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Does mercury contamination reduce body condition of endangered California clapper rails?

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ABSTRACT

We examined mercury exposure in 133 endangered California clapper rails (*Rallus longirostris obsoletus*) within tidal marsh habitats of San Francisco Bay, California from 2006 to 2010. Mean total mercury concentrations were 0.56 μg/g ww in blood (range: 0.15–1.43), 9.87 μg/g fw in head feathers (3.37–22.0), 9.04 μg/g fw in breast feathers (3.68–20.2), and 0.57 μg/g fww in abandoned eggs (0.15–2.70). We recaptured 21 clapper rails and most had low within-individual variation in mercury. Differences in mercury concentrations were largely attributed to tidal marsh site, with some evidence for year and quadratic date effects. Mercury concentrations in feathers were correlated with blood, and slopes differed between sexes (R^2 = 0.58–0.76). Body condition was negatively related to mercury concentrations. Model averaged estimates indicated a potential decrease in body mass of 20–22 g (5–7%) over the observed range of mercury concentrations. Our results indicate the potential for detrimental effects of mercury contamination on endangered California clapper rails in tidal marsh habitats.

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1. Introduction

The California clapper rail (*Rallus longirostris obsoletus*) is a secretive tidal marsh dependent bird listed as endangered by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service, 1973) and by the State of California (Leach et al., 1976). Historically, California clapper rails occurred along California's coastal marshes and were common in San Francisco Bay (Gill, 1979). However, their population size has declined over the past century and was recently estimated at 1403 individuals in 2005–2008 (Liu et al., 2009). Their range has become restricted to San Francisco Bay, where they are obligate residents of tidal marshes (Albertson and Evens, 2000). Tidal marsh habitats have been severely reduced in California by over 80% due to bayfill and diking for urban development, agriculture, and salt production (Goals Project, 1999), and this loss of habitat is thought to have contributed to the decline in the population of clapper rails (Albertson and Evens, 2000).

The loss of historic tidal marshes and prevalence of endangered species requiring this habitat has prompted expansive efforts to restore tidal marsh habitats in San Francisco Bay. Over 10,000 ha of the 13,000 ha of salt evaporation ponds that were constructed along the bay's margins during the late 1800s and early 1900s have been transferred to government ownership (Goals Project, 1999; http://www.southbayrestoration.org/). Federal and state agencies are implementing plans to convert 50–90% of these former salt ponds back into tidal marsh habitats, making this the largest wetland restoration project on the West Coast of the United States (Goals Project, 1999; http://www.southbayrestoration.org/).

Although this wetland restoration will likely benefit many tidal marsh dependent species, including the California clapper rail, a potential unintended consequence is enhanced mercury contamination of aquatic biota. San Francisco Bay has a legacy of mercury contamination from historic mercury mining and gold extraction from its tributaries (Davis et al., 2003). Tidal marshes in San Francisco Bay are thought to produce more methylmercury than open bay habitats (Marvin-DiPasquale et al., 2003), and there is concern that mercury could become more bioavailable within the estuary during large-scale landscape change and the restoration of tidal marsh habitats (Davis et al., 2003). This increase in the bioavailability of mercury could be problematic because mercury contamination within wildlife breeding in San Francisco Bay

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already is elevated, with several waterbird species considered to be at high risk to impaired reproduction (Schwarzbach et al., 2006; Ackerman et al., 2007, 2008a,b; Eagles-Smith et al., 2009).

We examined mercury exposure in endangered California clapper rails at several tidal marsh habitat sites in San Francisco Bay during a five-year period from 2006 to 2010. We evaluated factors influencing mercury bioaccumulation, and used recaptured clapper rails to assess changes in mercury exposure of individual birds over time. Using several tissue types (blood, feathers, and eggs), we evaluated the potential risk of mercury to clapper rails, and whether body condition was being negatively influenced by current levels of contamination.

2. Materials and methods

2.1. Study sites and bird sampling

We studied mercury contamination in adult California clapper rails seasonally, between October and March of 2006–2010. We sampled clapper rails at four tidal marsh study sites within the baylands of South San Francisco Bay (Fig. 1): Faber-Laumeister Marsh (36 ha; 37.47°N, 122.12°W), Colma Creek Marsh (25 ha; 37.64°N, 122.39°W), Arrowhead Marsh (10 ha; 37.74°N, 122.21°W), and Cogswell Marsh (60 ha; 37.63°N, 122.14°W). These tidal marshes represented both recently restored (Cogswell Marsh) and naturally developed (Colma Creek Marsh) marshlands created within the last 25 years, as well as tidal marshes older than 80 years (Faber-Laumeister and Arrowhead Marshes). Cogswell and Faber-Laumeister Marshes were characterized as mid- to high-elevation tidal marshes, which provided refugia for clapper rails during high tides throughout the year. Colma Creek and Arrowhead Marshes were characterized as lower-elevation tidal marshes, which may have had limited refugia for clapper rails during high tide conditions. Except for Faber-Laumeister Marsh, all marshes were dominated by a hybridized form of invasive Spartina (*Spartina alterniflora* × *foliosa*). Faber-Laumeister Marsh consisted of a mix of pickleweed (*Sarcocornia pacifica*), gumplant (*Grindelia* sp.), and Pacific cordgrass (*Spartina foliosa*).

We captured clapper rails with modified drop-door traps and dip-nets during high tides (Zembal and Massey, 1983; Conway et al., 1993; Albertson, 1995). We weighed each bird with a spring scale (to the nearest 5 g; Pesola Ag, Baar, Switzerland), and measured exposed culmen length and short tarsus (tarsometatarsus bone) length with digital calipers (to the nearest 0.01 mm; Fowler, Newton, Massachusetts, USA) and flattened wing length with a stopped wing rule. From each clapper rail, we collected fully grown breast and head feathers and stored them in Whirl-paks® (Nasco, Modesto, California, USA) prior to laboratory analysis. Because clapper rails are an endangered species, we collected blood samples only when

