evaluate the reproductive success, adult body condition, and migration of diving benthivores that over-winter in the estuary using satellite telemetry and stable isotopes.

We followed the spring migration of 10 PTT-marked female scoters (see Task I.3 for methods) from San Francisco Bay to their breeding areas in the Northwest Territories of Canada. The mean departure date of PTT-marked scoters from San Francisco Bay was 15 April 2005. Figures 19a and b each depict individual scoter movements indicating the major spring migration routes. Overall, 80% of scoters used the route depicted in Figure 19a and 20% followed route 19b. Since February 2005, we have maintained an interactive web-page showing migration and breeding ground locations of satellite-marked scoters (updated weekly). The public can track scoters and get information on the web (<http://www.werc.usgs.gov/scoter/2005>).

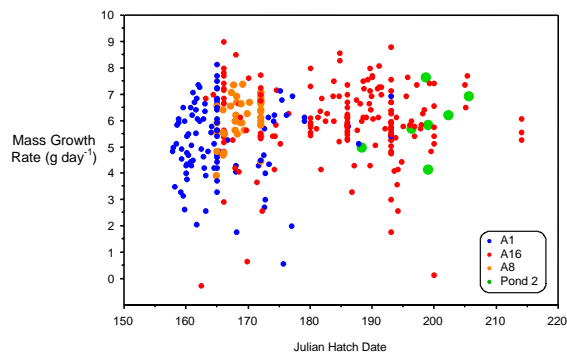


Figure 17. Forster's tern mass growth rates in relation to Julian hatch date for each colony.

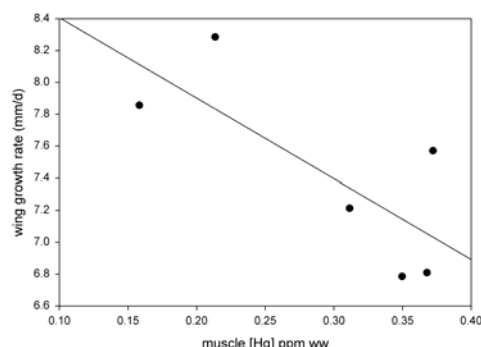


Figure 18. Relationship between wing growth rate and breast muscle Hg levels in Forster's tern chicks.

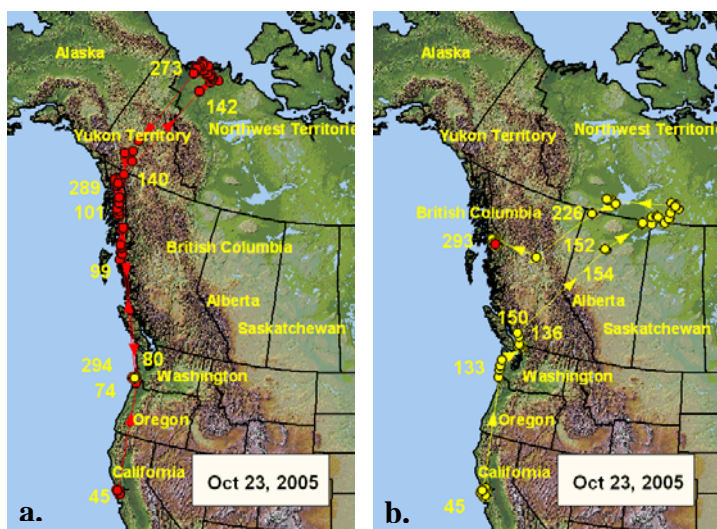


Figure 19 (a) PTT-marked scoter migrated from San Francisco Bay through Southeast Alaska and the Yukon Territory to the breeding area in the Northwest Territories, Canada. (b) PTT-marked scoter migrated through Puget Sound, WA and the Strait of Georgia, British Columbia. (c) Nest of PTT-marked San Francisco Bay scoter found in the NWT, Canada. Numbers indicate Julian date.

