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Avian influenza antibody prevalence increases with mercury contamination in wild waterfowl

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Environmental contamination is widespread and can negatively impact wildlife health. Some contaminants, including heavy metals, have immunosuppressive effects, but prior studies have rarely measured contamination and disease simultaneously, which limits our understanding of how contaminants and pathogens interact to influence wildlife health. Here, we measured mercury concentrations, influenza infection, influenza antibodies and body condition in 749 individuals from 11 species of wild ducks overwintering in California. We found that the odds of prior influenza infection increased more than fivefold across the observed range of blood mercury concentrations, while accounting for species, age, sex and date. Influenza infection prevalence was also higher in species with higher average mercury concentrations. We detected no relationship between influenza infection and body fat content. This positive relationship between influenza prevalence and mercury concentrations in migratory waterfowl suggests that immunotoxic effects of mercury contamination could promote the spread of avian influenza along migratory flyways, especially if influenza has minimal effects on bird health and mobility. More generally, these results show that the effects of environmental contamination could extend beyond the geographical area of contamination itself by altering the prevalence of infectious diseases in highly mobile hosts.

1. Introduction

Environmental pollution is widespread. Many contaminants, such as heavy metals and organic compounds, can persist in ecosystems for years or decades [1], leading to prolonged exposure of wildlife to potential toxicants. Exposure to contaminants can impact wildlife health, behaviour, survival and reproduction [2,3], but animals can detoxify and/or excrete many contaminants, and the physiological effects of contamination vary across species and ecological contexts [2,4].

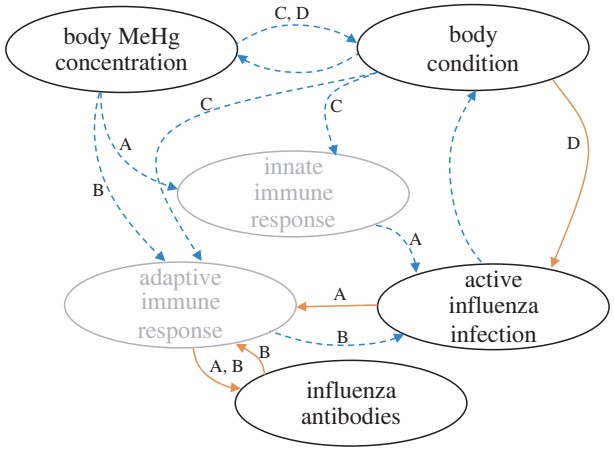

Figure 1. Hypothesized and predicted relationships among mercury concentrations, body condition, active infection and antibody detection. Dashed blue arrows represent negative relationships between variables and solid orange arrows represent positive relationships. Grey ovals are variables that are important mechanistically but were not measured directly in this study. In pathway A, MeHg immunotoxicity compromises the innate immune response to infection, leading to a higher probability of active infection, more antibody production and an increased probability of antibody detection. In B, MeHg immunotoxicity compromises the adaptive immune response, reducing antibody detection but increasing the probability of active infection. In C, MeHg toxicity reduces host body condition, reducing the energy available to mount an immune response and increasing infection probabilities. In pathway D, MeHg toxicity reduces host body condition, altering physiologic conditions for viral replication and decreasing the probabilities of both active infection and antibody detection. Feedbacks complicate these relationships, including disease-induced reductions in body condition following influenza infection and effects of body condition on MeHg concentrations (due to concentrating of body MeHg with mass loss). (Online version in colour.)

Understanding how and when contaminants most strongly affect wildlife is important for prioritizing monitoring, managing exposure and implementing mitigation measures.

Infection with pathogens and parasites is one important context in which contaminants might affect wildlife health and fitness [5]. Wildlife naturally experience infection over their lives, but the probability and effects of infection are context dependent. For example, the pathogens that cause chytridiomycosis in amphibians and white-nose syndrome in bats can cause massive die-offs, but in some contexts cause no disease [6,7]. This variation in pathogen impacts stems from differences in pathogen exposure, susceptibility (i.e. probability of infection given exposure) and immune responses across hosts. Exposure to contaminants could influence these pathways via changes to a host's behaviour, immune system and/or energetic state [8], but the relationships between contamination and infection are complex. For instance, contaminants could increase infection prevalence if they have immunosuppressive effects [9–11] (figure 1*a*,*b*), but could decrease infection prevalence if contamination reduces host competence [12] or parasite fitness [8] (figure 1*d*). Therefore, the influence of environmental contaminants on infection prevalence depends on the contaminant–pathogen combination, the host's physiological response to each, and the environmental context.