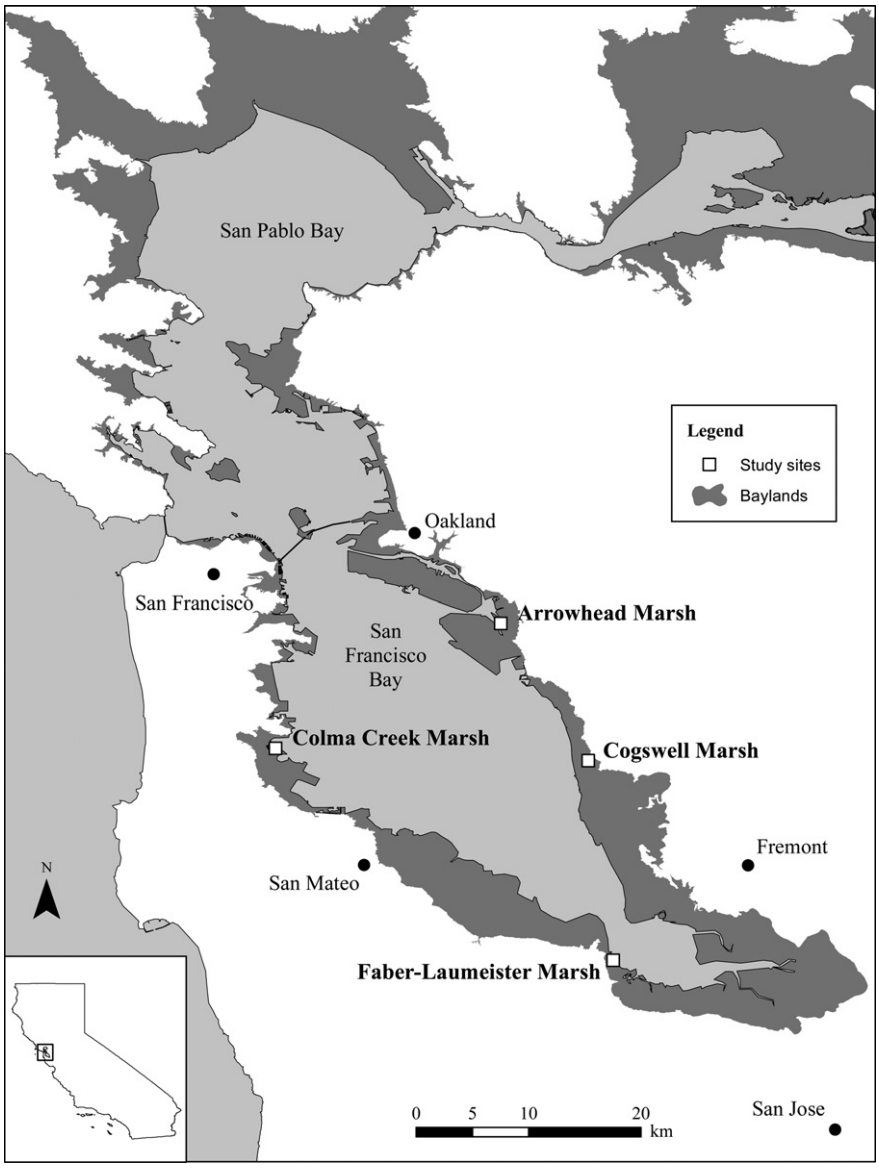

Fig. 1. Mercury contamination in California clapper rails was studied at four tidal marsh sites within the baylands of San Francisco Bay, California.

investigators with extensive bird-bleeding experience were present during captures. Therefore, we collected whole blood from a subset of live birds via the brachial or jugular vein using heparinized 26 or 28 gauge needles. We restricted the volume of blood collected to <1% of the bird's body mass (<3 ml). We immediately transferred whole blood to polypropylene cryovials and stored it on wet ice in the field until we transferred it to the laboratory for storage at −20 °C until it was analyzed. We determined the bird's sex using a discriminant function analysis (Overton et al., 2009) and confirmed the sex when necessary using genetic analysis (Zoogen Services, Davis, California, USA). We also salvaged California clapper rail eggs that were found abandoned during our routine field efforts for capturing and radio-tracking clapper rails.

We captured and marked California clapper rails under California Department of Fish and Game Scientific Collection permit (801203-03), Federal U.S. Fish and Wildlife Service permit (TE020548-9), and U.S. Geological Survey Bird Banding Laboratory permit (21142), and we conducted research under the guidelines of the U.S. Geological Survey, Western Ecological Research Center, Animal Care and Use Committee.

2.2. Sample processing and mercury determination

We processed and analyzed whole blood, entire feathers, and egg samples for total mercury (THg) as described in Ackerman et al. (2007, 2008a) and Ackerman and Eagles-Smith (2009). Prior research has demonstrated that >95% of the mercury in avian blood, feathers, and eggs is methylmercury (Thompson and Furness, 1989; Fournier et al., 2002; Schwarzbach et al., 2006). Following Environmental Protection Agency Method 7473 (United States Environmental Protection Agency, 2000), we analyzed each whole blood, feather, or egg sample for THg at the U. S. Geological Survey, Davis Field Station 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 blood averaged 101.0 ± 1.4% ($n = 11$) and 102.8 ± 1.6% ($n = 6$) for certified reference materials and matrix spikes, respectively. Recoveries for feathers averaged 100.5 ± 0.8% ($n = 25$) and 107.0 ± 8.8% ($n = 11$) for certified reference materials and matrix spikes, respectively. Absolute relative percent difference for all duplicates averaged 3.2 ± 0.8% for blood and 11.8 ± 2.3% for feathers.

2.3. Statistical analysis

We used general linear models in R (R Development Core Team, 2011) to analyze the variation in THg concentrations and body mass of California clapper rails. We evaluated the relative support of models and variables using a second-order Akaike Information Criterion (AIC*c*). For each stage of our analyses, we built an *a priori* candidate set of models and considered the model with the smallest AIC*c* to be the most parsimonious (Burnham and Anderson, 1998). We used the AIC*c* differences between the best model and the other candidate models ($\Delta AICc_i$) to determine the relative ranking of each model. We considered candidate models to be important when $\Delta AICc_i \leq 2.0$, and present only candidate models with $\Delta AICc_i \leq 7.0$ and the null model in tables. We used Akaike weights (w_i) to assess the weight of evidence that the selected model was actually the best model within the set of candidate models considered. We also calculated variable weights by summing Akaike weights across models that incorporated the same variable to assess the relative importance of each variable. Because the date effect can occur in several functional forms (linear, quadratic, cubic), we presented variable weights only for the functional form of date with the highest relative variable weight. We used evidence ratios to compare the relative weight of support between models. We used model averaging to calculate least-squared mean and 95% confidence intervals (CIs) for THg concentrations among sites, sexes, and years. We $\log_e$-transformed mercury concentrations (wet weight [hereafter, ww] for whole blood, fresh weight [hereafter, fw] for feathers, and fresh wet weight [hereafter, fww] for eggs) to improve normality, and back-transformed the data to report geometric means and 95% CIs.

In the first stage of our analysis, we examined the variables potentially influencing THg concentrations in California clapper rails. We built a candidate model set for each tissue type (blood, head feather, and breast feather) based on all combinations of the potential predictor variables capture site, capture date, capture date^2, capture date^3, sex, and year, and included a single two-way interaction. Interactions with site were excluded. A null model (intercept only) also was included in each candidate model set. Because clapper rails were sampled from early fall to late winter each season, spanning two calendar years, we standardized date to be the number of days after October 1 each season. Similarly, we standardized year to represent each winter sampling season (October–March), rather than calendar year.

Next, we examined whether THg concentrations in California clapper rails were correlated among tissues. We built a candidate model set for each tissue comparison (head feather vs. blood, breast feather vs. blood, breast feather vs. head feather) based on potential combinations of sex and THg concentrations, and the sex × THg concentration interaction. A null model (intercept only) also was included in each candidate model set.

