Mercury-Selenium Effects on Reproductive Success of Forster's Terns in San Francisco Bay

Annual Report

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U. S. GEOLOGICAL SURVEY

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EXECUTIVE SUMMARY

Problem Statement

- Mercury (Hg) concentrations of Forster's terns in San Francisco Bay are higher than any other waterbird species in the region, and a large proportion of breeding terns exceed levels associated with high risk to reproductive impairment.
- Forster's terns have recently been incorporated into the Regional Monitoring Program as a key biosentinel species for evaluating wildlife risk to Hg.
- To facilitate the use of Forster's terns as bioindicators of Hg contamination within the Bay, toxicity thresholds need to be established for tern reproduction. However, standard methods for deriving field-based thresholds are hampered by the high variability in egg Hg concentrations within and individual clutch, as well as potential interactions with selenium (Se).

Study Results

Objective 1. Link mercury concentrations in egg albumen to those of whole eggs.

- Total Hg (THg) concentrations in egg albumen was highly correlated with total mercury concentrations in whole-eggs ($n = 49$, $r^2 = 0.89$, $p < 0.0001$), indicating that albumen THg concentrations can be used to non-lethally sample for THg in Forster's tern eggs.

Objective 2. Microsample live Forster's tern eggs to determine the effect of mercury and selenium on hatching success.

- We microsampled 132 Forster's tern eggs in San Francisco Bay.
- Both the microsampled and control eggs hatched in 47% of nests, the microsampled egg failed and the control egg hatched in 5% of nests, and the control egg failed and the microsampled egg hatched in 6% of nests. The remaining 42% of nests were either abandoned (18%) or destroyed/depredated (24%).
- In comparison, we monitored but did not microsample 758 Forster's tern nests. All eggs hatched in 49% of nests, 1 or more eggs failed to hatch in 11% of nests, and the remaining 40% of nests were either abandoned (28%) or destroyed/depredated (12%).
- Albumen THg concentrations were not strongly correlated with albumen Se

concentrations.

- We found no relationship between hatching probability and albumen THg or Se concentrations.
- The albumen THg:Se molar ratio significantly influenced the probability of egg hatching when controlling for the variance associated with THg and Se concentrations. However, hatching probability increased with increasing molar ratios, suggesting that excess THg relative to Se can improve hatchability.

Conclusions and Management Implications

- In contrast to two prior studies where we found that (1) failed-to-hatch Forster's tern eggs had higher THg concentrations than in random, viable eggs, and (2) the probability of the remaining eggs successfully hatched decreased with THg concentrations in a randomly selected egg from the same clutch (i.e., the surrogate egg technique), we did not find evidence that THg concentrations in albumen influenced the probability of hatching.
- Our sample size in 2008 was relatively small for both contaminants (n = 50), and 2008 happened to have relatively low THg concentrations in eggs compared to the prior 4 years of sampling in the South Bay. These factors may have contributed to our results which suggested no effect of THg on the probability of an egg hatching successfully.
- Our on-going 2009 microsampling data for THg (only) will increase our effective sample size and should help provide for more robust conclusions. However, it will not allow for more detailed assessment of the effect that Se in combination with THg has on the probability of egg hatching.

MERCURY-SELENIUM EFFECTS ON EGG HATCHABILITY OF FORSTER'S TERNS IN SAN FRANCISCO BAY

Annual Report

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

INTRODUCTION

The San Francisco Bay has a well-documented legacy of mercury (Hg) contamination (Davis et al. 2003), resulting in its designation as an impaired water body according to Section 303d of the Federal Clean Water Act. Elevated Hg concentrations measured in fish and other biota have stemmed concern that high trophic level wildlife, such as fish-eating waterbirds, may be suffering from deleterious effects due to Hg bioaccumulation (Ackerman et al. 2008, Eagles-Smith et al. 2009). Indeed, recent work assessing Hg risk to waterbirds found high concentrations in tissues of several species, spanning multiple trophic guilds (Eagles-Smith et al. 2009). Forster's terns (*Sterna forsteri*) had the highest concentrations of all species studied, with blood concentrations in 48% of breeding Forster's terns exceeding 3.0 μg/g ww (Eagles-Smith et al. 2009), a threshold for reproductive impairment developed for the common loon (*Gavia immer*) in the wild (Evers et al. 2008).

Because Forster's terns forage at a high trophic level (on small fish) in shallow water habitats along the Bay's margins (Ackerman et al. 2008), such as tidal flats, tidal marshes, and salt ponds where Hg methylation is elevated (Marvin-DiPasquale et al. 2003), they are effective biosentinels for indicating mercury contamination and risk in San Francisco Bay wildlife. Accordingly, USGS Western Ecological Research Center has recently worked with the San Francisco Estuary Institute to incorporate Forster's tern egg sampling into the Regional Monitoring Program's Status and Trends monitoring to conduct triennial evaluations of contaminant levels in the Region's wildlife. However, sensitivity to MeHg is known to vary among bird species (Heinz et al. 2009), and currently there are no robust estimates of toxicity thresholds for at-risk waterbirds breeding in San Francisco Bay. To facilitate interpretation of risk based on Hg concentrations in Forster's tern eggs, appropriate benchmarks are necessary to

establish threshold concentrations where Forster's tern reproduction may become impaired.

