Environmental Science & Technology

ARTICLE

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Bird Mercury Concentrations Change Rapidly as Chicks Age: Toxicological Risk is Highest at Hatching and Fledging

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Supporting Information

ABSTRACT: Toxicological risk of methylmercury exposure to juvenile birds is complex due to the highly transient nature of mercury concentrations as chicks age. We examined total mercury and methylmercury concentrations in blood, liver, kidney, muscle, and feathers of 111 Forster's tern (*Sterna forsteri*), 69 black-necked stilt (*Himantopus mexicanus*), and 43 American avocet (*Recurvirostra americana*) chicks as they aged from hatching through postfledging at wetlands that had either low or high mercury contamination in San Francisco Bay, California. For each waterbird species, internal tissue, and wetland, total mercury and methylmercury concentrations changed rapidly as chicks aged and exhibited a quadratic, U-shaped pattern from hatching through postfledging. Mercury concentrations were highest immediately after hatching, due to maternally deposited mercury in eggs, then rapidly declined as chicks aged and diluted their mercury body burden through growth in size and mercury depuration into growing feathers. Mercury concentrations then increased during fledging when mass gain and feather growth slowed, while chicks continued to acquire dietary mercury. In contrast to mercury in internal tissues, mercury concentrations in chick feathers were highly variable and declined linearly with age. For 58 recaptured Forster's tern chicks, the proportional change in blood mercury concentration was negatively related to the proportional change in body mass, but not to the amount of feathers or wing length. Thus, mercury concentrations declined more in chicks that gained more mass between sampling events. The U-shaped pattern of mercury concentrations from hatching to fledging indicates that juvenile birds may be at highest risk to methylmercury toxicity shortly after hatching when maternally deposited mercury concentrations are still high and again after fledging when opportunities for mass dilution and mercury excretion into feathers are limited.

INTRODUCTION

Methylmercury is a global pollutant that biomagnifies through aquatic food chains and can be highly toxic to humans and wildlife.[1] Reproduction is among the most sensitive toxicity end points to methylmercury exposure in birds.[1] Several laboratory studies that dosed birds with experimental mercury concentrations have demonstrated that methylmercury can reduce egg hatching success [2−4] and impair chick behavior, health, growth, and survival.[5−7] However, few studies have been able to detect effects of methylmercury on chicks in the wild.[8−10]

Examining the effects of methylmercury exposure on juvenile birds is complicated due to the highly dynamic nature of mercury concentrations through time as chicks age from hatching to fledging.[11,12] For example, the half-life of methylmercury concentrations in blood of Cory's shearwater (*Calonectris diomedea*) chicks was shorter (5−6 days) than in adults (40−65 days; [13,14]). Similarly, the half-life of methylmercury concentrations in common loon (*Gavia immer*) chicks that were actively growing feathers was much shorter (3 days) than in older chicks (116 days) that had already completed feather growth.[15] Quantifying the rapid changes in mercury concentrations as chicks age would improve our understanding of the sensitive time periods when juvenile birds are at greatest risk to methylmercury toxicity.

At hatching, mercury concentrations in chicks are the result of maternally deposited methylmercury into the egg. Thereafter, mercury concentrations in juvenile birds are primarily influenced by methylmercury accumulation through the diet, as well as changes in bird size and sequestration of methylmercury into actively growing feathers. Although the total body burden of mercury may continue to increase in chicks as they age and acquire methylmercury through their diet, the concentrations of mercury in their tissues can decrease due to mass dilution resulting from the approximate 10-fold increase in size as they age from hatching to fledging. Simultaneously, growing chicks also are developing feathers which provide a temporary depuration pathway for methylmercury and results in a decrease in both internal tissue mercury concentrations and body burdens. Feathers

Received: February 24, 2011
Accepted: May 6, 2011
Revised: April 26, 2011
Published: May 18, 2011

can account for 34−65% of the total body burden of mercury in juvenile birds.[11−13,16] The importance of feather growth for reducing mercury concentrations in juvenile birds has been demonstrated often,[11−13,15,17] but few studies have quantitatively demonstrated the importance of mass dilution in birds. The complex interplay among methylmercury dietary accumulation, sequestration in feathers, and mass dilution as chicks age make it difficult to understand the mercury dynamics and toxicological risk to juvenile birds.

In this work, we identified the time periods for the greatest potential toxicity of methylmercury to juvenile birds by examining total mercury and methylmercury concentrations in chicks as they aged from hatching through postfledging. We did so in three waterbird species (Forster's tern, *Sterna forsteri*; black-necked stilt, *Himantopus mexicanus*; and American avocet, *Recurvirostra americana*) and in five tissues (blood, liver, kidney, muscle, and feathers), at wetlands known to have either low or high mercury contamination.[9,10] After we established the observed quadratic (U-shaped) pattern of mercury concentrations as chicks aged, we then assessed the relative importance of mass dilution and methylmercury depuration into feathers as causes for this pattern.

EXPERIMENTAL SECTION

Study Area. We studied free-living Forster's tern (hereafter tern), black-necked stilt (hereafter stilt), and American avocet (hereafter avocet) chicks during the 2005−2007 breeding seasons in San Francisco Bay, California. See Supporting Information for details.