In the third stage of our analysis, we examined whether body mass was related to THg concentrations in California clapper rails. We built a candidate model set for each tissue type (blood, head feather, and breast feather) based on a base model of sex and an index of the bird's structural size (PC1; see below). To these base models, we included all combinations of the other potential predictor variables capture date, capture date^2, capture date^3, year, THg concentrations for the specific tissue, and a single two-way interaction. We used the base model (sex and the bird's structural size; PC1) as the null model, and the base model also was included in each candidate model of this set. Date and year were standardized as described above. We used principal components analysis (PCA) of three structural body size measurements (length in mm of flattened wing, short tarsus, and exposed culmen) and the first principal component (PC1) as an index of a bird's structural body size. Because we accounted for sex and size related variation in body mass by including sex and PC1 of the bird's structural size as variables in each of the candidate models, we interpreted our results as factors influencing bird body condition.

3. Results

3.1. Mercury concentrations in clapper rails

We captured and collected feathers from 133 adult California clapper rails during the fall and winter from 2006 to 2010 (a total of 5 years of sampling). Of these, we also sampled blood from 67 birds. Overall, geometric mean mercury concentrations for California clapper rails in San Francisco Bay were 0.56 μg/g ww for blood (95% CI: 0.20–1.56; $n = 67$), 9.87 μg/g fw for head feathers (95% CI: 4.23–23.0; $n = 133$), and 9.04 μg/g fw for breast feathers (95% CI: 4.25–19.2; $n = 126$). We recaptured 21 California clapper rails at least once, of which 6 were recaptured a total of three times. For recaptured clapper rails, the within-individual variation in mercury concentrations was greatest for blood (63%; $n = 12$; 5 individuals; Fig. 2A), followed by head feathers (16%; $n = 43$; 19 individuals; Fig. 2B) and breast feathers (14%; $n = 41$; 18 individuals; Fig. 2C).

We found that the most parsimonious model explaining differences in blood mercury concentrations among clapper rails contained site and year, and had an Akaike weight of 0.27 (Table 1A). Two other models containing site also were reasonably supported by the data ($\Delta AICc < 2.0$). Using evidence ratios, the best model with site and year was 1.64 times more likely than the next top model containing site, date, and date^2, and 2.36 times more likely than the third top model containing site, sex, and year. We estimated the relative importance of individual variables and found that the data strongly supported site differences in blood mercury concentrations (relative variable importance = 1.0). In addition, there was some support for a quadratic date effect (0.44), as well as variation among years (0.61) and between sexes (0.36). Model averaged predictions of mercury concentrations in clapper rail blood was highest at Arrowhead Marsh (0.83 ± 0.20 μg/g ww), followed by Cogswell Marsh (0.56 ± 0.21 μg/g ww), Faber-Laumeister Marsh (0.46 ± 0.18 μg/g ww), and Colma Creek Marsh (0.27 ± 0.07 μg/g ww). Mercury concentrations in blood also varied among years (winters of 2007/2008: 0.47 ± 0.14 μg/g ww; 2008/2009: 0.59 ± 0.14 μg/g ww; 2009/2010: 0.54 ± 0.15 μg/g ww; 2010/2011: 0.39 ± 0.17 μg/g ww).

For head feathers, we found that the most parsimonious model explaining differences in mercury concentrations among clapper rails contained site, date, and date^2, and had an Akaike weight of 0.22 (Table 1B). Three other models containing site also were reasonably supported by the data. Using evidence ratios, the best model with site, date, and date^2 was 1.67 times more likely than the next top model containing only site, 2.18 times more likely than the third top model containing site, date, date^2, and sex, and 2.57 times more likely than the fourth top model containing site, date, date^2, date^3, and sex. Using relative variable weights, we found that the data strongly supported site differences in head feather mercury concentrations (1.0), followed by date^2 (0.59), sex (0.40), and year (0.19). Model averaged predictions of mercury concentrations in clapper rail head

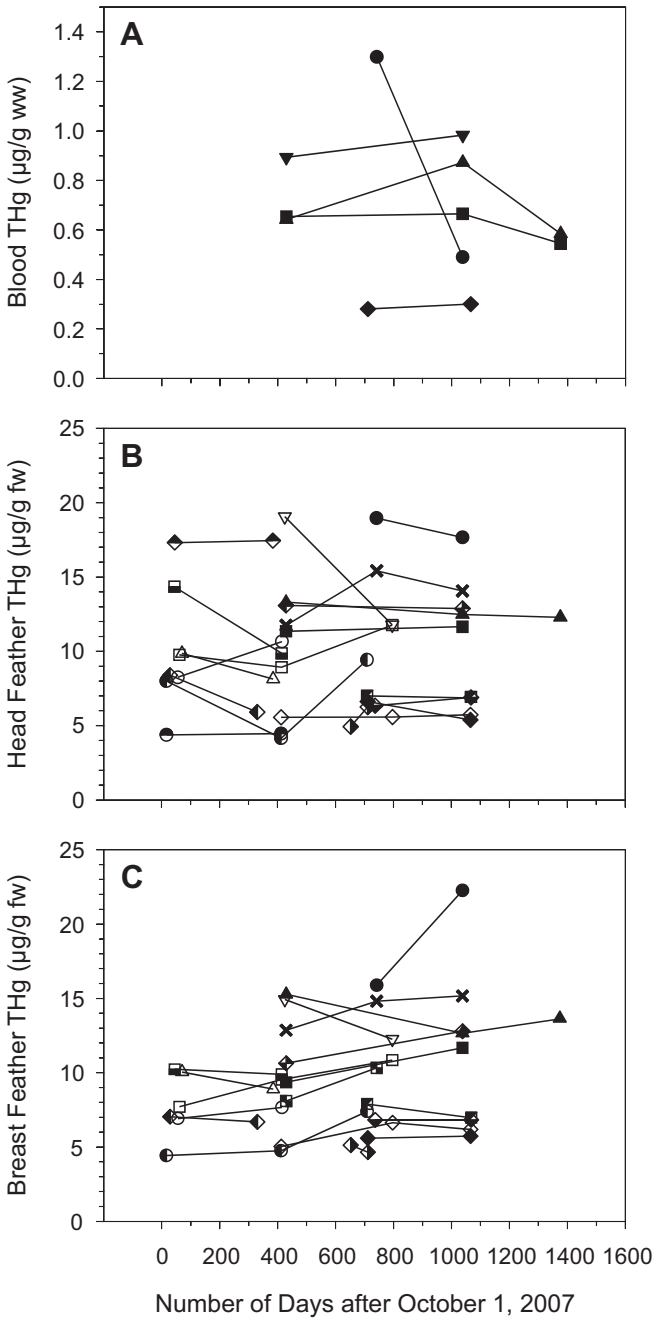

Fig. 2. Total mercury concentrations in (A) blood (THg µg/g wet weight, ww), (B) head feathers (THg µg/g fresh weight, fw), and (C) breast feathers (THg µg/g fw) of recaptured California clapper rails in San Francisco Bay, California. Different symbols represent different individual birds (blood: $n = 5$ individuals, head feathers: $n = 19$, and breast feathers: $n = 18$) and lines connecting symbols represent the number of days between sampling events.

feathers was highest at Arrowhead Marsh (14.2 ± 1.42 µg/g fw), followed by Cogswell Marsh (12.6 ± 1.33 µg/g fw), Faber-Laumeister Marsh (9.24 ± 0.85 µg/g fw), and Colma Creek Marsh (6.59 ± 0.55 µg/g fw).

