# Assessment of Ecological and Human Health Impacts of Mercury in the Bay-Delta Watershed

CALFED Bay-Delta Mercury Project

**Subtask 3B: Field assessment of avian mercury exposure in the Bay-Delta ecosystem.**

Draft Final Report

Submitted to

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## BACKGROUND

The Bay/Delta watershed has a legacy of mercury contamination resulting from mercury mining in the Coast Range and the use of this mercury in the amalgamation method for extraction of gold from stream sediments and placer deposits in the Sierra Nevada. Because mercury, and methylmercury in particular, strongly bioaccumulate in aquatic foodwebs there has been a reasonable speculation that widespread mercury contamination of the bay/delta from historic sources in the watershed could be posing a health threat to piscivorous wildlife. As a result this systematic survey of mercury exposure in aquatic birds was conducted in both San Francisco Bay and the Sacramento/San Joaquin Delta. The Delta component of the survey was subtask 3b of the CalFed mercury project. The San Francisco Bay component of the project was conducted at the behest of the California Regional Water Quality Control Board, Region 2, San Francisco Bay. Results of both projects are reported on here because of overlap in methods and species sampled, the interconnectedness of the Bay/Delta estuary and the need to address avian wildlife risk of mercury in the region as a whole.

Aquatic ecosystems tend to have higher rates of bioaccumulation and biomagnification than do terrestrial ecosystems (USEPA 1997). The most toxicologically significant transformation of mercury is the conversion to methylmercury. Sulfate reducing bacteria in sediment wetlands and aquatic ecosystems effectively convert inorganic Hg (II) to the methylated form. Methylmercury concentrations in fish are on the order of $10^6$ or $10^7$ times greater than the concentrations found in water. Consumption of aquatic life, primarily fish, is the primary pathway for methylmercury exposure fish and wildlife to mercury. We thus exclusively targeted aquatic birds for an assessment of mercury exposure.

EPA has developed scientific guidance for mercury concentrations in fish to protect human health (USEPA 2001). This EPA guidance states human health is protected by concentrations of methylmercury in fish less than 0.3 ppm, given certain standardized assumptions about the quantity of fish eaten. Concentrations of mercury in fish harmful to adult fish depend on the species being assessed, whether field or laboratory assessments are made and the toxic endpoint but harmful concentrations in fish muscle range between 5 and 20 ppm (Wiener and Spry, 1996). Adverse effects of mercury 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 mercury contamination of the aquatic environment (Evers et al., 2000). Avian reproduction of aquatic birds is perhaps the most sensitive component of ecosystem function affected by mercury contamination. Though recent toxicological work also shows that reproduction of fish can be a sensitive biological endpoint for methylmercury exposure (Drevnick and Sandheinrich in press). Fish mercury concentrations below the human health criteria of 0.3 ppm in fish may be harmful to reproduction in birds but this is a matter of ongoing research (Evers et al. 2001)

Documenting exposure of aquatic birds to mercury in a wide variety of species cannot rely solely upon assessments of fish tissue concentrations for several reasons. 1. Proportions of various fish species and sizes in the diet vary to an unknown degree between species and locations. 2. Mercury concentrations in fish vary with size and trophic position 3. Not all aquatic birds are exclusively piscivorous, and some are not even exclusively aquatic in their foraging habits.

To overcome the limitations of assessing bird exposure indirectly via imperfect diet estimates through fish mercury concentrations we documented avian mercury exposure directly through sampling of avian eggs. Mercury accumulates particularly in the egg-white proteins, which derive from serum proteins. Egg concentrations, therefore, more closely reflect mercury from recent dietary uptake than from accumulated tissue stores. There is also evidence that the ovalbumin fraction of egg white has a specific affinity for dietary mercury, while the ovoglobulin fraction tends to accumulate low levels of "nondietary" mercury. Because of the strong dietary connection, Walsh (1990) suggested that eggs provide a particularly good indicator of mercury exposure in the vicinity of the nesting site in the immediate pre-laying season. Monitoring of mercury in the avian egg thus has the advantage of providing both an environmental sampling relevancy and allows one to make direct inferences regarding embryotoxicity.

Methylmercury is generally considered the form most prevalent in avian eggs but this has been confirmed for wild species only in the loon (Barr 1986, Scheuhammer et al 2001). We sought to confirm methylmercury as the dominant form and quantify the proportion of total mercury found as methylmercury among a variety of piscivorous and non-piscivorous species. Because San Francisco Bay has known sources of selenium, is listed as impaired due to bioaccumulation of selenium in clams and diving ducks, and because selenium is embryo toxic and interacts with mercury, we sought to assess the concentrations of selenium as well.

**Summary of task objectives:**

1. Assess mercury and selenium concentrations in randomly collected bird eggs of a range of species in different geographic regions of the bay and delta to determine if concentrations exceed established Lowest Observed Adverse Effect Concentrations (LOAECs) for the avian egg (Currently the LOAEC in bird eggs for Hg is 0.5 ppm fresh wet weight. Species-specific LOAECs may ultimately be modified by subtask 3b.)

2. Assess mercury and selenium concentrations in fail-to-hatch bird eggs of endangered species nesting within the estuary to determine if concentrations exceed established Low effect concentrations in the avian egg.

3. Determine the proportion of methylmercury in bird eggs in a subset of those eggs analyzed for total mercury for each species assessed to evaluate whether methylmercury feeding studies are the appropriate toxicological model for all species evaluated.

4. Evaluate the species and geographic patterns of mercury concentrations and determine if birds or certain species of birds are good sentinels of local or regional mercury contamination.

5. Evaluate correlations of selenium and mercury and methylmercury in bird eggs of a range of species in different geographic regions of the bay and delta.

## METHODS

### *Analytical Chemistry*

Analytical chemistry of total mercury, methylmercury and selenium in avian eggs was performed by the California Department of Fish and Game Pollution Studies lab at Moss Landing California per the detailed protocols and quality assurance procedures in given in the methods appendix for task 3B. Total mercury analysis of avian eggs was conducted with atomic absorption spectrophotometry. Eggs analyzed for total mercury had a subsample of the homogenate digested with a 70:30 nitric/sulfuric acid mixture. Total mercury analyzed by this method includes but is not limited to inorganic forms such as Hg(II), $Hg_o$, HgS and organomercurials. A subset of 42 eggs was analyzed for methyl mercury. Eggs analyzed for methylmercury had a subsample digested with KOH/methanol and were analyzed using aqueous phase ethylation coupled with GC-CVAFS as described in the appendix (FGS-070). These eggs had a sub sample analyzed for total mercury as well. Methylmercury as defined by this method includes but is not limited to $CH_3Hg^+$, $CH_3HgCl$, $CH_3HgOH$, and $CH_3HgS$-R. Selenium analysis was done on a subsample of the whole egg homogenate using ICPMS with a Perkin Elmer Elan 5000. Digestion of the aliquot for selenium analysis was done with a microwave digestion in Nitric acid. The laboratory at Moss Landing participated in an external quality assurance comparison of laboratory results with Frontier Geosciences between March 2000 and November 2001. The results of Frontier’s analyses were compared against the participating laboratory’s results and the relative percentage difference (RPD) calculated. RPD values that exceeded 25% were considered out of compliance. Tissue samples were submitted to Frontier geosciences for Total mercury (n= 41) and mono-methylmercury analysis (n=2). For the total mercury analyses compliance was 95%, with two of the forty-four samples having RPD values of greater than 25%. The two out of compliance values were at 25.2% and 30.3%. For the mono-methylmercury analyses the RPD values of both samples were less than 25%. The paired values from the two labs were highly correlated ($R^2$ = 0.96). The mean RPD was 9%. The mean of Frontier Geosciences analyses for THg was actually only about 2% higher than the MLML mean as relative values were not biased above or below one another. Statistically there was no difference between the means from each lab, in a paired sample t-test (t = -.5, p = 0.6).

### *Field Collections and Methods*

Because of logistical constraints in finding nests avian egg collections were done in two breeding seasons between March and July of 2000 and 2001. The 2000 field season focused on the Suisun Bay and San Francisco Bay. The area of collection in 2001 included Suisun Bay, the Delta and two inland reference areas Stone Lakes and Davis and two sites near the Cosumnes River. We report mercury results in 2001 for three widely collected species including the double-crested cormorant (*Phalacrocorax auritus*), great egret (*Casmerodius albus*), and great blue heron (*Ardeia herodias)*. Double-crested cormorants were sampled at 4 locations in 2001 and four locations in 2000 with overlap in both years at one site in Suisun Bay Cormorants were the only species whose nests were found in abundance in the delta, the bay, and inland sites. Great egrets were collected at nine locations in 2001, and two locations in 2000. Nests of great egrets were primarily found at inland and freshwater tributary sites and at multiple sites around Suisun Bay. Great Blue herons were collected at 15 locations, mostly within the Delta and around Suisun Bay in the 2001 field season. From the 2000 field season within San Francisco and Suisun Bays we report on mercury concentrations in the eggs of Caspian Tern (*Sterna caspia*) three sites, Forster's Tern (*Sterna forsteri*) four sites, California Least Tern (*Sterna antillarum brownii*) one site, American Avocet (*Recurvirostra Americana)* four sites, Black-Necked Stilt (*Himantopus mexicanus)* 5 sites, Western Snowy Plover (C*haradrius alexandrinus nivosus)*, one site, Brandt's Cormorant (*Phalacrocorax penicillatus*) one site, California Clapper Rail (*Rallus longirostrus obsoletus*) one site, Black-Crowned Night Herons (*Nycticorax nycticorax*) four sites, Snowy Egret (*Egretta thula*) five sites, Western Gull (*Larus occidentalis*) one site, and California Gull (*Larus californicus*) one site.