Mark−recapture of Tern Chicks from Hatching to Fledging. We banded and recaptured tern chicks weekly from nest initiation (early May) until the last tern chick fledged (late August). We entered colonies weekly, marked and monitored each nest to determine fate, hand-captured every chick at the colony, and banded newly hatched chicks with stainless steel U.S. Geological Survey leg bands or recorded band numbers from recaptured chicks. At every visit, we weighed each chick with a spring scale (±1.0 g with 100-g or 300-g Pesola spring scales, Pesola Ag, Baar, Switzerland), and measured exposed culmen length and short tarsus (tarsometatarsus bone) length with digital calipers (±0.01 mm with Fowler electronic digital calipers, Newton, Massachusetts, U.S.) and flattened wing length with a wing board (±1.0 mm). When a chick was captured for the first time, we collected approximately 15 down feathers from the rump and mantle. Upon recapture of older chicks, we collected approximately 10 fully grown feathers from the breast. We stored feathers in Whirl-paks (Nasco, Modesto, California, USA) until mercury analysis. We held birds in shaded and screen-lined poultry cages (model 5KTC, Murray McMurray Hatchery, Webster City, Iowa, U.S.) and returned them back to the site of capture within 3 h.

Tern Chick Age Estimation. If we observed the hatching date for an individual chick during our routine nest monitoring visits, then we calculated the chick's age at every subsequent capture event by subtracting the date of recapture from the date of hatching. We considered chicks to have an age of one day if the chick was still located in or near the nest bowl, had eggshell membrane still attached to its down feathers or was still wet from hatching, and weighed ≤18 g. For chicks with unknown hatch dates, we estimated chick age using a model developed from 5 years and 1372 capture histories for 806 tern chicks with known hatching dates in San Francisco Bay (chick age in days = $20.7399-(3.3421 \times \log_e((42.05-\text{culmen})/\text{culmen})) - (5.6317 \times \log_e((279.63-\text{wing})/\text{wing}))$; $R^2 = 0.99$; J.T. Ackerman, unpublished data). We then calculated the hatching date by subtracting the chick's estimated age from the date on which it was captured and measured for the first time. For subsequent recaptures of the same chick, we estimated the chick's current age by subtracting the date it was recaptured from the estimated hatching date.

Stilt and Avocet Chick Age Estimation. We entered stilt and avocet nesting sites weekly, marked each new nest, and monitored nests to determine their fate. Unlike our methods for terns, we did not use mark−recapture methodology to track stilt and avocet chicks after they hatched and left the nest. Instead, we captured chicks with long-handled nets near nesting sites. We measured and weighed stilt and avocet chicks similarly to terns. There were no available models for estimating age of stilt and avocet chicks. Instead, we used exposed culmen length as an index of age because it is linearly correlated with known age in the related Hawaiian stilt (*Himantopus mexicanus knudseni* [18]).

Bird Collections, Sample Processing, and Chemical Determination. We collected tern, stilt, and avocet chicks throughout the period from hatching to just after fledging (approximately 28−35 days for terns,[19] 27−31 days for stilts,[20] and 27 days for avocets[21]). We also collected recently flighted chicks near nesting colonies with shotguns, using steel shot. We describe our methodology for bird necropsies, tissue processing, and chemical determination elsewhere.[22] Briefly, after a bird was collected, we immediately sampled whole blood via cardiac puncture, collected down feathers from the rump and mantle, collected fully grown feathers from the breast, and conducted necropsies on birds to excise the liver, kidney, and a sample of breast muscle tissue. For collected tern chicks, we also visually estimated the proportion of fully grown feathers present at the time of collection by averaging the estimates of two observers who independently examined each bird. To increase our sample size, we captured and released additional chicks after collecting whole blood via the brachial vein (0.5−1.5 mL, ≤1% of body mass). We determined total mercury (THg) concentrations via thermal decomposition and cold-vapor atomic absorption spectroscopy, and methylmercury (MeHg) concentrations on a subset of liver and kidney samples using cold vapor atomic fluorescence. See Supporting Information file for details on Hg determination and quality assurance.

Statistical Analysis. In the first stage of our analysis, we evaluated the effect of age on chick THg and MeHg concentrations in the three species and five tissues separately using analysis of covariance (ANCOVA; JMP version 8.0.2). The dependent variable was Hg concentration (either THg or MeHg in blood, liver, kidney, muscle, or feathers) and the fixed factors were wetland site, calendar date, age, and age-squared (hereafter age^2). We used $\log_e$-transformed THg and MeHg concentrations in dry weight (dw), except for blood where we used wet weight (ww). For stilts and avocets, culmen length was used as the index of age, and we included year as a fixed factor because chicks were collected at the same site in multiple years. For avocets, we assessed THg and MeHg concentrations only in blood, liver, and feathers due to a lack of muscle and kidney mass for samples from young avocet chicks, and therefore an incomplete time series of data. For all species and tissues, we assessed the relative fit of the linear age model versus the nonlinear (quadratic) age model using the likelihood ratio test. We then tested whether the quadratic pattern of Hg concentrations with age differed among

low and high Hg sites by using the likelihood ratio test to determine if adding the site interactions (age^2 × site and age × site) to the nonlinear age model improved model fit. To estimate the age when Hg concentrations stopped decreasing and began to increase, we calculated the point of inflection for the best quadratic model by solving for age when the derivative equaled zero. See Supporting Information file for details on graphical presentation of stilt and avocet data.