For breast feathers, we found that the most parsimonious model explaining differences in mercury concentrations among clapper rails contained site and year (similar to models for blood), and had an Akaike weight of 0.25 (Table 1C). Three other models containing site also were reasonably supported by the data. Using evidence ratios, the best model with site and year was 1.86 times more likely than the next top model containing site, date, and year, 2.47 times more likely than the third top model containing site, date, and date^2, and 2.67 times more likely than the fourth top model containing site, date, date^2, and date^3. Using relative variable weights, we found that the data strongly supported site differences in breast feather mercury concentrations (1.0), followed by year (0.64), date^2 (0.39), and sex (0.28). Model averaged predictions of mercury concentrations in clapper rail breast feathers was highest at Arrowhead Marsh (13.0 ± 1.34 µg/g fw), followed by Cogswell Marsh (10.2 ± 1.26 µg/g fw), Faber-Laumeister Marsh (7.96 ± 0.86 µg/g fw), and Colma Creek Marsh (6.02 ± 0.65 µg/g fw). Mercury concentrations in breast feathers also varied among years (winters of 2006/2007: 9.43 ± 0.69 µg/g fw; 2007/2008: 8.84 ± 0.70 µg/g fw; 2008/2009: 9.67 ± 0.69 µg/g fw; 2009/2010: 9.01 ± 0.92 µg/g fw; 2010/2011: 7.74 ± 1.44 µg/g fw).

3.2. Mercury concentrations in clapper rail eggs

During our routine capturing of clapper rails, we also salvaged clapper rail eggs that were abandoned. Geometric mean THg concentrations in abandoned clapper rail eggs were 0.57 µg/g fww (range: 0.15–2.70; $n = 13$). Four eggs were recovered from a flooded and abandoned nest in Cogswell Marsh (1.67 µg/g fww; range: 0.83–2.70; $n = 4$), two eggs from an abandoned nest in Cogswell Marsh (0.72 µg/g fww; range: 0.59–0.85; $n = 2$), five eggs from an abandoned nest in Faber-Laumeister Marsh (0.24 µg/g fww; range: 0.15–0.34; $n = 5$), and one abandoned egg each was found in Colma Creek Marsh (0.32 µg/g fww) and Corte Madera Marsh (1.08 µg/g fww). We recognize that eggs from a single nest are not independent, but we include them here because there is extremely limited data on mercury contamination in eggs of endangered species.

3.3. Mercury correlations between tissues

Mercury concentrations in head and breast feathers were highly correlated with mercury concentrations in blood (head feather vs. blood: females: $n = 37$, $R^2 = 0.76$, males: $n = 28$, $R^2 = 0.76$, Fig. 3A; breast feather vs. blood: females: $n = 38$, $R^2 = 0.58$, males: $n = 28$, $R^2 = 0.68$, Fig. 3B), and mercury concentrations in breast feathers were highly correlated with mercury concentrations in head feathers (breast feather vs. head feather: females: $n = 68$, $R^2 = 0.64$, males: $n = 57$, $R^2 = 0.80$, Fig. 3C). When comparing mercury concentrations among feather types, the best model for explaining mercury concentrations in breast feathers included only mercury concentrations in head feathers (AIC $w_i = 0.68$), without the sex or interaction terms (Table 2C). The best model was 2.79 times more likely than the second top model which included sex and mercury concentrations in head feathers ($\Delta AICc = 2.05$).

For comparisons between head or breast feather mercury concentrations and blood mercury concentrations, there was some evidence for sex and interaction effects (Table 2A,B). The top model for mercury concentrations in head feathers included blood mercury concentrations, sex, and their interaction (AIC $w_i = 0.49$). This top model for mercury concentrations in head feathers was 1.70 times more likely than the second top model ($\Delta AICc = 1.07$) which included only blood mercury concentrations (without sex) and 2.15 times more likely than the third top model ($\Delta AICc = 1.53$) which included blood mercury concentrations and sex. The best model for explaining mercury concentrations in breast feathers included only mercury concentrations in blood (AIC $w_i = 0.51$) without the sex or interaction terms. This top model for mercury concentrations in breast feathers was 1.87 times more likely than the second best model ($\Delta AICc = 1.26$) which included blood mercury concentrations, sex, and their interaction, and 2.31 times more likely than the

third top model ($\Delta AICc = 1.68$) which included blood mercury concentrations and sex. For each model, the relative importance of the other tissue's mercury concentration (breast feather vs. head feather: 1.0, head feather vs. blood: 1.0, breast feather vs. blood: 1.0), was higher than sex (breast feather vs. head feather: 0.32, head feather vs. blood: 0.71, breast feather vs. blood: 0.49). Therefore, the inclusion of the sex term provided some additional improvement for models with blood mercury concentrations.

The equations for predicting breast feather mercury concentrations (μg/g fw) from blood mercury concentrations (μg/g ww) were:

$$\text{Female}: \ln[\text{breast feather THg}] = 2.66 + 0.57 \times \ln[\text{blood THg}]$$

$$\text{Male}: \ln[\text{breast feather THg}] = 2.67 + 0.62 \times \ln[\text{blood THg}]$$

and, conversely,

Table 1
Ranking of candidate model set describing mercury concentrations in (A) blood, (B) head feathers, and (C) breast feathers of endangered California clapper rails in San Francisco Bay, California, USA during 2006–2010. Models are ranked by differences in Akaike's information criterion. Only candidate models with $\Delta AICc_i \leq 7.0$ and the null model are presented.

