Animal Behaviour 103 (2015) 153–160

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Risk-taking behaviours in zebra finches affected by mercury exposure

Megan E. Kobiela*, Daniel A. Cristol, John P. Swaddle

Institute for Integrative Bird Behavior Studies, Department of Biology, College of William & Mary, Williamsburg, VA, U.S.A.

ARTICLE INFO

Article history:
Received 18 October 2014
Initial acceptance 24 November 2014
Final acceptance 28 January 2015
Available online 29 March 2015
MS. number: A14-00834R2

Keywords:
environmental contaminant
mercury
predation
risk
starvation
trade-off
zebra finch

The trade-off between starvation and predation risk is of paramount importance to songbirds and many other small organisms. A contaminant with metabolic or neurological effects may hinder a bird's ability to manage these ecological risks, which are contingent on metabolic state and require cognitive assessments. Methylmercury (MeHg) is a ubiquitous pollutant that is associated with neurotoxicity, reproductive failure, altered behaviour and increased mortality in aquatic organisms. It was recently discovered that MeHg can enter terrestrial food webs and affect songbirds that eat contaminated invertebrates. Research on behavioural effects of environmentally relevant doses of MeHg in songbirds is a conservation priority as this pollutant is widespread, poorly regulated, increasing and understudied in terms of sublethal effects such as abnormal behaviours. To help close this knowledge gap, we examined how MeHg affects behavioural strategies in captive zebra finches, *Taeniopygia guttata*. We quantified the birds' responses to risk by measuring regulation of body mass, vigilance behaviour, willingness to move away from dense cover in search of food and reluctance to return to foraging after a disturbance. Dosed and undosed birds were placed in an experimental arena and were videorecorded over 3 days of increasing perception of predation risk. We found that MeHg-exposed birds, compared to control birds, (1) lost significantly more mass and (2) waited significantly longer to forage under the highest predation risk. Our results indicate that MeHg-exposed birds may react more strongly to threat of predation and thereby increase their risk of starvation. To our knowledge this is the first mechanistic study of how a pervasive pollutant may alter optimal decision making, and therefore potentially survival, in songbirds.

Foraging and antipredator vigilance are often mutually exclusive activities, thus most animals experience a trade-off between the risk of starvation and the risk of predation (Houston, McNamara, & Hutchinson, 1993; Lima & Dill, 1990). While this trade-off is an important determinant of behaviour in most animals, it has been extensively examined in small songbirds because they are vulnerable to many predators and have high costs of metabolic regulation and fat storage (Blem, 1990; Witter & Cuthill, 1993). For example, birds with more stored fat have a harder time escaping from predators (Witter, Cuthill, & Bonser, 1994), so, in response to increased predation risk, birds adaptively lower their body mass (Gentle & Gosler, 2001; Lilliendahl, 1997). If a bird does not eat enough, however, it can easily use up its fat reserves and may starve in a day or two (Ketterson & King, 1977).

There are three behaviours that a songbird can use to minimize its risk of predation. First, vigilance rate, or how often an individual lifts its head from foraging to scan for potential predators, influences detection of an incoming threat (Hart & Lendrem, 1984; Lima & Bednekoff, 1999). Second, time and distance away from protective cover, such as dense brush, increases a bird's likelihood of succumbing to predatory attack (Lima & Dill, 1990). Finally, reluctance to resume foraging after being disturbed by a potential predator (i.e. latency to forage) indicates a bird's willingness to expose itself to predation risk in order to eat (Seress, Bókony, Heszberger, & Liker, 2011). Under increased threat of predation, birds tend to increase their time spent vigilant, increase their time in protective cover and increase their latency to forage after disturbance (Lima & Dill, 1990). These behavioural changes all lead to a reduction in body mass in the short term, although birds may adaptively increase fat stores in the longer term to compensate (Witter, Swaddle, & Cuthill, 1995).

While behavioural trade-offs such as that between vigilance and foraging have been well studied, we know little about how these trade-offs may be affected by neurotoxins, specifically those that are environmental pollutants. Many such pollutants have been

* Correspondence and present address: M. E. Kobiela, Department of Ecology, Evolution, and Behavior, University of Minnesota, 100 Ecology Bldg, St Paul, MN 55108, U.S.A.
E-mail address: kobie003@umn.edu (M. E. Kobiela).

http://dx.doi.org/10.1016/j.anbehav.2015.02.024
0003-3472/

implicated in avian population declines, acute mortality events and sublethal effects on reproduction and behaviour (Mineau & Whiteside, 2013). Mercury is one well-known contaminant that is projected to increase globally due to fossil fuel combustion (Wang, Shen, & Ma, 2000), artisanal gold mining (Van Straaten, 2000) and climate change (Hooper et al., 2013). When elemental mercury enters water, it is methylated by sulphur-reducing bacteria (Boening, 2000) and forms methylmercury (MeHg). This organic form of mercury is more dangerous to organisms because of its ability to cross the intestinal wall and blood-brain barrier, making it a potent neurotoxin with effects throughout the central nervous system (Scheuhammer, 1987; Wolfe, Schwarzbach, & Sulaiman, 1998). Given that MeHg affects brain development and cognitive performance, there are most likely many understudied consequences on the development and expression of behaviour. MeHg has long been recognized as a teratogen (e.g. Harada, 1978), so there are pronounced effects of both short-term exposure during embryonic development and chronic adult exposure to bioaccumulated MeHg (Wolfe et al., 1998).