In addition to altering infection prevalence, the toxic effects of contaminants can increase disease severity by reducing the amount of energy available to mount an immune response (figure 1*c*). Body condition metrics (e.g. body mass or fat stores), which represent energy stores and overall health, are useful and common proxies for the severity of disease. However, relationships of body condition with infection or contamination are bidirectional (figure 1) [13]. For example, animals in poor body condition sometimes increase their feeding rates, which can increase their exposure to trophically transmitted pathogens and contaminants. Changes in body size can also concentrate or dilute contaminants in a host's body (figure 1), which could change their toxicity [14]. Body condition is therefore a measurement of overall health that depends on interacting ecological factors including disease and contamination, as well as stress, nutrition and reproductive status [13].

Waterfowl are natural hosts of avian influenza viruses (hereafter influenza) and exposure to environmental contaminants could affect the prevalence and disease severity of this common pathogen. Influenza is endemic in waterfowl populations, but infection prevalence is highest in late summer [15], in juveniles [16] and in dabbling and filter-feeding ducks [17,18]. Antibodies against influenza, which generally last months to over a year [19–21], are more prevalent in adults [16,22]. Influenza is transmitted via the environment and can persist in wetland environments for months or longer [23,24]. Infection occasionally affects body condition, behaviour and/or movement, but low pathogenic avian influenza infection is generally considered asymptomatic in wild birds [25]. However, co-exposure to immunotoxic contaminants could increase infection probabilities and magnify any negative health effects of infection. In addition, highly pathogenic strains of avian influenza viruses, which cause severe disease in poultry and occasionally humans and other mammals [26,27], are increasing in wild waterfowl [28]. Understanding the factors, including contaminant exposure, that influence influenza infection in wild birds is therefore important for informing assessments of risk to wildlife, livestock and human health.

Mercury contamination from natural and anthropogenic sources (e.g. atmospheric deposition from industrial outputs, gold mining [29]) is common in the aquatic habitats inhabited by waterfowl. Bacteria in aquatic soils convert inorganic mercury into methylmercury (MeHg), its toxic form, which is then acquired by animals through feeding and biomagnifies through food webs [30,31]. Animals can sequester and depurate mercury (e.g. via eggs and feathers), but it also bioaccumulates throughout individuals' lifetimes, so mercury concentrations in the internal tissues of wild animals reflect a combination of long-term and recent exposure [31]. Animals that feed at higher trophic levels (e.g. diving ducks as compared to dabbling ducks) tend to have higher mercury concentrations from increased dietary exposure [32]. MeHg exposure can reduce body condition [33,34], reproductive output [4,35] and survival [36]. MeHg can also alter immune function in mammals and birds [37], including compromising both the innate immune response (e.g. inflammation [9,38–41]) and the adaptive immune response (e.g. lymphocyte production [38,42,43], antibody titres [10]). Both innate and adaptive immunity influence the probability and severity of influenza infection [44,45], so mercury contamination could affect the prevalence of influenza infection and its impacts waterfowl populations.

Here, we study relationships among mercury contamination, influenza infection and body condition in 11 species of dabbling and diving ducks in the Pacific Flyway. The Central Valley and San Francisco Bay Estuary of California are hotspots of mercury contamination in North America [4,46] and are

Table 1. Sample sizes and prevalence of detected influenza antibodies or viral RNA in samples from ducks collected in the San Francisco Bay Estuary, California during winters of 2017–2018 and 2018–2019. All samples included in this table also included paired mercury data. Confidence intervals (CI) are derived from 10 000 bootstrapped samples.

common name	scientific name	antibody sample size (ELISA)	antibody prevalence [95% CI]	active infection sample size (rRT-PCR)	active infection prevalence [95% CI]
American green-winged teal	*Anas crecca carolinensis*	11	0.36 [0.09, 0.64]	89	0.08 [0.03, 0.13]
American wigeon	*Mareca americana*	22	0.50 [0.27, 0.68]	64	0.05 [0.00, 0.11]
canvasback	*Aythya valisineria*	70	0.84 [0.74, 0.91]	65	0.05 [0.00, 0.11]
cinnamon teal	*Anas cyanoptera*	7	0.71 [0.29, 1.00]	15	0.13 [0.00, 0.33]
gadwall	*Mareca strepera*	17	0.59 [0.35, 0.82]	33	0.03 [0.00, 0.09]
greater scaup	*Aythya marila*	24	0.83 [0.67, 0.96]	24	0.04 [0.00, 0.12]
lesser scaup	*Aythya affinis*	86	0.77 [0.67, 0.86]	88	0.09 [0.03, 0.16]
mallard	*Anas platyrhynchos*	50	0.80 [0.68, 0.90]	90	0.04 [0.01, 0.09]
northern pintail	*Anas acuta*	28	0.79 [0.64, 0.93]	61	0.08 [0.02, 0.15]
northern shoveler	*Spatula clypeata*	26	0.88 [0.73, 1.00]	89	0.11 [0.04, 0.18]
ruddy duck	*Oxyura jamaicensis*	96	0.65 [0.55, 0.74]	102	0.06 [0.02, 0.11]
total/mean		437	0.74 [0.70, 0.78]	720	0.07 [0.05, 0.09]