All eggs were collected under the appropriate State and Federal scientific collecting permits. Only one randomly selected egg was collected per nest sampled except in endangered species. In endangered species we collected all eggs that failed to hatch from a given nest, but we did not make a random collection of viable eggs. Eggs were collected from all three federally protected endangered bird species that nest within San Francisco Bay, California clapper rails (*Rallus longirostrus obsoletus*), Western snowy plovers (*Charadrius alexandrinus nivosus)* and California least terns ***(*** *Sterna antillarum brownii).*

Eggs were collected by hand or in some of the tree nests through the use of an aquarium net fastened to the end of a fiberglass boat hook, which increased the number of feasibly accessible nests in trees. Once collected eggs were labeled with a unique identifier that distinguished site and species and a sequential collection number within a given site. Eggs were stored on ice in the field and refrigerated till opened. All eggs had embryos were assessed for stage of development and the presence of gross abnormalities per the SOP for avian egg harvest and embryo examination provided in the task 3b methods appendix. Egg weight, length, breadth, shell weight and whole egg volume were measured and recorded. Once contents of eggs were removed from the shell, contents were stored frozen in chemically cleaned glass containers until digestion and chemical analysis.

*Analytes and Data Analysis*

Total mercury was measured in 321 eggs from the 15 species listed above. Methylmercury was analyzed in 42 eggs from 13 species. Species assessed for methylmercury in the egg were Double-Crested Cormorant, Brandt's Cormorant, Great Egret, Snowy Egret, Black-Crowned Night Heron, Great Blue Heron, Western Gull, Forster's Tern, Caspian Tern, American Avocet, Black-Necked Stilt, Mallard, and American Bittern. Mallards and bitterns were collected from an inland site near Davis in Yolo County and were included more for the taxonomic variation in methylmercury assessment they would provide rather than the geographic coverage. Only random fresh eggs were assessed for methylmercury to avoid the potential for aberrant results due to possible demethylation in decomposing embryo tissue of salvaged eggs. Methylmercury concentrations were compared with total mercury concentrations to calculate a percentage of total found as methyl.

Selenium was assessed in a subset of 74 eggs from eleven species in the eggs from the 2000 field season only. Egg selenium concentrations in this report are expressed in dry weight to compare with field and lab derived toxicity values, while egg mercury concentrations were expressed as fresh wet weight to compare with the established toxicological thresholds in laboratory investigations. Because eggs loose water during incubation and eggs were collected at a variety of incubation stages, wet weight mercury concentrations were adjusted to fresh wet weight concentrations via the method of Stickel et al.(1973).

Within each species sampled at multiple locations we used a one-way analysis of variance (ANOVA) to assess whether location means differed. Post-hoc comparisons to assess which specific locations differed from one another were made using Tukey's honest significant difference test for unequal sample sizes. Given the small sample sizes at some locations we set $\alpha$ to 0.1 and considered means statistically different if $p < 0.1$. Normality of the distribution of total mercury concentration data was assessed for each species where locations were compared. If data was non-normal within a species then data was log transformed and geometric means were used. Location means were also combined to form regional means. Regional means were also tested with ANOVA and post-hoc testing where appropriate.

Total mercury concentrations in silversides were available from five locations very near great blue heron colonies where egg mercury data was also collected. We used a Pearson Product Moment analysis to calculate a correlation coefficient a p value for mean dry weight concentration of mercury in silversides and fresh wet weight mean mercury in great blue herons at these five delta sites. The correlation between total mercury and methyl mercury concentrations in sediment with heron egg mercury at these five sites was also assessed. (Silverside and sediment mercury data were provided by other CalFed investigators, Stephenson and Slotton.)

## RESULTS

### MERCURY CONCENTRATIONS IN EGGS – DELTA SPECIES

*Double-Crested Cormorants.*

Double-Crested Cormorants were geographically the most widely sampled species. Double-Crested Cormorants were sampled at seven locations in the Bay/Delta. These included the Bay Bridge, the Richmond Bridge, Napa Marsh and Wheeler Island in the year 2000 and Wheeler Island, Venice Cut, Pelandini Ranch and Horseshoe Lake in the year 2001. Sampling locations for Cormorants are depicted in figure 1. A total of 46 cormorant eggs were collected with the number of eggs at individual sites varying between four and 11 eggs collected. Cormorant colonies at Pelandini Ranch and Horseshoe Lake are located near the Cosumnes River, the Venice Cut colony is in the Central Delta, the Wheeler Island colony is in Suisun Bay, the Napa Marsh colony is located between the Napa River and San Pablo Bay and the Richmond Bridge and Bay Bridge Colonies are within the north central portion of San Francisco Bay.

Mean mercury concentration in double crested cormorant eggs among all sites sampled was 0.31 ppm on a fresh wet weight basis. Mean mercury at individual sites ranged from a low of 0.24 at Pelandini Ranch to 0.55 ppm at Wheeler Island in 2000. There was a ten fold range in mercury concentrations among all cormorant eggs analyzed (0.06 to 0.62 ppm) but fresh wet weight mercury concentrations in most cormorant eggs were between 0.14 and 0.55 ppm (the $10^{th}$ and $90^{th}$ percentiles) and the data was normally distributed (Shapiro Wilks test, $p = 0.237$). Wheeler Island was the only site for which two years of egg mercury data were obtained for double-crested cormorants. Mercury concentrations at Wheeler Island in 2000 were the highest observed in cormorants at any site or year (year 2000 mean = 0.55 ppm, n = 3; year 2001 mean = 0.31 ppm, n = 8). A t-test comparison of cormorant egg mercury means in each year at Wheeler Island indicated these differences were statistically significant ($t = 2.78$, $p = .02$).

We grouped cormorant sampling locations into four sampling regions for further statistical comparisons (Cosumnes River, Central Delta, Suisun Bay and San Francisco Bay) to assess the hypothesis that cormorant mercury exposure differs regionally with greater power. Mercury concentrations in cormorants followed the pattern where concentrations at Suisun > SF Bay > Delta > Cosumnes River ( F = p = .09). When Suisun and SF Bay values were combined and compared in a t-test with Delta and Cosumnes sites combined a probability value <0.025 (t = 2.35) was found.

TABLE 1. Mercury Concentrations (fresh wet weight) in Double Crested Cormorant Eggs by colony locations and years.

| **Location (year)** | **Mean Hg ppm (fww)** | **N** | **Minimum** | **Maximum** |
|---|---|---|---|---|
| Bay Bridge 00 | 0.33 | 7 | 0.22 | 0.48 |
| Napa Marsh 00 | 0.35 | 5 | 0.16 | 0.49 |
| Richmond Bridge 00 | 0.30 | 4 | 0.14 | 0.55 |
| Venice Cut 01 | 0.27 | 5 | 0.18 | 0.39 |
| Pelandini Ranch 01 | 0.24 | 6 | 0.11 | 0.38 |
| Horseshoe Lake 01 | 0.27 | 8 | 0.06 | 0.58 |
| Wheeler Island 00 | 0.55 | 3 | 0.47 | 0.62 |
| Wheeler Island 01 | 0.33 | 8 | 0.11 | 0.54 |
| All Groups | 0.31 | 46 | 0.06 | 0.62 |

TABLE 2. Regional Minimum, Maximum and Mean Mercury Concentrations in Double-Crested Cormorant eggs on a fresh wet weight basis.

| **Location** | **Mean Hg ppm (fww)*** | **N** | **Minimum** | **Maximum** |
|---|---|---|---|---|
| Suisun Bay | $0.39^{b}$ | 11 | 0.11 | 0.61 |
| San Francisco Bay | $0.33^{ab}$ | 16 | 0.14 | 0.55 |
| San Joaquin/Central Delta | $0.27^{ab}$ | 5 | 0.18 | 0.39 |
| Cosumnes River | $0.25^{a}$ | 14 | 0.06 | 0.58 |

*Similar subscripts depict means that do not differ statistically at $\alpha = 0.10$.

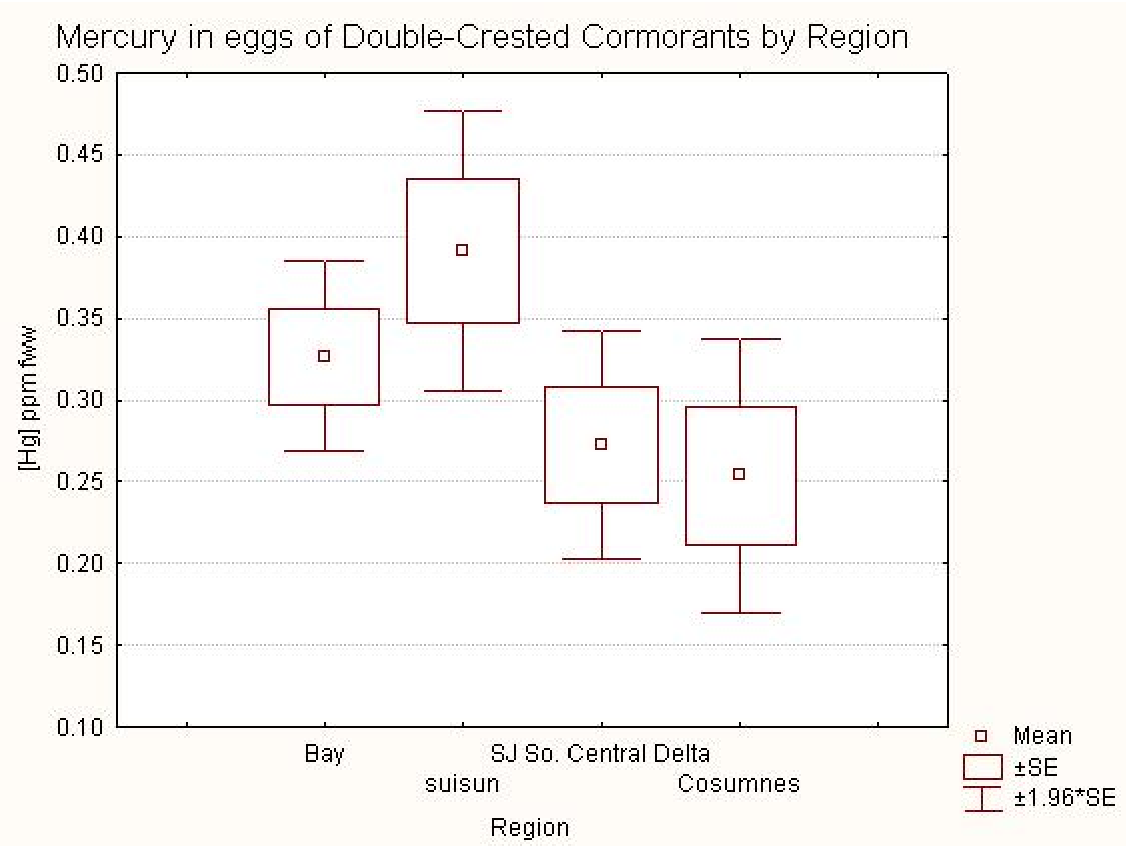


**FIGURE 1.**

*Great Blue Herons*

A total of 64 Great Blue Heron eggs were collected from 15 locations. Eleven of these 15 locations had at least 3 eggs collected. Two sites Van Sickle Island and Wheeler Island in the Suisun region had only one egg collected and two sites, Sand Mound Slough and Stone Lakes had only two eggs collected. Great Blue Heron eggs were more abundant at colonies within the delta than in Suisun Bay or tributaries and no great blue heron eggs were collected from San Francisco Bay.

