

***A Dual Life-Stage Approach to
Monitoring the Effects of Mercury
Concentrations on the Reproductive
Success of Forster's Terns in San
Francisco Bay***

Annual Report



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U. S. GEOLOGICAL SURVEY

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Prepared for:

San Francisco Estuary Institute
Regional Monitoring Program, Exposure and Effects Workgroup
Don Edwards San Francisco Bay National Wildlife Refuge
South Bay Salt Pond Restoration Project
California Department of Fish and Game
U. S. Fish and Wildlife Service
U. S. Geological Survey

Davis, California
[2008]

U.S. DEPARTMENT OF THE INTERIOR
Dirk Kempthorne, Secretary

U.S. GEOLOGICAL SURVEY
Mark Meyer, Director

Suggested citation:

Ackerman, J. T., and C. A. Eagles-Smith. 2008. A Dual Life-Stage Approach to Monitoring the Effects of Mercury Concentrations on the Reproductive Success of Forster's Terns in San Francisco Bay. Annual Report, U. S. Geological Survey, Western Ecological Research Center, Davis, CA 47 pp.

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Acknowledgments:

This research was funded from generous contributions by the Regional Monitoring Program's Exposure and Effects Workgroup administered by the San Francisco Estuary Institute, CALFED Bay-Delta Program's Ecosystem Restoration Program, and the USGS Western Ecological Research Center, and the efforts of Meg Sedlak and Jay Davis. We thank Sarah Stoner-Duncan, Joel Shinn, Kathy Baumberger, Leslie Yen, and Bronwyn McCulloh for field assistance. We also thank Meg Sedlak, Jay Davis, and the San Francisco Estuary Institute, Clyde Morris, Eric Mruz, Cheryl Strong, Joy Albertson, Mendel Stewart, Joelle Buffa, and the staff at the Don Edwards San Francisco Bay National Wildlife Refuge, Carl Wilcox, Larry Wyckoff, John Krause, and the staff of the Eden Landing Ecological Reserve (California Department of Fish and Game), Danielle Le Fer, San Francisco Bay Bird Observatory, Mark Herzog, PRBO Conservation Science, John Takekawa, and Nicole Athearn for logistical support.

EXECUTIVE SUMMARY

Problem Statement

- In order to facilitate long-term monitoring of mercury contamination in San Francisco Bay wildlife, the appropriate sampling tools and biosentinel species need to be developed that are indicative of local exposure conditions and are at risk to potentially impaired reproduction. Additionally, appropriate mercury toxicity thresholds which incorporate risks to multiple lifestages need to be developed.
- Forster's Terns are ideal and practical biosentinel species for monitoring mercury contamination in San Francisco Bay wildlife because they occupy a high trophic position, mainly forage along the Bay's margins where mercury methylation rates are elevated, and nest at high densities at several sites throughout the Bay making sampling eggs logistically feasible.
- Forster's Tern eggs have the highest mercury concentrations of any of the 17 aquatic birds sampled in San Francisco Bay to date; hence, continued monitoring of Forster's Terns is essential for examining risk to wildlife.

Study Results

Objective 1. Link mercury concentrations in eggs to those of down feathers in just-hatched chicks.

- Total mercury concentrations in down feathers were highly correlated with total mercury concentrations in the reconstructed fresh whole-egg homogenate ($n = 94$, $r^2 = 0.96$, $p < 0.0001$).
- Albumen mercury concentrations were correlated with mercury concentrations of down feathers from chicks found in the same nest ($n = 28$, $r^2 = 0.79$, $p < 0.0001$).
- Down feather mercury concentrations in recaptured chicks were correlated with mercury concentrations of down feathers sampled from the same chick during the first capture

event that occurred at hatching ($n = 88$, $r^2 = 0.74$, $p < 0.0001$).

- These results demonstrate the utility of using chick down feather mercury concentrations ($[\text{THg}]_{\text{df}}$; $\mu\text{g g}^{-1}$ fw) to predict concentrations in fresh whole eggs ($[\text{THg}]_{\text{we}}$; $\mu\text{g g}^{-1}$ fww), and vice versa, using the equation: $[\text{THg}]_{\text{we}} = (e^{-2.517 \pm 0.043}) \times [\text{THg}]_{\text{df}}^{0.962 \pm 0.020}$
- Our results demonstrate the utility of using down feathers of chicks up to 10 days of age to non-lethally predict mercury in eggs, and thus provide the ability to develop toxicity thresholds for eggs that incorporate *in ovo* mercury's effects on both egg hatchability and subsequent chick mortality.

Objective 2. Determine toxic thresholds in Forster's Tern eggs by comparing mercury concentrations in randomly collected eggs to concentrations in failed-to-hatch and abandoned eggs.

- In 2007, we monitored 683 Forster's Tern nests at 5 colony sites in San Francisco Bay.
- Forster's Tern nest success varied considerably among colonies from 13% to 72%, and hatching success ranged from 72% to 82%.
- Nest success tended to decline with the geometric mean egg mercury concentration estimated for each colony, but this trend was not statistically significant ($n = 5$ colonies, $r = -0.74$, $p = 0.15$).
- In 2007, fresh egg mercury concentrations ($\mu\text{g g}^{-1}$ fww) were highest in failed-to-hatch eggs (1.73 ± 0.13), followed by abandoned eggs (1.38 ± 0.09), and randomly sampled eggs from successful nests (1.27 ± 0.05 ; $F_{2,129} = 3.09$, $p < 0.05$).
- To improve our statistical power and account for the inherent temporal and spatial variability in mercury levels, we used our archived egg samples and incorporated nest data from our CALFED funded research in 2005 and 2006 to improve our sample size.

- For all 3 years of data, fresh egg mercury concentrations were higher in failed-to-hatch eggs ($1.74 \pm 0.13 \mu\text{g g}^{-1}$ fww) than in randomly sampled eggs from successful nests ($1.20 \pm 0.04 \mu\text{g g}^{-1}$ fww; $F_{2,341} = 13.58, p < 0.001$), but abandoned eggs ($1.43 \pm 0.07 \mu\text{g g}^{-1}$ fww) did not differ significantly from either failed-to-hatch or random eggs (all $p > 0.05$).

Objective 3. Examine effects of mercury on chick mortality by comparing mercury concentrations in down feathers of alive and dead Forster's Tern chicks.

- In 2007, we captured and banded 358 individual Forster's Tern chicks and found (salvaged) 73 dead chicks at ≤ 10 days of age.
- Colony-wide mortality of chicks tended to increase with mercury concentrations in live chick down feathers, but this trend was not statistically significant ($n = 5$ colonies, $r = 0.65, p = 0.27$).
- Geometric mean mercury concentrations in known-dead chicks ($20.64 \pm 1.07 \mu\text{g g}^{-1}$ dw) tended to be higher than those that were assumed to have died ($20.03 \pm 0.71 \mu\text{g g}^{-1}$ dw) or survived ($19.82 \pm 0.91 \mu\text{g g}^{-1}$ dw), but these results were not statistically significant ($F_{2,246} = 0.26, p = 0.77$).

Conclusions and Management Implications

- Forster's Terns were recently added to the San Francisco Bay Regional Monitoring Program's long-term plan for avian contaminant monitoring. In order for these egg monitoring results to be related to potential toxicity risk, egg toxicity levels for Forster's Terns should be established.
- Our results indicate that current mercury exposure is likely causing reduced hatching success in Forster's Terns nesting in San Francisco Bay. Approximately 27% of all Forster's Tern eggs sampled exceeded the geometric mean mercury concentration for failed-to-hatch eggs ($1.74 \pm 0.13 \mu\text{g g}^{-1}$ fww), and 97% of tern eggs were above the San Francisco Bay's current TMDL monitoring target for eggs ($0.50 \mu\text{g g}^{-1}$ ww).

- However, establishing the egg toxicity threshold is difficult due to high variability among years and colony sites, indicating the importance of defining the egg toxicity threshold over multiple years and sites.
- We also have shown a strong predictive link between mercury concentrations in chicks and eggs, suggesting that incorporating effects of mercury to both lifestages into a single tissue matrix – *eggs* – is possible once these thresholds are established.
- Continued assessment and monitoring of Forster's Tern mercury exposure and reproduction are warranted.

A DUAL LIFE-STAGE APPROACH TO MONITORING THE EFFECTS OF MERCURY CONCENTRATIONS ON THE REPRODUCTIVE SUCCESS OF FORSTER'S TERNS IN SAN FRANCISCO BAY

Annual Report

By Josh T. Ackerman and Collin A. Eagles-Smith

INTRODUCTION

The San Francisco Bay Estuary has a legacy of mercury contamination from historical mining activities (reviews by Davis et al. 2003, Wiener et al. 2003a) that has resulted in elevated mercury concentrations in several waterbird species. Reproduction is the most sensitive endpoint of mercury toxicity in birds, and it is thought that current levels may impair the reproduction of waterbirds breeding within the estuary (Schwarzbach et al. 2006, Ackerman et al. 2007a, 2008a,b).

Recent CALFED-funded research by the U. S. Geological Survey (USGS) has provided evidence that several waterbird populations are at high risk to mercury contamination (Ackerman et al. 2007a). For example, as many as 58% of the Forster's Terns breeding in the South San Francisco Bay are considered to be at high-risk or extra high-risk to mercury contamination due to present-day mercury concentrations in their blood (Figure 1).

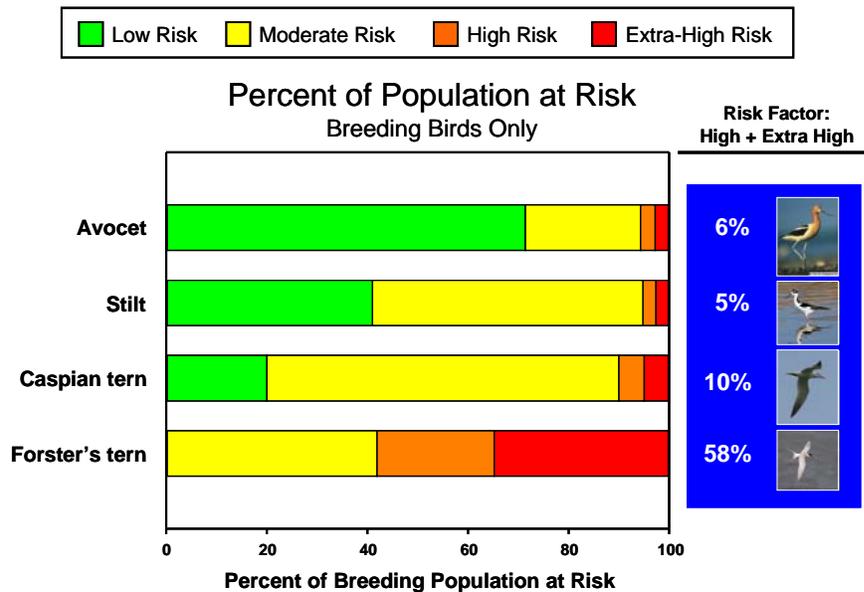


Figure 1. Percentage of waterbird breeding populations that are at risk to reduced reproductive success and declining populations due to mercury contamination in the South San Francisco Bay. Data are from birds captured on nests while incubating; blood was used as the sample matrix (Ackerman et al. 2007a).

Forster's Terns are ideal biosentinel species to indicate mercury contamination and risk in San Francisco Bay wildlife because, as fish eaters, they occupy a relatively high trophic position within the Bay's food web, and, as such, bioaccumulate mercury to potentially toxic levels. Additionally, Forster's Terns forage mainly within the Bay's margins in shallow water habitats, such as salt ponds and marshes (Figure 2, Ackerman et al. 2008b), where mercury methylation rates are elevated. Forster's Terns also nest at relatively high densities at several sites within the Bay, making sampling logistically feasible. Therefore, Forster's Terns are effective and practical biosentinel indicators of mercury contamination and risk to wildlife within the San Francisco Bay.