important sites for overwintering waterfowl; approximately 60% of the migratory waterfowl in the Pacific Flyway overwinter in this region annually [47,48]. Multiple waterfowl species co-occur at overwintering sites in the region, which enables cross-species influenza transmission and viral reassortment [49,50], and waterfowl occasionally use wetlands and agricultural habitats near and within poultry facilities [51]. This combination of mercury contamination, high waterfowl abundance and intensive poultry production makes northern California an important region for understanding how mercury contamination affects influenza prevalence and wildlife health.

We hypothesized that influenza infection would be positively related to mercury concentrations due to the immunotoxic effects of mercury [9,31] (figure 1), even while accounting for factors that could influence both infection and mercury concentrations, including age [16,31], species [4,17], sex [11,52] and body condition [33,53]. We also hypothesized that influenza infection would have sublethal effects on body condition [25,54], and that these effects would be exacerbated by mercury, especially at high mercury concentrations [55]. We explored these relationships for both active infection (i.e. PCR analysis of cloacal and oropharyngeal swabs) and for detectable antibodies (i.e. ELISA assay of blood samples, hereafter 'prior infection'). Antibodies to influenza are estimated to last 6 months-1.5 years [19–21] but usually peak within three weeks of infection [19,20]. The strength of the antibody response and the probability of detecting antibodies following infection vary across age classes and species [20,56]. Antibody assay results therefore represent probable prior infection within the last 1.5 years, but most likely within the last six months [57], and depend on individual traits.

2. Methods

(a) Study system and data collection

Dabbling and diving ducks were collected lethally by scientists during the non-breeding season (October–March) in 2017–2018 and 2018–2019 from two major bays in the San Francisco Bay Estuary of California (table 1). Cloacal and oropharyngeal swabs and a cardiac venipuncture, which supplied blood samples for both antibody and mercury analysis, were taken within 5 min of collection. We determined age (juvenile or adult) and sex using plumage characteristics [58–60]. In the laboratory, we measured body mass, extracted sera for blood mercury analysis, dissected carcasses to extract samples of liver and muscle for mercury analysis [61] and validated field ageing and sexing techniques [62,63]. For more detail, see electronic supplementary material, methods.

(b) Mercury concentrations

We measured total mercury (THg) concentrations in blood, liver and muscle (electronic supplementary material, methods). We used whole blood THg concentration to represent body MeHg concentration, because 95% of THg in blood is MeHg [31,64], and because studies of blood mercury are common [34], allowing us to compare our results directly to those from other studies. For the 88 birds (12% of data) for which blood mercury values were unavailable, we estimated blood THg concentrations using the strong relationships between mercury concentrations in blood and those in muscle and liver tissue [61] (electronic supplementary material, methods). We repeated analyses without these imputed data and found qualitatively similar results.

(c) Influenza laboratory analysis

Cloacal and oropharyngeal swabs were tested for the presence of influenza RNA using real-time reverse transcriptase-PCR (rRT-PCR) targeting the matrix gene [65] (electronic supplementary material, methods). We considered a sample to indicate active influenza infection if the cycle threshold (Ct) value was less than or equal to 45 [66]. All rRT-PCR-positive samples tested negative for highly pathogenic H5 clade 2.3.4.4 viruses [66], the only highly pathogenic influenza lineage previously identified in North American wild birds, and thus most likely represent infection with low pathogenic influenza viruses. Sera samples were tested for the presence of influenza A antibodies using commercially available blocking enzyme-linked immunoassay

(ELISA; AI MultiS-Screen Avian Influenza Virus Antibody Test Kit; IDEXX Laboratories, Westbrook, Maine, USA). We considered samples with a signal-to-noise ratio less than 0.5 to be positive for the presence of influenza antibodies [45,67].

(d) Body condition and composition

We used percentage crude fat from a sample of the whole body, as determined by body composition analysis (electronic supplementary material, methods), to measure body condition. For data analysis, we standardized percentage fat within species, age class and/or sex, which accounted for differences in average fat content among groups that were unrelated to condition. To do so, we subtracted the mean and divided by the standard deviation within each group (i.e. *z*-score scaling). For most species, we standardized percentage fat within three groups: adult male, adult female and juveniles of both sexes; for the two species that had fewer than eight samples within a group (cinnamon teal and greater scaup), percentage fat was scaled across all individuals in the species. Because we used percentage fat (rather than fat mass), our measurement of body condition accounted for body mass.