Model structure[a]	k[b]	$-2\text{Log}L$	AICc[c]	ΔAICc[d]	Akaike weight (w_i)[e]	Evidence ratio[f]
A) Blood mercury concentrations ($n = 67$)						
Site + Year	8	34.75	53.23	0.00	0.27	1.00
Site + Date + Date^2	7	38.32	54.22	0.99	0.17	1.64
Site + Sex + Year	9	33.79	54.95	1.71	0.12	2.36
Site + Date + Year	9	34.56	55.71	2.48	0.08	3.46
Site + Date + Date^2 + Sex	8	37.32	55.81	2.57	0.08	3.62
Site + Date + Date^2 + Date^3	8	37.43	55.91	2.68	0.07	3.81
Site + Date + Sex + Year	10	33.62	57.55	4.31	0.03	8.64
Site + Date + Date^2 + Date^3 + Sex	9	36.47	57.62	4.39	0.03	8.98
Site + Date + Date^2 + Sex + Sex × Date^2	9	36.90	58.05	4.82	0.02	11.13
Site + Date + Date^2 + Sex + Sex × Date	9	37.26	58.42	5.18	0.02	13.34
Site + Date + Date^2 + Year	10	34.54	58.47	5.24	0.02	13.72
Site + Sex + Year + Sex × Year	12	28.88	58.66	5.43	0.02	15.07
Site + Date + Year + Date × Year	11	32.18	58.98	5.75	0.02	17.70
Site + Date + Sex + Year + Sex × Date	11	33.13	59.93	6.69	0.01	28.43
Intercept Only (null)	2	100.95	105.13	51.90	0.00	1.86E + 11
B) Head feather mercury concentrations ($n = 133$)						
Site + Date + Date^2	7	61.55	76.45	0.00	0.22	1.00
Site	5	67.00	77.48	1.03	0.13	1.67
Site + Date + Date^2 + Sex	8	60.85	78.01	1.56	0.10	2.18
Site + Date + Date^2 + Date^3	8	61.18	78.34	1.89	0.08	2.57
Site + Sex	6	66.33	79.00	2.55	0.06	3.58
Site + Date + Date^2 + Sex + Sex × Date	9	59.81	79.27	2.82	0.05	4.10
Site + Year	9	59.91	79.37	2.92	0.05	4.31
Site + Date + Date^2 + Sex + Sex × Date^2	9	59.94	79.41	2.96	0.05	4.40
Site + Date	6	66.86	79.53	3.08	0.05	4.67
Site + Date + Date^2 + Date^3 + Sex	9	60.55	80.01	3.57	0.04	5.95
Site + Date + Year	10	58.32	80.12	3.67	0.03	6.27
Site + Sex + Year	10	59.07	80.87	4.42	0.02	9.12
Site + Date + Sex	7	66.21	81.10	4.66	0.02	10.27
Site + Date + Date^2 + Year	11	57.09	81.28	4.83	0.02	11.18
Site + Date + Sex + Year	11	57.34	81.53	5.08	0.02	12.68
Site + Date + Sex + Sex × Date	8	65.59	82.75	6.30	0.01	23.37
Site + Date + Date^2 + Sex + Year	12	56.17	82.77	6.33	0.01	23.65
Site + Date + Date^2 + Date^3 + Year	12	56.81	83.41	6.96	0.01	32.44
Intercept Only (null)	2	152.92	157.02	80.57	0.00	3.13E + 17
C) Breast feather mercury concentrations ($n = 126$)						
Site + Year	9	2.93	22.48	0.00	0.25	1.00
Site + Date + Year	10	1.81	23.72	1.25	0.14	1.86
Site + Date + Date^2	7	9.33	24.28	1.80	0.10	2.47
Site + Date + Date^2 + Date^3	8	7.21	24.44	1.96	0.09	2.67
Site + Sex + Year	10	2.93	24.84	2.36	0.08	3.25
Site + Date + Date^2 + Year	11	1.54	25.86	3.38	0.05	5.43
Site + Date + Sex + Year	11	1.80	26.12	3.64	0.04	6.18
Site	5	15.73	26.23	3.75	0.04	6.53
Site + Date + Date^2 + Sex	8	9.20	26.43	3.95	0.03	7.22
Site + Date + Date^2 + Date^3 + Sex	9	7.00	26.55	4.07	0.03	7.65
Site + Date + Date^2 + Date^3 + Year	12	0.38	27.14	4.66	0.02	10.27
Site + Date	6	15.25	27.96	5.48	0.02	15.48
Site + Date + Date^2 + Sex + Year	12	1.54	28.30	5.82	0.01	18.39
Site + Sex	6	15.63	28.34	5.86	0.01	18.71
Site + Date + Sex + Year + Sex × Date	12	1.77	28.53	6.06	0.01	20.66
Site + Date + Date^2 + Sex + Sex × Date^2	9	9.06	28.61	6.13	0.01	21.46
Site + Date + Date^2 + Sex + Sex × Date	9	9.07	28.62	6.14	0.01	21.59
Intercept Only (null)	2	116.39	120.49	98.01	0.00	1.92E + 21

[a] The + denotes an additive effect and the × denotes an interaction.
[b] The number of parameters in the model, including the intercept and variance.
[c] Akaike's Information Criterion (AICc).
[d] The difference in the value between AICc of the current model and the value for the most parsimonious model.
[e] The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).
[f] The weight of evidence that the top model is better than the selected model, given the candidate model set.

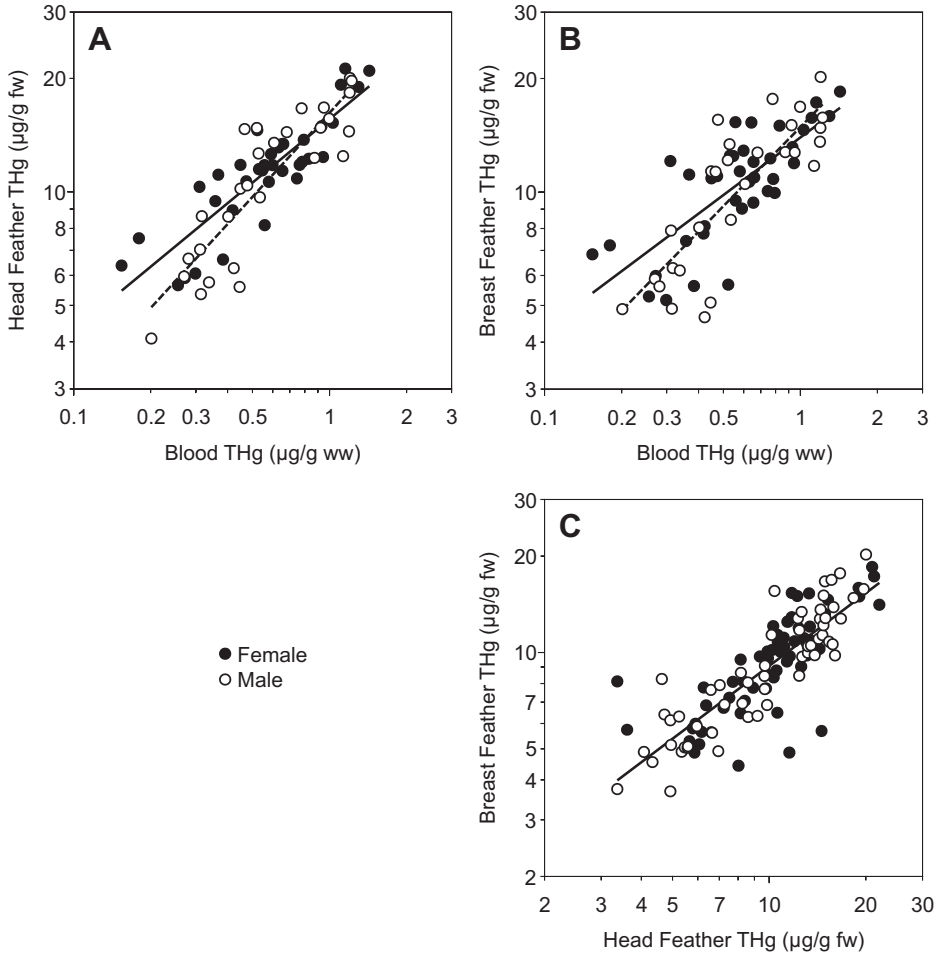

Fig. 3. Total mercury concentrations in (A) head and (B) breast feathers (THg µg/g fresh weight, fw) were correlated with total mercury concentrations in blood (THg µg/g wet weight, ww) of endangered California clapper rails in San Francisco Bay, California. (C) Head feather mercury concentrations also were correlated with breast feather mercury concentrations. Solid symbols and lines represent females and open symbols and dashed lines represent males in figures A and B.