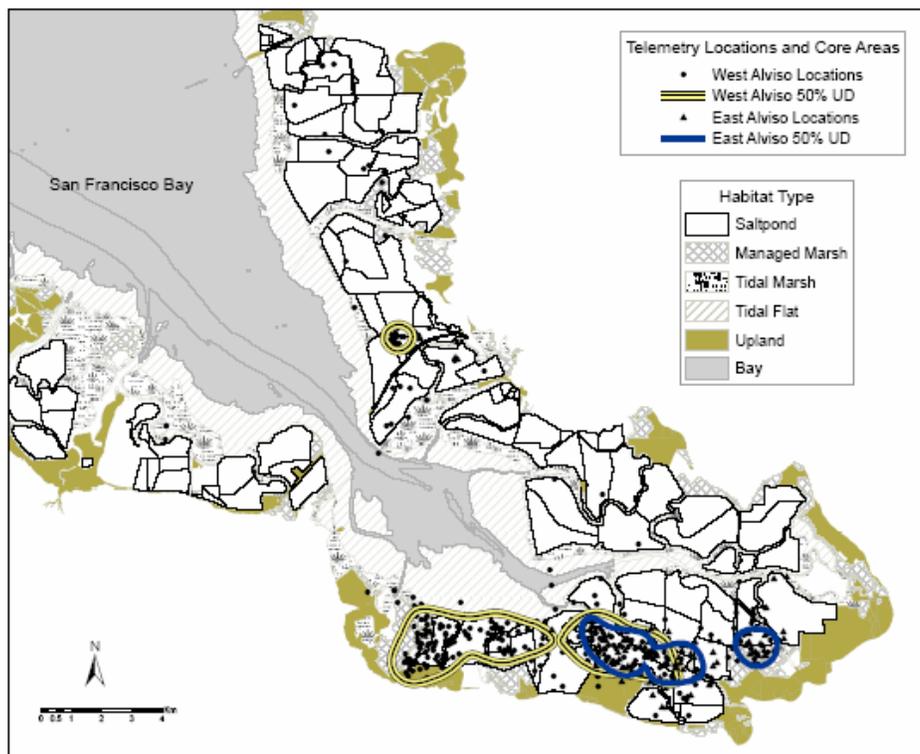


Figure 2. Foraging locations of Forster's Terns in the South San Francisco Bay in 2006. Terns mainly foraged within salt pond and marsh habitats along the Bay's margins. Modified from Ackerman et al. 2008b.

Furthermore, Forster's Tern egg mercury concentrations are the highest of any aquatic bird sampled in the San Francisco Bay (Figure 3). As this report will demonstrate, current mercury concentrations are likely causing reduced egg hatchability and may be reducing chick survival as well. Therefore, any monitoring program within the San Francisco Bay Estuary should incorporate those species that biomagnify mercury to the highest concentrations and are currently

experiencing toxicological effects. Forster's Terns also are related to the endangered California Least Tern, and can act as a biosentinel for exposure to these endangered species which are identified in the San Francisco Bay methylmercury TMDL as species of critical concern that are thought to be at especially high risk (California Regional Water Quality Control Board San Francisco Bay Region 2006). Thus, continued monitoring of Forster's Tern mercury concentrations is essential for examining risk to wildlife within the San Francisco Bay Estuary.

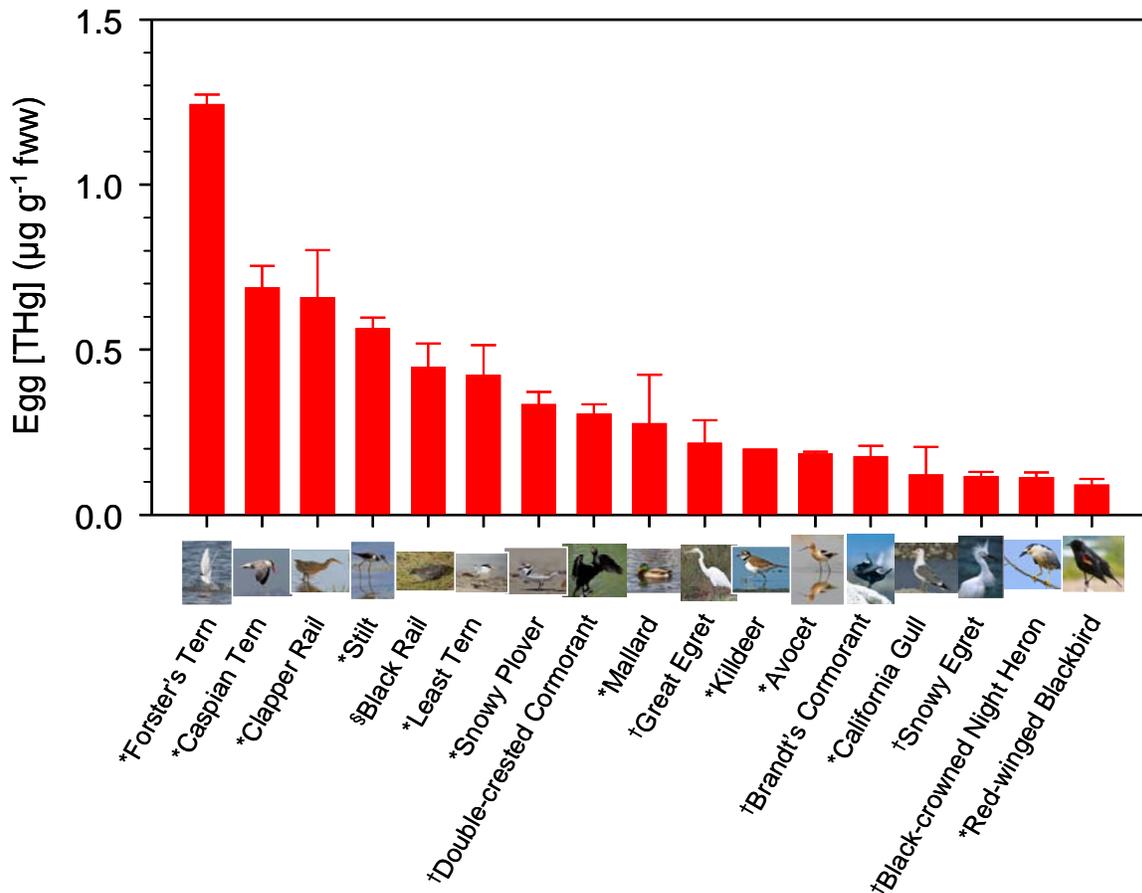


Figure 3. Geometric \pm SE mean mercury concentrations in 17 species of aquatic bird eggs ($\mu\text{g g}^{-1}$ fresh wet weight [fww]) in the San Francisco Bay Estuary, California. Of the birds studied, Forster's Terns have the highest egg mercury concentrations, other fish and invertebrate eating waterbirds have moderate mercury concentrations, and aquatic dependent songbirds have the lowest mercury concentrations. *unpublished data from Ackerman and Eagles-Smith. †data from Schwarzbach and Adelsbach 2003. §data from Tsao et al. 2008.

Unfortunately, the effects of mercury on avian reproductive success are difficult to quantify in the wild and have been little studied, particularly within the San Francisco Bay estuary.

Moreover, the development of an appropriate monitoring matrix that encompasses sensitive

reproductive endpoints, such as egg hatchability and chick survival, has yet to be accomplished.

OBJECTIVES

In order to facilitate long-term monitoring of mercury contamination in San Francisco Bay wildlife and link exposure to effects on avian reproduction, we need to develop sampling tools for biosentinel species that can be effectively applied in the field as well as that relate mercury concentrations to toxic threshold levels in sensitive life-stages, such as eggs and chicks.

Together, the three objectives detailed below will allow scientists and managers to monitor mercury concentrations in one tissue-matrix – *eggs* – and assess potential effects on both egg hatchability and chick survival.

Objective 1. Link mercury concentrations in eggs to those of down feathers in newly-hatched chicks.

Egg toxicity thresholds typically have been based on egg hatchability (Fimreite 1971, Heinz and Hoffman 2003a, Albers et al. 2007, Heinz et al. 2008). Yet, *in ovo* mercury exposure not only can affect egg survival, but it can also effect subsequent chick growth, behavior, and survival after hatching (Heinz 1974, Heinz 1979). For example, Kenow et al. (2003) conducted a dose-response study to quantify the effects of mercury exposure on chick development, although they found no effects of dietary mercury exposure on chick growth. Instead, they concluded that the observed reductions in asymptotic chick mass and health indices likely were due to *in ovo* mercury exposure (Kenow et al. 2003, 2007a). Incorporating the effects of *in ovo* mercury exposure on egg hatchability and subsequent chick survival into a single tissue matrix is difficult because it involves translating mercury concentrations from one avian life-stage to another. If this were possible, then egg toxicity thresholds potentially could be refined to incorporate mercury effects on both eggs and chicks.

Chicks are especially vulnerable to the effects of residual *in ovo* mercury exposure shortly after hatching when maternally deposited mercury levels are still relatively high. Thereafter, chick mercury concentrations rapidly decline as chicks age and dilute their body burden of mercury through growth in size and depuration into growing feathers (Monteiro and Furness 2001,

Fournier et al. 2002, Ackerman et al. 2007a, Kenow et al. 2007b). Chick mortality associated with mercury contamination often occurs within the first week after hatching (Heinz 1974, Finley and Stendell 1978, Ackerman et al. 2008a), indicating that *in ovo* mercury exposure can influence post-hatch survival. Incorporating this early chick mortality into egg toxicity thresholds is hampered by our inability to translate mercury concentrations in chicks to equivalent concentrations in eggs.

Down feathers of newly hatched chicks are potentially useful tools for estimating mercury concentrations in the eggs from which they hatched. Down feathers are grown *in ovo* during the embryonic phase and contain about 38% of the total body burden of mercury in newly hatched chicks (Becker et al. 1993, 1994). Mercury concentrations in down feathers can be correlated with whole-body mercury burdens in chicks (Becker et al. 1993), and therefore may also be useful for estimating whole-egg mercury concentrations. If down feather mercury concentrations are, in fact, correlated with concentrations in the whole-egg, then down feathers can be sampled non-lethally as a proxy for egg mercury concentrations in studies assessing the effect of *in ovo* mercury exposure on subsequent chick mortality.

In *Objective 1*, we developed equations to predict mercury concentrations in eggs using mercury concentrations in chick down feathers, and vice versa.

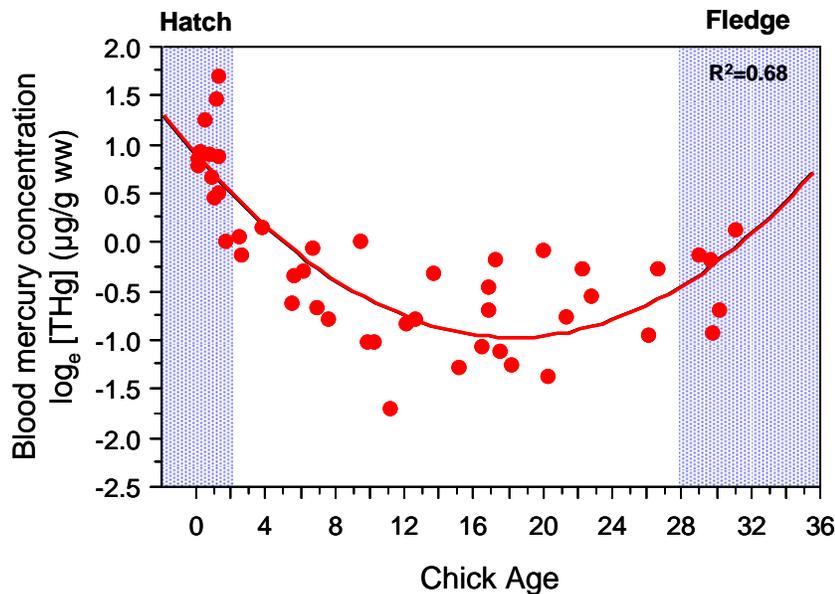


Figure 4. Forster's Tern chick blood mercury concentrations in relation to chick age. Note: Mercury concentrations are log transformed. Ackerman et al. 2007a.

Objective 2. Determine toxic thresholds in Forster's Tern eggs by comparing mercury concentrations in randomly collected eggs to concentrations in failed-to-hatch and abandoned eggs.

