Environmental Science & Technology

Article

pubs.acs.org/est

Mercury Exposure May Suppress Baseline Corticosterone Levels in Juvenile Birds

Garth Herring,*[‡,†] Joshua T. Ackerman,[‡] and Mark P. Herzog[‡]

[‡]U.S. Geological Survey, Davis Field Station, Western Ecological Research Center, University of California, Davis, One Shields Avenue, Davis, California 95616, United States

Supporting Information

ABSTRACT: Mercury exposure has been associated with a wide variety of negative reproductive responses in birds, however few studies have examined the potential for chick impairment via the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis regulates corticosterone levels during periods of stress. We examined the relationship between baseline fecal corticosterone metabolite concentrations and mercury concentrations in down feathers of recently hatched (<3 days) and blood of older (15−37 days) Forster's tern (*Sterna forsteri*) chicks in San Francisco Bay, California. Baseline fecal corticosterone metabolite concentrations were negatively correlated with mercury concentrations in blood of older chicks (decreasing by 81% across the range of observed mercury concentrations) while accounting for positive correlations between corticosterone concentrations and number of fledgling chicks within the colony and chick age. In recently hatched chicks, baseline fecal corticosterone metabolite concentrations were weakly negatively correlated with mercury concentrations in down feathers (decreasing by 45% across the range of observed mercury concentrations) while accounting for stronger positive correlations between corticosterone concentrations and colony nest abundance and date. These results indicate that chronic mercury exposure may suppress baseline corticosterone concentrations in tern chicks and suggests that a juvenile bird's ability to respond to stress may be reduced via the downregulation of the HPA axis.

INTRODUCTION

Mercury contamination has been associated with myriad of negative reproductive consequences in birds. Established effects of mercury exposure on breeding birds vary from sublethal effects on adults,[1] impaired breeding behavior,[2] decreased clutch size,[3] decreased hatching rates,[4] and an increased chance of embryos being malpositioned.[6] There also are effects on chicks, including altered behavior,[7] reduced growth rates,[8] impaired immune function,[9] and decreased survival.[10] One area that has received relatively little attention is how mercury contamination may produce deleterious effects via the hypothalamic−pituitary−adrenal (HPA) axis in birds. There are a limited number of studies that have assessed effects of mercury exposure on the HPA axis in birds and the results are mixed ranging from no effect[11,12] to both positive[13] and negative effects.[14,15]

The HPA axis regulates circulating concentrations of corticosterone in individuals during periods of stress.[16,17] The physiological and behavioral modifications that are mediated by increased concentrations of circulating corticosterone make it possible for individuals to limit the negative effect of short-term environmental stressors, such as inclement weather, risk of predation, and starvation.[16,18] Beneficial over the short-term, elevated corticosterone concentrations in juvenile birds can have long-term negative consequences including impaired cognitive abilities,[19] reduced mate attractiveness,[20] and decreased survival.[21]

We evaluated the influence of mercury exposure on corticosterone levels in Forster's tern (*Sterna forsteri*, hereafter tern) chicks in San Francisco Bay, California. Recent studies have found elevated concentrations of mercury in waterbirds breeding in San Francisco Bay.[1,22] We focused our corticosterone research on juvenile birds, and we expected the greatest stress response (either positive or negative) during the time periods when mercury concentrations are highest: (1) recently hatched chicks and (2) older chicks just prior to fledging.[23] Mercury concentrations in recently hatched chicks represent maternal mercury exposure and deposition during egg development.[24] Shortly after hatching, chicks grow rapidly and dilute their body burden of mercury through both mass dilution and depuration into actively growing feathers.[23] Near the time of fledging, mercury concentrations begin to increase

Received: February 17, 2012
Revised: May 9, 2012
Accepted: May 12, 2012
Published: May 12, 2012

 © 2012 American Chemical Society dx.doi.org/10.1021/es300668c | Environ. Sci. Technol. 2012, 46, 6339−6346

in body tissues again as both somatic growth and feather production slows.[23]

We therefore used separate indices of mercury exposure for either recently hatched or old chicks. For recently hatched chicks (<3 days of age), we used mercury concentrations in down feathers as an index of mercury exposure to the developing embryo. Down feathers in Forster's terns are grown in ovo,[25] and their mercury concentrations are highly correlated with egg mercury concentrations.[24] Therefore, down feathers represent maternal exposure to mercury and its subsequent deposition into eggs. For older chicks (15−37 days of age), we measured mercury concentrations in whole blood because it more accurately reflects internal tissue changes in mercury concentrations that are associated with mass dilution and feather growth, and blood is not subject to the high degree of variability in mercury concentrations observed in fully grown feathers.[23] To measure baseline stress levels in both recently hatched and old chicks, we used fecal corticosterone concentrations because it reflects an integrated baseline measure of plasma corticosterone concentrations and associated stress over a period of 1−3 days.[26,27] Therefore, in our study, fecal corticosterone concentrations in recently hatched chicks was used to indicate stress associated with in ovo mercury levels caused by maternal exposure to mercury and depuration into the egg. For older chicks, fecal corticosterone concentrations were used to indicate potential stress associated with circulating blood mercury concentrations from within 1−3 days of sampling the chick.

■ EXPERIMENTAL SECTION

Study Area. We examined the physiological condition of tern chicks in relation to mercury exposure at eight colony sites within the Don Edwards San Francisco Bay National Wildlife Refuge, San Francisco Bay, California (37°26′N 121°58′W) between 23 May and 1 August 2010. Terns predominantly nest on islands within former salt evaporation ponds, with nesting colonies ranging in size from 14 to 266 nests in 2010 (mean, 129 nests).

Chick Sampling. We conducted routine nest monitoring and chick banding during visits to each colony once a week. We sampled tern chicks when they were at two different developmental stages: recently hatched (<3 days old, n = 22) and older fledglings that were 15−37 days of age (mean = 22 days, n = 43; hereafter old chicks). We estimated tern chick age following the methods and models of Ackerman et al.[23] Specifically, we either observed the chick hatching, and thus calculated the chick's age when it was sampled by subtracting the date the chick was sampled from its observed hatching date, or, if we did not observe the hatching date, we used a model[23] to estimate the chick's age when it was first found and then added the number of days between the date when it was sampled and the date when it was found to calculate the chick's age when it was sampled.