In the second stage of our analysis, we used tern chicks to evaluate whether body mass and feather production contributed to the observed quadratic pattern of Hg concentrations as chicks aged. We tested whether the proportional change in an individual's blood THg concentration was related to the proportion of fully grown feathers and the proportional change in its body mass over the same time period using ANCOVA. The dependent variable was $\log_e$-transformed proportional change in blood THg concentration, and fixed factors in the model included wetland site, calendar date, age, age^2, $\log_e$-transformed proportion of fully grown feathers or wing length, and $\log_e$-transformed proportional change in body mass. The proportional change in body mass was calculated as chick mass at the final Hg sampling event divided by chick mass at the initial Hg sampling event. Similarly, the proportional change in an individual's blood THg concentration was calculated as blood THg concentration at the time of the final mass measurement divided by blood THg concentration at the time of the initial mass measurement. For this analysis, we used only those tern chicks that were initially captured at ≤8 days of age. Because we could not sample enough volume of blood from newly hatched chicks without potentially influencing subsequent growth, we developed a model to predict THg concentrations in blood from THg concentrations in down feathers. To develop this regression model, we used a subset of chicks that were collected at ≤8 days of age. THg concentration in blood was the dependent variable and THg concentration in down feathers and chick age (linear; from 0 to 8 days) were independent variables. We then used this resulting equation (see Results) to estimate THg concentrations in blood for chicks that were ≤ 8 days of age at the time of their first capture (initial blood and mass sample) and that were subsequently sampled at older ages (final blood and mass sample).

RESULTS

Mercury Concentrations as Chicks Aged: Terns. We sampled 111 tern chicks from two breeding colonies in salt ponds A16 and N7, representing a relatively high and low Hg site, respectively. THg concentrations in blood of tern chicks were 1.95 times higher at A16 then at N7. Accounting for calendar date and site, THg concentrations in blood were significantly related to tern chick age (age^2: $F_{1,106} = 115.96$, $p < 0.0001$; age: $F_{1,106} = 168.58$, $p < 0.0001$; site: $F_{1,106} = 36.30$, $p < 0.0001$; date: $F_{1,106} = 0.69$, $p = 0.41$). Using the likelihood ratio test, we found that the model containing a quadratic age coefficient better explained the data than did a similar model with only a linear age coefficient ($n = 111$, $\chi^2 = 82.03$, $p < 0.0001$). In order to test whether the quadratic pattern of THg concentrations with age differed among sites, we conducted a third iteration of the model which included interactions age^2 × site ($F_{1,104} = 1.36$, $p = 0.25$) and age × site ($F_{1,104} = 2.45$, $p = 0.12$), and found that the model with the interactions did not improve model fit ($n = 111$, $\chi^2 = 4.00$, $p = 0.14$). On the basis of the inflection point of the best model, THg concentrations in chick blood rapidly decreased with age until 20 days of age and then increased thereafter (Figure 1A).