Table 2
Ranking of candidate model set describing correlations between mercury concentrations in tissues of endangered California clapper rails in San Francisco Bay, California, USA during 2006–2010. Models are ranked by differences in Akaike's information criterion. Only candidate models with $\Delta AICc_i \leq 7.0$ and the null model are presented.

Model structure[a]	k[b]	$-2\text{Log}L$	AICc[c]	ΔAICc[d]	Akaike weight (w_i)[e]	Evidence ratio[f]
A) Head feather mercury concentrations vs. blood mercury concentrations ($n = 65$)						
Blood Mercury + Sex + Sex × Blood Mercury	5	−32.83	−21.81	0.00	0.49	1.00
Blood Mercury	3	−27.14	−20.74	1.07	0.29	1.70
Blood Mercury + Sex	4	−28.94	−20.28	1.53	0.23	2.15
Intercept Only (null)	2	60.50	64.70	86.51	0.00	$6.09E+18$
B) Breast feather mercury concentrations vs. blood mercury concentrations ($n = 66$)						
Blood Mercury	3	−1.04	5.34	0.00	0.51	1.00
Blood Mercury + Sex + Sex × Blood Mercury	5	−4.40	6.60	1.26	0.27	1.87
Blood Mercury + Sex	4	−1.63	7.02	1.68	0.22	2.31
Intercept Only (null)	2	62.17	66.36	61.02	0.00	$1.78E+13$
C) Breast feather mercury concentrations vs. head feather mercury concentrations ($n = 125$)						
Head Feather Mercury	3	−47.78	−41.58	0.00	0.68	1.00
Head Feather Mercury + Sex	4	−47.86	−39.53	2.05	0.24	2.79
Head Feather Mercury + Sex + Sex × Head Feather Mercury	5	−47.89	−37.39	4.19	0.08	8.13
Intercept Only (null)	2	116.39	120.48	162.07	0.00	$1.56E+35$

[a] The + denotes an additive effect and the × denotes an interaction.
[b] The number of parameters in the model, including the intercept and variance.
[c] Akaike's Information Criterion (AICc).
[d] The difference in the value between AICc of the current model and the value for the most parsimonious model.
[e] The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).
[f] The weight of evidence that the top model is better than the selected model, given the candidate model set.

$$\text{Female}: \ln[\text{blood THg}] = -3.03 + 1.05 \times \ln[\text{breast feather THg}]$$

$$\text{Male}: \ln[\text{blood THg}] = -2.97 + 1.04 \times \ln[\text{breast feather THg}]$$

The equations for predicting head feather mercury concentrations (μg/g fw) from blood mercury concentrations (μg/g ww) were:

$$\text{Female}: \ln[\text{head feather THg}] = 2.76 + 0.60 \times \ln[\text{blood THg}]$$

$$\text{Male}: \ln[\text{head feather THg}] = 2.77 + 0.69 \times \ln[\text{blood THg}]$$

and, conversely,

$$\text{Female}: \ln[\text{blood THg}] = -3.57 + 1.24 \times \ln[\text{head feather THg}]$$

$$\text{Male}: \ln[\text{blood THg}] = -3.19 + 1.10 \times \ln[\text{head feather THg}]$$

The equation for predicting breast feather mercury concentrations (μg/g fw) from head feather mercury concentrations (μg/g fw) was:

$$\text{Sexes combined}: \ln[\text{breast feather THg}] = 0.38 + 0.79 \times \ln[\text{head feather THg}]$$

and, conversely,

$$\text{Sexes combined}: \ln[\text{head feather THg}] = 0.27 + 0.92 \times \ln[\text{breast feather THg}]$$

3.4. Mercury's influence on body condition

The best model explaining differences in body condition among clapper rails contained the influence of mercury concentration for each of the three tissue types (Fig. 4). Within the candidate model set for blood, the most parsimonious model for predicting variation in body mass among birds included mercury concentrations in blood, date, date^2, sex, bird's structural size PC1, and the sex × bird's structural size PC1interaction, and had an Akaike weight of 0.30 (Table 3A). The second top model included date, date^2, sex, bird's structural size PC1, and the sex × bird's structural size PC1 interaction, and also was reasonably supported by the data ($\Delta AICc = 1.65$). Using evidence ratios, the best model which included mercury concentrations in blood was 2.28 times more likely than the second top model, 2.45 times more likely than the third top model containing mercury concentrations in blood, date, year, sex, bird's structural size PC1, and the sex × bird's structural size PC1 interaction, and 2.62 times more likely than the fourth top model containing mercury concentrations in blood, sex, bird's structural size PC1, and the sex × bird's structural size PC1 interaction. We estimated the relative importance of individual variables and found that, besides bird's sex and structural size PC1, the data supported an influence of blood mercury concentrations (0.77) on body mass. In addition, there was some support for a quadratic date effect (0.49) and less support for year effect (0.34).

For both feather types, the most parsimonious models explaining variation in body mass were the same, and included mercury concentrations in head or breast feathers, date, year, sex, bird's structural size PC1, and the sex × bird's structural size PC1interaction, and had an Akaike weight of 0.40 and 0.55, respectively (Table 3B,C). No other models in either of the candidate model sets for head and breast feathers were reasonably supported with these data ($\Delta AICc > 2.00$), and the best models were 3.30 and 3.35 times more likely than the next top models in each candidate set, respectively. We estimated the relative importance of individual variables for each of the head and breast feather candidate model data sets and found that, besides bird's sex and structural size PC1, the data supported an influence of feather mercury concentrations (0.94 and 0.90, respectively) on body mass. In addition, there was strong support for a year effect