To measure mercury concentrations in recently hatched chicks, we collected 15−20 down feathers from each chick's rump. Down feathers were stored in labeled Whirl-paks (Nasco, Modesto, California, USA) until mercury analysis. To measure mercury concentrations in old chicks, we collected whole blood from the brachial vein using a 27 gauge syringe, stored it in labeled cryovials, placed it on wet ice in the field, and later froze it in the lab at −20 °C until mercury analysis was conducted.

We used noninvasive fecal sampling to measure corticosterone metabolite concentrations in tern chicks. Corticosterone metabolite concentrations in fecal material represent an integrated baseline measure of steroid concentrations from within 1−3 days of sampling[27] and takes 2−8 h to become elevated after an individual stress event.[28] Baseline fecal corticosterone metabolites have been shown to be effective biomarkers of mercury-associated stress in birds.[13,29] We did not use corticosterone concentrations in blood plasma because of the short time frame that blood samples represent and the logistical difficulties associated with sampling all chicks within 3 min of breeding colony disturbance.[30]

For each sampled chick, we measured exposed culmen, short tarsus, and flattened wing lengths (to the nearest 0.01 mm using digital calipers) and mass (to the nearest 0.1 g using a digital scale; Ohaus model Scout Pro, Ohaus Parsippany New Jersey, USA). Chicks were held in shaded and screen-lined poultry cages until processing within 30 min of capture (model 5KTC, Murray McMurray Hatchery, Webster City, Iowa, USA). From each chick, we collected approximately 1 mL of fecal material. For recently hatched chicks we placed each chick on a clean piece of tin foil until they defecated and then transferred the defecation using a clean pipet, whereas we extracted fecal material directly from the cloaca of older chicks using a 2 mL pipet. We sampled chicks within the same 4 h time period each day to minimize any diel variation in corticosterone metabolite concentrations.[31] Each chick was banded with a uniquely numbered aluminum U.S. Geological Survey leg band to facilitate identification if recaptured and prevented sampling the same chick multiple times. Fecal samples were stored in labeled cryovials, placed on wet ice in the field, and later frozen in the lab at −20 °C until corticosterone analysis was conducted within 78 days.

Mercury Determination. We processed and analyzed whole blood and entire feathers for total mercury as described in Ackerman et al.[22] Typically, 95% of the total mercury in avian blood and feathers is in the methylmercury form.[32] We pipetted 50 μL of blood into a quartz sample vessel and weighed it (to the nearest 0.0001 g; Ohaus Adventurer Balance, model AR0640, Ohaus Corporation, Parsippany, New Jersey, USA). For feathers, we washed each feather in a 1% Alconox solution (Alconox, White Plains, New York, USA) while manually scrubbing each feather to remove surface debris. We then dried feathers at 60 °C for 24 to 48 h, weighed them to the nearest 0.0001 g (Mettler Toledo, Model AT201, Greifensee, Switzerland), and transferred each feather into a quartz sample vessel.

Following U.S. Environmental Protection Agency method 7473,[33] we analyzed each blood or feather sample for total mercury at the U.S. Geological Survey, Davis Field Station's Mercury Lab on a Milestone DMA-80 direct mercury analyzer (Milestone, Monroe, Connecticut, USA). Quality assurance measures included analysis of two certified reference materials per batch (either dogfish muscle tissue [DORM-2], dogfish liver [DOLT-3], or lobster hepatopancreas [TORT-2] by the National Research Council of Canada, Ottawa, Canada). Recoveries for certified reference materials and matrix spikes in feathers averaged 101.3 ± 1.8% (n = 20) and 99.1 ± 0.3% (n = 8), respectively. Recoveries for certified reference materials and matrix spikes in blood averaged 97.6 ± 1.3% (n = 27) and 97.7 ± 0.6% (n = 12), respectively. Absolute relative percent difference for all duplicates averaged 2.4% ± 1.5% (n = 8) for feathers and 2.1 ± 1.5% (n = 10) for blood.

Validation Study. To validate the determination of excreted corticosterone metabolites in our Correlate-EIA™

Corticosterone Enzyme Immunoassay kit (EIA), we used a capture-and-handling stress protocol.[34] Briefly, five tern chicks were held for an additional 60 min after their initial handling for measurements and fecal sampling before release to increase their potential for elevated fecal corticosterone metabolite concentrations in response to stress. We then recaptured each of these chicks separately at 8 and 24 h after the initial capture and stress event. At each of the two subsequent recaptures, we resampled their fecal material to measure corticosterone metabolite concentrations. Chicks were released at their capture location.

Corticosterone Metabolite Determination. Corticosterone concentrations were measured following procedures outlined by Möstl et al.[35] and Palme.[36] Briefly, wet fecal samples were dried to constant mass at 40 °C and then ~0.50 g subsamples were homogenized for 1 min and then mixed with 5.0 mL of 95% ethanol and vortexed for 30 min. To reduce bias associated with differences between fecal and urinary corticosterone concentrations,[35] we excluded samples that were exclusively urinary liquids. Further, variation in fecal samples was likely minimal because the forms of excreta are mixed in the cloaca of birds.[37] Samples were then centrifuged (15 min, 2500 × g), the supernatant was transferred to a new vial, and was then evaporated under nitrogen gas. Corticosterone metabolites were then resuspended in diluted extraction buffer and measured using the EIA following the manufacturer's instructions (Assay Design, Inc., Ann Arbor, Michigan, USA). The Correlate-EIA™ Corticosterone kit used a donkey antisheep IgG microtiter plate, and a sheep polyclonal corticosterone EIA antibody as a standard. Cross reactivity for these EIA kits are considered low and reported for desoxycorticosterone (12.3%), progesterone (0.2%), tetrahydrocorticosterone (0.7%), aldosterone (0.6%), cortisol (0.4%), progesterone (0.2%), cortisone (<0.1%), and estradiol (<0.1%; Assay Design, Inc., Ann Arbor, Michigan, USA). The minimum detectable level of corticosterone metabolites as reported in the manufacturer's manual was 0.02 ng/g (Assay Design, Inc., Ann Arbor, Michigan, USA).