Egg hatchability is the most commonly measured endpoint of the effect of mercury on impaired reproduction (Heinz and Hoffman 2003a, Heinz et al. 2008). However, mercury also has been shown to affect parental nest attendance and tenacity, and may cause increased nest abandonment rates (Evers et al. 2004). Despite considerable laboratory work to develop toxicity thresholds for bird eggs (Fimreite 1971, Heinz and Hoffman 2003a, Albers et al. 2007, Heinz et al. 2008), there still are no defined thresholds where mercury concentrations negatively affect egg hatchability for birds breeding in San Francisco Bay. Furthermore, toxicity thresholds developed using laboratory egg-injection techniques for other species are considered inappropriate for application to wild birds because the injected mercury is thought to be much more toxic than maternally derived mercury (Heinz et al. 2008). Instead these laboratory techniques are most useful in assessing the relative sensitivities of avian embryos to mercury toxicity among species (Heinz et al. 2008).

Heinz et al. (2008) assessed the relative sensitivities of 26 species of birds to methylmercury chloride injected into the egg. Although they did not test embryo sensitivity to mercury specifically for Forster's Terns, they did find that three closely related tern species (Common Tern, Royal Tern, and Caspian Tern) were at moderate sensitivity to mercury exposure. The LC_{50} for Common Terns was $0.87 \mu\text{g g}^{-1} \text{ ww}$ and $0.40 \mu\text{g g}^{-1} \text{ ww}$ for Royal Terns (there was not enough data for an accurate LC_{50} estimate for Caspian Terns). These toxic concentrations are far below the geometric mean \pm SE mercury concentrations observed in all Forster's Terns eggs in San Francisco Bay between 2005 and 2007 ($1.29 \pm 0.02 \mu\text{g g}^{-1} \text{ fww}$; Ackerman and Eagles-Smith, unpublished data). Hence, there is cause for concern that mercury may be impairing the hatchability of tern eggs in the Bay.

In *Objective 2*, we compared mercury concentrations in randomly collected eggs from successful nests to concentrations in failed-to-hatch and abandoned eggs.

Objective 3. Examine effects of mercury on chick mortality by comparing mercury concentrations in down feathers of alive and dead Forster's Tern chicks.

Chicks also are a sensitive life-stage for mercury toxicity, and *in ovo* mercury concentrations that do not impact hatchability may still impair subsequent chick growth, behavior, and survival (Heinz 1974, 1979; Kenow et al. 2003). Data from our CALFED-funded study demonstrated that mercury concentrations in Forster's Tern chick's blood are extremely elevated during the first few days after hatching and then rapidly decline as chicks age and dilute their body burden of mercury through growth in size and depuration into growing feathers (Figure 4; Ackerman et al. 2007a). Mercury concentrations in chick's blood begin to rapidly increase again when feather and body growth slows near the age of fledgling (about 28 days old). Our research also has shown that chick mortality mainly occurs at ages where mercury is elevated in blood – either just after hatching or during fledging. Chick down feathers are a valuable sample matrix because they are formed *in ovo* during embryo development and, hence, may be related to egg mercury concentrations (*Objective 1*). Therefore, down feathers likely provide a valuable link between egg mercury concentrations and chick survival.

In *Objective 3*, we compared mercury concentrations in down feathers of alive, apparently healthy chicks to concentrations in dead chicks.

STUDY AREA

We studied mercury concentrations in eggs and chicks of Forster's Terns during the 2005-2007 nesting seasons (April to August) in South and North San Francisco Bay, California (Figure 5). Our main study site was in the South Bay at the Don Edwards San Francisco Bay National Wildlife Refuge (37.4° N, 122.0° W). Most tern colonies occurred on islands within former salt evaporation ponds within the Alviso (Ponds A5, A7, A8, A16), Newark (Ponds N6, N7) and Moffett (Pond A1) salt pond complexes of the Don Edwards San Francisco Bay National Wildlife Refuge, Eden Landing Ecological Reserve (Ponds B4, B7), or Napa-Sonoma Marsh Wildlife Area (Pond 2).

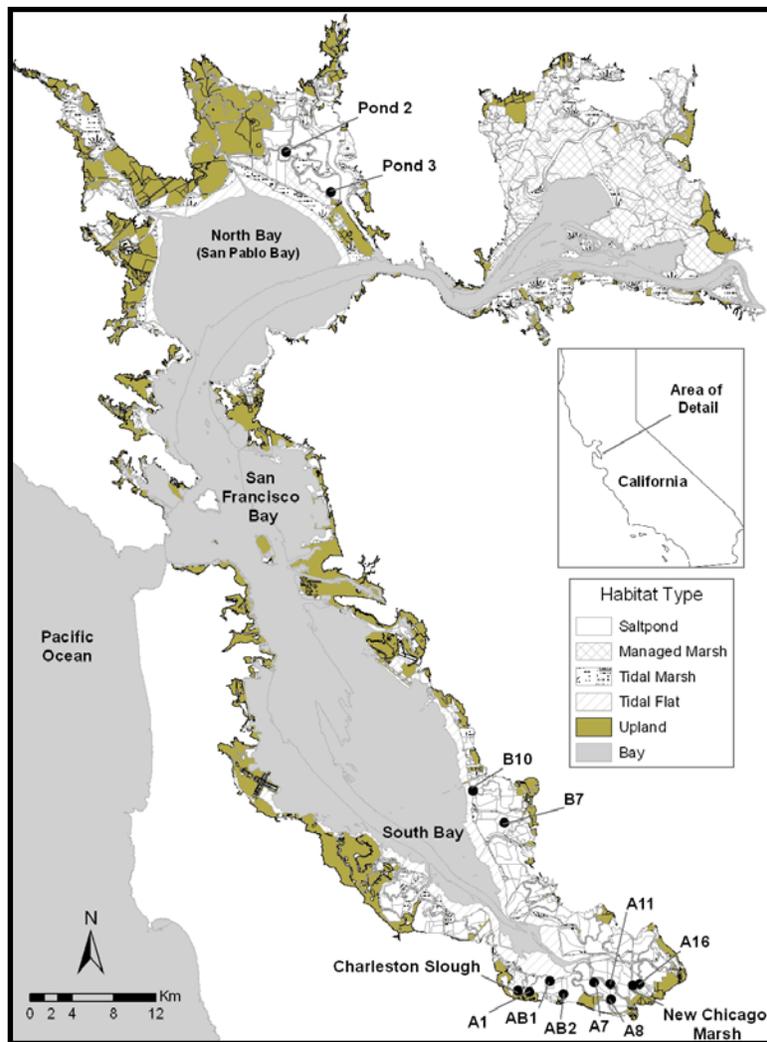


Figure 5. Map of Forster's Tern colonies monitored for eggs and chicks in San Francisco Bay. Additional tern colonies are located throughout the bay in several other locations, but were not monitored.

METHODS

Objective 1. *Link mercury concentrations in eggs to those of down feathers in newly-hatched chicks.*

We significantly expanded *Objective 1* beyond the original proposal's scope in three ways. First, we completed this objective for three species (including American Avocets and Black-necked Stilts), rather than just for Forster's Terns. Including the two additional species allowed us to observe the egg to chick correlations over a much wider range of mercury concentrations than had we only sampled terns. Second, we conducted a similar egg to chick study by using

subsampled egg albumen and newly hatched chicks. This further strengthened our primary (egg to chick) result by alleviating any concerns about our sampling eggs just before they hatched at the pipping stage. Third, we assessed whether mercury concentrations in down feathers changed as chicks age, possibly due to the continued production of down feathers after hatching, in order to identify the appropriate age(s) for sampling feathers from chicks. Together, these three studies within *Objective 3* provide a robust equation to estimate egg mercury concentrations using chick down feather samples.

Whole Egg and Chick Down Feather Mercury Concentrations

To link mercury concentrations in down feathers of chicks to whole-egg concentrations, we collected eggs immediately before they hatched. We attempted to collect eggs of each species at several nesting sites to yield a range of mercury concentrations common in San Francisco Bay (Ackerman et al. 2007a, Ackerman et al. 2008a, Ackerman et al. 2008b). We entered colonies weekly and marked each new nest we found with a uniquely numbered anodized aluminum tag (Ben Meadows Company, Janesville, WI, USA) placed at the nest and a colored pin flag placed 2 m from the nest. We recorded Universal Transverse Mercator coordinates of each nest site (Garmin GPSMAP 76, Garmin International Inc., Olathe, KS, USA) to facilitate re-location of the nest. Each nest was re-visited once every seven days, the stage of embryo development was determined by floating (Hays and LeCroy 1971, Alberico 1995), and clutch size and nest fate (hatched, depredated, or abandoned) were determined. For those nests that were nearing the full incubation term (about 24 days), we randomly collected one egg from nests that were at the 1- to 4-star pipping stage (also called pip to ring stage; about 21-24 days in incubation; Robinson et al. 1997, Robinson et al. 1999, McNicholl et al. 2001). Collecting pipping eggs within three days of hatching ensured that we could assess mercury concentrations in both the whole-egg homogenate and down feathers from the fully developed embryo. We stored collected eggs in the refrigerator for less than 10 days prior to processing. We opened each egg with acid-rinsed, stainless steel scissors and removed all egg contents into a 2-ounce polypropylene jar. Using tweezers, we removed about 15 down feathers (0.03 ± 0.01 [SD] g) from the mantle of each chick and placed them in polypropylene cryovials. We then immediately froze both the egg and feathers until processing and analysis of mercury concentrations.

Micro-Sampled Egg and Chick Down Feather Mercury Concentrations

Because mercury concentrations in down feathers collected from pipping chicks, still in the egg, could be different than mercury concentrations in down feathers collected from chicks once they had hatched, we also used a non-lethal egg sampling technique, called micro-sampling, to verify that egg mercury concentrations were correlated with down feathers in recently hatched chicks. We have described the methodological details of this technique elsewhere (Stebbins et al., in press), so we will only briefly describe it here. During our routine nest monitoring procedures (described above), we selected nests where all eggs were ≤ 3 days in incubation as determined from floating (Hays and LeCroy 1971). We randomly selected one egg from the clutch for micro-sampling, dipped the egg in a dilute betadine (1%) solution, and wiped it clean with isopropanol. Using a handheld cordless rotary tool (Dremel Rotary Tool, 7.2V Cordless MultiPro, Racine, WI, USA) with a diamond-tipped grinding bit, we breached the egg shell in two locations: one hole was placed at the top of the egg above the air cell to act as a vent during albumen extraction and the other hole was placed one-third of the way up from the bottom of the egg as a site for albumen extraction. We then used a sterile 20-gauge needle attached to a 1-ml sterile syringe to carefully extract 200 μl (Forster's terns and stilts; about 1.1% of egg content fresh mass) or 300 μl (avocets; about 1.1% of egg content fresh mass) of albumen from the egg. We quickly sealed the extraction hole and the vent hole with a cordless hot glue gun (ColdHeat™ Cordless Glue Gun, Bellevue, WA, USA) and then applied a layer of cyanoacrylate glue over the hot glue to ensure an adequate seal when parents rotated the egg during incubation. We marked the egg we sub-sampled with a blue permanent marker and then returned the egg to its nest once the glue had dried. The albumen micro-sample was transferred to a clean cryovial and stored on ice until it was returned to the laboratory within 5 hrs. Thereafter, the albumen was stored in a freezer at -20°C until mercury analysis.

To match the albumen sample with a down feather sample from the same chick, we returned to the nest weekly to monitor the embryo's development. When the clutch hatched, we attempted to collect down feathers (mantle) from the same chick that hatched from the micro-sampled egg. However, this was not always possible, especially when multiple eggs hatched in the clutch before we had revisited the nest. Therefore, we categorized the down feather sample into one of three groups: feathers known to be sampled from the chick hatching from the micro-sampled

egg, feathers known to be sampled from a sibling (not micro-sampled) egg, and unknown feathers sampled either from the micro-sampled or sibling egg. We considered the sibling and unknown feather samples still to be useful, since sibling egg mercury concentrations are often highly correlated, however we acknowledge that intra-clutch variation in mercury concentrations in down feathers can be 10-39% (Becker 1992, Becker et al. 1994).

Mercury Concentrations in Down Feathers as Chick Age

In order to use down feather mercury concentrations to predict egg mercury concentrations, it is necessary to understand if and how mercury concentrations change in down feathers as chicks age. Therefore, we used Forster's tern chicks and mark-recapture methodology to assess whether mercury concentrations in down feathers changed as chicks aged. Monitoring methods for chicks are described below in *Objective 3*. Our weekly colony monitoring yielded two down feather samples taken from the same individual separated by 7 days. For this analysis, we only used those chicks that were first captured at 0-3 days of age when they were first found, thus the maximum age of chicks during the subsequent recaptures were 7-10 days of age.