Because mercury methylation occurs in aquatic ecosystems, much attention has been paid to its effects on aquatic organisms, especially large predatory fish and piscivorous mammals and birds (Scheuhammer, Meyer, Sandheinrich, & Murray, 2007). It has recently been discovered, however, that methylmercury can enter terrestrial food webs and accumulate in songbirds (Cristol et al., 2008; Rimmer, Miller, McFarland, Taylor, & Faccio, 2010). As in aquatic taxa, environmental mercury has sublethal effects on songbird reproduction (Bouland, White, Lonabaugh, Varian-Ramos, & Cristol, 2012; Hallinger & Cristol, 2011; Varian-Ramos, Swaddle, & Cristol, 2013), endocrine physiology (Wada, Cristol, McNabb, & Hopkins, 2009) and immune competence (Hawley, Hallinger, & Cristol, 2009; Lewis, Cristol, Swaddle, Varian-Ramos, & Zwollo, 2013). While behavioural end points have become routine for ecotoxicologists (e.g. Blocker & Ophir, 2013; Walker, 2003), animal behaviourists have been slow to conduct robust experiments quantifying effects of pollutants on behaviours (Clotfelter, Bell, & Levering, 2004; Montiglio & Royauté, 2014; but see Bean et al., 2014). Furthermore, it is important for behavioural ecologists to recognize that ubiquitous and persistent environmental contaminants, such as MeHg, are likely present in many study populations and could influence the results of basic behavioural studies.

We examined the effect of lifelong sublethal dietary MeHg exposure on the trade-off between starvation and predation risk in a model songbird, the zebra finch, *Taeniopygia guttata*. Because of the various effects of MeHg on animals, we had two competing hypotheses. First, MeHg could increase a bird's risk of predation by causing aberrant antipredator behaviours (as shown in fish, e.g. Webber & Haines, 2003) or affecting their senses (i.e. vision and hearing, as shown in primates: Burbacher, Grant, Mayfield, Gilbert, & Rice, 2005; Rice & Gilbert, 1992), and, therefore, their ability to detect and assess risk. Alternatively, MeHg could increase a bird's risk of starvation through several possible mechanisms. Birds dosed with mercury have been shown to have a reduced appetite, and therefore may have a reduced motivation to forage (Bouton, Frederick, Spalding, & McGill, 1999); there is also some evidence that mercury may affect foraging efficiency (Adams & Frederick, 2008). In addition, MeHg may cause birds to be hypersensitive to a perceived predatory threat (Heinz, 1979) and overreact to stimuli by increasing their latency to forage and losing more body mass than undosed birds. If mercury affects a bird's perceived predation risk, we predicted that MeHg-dosed birds would spend less time in cover, be less vigilant, not wait as long to forage and be heavier compared to controls. However, if mercury causes an increased starvation risk, we predicted that MeHg-dosed birds would spend more time in cover, be more vigilant, wait longer to forage and lose more body mass in response to a predatory threat compared to control birds.

METHODS

We conducted this experiment in an aviary with captive-bred zebra finches exposed to chronic sublethal dietary MeHg from the embryo stage through the rest of their lives. Control birds were hatched and raised by parents receiving no MeHg, while the MeHg-treated subjects were raised by parents receiving a diet of 1.2 μg/g MeHg-cysteine (wet weight, equivalent to 1.39 μg/g dry weight) for 10 weeks before they were allowed to breed. This mercury level simulates exposure of wild songbirds at a highly contaminated industrial site (see Varian-Ramos, Swaddle, & Cristol, 2014). At such a site, exposure would begin as an embryo, because females deposit MeHg into their eggs (Wolfe et al., 1998), and continue as a nestling, because parents provision them with contaminated food. Developmental exposure to neurotoxins typically has more impact than exposure later in life (Harada, 1978). To achieve proper MeHg concentration in the diet, an aqueous MeHg-cysteine solution was added to commercial zebra finch food (ZuPreem FruitBlend) and homogenized as described in Lewis et al. (2013). Food was tested on a direct mercury analyser (DMA-80; Milestone, Shelton, CT, U.S.A.) to ensure that total mercury concentrations were within 10% of 1.2 μg/g (or contained no detectable mercury in the case of control food, which was mixed only with aqueous cysteine).