The development of contaminant toxicity thresholds of reproductive endpoints for animals in the wild can be complicated by other factors that may influence reproductive success, such as predation and food availability. Thus, threshold derivation requires a multi-stage process to reduce variability and increase predictive power. Recent work on Forster's terns in the San Francisco Bay initiated this effort. Ackerman and Eagles-Smith (2008) found that between 2005 and 2007, total Hg (THg) concentrations (geometric mean ± SE) in failed-to-hatch Forster's tern eggs (1.74 ± 0.13 μg/g fww) were significantly higher than concentrations in randomly sampled eggs from successful nests (1.20 ± 0.04 μg/g fww). In addition, approximately 27% of all Forster's tern eggs sampled between 2005 and 2007 exceeded the geometric mean THg concentration in failed-to-hatch eggs (Ackerman and Eagles-Smith 2008). These results suggest that Hg is impairing egg hatchability in San Francisco Bay, but do not provide a robust dose-response threshold. Traditionally, dose-response effects of contaminants on hatching success have either been evaluated in the lab through egg injections and controlled feeding studies (Heinz et al. 2009, Albers et al. 2007), or in the field using the surrogate egg technique, where one egg is removed from a nest as an index of contamination in the other eggs, and the remaining eggs are monitored to determine hatchability (Blus 1982, 1984). The lab-based approaches for Hg are hampered by either enhanced toxicity of injected MeHg (Heinz et al. 2006), or lack of applicability to wild populations in the controlled feeding studies. In turn, the field-based surrogate egg studies are confounded by high variability of Hg concentrations in eggs within an individual clutch (Kennamer et al. 2005). In addition to these factors, the toxicity of Hg is known to interact with selenium (Se). Selenium is generally protective against Hg toxicity in adult birds (Cuvin-Aralar and Furness 1991, Eagles-Smith et al. 2009), but may increase Hg toxicity in avian eggs depending on the relative amounts of each contaminant (Heinz and Hoffman 1998).

To evaluate the effects of Hg on the hatching success of ***individual*** Forster's tern eggs, we employed a novel method – the microsampling technique – that we recently developed with our collaborators at the USGS Patuxent Wildlife Research Center (Stebbins et al. 2009). Briefly, this technique entails sampling a small amount of egg albumen from a tiny hole drilled through the shell of a freshly laid egg, sealing the egg with glue, and following its fate as it is naturally

incubated by its parents. We can then relate contaminant concentrations in an individual egg to its own subsequent fate (hatch or fail).

OBJECTIVES

In order to maximize the utility of monitoring Hg in biosentinel Forster's terns, we need to develop toxic threshold levels for sensitive reproductive endpoints, such as egg hatchability and chick survival. The objectives detailed below allow scientists and managers to non-lethally monitor Hg concentrations in avian eggs and assess potential effects of Hg and Se on egg hatchability.

Objective 1. Link mercury concentrations in egg albumen to those of whole eggs.

THg concentrations in albumen of mallard (*Anas platyrhynchos*) eggs from hens fed a range of dietary MeHg concentrations were significantly correlated with THg concentrations in the whole egg (Stebbins et al. 2009). However, the yolk:albumen mass ratios of eggs can vary substantially among species, and this may alter the relationship between THg concentrations in albumen and whole eggs. Thus, in order to determine a whole egg toxicity threshold for Hg using the microsampling technique, a quantitative relationship between THg in albumen and THg in whole eggs is required on a species-specific basis. Therefore, we developed an equation to estimate whole egg THg concentrations from albumen in Forster's tern eggs.

Objective 2. Microsample live Forster's tern eggs to determine the effect of mercury and selenium on hatching success.

Mercury is known to impair egg hatchability which is an effective endpoint for quantifying the effect of contaminants on avian reproduction (Heinz and Hoffman 2003a, Heinz et al. 2009). However, there are currently no robust thresholds for Hg concentrations that negatively affect egg hatchability for birds breeding within San Francisco Bay. Toxicity thresholds developed using laboratory egg-injection techniques are inappropriate for application to wild birds because the injected Hg is thought to be much more toxic than maternally derived Hg (Heinz et al. 2006). The surrogate-egg technique that is often used to assess hatchability effects in the field is hampered by the high intra-clutch variability of egg Hg concentrations (Kennamer et al. 2005, Authors' unpublished data). For example, the intra-clutch variation in egg contaminant

concentrations can be as high as 50% for Hg (Authors, unpublished data) which can limit the detection of contaminant effects on reproductive success. Additionally, intra-clutch variability increases at high Hg concentrations where effects are likely to occur. Thus, the surrogate egg technique might mask true effects if Hg concentrations within a clutch overlap the hatchability threshold.

Another complicating factor when determining Hg-induced hatchability thresholds in the field is interactions with other contaminants. Selenium is of particular importance with respect to Hg toxicity, because the two contaminants are strongly associated. Selenium is known to be protective of Hg toxicity in adult birds (Eagles-Smith et al. 2009, Heinz and Hoffman 1998), but may be both synergistic and antagonistic in eggs depending on the relative concentrations of the two contaminants (Ackerman et al. 2007, Gary Heinz unpublished data). However, the interaction mechanisms are still not understood, making predictive modeling difficult. As such, it is important to evaluate both contaminants simultaneously in order to properly quantify the toxicity of either one. In *Objective 2*, we employed a novel egg microsampling technique to evaluate the toxicity of Hg and Se simultaneously, on an individual basis.

STUDY AREA

We conducted our microsampling study on Forster's tern eggs during the 2008 nesting season (April to August) in South San Francisco Bay, California (Figure 1). Our study sites were located at the Don Edwards San Francisco Bay National Wildlife Refuge (37.4º N, 122.0º W), where tern colonies occurred on islands within former salt evaporation ponds within the Alviso (Ponds A7, A8, A12, and A16) and Moffett (Ponds A1, A2W, and AB1) salt pond complexes (Figure 1).