We conducted a standard assay validation, which included an assessment of parallelism, recovery of exogenous corticosterone, and intra- and interassay precision to confirm that the EIAs accurately measured corticosterone metabolites in fecal samples.[38] All fecal samples were analyzed within 78 days of collection to avoid any issues with changes in metabolite concentrations associated with longer storage times.[39]

Statistical Analyses. To validate ELISA test kits for fecal corticosterone metabolites, we performed a serial dilution of fecal corticosterone metabolites from tern chicks and tested for equality of slopes (parallelism) between the tern fecal corticosterone metabolites and the corticosterone standards using a general linear model. Similar slopes between serially diluted samples and standards demonstrated that the EIA kits were in fact measuring the parameter as purported by the manufacturer (Results).

We used an information-theoretic approach[40] and general linear models in R[41] to determine which variables influenced Forster's tern chick fecal corticosterone metabolite concentrations. We built and ranked competing candidate a priori models for recently hatched and old chicks separately. Recently hatched chick candidate models included rump feather mercury concentration, date, colony nest abundance (the total number of nests at this colony site over the entire 2010 breeding season), culmen length (a measure of chick size and age), chick mass, and colony site (island). We included colony nest abundance as a potential measure of colony-induced stress to females during egg laying when they deposit corticosterone into the egg.[42,43] We included culmen length in all models that included mass to account for variation in a recently hatched chick's structural size; therefore the mass variable should be interpreted as an index of the chick's body condition relative to its size. Similarly, candidate models for old chicks included blood mercury concentration, date, number of chicks (from recently hatched to fledged) in the colony during the specific time-period (visit) when a chick was sampled, chick age, chick mass, and colony site (island). We included the number of chicks in a colony as a potential measure of colony-induced stress to chicks. For models within the candidate model set for older chicks, we included age in all models that included mass to account for variation in a chick's structural size. Therefore, the mass variable should be interpreted as an index of the chick's body condition relative to its size. For both young and old chick model candidate sets, we also included an intercept only model and global model that included all variables and all possible two-way interactions. We $\log_e$-transformed corticosterone metabolite concentrations to improve normality. Mercury concentrations are reported as micrograms per gram fresh weight (μg/g fw) in feathers and as wet weight (μg/g ww) in whole blood. Fecal corticosterone metabolite concentrations are reported as nanograms per gram dry weight (ng/g dw).

We used Akaike's Information Criterion adjusted for small samples sizes (AIC_c) and considered the model with the lowest AIC_c value to be the most parsimonious.[40] We determined the relative ranking of each model by subtracting the AIC_c value from the best model from each candidate model's AIC_c value (ΔAIC_c). Candidate models were considered to be important when $\Delta AIC_c \leq 2.0$. We calculated Akaike weights (w_i) to assess the weight of evidence that the selected model was the best candidate model.[40] We also calculated variable weights by summing Akaike weights across all models that included the variable to assess the relative importance of each variable. We calculated model-averaged beta coefficient estimates from all candidate models.[40]

RESULTS

Mean mercury concentrations in recently hatched chick down feathers was 20.8 μg/g fw (95% confidence interval [CI] = 17.26−23.68, n = 22), and 0.45 μg/g ww (95% CI = 0.38−0.52, n = 43) in blood of older chicks. Mean fecal corticosterone concentrations for recently hatched chicks was 2.38 ng/g dw (95% CI = 1.45−3.51, n = 22) and 3.22 ng/g dw (95% CI = 2.18−4.39, n = 43) for older chicks.

Corticosterone Metabolite Validation Studies. Serial dilutions of tern chick fecal corticosterone metabolite extracts yielded displacement parallel to the standard corticosterone curve ($F_{3,9}$ = 3.15, P = 0.11) demonstrating that the EIA kits were measuring metabolites correctly. Mean recovery of the added exogenous corticosterone spike was 89.5 ± 8.1% (SE). Inter- and intra-assay coefficients of variation for corticosterone internal standards were 7% and 9%, respectively. Corticosterone metabolite concentrations of chicks captured and handled to induce a stress response (following our stress induction protocol) all increased over baseline metabolite concentrations after 8 h and then returned to near baseline concentrations after 24 h from their initial capture (Figure S1 of the Supporting Information) .

Table 1. Ranking of Candidate Models Describing Variables Influencing Fecal Corticosterone Metabolite Concentrations in Recently Hatched and Old Forster's Tern Chicks.[f]

model structure	n	K^a	−2 Log L	$AIC_C{}^b$	$\Delta AIC_C{}^c$	$w_i{}^d$	evidence ratio[e]
Recently Hatched Tern Chicks							
mercury + nest abundance + date	22	5	42.820	56.570	0.000	0.35	1.00
nest abundance + date	22	4	46.563	56.916	0.346	0.30	1.19
mercury + date	22	4	48.499	58.852	2.283	0.11	3.13
nest abundance + date + culmen	22	5	45.931	59.681	3.112	0.07	4.74
mercury + nest abundance + date + culmen	22	6	42.676	60.276	3.707	0.06	6.38
date	22	3	53.716	61.050	4.480	0.04	9.39
mercury + date + culmen	22	5	48.436	62.186	5.617	0.02	16.58
nest abundance + date + culmen + mass	22	6	45.907	63.507	6.938	0.01	32.10
intercept	22	2	61.116	65.748	9.178	0.00	98.41
Old Tern Chicks							
mercury + number of chicks + age	43	5	118.488	130.109	0.000	0.28	1.00
mercury + number of chicks + age + mass	43	6	117.864	132.197	2.088	0.10	2.84
mercury + age	43	4	123.291	132.344	2.234	0.09	3.06
mercury + number of chicks + age + date	43	6	118.428	132.761	2.652	0.07	3.77
number of chicks + age + mass	43	5	122.101	133.723	3.613	0.05	6.09
mercury + age + date	43	5	123.086	134.708	4.598	0.03	9.97
mercury + number of chicks + age + mass + age × mass	43	7	117.516	134.716	4.606	0.03	10.01
mercury + age + mass	43	5	123.269	134.891	4.781	0.03	10.92
number of chicks + age	43	4	125.897	134.949	4.840	0.02	11.25
mercury + number of chicks + age + date + mass	43	7	117.841	135.041	4.931	0.02	11.77
mercury + number of chicks + age + date + number of chicks × date	43	7	117.917	135.117	5.007	0.02	12.23
mercury + number of chicks + age + date + age × date	43	7	118.049	135.249	5.140	0.02	13.06
age	43	3	129.029	135.645	5.535	0.02	15.92
number of chicks	43	3	129.166	135.781	5.672	0.02	17.04
number of chicks + age + date + age × mass	43	6	121.802	136.135	6.026	0.01	20.35
number of chicks + age + date + mass	43	6	121.901	136.234	6.124	0.01	21.38
mercury + number of chicks	43	4	127.354	136.407	6.297	0.01	23.30
age + mass	43	4	127.381	136.434	6.325	0.01	23.62
number of chicks + colony	43	12	102.656	137.056	6.947	0.01	32.25
intercept	43	2	137.501	141.801	11.692	0.00	345.82