Statistical Analyses for Objective 1

We reconstructed mercury concentrations in the whole egg by combining mercury concentrations determined for the down feathers sampled from pipping chicks and the remaining whole egg-homogenate. To do so, we weighed (dry weight; dw) the entire sample of down feathers removed from the embryo (M_{df}) and the remaining whole-egg homogenate (M_{eh}) separately before determining their respective mercury concentrations (dw; accuracy to 0.0001 g). We then multiplied the weight of the down feathers removed from the embryo by its specific mercury concentration ($[THg]_{df}$) and added the product of the weight of the remaining whole-egg homogenate (dw) and the average mercury concentration of three sub-samples of the whole-egg homogenate ($[THg]_{eh}$). This resulted in the total mercury burden in the whole egg, and we divided this quantity by the combined mass (dw) of the removed down feathers and the remaining whole-egg homogenate to yield the mercury concentration of the reconstructed whole-egg homogenate at pipping ($[THg]_{we}$ dry weight at pipping; Equation 1).

$$\text{Equation 1: } [THg]_{we} \text{ dry weight at pipping} = ((M_{df})([THg]_{df}) + (M_{eh})([THg]_{eh})) / (M_{df} + M_{eh})$$

Next, we converted the mercury concentration of the reconstructed whole-egg homogenate at pipping from a dry weight to a wet weight (ww) basis using Equation 2:

$$\text{Equation 2: } [\text{THg}]_{\text{we}} \text{ wet weight at pipping} = ([\text{THg}]_{\text{we}} \text{ dry weight at pipping}) \times (1 - [\% \text{ moisture}/100])$$

Since pipping eggs may have lost a substantial amount of mass from the time of laying (due to respiration and moisture loss), we then adjusted the wet weight mercury concentration of the reconstructed whole-egg homogenate at pipping ($[\text{THg}]_{\text{we}}$ wet weight at pipping) to a fresh egg wet weight mercury concentration (fww) by dividing the total mass (ww) of the egg content at processing (M_{ec}) by the predicted fresh egg mass (ww) at laying (M_{fe}) and multiplying that value by the wet weight mercury concentration at pipping (following Stickel et al. 1973; Equation 3):

$$\text{Equation 3: } [\text{THg}]_{\text{we}} \text{ fresh wet weight} = [\text{THg}]_{\text{we}} \text{ wet weight at pipping} \times (M_{\text{ec}}/M_{\text{fe}})$$

The fresh egg mass (ww) was estimated using egg morphometrics following Hoyt (1979) using Equation 4:

$$\text{Equation 4: } M_{\text{fe}} = 0.548 \times \text{egg length} \times \text{egg width}^2$$

We used Analysis of Covariance (ANCOVA, JMP® version 4.0.4; Sall et al. 2001) to examine whether there was an interaction between the effects of species and the reconstructed whole-egg homogenate mercury concentrations on down feather total mercury concentrations. We then used linear regression to test whether: 1) mercury concentrations in down feathers were correlated with concentrations in the reconstructed fresh whole-egg homogenate, 2) mercury concentrations in albumen were correlated with concentrations in down feathers sampled from chicks found in the same nest, and 3) mercury concentrations in down feathers sampled from recaptured chicks were correlated with concentrations in down feathers sampled from chicks captured for the first time. We used t-tests to determine whether regression slope coefficients differed from a value of one. Lastly, we used ANCOVA to examine whether fresh wet weight mercury concentrations differed between pipping eggs and randomly sampled eggs, and we

controlled for colony site and calendar date statistically by including them as main effects in the model. All data were \log_e -transformed for analysis, and we report all egg concentrations in fresh wet weight (fww); the mean (\pm SE) moisture content in pipping eggs was $74.85 \pm 0.20\%$ (avocet), $74.03 \pm 0.44\%$ (stilt), and $78.71 \pm 0.18\%$ (Forster's terns). Albumen is reported in wet weight (ww), and down feathers are reported in fresh weight (fw).

Objective 2. Determine toxic thresholds in Forster's Tern eggs by comparing mercury concentrations in randomly collected eggs to concentrations in failed-to-hatch and abandoned eggs.

We monitored Forster's Tern nests at several sites from late April to August in 2007. We entered colonies weekly and each new nest we found was marked with a uniquely numbered anodized aluminum tag (Ben Meadows Company, Janesville, WI) placed at the nest and a colored pin flag placed 2 m from the nest. We recorded Universal Transverse Mercator coordinates of each nest site (Garmin GPSMAP 76, Garmin International Inc., Olathe, KS) to facilitate re-location of the nest. Each nest was re-visited once every seven days, the stage of embryo development was determined by floating (Hays and LeCroy 1971, Alberico 1995), and clutch size, overall nest fate (hatched, failed, abandoned, or depredated), and the fate of each individual egg (hatched, failed-to-hatch, abandoned, or depredated) was determined.

To assess whether mercury concentrations in failed-to-hatch and abandoned eggs were higher than expected, we randomly collected one egg from several nests at the 9 to 12 day incubation stage. We then followed the fate of the remaining eggs in the nest (average clutch size is 2.9 eggs) and classified the nest as successful if ≥ 1 of the remaining eggs hatched. For our random egg sample, we used only those random eggs that were collected from successful nests. In addition, we collected failed-to-hatch and abandoned eggs during routine nest monitoring. We defined failed-to-hatch eggs as those eggs that did not successfully hatch despite the fact that other siblings within the clutch successfully hatched. Importantly, eggs from nests that were depredated, abandoned, or where all eggs were either infertile or dead were excluded from our definition of failed-to-hatch eggs. We defined abandoned eggs as those clutches that were naturally abandoned by their parents without any obvious sign of depredation, disturbance, or

flooding. Additionally, we excluded from our analyses any eggs that contained signs of physical damage, such as cracks or dents in the shell, and any heavily decomposed eggs or highly desiccated eggs with moisture content as later determined to be below 70%, to ensure that comparable samples were used across all egg categories.

After egg collection, we stored eggs in the refrigerator until processing within three months. Before processing we measured the length and breadth of each egg (± 0.01 mm) with digital calipers, and the whole mass of each egg (± 0.01 g) with a digital balance. We opened each egg with scissors, removed all egg contents into a tared two ounce polypropylene jar, and recorded egg content mass. We then immediately froze the egg until mercury analysis which was completed within seven months of egg collection.

Statistical Analyses for Objective 2

We used ANCOVA to test whether egg mercury concentrations differed among the three experimental groups: random eggs from successful nests, failed-to-hatch eggs, and abandoned eggs. We included colony site and year as covariates to control for their potential effects on egg hatchability and mercury levels.

Objective 3. Examine effects of mercury on chick mortality by comparing mercury concentrations in down feathers of alive and dead Forster's Tern chicks.

During the chick rearing period, we accessed Forster's Tern colonies weekly. At each visit, we hand-captured every chick at the colony, banded newly hatched chicks with stainless steel U. S. Geological Survey leg bands or recorded band numbers from recaptured chicks, and collected 10-15 down feathers from the chick's rump for mercury analysis. We also measured the structural size of chicks (mm) at each visit in order to estimate their age using an equation we developed based on Forster's Tern chicks with known hatching dates ($\text{chick age [days]} = [0.11 \times \text{wing chord}] + [1.11 \times \text{culmen}] - [0.018 \times \text{culmen}^2] + [1.34 \times \text{tarsus}] - [0.035 \times \text{tarsus}^2] - 22.15$; $n = 472$, $r^2 = 0.98$; J. T. Ackerman, unpublished data). We measured exposed culmen and short tarsus (tarsometatarsus bone) lengths with digital calipers (± 0.01 mm with Fowler® electronic digital calipers, Newton, Massachusetts, USA) and flattened wing length with a wing board

(± 1.0 mm). We also salvaged all the dead tern chicks we found on or near the nesting colonies, and sampled their down feathers and measured their structural size to estimate their age as described above. We evaluated the fate of each banded chick and categorized fates as: survived, assumed dead, known dead, and unknown. We considered a chick to have survived if its age at its last capture was ≥ 18 days, since on the following visit 7-days later it would have been 25 days of age or older and they fledge at 25-28 days of age. We assumed a chick to have died if it was ≤ 10 days old at their initial capture and it was never recaptured again. We considered a chick to have definitively died if its body was recovered at any point after banding. For chicks that were recaptured at least once, but whose age at last capture was ≤ 18 days, we classified their fate as unknown.

Statistical Analyses for Objective 3

We used ANCOVA to test whether mercury concentrations in down feathers differed among three experimental groups: survived, assumed dead, and known dead. We included colony site and date as covariates to control for their potential effects on chick survival. We controlled for the potential effect of age by including only those chicks that were ≤ 10 days of age.

Mercury Determination

We analyzed down feathers and remaining whole-egg homogenate samples for total mercury (U. S. Geological Survey, Davis Field Station Mercury Lab), since more than 95% of mercury in avian eggs and feathers is methyl mercury (Thompson and Furness 1989, Heinz and Hoffman 2004, Evers et al. 2005). Prior to analysis, we thawed albumen samples to room temperature and ensured sample homogeneity by inverting the cryovials several times and thoroughly mixed the albumen by stirring with a clean pipette tip. We pipetted 50-100 μl of albumen from each cryovial and weighed (to the nearest 0.0001 g, Ohaus Adventurer Balance, model AR0640, Ohaus Corporation, Pine Brook, New Jersey, USA) each aliquot into a nickel or quartz sample vessel. For eggs, we measured the length and breadth of each egg to the nearest 0.01 mm using digital calipers and measured a total egg weight to the nearest 0.01 g on a digital balance. We then carefully cut an approximately 25 mm diameter hole in the top of the egg using clean, stainless steel scissors and removed the embryo and any remaining contents into a clean, glass petri dish with stainless steel forceps. Total content mass was measured with a digital balance to

the nearest 0.01 g. After sub-sampling down feathers, the embryo and remaining egg contents were stored frozen as described above. Prior to analysis, we dried the entire egg contents at 50-60°C for 48 hrs or until completely dried and re-weighed the egg contents to determine moisture content. We then ground the dried egg contents to a powder in a Wiley mill, followed by further grinding in a mortar and pestle. Just prior to analysis we weighed 0.02-0.05 g of homogenized sample into a nickel sample vessel to the nearest 0.0001 g. For feathers, we washed and mechanically scrubbed each feather in a 1% Alconox solution (Alconox, White Plains, NY, USA) to remove surface debris. We then dried the feathers at 60°C for 24 hrs, weighed them to the nearest 0.0001 g (Mettler Toledo, Model AT201, Greifensee, Switzerland) and transferred each feather sample into a quartz sample vessel. Following EPA Method 7473 (U. S. EPA 2000), we analyzed each albumen, egg, and feather sample for total mercury on a Milestone DMA-80 Direct Mercury Analyzer (Milestone Inc., Monroe, Connecticut, USA) as described in Ackerman et al. (2007b, 2008c). Quality assurance measures included analysis of two certified reference materials, two system and method blanks, two duplicates, one matrix spike, and one matrix spike duplicate per sample batch. Recoveries of certified reference materials, calibration checks, and matrix spikes, respectively, averaged (\pm SE) $98.47 \pm 0.78\%$ ($n = 33$), $102.36 \pm 1.09\%$ ($n = 49$), and $103.02 \pm 1.51\%$ ($n = 17$). Absolute relative percent difference for all duplicates and matrix spike duplicates, respectively, averaged (\pm SE) $5.23 \pm 1.54\%$ ($n = 7$) and $4.86 \pm 2.10\%$ ($n = 4$) for down, and $3.65 \pm 0.66\%$ ($n = 25$) and $2.44 \pm 0.45\%$ ($n = 13$) for eggs.

RESULTS AND DISCUSSION

Objective 1. Link mercury concentrations in eggs to those of down feathers in newly-hatched chicks.