For the cross-seasonal component of our project, we conducted fieldwork in the Northwest Territories (NWT), Canada, from 25 May – 15 August 2005. We located the nest of one PTT-marked female and collected two eggs (Fig. 19c). We froze albumen and yolk of each egg separately for THg, MeHg, SI, and other COC analyses. We also used locations of PTT-marked birds to search for nests of VHF-marked scoters. Several VHF-marked females were located and appeared to be nesting; however, subsequent trips to find nests revealed that these birds had moved from their original positions. We suspect that their nests had been depredated, particularly since high nest depredation rates were noted by other researchers in the NWT (S. Slattery, Ducks Unlimited Canada). Despite the low reproductive output this year, we were successful at locating VHF-marked birds and found breeding ground concentrations to narrow searches for the coming two field seasons.

### **Objective III. Laboratory Studies of Hg Effects on Birds**

*Establish and refine dose-response relationships and threshold concentrations of MeHg 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.*

Our objective in this task is to compare the sensitivities of the embryos of different species of birds to MeHg. To accomplish this comparison we are using egg injections as the means of depositing graded concentrations of Hg into the eggs. In brief, cooperators collect eggs from the wild and we inject them with graded doses of MeHg dissolved in corn oil. The eggs of all species are injected at an embryonic stage equivalent to a 3-day-old chicken embryo. Eggs are randomized into various treatment groups. We try to have a minimum of 10 eggs per treatment, with one group receiving the injection solvent, corn oil, without any added MeHg, and other groups receiving different concentrations of Hg in the corn oil. When we have enough eggs, we also have a group that does not even receive the pure corn oil. We inject 1  $\mu$ l of corn oil per gram of egg contents. We swab the cap (blunt) end of the egg with alcohol, drill a hole in the cap with a rotary drill, and inject the corn oil into the air cell of the egg. The injected eggs are placed on their sides in an incubator set at 37.5°C and are rolled through 180 degrees of turn each hour. The relative humidity is adjusted throughout incubation so as to produce an average weight loss in eggs up until the time of pipping of about 16%. At least twice a week we candle all eggs to follow embryo mortality. Dead eggs are removed from the incubator and opened to examine the embryos for deformities. Any unhatched eggs are examined for deformities. Based on the appearance of the embryos we can estimate how close they came to hatching. Because even artificially incubated control eggs of wild birds generally survive well up to the time they are placed in the hatching unit, but often do not hatch as well as when incubated by the parents, the use of hatching success as the measure of the toxic effects of MeHg weakens statistical tests. Therefore, we calculate the number of embryos that survived through 90% of the incubation period and use this as our measure of survival. Survival of controls through 90% of incubation can then be compared to that of the Hg-treated groups by a test such as the Fisher's exact probability test, and dose response curves and LC50s also can be calculated and compared among different species.

Thus far, we have injected the eggs of 23 species of birds, including Pelecaniformes (3 species), Ciconiiformes (4), Charadriiformes (4), Galliformes (2), Gruiformes (2), Anseriformes (4), Passeriformes (2), and Falconiformes (2). One of the objectives in testing different species from many different orders of birds is to develop a database that reveals how different the

embryos of various species of birds can be in their sensitivity to MeHg. Data analysis is currently in various stages for each of these species. With some species, either sample sizes are small or hatching success of controls was poor. These include royal tern, Caspian tern, hooded merganser, and osprey. With these species, additional egg collections will be necessary for the species to yield satisfactory results. In addition, we injected the eggs of stilts and avocets, but had very poor survival of embryos, even among the controls. The embryos looked healthy upon arrival from California, but died after a few days in the incubator. We are not certain why the embryos died, but one cause could have been rough handling during shipping. As a precaution, next spring (2006) we are considering having someone hand-carry the eggs on board a plane from California to Maryland. This would insure safer handling. Next year, in addition to testing more stilt and avocet eggs, we plan to inject the eggs of at least one species of tern. By completing MeHg injections of the eggs of a diving duck species (lesser scaup), two species of recurvirostrids (avocet and stilt), and a tern species, we will have accomplished a major goal of the laboratory work, which was to compare the toxicity of MeHg to species in the same three feeding guilds as are being studied in the field.