[a]Number of estimated parameters in the model including the intercept and variance. [b]Second-order Akaike's information criterium (AIC_c). [c]The difference in the value between AIC_c of the current model and the value of the most parsimonious model. [d]Likelihood of the model given the data, relative to models in the candidate set. [e]The weight of the evidence that the top model is better than the selected model, given the candidate model set. [f]Chicks were sampled at eight nesting colonies in San Francisco Bay, California. Variables considered for recently hatched chicks included rump feather mercury concentrations, nest abundance, date, chick mass, culmen length, and colony site. For old chicks variables considered included blood mercury concentrations, number of chicks in colony, date, chick age, chick mass, and colony site. Models presented include only those that were within 7 AIC_C units of the top model ($\Delta AIC_C = 0$) and the null (intercept) models.

Factors Influencing Fecal Corticosterone Concentrations. *Recently Hatched Chicks.* The most parsimonious model explaining differences in recently hatched chick fecal corticosterone metabolite concentrations included down feather mercury concentrations, colony nest abundance, and date, with a model Akaike weight of 0.35 (Table 1). A second model containing the variables colony nest abundance and date also had reasonable support (<2 ΔAIC_C units, Table 1). The weight of evidence suggested that the model containing down feather mercury concentrations, date, and colony nest abundance was 1.2 times more likely than the next model with colony nest abundance and date (Table 1). Using variable weights to assess variable importance,[40] we found that models containing date had a combined AIC_C weight of 0.98, followed by colony nest abundance (0.80), and down feather mercury concentrations (0.56), with little evidence for effects of culmen length (0.18), chick mass (0.02), or colony site (0.00). The model averaged beta coefficient estimate indicated some evidence that down feather mercury concentration ($\beta = -0.024 \pm 0.016$ SE) was negatively correlated with fecal corticosterone metabolite concentrations. However, the 95% confidence interval overlapped zero, and thus this hypothesis was not strongly supported. However, model-averaged coefficients for covariates such as date ($\beta = 0.043 \pm 0.012$) and colony nest abundance ($\beta = 0.004 \pm 0.002$) had stronger support for influencing corticosterone metabolite concentrations. Model-averaged estimates for the effect of mercury on fecal corticosterone metabolite concentrations indicated a decrease in corticosterone concentrations of 45% over the observed range of down feather mercury concentrations (range = 11.16−35.75 μg/g dw, parts A−C of Figure 1). Fecal corticosterone metabolite concentrations also increased with date by 519% between 1 June and 13 July, 2010, and colony nest abundance by 153% across the range of colony sizes observed (41−249 nests, parts A−C of Figure 1).

Older Chicks. Candidate models for older chicks were similar to those for younger chicks, except that culmen length was replaced by chick age in the candidate models, and season-long colony nest abundance was replaced by weekly chick abundance. The most parsimonious model explaining differences in older chick fecal corticosterone metabolite concentrations included blood mercury concentrations, the number of