Whole Egg and Chick Down Feather Mercury Concentrations

We collected 94 pipping eggs at 22 ± 1.5 (SD) days in incubation (Figure 6). Total mercury concentrations (mean \pm SD) in down feathers were $2.88 \pm 2.06 \mu\text{g g}^{-1}$ fw for avocets (range: 0.47 - $8.75 \mu\text{g g}^{-1}$ fw), $5.96 \pm 2.97 \mu\text{g g}^{-1}$ fw for stilts (range: 2.61 - $13.69 \mu\text{g g}^{-1}$ fw), and $18.32 \pm 5.60 \mu\text{g g}^{-1}$ fw for terns (range: 9.20 - $32.39 \mu\text{g g}^{-1}$ fw). We sub-sampled the remaining whole-egg homogenate and determined mercury concentrations in each of the three sub-samples to reduce

any variation that might occur due to the advanced stage of embryo development and mercury partitioning among different tissues. However, we found little variation among our three sub-samples of the remaining whole-egg homogenate; the coefficient of variation among the three egg sub-samples averaged 2.5% (range: 0.1-9.6%). Total mercury concentrations (mean \pm SD) in the reconstructed fresh whole-egg homogenate was $0.23 \pm 0.15 \mu\text{g g}^{-1}$ fww for avocets (range: 0.04-0.62 $\mu\text{g g}^{-1}$ fww), $0.43 \pm 0.21 \mu\text{g g}^{-1}$ fww for stilts (range: 0.15-0.95 $\mu\text{g g}^{-1}$ fww), and $1.35 \pm 0.47 \mu\text{g g}^{-1}$ fww for terns (range: 0.69-2.79 $\mu\text{g g}^{-1}$ fww).



Figure 6. Forster's Tern nest with a pipping egg.

To assess the relationship between total mercury concentrations in down feathers and those in the reconstructed fresh whole-egg homogenate (fww), we first tested whether there were species differences in the relationships. There was no interaction between species and reconstructed whole-egg homogenate mercury concentrations on down feather total mercury concentrations (ANCOVA: species x egg: $F_{2,88} = 1.44$, $p = 0.24$; species: $F_{2,88} = 5.31$, $p = 0.01$; egg: $F_{1,88} = 306.06$, $p < 0.0001$). We therefore pooled all data among species to test whether total mercury concentrations in down feathers were correlated with concentrations in the reconstructed whole-egg homogenate. Total mercury concentrations in down feathers were highly correlated with total mercury concentrations in the reconstructed fresh whole-egg homogenate (linear regression: $n = 94$, $r^2 = 0.96$, $p < 0.0001$; Figure 7). After back-transforming the linear regression equations, the equations for predicting chick down total mercury concentrations ($[\text{THg}]_{\text{df}}$; $\mu\text{g g}^{-1}$ fw) from fresh whole-egg total mercury concentrations ($[\text{THg}]_{\text{we}}$; $\mu\text{g g}^{-1}$ fww) were:

$$\text{Equation 5: } [\text{THg}]_{\text{df}} = (e^{2.590 \pm 0.025}) \times [\text{THg}]_{\text{we}}^{1.000 \pm 0.021}$$

and, conversely,

$$\text{Equation 6: } [\text{THg}]_{\text{we}} = (e^{-2.517 \pm 0.043}) \times [\text{THg}]_{\text{df}}^{0.962 \pm 0.020}$$

The slope coefficient (from Equation 5) of 1.000 ± 0.021 (SE) did not differ from 1.0 ($t_{92}=0.01$, p

= 0.99).

To examine the potential for differential mercury partitioning into down feathers, we also performed the linear regression analysis using the dry weight total mercury concentration of the reconstructed whole-egg homogenate at pipping, rather than the fresh wet weight egg mercury concentration. The linear regression equation (linear regression: $n = 94$, $r^2 = 0.97$, $p < 0.0001$) to predict chick down total mercury concentrations ($[\text{THg}]_{\text{df}}$; $\mu\text{g g}^{-1}$ fw) from whole-egg total mercury concentrations at pipping ($[\text{THg}]_{\text{we}}$; $\mu\text{g g}^{-1}$ dw at pipping) was:

$$\text{Equation 7: } [\text{THg}]_{\text{df}} = (e^{0.971 \pm 0.025}) \times [\text{THg}]_{\text{we}}^{0.940 \pm 0.017}$$

The slope coefficient of 0.940 ± 0.017 (SE) differed from 1.0 ($t_{92}=3.53$, $p \leq 0.001$).

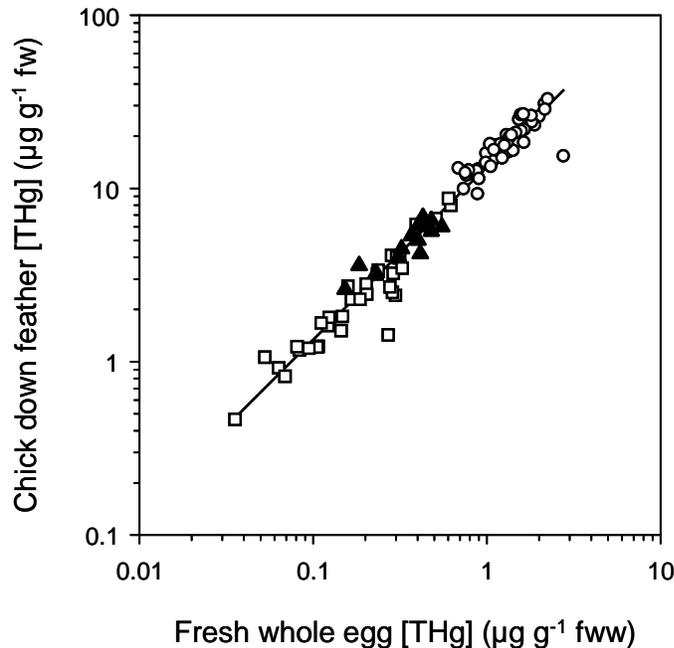


Figure 7. Mercury concentrations in down feathers ($\mu\text{g g}^{-1}$ fresh weight [fw]) of pipping chicks were highly correlated ($r^2 = 0.96$) with mercury concentrations in the reconstructed fresh whole-egg homogenate ($\mu\text{g g}^{-1}$ fresh wet weight [fww]) for Forster's terns (circles), American avocets (squares), and black-necked stilts (triangles) in South San Francisco Bay, California, USA. The linear regression equation describing the data was: $\log_e Y = 2.590 + 1.000(\log_e X)$.

Lastly, we compared fresh wet weight mercury concentrations in pipping eggs to randomly

sampled eggs to verify that pipping eggs were representative of the population. Mercury concentrations in pipping eggs did not differ from randomly sampled eggs for avocets ($F_{1,264} = 0.46, p = 0.50$), stilts ($F_{1,50} = 0.09, p = 0.77$), or Forster's terns ($F_{1,173} = 0.01, p = 0.93$).

Micro-Sampled Egg and Chick Down Feather Mercury Concentrations

We pooled data among all three species for these statistical analyses because we did not have a large enough sample of nests to test for interactions among species (stilts: $n = 1$, avocets: $n = 5$, terns: $n = 22$). We also randomly selected a down feather sample to correlate with the albumen sample when multiple chicks had hatched from the same nest containing the micro-sampled egg. Albumen mercury concentrations were correlated with mercury concentrations of down feathers from chicks found in the same nest (linear regression: $n = 28, r^2 = 0.79, p < 0.0001$; Figure 8). We found similar results when we used only those samples where we were able to positively match the micro-sampled egg and chick as the same individual (linear regression: $n = 6, r^2 = 0.77, p = 0.02$). After back-transforming the linear regression equation from the full dataset, which was based on \log_e -transformed data (\pm SE), the equation for predicting chick down feather total mercury concentrations ($\mu\text{g g}^{-1}$ fw) from albumen total mercury concentrations ($[\text{THg}]_{\text{alb}}$; $\mu\text{g g}^{-1}$ ww) was:

$$\text{Equation 8: } [\text{THg}]_{\text{df}} = (e^{2.291 \pm 0.085}) \times [\text{THg}]_{\text{alb}}^{0.888 \pm 0.090}$$

and, conversely,

$$\text{Equation 9: } [\text{THg}]_{\text{alb}} = (e^{-1.919 \pm 0.261}) \times [\text{THg}]_{\text{df}}^{0.889 \pm 0.090}$$

The slope coefficient (from Equation 8) of 0.888 ± 0.090 (SE) did not differ from 1.0 ($t_{26} = 1.24, p = 0.22$).

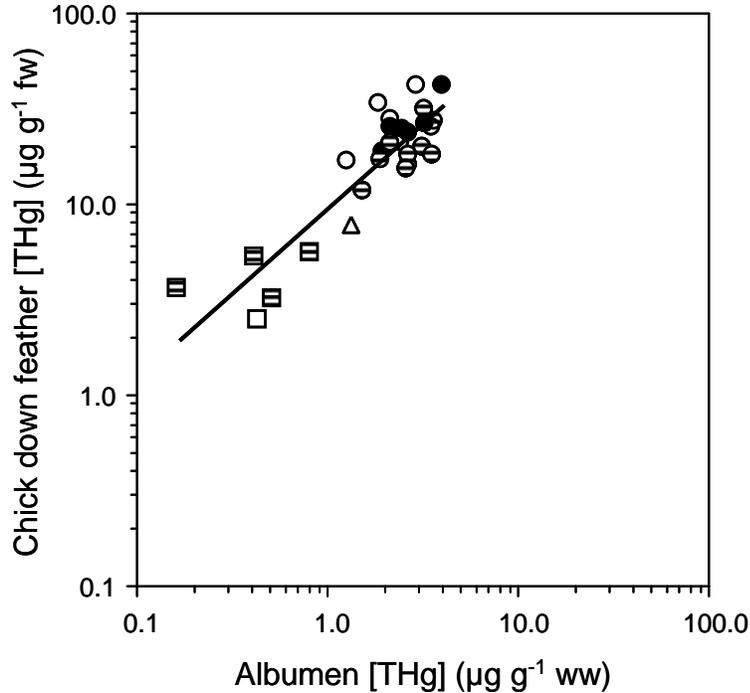


Figure 8. Mercury concentrations in down feathers ($\mu\text{g g}^{-1}$ fresh weight [fw]) of newly hatched chicks found in the nest were correlated ($r^2 = 0.79$) with albumen mercury concentrations ($\mu\text{g g}^{-1}$ wet weight [ww]) micro-sampled from an egg in the same nest when the eggs were ≤ 3 days incubated in South San Francisco Bay, California, USA. Symbol patterns (Forster's terns [circles], American avocets [squares], and black-necked stilts [triangles]) indicate whether the feathers were sampled from: the same chick that hatched from the albumen micro-sampled egg (filled), a sibling chick from the same nest that was not micro-sampled during incubation (partially filled), or an unknown chick from the same nest sampled either from the micro-sampled or sibling egg (open). The linear regression equation describing the data was: $\log_e Y = 2.291 + 0.888(\log_e X)$.

Mercury Concentrations in Down Feathers as Chick Age

To examine whether down feather mercury concentrations changed with age after hatching, we compared mercury concentrations in down feathers collected from chicks that were first captured when they were ≤ 3 days of age to their down feather mercury concentrations upon their next capture 7-days later (≤ 10 days of age). Down feather mercury concentrations in recaptured chicks were correlated with mercury concentrations of down feathers sampled during the first capture event from recently hatched chicks (linear regression: $n = 88$, $r^2 = 0.74$, $p < 0.0001$; Figure 9). After back-transforming the linear regression equation, which was based on \log_e -transformed data (\pm SE), the equation for predicting recaptured chick down feather total mercury concentrations ($[\text{THg}]_{\text{df-recapture}}$; $\mu\text{g g}^{-1}$ fw) from first-captured chick down feather total mercury concentrations ($[\text{THg}]_{\text{df-first capture}}$; $\mu\text{g g}^{-1}$ fw) was:

Equation 10: $[\text{THg}]_{\text{df-recapture}} = (e^{0.384 \pm 0.166}) \times [\text{THg}]_{\text{df-first capture}}^{0.798 \pm 0.052}$

and, conversely,

Equation 11: $[\text{THg}]_{\text{df-first capture}} = (e^{0.485 \pm 0.176}) \times [\text{THg}]_{\text{df-recapture}}^{0.922 \pm 0.060}$

The slope coefficient (from Equation 10) of 0.798 ± 0.052 (SE) differed from 1.0 ($t_{86}=3.88$, $p \leq 0.0001$).