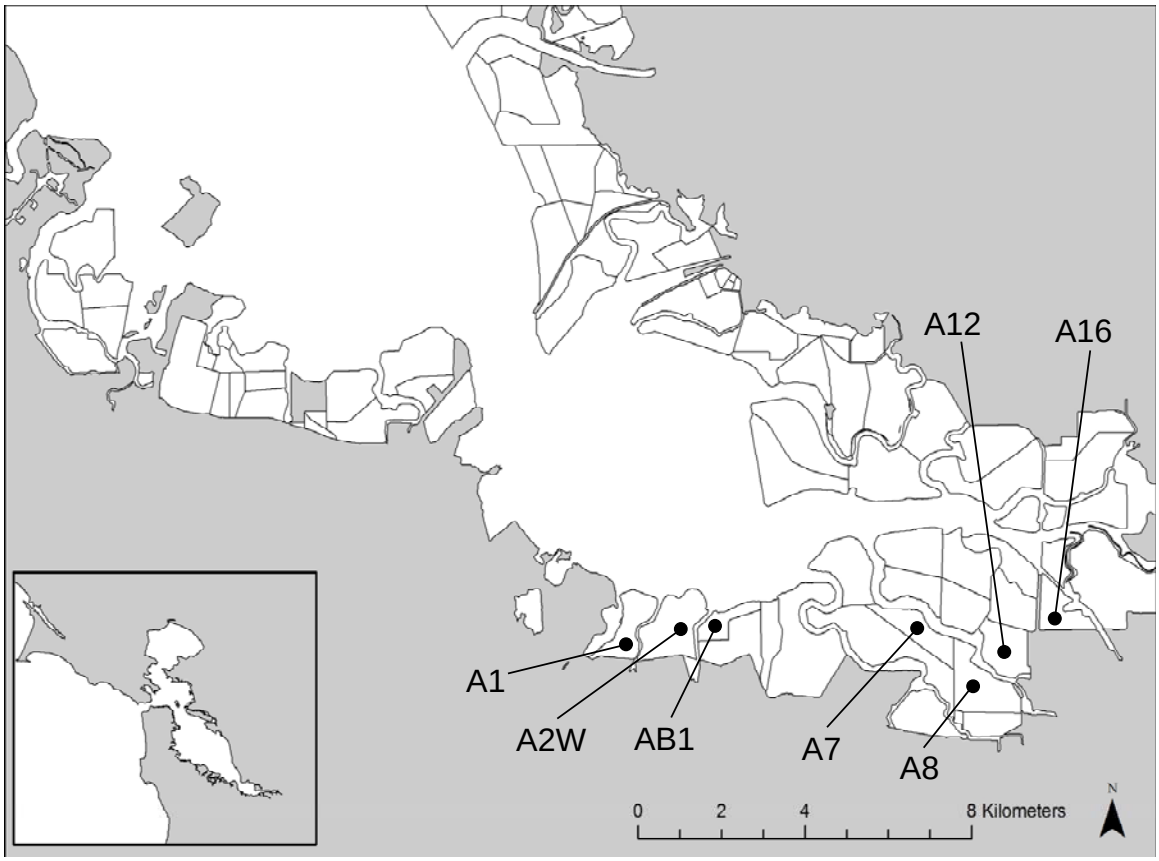

Figure 1. Map of Forster's tern colony locations microsampled in 2008 in San Francisco Bay.

METHODS

Objective 1. Link mercury concentrations in egg albumen to those of whole eggs.

Whole Egg and Albumen Mercury Concentrations

To link THg concentrations in microsampled egg albumen to whole-egg concentrations, we collected eggs immediately after they were laid during the 2007-2008 nesting seasons. We attempted to collect eggs at several nesting sites to yield a range of THg concentrations common in San Francisco Bay, and to increase our predictive range of converting THg concentrations from one matrix to another without requiring extrapolation. 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. At the initial nest visit, the stage of embryo development was determined by floating

(Hays and LeCroy 1971, Alberico 1995), and clutch size was recorded. We randomly collected one egg from a subset of nests where the clutch size was greater than one and all eggs had been incubated for <3 days. These criteria were established to duplicate our protocol for the microsampling study (*see below*), to ensure that we compared albumen and whole-egg concentrations in similar eggs between objectives. 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. Within 3-5 hours of egg collection, we sampled albumen from each egg following our micro-sampling protocol (Ackerman and Eagles-Smith 2009, Stebbins et al. 2009) to remove albumen from each egg to simulate our methodology in the field (Figure 2). We then opened each egg with scissors, removed all egg contents into a two ounce polypropylene jar, and recorded egg content mass. We immediately froze the egg contents and albumen aliquots at -20°C until THg analysis, which was completed within five months of egg collection.

Figure 2. Photo sequence of egg microsampling technique.

Our microsampling protocol was conducted as follows. Briefly, we dipped each egg in a dilute betadine (1%) solution and wiped them 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 at the top of the egg above the air cell to act as a vent during albumen extraction and one-third of the way up from the bottom of the egg for albumen extraction. Using a sterile 20-gauge needle attached to a 1-ml sterile syringe we

carefully extracted 200-300 µl (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. The albumen microsample was immediately transferred to a clean cryovial and stored in a freezer at -20˚C until THg analysis.