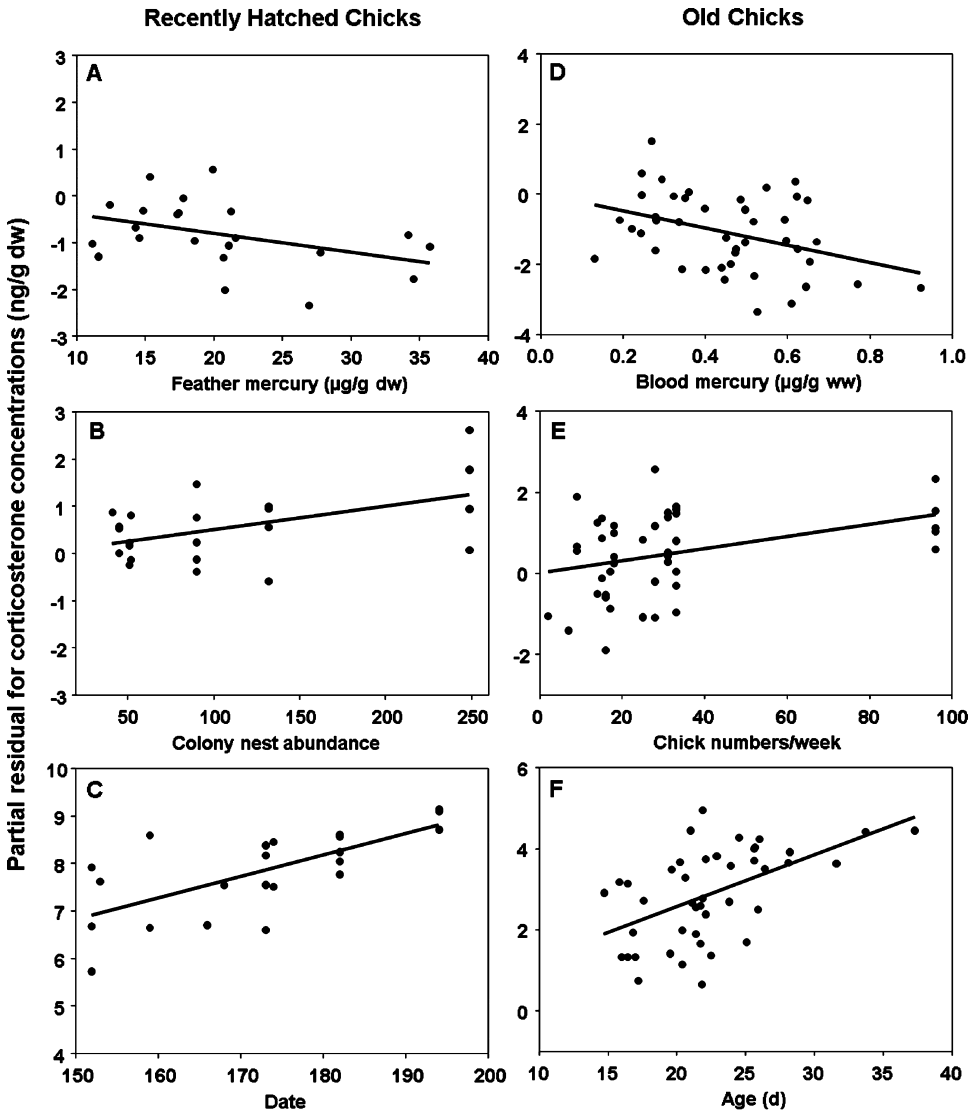

Figure 1. Relationship between partial residuals of corticosterone concentrations (ng/g dw) and (A) down feather mercury concentrations (μg/g fw), (B) colony nest abundance, and (C) date in recently hatched chicks ($\leq$3 days), and (D) blood mercury concentrations (μg/g ww), (E) chick abundance at colony sites, and (F) chick age (days) in older chicks (15−37 days) for Forster's terns in San Francisco Bay, California.

chicks during weekly visits in the colony, and chick age, with a model Akaike weight of 0.28 (Table 1). No other models were found to be competitive (>2 ΔAIC_C units) in explaining fecal corticosterone concentrations in old chicks, and all of the top four models had blood mercury concentrations in them (Table 1). The weight of evidence suggested that the model containing blood mercury concentrations, number of chicks in the colony, and chick age was 2.8 times more likely than the next model with blood mercury concentrations, number of chicks, mass, and age (Table 1). Using variable weights to assess variable importance, we found that models containing chick age had a combined AIC_C weight of 0.93, followed by chick blood mercury concentrations (0.77), and number of chicks in the colony (0.75), with little evidence for effects of chick mass (0.33), date (0.29), or colony site (0.03). Fecal corticosterone metabolites in older chicks decreased by 81% across the observed range of blood mercury concentrations (range = 0.13−1.08 μg/g ww, part D of Figure 1). Although the number of chicks in a colony and chick age had high variable weights, the model averaged beta coefficient estimates for these variables had 95% confidence intervals that overlapped zero indicating that both the number of chicks in a colony ($\beta = 0.025 \pm 0.037$) and chick age ($\beta = 0.073 \pm 0.181$) had much smaller effects on an older chick's fecal corticosterone metabolite concentrations than did its blood mercury concentrations ($\beta = -1.765 \pm 0.884$, parts D−F of Figure 1).

■ DISCUSSION

We found strong support for a negative relationship between fecal corticosterone metabolite concentrations and blood mercury concentrations in older Forster's tern chicks and some support for a similar negative relationship between corticosterone concentrations and down feather mercury concentrations in recently hatched chicks as well. Across the observed range of blood and down feather mercury concentrations, we found an 81% and 45% reduction in fecal corticosterone metabolite concentrations in old and recently hatched chicks, respectively. Similarly, Franceschini et al.[15] observed a negative correlation between baseline plasma corticosterone concentrations and whole blood mercury concentrations in both nestling and breeding adult tree

swallows (*Tachycineta bicolor*). Jayasena[29] also observed decreased baseline fecal corticosterone concentrations during reproduction in experimentally dosed adult white ibis (*Eudocimus albus*). Wada et al.[14] observed decreased stress-induced plasma corticosterone concentrations relative to control sites in late stage nestling tree swallows. Adams et al.[13] found more equivocal results in juvenile white ibis that were experimentally dosed with mercury. Baseline fecal corticosterone concentrations were higher for birds in each of three dosing treatment levels relative to birds in the control group after 50 days, but before 50 days of treatment results varied in a nonlinear dose−response relationship.

Baseline fecal corticosterone concentrations may be reduced by mercury exposure due to the down-regulation of adenocorticotropin (ACTH) receptors, followed by a decreased ability to produce the hormone, and result in a reduction in corticosterone concentrations.[44] A controlled down-regulation of the HPA response to chronic stress may function to minimize any negative effects of chronic corticosterone release.[44] Lower baseline corticosterone concentrations may also be due to mercury impairing the endocrine system.[45] For example, an experimental study that injected mercury into rats found that mercury accumulated in the adrenal gland, produced alterations in heme and hemoprotein metabolism, and resulted in abnormal steroidogenic activities and decreased corticosterone production.[46] Impairment of the endocrine system could also lead to down-regulation of the ACTH hormone receptors resulting in a decrease in hormone production.[47] Regardless of the mechanism, our results support a growing body of evidence that indicates that chronic mercury exposure may actually suppress baseline corticosterone concentrations and, potentially, the ability of birds to respond to stress.

The long-term consequences of reduced baseline corticosterone concentrations are unclear because there is a paucity of studies that have demonstrated this negative HPA response in association with mercury exposure, particularly in juvenile birds. Baseline corticosterone concentrations reflect the naturally circulating concentrations of corticosterone hormone available for basic metabolic functions, which include sustaining standard concentrations of blood glucose and maintaining anti-inflammatory and immunosuppressant functions.[48,49] In the case of fecal corticosterone, concentrations reflect an integrated measure of baseline corticosterone levels from a period of time lasting up to 3 days.[27] Our results suggest that mercury exposure suppresses the HPA stress response in juvenile birds and indicates the potential for altering basic metabolic functions. When birds are faced with a stressful event, the body responds by elevating circulating corticosterone concentrations. Whereas short-term increases in corticosterone concentrations are considered to be immune enhancing, chronic elevation of corticosterone concentrations are considered to be an immune suppressant.[50] Therefore, any substantial chronic reduction in baseline corticosterone concentrations would also be expected to impair the ability of juvenile birds to resist and overcome infections. A reduction in baseline corticosterone may limit the ability of juvenile birds to mount the appropriate level of stress response to environmental stimuli and could impact survival.[49]