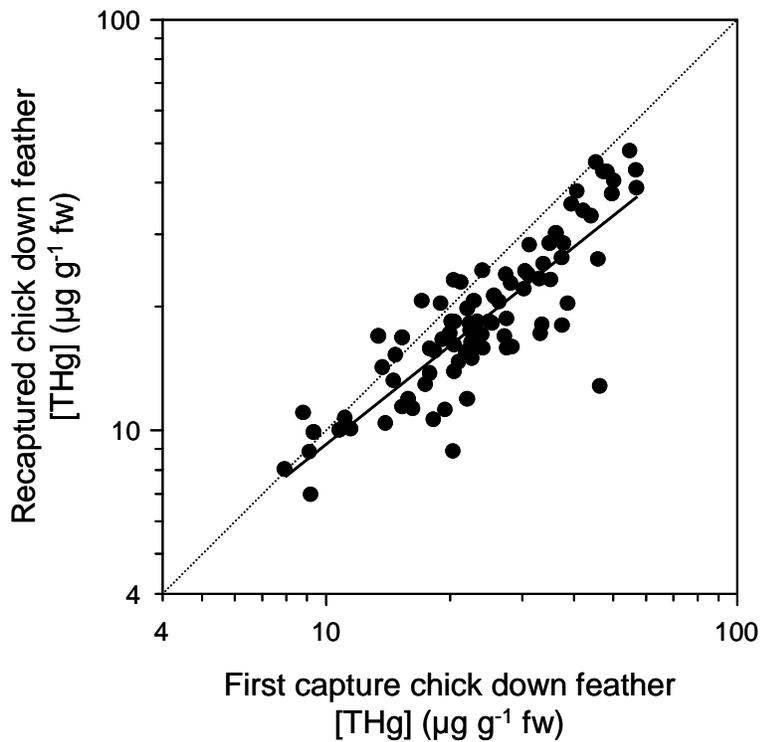


Figure 9. Mercury concentrations in down feathers ($\mu\text{g g}^{-1}$ fresh weight [fw]) of recaptured Forster's tern chicks (≤ 10 days of age) were correlated ($r^2 = 0.74$) with mercury concentrations in down feathers of the same chicks sampled just after they hatched (≤ 3 days of age) in South San Francisco Bay, California, USA. The stippled line indicates a one-to-one line. The linear regression equation describing the data was: $\log_e Y = 0.384 + 0.798(\log_e X)$.

Conclusions: Objective 1

Together, our results in *Objective 1* demonstrate the utility of using chick down feather mercury concentrations to predict concentrations in eggs, and vice versa. These results have several applications, including for mercury monitoring programs as well as in research for assessing toxicity thresholds. Often mercury monitoring programs are based on sampling eggs (Evers et

al. 2003, Hill et al. 2008), however sampling wild bird eggs is sometimes not possible due to permitting restrictions, especially with endangered species (Schwarzbach et al. 2006), or not desired since it necessarily results in the destruction of eggs. Currently, there are only a few ways to sample mercury concentrations in eggs non-lethally. These techniques include using chorioallantoic membranes left behind in the eggshell post-hatch (Heinz and Hoffman 2003b) and micro-sampling a viable egg by extracting a small amount of albumen (Ackerman and Eagles-Smith, unpublished data). However, chorioallantoic membranes can be difficult to find and should be collected shortly after hatching (Cobb et al. 1994, G. H. Heinz, pers. comm.) and albumen micro-sampling requires considerable training and must be done within a short time window when eggs have been incubated for ≤ 3 days (Ackerman and Eagles-Smith, unpublished data). In contrast, sampling down feathers of chicks can occur over a longer time period (up to 10 days post-hatch) and is relatively easy.

In addition to the value of this technique for monitoring mercury non-lethally, the ability to predict mercury concentrations in eggs from chick down feathers, or vice versa, can improve our assessment of egg toxicity thresholds. Because avian embryos are especially sensitive to methylmercury (reviews by Scheuhammer et al. 2007, Thompson 1996, Wolfe et al. 1998, Wiener et al. 2003b), egg toxicity thresholds traditionally have been developed by examining effects of *in ovo* mercury concentrations on egg survival (Fimreite 1971, Heinz and Hoffman 2003a, Albers et al. 2007, Heinz et al. 2008). However, chick growth (Spalding et al. 2000b, Longcore et al. 2007), behavior (Bouton et al. 1999), and survival (Heinz 1976, Finley and Stendell 1978, Albers et al. 2007, Burgess and Meyer 2008, Evers et al. 2008) also can be affected by methylmercury. The ability to combine both of these sensitive reproductive endpoints into a single tissue matrix, could improve our understanding of mercury toxicity levels that cause reproductive impairment. For instance, *in ovo* mercury concentrations that impair egg hatchability are likely to be higher than those concentrations that could impair subsequent chick growth and survival, since the chick must first hatch before it has the opportunity for its growth and survival to be affected by residual mercury exposure.

To illustrate the utility of this tissue conversion equation, we used the mercury concentration in eggs commonly associated with impaired hatchability to estimate the corresponding mercury

concentration that would occur in down feathers of a newly hatched chick. Egg mercury concentrations $>1.0 \mu\text{g g}^{-1}$ ww often cause impaired hatchability and embryonic mortality in birds (review by Scheuhammer et al. 2007). Using our equation developed in this report (Equation 5), a concentration of $1.0 \mu\text{g g}^{-1}$ ww in the whole fresh egg is equivalent to 13.3 (13.0-13.7) $\mu\text{g g}^{-1}$ fw in down feathers of recently hatched chicks. There is limited data on chick toxicity to compare this value to, but Ackerman et al. (2008a) found that mercury concentrations (geometric mean \pm SE) in down feathers of dead stilt chicks at hatching ($16.43 \pm 2.19 \mu\text{g g}^{-1}$ fw) were higher than levels in randomly-sampled live chicks of similar age ($9.98 \pm 0.77 \mu\text{g g}^{-1}$ fw). Down feather mercury concentrations in live stilt chicks correspond to a predicted fresh egg concentration of $0.74 \mu\text{g g}^{-1}$ fww, whereas down feathers from dead stilt chicks correspond to a predicted fresh egg concentration of $1.19 \mu\text{g g}^{-1}$ fww (Equation 6). These data suggest that although eggs with mercury concentrations above $>1.0 \mu\text{g g}^{-1}$ ww can still hatch, the residual effects of maternally derived mercury on early chick mortality may continue to impair overall reproduction. This is particularly important because it means that commonly used endpoints, such as egg hatchability, likely underestimate the risk of mercury contamination to avian reproduction. However, by using the equation we derived between mercury concentrations in down feathers and eggs, inclusion of early chick mortality into the egg toxicity criterion allows us to integrate toxicity risk for two life-stages into a single tissue matrix.

Furthermore, toxicity thresholds for egg hatchability are often estimated using lab experiments where a measured amount of methylmercury chloride is dissolved in corn oil and injected into the egg's aircell and allowed to diffuse into the embryo (Heinz et al. 2006). The egg is then artificially incubated and hatchability is compared to concentrations of methylmercury that were injected. Whereas this technique has proven useful in estimating the relative sensitivities of avian embryos to mercury toxicity among species, the injected mercury is thought to be more toxic than maternally derived mercury (Heinz et al. 2008). Moreover, the amount of injected mercury that is actually assimilated by the growing embryo is not fully known (Heinz et al. 2006; G. H. Heinz, pers. comm.). Perhaps sampling down feathers from successfully hatched and failed-to-hatch chicks in these lab studies could refine egg toxicity thresholds by comparing assimilated- to injected-egg mercury concentrations.

Objective 2. Determine toxic thresholds in Forster's Tern eggs by comparing mercury concentrations in randomly collected eggs to concentrations in failed-to-hatch and abandoned eggs.

We monitored 683 Forster's Tern nests at several sites in San Francisco Bay in 2007, including Ponds A1, A7, A16, Eden Landing Ecological Reserve, and Moffett. Mercury concentrations in randomly collected eggs differed among colonies (ANOVA: $F_{5,129} = 2.71$, $p = 0.02$; Table 1). Mayfield nest success (Mayfield 1975, Klett et al. 1986) varied considerably among colonies from just 13% in Moffett to 72% in Pond A16 (Table 1). Nest success tended to decline with the geometric mean egg mercury concentration estimated for each colony using randomly sampled eggs, but this trend was not significant ($n = 5$, $r = -0.74$, $p = 0.15$; Figure 10). Instead, the greatest factor influencing nest success was depredation. The proportion of eggs hatching in a successful nest (i.e., ≥ 1 egg hatched) ranged from 72% to 82%, and was reduced by both partial clutch depredation and failed-to-hatch eggs.

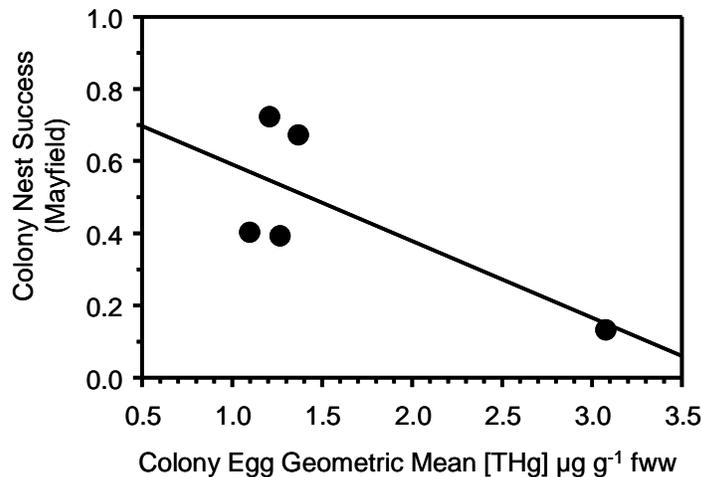


Figure 10. Colony nest success tended to decline with the geometric mean egg mercury concentration for each colony.

Table 1. Mercury concentrations in random eggs from successful nests, abandoned, and failed-to-hatch Forster's Tern eggs, colony nest success, and egg hatching success by colony in South San Francisco Bay, CA in 2007.

Colony	# Nests Monitored	Random Eggs [THg] $\mu\text{g g}^{-1}$ fww	Abandoned Eggs [THg] $\mu\text{g g}^{-1}$ fww	Failed-to-Hatch Eggs [THg] $\mu\text{g g}^{-1}$ fww	Nest Success (Mayfield)	Hatching Success (in successful nests)
A1	185	1.37 \pm 0.08	1.32 \pm 0.10	1.56 \pm 0.19	67%	72%
A7	103	1.27 \pm 0.13	1.62 \pm 0.45	1.42	39%	74%
A8	0	na	na	1.62	na	na
A16	258	1.21 \pm 0.06	1.56 \pm 0.17	1.95 \pm 0.03	72%	74%
Eden Landing	119	1.11 \pm 0.19	1.02 \pm 0.19	2.62	40%	82%
Moffett	18	3.08	2.43 \pm 0.38	na	13%	75%
Total	683	1.27 \pm 0.05	1.38 \pm 0.09	1.73 \pm 0.13	---	---

In 2007, mercury concentrations in Forster's Tern eggs differed among random eggs from successful nests, abandoned, and failed-to-hatch eggs, after controlling for potential effects of colony site (Figure 11: top panel; ANCOVA: egg type: $F_{2,129} = 3.09$, $p < 0.05$, colonies: $F_{5,129} = 2.72$, $p = 0.02$). For all sites combined in 2007, mercury concentrations (geometric mean \pm SE) were highest in failed-to-hatch eggs ($1.73 \pm 0.13 \mu\text{g g}^{-1}$ fww), followed by abandoned eggs ($1.38 \pm 0.09 \mu\text{g g}^{-1}$ fww), then randomly collected eggs from successful nests ($1.27 \pm 0.05 \mu\text{g g}^{-1}$ fww). However, abandoned eggs did not differ from failed-to-hatch eggs or random eggs from successful nests, due in part to limited sample sizes and relatively low statistical power (0.59) in 2007. To improve our statistical power and account for the inherent temporal and spatial variability in mercury levels, we used our archived egg samples and evaluated nest data from our CALFED funded research in 2005 and 2006 (Ackerman et al. 2007a) to increase our sample size. We went through our nest records and classified eggs from 2005 and 2006 similarly to our approach in 2007.