Statistical Analyses for Objective 1

We reconstructed THg concentrations in the whole egg by combining THg concentrations determined for the albumen sampled from the fresh egg and the remaining whole egg-homogenate. To do so, we weighed (wet weight; ww) the entire sample of albumen removed from the fresh egg and the remaining whole-egg homogenate separately before determining their respective THg concentrations (ww; accuracy to 0.0001 g). We then multiplied the weight of the albumen removed from the embryo by its specific THg concentration and added the product of the weight of the remaining whole-egg homogenate and the THg concentration of the whole-egg homogenate. This resulted in the total THg burden in the whole egg, and we divided this quantity by the combined mass (ww) of the removed albumen and the remaining whole-egg homogenate to yield the THg concentration of the reconstructed whole-egg homogenate at sampling.

Although these eggs were sampled soon after being laid, it is unknown how much moisture loss may have occurred prior to sampling. Thus, we adjusted the wet weight THg concentration of the reconstructed whole-egg homogenate to a fresh egg wet weight THg concentration (fww) by dividing the total mass (ww) of the egg content at processing by the predicted fresh egg mass (ww) at laying, and multiplying that value by the wet weight THg concentration (following Stickel et al. 1973). The fresh egg mass (ww) was estimated using egg morphometrics following Hoyt (1979).

We used linear regression (JMP® version 8.0) to test whether THg concentrations in albumen were correlated with THg concentrations in the reconstructed fresh whole-egg homogenate. All data were natural-log transformed for analysis, and we report all egg THg concentrations in fresh

wet weight (fww); the mean (± SE) moisture content in eggs was 77.57 ± 0.09%. Albumen is reported in wet weight (ww).

Objective 2. Microsample live Forster's tern eggs to determine the effect of mercury and selenium on hatching success.

We monitored Forster's tern nests at several colonies from late April to August in 2008. During weekly colony visits 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. For each new nest, we also evaluated the suitability for microsampling using very strict criteria (Stebbins et al. 2009). Specifically, each nest needed to contain at least 2 eggs, and each egg in the nest was required to be <3 days in incubation. All nests that met these criteria and that could be microsampled within a limited time frame to reduce time spent on colonies were selected for microsampling, whereas all other nests remained un-manipulated. This protocol meant that we had to search for, find, and monitor approximately 10 times the number of nests needed for the microsampling study. The specifics of our microsampling protocol were described in *Objective 1* and Stebbins et al. (2009). If a microsampled nest contained only two eggs, then one egg was randomly selected for microsampling and the other was used as a control where it received the same treatment as the microsampled egg, but the shell was not breached in any way. In nests with more than two eggs, we randomly selected one egg for microsampling, one egg as a control, and one egg as a microsampling sham. The sham egg was breached and sealed in an identical fashion to the microsampled egg, but no albumen was withdrawn from the egg. During nest re-visits, 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.

Statistical Analyses for Objective 2

We evaluated the influence of THg and Se concentrations on egg hatching success using two different approaches. First, we used analysis of covariance (ANCOVA) to simultaneously test

for differences in albumen THg or Se concentrations among colonies, sampling date, and among egg fates (microsampled and control eggs hatched, microsampled egg failed and control egg hatched, microsampled egg hatched and control egg failed, and both microsampled and control eggs failed). Second, we used logistic regression to test whether albumen THg concentrations, albumen Se concentrations, or their molar ratio influenced the hatching probability of Forster's tern eggs. We included colony site as a cofactor to control for their potential effects on egg hatchability and THg levels.

Contaminant Determination

Mercury

Because most of the Hg in eggs and egg albumen is comprised of MeHg (Kennamer et al. 2005), we analyzed albumen and egg samples for THg (U. S. Geological Survey, Davis Field Station Mercury Lab). 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 each aliquot into a quartz sample vessel. For eggs, we dried the entire egg contents at 50°C for 48 hrs or until completely dried and re-weighed the egg contents to determine moisture content prior to analysis. We then ground the dried egg contents to a powder in a Wiley mill and/or porcelain mortar and pestle. Following EPA Method 7473 (U. S. EPA 2000), we analyzed each albumen and egg sample for THg on a Milestone DMA-80 Direct Mercury Analyzer (Milestone Inc., Monroe, Connecticut, USA) as described in Ackerman and Eagles-Smith (2009). 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, and calibration checks, respectively, averaged (± SE) 101.40 ± 1.92% (n = 13), 101.15 ± 1.33% (n = 19). Absolute relative percent difference for all duplicates averaged (± SE) 4.38 ± 1.30% (n = 13).

Selenium

All Se analyses were performed by the Trace Element Research Lab at Texas A&M University. Prior to Se analysis, albumen samples were digested with nitric acid, hydrogen peroxide, and hydrochloric acid. The digestion included a method blank for each set of 20 or fewer samples, a

spiked blank and SRM (NIST 2976) for evaluating accuracy, and a duplicate and spiked sample. Samples were run on an inductively coupled plasma-mass spectrometer (ICP-MS) equipped with a reaction cell to remove interferences (Perkin Elmer DRC 2). Ammonia was used as the reaction cell gas. Recoveries of certified reference materials, calibration checks, and matrix spikes, respectively, averaged (± SE) 94.33 ± 3.18% (n = 3), 95.00 ± 2.31% (n = 3), and 96.67 ± 1.86% (n = 3). Absolute relative percent difference for all duplicates averaged (± SE) 1.67 ± 0.67% (n = 3).

RESULTS

Objective 1. Link mercury concentrations in egg albumen to those of whole eggs.