The mean mercury concentration among all 64 Great-Blue Herons eggs collected was 0.12 ppm on a fresh wet weight basis. Mercury concentrations throughout the delta varied nearly 30 fold between a minimum of 0.013 in an egg from Middle River and 0.37 ppm at Clifton Court forebay in the south delta. Mercury concentrations in great blue heron eggs were not normally distributed (Shapiro Wilks Test, $p = 0.00064$) so values were log transformed for statistical analysis. The geometric mean concentration was 0.09 ppm.

Geometric mean concentrations of mercury differed between locations and regions in Great Blue Heron eggs. ( $F = 8.34$, $p = .000005$). Egg mercury results for great blue heron eggs by are summarized by region in table 3. A regional mercury pattern within the delta was apparent in Great Blue Heron eggs. The highest mean concentrations occurred in the Sacramento River portion of the delta and in Suisun while lower mean concentrations were found in eggs from the San Joaquin River portion of the central delta. The lowest concentrations in Great Blue Heron eggs was found in colonies near the Consumnes River and Stone Lakes National Wildlife Refuge.

TABLE 3. Minimum, Maximum and Geometric Mean Mercury Concentrations in Great Blue Heron Eggs from different bay/delta regions.

| **Location (year)** | **Geo. Mean Hg ppm (fww)*** | **N** | **Minimum** | **Maximum** |
|---|---|---|---|---|
| Sac River/Yolo Bypass | 0.146[a] | 25 | .050 | .321 |
| Suisun | 0.125[ac] | 2 | .103 | .154 |
| Sherman | 0.106[ac] | 3 | .069 | .141 |
| SJ River So. Delta | 0.072[cb] | 29 | .013 | .375 |
| Cosumnes | 0.024[cb] | 3 | .014 | .057 |
| Stone Lakes | 0.017[b] | 2 | .016 | .017 |

*Similar subscripts depict means that do not differ statistically at $\alpha = 0.10$.

*Great Egrets*

The mean fresh wet weight concentration among the 74 egret eggs collected was 0.16 ppm. Concentrations in great egret eggs varied by nearly 12 fold from 0.04 ppm in an egg from Grizzly Island to 0.45 ppm. Mercury concentrations in great egret eggs were not normally distributed (Shapiro Wilks Test, $p = 0.00007$) so values were log transformed for statistical analysis of location differences for comparisons within this species. The geometric mean concentration of mercury in great egret eggs among all eggs collected was 0.13 ppm. Geometric mean mercury concentrations differed among locations ($F = 2.47$, $p = 0.0144$) and regions ($F = 4.289$, $p = 0.00189$).

Two years of mercury data were available for great egret eggs at only one site, Montezuma Slough, where sample sizes of 5 and 6 eggs were obtained for each year. No difference in geometric mean mercury concentration between years was observed for great egrets at this location. Geometric mean concentration of mercury was 0.12 and 0.11 ppm Hg (fww) for the years 2000 and 2001, respectively.

TABLE 4. Minimum, Maximum and Geometric Mean Mercury Concentrations in Great Egret Eggs from different bay/delta regions.

| **Location (year)** | **Geo. Mean Hg ppm (fww)*** | **N** | **Minimum** | **Maximum** |
|---|---|---|---|---|
| SF Bay | 0.22[a] | 6 | 0.07 | 0.39 |
| Suisun Bay | 0.16[ab] | 34 | 0.04 | 0.45 |
| Sherman | 0.16[ab] | 6 | 0.05 | 0.24 |
| Davis | 0.10[ab] | 5 | 0.07 | 0.20 |
| Cosumnes River | 0.09[ab] | 17 | 0.04 | 0.27 |
| Stone Lakes | 0.08 [b] | 6 | 0.04 | 0.19 |

*Similar subscripts depict means that do not differ statistically at $\alpha = 0.10$.

*Species Comparisons among herons egrets and double crested cormorants in the delta region*

We found Great Blue Heron Nests and Great Egret nests co-occurred at four colonies Van Sickle Island, Stone Lakes, Horseshoe Lake, and the East side of Sherman Lake). Egg mercury concentrations among herons and egrets were highly correlated at these four sites ($r^2 = .986$, $p = 0.0067$). The mean mercury concentration in eggs of Great Egrets (0.15) ppm ) however was nearly 2 fold greater at these four sites than was the mean mercury concentration in great blue herons ( 0.08 ppm).

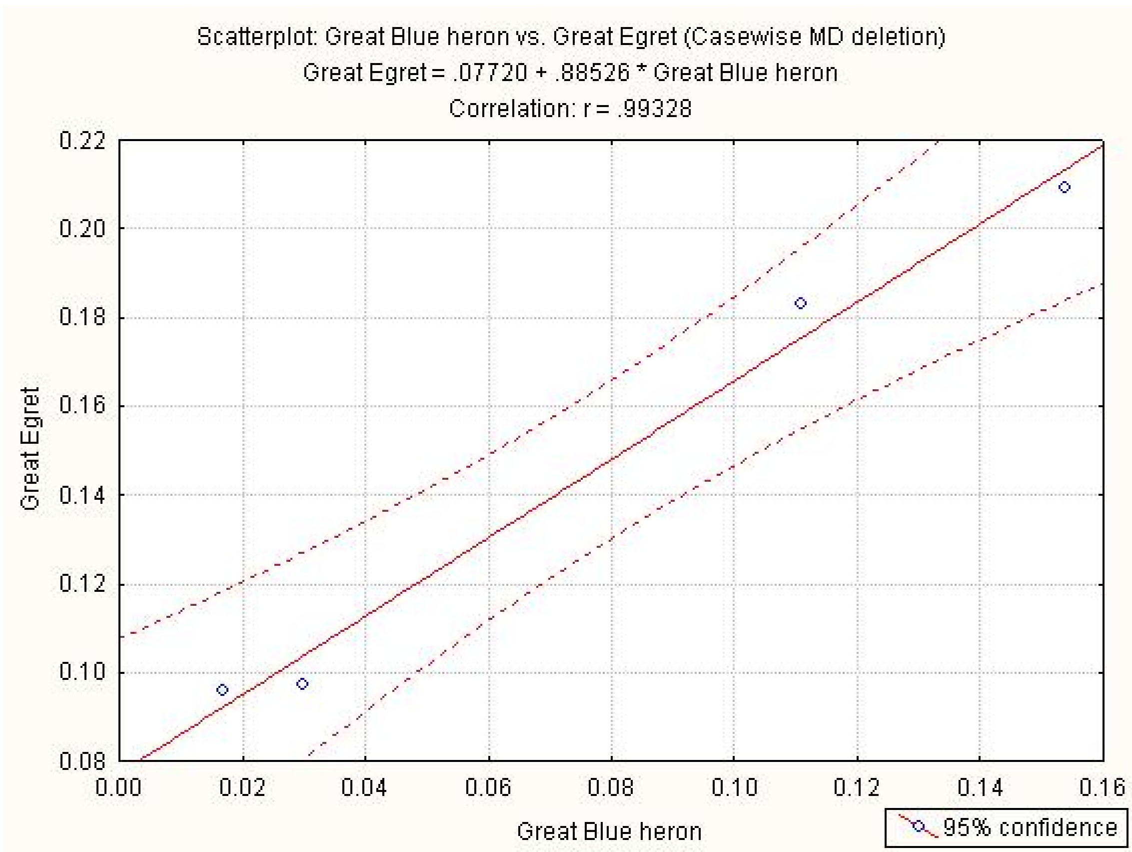


**FIGURE 2.**

Great blue heron and double-crested cormorant nests were co-located at three colony locations, Wheeler Island, Horseshoe Lake, and Venice Cut. Mean concentrations among these three sites were not well correlated statistically ($r^2 = 0.5$, $p = 0.5$) although the maximum means occurred at the same location. Mean mercury concentrations for these three colonies in eggs from Double- crested Cormorants (0.309 ppm) were 3 times greater than those found in great blue heron eggs (0.103).

Great Egrets and Double-Crested Cormorant nests were co-located at two colonies; Pelandini Ranch and Horseshoe Lake, both near the Cosumnes River. These two locations had the lowest mean mercury concentrations and the lowest minimums among all locations where double crested cormorants were sampled, and were also among the very lowest sites for egg mercury concentrations in Great Egrets.
The Wheeler Island colony in 2000 had the maximum mean mercury concentration for Double-Crested Cormorants among all samples and years. The two Great Egret colonies nearest Wheeler Island were Van Sickle Island and Montezuma Slough. Egret egg mercury was greater at these sites than at Cosumnes River sites but was again consistently less than found in Double-Crested Cormorants.