After pooling all 3 years of data, mercury concentrations differed significantly among egg collection status ($F_{2,341} = 13.58$, $p < 0.001$), colony ($F_{8,341} = 12.18$, $p < 0.0001$), and year ($F_{2,341} = 12.48$, $p < 0.001$). Mercury concentrations (geometric mean \pm SE) were higher in failed-to-hatch eggs ($1.74 \pm 0.13 \mu\text{g g}^{-1}$ fww) than in randomly sampled eggs from successful nests ($1.20 \pm 0.04 \mu\text{g g}^{-1}$ fww), but abandoned eggs ($1.43 \pm 0.07 \mu\text{g g}^{-1}$ fww) did not differ significantly from either failed-to-hatch or random eggs from successful nests (Figure 11: bottom panel; Tukey's multiple comparisons tests: both $p > 0.05$). These results indicate the importance of collecting data across multiple years and colony sites in the derivation of effects thresholds.

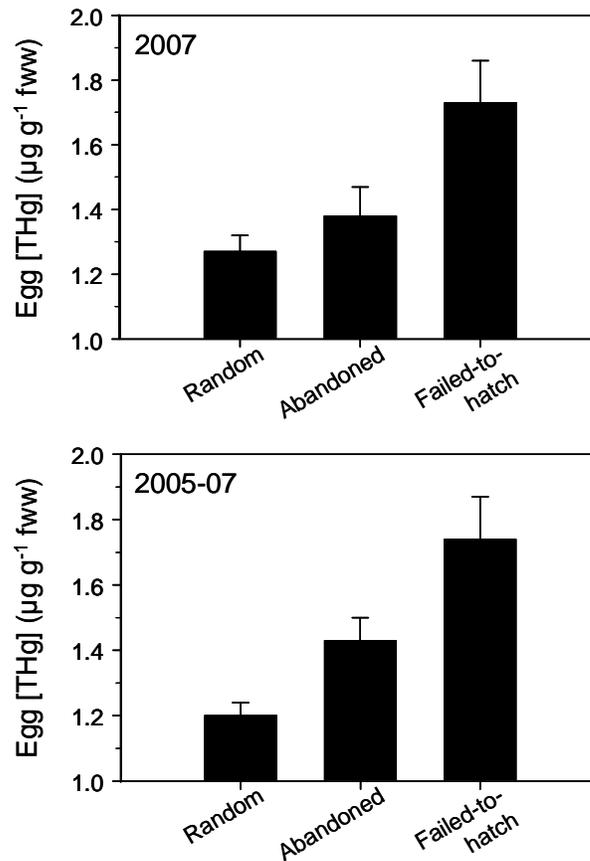


Figure 11. Mercury concentrations in failed-to-hatch Forster's Tern eggs were higher than randomly sampled eggs from successful nests during 2007 and for all 3 years combined.

Conclusions: Objective 2

Together, our results in *Objective 2* indicate that current mercury concentrations may be reducing hatching success of Forster's Tern eggs and suggest that the large variability among years and colonies make determining toxic threshold concentrations in eggs difficult. Defining a threshold mercury concentration for reduced egg hatchability in the wild is complicated by external factors such as disturbance, predation, and food availability. Thus, studies should span multiple colony sites and years to account for the inherent variability in reproductive success.

We found that mercury concentrations in failed-to-hatch eggs were higher than in random eggs during 2007 as well as during 2005-2007 using the expanded dataset. Additionally, we found that colony-specific nest success tended to decline with increasing colony-wide egg-mercury

concentrations. Importantly, we found that mercury concentrations in failed-to-hatch eggs were about 45% higher than in randomly collected eggs from successful nests (2005-2007). Approximately 27% of all Forster's Tern eggs sampled between 2005 and 2007 exceeded the geometric mean mercury concentration for failed-to-hatch eggs ($1.74 \pm 0.13 \mu\text{g g}^{-1}$ fww), suggesting that hatchability of Forster's Tern eggs in San Francisco Bay may currently be affected by mercury exposure and bioaccumulation. However, the degree to which mercury itself is affecting tern reproduction is still unclear. Depredation was generally the greatest factor influencing nest success, and variability in mercury levels, predation pressure, and disturbance among years and colony sites can confound interpretation of potential effects, as well as the development of toxicity thresholds. To account for this inter-annual and inter-colony variability, egg toxicity thresholds should be developed over multiple years.

Objective 3. Examine effects of mercury on chick mortality by comparing mercury concentrations in down feathers of alive and dead Forster's Tern chicks.



Figure 12. Forster's Tern chicks were banded soon after hatching (left panel), their down feathers sampled for mercury, and compared to chicks found dead (right panel) at the colony during weekly visits.

We captured and banded 358 individual Forster's Tern chicks (Figure 12, Table 2), 341 of which were ≤ 10 days old at their first capture. Through a mark-recapture methodology, we followed their fate to fledging (survived or died). We considered a chick to have died if it was ≤ 10 days old at their initial capture and it was never recaptured again. We considered a chick to have survived if its age at its last capture was ≥ 18 days, since on the following visit 7-days later it would have been 25 days of age or older and they fledge at 25-28 days of age. For chicks that

were recaptured at least once, but whose age at last capture was ≤ 18 days, we classified their fate as unknown and excluded them from analyses. Of the 341 chicks that we banded at ≤ 10 days of age, 141 individuals were assumed to have died, 41 were known to have died, 76 survived, we could not assign a fate to 40 individuals, and we excluded 43 chicks that were manipulated (blood sampled). In total, we found 73 dead Forster's Tern chicks at ≤ 10 days of age on colonies (Figure 12: right panel), 41 were previously banded and 32 were not banded (they had hatched between colony visits and died before our next colony visit).

Table 2. Mercury concentrations in alive and dead Forster's Tern chicks and three estimates of chick mortality by colony in South San Francisco Bay, CA in 2007.

Colony	# Chicks Monitored	Alive Chick Down [THg] $\mu\text{g g}^{-1}$ fw	Dead Chick Down [THg] $\mu\text{g g}^{-1}$ fw	Chick Mortality (# never recaptured / total # banded)	Chick Mortality (total # found dead / total # handled)	Chick Mortality (# banded found dead / total # banded)
A1	136	20.76 \pm 0.69	18.24 \pm 1.45	31%	23%	17%
A7	32	21.60 \pm 1.67	29.08 \pm 1.83	47%	14%	3%
A8	0	26.45 \pm 3.84	na	---	---	---
A16	148	19.51 \pm 0.73	22.18 \pm 2.01	41%	16%	10%
Eden Landing	42	18.07 \pm 0.75	23.21 \pm 2.77	50%	5%	5%
Moffett	0	43.45	28.12	---	---	---
Total	358	20.06 \pm 0.44	20.76 \pm 1.15	39%	19%	12%

We estimated colony-wide chick mortality in three ways, including the number of chicks banded that were never recaptured again, the number of chicks handled (banded or found dead unbanded) that were found dead, and the number of chicks banded that were later found dead (Table 2). In general, colony-wide mortality of chicks tended to increase with mercury concentrations in live chick down feather, but this trend was not significant (Figure 13; $n = 5$, $r = 0.65$, $p = 0.27$) and most apparent for the second chick mortality estimate (i.e., number handled that were found dead). Future research should use more sophisticated mark-recapture models (Program MARK) to estimate survival rates, but that is beyond the scope of this study.

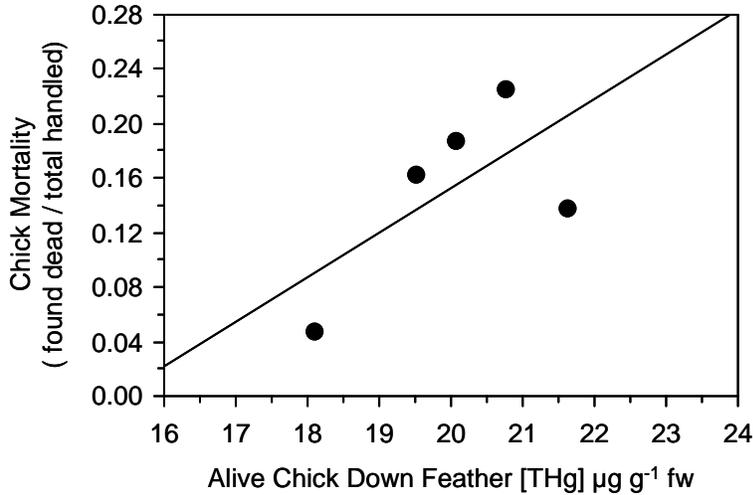


Figure 13. Colony-wide chick mortality tended to increase with mercury concentrations in live chick down feathers.

As expected based on our results from *Objective 1*, colony-wide mercury concentrations in live chick down feathers was positively correlated with colony-wide geometric mean mercury concentrations in eggs ($n = 5$, $r = 0.99$, $p = 0.0002$; Figure 14).

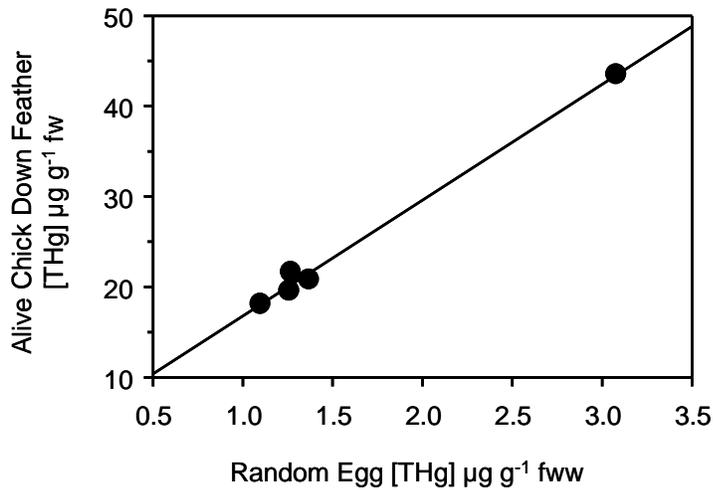


Figure 14. Colony-wide mercury concentrations in live chick down feathers were positively correlated with colony-wide (randomly collected) egg mercury concentrations.

We analyzed total mercury concentrations in down feathers of each assumed-dead, known-dead, and assumed-survived chicks. While controlling for the potential effects of date ($F_{1,246} = 0.04$, $p = 0.83$) and colony site ($F_{5,246} = 1.81$, $p = 0.11$), we found no differences in down feather mercury concentrations among chick fates ($F_{2,246} = 0.26$, $p = 0.77$). However, geometric mean

mercury concentrations in known-dead chicks ($20.64 \pm 1.07 \mu\text{g g}^{-1} \text{ dw}$) were slightly higher than those that were assumed to have died ($20.03 \pm 0.71 \mu\text{g g}^{-1} \text{ dw}$) or survived ($19.82 \pm 0.91 \mu\text{g g}^{-1} \text{ dw}$; Figure 15).

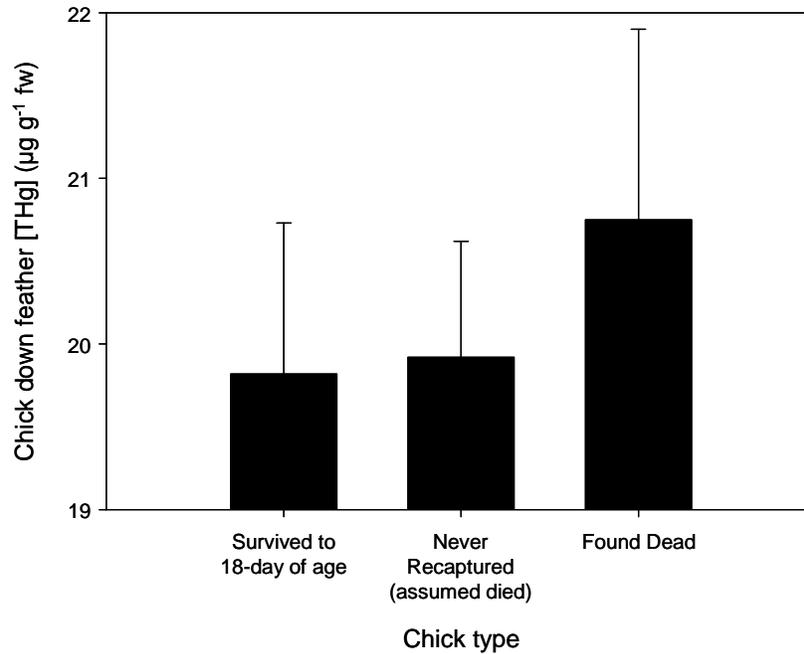


Figure 15. Mercury concentrations in down feathers of Forster's Tern chicks found dead at colonies were not statistically different than chicks that survived to ≥ 18 days of age (near fledging). Only chicks that were ≤ 10 days of age were included in the analyses.