Whole Egg and Egg Albumen Mercury Concentrations

We sampled albumen from 49 freshly laid eggs at 1.6 ± 0.2 (SE) days in incubation. THg concentrations (mean ± SD) were 2.32 ± 1.00 µg/g ww in albumen (range: 0.96-6.53 µg/g ww), and 1.44 ± 0.50 µg/g fww in the associated whole egg (range: 0.69-3.33 µg/g fww). These results are consistent with the fact that most of the egg volume is made of albumen, and with other studies that have shown that most of the Hg in avian eggs resides in the albumen (Kennamer et al. 2005).

THg concentrations in albumen were highly correlated with THg concentrations in the reconstructed fresh whole-egg homogenate (linear regression: n = 49, r^2 = 0.89, p < 0.0001; Figure 3). These results indicate that albumen THg concentrations can be used as a direct proxy for whole-egg THg concentrations.

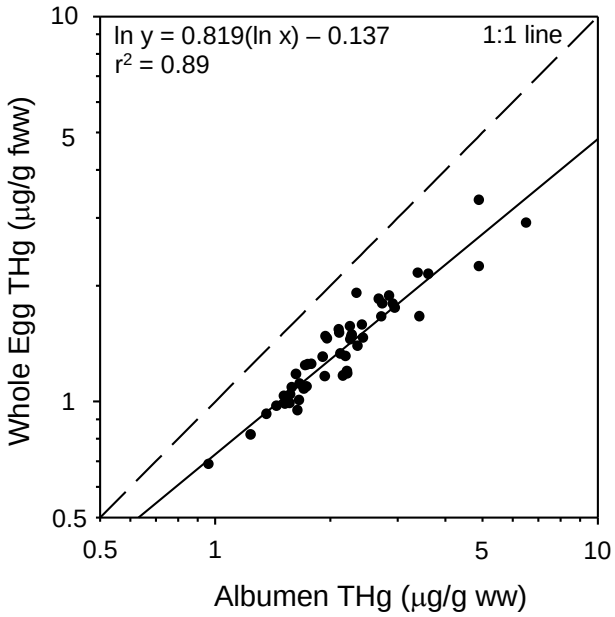

Figure 3. Total mercury (THg) concentrations in whole Forster's tern eggs are highly correlated with THg concentrations in egg albumen from the same eggs.

Objective 2. Microsample live Forster's tern eggs to determine the effect of mercury and selenium on hatching success.

We identified 940 Forster's tern nests at several sites in San Francisco Bay in 2008, including Ponds A1, AB1, A7, A8, A12, AB2, and A16. Of the 940 tern nests we found, 142 met our criteria (>1 egg in nest, all eggs incubated for <3 days) for microsampling. Of these, we successfully microsampled 132 nests. Of the 132 nests that we microsampled, 31 (23%) were depredated or otherwise destroyed and 24 (18%) were abandoned. For the remaining nests, both the microsampled and control eggs hatched in 62 (47%) nests, the microsampled egg hatched and the control egg failed for 7 (5%) nests, and the microsampled egg failed and the control egg hatched for 8 (6%) nests. Of the nests that we monitored but did not microsample (N = 758), 90 (12%) were depredated or destroyed and 217 (29%) were naturally abandoned. All eggs hatched in 370 (49%) nests, and at least 1 egg failed-to-hatch in 81 (11%) nests (Table 1). We analyzed THg concentrations in albumen (n = 77) of all microsampled eggs that were not subsequently destroyed or abandoned and Se concentrations for 50 samples. For four of the samples sent for Se analysis, the analytical laboratory did not have adequate sample mass for Se determination after THg determination. Thus, our effective sample size was 77 nests for THg and 46 for Se.

Table 1. Summary of fates for microsampled and undisturbed Forster's tern nests in San Francisco Bay, CA. during the 2008 nesting season.

Nest Type	Microsampled	Not Microsampled
Total # of nests	132	758
All eggs hatch	62 (47%)	370 (49%)
1 or more eggs fail to hatch	15 (11%)	81 (11%)
Nest abandoned	24 (18%)	217 (28%)
Nest depredated or destroyed	31 (24%)	90 (12%)

THg concentrations (mean ± SE) in albumen from microsampled eggs were 1.56 ± 0.08 μg/g ww (range: 0.33 – 3.58 μg/g ww), which is equivalent to an egg concentration of 1.26 μg/g (fww). This is similar to the average THg concentration of randomly sampled Forster's tern whole-eggs collected during the 2007 nesting season (1.27 ± 0.05 μg/g fww; Ackerman and Eagles-Smith 2008). Se concentrations (mean ± SE) in albumen from microsampled eggs were 0.58 ± 0.31 μg/g ww (range: 0.08 – 2.43 μg/g ww). It was beyond the scope of this study to develop an equation relating albumen Se concentrations with whole egg Se concentrations, but using a relatively robust relationship (r^2 = 0.94, n = 42) developed for Kildeer (Detwiler 2002), we estimate that Se concentrations in whole eggs averaged approximately 0.53 μg/g (fww). Neither THg nor Se concentrations in Forster's tern albumen differed among colonies or with date (Figure 4).