The importance of the egg injection research lies in the fact that nothing is known about the sensitivity of most bird embryos to MeHg, and the egg injection procedure provides a cost effective means of acquiring such data. For decades, data from mallard feeding studies have been used as a surrogate for other species that have never been tested in controlled laboratory studies. The toxic threshold of Hg in mallard eggs has been used as a default for what similar concentrations of Hg might do to the embryos of other birds. However, until now there has been no good way to compare the sensitivity of mallard embryos with the sensitivity of the embryos of other birds. As an example of our egg injection findings, in Figure 20 we compare the survival of mallard and white ibis embryos to the same doses of MeHg. With the ibis eggs, survival of embryos was decreased by as little as 0.1 ppm Hg, whereas with mallards the same level of harm did not occur until eggs were injected with 1.6 ppm Hg.

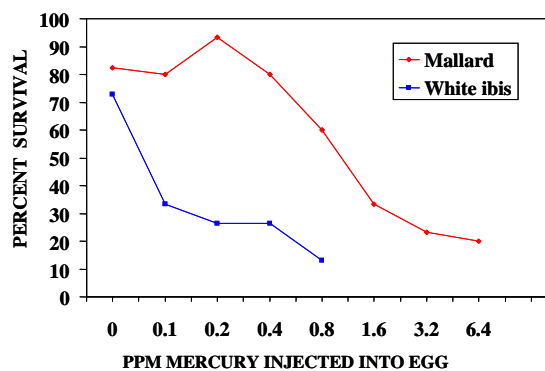


Figure 20. A comparison of the toxicity of methyl-Hg injected into mallard and white ibis eggs.

**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.

This task will begin in 2006.

**Task III.3** – Conduct a controlled feeding study using mallard ducks 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.

This task will begin in 2007.

## **E. POTENTIAL MANAGEMENT IMPLICATIONS OF FINDINGS TO DATE**

Initial results suggest that our study design is elucidating the biological processes creating Hg bioaccumulation in avian species of the estuary. To identify trophic pathways leading to Hg accumulation in eggs, we completed some of the first pre-breeding studies ever conducted in waterbirds, documenting habitat use by these guilds. Combined with collections and contaminant analyses, these results should indicate not only where, but when species are most likely to accumulate Hg. For example, total Hg increased in the few weeks separating the pre-breeding and breeding seasons for Forster's terns, indicating it most likely comes from estuary sources. Similarly, despite their potential for wide-ranging movements, our results suggest that birds have enough site fidelity to show variation in Hg accumulation by regions of the estuary. Our studies of fish and invertebrate prey in home ranges are providing a much clearer understanding of linkages from bioaccumulation in birds to their environment. Although avian productivity is dominated by predation effects, our approach to examining eggs that survive and chick growth rates should reveal any significant abnormalities. Habitat restoration or remediation projects may need to include an analysis of the potential for bioaccumulation by different guilds, based upon results from this study. Also, for the large numbers of migratory birds that only use the estuary in the winter, our studies with satellite-marked scoters should indicate the degree to which contamination is carried to remote breeding areas. With greater understanding of these processes, we should be able to better predict likely Hg risks to biota in many regions of the estuary.

## **F. PRODUCTS TO DATE**

### **Journal Articles**

Heinz, GH, DJ Hoffman, SL Kondrad, and CA Erwin. 2005. Factors affecting the toxicity of methylmercury injected into eggs. *Archives of Environmental Contamination and Toxicology* (in press).

### **Popular Articles**

Ackerman, JT, CM Marn, and JY Takekawa. 2005. Life and death on a salt pond: avocets and stilts survive amidst mercury pollution and invasive gulls. *Tideline*, in press.

Takekawa, J. Y. 2005. Finding a needle in a big haystack – locating surf scoter nests in the Northern Boreal Forest. *Sound Waves* 75: 1-2.

Wainwright-De La Cruz, S.E. and J.Y. Takekawa. 2005. Scooping for scoters on San Francisco Bay. *Tideline*, Spring Issue.