Comparisons of mean egg mercury concentrations between all three avian species were possible for two regions, Suisun and Cosumnes. In all three species we found eggs from the Suisun region had greater mercury concentrations than the Cosumnes Region. We also found a consistent species pattern for mercury concentrations where Double-Crested Cormorants > Great Egrets > Great Blue Herons.

TABLE 5. Comparison of Mercury concentrations between species for two regions.

| Species | Suisun Region Mean Hg ppm (fww) (N) | Cosumnes Region Mean Hg ppm (fww) (N) |
|---|---|---|
| **Double Crested Cormorant** | **0.39 [a] (11)** | **0.25 [a] (14)** |
| **Great Egret** | **0.19 [b] (28)** | **0.11 [b] (17)** |
| **Great Blue Heron** | **0.13 [b] (2)** | **0.03 [b] (3)** |

*Similar subscripts depict means that do not differ statistically at $\alpha = 0.10$ as assessed by Tukey's HSD for unequal Ns.

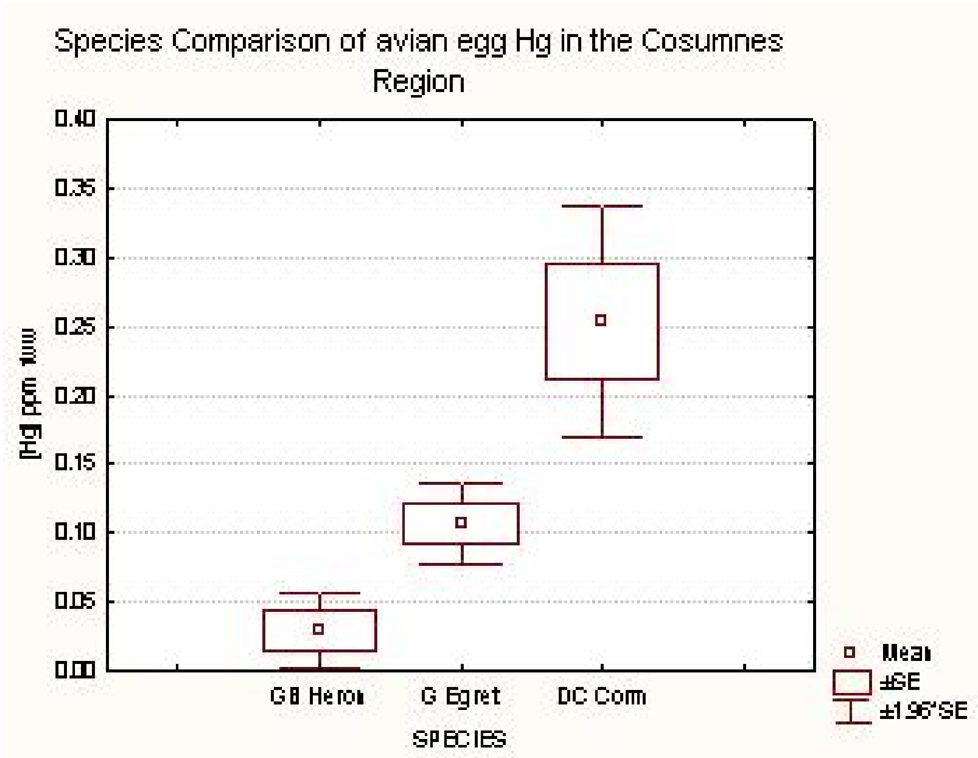


**FIGURE 3.**

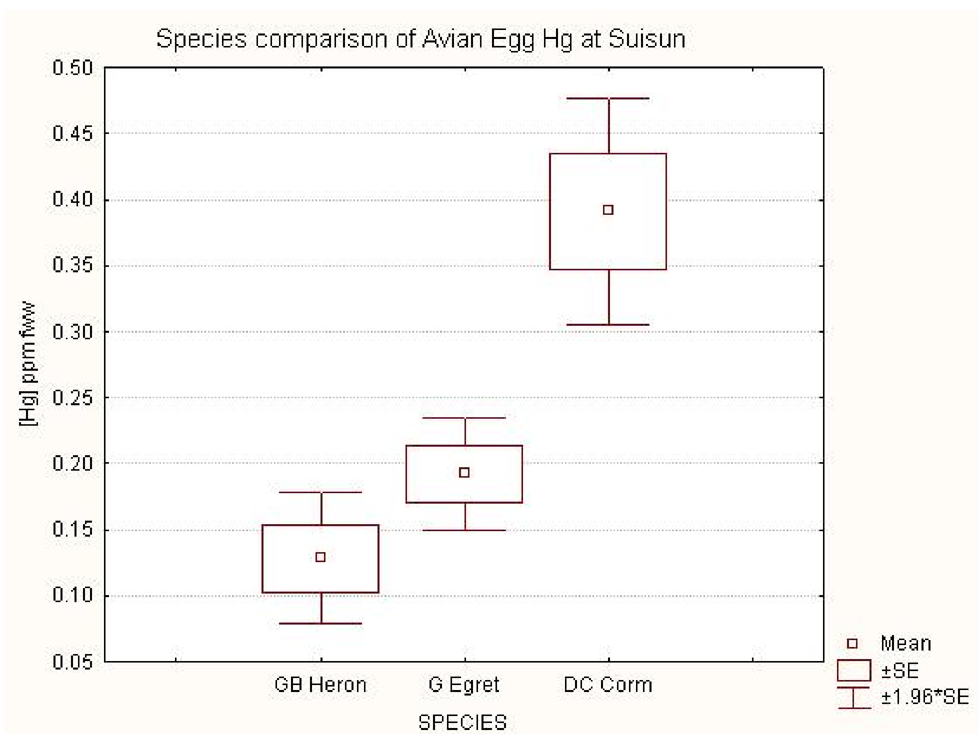


**FIGURE 4.**

## Recurvirostrids

Avocet eggs from four locations, Charleston Slough, Grizzly Island, Salt Pond A16, and Hayward marsh, were analyzed for mercury. Mean mercury concentrations varied by five fold among the four sites, from 0.06 at Charleston Slough to 0.3 ppm (fww) at the A16 pond site. Intermediate concentrations of mercury in eggs were found at Hayward Marsh and Grizzly Island. Mean concentrations in avocet eggs were statistically different between sites in the bay and post hoc testing revealed avocet mean egg mercury at Charleston slough to be distinct from the elevated mean egg mercury concentrations pond A16 site.

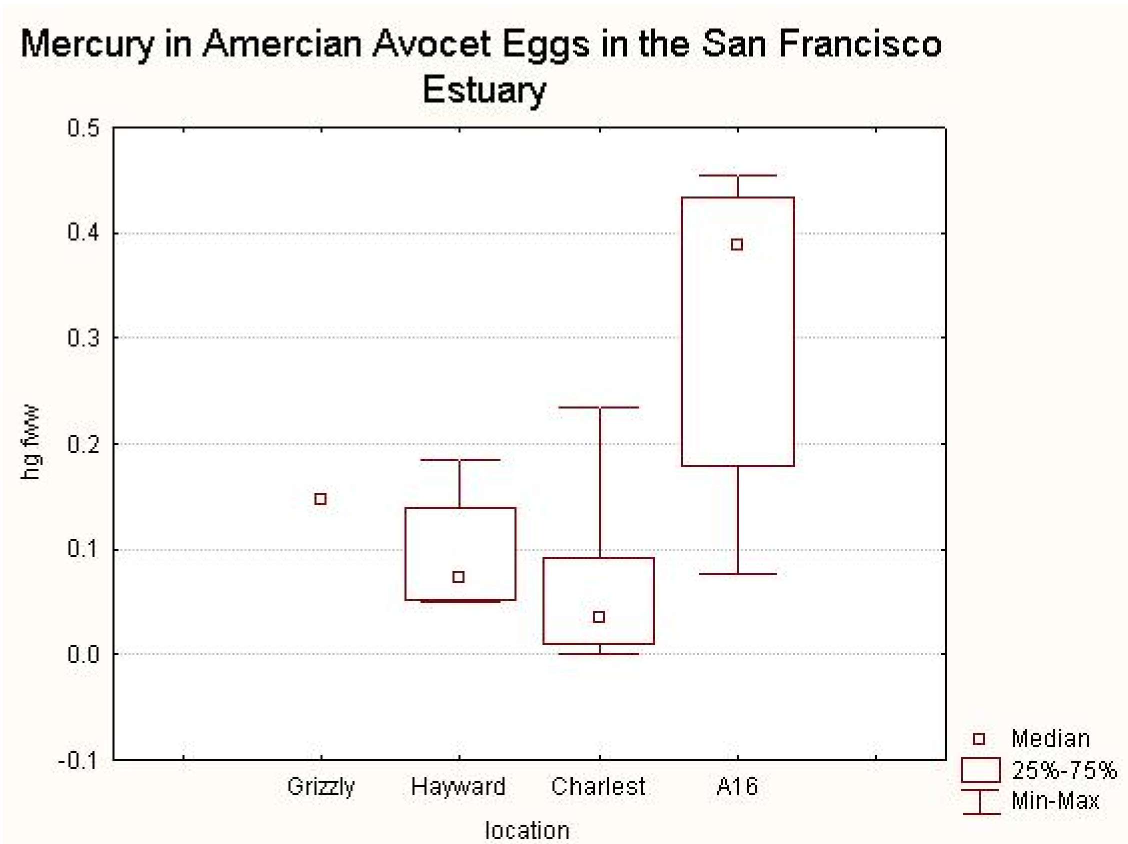


**Figure 5.**

Black-Necked Stilt eggs were sampled at four locations, Wildcat Marsh in the Central bay, and at three salt ponds in the south bay near Baumberg, Hayward and Moffitt field. As observed in Avocets, the southernmost colony at a salt pond associated with water intakes from Alviso Slough had the highest mean mercury concentrations. Table x summarizes stilt and avocet mean egg mercury concentrations by location.