In addition to the effects of mercury on baseline corticosterone concentrations, we also found that colony size and date influenced fecal corticosterone metabolite concentrations. Interestingly, these other variables were more important factors (based on parameter weights) than mercury influencing corticosterone concentrations in recently hatched chicks, whereas they were less important than the effect of mercury on older chicks. For example, in recently hatched chicks, we observed a positive relationship between corticosterone concentrations and date while accounting for the negative effects of mercury and positive effects of colony nest abundance. Egg laying date is often correlated with physiological condition in birds; subsequently females that lay eggs later in the season are often in inferior physiological condition[51] and subsequently are stressed.[52] Stressed females deposit more corticosterone into eggs[42,43] resulting in chicks with elevated corticosterone levels.[53] Increased corticosterone concentrations at a young age can decrease chick growth rates,[42] reduce the aggressiveness of chick begging behaviors,[54] and increase activity of the HPA axis in response to stress as adults.[42]

We also observed a positive relationship between corticosterone concentrations and nest abundance (colony size) in recently hatched chicks while accounting for the effects of mercury and date. Other studies of birds have found both positive and negative relationships between corticosterone concentrations and colony size, although it is unclear if these relationships had any effect on chicks. Silverin[55] found that corticosterone concentrations in breeding male pied flycatchers (*Ficedula hypoleuca*) increased with breeding numbers, whereas Love et al.[56] found that corticosterone concentrations decreased in European starling (*Sturnis vulgaris*) egg yolks in colonies with large nest numbers. Elevated concentrations of corticosterone in recently hatched chicks may be associated with increased stress levels in females during egg development,[42,43] who are exposed to increased territorial challenges[54,55] and competition for food (terns forage close to breeding colonies[57]) when breeding in large colonies). However, colonial nesting is a common life history strategy that often can increase reproductive success via several mechanisms, including colony attentiveness to predators,[58] minimizing the threat of predation[59] and supplying foraging information.[60] Thus, an apparent trade-off may exist between the benefits of colonial nesting and the increased baseline corticosterone levels in recently hatched and, to a lesser extent, older chicks that may have longer-term consequences. However, the response of increased corticosterone concentrations in juvenile birds being raised in larger colonies may be within the normal range for this life history strategy and may not necessarily signify an impaired state.[48] Regardless of the influence of colony size and date on corticosterone concentrations in chicks, exposure to mercury contamination appears to have an additional effect on corticosterone levels such that polluted wetlands may suppress the baseline corticosterone concentrations of juvenile birds.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results of capture and handling stress protocol illustrating baseline (0 h) and stress-induced (>0 h) fecal corticosterone metabolite concentrations of chicks. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 541-231-0543, e-mail: gherring@usgs.gov.

Present Address

†U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, 3200 SW Jefferson Way, Corvallis, OR 97331, USA

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the U.S. Geological Survey. We appreciate the logistical support and cooperation of the Don Edwards San Francisco Bay National Wildlife Refuge. All birds were captured, sampled, and banded under U.S. Fish and Wildlife Service and California Department of Fish and Game permits. We thank R. Keister, J. LaCoss, C. Thao, S. Peterson, K. Ruskin, C. Schacter, and D. Spatz for field and lab assistance. J. Stott and M. Blanchard provided access to a plate reader and valuable advice on running EIA plates. 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.

■ REFERENCES

(1) Eagles-Smith, C. A.; Ackerman, J. T.; Yee, J. L.; Adelsbach, T. L. Mercury demethylation in waterbird livers: dose-response thresholds and differences among species. *Environ. Toxicol. Chem.* **2009**, *28*, 568−577.

(2) Evers, D. C.; Savoy, L. J.; DeSorbo, C. R.; Yates, D. E.; Hanson, W.; Taylor, K. M.; Siegel, L. S.; Cooley, J. H., Jr.; Ban, M. S.; Major, A.; Munney, K.; Mower, B. F.; Vogel, H. S.; Schoch, N.; Pokras, M.; Goodale, M. W.; Fair, J. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* **2008**, *17*, 69−81.

(3) Albers, P. H.; Koterba, M. T.; Rossmann, R.; Link, W. A.; French, J. B.; Bennett, R. S.; Bauer, W. C. Effects of methylmercury on reproduction in American kestrels. *Environ. Toxicol. Chem.* **2007**, *26*, 1856−1866.

(4) Heinz, G. H.; Hoffman, D. J. Embryotoxic thresholds of mercury: estimates from individual mallard eggs. *Arch. Environ. Contam. Toxicol.* **1998**, *44*, 257−264.

(5) Heinz, G. H.; Hoffman, D. J.; Klimstra, J. D.; Stebbins, K. R.; Kondrad, S. L.; Erwin, C. A. Species differences in the sensitivity of avian embryos to methylmercury. *Arch. Environ. Contam. Toxicol.* **2009**, *56*, 129−138.

(6) Herring, G.; Ackerman, J. T.; Eagles-Smith, C. A. Embryo malposition as a potential mechanism for mercury-induced hatching failure in bird eggs. *Environ. Toxicol. Chem.* **2010**, *29*, 1788−1794.

(7) Heinz, G. H. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. *J. Wildl. Manage.* **1979**, *43*, 394−401.

(8) Spalding, M. G.; Frederick, P. C.; McGill, H. C.; Bouton, S. N.; Richey, L. J.; Schumacher, I. M.; Blackmore, C. G. M.; Harrison, J. Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. *J. Wildl. Dis.* **2000**, *36*, 423−435.

(9) Kenow, K. P.; Grasman, K. A.; Hines, R. K.; Meyer, M. W.; Gendron-Fitzpatrick, A.; Spalding, M. G.; Gray, B. R. Effects of methylmercury exposure on the immune function of juvenile common loons (*Gavia immer*). *Environ. Toxicol. Chem.* **2007**, *26*, 1460−1469.

(10) Ackerman, J. T.; Takekawa, J. Y.; Eagles-Smith, C. A.; Iverson, S. A. Mercury contamination and effects on survival of American avocet and black-necked stilts in San Francisco Bay. *Ecotoxicology* **2008**, *17*, 103−116.