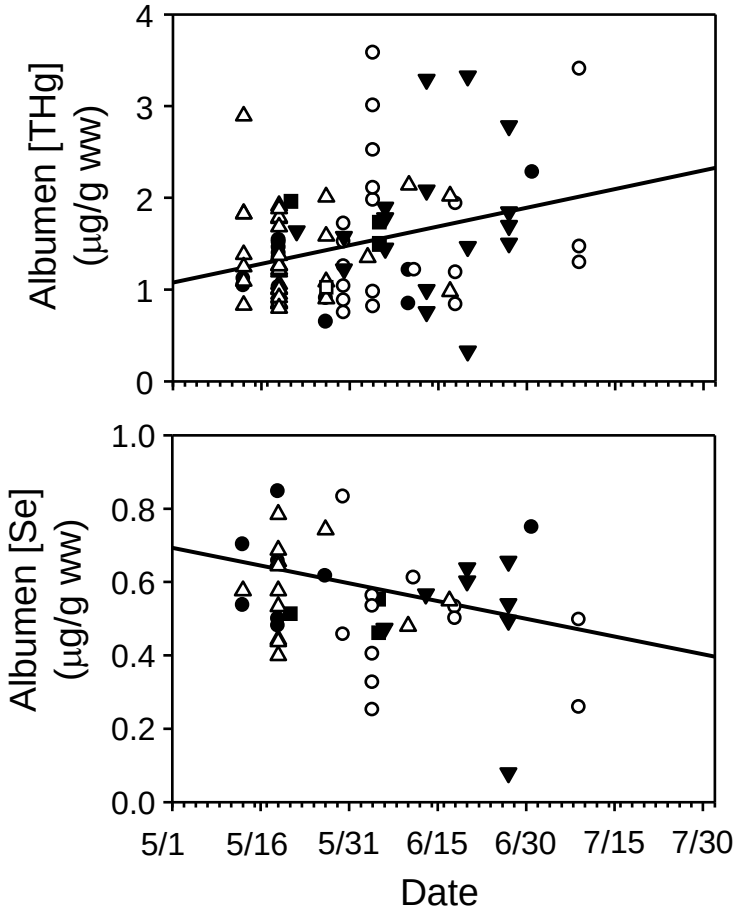

Figure 4. Total mercury (THg) and selenium (Se) concentrations in Forster's tern albumen was not significantly correlated with sampling date and did not vary among colonies. Colony key: ● = Pond A1, ○ = Pond A16, ▼ = Pond A7, △ = Pond AB1, ■ = Pond A12, □ = Pond A2W.

THg concentrations also did not differ among nests where either: 1) the microsampled egg failed to hatch but the control egg hatched, the microsampled egg hatched but the control egg failed to hatch, or both the microsampled and control eggs hatched (THg: Fate: $F_{2,64} = 1.40$, $p = 0.25$; Colony: $F_{5,64} = 1.05$, $p = 0.40$; Date: $F_{1,64} = 0.75$, $p = 0.39$; Se: Fate: $F_{2,37} = 0.51$, $p = 0.60$; Colony: $F_{4,37} = 0.17$, $p = 0.95$; Date: $F_{1,37} = 0.71$, $p = 0.40$; Figure 5). We found a weak inverse relationship between THg and Se concentrations in Forster's tern eggs (Linear Regression: $r^2 = 0.11$, $p = 0.03$, $n = 46$; Figure 6),

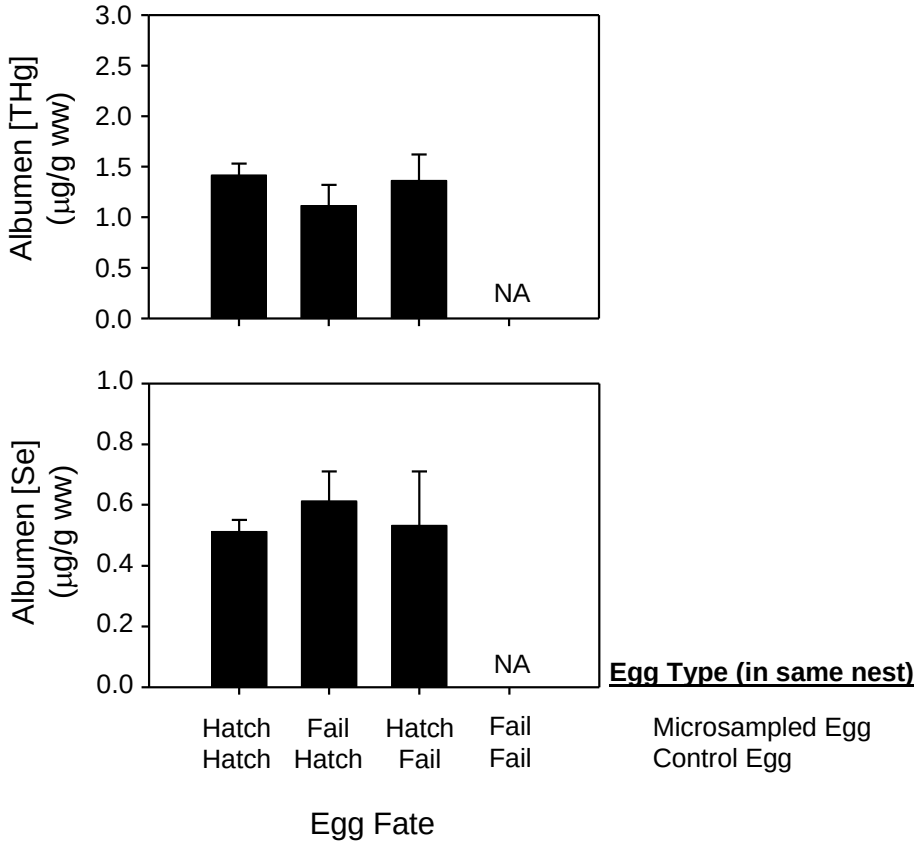

Figure 5. Total mercury (THg) and selenium (Se) concentrations from microsampled nests where (1) both the microsampled and control eggs hatched, (2) the microsampled egg failed and the control egg hatched, (3) the microsampled egg hatched and the control egg failed, and (4) both the microsampled and control eggs failed.