(e) Statistical analysis

We first examined relationships between blood mercury concentrations and influenza prevalence at the species level using univariate generalized linear models (GLMs). We modelled antibody prevalence and active infection prevalence separately; each GLM used a logit link function where the response variable was the number of positive and negative samples for each species and the predictor variable was the species' geometric mean blood mercury concentration ($\log_{10}$-transformed).

Next, we studied relationships between influenza infection probabilities, mercury and ecological variables at the individual level. We transformed the date of sampling (hereafter date) to a fraction of the year beginning on 1 July so that it would be continuous throughout the winter. To do so, we converted each date to the day of year, subtracted 365 from any day after 180 (30 June) and divided these numbers by 365. In our dataset, this variable ranged from −0.21 (16 October) to 0.24 (30 March), where 0 was 1 January.

We used GLMs and generalized additive models (GAMs) and AIC_c-based multi-model inference [68,69] to analyse relationships among influenza infection, mercury contamination, body condition and ecological variables. First, we built a GLM predicting the influenza antibody status of an individual (a binary variable) from the following predictors: species (11 species, a categorical variable), age class (adult or juvenile, categorical), sex (male or female, categorical), blood mercury concentration ($\log_{10}$ µg g^{-1} wet weight (ww)), percentage crude fat (scaled), date (scaled), $date^2$ and year (2017 or 2018, categorical). The quadratic effect of date was included because influenza prevalence is higher in mid-winter than during fall and spring migration in many ducks [70]. We also included the interaction between age and date to test for age-specific changes in the probability detecting antibodies over the season, as well as pairwise interactions of blood mercury concentration with species, age and sex, to examine differences in each group's response to mercury. The GLM used a logit link.

For model selection, our candidate model set included most model subsets, but included interactions and quadratic effects only when main effects were also included. Model selection was implemented using the MuMIn package in R [71,72]. Because there were many models with similar AIC_c values, indicating uncertainty in our ability to identify a single best model, we used model averaging on the full candidate set to estimate the overall effect of each variable. We used conditional averaging, which averages parameters from models in which they appear [69]. We report results from the averaged model following guidelines for reporting statistical evidence from Muff *et al.* [73] and using predictions from the averaged model [74]. We report 85% confidence intervals (CIs), which are consistent with model selection criteria [75], and provide 95% CIs for reference. In addition, we calculated relative variable importance using Akaike weights ($w = e^{-0.5\Delta AIC_c}$) and an adjusted metric of relative variable importance [76], in which variables with a relative importance greater than 0 have a higher weight than expected based on their inclusion in the candidate set (electronic supplementary material, methods). We also measured relative support for pairs of models in the model set using evidence ratios (i.e. ratios of Akaike weights [69]) and differences in R^2 values.

We used the same procedure and the same set of predictor variables to fit, evaluate and average models for active influenza infection status ($Ct \leq 45$).

Finally, we used a similar procedure to model body condition, measured using percentage crude fat (scaled, described above). For this model set, we tested both linear models and GAMs, which can model complex nonlinear relationships [77], because avian endogenous reserves can change rapidly and nonlinearly throughout the non-breeding season [78]. We modelled body condition as a normally distributed (Gaussian) variable. Predictors in the LMs were blood mercury concentration, antibody status, active infection status, date, year and the interaction of blood mercury with antibody status, active infection status, age, sex and species. The GAMs included smoothed effects of all continuous variables (mercury, date and the interactions of mercury) instead of their parametric terms, and the same categorical variables as the LMs (electronic supplementary material, methods). We performed model averaging using the model set (LMs or GAMs) with the lowest AIC_c.

3. Results

We analysed 437 influenza antibody samples and 720 active influenza infection samples with paired mercury data from 749 individuals across 11 species of dabbling and diving ducks (table 1). Mean blood mercury concentrations ranged from 0.007 µg g^{-1} ww in American wigeon to 0.566 µg g^{-1} ww in greater scaup. Antibody prevalence ranged from 0.364 (i.e. 36.4%) in American green-winged teal to 0.885 in northern shoveler. Across species, antibody prevalence increased with blood mercury concentrations (figure 2*a*). Active influenza infection prevalence ranged from 0.030 in gadwall to 0.133 in cinnamon teal; active infection prevalence was unrelated to average blood mercury concentrations at the species level (figure 2*b*).

(a) Prior infection

At the individual level, the most important variables predicting prior influenza infection (i.e. antibody detection) were age, date, $date^2$, blood mercury and the interaction between date and age. The averaged model showed a positive effect of blood mercury concentration on antibody status (figure 3; electronic supplementary material, table S1); the evidence for this effect was relatively weak, but its effect size was substantial (odds ratio (OR): 1.723; 85% CI: [1.087, 2.729]; $p = 0.089$), indicating that the odds of prior infection increased 1.7 times for every 10-fold increase in mercury concentrations, and that the predicted probability of infection increased 1.2- to 3.7-fold across the observed range of mercury concentrations (0.001–1.623 µg g^{-1} ww), depending on other variables in the model.