(11) Bowerman, W. W.; Mehne, C. J.; Best, D. A.; Refsal, K. R.; Lombardini, S.; Bridges, W. C. Adrenal corticotrophin hormone and nestling bald eagle corticosterone levels. *Bull. Environ. Contam. Toxicol.* **2002**, *68*, 355−360.

(12) Wayland, M.; Gilchrist, H. G.; Marchant, T.; Keating, J.; Smits, J. E. Immune function, stress response, and body condition in Arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environ. Res. Sect. A* **2002**, *90*, 47−60.

(13) Adams, E. M.; Frederick, P. C.; Larkin, I. L. V.; Guillette, L. J. The effects of chronic methylmercury exposure upon fecal testostosterone, estradiol, and corticosterone levels in juvenile White Ibises (*Eudocimus albus*). *Environ. Toxicol. Chem.* **2009**, *28*, 982−989.

(14) Wada, H. D.; Cristol, A.; McNabb, F. M. A.; Hopkins, W. A. Suppressed adenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. *Environ. Sci. Technol.* **2009**, *43*, 6031−6038.

(15) Franceschini, M. D.; Lane, O. P.; Evers, D. C.; Reed, J. M.; Hoskins, B.; Romero, L. M. The corticosterone stress response and mercury contamination in free-living tree swallows, *Tachycineta bicolor*. *Ecotoxicology* **2009**, *18*, 514−521.

(16) Wingfield, J. C. Corticosterone and behavioural responses to unpredictable events. In *Perspectives in Avian Endocrinology*; Harvey, S, Etchen, R. J., Eds.; Sinauer, CT, 1997; pp 267−278.

(17) Wingfield, J. C.; Ramenofsky, M. Hormones and the behavioral ecology of stress. In *Stress Physiology in Animals* Balm, P. H. M., ed.; Sheffield Academic Press, 1999; pp 1−51.

(18) Wingfield, J. C. Influence of weather on reproductive function in male song sparrows (*Melospiza melodia*). *J. Zool.* **1985**, *205*, 525−544.

(19) Kitaysky, A. S.; Kitaiskaia, E. V.; Piatt, J. F.; Wingfield, J. C. A mechanistic link between chick diet and decline in seabirds? *Proc. R. Soc. Lond. B. Biol. Sci. B* **2006**, *273*, 445−450.

(20) Naguib, M.; Heim, C.; Gil, D. Early developmental conditions and mate attractiveness in zebra finches. *Ethology* **2008**, *114*, 255−261.

(21) Blas, J.; Bortolotti, G. R.; Tella, J. L.; Baos, R.; Marchant, T. A. Stress response during development predicts fitness in wild, long lived vertebrate. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8880−8884.

(22) Ackerman, J. T.; Eagles-Smith, C. A.; Takekawa, J. Y.; Demers, S. A.; Adelsbach, T. A.; Bluso, J. D.; Miles, A. K.; Warnock, N.; Suchanek, T. H.; Schwarzbach, S. E. Mercury concentrations and space use of pre-breeding American avocets and black-necked stilts in San Francisco Bay. *Sci. Total Environ.* **2007**, *384*, 452−466.

(23) Ackerman, J. T.; Eagles-Smith, C. A.; Herzog, M. P. Bird mercury concentrations rapidly change with chick age: toxicological risk is highest at hatching and fledging. *Environ. Sci. Technol.* **2011**, *45*, 5418−5425.

(24) Ackerman, J. T.; Eagles-Smith, C. A. Integrating toxicity risk in bird eggs and chicks: using chick down feathers to estimate mercury concentrations in eggs. *Environ. Sci. Technol.* **2009**, *43*, 2166−2172.

(25) Nice, M. M. Development of behavior in precocial birds. *Trans. Linnaean Soc. N.Y.* **1962**, *8*, 1−212.

(26) Sheriff, M. J.; Krebs, C. J.; Boonstra, R. Assessing stress in animal populations: do fecal and plasma glucocorticoids tell the same story? *Gen. Comp. Endrocrinol.* **2010**, *166*, 614−619.

(27) Millspaugh, J. J.; Washburn, B. E. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for applications and interpretation. *Gen. Comp. Endrocrinol.* **2004**, *138*, 189−199.

(28) Wasser, S. K.; Bevis, K.; King, G.; Hanson, E. Noninvasive physiologic measures of disturbance in the northern spotted owl. *Con. Biol.* **1997**, *11*, 1019−1022.

(29) Jayasena, N. Effects of chronic methylmercury exposure on reproductive success, behavior, and steroid hormones of the white ibis (*Eudocimus albus*). Ph.D. Dissertation, University of Florida: Gainsville, FL, 2010.

(30) Romero, M. L.; Reed, J. M. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp. Biochem. Physiol. A.* **2005**, *140*, 73−79.

(31) Touma, C.; Palme, R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a biological validation. *Ann. N.Y. Acad. Sci.* **2005**, *1046*, 54−74.

(32) Thompson, D. R.; Furness, R. W. Comparison of the levels of total and organic mercury in seabird feathers. *Mar. Pollut. Bull.* **1989**, *20*, 577−579.

(33) U.S Environmental Protection Agency. Method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation, and