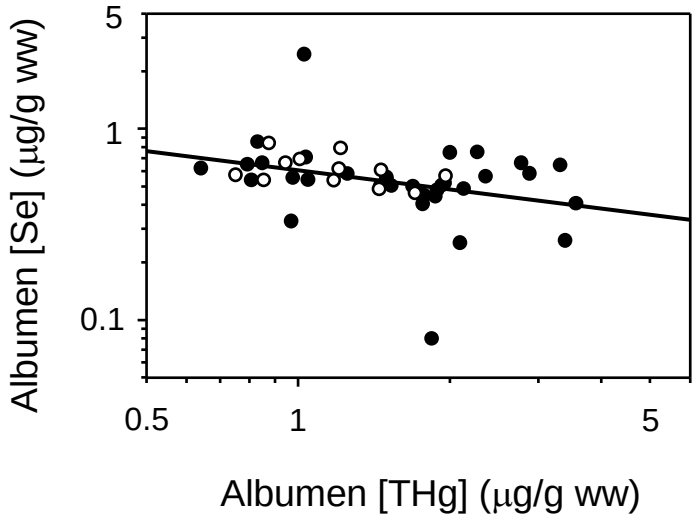

Figure 6. Selenium (Se) and total mercury (THg) concentrations in microsampled Forster's tern eggs were weakly correlated ($r^2 = 0.11$, $p = 0.03$, $n = 46$). Open circles are unsuccessful (failed) eggs and closed circles are successful (hatched) eggs.

We used logistic regression models to assess the effects of albumen THg and Se concentrations on the probability of microsampled eggs hatching. In our initial model we included albumen [THg], albumen [Se], the albumen [THg] × albumen [Se] interaction, and colony as factors. Because the interaction was not significant ($\chi^2_2 = 2.03$, $p = 0.15$; $n = 46$), it was excluded from our final model which contained [THg], [Se], and colony site. We did not find any effects of these factors on the likelihood of an egg hatching (albumen THg: $\chi^2_1 = 3.11$, $p = 0.08$; albumen Se: $\chi^2_1 = 0.84$, $p = 0.36$; colony: $\chi^2_4 = 3.70$, $p = 0.45$; $n = 46$).

Interpreting the toxicity of Hg and Se together can be confounded when both contaminants are evaluated solely on a concentration basis because Hg and Se can bind together at the molecular level to form an insoluble Hg-Se complex, which may alter the toxicity of one or both contaminants (Cuvin-Aralar and Furness 1991). Since the mass of Hg (200.59 g/mol) is approximately 2.5 times greater than Se (78.96 g/mol), interpreting their potential interaction requires evaluating their molar ratio (mol Hg/g egg: mol Se/g egg) to determine if there is an excess of one contaminant relative to the other. We found a wide range of THg:Se molar ratios in Forster's tern albumen, ranging from 0.17 to 9.18. The mean (±SE) ratio was 1.44 ± 0.22, indicating that, on average, there was a slight excess of Hg in the albumen relative to Se. The THg:Se molar ratio was positively correlated with albumen THg concentrations (linear regression: $r^2 = 0.66$, $p < 0.0001$, $n = 46$, Figure 7) and negatively correlated with albumen Se concentrations ($r^2 = 0.68$, $p < 0.0001$, $n = 46$; Figure 7), indicating that since a change in either Hg or Se concentrations can alter the molar ratio of the two, the variance associated with both contaminants should be statistically controlled when evaluating the influence of molar ratio on hatching success.

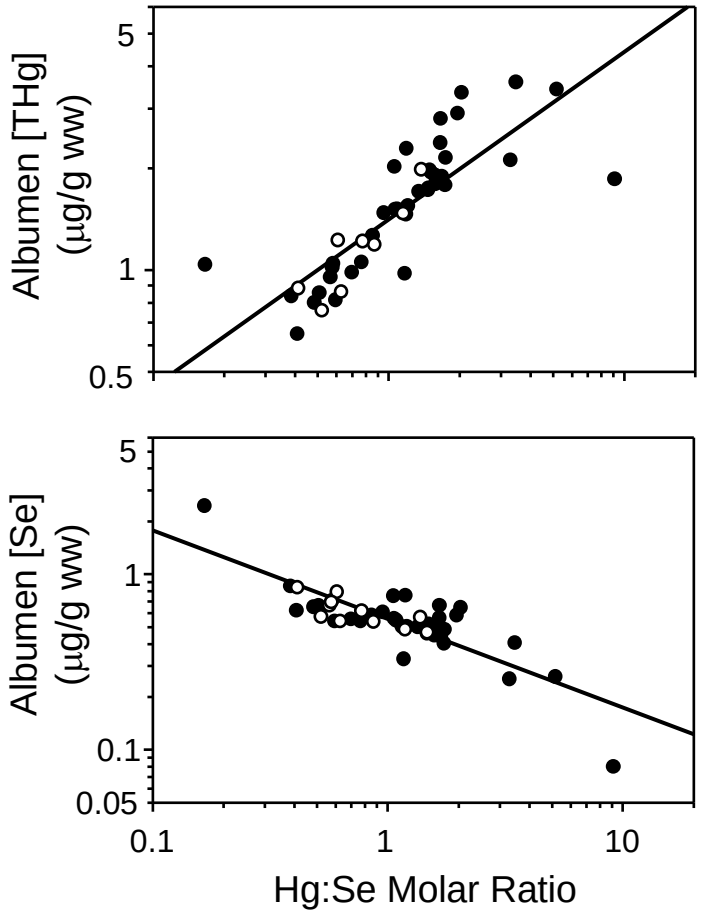

Figure 7. Linear correlation between Forster's tern egg albumen total mercury (THg; top panel) and selenium (Se; bottom panel) concentrations and the Hg:Se molar ratio. Open circles are unsuccessful (failed) eggs and closed circles are successful (hatched) eggs.