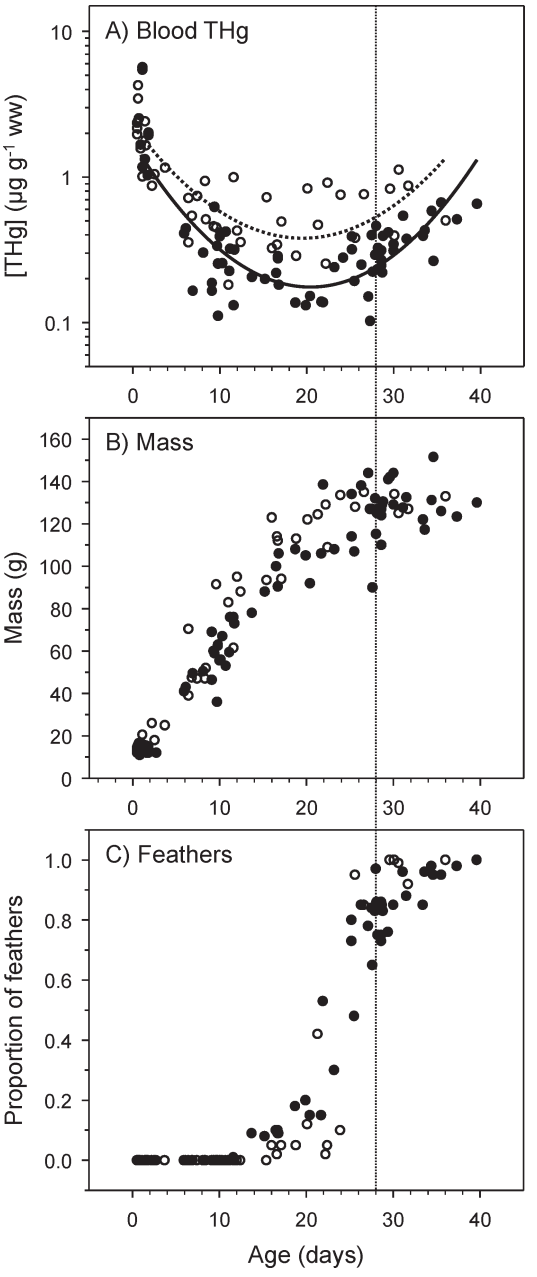

Figure 1. (A) Total mercury (THg) concentrations in blood, (B) chick mass (g), and (C) proportion of fully grown feathers as Forster's tern chicks age from hatching (0 days) to postfledging in San Francisco Bay, California. Open symbols indicate the higher Hg contaminated wetland (A16) and closed symbols indicate the lower Hg contaminated wetland (N7). The stippled vertical line indicates the approximate age when Forster's tern chicks become flighted (28 days; [23]).

We performed the same analyses for the influence of age on THg and MeHg concentrations in other internal tissues for a subset of tern chicks sampled at N7 (Figures 2A–E). Similar to our results for blood, THg concentrations in liver, kidney, and muscle and MeHg concentrations in liver and kidney were significantly nonlinearly related to chick age (all $p < 0.01$ for age^2 effect; Table S1 of the SI). In all cases, the model containing the quadratic age coefficient better explained the data than a

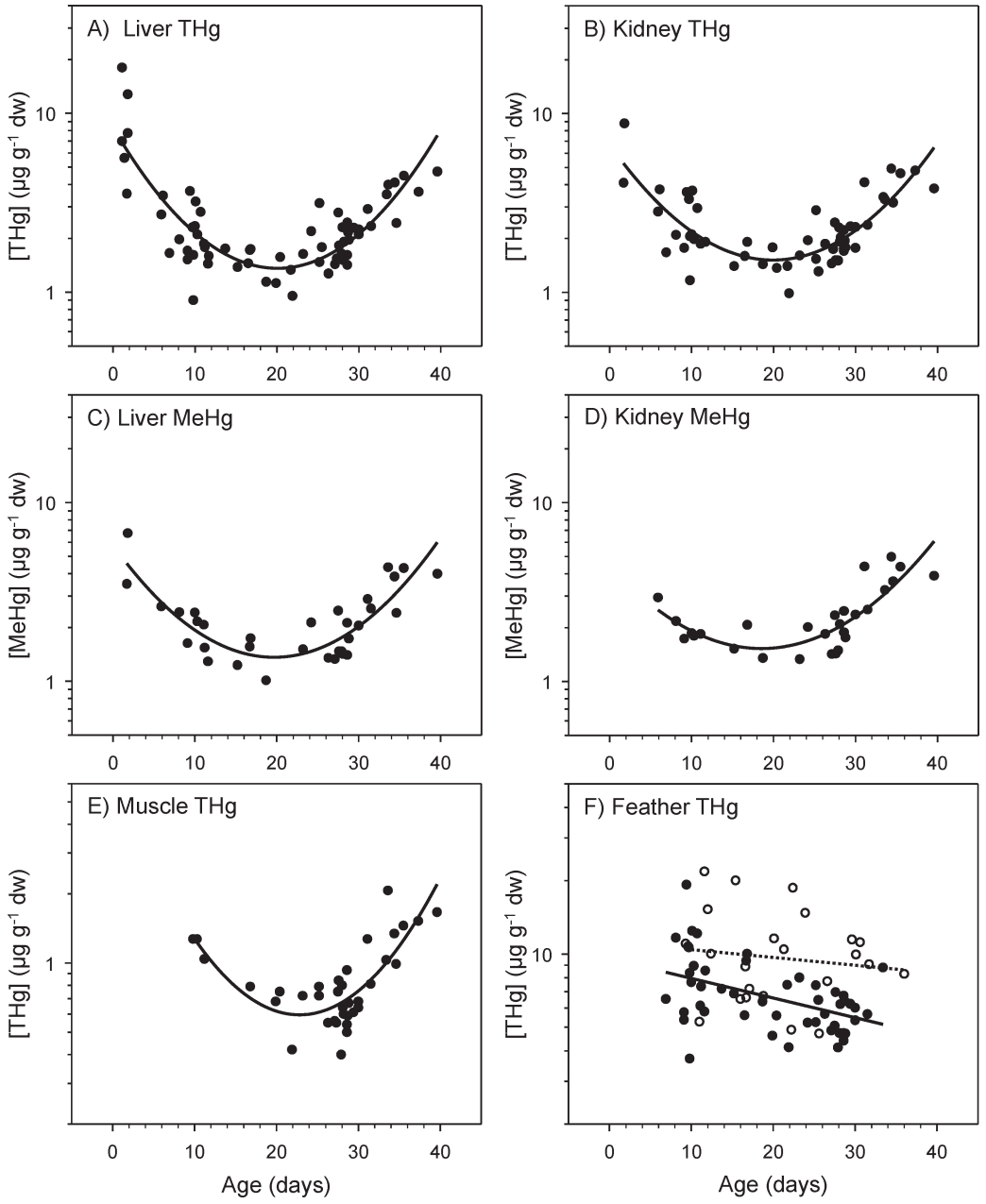

Figure 2. Total mercury (THg) concentrations in (A) liver, (B) kidney, (E) muscle, and (F) fully grown feathers, and methylmercury (MeHg) concentrations in (C) liver and (D) kidney as Forster's tern chicks age from hatching (0 days) to postfledging (chicks fledge at 28 days [23]) in San Francisco Bay, California. Open symbols indicate the higher Hg contaminated wetland (A16) and closed symbols indicate the lower Hg contaminated wetland (N7).