atomic absorption spectrophotometry, test methods for evaluating solid waste, physical/chemical methods SW 846, Update IVA. U.S. Government Printing Office, Washington, DC, 2000.
(34) Herring, G.; Ackerman, J. T. California gull chicks raised near colony edges have elevated stress levels. *Gen. Comp. Endrocrinol.* **2011**, *173*, 72−77.
(35) Möstl, E.; Rettenbacher, S.; Palme, R. Measurement of corticosterone metabolites in bird's droppings: an analytical approach. *Ann. N.Y. Acad. Sci.* **2005**, *1046*, 17−34.
(36) Palme, R. Measuring fecal steroids: guidelines for practical application. *Ann. N.Y. Acad. Sci.* **2005**, *1046*, 75−80.
(37) Klasing, K. C. Potential impact of nutritional strategy on noninvasive measurements of hormones in birds. *Ann. N.Y. Acad. Sci.* **2005**, *1046*, 5−16.
(38) Goymann, W. Noninvasive monitoring of hormones in bird droppings − physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. *Ann. N.Y. Acad. Sci.* **2005**, *1046*, 35−53.
(39) Herring, G.; Gawlik, D. E. Stability of avian fecal corticosterone metabolite levels in frozen avian feces. *J. Wildl. Manage.* **2009**, *73*, 1010−1013.
(40) Burnham, K. P.; Anderson, D. R. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd ed. Springer-Verlag, New York, 2002.
(41) R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3−900051−07−0, URL http://www.R-project.org/, 2011.
(42) Hayward, L. S.; Wingfield, J. C. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and phenotype. *Gen. Comp. Endrocrinol.* **2004**, *135*, 36.
(43) Saino, N.; Romano, M.; Ferrari, R. P.; Martinelli, R.; Moller, A. P. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool. A. Comp. Exp. Biol.* **2005**, *303*, 998−1006.
(44) Rich, E. L.; Romero, L. M. Exposure to chronic stress downregulates corticosterone responses to acute stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2005**, *288*, R1628−R1636.
(45) Hontela, A.; Dumont, P.; Duclos, D.; Fortin, R. Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. *Environ. Toxicol. Chem.* **1995**, *14*, 725−731.
(46) Veltman, J. C.; Maines, M. D. Alterations of heme, cytochrome P-450, and steroid metabolism by mercury in rat adrenal. *Arch. Biochem. Biophys.* **1986**, *248*, 467−78.
(47) Norris, D. O.; Donahue, S.; Dores, R. M.; Lee, J. K.; Maldonado, T. A.; Ruth, T.; Woodling, J. D. Impaired adrenocortical response to stress by brown trout, *Salmo trutta*, living in metal-contaminated waters of the Eagle River, Colorado. *Gen. Comp. Endrocrinol.* **1999**, *113*, 1−8.
(48) Munck, A.; Guyre, P. M.; Holbrook, N. J. Physiological functions of glucorticoids in stress and their relationship to pharmacological actions. *Endocr. Rev.* **1984**, *5*, 25−44.
(49) Landys, M. M.; Ramenofsky, M.; Wingfield, J. C. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Edocrinol.* **2006**, *148*, 132−145.
(50) Dhabhar, F. S.; McEwen, B. S. Acute stress enhances while chronic stress suppresses cell-mediated immunity *in vivo*: a potential role for leukocyte trafficking. *Brain Behav. Immun.* **1997**, *11*, 286−306.
(51) Astheimer, L. B. Egg formation in Cassin's Auklet. *Auk.* **1986**, *103*, 682−693.
(52) Love, O. P.; Chin, E. H.; Wynne-Edwards, K. E.; Williams, T. D. Stress hormones link between maternal condition and sex-biased reproduction investment. *Am. Nat.* **2005**, *166*, 751−766.
(53) Lay, D. C.; Wilson, M. E. Development of the chicken as a model for prenatal stress. *J. Anim. Sci.* **2002**, *80*, 1954−1961.
(54) Rubolini, D.; Romano, M.; Boncoraglio, G.; Ferrarri, R. P.; Martinelli, R.; Galeotti, P.; Fasola, M.; Saino, N. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Horm. Behav.* **2005**, *47*, 592−605.
(55) Silverin, B. Territorial behavior and hormones of pied flycatchers in optimal and suboptimal habitats. *Anim. Behav.* **1998**, *56*, 811−818.
(56) Love, O. P.; Wynne-Edwards, K. E.; Bond, L.; Williams, T. D. Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. *Horm. Behav.* **2008**, *53*, 104−111.
(57) Bluso-Demers, J.; Colwell, M. A.; Takekawa, J. Y.; Ackerman, J. T. Space use by Forster's tern breeding in South San Francisco Bay. *Waterbirds* **2008**, *31*, 357−364.
(58) Burger, J.; Gochfeld, M. Predation and effects of humans on island nesting seabirds. *BirdLife. Conserv. Ser.* **1994**, *1*, 39−67.
(59) Aebisher, N. J.; Coulson, J. C. Survival of the kittiwake in relation to sex, year, breeding experience and position in the colony. *J. Anim. Ecol.* **1990**, *59*, 1063−1071.
(60) Ward, P.; Zahavi, A. The importance of certain assemblages of birds as 'information centers' for food finding. *Ibis* **1973**, *115*, 517−534.

Supporting Information for

Mercury Exposure May Suppress Baseline Corticosterone Levels in Juvenile Birds

by Garth Herring, Joshua T. Ackerman, and Mark P. Herzog
Total pages (including cover): 2
Number of Figures: 1

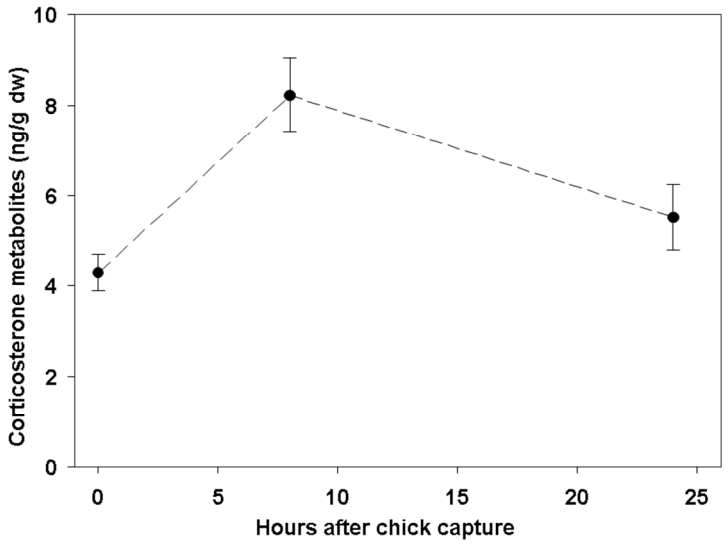

Figure S1. Baseline (0 hours) and stress-induced (>0 hours) fecal corticosterone metabolite concentrations (ng/g dw) of Forster's tern chicks in San Francisco Bay, California. Chicks were stressed by capturing and holding them for 1 hr, then releasing and re-capturing them at 8 and 24 hrs after their initial capture.