We tested young adult female finches between 100 and 200 days old that had been maintained on the same diet as their parents ($N = 20$ in the embryonically and chronically exposed 1.2 μg/g MeHg treatment; $N = 20$ controls). Birds were housed in groups of four in 75 × 45 × 45 cm wire enclosures ('home cages') with ad libitum food and water until trials. Because zebra finches are highly social (Zann, 1996), they exhibit fearful behaviour when alone in a novel arena, and thus we conducted each trial with one focal and one nonfocal companion bird, with both birds coming from the same treatment group. To reduce animal use as much as possible while maintaining independent samples, we used each bird in two trials (described below), once as a focal, and once as a nonfocal, individual. At least 2 weeks passed between each bird's two trials and we assigned trials such that no bird was in the arena twice with the same companion. Hence, half of the birds were the focal subject the first time they entered the arena, and the other half were nonfocal during the first trial and focal in their second time in the arena. Both birds in the pair were from the same treatment for practical reasons (since they had to consume the same control or dosed food while in the arena) and because one would expect that, in the wild, all birds (within an age class) in the same location would have similar levels of mercury exposure.

Experimental Arena

We created two identical arenas in two 4.3 × 4.3 × 2.7 m rooms (Fig. 1a). We constructed an observation blind (1.2 × 1.5 × 2.7 m) around the entrance door and delineated two experimental patches (84 × 84 cm), each 1.5 m from the blind and 3 m apart. These patches contained the dense cover (provided by artificial evergreen trees), water dishes and food dishes where the birds foraged. The food dishes were pie pans situated within larger, high-rimmed 35 × 25 × 6 cm aluminium trays that reduced the birds' ability to be vigilant while their heads were down during foraging. Food dishes contained ad libitum food, control or dosed, mixed with inedible dried black beans to increase difficulty of foraging. We placed a 1.5 m high exposed perch constructed from PVC pipe and wooden dowels approximately 2.5 m from either patch to give birds another perching option outside of cover. Two video cameras

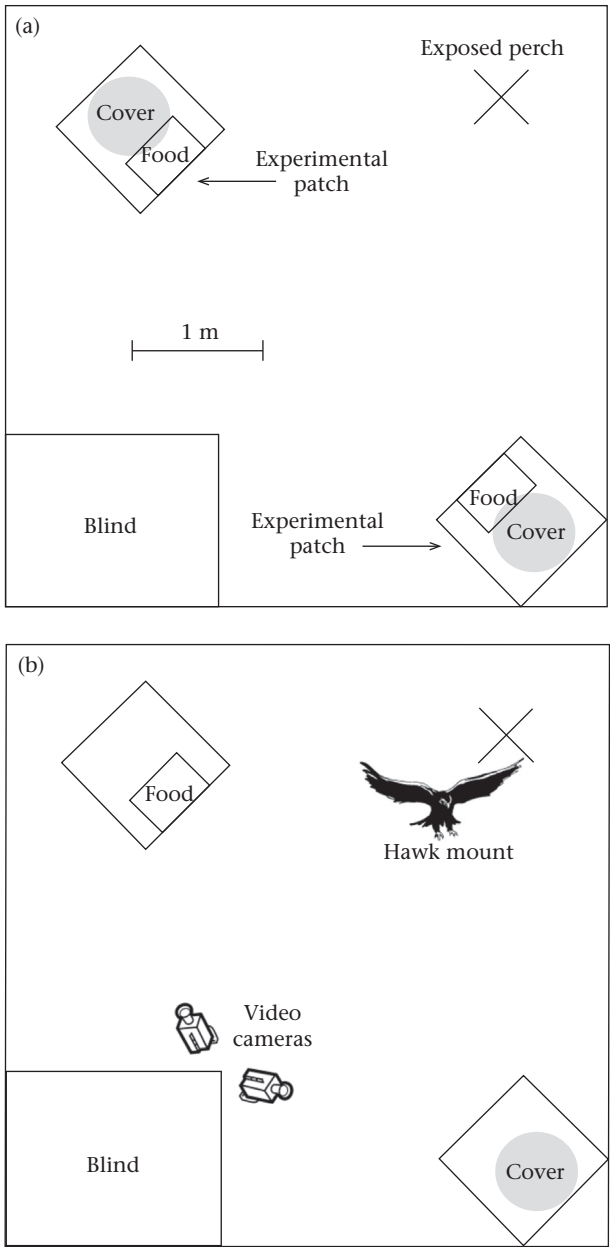

Figure 1. Plan view of the experimental arena layout (a) at the beginning of each trial and (b) for moderate- and high-risk days.

recorded the birds' behaviour, with one pointing at each experimental patch (Fig. 1b). Birds were housed and tested on a 14:10 h light:dark cycle, with the lights turning on at 0800 hours each morning.