similar model with only a linear age coefficient (THg liver: $n = 65$, $\chi^2 = 62.30$, $p < 0.0001$; THg kidney: $n = 56$, $\chi^2 = 38.97$, $p < 0.0001$; THg muscle: $n = 36$, $\chi^2 = 26.98$, $p < 0.0001$; MeHg liver: $n = 33$, $\chi^2 = 26.00$, $p < 0.0001$; MeHg kidney: $n = 28$, $\chi^2 = 7.91$, $p = 0.01$). We also assessed the relationship between THg concentrations in fully grown feathers and age for chicks >8 days old when feathers began appearing (Figure 2F). For feathers, the quadratic age coefficient did not improve the linear age model's fit to the data ($n = 72$, $\chi^2 = 2.63$, $p = 0.10$) and THg concentrations in fully grown feathers tended to decline with age but was not significant ($p = 0.14$ for age effect; Table S1 of the SI).

Mercury Concentrations as Chicks Aged: Stilts. We sampled 69 stilt chicks from two wetlands representing a high and low Hg site. THg concentrations in blood of stilt chicks were 2.33 times higher at New Chicago Marsh than at Eden Landing Ecological Reserve. Accounting for calendar date, year, and site, THg concentrations in blood were significantly related to the index of stilt chick age (age^2: $F_{1,62} = 51.52$, $p < 0.0001$; age: $F_{1,62} = 59.79$, $p < 0.0001$; site: $F_{1,62} = 26.91$, $p < 0.0001$; date: $F_{1,62} = 0.55$, $p = 0.46$; year: $F_{2,62} = 7.62$, $p = 0.001$). The model with the quadratic age coefficient better explained the data than did a similar model with only a linear age coefficient ($n = 69$, $\chi^2 = 41.74$, $p < 0.0001$), and the pattern was similar among sites such that adding the interactions (age^2 × site: $F_{1,60} = 1.98$, $p = 0.17$; age × site: $F_{1,60} = 2.16$, $p = 0.15$) did not improve model fit ($n = 69$, $\chi^2 = 2.51$, $p = 0.28$). On the basis of the inflection point of

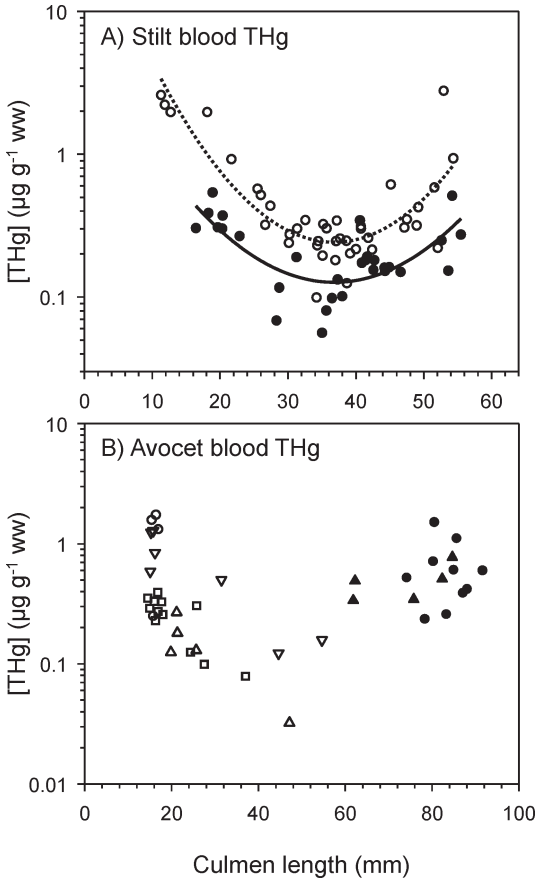

Figure 3. Total mercury (THg) concentrations in the blood of (A) black-necked stilt and (B) American avocet chicks as they age from hatching to postfledging in San Francisco Bay, California. Exposed culmen length (mm) was used as an index of age. Culmen length is approximately (A) 12 mm at hatching and 41 mm at fledging for stilts and (B) 16 mm at hatching and 55 mm at fledging for avocets. For stilts (A), open symbols indicate the higher Hg contaminated wetland (New Chicago Marsh) and closed symbols indicate the lower Hg contaminated wetland (Eden Landing Ecological Reserve). For stilts (A), data from years 2005 and 2007 were standardized to 2006 (see Supporting Information). For avocets (B), different symbol shapes indicate different wetlands (circles are Alviso, triangles are Eden Landing Ecological Reserve, upside-down triangles are New Chicago Marsh, and squares are Newark wetlands) and open symbols indicate 2006 and closed symbols indicate 2005.

the quadratic model, we found that THg concentrations in stilt chick blood rapidly decreased until their culmen length was 37 mm and then THg concentrations increased thereafter (Figure 3A).