TABLE 6. Mean mercury concentrations in Recurvirostrid eggs.

| **Region** | **Location** | **B-N Stilt Mean ppm Hg fww (N)** | **Am. Avocet Mean ppm Hg fww (N)** |
|---|---|---|---|
| **Suisun Bay** | Grizzly Island | | 0.15 (1) |
| **Central SF Bay** | Wildcat Marsh | 0.37ab (2) | |
| **South East SF Bay** | Hayward Marsh | 0.11b (4) | 0.09b (4) |
| | Baumberg salt ponds | 0.41ab (3) | |
| **South West SF Bay** | Charleston Slough | | 0.07b (6) |
| **Extreme South SF Bay** | Moffitt salt ponds | 0.45a (4) | |
| | A16 salt pond | | 0.31a (5) |
| **Bay Wide** | | 0.31 | 0.19 |

*Similar subscripts depict means that do not differ statistically (at $\alpha = 0.10$) as assessed by Tukey's HSD for unequal Ns.

CALIFORNIA CLAPPER RAIL

Six fail-to-hatch eggs of the California Clapper Rail were salvaged from an intertidal marsh in the Central Bay near Castro Cove called Wildcat Marsh during the spring of 2000. The geometric mean concentration of mercury in these eggs was 0.81 ppm on a fresh wet weight basis. Mercury concentrations in these eggs ranged from 0.60 to 1.06 ppm. This geometric mean mercury concentration exceeded that found in 9 failed eggs in this marsh in 1998 and 1999 of 0.5 ppm (Schwarzbach in prep) and the geometric mean in 42 rail eggs from the south bay in 1992 of 0.56 ppm.

Table 7. Mercury concentrations in Tern eggs in San Francisco Bay by locations and region (in ppm on a fresh wet weight basis)

| **Region** | **location** | **Least Tern** | **Forster's tern** | **Caspian Tern** |
|---|---|---|---|---|
| **North SF Bay** | Napa Marsh | | 0.64b (6) | 0.90b (5) |
| **Central SF Bay** | | | | |
| | Brooks Island | | | 0.72b (5) |
| | Alameda Naval Air Station | 0.3 (3) | | |
| **South SF Bay** | | | | |
| | Charleston Slough | | 0.59b (5) | |
| | Hayward | | 0.50b (5) | |
| | Salt Pond A16 | | 1.62a (5) | |
| | Salt Pond A7 | | | 1.18a (5) |
| **Bay Wide average** | | 0.3 | 0.83 | 0.93 |

TERNS

Mean mercury concentrations in tern eggs varied with species and location. Results are summarized in table 7. Overall for the bay as whole mercury concentrations in tern eggs were greater in the larger species of terns. A plot of egg mercury vs. log body weight of the adult was highly correlated ($r^2 = .98$). Despite this strong trend between species location was very important also in determining concentrations of mercury. Eggs of Forster's Terns nesting and foraging near south bay salt ponds at Cargill's A16 pond had the highest mean concentration of any bird at any location sampled in this study and three times higher than the next nearest colonies of Forster's terns at Charleston Slough or Hayward. The next highest mean was found in Caspian terns nesting at Cargill's A7 pond near Alviso Slough.

Snowy Egret and Black-Crowned Night Heron

TABLE 8. Mean mercury concentrations in Snowy Egrets and Black Crowned Night Herons

| REGION | LOCATION | B-CN HERON | SNOWY EGRET |
|---|---|---|---|
| YOLO COUNTY | | | |
| | CO. RD. 103 | 0.13<br>(9) | 0.21<br>(4) |
| CENTRAL SF BAY | | | |
| | WEST MARIN ISLAND | 0.19<br>(5) | 0.17<br>(5) |
| | ALCATRAZ ISLAND | 0.13<br>(10) | 0.09<br>(1) |
| SOUTH SF BAY | | | |
| | REDWOOD SHORES | 0.07<br>(4) | 0.10<br>(5) |
| | HAYWARD | | 0.11<br>(3) |

The geometric mean for all 18 snowy egret eggs analyzed was 0.16 ppm on a fresh wet weight basis, with concentrations varying between 0.05 and 0.33 ppm. The greatest concentrations in snowy egret eggs were found in eggs from Yolo County and West Marin Island. Mean concentrations in eggs of both snowy egrets and black-crowned night herons did not statistically differ between locations, however a similar geographic pattern is apparent for both species where West Marin Island eggs have roughly twice the mercury as in eggs from Redwood Shores. To assess locational differences we combined egret and heron data and computed a one way ANOVA test. The increased sample size produced statistically different means of 0.18 for herons and egrets from West Marin Island and 0.09 for eggs from Redwood shores ($p = 0.046$).

BRANDT'S CORMORANTS

Brandt's Cormorants were sampled only at Alcatraz Island in the central portion of San Francisco Bay. Most Brandt's Cormorants nest on the outer coast. The Alcatraz colony is the most landward nesting colony of Brandt's Cormorants in California and the only colony in San Francisco Bay. Mean mercury concentrations in eggs of Brandt's Cormorants nesting at Alcatraz were 0.19 ppm (fww). Brandt's Cormorants had higher mercury concentrations than the other two species nesting at Alcatraz, Snowy egrets and

Black-Crowned night Herons, but lower mean mercury than Double-Crested Cormorants at either the Bay Bridge or the Richmond Bridge. Within San Francisco Bay Brandt's Cormorants forage near the extreme western portion of the central bay between the Golden Gate and Angel Island (Bill Sydeman, personal communication). The lower mercury in Brandt's Cormorants as compared with Double Crested Cormorants on the Richmond and Bay Bridge likely reflects the tidal dilution that occurs near the mouth of the bay.

### GULLS

Gulls were sampled on a limited basis from two locations in San Francisco Bay. Four Western gull eggs from West Marin Island in the Central Bay and two eggs from California Gulls from the Knapp property in the South bay were analyzed for mercury. Western Gulls from West Marin Island had the cleanest eggs in the bay with a mean concentration of 0.05 ppm Hg (fww). Western gull eggs ranged from below method detection limits in one egg of 0.0197 ppm ww to 0.09 ppm. In contrast black-crowned night herons and snowy egrets both had bay-wide single egg maximums of 0.29 in eggs from West Marin Island, but obviously foraging strategies for Western Gulls were quite different from herons and egrets

Two California Gull eggs from the Knapp property in the extreme south San Francisco Bay contained more mercury than western gull eggs with a mean concentration of 0.1 ppm, but not statistically more and not as much mercury as their extreme south bay counterparts avocets, plovers and terns which had an order magnitude more mercury in their eggs. This suggests that California gulls were not feeding in the south bay salt ponds or perhaps not even in the aquatic system of the south bay, preferring garbage and landfills as foraging sites.

### WESTERN SNOWY PLOVERS

Three western snowy plover eggs that failed to hatch were analyzed for mercury. These eggs were from nests on salt pond levees in the extreme south bay. Concentrations in these eggs ranged from 0.33 to 0.66 ppm, with a mean of 0.45 ppm.