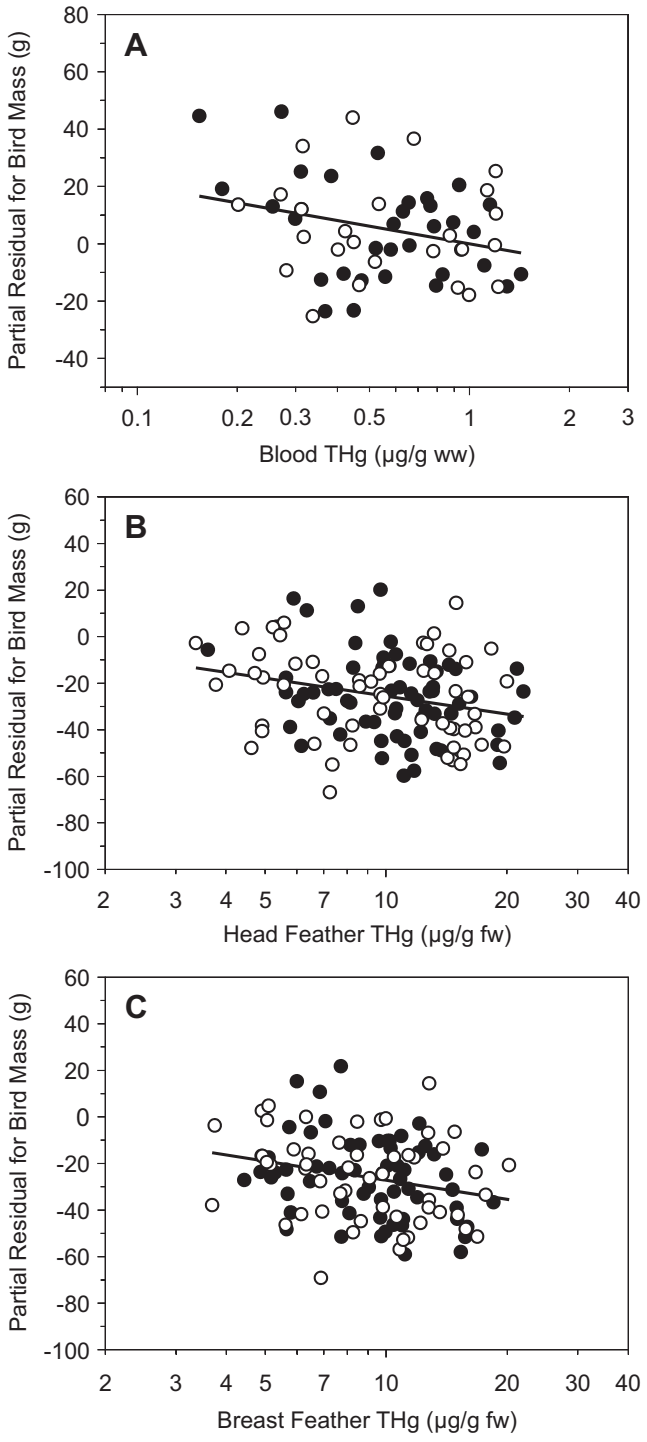

Fig. 4. Partial residual plots showing the partial correlations between California clapper rail mass (g) and total mercury concentrations in (A) blood (THg μg/g wet weight, ww), (B) head feathers (THg μg/g fresh weight, fw), and (C) breast feathers (THg μg/g fw) in San Francisco Bay, California, after accounting for the other predictor variables in the best models (see Table 1). Solid symbols represent females and open symbols represent males. The linear regression equations describing the partial residual plots were (A) $\ln Y = -8.84 \times (\ln X)$, (B) $\ln Y = -11.09 \times (\ln X)$, and (C) $\ln Y = -11.81 \times (\ln X)$.

Table 3
Ranking of candidate model set describing body mass of endangered California clapper rails in San Francisco Bay, California, USA during 2006–2010. The base model includes sex and the first principal component of a bird's structural size (PC1), so model results should be interpreted as describing bird body condition. Models are ranked by differences in Akaike's information criterion. Only candidate models with $\Delta AICc_i \leq 7.0$ and the null model are presented.

Model structure[a]	k[b]	$-2\text{Log}L$	AICc[c]	ΔAICc[d]	Akaike weight (w_i)[e]	Evidence ratio[f]
A) Model set with blood mercury concentrations ($n = 58$)						
Sex + PC1 + Blood Mercury + Date + Date^2 + Sex × PC1	8	491.08	510.02	0.00	0.30	1.00
Sex + PC1 + Date + Date^2 + Sex × PC1	7	495.43	511.67	1.65	0.13	2.28
Sex + PC1 + Blood Mercury + Date + Year + Sex × PC1	10	487.14	511.82	1.80	0.12	2.45
Sex + PC1 + Blood Mercury + Sex × PC1	6	498.30	511.95	1.93	0.11	2.62
Sex + PC1 + Blood Mercury + Year + Sex × PC1	9	490.31	512.06	2.03	0.11	2.77
Sex + PC1 + Blood Mercury + Date + Sex × PC1	7	496.27	512.51	2.49	0.09	3.47
Sex + PC1 + Blood Mercury + Date + Date^2 + Year + Sex × PC1	11	486.12	513.85	3.83	0.04	6.79
Sex + PC1 + Date + Year + Sex × PC1	9	492.26	514.01	3.98	0.04	7.33
Sex + PC1 + Sex × PC1	5	504.51	515.67	5.65	0.02	16.83
Sex + PC1 + Date + Sex × PC1	6	502.14	515.79	5.76	0.02	17.86
Sex + PC1 + Date + Date^2 + Year + Sex × PC1	10	491.62	516.30	6.28	0.01	23.07
Sex + PC1 + Year + Sex × PC1	8	497.73	516.67	6.64	0.01	27.70
Sex + PC1 (null)	4	517.44	526.19	16.17	0.00	3248.99
B) Model set with head feather mercury concentrations ($n = 122$)						
Sex + PC1 + Head Feather Mercury + Date + Year + Sex × PC1	11	1045.34	1069.74	0.00	0.40	1.00
Sex + PC1 + Head Feather Mercury + Date + Date^2 + Year + Sex × PC1	12	1045.26	1072.12	2.39	0.12	3.30
Sex + PC1 + Head Feather Mercury + Year + Sex × PC1	10	1050.81	1072.79	3.05	0.09	4.60
Sex + PC1 + Head Feather Mercury + Date + Year	10	1051.55	1073.54	3.80	0.06	6.69
Sex + PC1 + Head Feather Mercury + Date + Year + Date × Head Feather Mercury	11	1049.87	1074.27	4.54	0.04	9.66
Sex + PC1 + Head Feather Mercury + Date + Year + PC1 × Date	11	1050.04	1074.44	4.71	0.04	10.53
Sex + PC1 + Date + Year + Sex × PC1	10	1053.16	1075.15	5.41	0.03	14.96
Sex + PC1 + Head Feather Mercury + Date + Year + PC1 × Head Feather Mercury	11	1051.13	1075.53	5.79	0.02	18.11
Sex + PC1 + Head Feather Mercury + Date + Year + Sex × Date	11	1051.45	1075.85	6.12	0.02	21.30
Sex + PC1 + Head Feather Mercury + Date + Date^2 + Year	11	1051.54	1075.94	6.20	0.02	22.20
Sex + PC1 + Head Feather Mercury + Date + Year + Sex × Head Feather Mercury	11	1051.55	1075.95	6.22	0.02	22.37
Sex + PC1 + Head Feather Mercury + Date + Date^2 + Year + Date × Head Feather Mercury	12	1049.87	1076.73	7.00	0.01	33.04
Sex + PC1 + Head Feather Mercury + Year	9	1057.13	1076.74	7.00	0.01	33.16
Sex + PC1 (null)	4	1090.94	1099.28	29.54	0.00	2.60E + 06
C) Model set with breast feather mercury concentrations ($n = 114$)						
Sex + PC1 + Breast Feather Mercury + Date + Year + Sex × PC1	11	972.71	997.29	0.00	0.55	1.00
Sex + PC1 + Breast Feather Mercury + Date + Date^2 + Year + Sex × PC1	12	972.62	999.71	2.42	0.16	3.35
Sex + PC1 + Breast Feather Mercury + Year + Sex × PC1	10	978.64	1000.77	3.48	0.10	5.69
Sex + PC1 + Date + Year + Sex × PC1	10	979.33	1001.47	4.17	0.07	8.06
Sex + PC1 + Date + Date^2 + Year + Sex × PC1	11	979.33	1003.92	6.63	0.02	27.47
Sex + PC1 + Breast Feather Mercury + Date + Year	10	982.16	1004.29	7.00	0.02	33.11
Sex + PC1 (null)	4	1017.69	1026.06	28.76	0.00	1.76E + 06

[a] The + denotes an additive effect and the × denotes an interaction.
[b] The number of parameters in the model, including the intercept and variance.
[c] Akaike's Information Criterion (AICc).
[d] The difference in the value between AICc of the current model and the value for the most parsimonious model.
[e] The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).
[f] The weight of evidence that the top model is better than the selected model, given the candidate model set.