When the THg:Se molar ratio was added to our logistic model of hatching probability containing colony, albumen [THg], and albumen [Se], we found that Hg concentrations, Se concentrations, and the Hg:Se molar ratio all significantly influenced the probability of egg hatching (albumen THg: $\chi^2_1 = 6.86$, $p = 0.009$; albumen Se: $\chi^2_1 = 5.95$, $p = 0.015$; colony: $\chi^2_4 = 7.24$, $p = 0.12$; molar ratio: $\chi^2_1 = 10.01$, $p = 0.002$, $n = 46$). These results indicate that the probability of hatching was positively influenced by the THg:Se molar ratio. Although the THg:Se molar ratio was the most important factor influencing hatching success, albumen THg concentrations also influenced the probability of hatching such that the residual variance in THg that was not explained by the THg:Se molar ratio negatively influenced egg hatching. This suggests that THg concentrations statistically reduced the probability of egg hatching successfully, but that the THg:Se molar ratio positively influenced egg hatching to a larger degree.

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

In contrast to our recent, previous studies of Hg effects on Forster's tern egg hatchability (Ackerman and Eagles-Smith 2008, Authors' unpublished data), we found no strong evidence that Hg or Se (individually or in conjunction) influenced egg hatchability using the microsampling technique. The reason for this discrepancy is unclear, but may be due to the fact that Forster's tern egg Hg concentrations in San Francisco Bay in 2008 were substantially lower than in any of the previous four years (Figure 7).

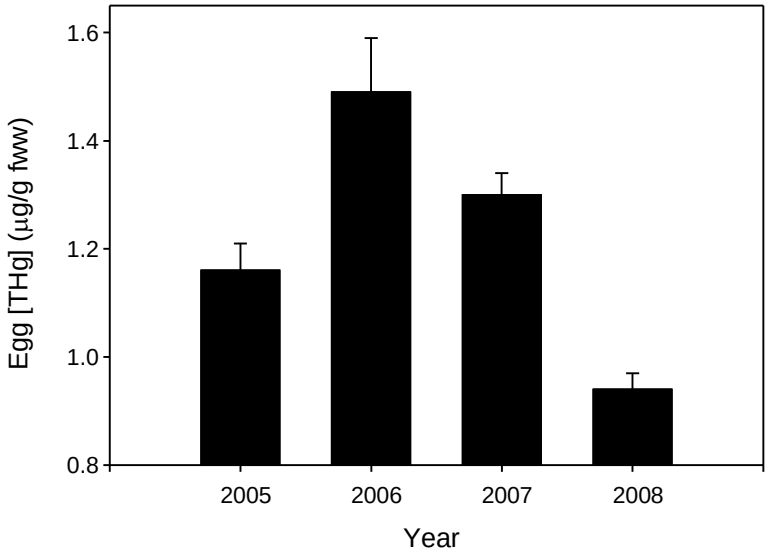

Figure 7. Annual trend in Forster's tern egg total mercury (THg) concentrations in San Francisco Bay from 2005 to 2008.

In fact, the mean THg concentration of randomly sampled Forster's tern eggs in 2008 was below 1.0 µg/g fww, a commonly cited concentration above which egg hatchability can be impaired in other waterbird species (Scheuhammer et al. 2007). Similarly, Ackerman and Eagles-Smith (2008) did not find differences between random and failed to hatch eggs using data from a single year (2007), but found that Hg concentrations were significantly higher in failed-to-hatch eggs than in randomly sampled eggs when the data set was expanded to include two additional years (2005 and 2006). Thus, it is likely that temporal and spatial variability in factors such as egg Hg concentrations, food availability, and predation pressure confound the relationship between Hg exposure and egg hatchability in the wild, and data across multiple years are required to

statistically control for these factors with adequate power.

We also acknowledge that the use of the microsampling technique may have influenced our results, though we found no evidence that this method biased our data in any way. In fact, failure rate of microsampled eggs (6%) was nearly identical to control eggs from our microsampled nests (5%). In addition, a similar proportion of microsampled nests hatched all eggs in the clutch in comparison to nests that were not microsampled. Finally, we found a very robust linear relationship between THg concentrations in egg albumen and THg concentrations in the whole-eggs from which albumen was microsampled, indicating that albumen THg concentrations are adequately representative of THg concentrations in the whole egg.

Selenium is known to interact with Hg with regards to toxic endpoints, and it is generally thought that Se ameliorates the toxicity of Hg, though there is some evidence that the two contaminants can be synergistic, causing much reduced hatching success at higher concentrations. As with Hg, we found no direct influence of albumen Se concentrations on egg hatchability, nor did we find an interaction between the two contaminants. However, we did find a significant effect of the Hg:Se molar ratio on egg hatchability when controlling for the variance associated with both THg and Se. These results suggest that the toxicity of either contaminant is strongly mediated by the relative excess of Hg or Se. Unfortunately, we lacked statistical power to fully explore this possibility (n = 46), and larger sample sizes may provide more robust conclusions. Thus, it is important for field-based studies of toxic effects to incorporate substantially large sample sizes to reach an appropriate level of power for developing toxicity thresholds.

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