Experimental Trials

We ran each pair of birds through an experimental trial that lasted 5 consecutive days. The arena conditions changed each day to increase the birds' perception of predation risk. On the first day of each trial, we removed the birds from their smaller home cages, attached coloured plastic leg rings for identification and weighed them before 0800 hours to obtain their pre-feeding body mass. We placed the birds in an arena, in which both patches contained dense cover, food dishes and water dishes (Fig. 1a). The birds were allowed to acclimate to the arena for the first day. On the morning of the second day, we captured the birds to measure body mass prior to 0800 hours (i.e. before the lights turned on) and then replaced them in the arena in cover. We videorecorded their behaviours from 0800 to 1100 hours, providing a record of behaviour during a 'low-risk' situation. Between 1100 and 1500 hours on the same day, we entered the arena and altered the patch composition such that the artificial cover was removed from one patch and the food dish was removed from the other. This created a distance of 3 m between the food and the dense cover, which produced a 'moderate-risk' situation (Fig. 1b). Before 0800 hours on the third morning, we again measured the birds' pre-feeding mass and videorecorded their behaviours in the moderate-risk situation from 0800 to 1100 hours. Because the birds were always weighed pre-feeding, their mass on one morning reflected their response to the previous day's treatment (e.g. the mass of the birds on the morning of the moderate-risk day reflected their behaviours and metabolism on the previous, low-risk, day).

On the fourth morning, the birds were captured, weighed and returned to the arena once again. At 0805 hours, we brought a taxidermic red-tailed hawk, *Buteo jamaicensis*, mounted in a flight position into the arena and hung it from the ceiling 2.5 m from both patches (Fig. 1b). While the hawk was on display, we broadcasted calls of two natural predators that wild zebra finches encounter in Australia (Zann, 1996): black kite, *Milvus migrans*, and pied butcherbird, *Cracticus nigrogularis*, acquired from the Macaulay Library of Natural Sounds (ML numbers 1520 and 57224; Cornell University, Ithaca, NY, U.S.A.). We removed the hawk mount from the arena at 0905 hours. The video recorded between 0800 and 1200 hours showed the behaviours exhibited in this 'high-risk' situation. We recorded the behaviours for 4 h in the high-risk situation, instead of 3 h, to encompass both the hour of the hawk's presence and the 3 h following its removal. We kept the birds in the arena until the morning of the fifth, 'post-predator', day to acquire a final pre-feeding mass reflecting the high-risk situation, and then returned them to their home cages. A summary of the data collected on each experimental day appears in Fig. 2. We attempted to weigh the food pans to quantify the amount of food consumed each day, but the food pellets unfortunately absorbed humidity over time and were occasionally contaminated by faeces, precluding accurate and useful measurements.

We decided to systematically increase the risk status of birds over sequential days, rather than randomizing the order of risk treatments, as we predicted that birds' behaviour on days following the high-risk treatment could be altered. This carryover effect would mask any behavioural alteration on low- and moderate-risk days if the high-risk day was presented first. The repeated measures design was also chosen for ethical reasons, as it allowed us to use many fewer animals in the study than if we had randomized the order of treatments. Hence, we acknowledge that 'day in the arena' is confounded with increasing risk status, but this does not alter the tenet of our overall conclusions.

Video Analysis

We analysed videos for three behaviours: proportion of time spent in each experimental patch, proportion of time spent vigilant while not in dense cover and latency to forage. One observer (M.E.K.) analysed all video. We determined the proportion of time spent in each experimental patch by using KMPlayer media software to advance each video at 30 s intervals and recording whether the focal bird was in the patch or not. If the focal bird was in the food dish during that 30 s snapshot, we recorded whether the bird's head was up (vigilant) or down (not vigilant). We determined latency to forage by noting how long the focal bird took to begin eating after 0800 hours on the low- and moderate-risk days and

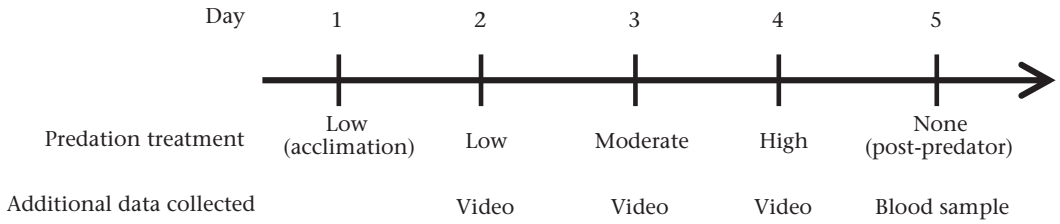

Figure 2. Timeline of each experimental trial. Trials lasted 5 days; both birds were weighed every morning and the focal bird's behaviour was videorecorded during days 2–4. Each bird experienced two trials, once as the focal bird and once as a nonfocal companion, assigned in random order.

after the hawk was introduced to the arena on the high-risk day. Unfortunately, one set of videos for a dosed focal bird was corrupted before analysis, reducing the sample size by one. The total amount of video recorded over the 39 successful trials was 390 h per camera.