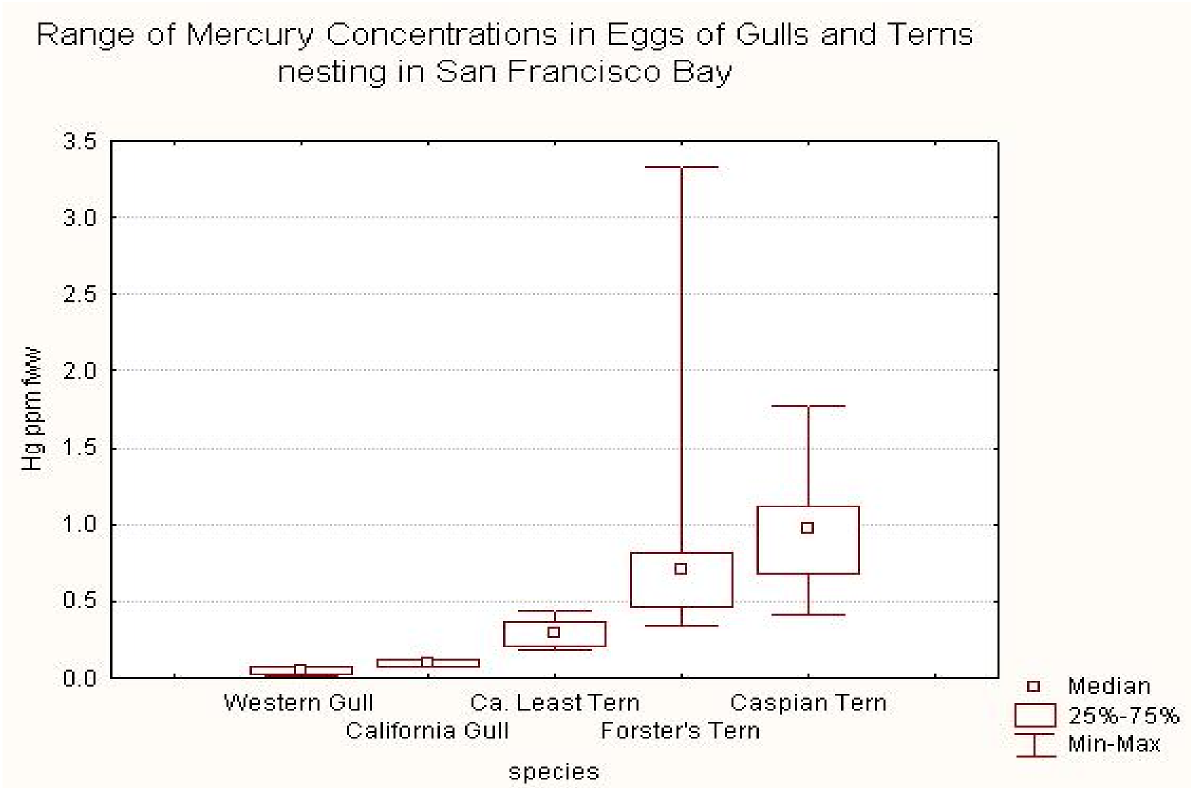


**FIGURE 6.**

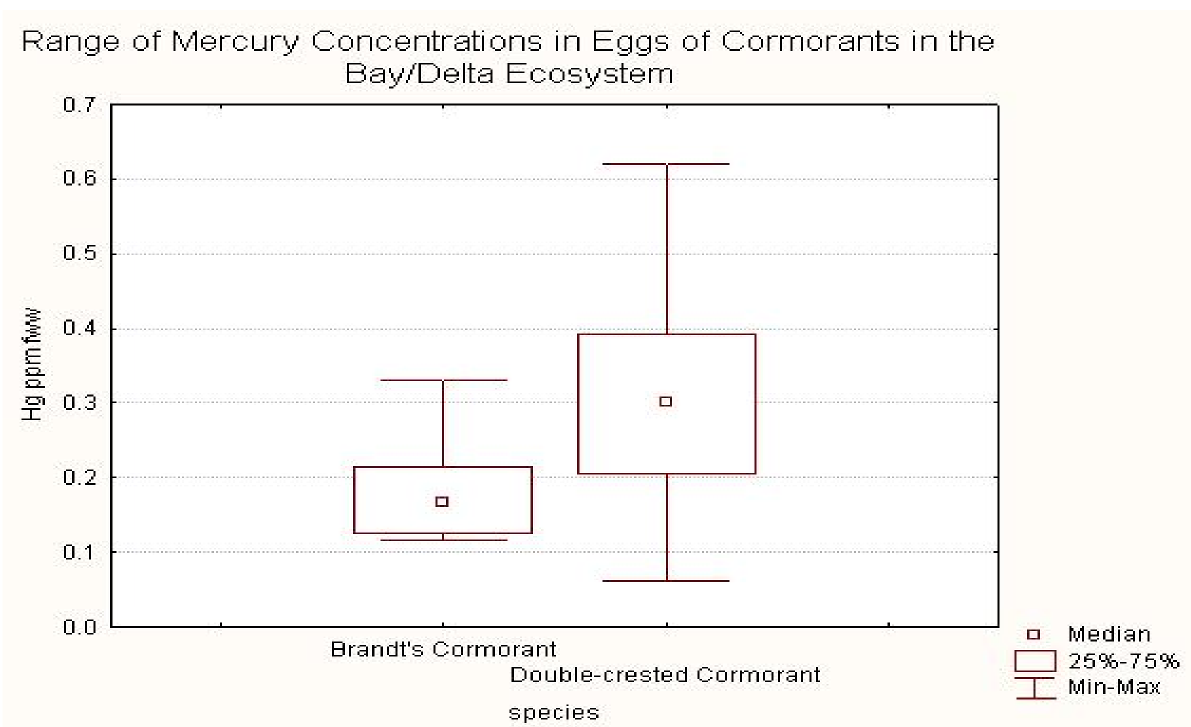


**FIGURE 7.**

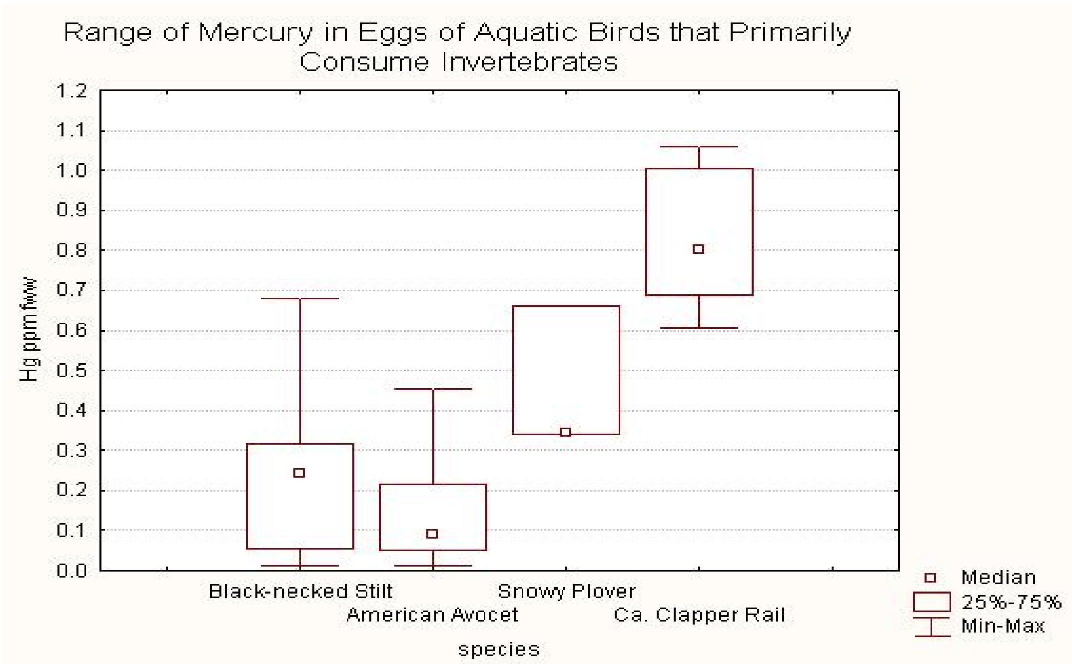


**FIGURE 8.**

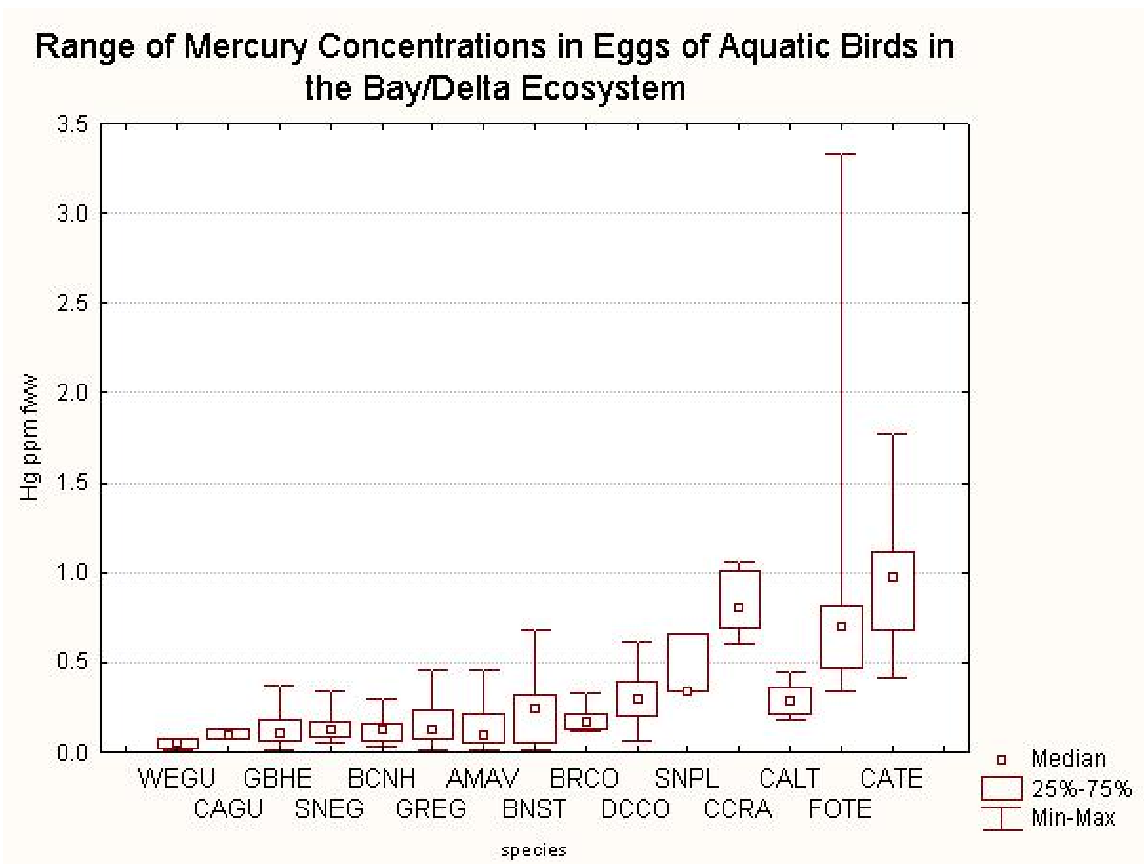


**FIGURE 9.**

*Methylmercury in the Avian Egg*

We found the mean percentage methyl mercury in the 42 eggs assessed was 100.4 percent with a 95 percent confidence interval of 97.4 to 103.4 percent. Methylmercury varied as a percentage of total mercury from 79.6 percent to 122.2 percent. Percent methylmercury values greater than 100% are not unexpected for split biological samples (Bloom, 1992). Methylmercury was strongly correlated with total mercury concentrations ($R^2 = 0.97$, $p < .001$). There did appear to be some possible species differences in the mean percentage methylmercury as great egrets and great blue herons had lower mean percentages of 90.3 and 81.5 percent, respectively, but this was based only upon four eggs and analytical variability and small sample size confounds our ability to detect small percentage differences in the proportion of methylmercury between species. A statistically significant but weak negative correlation between total mercury and the percentage methyl mercury was found ($r^2 = -.3147$, $p = .042$).