We performed the same analyses for the influence of age on THg and MeHg concentrations in other internal tissues for a subset of stilt chicks. THg concentrations in liver, kidney, and muscle and MeHg concentrations in liver were significantly nonlinearly related to chick age (all $p < 0.04$ for age^2 effect; Table S1 of the SI). Except for MeHg concentrations in kidneys, the models containing the quadratic age coefficient better explained the data than similar models with only a linear age coefficient (THg liver: $n = 63$, $\chi^2 = 31.73$, $p < 0.0001$; THg kidney: $n = 63$, $\chi^2 = 25.60$, $p < 0.0001$; THg muscle: $n = 49$, $\chi^2 = 5.03$, $p = 0.02$; MeHg liver: $n = 44$, $\chi^2 = 17.47$, $p < 0.0001$; MeHg kidney: $n = 37$, $\chi^2 = 0.47$, $p = 0.49$). Similar to terns, the quadratic age coefficient did not improve the linear age model's fit to the data for feathers ($n = 54$, $\chi^2 = 0.16$, $p = 0.69$) and THg concentrations in fully grown feathers tended to decline with age ($p = 0.07$ for age effect; Table S1 of the SI).

Mercury Concentrations as Chicks Aged: Avocets. Due to their low availability, it was necessary to sample avocet chicks at many different wetlands to obtain a sample of 43 chicks. THg concentrations in blood were significantly related to the index of avocet chick age, while accounting for calendar date, year, and site (age^2: $F_{1,35} = 8.01$, $p = 0.01$; age: $F_{1,35} = 24.64$, $p < 0.0001$; site: $F_{3,35} = 8.72$, $p = 0.001$; date: $F_{1,35} = 1.73$, $p = 0.20$; year: $F_{1,35} = 20.21$, $p < 0.0001$). The model with the quadratic age coefficient better explained the data than did a similar model with only a linear age coefficient for THg concentrations in blood ($n = 43$, $\chi^2 = 8.86$, $p = 0.01$) and liver ($n = 44$, $\chi^2 = 4.91$, $p = 0.03$), and MeHg concentrations in liver ($n = 29$, $\chi^2 = 6.83$, $p = 0.01$). The quadratic pattern of THg concentrations in chick blood with age was similar among sites such that adding the interactions (age$^2 \times$ site: $F_{3,29} = 0.25$, $p = 0.86$; age $\times$ site: $F_{3,29} = 0.18$, $p = 0.91$) did not improve model fit ($n = 43$, $\chi^2 = 11.02$, $p = 0.09$). Similar to terns and stilts, the quadratic age coefficient did not improve the linear age model's fit to the data for feathers ($n = 23$, $\chi^2 = 0.06$, $p = 0.80$) and THg concentrations in fully grown feathers tended to decline with age but was not significant ($p = 0.08$ for age effect; Table S1 of the SI). On the basis of the inflection points of the quadratic models for THg and MeHg concentrations in blood and liver, Hg concentrations in avocet chicks decreased until their culmen length was approximately 64−81 mm and then increased thereafter (Figure 3B).

Correlation between Mercury Concentrations in Blood and Down Feathers for Tern Chicks. Using only tern chicks that were ≤8 days of age ($n = 33$), THg concentrations in blood were strongly correlated with THg concentrations in down feathers while accounting for the linear effect of age (THg down feathers: $F_{1,30} = 55.37$, $p < 0.0001$; age: $F_{1,30} = 175.69$, $p < 0.0001$; Figure S1 of the SI). Using this model (R^2=0.91; $\log_e$-THg concentrations in blood = $-1.4485+[0.7972 \times \log_e$-THg concentrations in down feathers$] + [-0.19945 \times$ age$]$), we then estimated THg concentrations in blood from THg concentrations in down feathers for chicks sampled at ≤8 days of age that were subsequently recaptured and bled at an older age.

Influence of Mass and Feather Growth on Mercury Concentrations in Tern Chicks. Tern chicks gained mass rapidly until just before they fledged (28 days), increasing in size from hatching by nearly 10-fold (Figure 1B). Simultaneously, tern chicks were mostly covered with down feathers up until 10 days of age, at which time the amount of fully grown feathers increased from <1% to >80% at the time of fledging (Figure 1C).