Analysis of Mercury Levels

We sampled blood from each bird at the end of the 5-day trial period and determined total mercury concentration with our direct mercury analyzer, following protocols described in Cristol et al. (2008). Control birds had mean ± SD blood mercury concentration, on a wet weight basis, of 0.07 ± 0.09 μg/g (range 0.01–0.33 μg/g) and dosed birds averaged 13.93 ± 3.60 μg/g (range 7.96–24.79 μg/g).

Statistical Analyses

All statistical analyses were conducted in SPSS for Windows v20 (SPSS Inc., Chicago, Illinois, U.S.A.) and we report averages ± SEs. Because we measured the birds' masses and behaviours over multiple consecutive days, we used repeated measures ANOVA, with day in the arena (i.e. increasing perceived predation risk) as a within-subjects factors and mercury treatment as an among-subjects factor, to assess the effect of the risk treatments on each behavioural metric (vigilance, latency to forage) and on body mass. Because birds spent the vast majority of their time near dense cover on the low-risk day, comparisons for time spent in cover were made between the moderate- and high-risk days only (when birds had to choose to forage away from cover). We found no significant interaction between risk treatment and mercury treatment on any of our dependent variables. Therefore, we made post hoc comparisons between the control and dosed birds in the high-risk environment (day 4) only. Examining the birds' responses to a direct threat of predation allowed us to determine whether MeHg causes birds to be more prone to starvation or predation on the trade-off continuum.

We first present the overall results with control and treated birds combined to assess the effect of changing perceived risk and then show the comparisons between treatments for each metric (mass, latency to forage, time spent in cover, vigilance). We collected data on body mass for every bird each time they were in the arena, regardless of whether they were the focal or companion bird, so mass data is described for both first and second trials. There was no effect of whether it was the bird's first or second time in the experimental arena on any of the focal birds' behavioural metrics.

Ethical Note

The birds in the mercury treatment were exposed to a lifelong diet of 1.2 μg/g MeHg. To provide perspective from a human health context, this could be the equivalent of a human eating a lifetime diet consisting entirely of swordfish, which is one of the more mercury-contaminated diet choices available (Jinadasa, Edirisinghe, & Wickramasinghe, 2013). Unfortunately, the zebra finch mercury concentrations cannot be accurately converted to human health benchmarks, which are based on blood volume rather than weight.

Ideally, we would have performed a pilot dose-response study with a range of mercury treatments and behavioural end points to identify the lowest dose that would be predicted to affect the risk-taking behaviour of zebra finches. The large number of required animal subjects would make such a study antithetical to the 'reduction' criterion for ethical use of animals in research. Therefore, we relied on recent studies from our own zebra finch colony in which dose-response curves were generated for mercury-exposed birds. In one study, Lewis et al. (2013, their Figure 4c) found that immune response was delayed in mercury-exposed birds, with the effect being statistically detectable in birds eating food with 1.0 μg/g mercury, but not the lower dose of 0.5 μg/g (Lewis, 2012). Moore, Cristol, Maddux, Varian-Ramos, and Bradley (2014, their Figure 2b) showed that a surge in corticosterone, which is an important response to acute stress in birds, was suppressed at doses of 0.6, 1.2 and 2.4 μg/g, but not at 0.3 μg/g. A third study, Henry, Cristol, Varian-Ramos, and Bradley (2015, their Figure 2a) provided evidence of oxidative damage to the liver only at blood mercury levels above 15 μg/g, which corresponds with the 1.2 μg/g dietary dose. Finally, a study on the reproductive success of the same birds used in the previous two studies found reduction in various measures of reproductive success that was apparent at all doses and ranged from, for example, 16% fewer offspring at 0.3 μg/g to 50% fewer at 2.4 μg/g (Varian-Ramos et al., 2014), while none of the doses affected adult survival. We ruled out the lowest two doses (0.3 μg/g and 0.6 μg/g) because they did not show consistent effects across all four studies.

Understanding that these studies on physiology and reproductive success are not necessarily precise predictors of effects of mercury on complex behaviours, we also considered our unpublished data from behavioural studies and personal observations on the effects of dietary doses of approximately 1.2 μg/g on zebra finches. We concluded that the lowest dose that would provide a valid test of our hypothesis that mercury affects risk-taking behaviour, and thus justifies the use of animals for this study, was 1.2 μg/g. Using lower doses risked the use of animals for a study that would not be as likely to answer the questions posed. Anecdotally, we have observed no overt signs of increased frightfulness, discomfort or illness at doses of 1.2 μg/g.

In addition, the daily capture and brief handling required to weigh the finches for 5 consecutive days was only mildly stressful; McGraw, Lee, and Lewin (2011) found that female zebra finches do not lose mass after 4 weeks of daily handling and only experience short-term elevations in circulating corticosterone. All finches were returned to the research aviary after the study and used in additional experiments on the effects of mercury on songbird behaviour and reproduction. All procedures were approved by the College of William & Mary's Institutional Animal Care and Use Committee (protocol number IACUC-IBC-2012-05-23-7982).