(0.99 and 0.99) and some support for a linear date effect (0.89 and 0.89).

4. Discussion

Similar to several other studies on mercury contamination in waterbirds within the San Francisco Bay (Ackerman et al., 2007, 2008a,b,c; Eagles-Smith et al., 2009), we found that differences in mercury concentrations among adult California clapper rails were largely attributed to site. Despite nearly a 10-fold difference in blood mercury concentrations among individuals, the majority of recaptured clapper rails exhibited low within-individual variation, indicating that mercury exposure was relatively stable for most individuals. Clapper rails have extremely small yearly home ranges, averaging 3.1 ha, and are even more restricted in their movements during the breeding season to 1.8 ha (Rohmer, 2010). Moreover, clapper rails are non-migratory and have limited dispersal (Casazza et al., 2008). Thus, small-scale site-specific exposure is expected to drive mercury contamination in clapper rails.

Mercury concentrations in California clapper rails were considered elevated (geometric mean: 0.56 μg/g ww for blood, 9.87 μg/g fw for head feathers, and 9.04 μg/g fw for breast feathers) and some individuals had mercury concentrations above levels that have been associated with reproductive impairment. In comparison, California black rail (*Laterallus jamaicensis coturniculus*) mercury concentrations in marshes north of San Francisco Bay were 0.38 μg/g ww in blood and 6.94 μg/g fw in feathers (Tsao et al., 2009). Overall, 15% of blood and 56–63% of feather samples from clapper rails were over 1.0 μg/g ww and 9.0 μg/g fw, respectively. These mercury concentrations are considered to put birds at risk to potentially impaired reproduction (Evers et al., 2004; Burger and Gochfeld, 1997). Although our sample size for eggs was small due to restrictions on collecting viable eggs from an endangered species, we found that 31% of abandoned clapper rail eggs were considered at high risk (>1.0 μg/g fww) to potential reproductive impairment based on toxicity endpoints for other species (Evers et al., 2003; Scheuhammer et al., 2007). The egg mercury concentrations we observed in 2007–2010 (geometric mean: 0.57 μg/g fww), were similar to those found for clapper rails in the South San Francisco Bay in 1986–1987 (0.55 μg/g fww; Lonzarich et al., 1992) and 1991–1992 (0.54 μg/g fww; Schwarzbach et al., 2006). Substantial variability exists among bird species in their sensitivity to methylmercury, and clapper rails were among the most

vulnerable of 26 species studied (Heinz et al., 2009). Therefore, our results suggest that California clapper rails may be at risk to impaired reproduction due to mercury contamination.

Body condition of California clapper rails was negatively related to mercury concentrations in each of the three tissues sampled. Model averaged estimates for the effect of mercury on body condition indicated a potential decrease in body mass of 20–22 g over the observed range of mercury concentrations in clapper rails, which translates into a body mass loss of 5–6% for males and 6–7% for females. A few other field studies also have found negative correlations between indices of bird condition and tissue mercury concentrations (Takekawa et al., 2002; Wayland et al., 2003). Captive studies where birds have been fed diets treated with methylmercury have shown that mercury exposure can make birds less motivated to hunt for food (Bouton et al., 1999), and can reduce foraging efficiency (Adams and Frederick, 2008), appetite (Spalding et al., 2000), and body condition (Spalding et al., 2000). It is unclear whether this decline in body condition could influence clapper rail survival or reproduction; however clapper rails are known to have extremely low adult annual survival rates (32%; C. Overton, U.S. Geological Survey, unpublished) and moderate nest survival rates (45% apparent nest success; Schwarzbach et al., 2006). Reduced body condition may exacerbate already high mortality rates and may be indicative of other sublethal effects.

Although we found a strong correlation between bird mass and mercury concentrations, an alternate possibility is that clapper rails lost mass due to other reasons, resulting in mercury concentrating within body tissues. Mass dilution can dramatically reduce mercury concentrations in rapidly growing bird chicks (Ackerman et al., 2011) and juvenile fish (Ward et al., 2010). Diluting mercury concentrations via mass growth has typically been demonstrated in fast-growing juvenile animals, so whether it may also be a likely scenario for adult birds is unclear. Presumably any organism undergoing fluctuations in body mass in which the trajectory of mass loss or gain diverges from the trajectory of mercury depuration or accumulation from the diet, will exhibit changes in tissue mercury concentrations. Thus, it is unclear whether our finding of reduced body mass with increased mercury concentrations represents a causal, physiological effect of mercury on clapper rails, or is simply the result of mercury concentrating in the body tissues as body mass declined due to other reasons. The fact that clapper rail body mass was negatively correlated with head and breast feather mercury concentrations, as well as blood mercury concentrations, provides additional support for a physiological effect of mercury on clapper rails. Fully-grown feathers were sampled from two different feather tracts (head and breast) and represent mercury concentrations from temporally distant and distinct time periods (molting) from the time when body mass was measured. Therefore, it is possible that chronic mercury exposure may reduce bird body condition over time. Additional research is needed to further elucidate the physiological relationship between bird body condition and mercury concentrations.

5. Conclusion

In general, our results indicate the potential for detrimental effects of mercury contamination on endangered California clapper rails. Clapper rail populations have become restricted to small, isolated tidal marshes throughout San Francisco Bay (Albertson and Evens, 2000) and exhibit little movement within (Rohmer, 2010) and among sub-populations (Casazza et al., 2008). This fragmented population, coupled with the fact that site is the most important predictor of mercury contamination in waterbirds in San Francisco Bay (Ackerman et al., 2007, 2008a,b,c; Eagles-Smith et al., 2009), suggests that monitoring programs for clapper rails should be spatially widespread so that local mercury hotspots can be identified for appropriate conservation and mitigation actions. Fully-grown feathers can be relatively poor predictors of internal tissue mercury concentrations in free-living chicks (Ackerman et al., 2011) and adults (Eagles-Smith et al., 2008), particularly in migratory birds. Therefore, feathers typically are not recommended for mercury monitoring programs. However, in the case of California clapper rails, feather mercury concentrations were more highly correlated with blood mercury concentrations while accounting for sex differences ($R^2 = 0.58–0.76$) than in other waterbirds that are more vagile ($R^2 = 0.14–0.76$; Eagles-Smith et al., 2008). Therefore, feathers may provide a viable sampling tool for assessing mercury contamination of endangered California clapper rails if more invasive sampling protocols for preferred tissues, such as blood or eggs, are restricted. We have provided the necessary equations to predict mercury concentrations in blood (which are more interpretable in regards to toxicology) from those in head or breast feathers (which can be sampled non-destructively).

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