The averaged model also provided strong evidence for a positive effect of age on antibody status, such that the odds of prior infection were 2.7 times greater in adults than

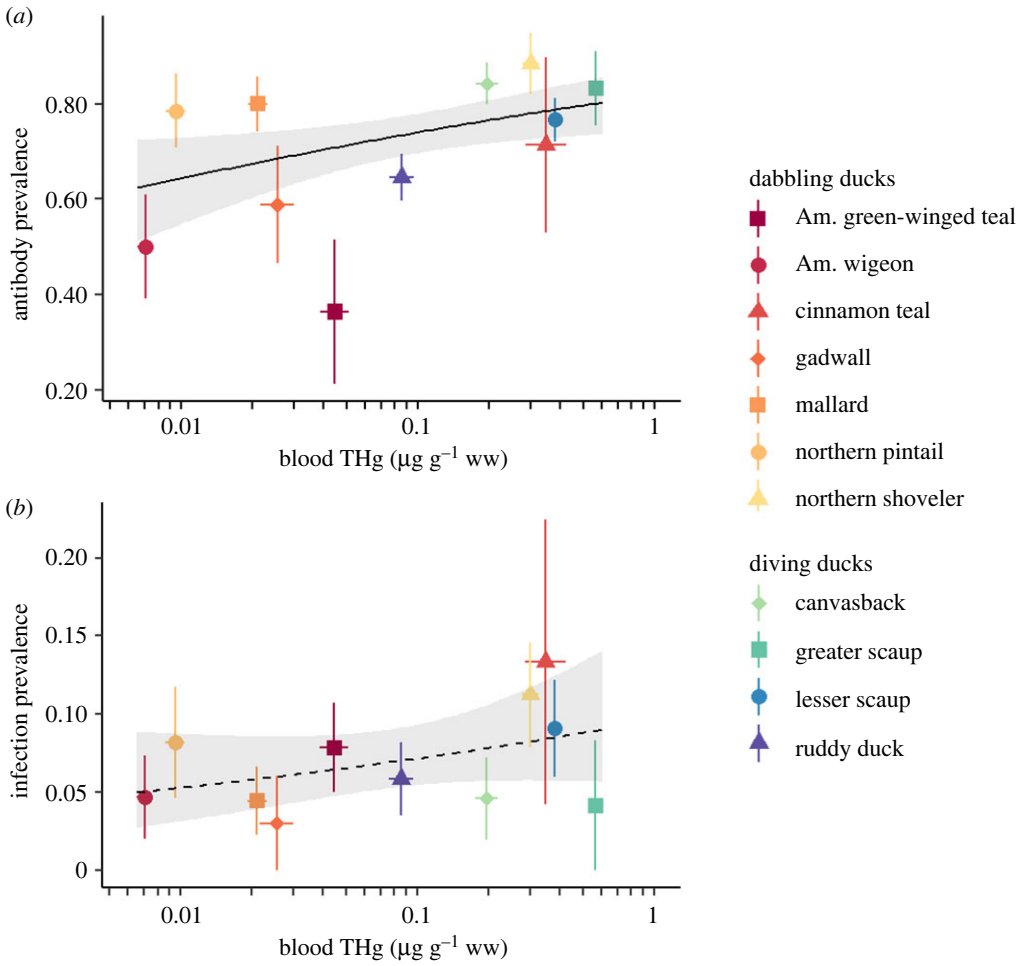

Figure 2. Species-level relationships between influenza prevalence and average blood mercury concentration. Points and error bars show geometric mean and standard error for each variable in raw data. Black lines show the fitted relationship from univariate GLMs; shaded areas show 95% CIs. Note the log scale of the *x*-axis. (*a*) Antibody prevalence increases with blood mercury concentrations ($\beta = 0.198$, $p = 0.013$). (*b*) There is little evidence that infection prevalence increases with blood mercury concentrations ($\beta = 0.140$, $p = 0.190$). (Online version in colour.)

juveniles (OR: 2.697; 85% CI: [1.564, 4.651]; $p = 0.009$) (figure 3). For juveniles, the probability of prior infection (i.e. antibody detection) increased over the course of the winter, whereas prior infection probabilities in adults changed relatively little over time and were highest in mid-winter (electronic supplementary material, figure S1). The full model (i.e. containing all predictor variables) had an R^2 of 0.15 (electronic supplementary material, table S2).