RESULTS

All Subjects Combined

Mass loss

Before entering the arena for the first time, the birds weighed 15.06 ± 0.19 g, on average. At the end they weighed 13.95 ± 0.14 g, so they had lost 1.11 g on average, or 7.4% of their body mass over the course of their first trial. Body mass declined significantly with increasing predation risk, which is confounded with day in the arena within each trial (Greenhouse–Geisser corrected repeated measures ANOVA: $F_{1.83,71.5} = 6.88$, $P = 0.002$; Fig. 3a). Only half of the birds were the focal subject during their first time in the arena, but changes in body mass were qualitatively similar for birds running through the sequence of trials in the arena for the second time (an average of 0.91 g, or 6%, loss of body mass across all 40 birds).

Latency to forage

Focal birds ($N = 39$) waited on average 20.3 ± 5.3 min after dawn to forage on the low-risk day (day 2) and 22.3 ± 3.5 min on the moderate-risk day (day 3). After the addition of the hawk into the experimental arena, focal birds waited on average 37.4 ± 3.8 min to forage on day 4. Overall, latency to forage increased significantly over the course of the experimental trials (repeated measures ANOVA: $F_{2,76} = 6.112$, $P = 0.003$; Fig. 3b).

Time spent in dense cover

With an artificial tree in both experimental patches on the low-risk day, focal birds spent an average of 93.2 ± 0.02% of their time in dense cover. Birds spent significantly more time in cover during the high-risk situation (61.7 ± 4.3%) than during moderate risk (53.5 ± 5.3%; paired *t* test: $t_{36} = 3.21$, $P = 0.003$; Fig. 3c). Sample size was slightly reduced for this metric ($N = 37$) because, for two focal birds' videos, the camera frame did not capture the entire artificial evergreen tree.

Comparison between Mercury and Control Treatments

Mass during the first trial

Before entering the arena the first time, control birds weighed 14.62 ± 0.25 g and treatment birds weighed 15.5 ± 0.24 g, on average. A post hoc sample of ulna length in 15 control and 17 mercury-dosed birds indicated no significant difference in body mass corrected for skeletal size (two tailed t test: $t_{30} = 0.225$, $P = 0.824$). There was not a significant effect of mercury treatment on the pattern of mass loss over the first trial (Greenhouse–Geisser corrected repeated measures ANOVA: $F_{1.87,70.9} = 2.29$, $P = 0.115$). However, post hoc analysis showed that there was a significant difference in mass lost after exposure to the hawk (day 4 minus day 5): mercury-dosed birds lost, on average, 0.85% of their mass while control birds remained at the same weight (one-way ANOVA: $F_{1,38} = 5.549$, $P = 0.024$; Fig. 4). This pattern held when the birds' second trials were included (no interaction between MeHg treatment and trial number; two-way ANOVA: $F_{1,75} = 1.868$, $P = 0.176$).

Latency to forage

There was no significant effect of mercury treatment on the change in latency to forage over the days in the arena (repeated measures ANOVA: $F_{2,74} = 1.55$, $P = 0.221$). However, post hoc analysis revealed that there was a significant effect of treatment on latency to forage in the high-risk situation (one-way ANOVA: $F_{1,37} = 6.381$, $P = 0.016$; Fig. 5), with mercury-dosed birds waiting 18 min longer than control birds, on average. In addition, 7 of 19 mercury-dosed birds failed to forage in the arena during the hour that the hawk was present, as opposed to only 2 of 20 controls ($\chi^2_{39} = 3.954$, $N = 39$, $P = 0.0467$).