TABLE 9. Methylmercury as a percentage of total mercury by species in the egg.

| Species | Mean Percent methyl Hg | Lower 95% CI | Upper 95% CI | N | SD | Min | Max | Mean THg (dw) |
|---|---|---|---|---|---|---|---|---|
| Forster's Tern | 94.0 | 83.1 | 104.9 | 6 | 10.4 | 79.6 | 104.9 | 7.30 |
| Caspian Tern | 101.3 | 89.9 | 112.7 | 3 | 4.6 | 98.4 | 106.6 | 4.66 |
| Double – Crested Cormorant | 103.0 | 99.3 | 106.8 | 6 | 3.6 | 98.2 | 108.5 | 2.70 |
| All species | 100.4 | 97.4 | 103.4 | 42 | 9.7 | 79.6 | 122.2 | 2.40 |
| Black-Necked Stilt | 93.0 | 83.9 | 102.1 | 3 | 3.7 | 90.6 | 97.2 | 1.73 |
| Great Egret | 90.3 | 69.8 | 110.8 | 3 | 8.2 | 81.2 | 97.2 | 1.60 |
| Brandt's Cormorant | 109.2 | 79.4 | 139.1 | 3 | 12.0 | 98.4 | 122.2 | 1.58 |
| American Avocet | 111.0 | 98.2 | 123.8 | 3 | 5.1 | 108.1 | 117.0 | 1.14 |
| Snowy Egret | 100.6 | 82.4 | 118.9 | 3 | 7.3 | 93.7 | 108.3 | 0.94 |
| American Bittern | 97.0 | 78.7 | 115.3 | 2 | 2.0 | 95.6 | 98.4 | 0.82 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Black-Crowned Night Heron | 95.7 | 78.7 | 112.7 | 3 | 6.9 | 88.1 | 101.4 | 0.80 |
| Great Blue Heron | 81.5 | | | 1 | | 81.5 | 81.5 | 0.67 |
| Western Gull | 109.8 | 103.8 | 115.8 | 3 | 2.4 | 108.2 | 112.6 | 0.28 |
| Mallard | 108.7 | 82.9 | 134.5 | 3 | 10.4 | 99.3 | 119.9 | 0.15 |

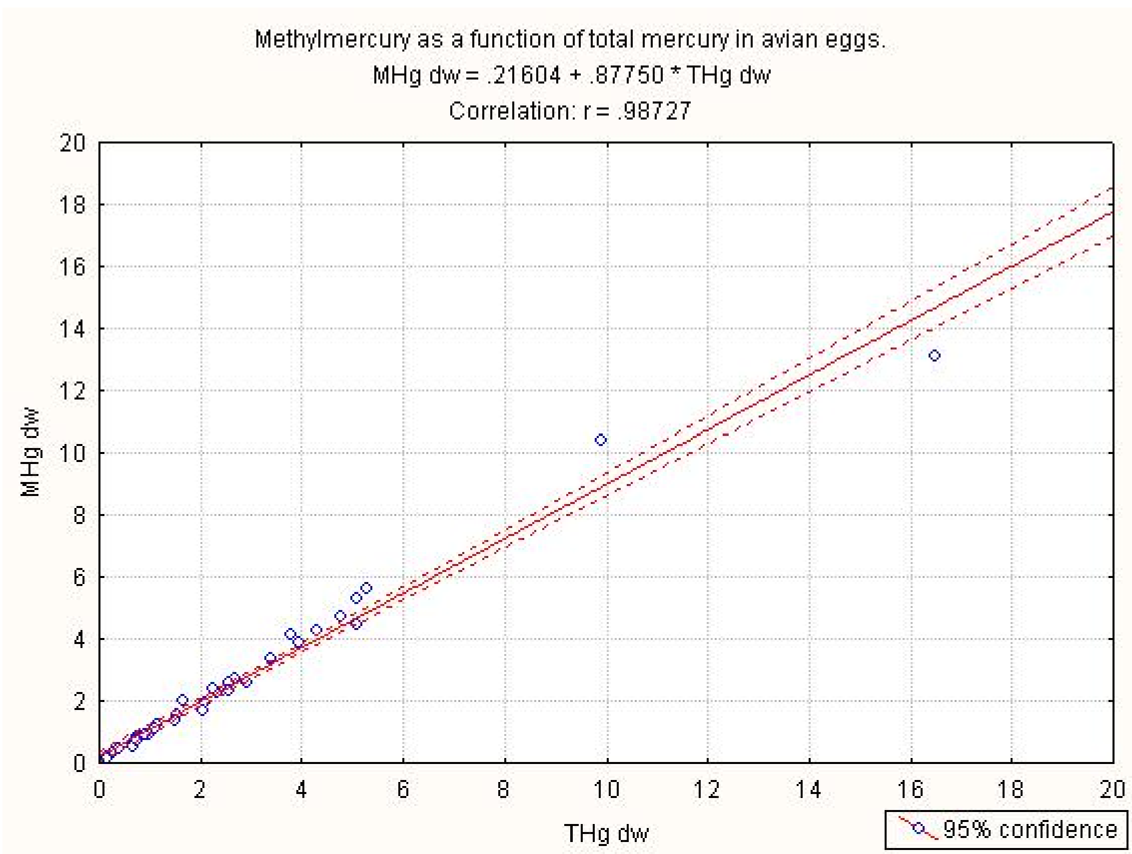


**FIGURE 10.**

*Selenium in avian eggs.*

Selenium concentrations were determined in 74 eggs of aquatic birds from San Francisco Bay and Suisun Bay. The average concentration of selenium in all eggs was 2.7 mg/kg (dry weight). Selenium concentrations ranged from 0.86 mg/kg to 7.76 mg/kg and appeared unrelated to mercury concentrations. Selenium concentrations were generally much less variable than mercury concentrations and were normally distributed. The highest selenium concentrations were seen in great egrets and snowy egrets from West Marin Island and black-crowned night herons nesting at Alcatraz. Eggs of aquatic birds with concentrations of selenium less than 3ppm on a dry weight basis are not considered elevated. There is however a steep dose response curve for selenium concentrations in bird eggs. Thresholds for selenium effects upon hatchability and terratogenesis vary between different species with mallards being more sensitive than stilts which are more sensitive than avocets (USDOI, 1998). Thresholds range for selenium toxicity in avian eggs range between 6 and 10 ppm on a dry weight basis.

TABLE 10. Selenium concentrations in eggs of aquatic birds of San Francisco and Suisun Bays.

| **Species** | **Mean Se ppm (dw)** | **N** | **SD** | **Min** | **Max** | **10%ile** | **90%ile** |
|---|---|---|---|---|---|---|---|
| Snowy Plover | 1.5 | 3 | 0.6 | 0.9 | 2.0 | 0.9 | 2.0 |
| American Avocet | 1.8 | 6 | 0.3 | 1.3 | 2.2 | 1.3 | 2.2 |
| California Clapper Rail | 1.6 | 6 | 0.1 | 1.3 | 1.7 | 1.3 | 1.7 |
| Brandt's Cormorant | 2.1 | 2 | 0.3 | 1.9 | 2.3 | 1.9 | 2.3 |
| Forester's Tern | 2.4 | 6 | 0.3 | 2.0 | 2.9 | 2.0 | 2.9 |
| Black-Necked Stilt | 2.4 | 2 | 0.3 | 2.2 | 2.6 | 2.2 | 2.6 |
| Double-Crested Cormorant | 2.6 | 8 | 0.3 | 2.3 | 3.0 | 2.3 | 3.0 |
| California Least Tern | 2.8 | 6 | 0.4 | 2.4 | 3.3 | 2.4 | 3.3 |
| Black-Crowned Night Heron | 3.5 | 11 | 0.7 | 2.5 | 4.6 | 2.6 | 4.3 |
| Snowy Egret | 4.2 | 9 | 0.5 | 3.7 | 4.9 | 3.7 | 4.9 |
| Great Egret | 3.0 | 15 | 1.4 | 2.0 | 7.8 | 2.0 | 3.4 |
| All groups | 2.8 | 74 | 1.1 | 0.9 | 7.8 | 1.7 | 4.2 |

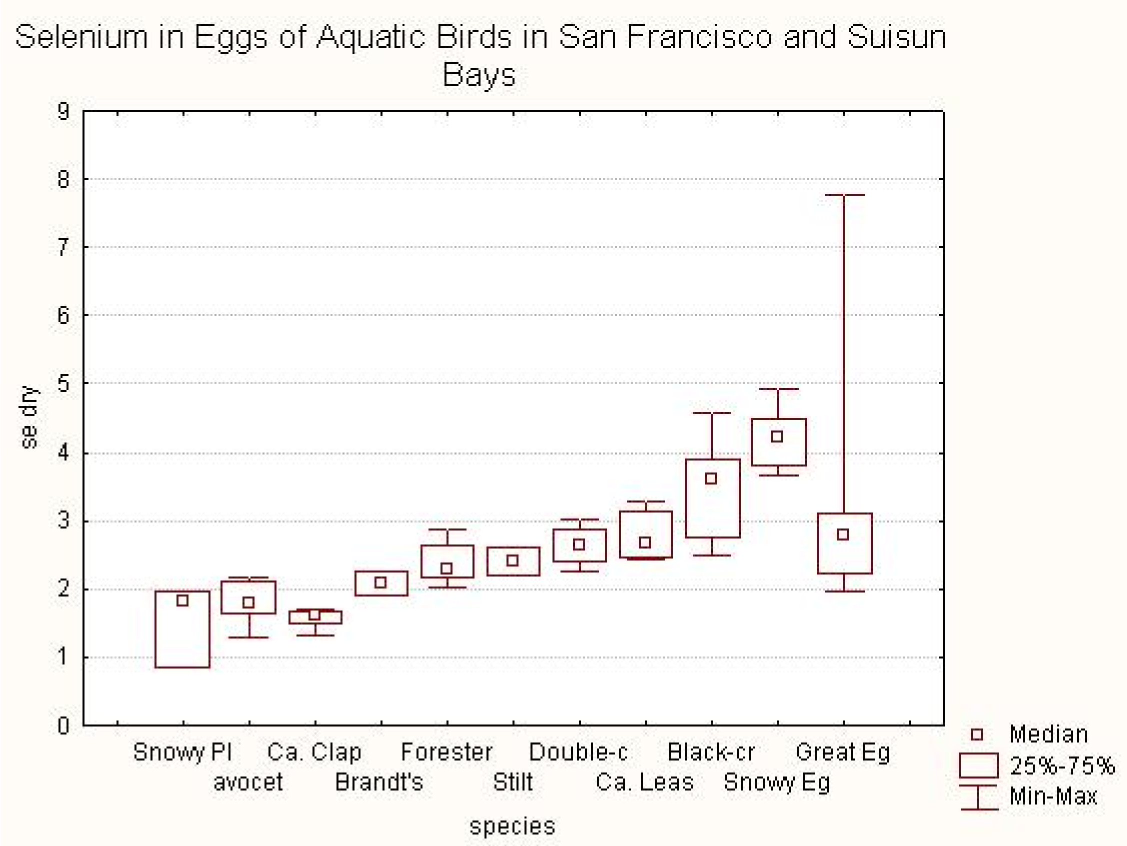


**FIGURE 11.**

## DISCUSSION

*Methylmercury in wild bird eggs*

When methylmercury is fed to birds in laboratory settings nearly all the mercury deposited in the egg is methylmercury (Tejning 1967). Methylmercury has been established as the prevalent form in the wild in eggs of common loons (Scheuhammer et al. 2001), and black terns (Weseloh et al., 1997) both of which are nearly exclusively piscivorous. Our study confirmed that methylmercury is the predominant form of mercury wild bird eggs among 13 aquatic species assessed regardless of degree of piscivory. Consequently we feel well justified in applying toxicological thresholds derived from methylmercury feeding studies to the interpretation of total mercury concentrations in bird eggs.