Most models with substantial support (i.e. low AIC_c) contained terms for blood mercury and/or species (electronic supplementary material, table S2). The model with the lowest AIC_c included both, but models without each variable were competitive ($\Delta AIC_c < 2$). Evidence for the nested model with blood mercury only (i.e. age, date, date^2, blood mercury and age × date) was 1.5 times stronger than for the model with species only, but this model explained 3% less of the variance in antibody status (R^2 of 0.08 versus 0.11, electronic supplementary material, table S2). Together, these patterns suggest prior infection is positively associated with blood mercury concentrations and that blood mercury concentrations explain a small portion of the variation in antibody detection, both within and across species.

(b) Active infection

Our ecological predictors explained very little of the variation in active infection status; the full model had an R^2 of only 0.08, there was no strong evidence for an effect of any variable in the averaged model, and the intercept-only model was competitive ($\Delta AIC_c = 1.490$, electronic supplementary material, table S3), indicating that the other parameters were uninformative [75]. The only variables with positive relative importance scores were age and the interaction between age and date (electronic supplementary material, table S4). Nevertheless, the direction of each effect in the averaged model was consistent with our hypotheses and with results from the antibody status models (electronic supplementary material, figure S3): infection probabilities tended to increase with blood mercury concentrations (OR: 1.609; 85% CI: [1.021, 2.536]; $p = 0.132$); adults were generally less likely to be infected than juveniles (OR: 0.471; 85% CI: [0.220, 1.006]; $p = 0.153$); and infection probabilities tended to decrease through the winter for juveniles (electronic supplementary material, figure S3).

(c) Body condition

GAMs performed better than linear models for predicting body condition. Across all GAMs, date was the only variable with a positive relative importance score (electronic supplementary material, table S5); body condition decreased nonlinearly through the winter. The R^2 of the full GAM was 0.11. In the averaged model, there was no evidence for an effect of active influenza infection status or influenza