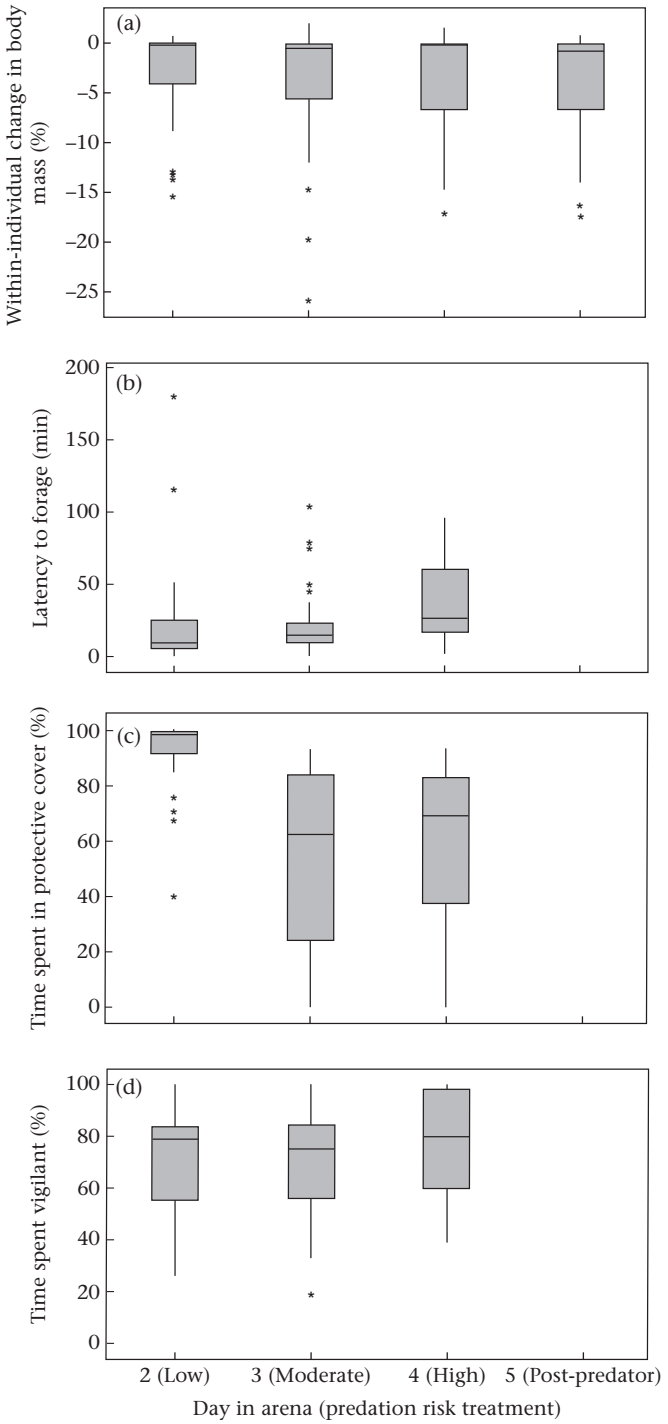

Figure 3. (a) Within-individual change in percentage of body mass with day in the arena (data are from the first set of trials in the arena for each bird, $N = 40$); (b) latency to forage; (c) percentage of time spent in dense cover; (d) percentage of time spent with head up (vigilant) during the first hour of each experiment day. Graphs in (b–d) report data for the focal birds in each trial over all three risk treatments ($N = 39$ for (b) and (d); $N = 37$ for (c); see note in Methods). For all graphs, the middle line represents the median, the box represents the interquartile range, the whiskers are 95th percentile limits and asterisks are outlying data points.

Time spent in dense cover and vigilance

Focal birds ($N = 39$) spent on average 65.5 ± 0.03% of their time vigilant in the low-risk situation and 69.1 ± 0.02% in the moderate-risk situation, both during the 3 h video recording period. On the high-risk day, birds were vigilant 65.5% ± 0.02 over the entire 4 h

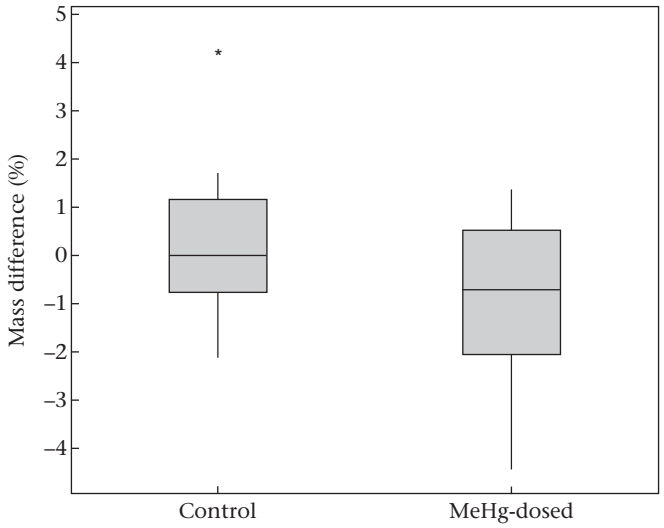

Figure 4. Mass changes in response to the high-risk situation. Box plots show the percentage of change in mass for zebra finches between day 4 (high risk) and day 5 for each treatment, with a value of 0% indicating no change in a bird's mass between the two measurements ($N = 20$ for both the control and MeHg groups). The middle line represents the median, the box represents the interquartile range, the whiskers are 95th percentile limits and asterisks are outlying data points.

video recording period (Fig. 3d). There were no significant effects of mercury treatment either on the time spent in dense cover (Greenhouse–Geisser corrected repeated measures ANOVA: $F_{1.45,50.8} = 0.954$, $P = 0.37$) or on vigilance ($F_{1.56,57.7} = 0.197$, $P = 0.767$) over the days in the arena.