The lowest three mean percentages of methylmercury were found in eggs of the black-necked stilt, (93%) the great egret (90.3%) and the great blue heron (81%). For the stilt and great egret the 95% confidence interval for mean percentage methyl mercury bounded 100%. For the heron only one egg was available for analysis and a confidence interval was not obtainable. For many species the mean percentage of mercury as methylmercury was greater than 100%. This was due to combining measurements of methyl mercury and total mercury from the same egg without separately quantifying inorganic mercury.  Separate quantification of inorganic and methyl mercury would have produced a total mercury result in which the percentage as methyl would not have exceeded 100%.  If methyl mercury is indeed nearly 100% of the total mercury then the result of combining two separate measures will be to combine the inherent measurement errors of two methods to estimate total mercury.  We thus could not distinguish whether the small deviations from 100% we observed were due to measurement precision differences between the two methods for total mercury and methylmercury or whether small amounts of inorganic mercury are actually present in the egg. Further studies to assess whether less piscivorous species such as stilts and herons have a fraction (10% to 20%) of mercury occurring, as the inorganic form would benefit from direct measures of the inorganic form.

*Toxicological significance of observed mercury concentrations in bird eggs*

Embryos of birds are considered among the most sensitive life stages to the adverse effects of mercury (Schuehammer, 1991) however comparatively little is known about the differences between species in the sensitivity of avian embryos to methylmercury.   In predicting the adverse effects of egg mercury concentrations on birds in the field avian researchers typically rely upon a few benchmark studies that have established effects 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 methylmercury which have demonstrated reduced hatchability of avian eggs include Fimreite (1971) which found only 10% hatchability in pheasant eggs at egg mercury concentrations of 0.5 to 1.5 µg/g (fww) in pheasants and Heinz (1979) which found moderate to low effects upon hatchability were associated with average egg concentrations of approximately 0.8 µg/g (fww) in mallard ducks.   A key uncertainty for evaluating the importance of mercury in limiting avian reproduction in the bay delta is the relative applicability of pheasant and mallard mercury toxicity thresholds to piscivorous birds inhabiting the bay/delta region. The Heinz egg injection experiment demonstrated that relative to mallards, pheasants were much less sensitive and clapper rails and tricolored herons were more sensitive.  These results suggest that mean mercury concentrations in clapper rails of 0.82 ppm (fww) in fail-to-hatch clapper rail eggs in this study should be considered embyrotoxic, while concentrations in cormorant that did not exceed 0.61 ppm (fww) should be considered minimally embyrotoxic.  Heron and egret concentrations were generally lower than cormorant and the rail. It is unclear whether any concentrations in these species were embyrotoxic.  Concentrations in western gulls, California gulls, Brandt's Cormorants in the bay should be considered to be below toxic levels for the embryo.  Avocets, plovers and stilts had variable concentrations with some high eggs at salt pond sites in the south bay that exceeded the threshold concentrations though means were just under 0.5 ppm.  Data for the interpretation of tern eggs is limited to a non-systematic field study of common terns that suggested normal hatchability of tern eggs at mean concentrations of around 1 ppm but severe impairment by mercury to reproduction at concentrations of 3.65 ppm (Fimreite, 1974). Laboratory data is needed for better interpretation of the hazards posed by mercury concentrations found in the bay but terns have demonstrated they are the effective avian mercury bioaccumulators in the bay.

**CONCLUSIONS:**

- **Geographic variability in the bioavailability of mercury in the bay/delta system can be monitored effectively by measuring mercury in aquatic bird eggs.**

  FINDINGS: Significant species and location differences were found in mercury bioaccumulation within both the delta and the bay. Mercury concentrations varied by two orders of magnitude, from less than 0.02 to 3.33 ppm on a fresh wet weight basis, in 328 bird eggs in the bay/delta ecosystem. Statistical differences in species mean egg mercury between locations or regions in the Bay Delta were found within Double-Crested Cormorants, Great Blue Herons, Great Egrets, Caspian Terns, Forster's Terns, Black-Necked Stilts, and American Avocets. Statistical differences in locations were not found in Black-Crowned Night Herons, or Snowy Egrets. Clapper Rails, Snowy Plovers, California Gulls, and Western Gulls were only opportunistically sampled at single locations and could not be tested for location effects.

- **Great Blue Heron egg mercury was correlated with silverside mercury concentrations in the Delta.**

  FINDINGS: Great Blue Herons were the nesting bird species with the best in-delta geographic coverage. Egg mercury from this species illustrated a within delta pattern of mercury bioavailability that was similar to that found in sport fish as well as silversides where concentrations in the San Joaquin River and Central Delta region were significantly lower than other locations such as Suisun Bay, Prospect Slough and the Sacramento River. Great Blue Heron egg mercury was significantly correlated with silverside mercury results at five delta locations ( $R^2 = 0.78$, $P = 0.046$). Great Blue Heron egg mercury within the delta was not correlated with total mercury or methylmercury concentrations in sediment or clams.

- **Piscivorous birds had the highest concentrations of egg mercury, but some non-piscivorous birds also had dangerously high egg mercury concentrations.**

  FINDINGS: In the delta, degree of piscivory strongly influenced species egg mercury patterns as double crested cormorants consistently had more mercury than great egrets which had more mercury than great blue herons when two or more of these species were sampled from the same locations. Terns, exhibited a bay-wide pattern of egg mercury where log adult body weight was strongly correlated with mean egg mercury and at only one location in the extreme south bay did the smaller Forster's tern have greater mercury in eggs than the much larger Caspian Tern. Findings of elevated egg mercury was not restricted to piscivorous birds however as high mercury concentrations were found in non-piscivorous birds nesting near salt ponds or in tidal marshes. Non-piscivorous birds with elevated mercury concentrations  included California Clapper Rails (0.83 ppm at Wildcat Marsh), stilts (0.45 ppm at salt ponds near Moffitt), Avocets (0.31 ppm at pond a16) snowy plovers (0.45 ppm at Pond A22).  These concentrations all exceeded those found in the exclusively piscivorous  Brandt's Cormorants (0.19 ppm) nesting at Alcatraz Island.  The findings of high mercury in the California Clapper Rail may be related to the fact that methylmercury production is greatest in sediments of tidal wetlands, the preferred foraging substrate of rails, and the fact that rails do not migrate out of bay during their life cycle.

- **Egg mercury concentrations in some avian species were elevated above the known embryotoxic thresholds the mallard (0.8 ppm) or the pheasant (0.5 ppm).**

  FINDINGS:  Among species sampled in the Delta (DCCO, GBHE, GREG) only Double-Crested Cormorants had a location mean concentration above a known toxic threshold, and this was in Suisun bay not the delta proper (0.54 ppm at Wheeler Island in 2000). Among species sampled in San Francisco Bay, three species had location means above the mallard toxic thresholds.  These were the Caspian Tern, which had location means ranging from 0.7 to 1.2 ppm, the Forster's Terns, which had location means between 0.5 and 1.63 ppm, and the California Clapper Rail, which had a mean of 0.82.  Two other species, the snowy plover and black-necked stilt had a location mean concentration just below 0.5 but had some eggs between the 0.5 and 0.8 thresholds.  The egg injection work of Heinz seems to indicate the cormorant is less sensitive than the mallard so the threshold exceedance in this species is probably not indicative of a mercury problem for cormorant hatchability.  Heinz work also indicated clapper rails were more sensitive than the mallard and the pheasant and the high concentrations found in fail-to-hatch rail eggs were likely embyrotoxic.  Preliminary data in tricolored herons suggest they may not be as insensitive as the cormorant however more data is needed for herons and egrets to provide confidence in interpreting egg mercury concentrations in these species.  There is not yet data available to provide species specific interpretation of concentrations in the stilts, plovers and terns but concentrations over 1 ppm in fish eating birds and over 0.5 ppm in non-piscivorous species should be probably be considered elevated.

- **Selenium was below embyrotoxic thresholds and either not correlated or weekly negatively correlated with total mercury concentrations.**

  FINDINGS:  Selenium concentrations were not positively correlated with mercury concentrations in eggs.   The mean selenium concentration in 74 eggs of 10 aquatic bird species from San Francisco Bay and Suisun Bay was 2.7 mg/kg (dry weight). Individual eggs ranged from 0.86 mg/kg  to 7.76 mg/kg.  The highest selenium concentrations were seen in great egrets and snowy egrets from West Marin Island and black-crowned night herons nesting at Alcatraz. Baywide mean concentrations of selenium in eggs for all eleven species sampled were below known avian embryotoxic thresholds for birds.

- **For 13 aquatic bird species assessed nearly all of the mercury in avian eggs was methylmercury**.

  FINDINGS: Methylmercury was estimated to be100.4% of the total mercury $\pm$ 3%. Herons and egrets may have had slightly lower percentages of mercury as methylmercury but more data is needed to confirm or deny this. Methylmercury was strongly correlated with total mercury concentrations among the 42 eggs assessed for methylmercury ($R^2 = 0.97$, $p <.001$).

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