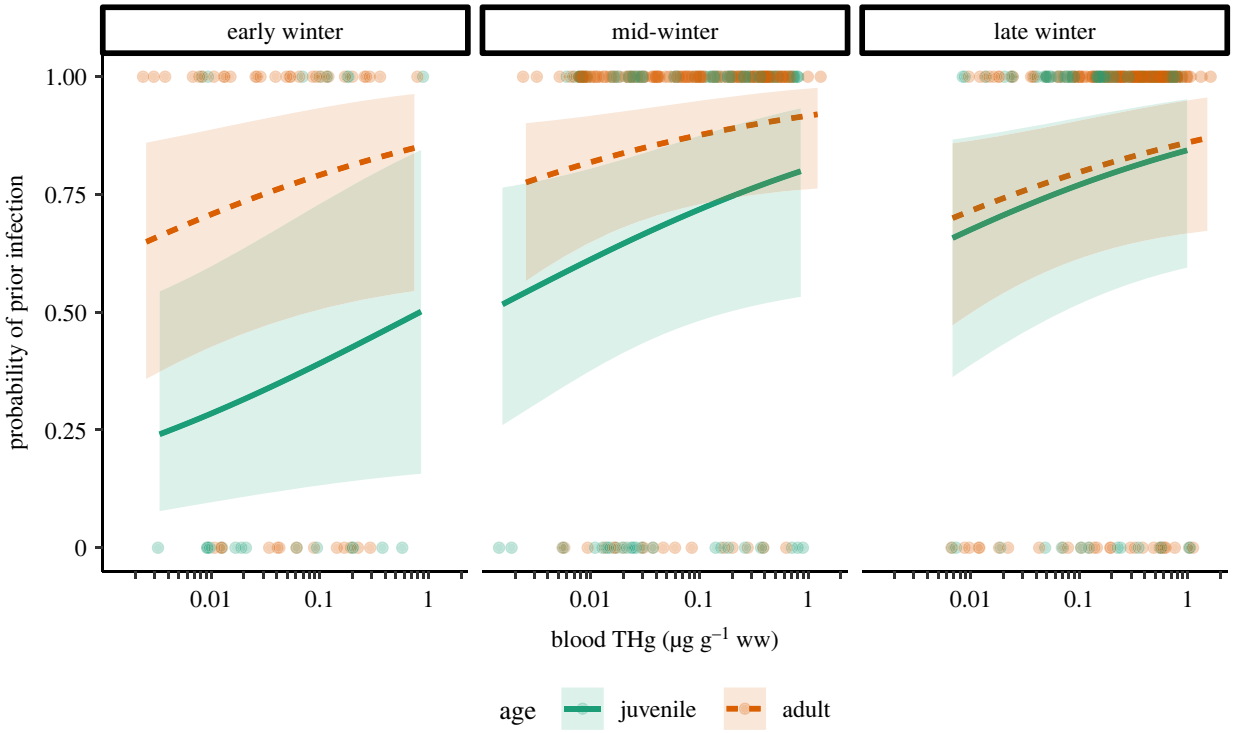

Figure 3. The probability of prior influenza infection increases with blood mercury concentrations. Lines show mean predictions and shaded areas show 85% CIs of predictions in early (16 October–10 December), mid (11 December–2 February) and late (3 February–16 March) winter. Points show raw data (i.e. points at bottom are birds without antibodies; points at top are birds with antibodies). Prediction lines for each panel are based on parameters for 16 October, 1 January and 16 March, respectively, and extend only within the observed range of mercury values for each age class and date. Lines show predicted values for a male northern pintail and all other variables held at their mean values across the dataset, but points for all individuals are shown. For 95% CIs, see electronic supplementary material, figure S2. (Online version in colour.)

antibody status on body condition, and no evidence for an interaction of either with mercury concentration.

4. Discussion

Environmental contaminants can influence wildlife health by mediating the prevalence of pathogens and the severity of disease. Here, we found evidence for a positive relationship between the probability of prior influenza infection and blood mercury concentrations across 11 species of waterfowl wintering in California's San Francisco Bay Estuary. The odds of prior infection (i.e. antibody detection) increased 5.2 times across the observed range of blood mercury concentrations (0.001–1.623 µg g^{-1} ww) and the odds of active influenza infection increased 5.1 times (0.001–2.023 µg g^{-1} ww). We found no effect of influenza infection on body fat stores, even in an interaction with mercury. Our results also confirm established age-specific patterns of influenza infection in wild waterfowl, including higher probabilities of prior infection and lower probabilities of active infection in adults as compared to juveniles [21].

The positive relationship between prior infection and mercury concentrations suggests that immunotoxic effects of chronic mercury exposure could increase the probability of influenza infection. Mercury contamination has documented impacts on immune function in multiple avian taxa [9,10,37,42,55], but has rarely been linked to actual infections (but see [11]). Blood mercury concentrations represent a combination of chronic and acute mercury exposure that could affect both long- and short-term infection dynamics [79,80], and our results showed that the positive relationship between influenza infection and mercury concentrations was stronger when measuring antibodies indicative of prior infection than for active infection. This pattern suggests that short-term active infection risk might depend on ecological drivers of influenza exposure (e.g. habitat use, local density or stochasticity [81]), whereas chronic mercury contamination could increase influenza infection risk over the long term by increasing susceptibility to infection upon exposure [42]. Further, while influenza antibody prevalence was related to average mercury concentrations across species, this relationship persisted at the individual level, suggesting that interspecific differences in habitat use, diet and immunology can explain some, but not all, of the relationship between mercury and influenza infection probability. Together, these results provide compelling evidence that the immunotoxic effects of mercury might increase the prevalence of influenza antibodies across waterfowl host species.

Although the probability of detecting antibodies increased through the winter, suggesting that influenza transmission was ongoing, most of the variation in active infection status remained unexplained by our covariates. Even variables known to affect influenza prevalence in wild birds (e.g. species and age [17,49]) were only weakly related to infection status. Influenza prevalence in wild ducks usually peaks in autumn in the Northern Hemisphere [15] and infected birds shed influenza for only 5–11 days [54,82]; this combination of low infection prevalence during winter and the short infectious period produced an infection prevalence of only 7% in our dataset, which limited the statistical power to detect any effects. By contrast, detectable antibodies are

estimated to last months to over a year [19–21] and antibody prevalence was 74%. The substantial increase in antibody detection through the winter among juvenile birds implies that infections were occurring but difficult to detect, whereas antibodies left longer lasting evidence of prior infection. Alternatively, individual traits such as age and species could produce an apparent relationship between antibody detection and mercury concentrations; for example, if adults exhibit both longer antibody persistence and higher contaminant concentrations [31,57], the relationship between antibody detection and contaminants could be driven primarily by age. However, our results suggest that antibody–mercury relationships persist even within age and species groups. These results highlight the value of analysing antibody data alongside samples for active infection, especially for pathogens like influenza, where infection is only detectable for a short duration but antibodies last much longer.