We assessed the change in an individual chick's THg concentration in relation to their change in body mass and fully grown feathers for 58 tern chicks where we were able to resample them ≥7 days (14.4 ± 6.8 days [mean ± SD]) after their initial sampling at ≤8 days of age. The proportional change in an individual chick's blood THg concentration was negatively related to the proportional change in its body mass, but not fully grown feathers (proportional mass change: $F_{1,51} = 56.09$, $p < 0.0001$; proportion fully grown feathers: $F_{1,51} = 0.03$, $p = 0.86$; age^2: $F_{1,51} = 0.12$, $p = 0.73$; age: $F_{1,51} = 1.77$, $p = 0.19$; site: $F_{1,51} = 2.84$, $p = 0.10$; date: $F_{1,51} = 0.69$, $p = 0.41$), such that THg concentrations declined more in chicks which gained more mass between sampling events (Figure 4). We also conducted the same analysis using a second index of feather production (wing length as opposed to fully grown feathers) with similar results

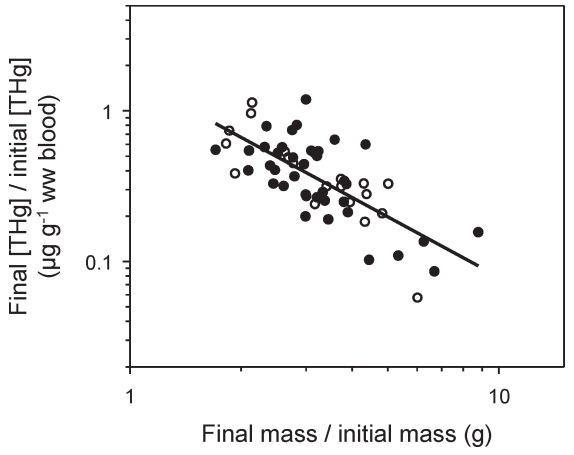

Figure 4. The proportional change in an individual's blood total mercury (THg) concentration (final THg concentration divided by initial THg concentration) was negatively related to the proportional change in its body mass (final mass divided by initial mass) between sampling events for Forster's tern chicks in San Francisco Bay, California. Leverage plot accounting for the potential influence of age^2, age, site, date, and proportion of fully grown feathers, illustrates that Hg concentrations declined more in chicks which gained more mass between sampling events. Initial blood THg concentrations were estimated using THg concentrations in down feathers (see Results). Open symbols indicate the higher Hg contaminated wetland (A16) and closed symbols indicate the lower Hg contaminated wetland (N7).

(proportional mass change: $F_{1,51} = 56.00$, $p < 0.0001$; wing length: $F_{1,51} = 0.05$, $p = 0.83$; age^2: $F_{1,51} = 0.03$, $p = 0.87$; age: $F_{1,51} = 1.26$, $p = 0.27$; site: $F_{1,51} = 3.19$, $p = 0.08$; date: $F_{1,51} = 0.79$, $p = 0.38$).

DISCUSSION

We found that THg and MeHg concentrations in juvenile birds changed rapidly as they aged, and varied by more than an order of magnitude over this short time period (~40 days). Chick Hg concentrations exhibited a quadratic, U-shaped pattern from hatching through postfledging at both low and high Hg contaminated sites, where blood THg concentrations differed by 2.0 (terns) to 2.3 (stilts) times. We found a similar U-shaped pattern in Hg concentrations as chicks aged for all four internal tissues and in each of three waterbird species, which represented birds with both precocial young (stilts and avocets;[20,21]) and semiprecocial, nidiculous young (Forster's terns[19]) that are fed by their parents at nesting colonies until they fledge at about 28 days of age.[23] These similar results among tissues and species indicate that this U-shaped pattern may be ubiquitous among birds. Other studies have documented a general decrease and then increase in Hg concentrations as juvenile birds aged,[6,11,12] but none have documented such rapid changes in Hg concentrations over a short time period.

This U-shaped pattern of Hg concentrations as chicks aged was likely driven by the increase in body mass and the development of feathers that are inherently correlated with age. Tern chicks gained mass rapidly until just before they fledged at approximately 28 days of age,[23] increasing in size from hatching by nearly 10-fold. Simultaneously, tern chicks were mostly covered with down feathers up until 10 days of age, at which time the amount of fully grown feathers increased from <1% to >80% at the time of fledging. Accordingly, Hg concentrations in chicks were highest immediately after hatching, due to in ovo exposure from maternally deposited Hg into the egg, then they rapidly declined as chicks aged and diluted their body burden of Hg through growth in size and Hg depuration into growing feathers. Hg concentrations then began to increase just before and during fledging when body mass growth and feather production slowed, while chicks presumably continued to acquire Hg through their diets. Chick age at the inflection point when Hg concentrations stopped declining and started to increase occurred shortly before chicks became flighted (approximately 20 days after hatching for terns and at a culmen length of 37−39 mm for stilts). In the week prior to fledging, mass growth and feather production slowed such that Hg concentrations were no longer reduced by mass dilution and Hg depuration into feathers, because Hg acquired through their diet likely exceeded dilution and excretion pathways. Overall, THg concentrations in tern chick blood decreased by 86% from hatching to 20 days of age and then increased by 42% from 20 to 28 days of age at the time of fledging, resulting in an 80% decrease in THg concentrations from hatching to fledging.