### **Reports**

Schwarzbach, SE, TH Suchanek, GH Heinz, JT Ackerman, CA Eagles-Smith, TL Adelsbach, JY Takekawa, AK Miles, DJ Hoffman, SE Wainwright-De La Cruz, SE Spring, and T Maurer. 2005. Mercury in birds of the San Francisco Bay-Delta: trophic pathways, bioaccumulation and ecotoxicological risk to avian reproduction. Unpublished 2005 annual report, U. S. Geological Survey, Western Ecological Research Center, and U. S. Fish and Wildlife Service, Environmental Contaminants Division, Sacramento, CA 17 pp.

### **Presentations**

Ackerman, JT, and JY Takekawa. 2005. Impacts of gulls: predation on shorebird and tern nests and chicks. South Bay Salt Pond Restoration Project, Bird Workshop 3: Invasive and Nuisance Species, Don Edwards San Francisco Bay National Wildlife Refuge, California, November 18. (talk)

Ackerman, JT, JY Takekawa, and C Marn. 2005. Survival of American avocet and black-necked stilt chicks in the South San Francisco Bay: variable risks of gull predation. The 7th Biennial State of the Estuary Conference, Oakland, California, October 4–6. (poster)

Ackerman, JT, TL Adelsbach, and CA Eagles-Smith. 2005. Growth rates of Forster's tern chicks at four nesting colonies in the San Francisco Bay. The 7th Biennial State of the Estuary Conference,



- Oakland, California, October 4–6. (poster)
- Bluso, JB, M Colwell, JY Takekawa, and JT Ackerman. 2005. Space use of foraging Forster's terns in South San Francisco Bay, California. The 7th Biennial State of the Estuary Conference, Oakland, California, October 4–6. (poster)
- Demers, SA, M Colwell, JY Takekawa, and JT Ackerman. 2005. Space use of breeding female American avocets in the San Francisco Bay Estuary. The 7th Biennial State of the Estuary Conference, Oakland, California, October 4–6. (poster)
- Eagles-Smith, CA, TL Adelsbach, JT Ackerman, SE Schwarzbach, TH Suchanek, GH Heinz, JY Takekawa, AK Miles, SE Wainwright-De La Cruz, JD Henderson, SE Spring, L Bowen, JD Bluso, SA Demers, M Ricca, C Marn, C Strong, N Warnock, M Wilson, and T Maurer. 2005. Mercury in birds of the San Francisco Bay-Delta: trophic pathways, bioaccumulation and ecotoxicological risk to avian reproduction. The 7th Biennial State of the Estuary Conference, Oakland, California, October 4–6. (poster)
- Heinz, GH, and DJ Hoffman. 2005. Mercury and birds: Future directions in research. The Wildlife Society 12th Annual Conference, Madison, Wisconsin, September 25-29. (talk)
- Heinz, GH, and DJ Hoffman. 2005. Species differences in sensitivity of birds to methylmercury. Society of Environmental Toxicology and Chemistry 26th Annual Meeting, Baltimore, Maryland, November 13-17. (poster)
- Heinz, GH, and DJ Hoffman. 2005. The use of wild bird eggs to measure the sensitivity of avian embryos to methylmercury. Joint Meeting of the Wilson Ornithological Society and Association of Field Ornithologists, Beltsville, Maryland, April 21–24. (talk)
- Schwarzbach, SE, JT Ackerman, CA Eagles-Smith, TL Adelsbach, TH Suchanek, GH Heinz, JY Takekawa, AK Miles, DJ Hoffman, SE Wainwright-De La Cruz, SE Spring, and T Maurer. 2005. Mercury in birds of the San Francisco Bay-Delta: trophic pathways, bioaccumulation and ecotoxicological risk to avian reproduction. CALFED review panel and California Bay-Delta Authority, Sacramento, California, November 29-December 1. (talk)
- Wainwright-De La Cruz, SE, M Wilson, JY Takekawa, D Nysewander, J Evenson, D. Esler, S. Boyd, D Rosenburg, D Ward, and J Eadie. 2005. Spring migration chronology and breeding areas of surf scoters: A synthesis of Pacific Coast Population Studies, Sea Duck Joint Venture Conference, Annapolis, MD, November 7-11. (talk)