Despite the positive relationship between the probability of prior influenza infection and mercury concentrations, we found no evidence that body condition was related to influenza infection, even in combination with mercury. This pattern could indicate that low pathogenic influenza has no effect on bird body condition (and vice versa), or that unmeasured variables (e.g. time since infection) or sampling biases (e.g. contaminant- or infection-induced changes in behaviour and mortality) obscured the true relationship between fat stores and influenza infection [13]. Most prior studies have concluded that low pathogenic influenza infection has no effects on wild waterfowl body condition [25], but at least one field study found that influenza-infected swans fed at reduced rates, suggesting that they had lower energy intake [83]. While reduced energy intake would eventually affect fat stores, behavioural adaptations to short-term infection, such as reducing flight activity (an energetically demanding behaviour [84]), could offset any energy imbalance from infection-induced reduced feeding rates. Further, mercury concentrations in most ducks we studied were below benchmarks for effects of mercury on reproductive success and mortality (less than 1.0 $\mu g\ g^{-1}$ ww blood) [4]. However, sublethal effects of mercury can occur as low as 0.2 $\mu g\ g^{-1}$ ww blood in some birds [4]. For comparison, blood mercury concentrations in our data ranged from 0.001 to 2.02 $\mu g\ g^{-1}$ ww, with a geometric mean of 0.07 $\mu g\ g^{-1}$ ww. Longitudinal or experimental studies that control for infection timing, body condition prior to infection, and behavioural responses to infection could further explore how influenza infection and environmental contamination interact to affect body condition.

Even in the absence of direct effects on wild bird health, the positive relationship between low pathogenic influenza infection and mercury concentrations could have important implications for the epidemiology of influenza viruses and, ultimately, avian health. All 11 species we studied can be medium- or long-distance migrants, some of which breed as far north as Alaska and northern Canada. Migrants can spread viruses over large spatial scales, introduce influenza viruses of diverse origins into resident populations [16,50] and amplify local viral transmission [16]. Immunotoxic effects of mercury could therefore promote the spatial spread of influenza viruses by increasing infection prevalence in migrants, especially if neither infection nor mercury contamination impairs their long-distance movements. While most influenza viruses have minimal impacts on wild waterfowl, highly pathogenic strains, which are an emerging disease threat for wild birds, can cause significant morbidity and mortality [28]. Further, influenza infection can cause mass mortality in poultry [26], so increased susceptibility to highly pathogenic avian influenza in wild birds could pose a significant health risk to both wild and domestic birds as well as economic risk to the agriculture sector.

Beyond its implications for the mercury-influenza system, this study highlights the potential strength of the relationships among contaminants, infection and wildlife health. The increase in antibody detection associated with mercury was as large as some species-specific differences in influenza prevalence [17,18], suggesting that contaminant-induced susceptibility to pathogens could be a major contributor to differences in infection prevalence across species. Moreover, we observed these relationships at relatively low mercury concentrations, consistent with a prior study of bat immune function [38], and suggesting that infection risk might be relatively sensitive to contaminant exposure. In addition, although we found no association between infection and body condition, environmental contaminants might have more significant population impacts when interacting with more virulent and emerging pathogens. The immunotoxic effects of environmental contaminants could also scale up to affect population or community health; infectious diseases spread between individuals, across space and among species, meaning that hosts with minimal contaminant exposure could experience negative impacts of contaminants via increased prevalence of infectious diseases. Understanding when and where these effects appear is particularly important because environmental contaminants can persist in the environment for years after emissions end [1,29].

Ethics. Ducks were collected under federal migratory bird permits MB102896–1 and MB57358C-0; California state Scientific Collection Permits SC-003855, 007908 and SC-8090; and USGS Federal Bird Banding Permit 21142. Sampling procedures were reviewed by the Animal Use and Care committee at the USGS Western Ecological Research Center on 11/08/2017 (diving ducks) and through the permit 'Breeding and Wintering Ecology of Waterfowl April 2015' (dabbling ducks).

Data accessibility. Data are available at the US Geological Survey's ScienceBase: https://doi.org/10.5066/P9QC53G9 [85]. Code to reproduce the results are archived at Zenodo: https://doi.org/10.5281/zenodo.6985261 [86].

The data are provided in the electronic supplementary material [87].

Authors' contributions. C.S.T.: formal analysis, methodology, software, validation, visualization, writing—original draft and writing—review and editing; J.T.A.: conceptualization, funding acquisition, methodology, project administration, resources, supervision and writing—review and editing; M.A.H.: investigation and writing—review and editing; J.M.S.: investigation; M.L.C.: conceptualization, funding acquisition, methodology, resources and writing—review and editing; S.E.W.D.L.C.: conceptualization, funding acquisition, methodology, resources and writing—review and editing; W.M.B.: investigation, resources and writing—review and editing; E.J.B.: data curation and writing—review and editing; J.M.E.: resources and supervision; M.P.H.: investigation, methodology and writing—review and editing; E.L.M.: data curation, investigation and writing—review and editing; C.T.O.: data curation, investigation and writing—review and editing; S.H.P.: data curation, investigation, methodology and writing—review and editing; M.P.: investigation and writing—review and editing; A.M.R.: conceptualization, methodology and writing—review and editing; J.D.S.: data curation and writing—review and editing; D.J.P.: conceptualization, funding acquisition, methodology, project administration, resources, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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