Whereas several studies have demonstrated the importance of feather growth for reducing Hg concentrations in juvenile birds,[11−13,15,17] few studies have quantitatively demonstrated the importance of mass dilution. We found that the proportional change in an individual chick's blood THg concentration was strongly related to the proportional change in its body mass, such that THg concentrations declined more in those chicks that gained proportionately more mass between sampling events. In fact, we found more support for the influence of mass dilution than for Hg excretion into feathers as the main mechanism reducing Hg concentrations in aging chicks. However, it must be noted that chick mass and feather growth are inherently correlated with age, and thus not finding a statistically significant effect of the feather production index (proportion of fully grown feathers or wing length) on chick Hg concentrations does not indicate that Hg depuration into feathers was not biologically important for reducing chick Hg concentrations. On the contrary, these results indicate that the residual effect of mass was more important than the residual effect of feather production after the aggregate effect of age on Hg concentrations was taken into account. The importance of Hg excretion into growing feathers often has been demonstrated, with the final plumage of chicks accounting for 34−42% of the total body burden of Hg in common loon chicks,[12] 42−60% in Cory's shearwater chicks,[13] 48% in eastern great white egret chicks (*Egretta alba modesta*[11]), and 65% in black-headed gull chicks (*Larus ridibundus*[16]). Decoupling the effects of mass growth and feather production on reducing Hg concentrations in chicks during the time period from hatching to fledging is difficult, and their independent effects on Hg concentrations are more easily observed at other age classes. For example, the effect of mass dilution on reducing Hg concentrations was evident for recently hatched tern chicks before they had begun producing most of their body feathers (0−10 days). Alternatively, the importance of feather production for reducing Hg concentrations in juvenile birds can be observed during the first feather molt when body mass is relatively stable (e.g.,[11,17]).

It appears that Hg concentrations in tern chicks were largely driven by mass dilution during the initial time period after hatching when fully grown feathers had not yet developed (~0−10 days in tern chicks). Thereafter, Hg excretion into growing feathers likely helped to reduce Hg concentrations

further as did continued mass dilution. Near the time of fledging when mass growth and feather development slowed, Hg concentrations in chicks began to increase again as Hg excretion and dilution pathways could not compensate for the continued intake of Hg through the diet. Another possibility is that an ontogenetic shift in chick diet may have contributed to the U-shaped pattern of Hg concentrations as chicks aged. Although our data are limited to ≤40 days after hatching, it is likely that Hg concentrations in juveniles continued to increase as they aged without the opportunity for growth dilution and excretion of Hg into growing feathers. The next major opportunity for juvenile terns to reduce Hg concentrations would occur during the fall and winter at the first feather molt.[19]

THg concentrations in fully grown breast feathers did not exhibit the same U-shaped pattern with age as we found with THg and MeHg concentrations in the four internal tissues. Instead, feather THg concentrations were highly variable and tended to decline linearly with age. These results illustrate that fully grown feathers are relatively poor predictors of internal tissue Hg concentrations in free-living chicks, as they are in adult birds.[22] In contrast, THg concentrations in chick down feathers were highly correlated with both THg concentrations in blood of recently hatched chicks (this paper) and THg concentrations in the egg before hatching.[24] THg concentrations in feathers represent THg concentrations in blood at the time of feather growth.[25] However, the timing of feather growth is often not known for fully grown feathers, whereas down feathers of precocial young grow in ovo during embryo development and can be more accurately compared to internal tissue concentrations of recently hatched chicks. Therefore, down feathers may have more utility than fully grown feathers for estimating internal tissue Hg concentrations in chicks.

The general U-shaped pattern of Hg concentrations in juvenile birds from hatching to fledging has important toxicological implications. Assuming similar sensitivities among ages, juvenile birds are at highest risk to Hg toxicity shortly after hatching when maternally deposited Hg concentrations are still high and again after fledging when opportunities for mass dilution and excretion of Hg into feathers are limited. The hatching and postfledging stages (especially just before a juvenile's first feather molt) are therefore hypothesized to be an especially sensitive time period for survival of juvenile birds. For example, Ackerman et al. [9] found that Hg concentrations in down feathers of newly hatched stilt chicks were higher in those discovered dead than those that were still alive, but they did not find an effect of Hg on stilt and avocet chick survival during the subsequent time period from after hatching through fledging. These results illustrate the complexity of understanding the timing of Hg effects on juvenile birds because Hg concentrations and the potential for Hg toxicity vary substantially during the short time period from hatching to fledging due to rapid mass growth and feather development. In addition to the rapid changes in Hg concentrations in growing chicks, there could be differences in the toxicological sensitivity of chicks to MeHg as they age.

■ ASSOCIATED CONTENT

Supporting Information. Detailed analysis of covariance results for species and tissue-specific models evaluating effects of variables on THg and MeHg concentrations in chicks (Table S1); correlation between THg concentrations in blood and down feathers (Figure S1); explanation of the graphical presentation of stilt and avocet data; study sites; and mercury determination and quality assurance methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This research was funded by the CALFED Ecosystem Restoration Program and USGS Western Ecological Research Center. We thank the U.S. Fish and Wildlife Service, California Department of Fish and Game, Don Edwards San Francisco Bay National Wildlife Refuge, and Eden Landing Ecological Reserve for logistical support; T. Adelsbach, S. Stoner-Duncan, C. Strong, T. Maurer, and many others for assistance; and Gary Heinz and Mark Ricca for manuscript review. The use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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