Conclusions: Objective 3

Together, our results in *Objective 3* are inconclusive as to whether current mercury concentrations *in ovo* have an important effect on chick survival. Although colony-wide mortality trended higher with chick down mercury levels, this result was not significant. Additionally, chicks that were found dead tended to have slightly higher mercury concentrations than chicks that were known to have survived to ≥ 18 days of age, but this difference was not statistically different.

Examining the effects of mercury exposure on chick survival is complicated due to high variability in mercury concentrations among chicks of differing ages (Karasov et al. 2007). Any effects of mercury exposure on chick survival are likely to occur shortly after hatching or during fledging when blood concentrations are at their highest levels. Blood mercury concentrations in chicks are relatively high immediately after hatching, due to *in ovo* exposure, then rapidly

decline as chicks age and dilute their body burden of mercury through growth in size and depuration into growing feathers (Monteiro and Furness 2001, Fournier et al. 2002). Blood mercury concentrations then begin to increase just before and during fledging when body growth and feather production slows, while chicks continue to acquire mercury through their diets. This U-shaped pattern of blood mercury dilution followed by accretion as chicks age has been observed in several species (Spalding et al. 2000b, Kenow et al. 2003), including Forster's Terns (Ackerman et al. 2007a, Figure 4). Therefore, juvenile terns may experience a period of higher risk to mercury toxicity shortly after hatching and again at the time of fledging when feather production ceases.

Elsewhere we did not detect an effect of blood mercury concentrations on survival of postfledging (>28 days of age) Forster's Terns using radio-telemetry, instead size-adjusted mass had an overwhelming influence on survival (Ackerman et al. 2008c). In fact, cumulative survival probability was 61% lower for terns with the lowest, compared to the highest, observed masses (Ackerman et al. 2008c). Mercury exposure *in ovo* or soon after hatching can influence chick growth rates and fledging mass, therefore *in ovo* mercury may have had a residual effect on postfledging tern survival via reduced growth rates and fledging masses. If so, then future work should focus on examining whether *in ovo* mercury concentrations are having a detrimental effect on Forster's Tern chick growth rates.

MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

Mercury contamination in San Francisco Bay has necessarily been a main focus of water quality agencies in the San Francisco Bay Region, and protection of wildlife is among the top issues of concern. In fact, the San Francisco Bay Regional Water Quality Control Board recently included a bird egg monitoring target into its methylmercury TMDL (California Regional Water Quality Control Board San Francisco Bay Region 2006). However, sensitivity to mercury exposure can vary widely among bird species (Heinz et al. 2008) and lifestages, and little information is known about the sensitivity of high-risk San Francisco Bay species, such as Forster's Terns. As such, there is a need to link mercury exposure to adverse effects in species that breed in the Bay as the first step in evaluating population level impacts of mercury, and ultimately developing a compensation strategy for any potential reduced reproduction.

In particular, Forster's Terns were recently added to the Regional Monitoring Program's long-term plan for avian contaminant monitoring, along with Double-crested Cormorants (J. Davis, pers. comm.). Starting in 2009, Forster's Tern eggs will be monitored for contaminant exposure every three years as part of this long-term monitoring plan. In order for these egg monitoring results to be related to potential toxicity risk, egg toxicity levels for mercury should be established.

Effects of mercury on avian reproduction can impact several lifestages, and post-hatch effects on chick survival and growth are important considerations for reproductive impairment. Thus, there is great value in incorporating effects of multiple, sensitive lifestages into a single threshold value. Our dual-lifestage approach can serve as a predictive tool for simultaneously estimating potential impacts across lifestages. The avian egg is an extremely valuable indicator of mercury exposure, and potential effects across multiple lifestages, because it links maternal exposure to hatchability and early-chick survival (Figure 16).

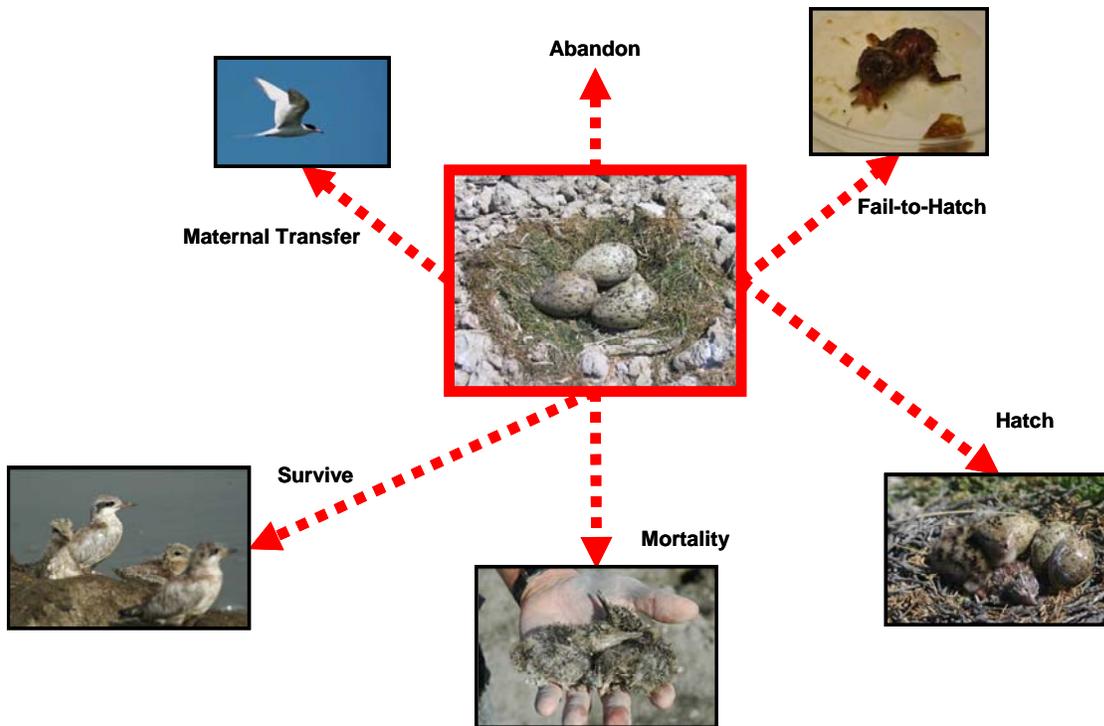


Figure 16. Conceptual model demonstrating the utility of using eggs as a monitoring tool for multiple lifestages, incorporating effects to adults, chicks, and eggs into a single tissue monitoring matrix – *eggs*. Once toxicity thresholds are developed for each lifestage shown, they can be translated into equivalent concentrations in eggs. Thereafter, toxicity thresholds for eggs will incorporate mercury's effect on hatchability, chick growth and survival, and the probability of adult nest abandonment.

Our results indicate that current mercury exposure is likely causing reduced hatching success in Forster's Terns nesting in San Francisco Bay and continued assessment and monitoring of their mercury exposure and reproduction are warranted. In particular, geometric mean mercury concentrations observed in Forster's Terns eggs in San Francisco Bay ($1.29 \mu\text{g g}^{-1}$ fww) are above mercury toxicity levels developed in the lab for two closely related species: Common Terns LC_{50} was $0.87 \mu\text{g g}^{-1}$ ww and Royal Terns LC_{50} was $0.40 \mu\text{g g}^{-1}$ ww. Although injected mercury is likely more toxic than maternally derived mercury, current mercury concentrations in Forster's Tern eggs in the Bay are considered high. For example, 99% of Forster's Tern eggs we sampled were above the toxic threshold concentration calculated for Royal Terns, 97% were above the San Francisco Bay's TMDL monitoring target for eggs, 80% were above the toxic threshold concentration calculated for Common Terns, and 27% were above the geometric mean mercury concentrations in failed-to-hatch eggs that we observed in San Francisco Bay (Figure 17).

We also have shown a strong link in mercury concentrations between chick and eggs, suggesting that incorporating effects using a dual-lifestage approach is possible. We found only limited evidence that chick survival is being impaired by mercury. However, the inherent variability in mercury concentrations and reproductive success across years make defining a clear toxic threshold difficult, and data spanning multiple years are necessary to control for this variability and establish more definitive thresholds. Additionally, wildlife in San Francisco Bay are exposed to potentially deleterious levels of other contaminants of concern, such as selenium, PCBs, and PBDEs. These bioaccumulative pollutants may interact with mercury to enhance or reduce its effect on avian reproduction, thus confounding the interpretation of risk from mercury alone. A robust assessment of compounding effects of these multiple contaminants together will be necessary to partition risk among the different chemical classes. We recommend that support of additional research to achieve this goal be a priority in San Francisco Bay.

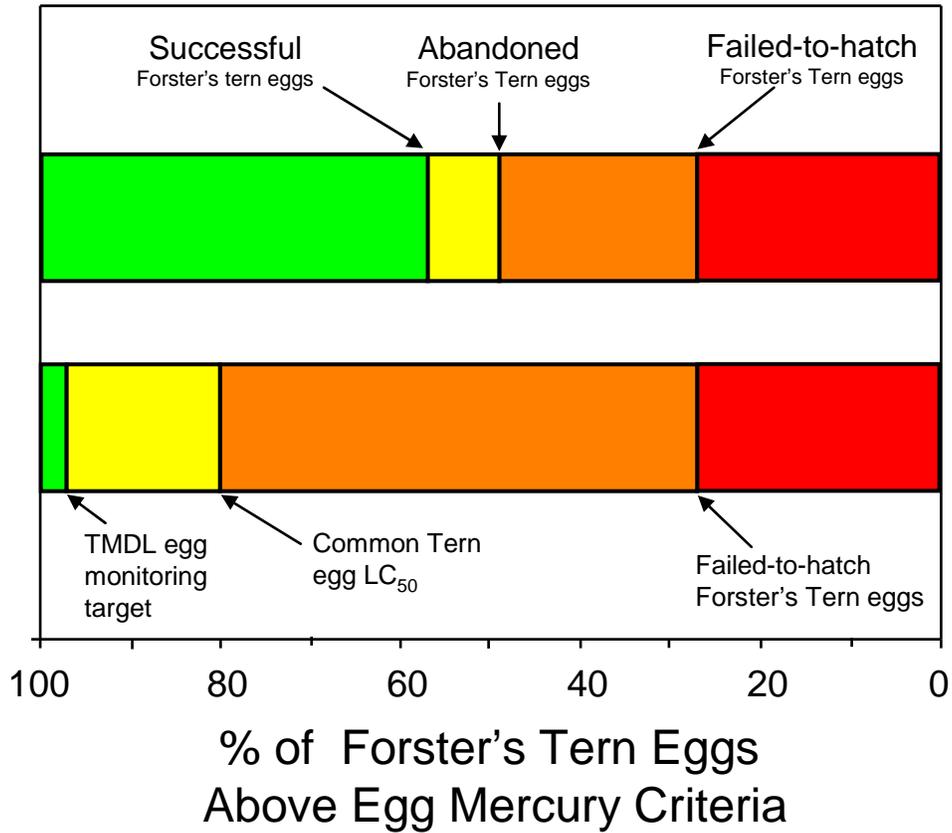


Figure 17. The percentage of Forster's Tern eggs we sampled in San Francisco Bay that were above various toxic threshold concentrations. Overall, 97% of Forster's Tern eggs were above the San Francisco Bay's TMDL monitoring target for eggs ($0.50 \mu\text{g g}^{-1}$ fww; California Regional Water Quality Control Board San Francisco Bay Region 2006), 80% were above the toxic threshold concentration calculated for Common Terns ($0.87 \mu\text{g g}^{-1}$ ww; Heinz et al. 2008), and 27% were above the geometric mean mercury concentrations in failed-to-hatch eggs that we observed in San Francisco Bay ($1.74 \mu\text{g g}^{-1}$ fww; this report).

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