DISCUSSION

Our sequential treatments were intended to increase perceived predation risk, and for all subjects combined, appear to have produced the behavioural and body mass changes expected, based on comparable findings from wild birds. Zebra finches under greater predatory threat were more hesitant to leave cover to feed and, as a result, experienced a loss of body mass. Comparing treatments, it is clear that lifelong exposure to mercury (from the embryo onward) altered the birds' responses to our experimentally simulated predatory threats. Finches dosed with mercury reduced their body mass more in response to the high-risk situation (i.e. the hawk exposure) and waited longer to forage in the presence of this simulated predatory threat, relative to control birds. These results are consistent with our second hypothesis, where MeHg could lead to an increased risk of starvation and hypersensitivity to predatory stimuli.

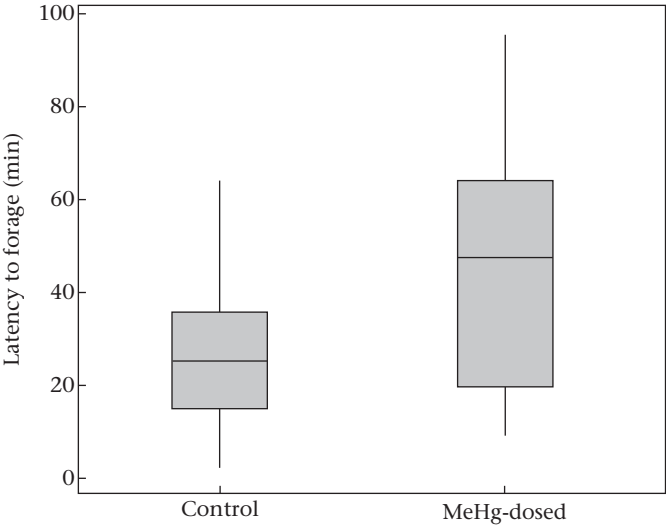

Figure 5. Time between 0800 hours (lights on) and when the focal bird first foraged under high risk by treatment (birds fed control, $N = 20$, or mercury-dosed food, $N = 19$).

For all subjects combined, as birds experienced treatments that simulated increased predation risk, they significantly reduced their body mass, increased their latency to forage and increased their time spent in dense cover. These changes are consistently predicted by increased predation risk and similar changes have been observed in many avian taxa (Lima & Dill, 1990). Overall, mass loss was about 7% over the 4 days in the arena, which is a biologically important amount for zebra finches (Rashotte, Sedunova, Johnson, & Pastukhov, 2001) and other small passerines (Ketterson & King, 1977; Stuebe & Ketterson, 1982) as these species can only lose approximately 20% of their body mass before dying of starvation. All of the birds waited on average 15 min longer to forage in the presence of the hawk (high risk) than they had on the previous day (moderate risk), which is on par with or even longer than similar studies of latency to forage under the threat of predation (e.g. Seress et al., 2011).

Although we predicted that the proportion of time spent vigilant would change among the risk situations, it did not. It is possible that our method of quantifying vigilance through 30 s snapshots did not account for variation in rate of vigilance (Cresswell, Quinn, Whittingham, & Butler, 2003) or amount of side-to-side head movement (Jones, Krebs, & Whittingham, 2007) that can be important in how birds assess their surroundings. Also, the dichotomy of head-up versus head-down position may not entirely encapsulate a bird's assessment of risk in the surrounding environment. Lima and Bednekoff (1999) showed that birds could still detect an oncoming hawk model at a distance of approximately 10 m even when their view while head-down and feeding was obstructed; it is quite possible that the birds in our experiment positioned themselves to keep the stationary hawk model in view even when they were eating, and thus did not show significant change in the amount of time in the head-up position.

Response to risk of predation was heightened in mercury-exposed birds, in terms of mass loss and reluctance to leave cover after perceiving a predator. There are two potential mechanisms to explain these results. First, birds exposed to mercury may have had reduced motivation to forage. Great egrets, *Ardea alba*, dosed with methylmercury had reduced appetites, consuming less food per weight than control birds (Spalding, Frederick, McGill, Bouton, & McDowell, 2000), and spent less time hunting and eating fish than controls (Bouton et al., 1999). We did observe anecdotally that two dosed birds failed to forage at all on the first day of their first trial, leading to one death on the second morning despite cessation of the trial and return to the home cage. However, if reduced motivation to forage were the mechanism driving our results, we would expect to see a marked difference in the treatment birds in their latency to forage in all risk situations, not just during high risk (day 4).

Alternatively, mercury-exposed birds may be in overall poor health (Scheuhammer et al., 2007) and may have been reducing their predation risk because their escape responses were compromised. Reduction in body mass (including reduced fat, organ size, or total body mass) has been associated with mercury exposure on naturally contaminated sites (Ackerman et al., 2012; Takekawa, Wainwright-De La Cruz, Hothem, & Yee, 2002). While the birds used for this study did not differ in size-corrected body mass in

their home cages, there is evidence that mercury exposure reduces take-off flight performance in another songbird species (Carlson, Cristol, & Swaddle, 2014). In addition, hypersensitivity to startle stimuli has been observed in mallard, *Anas platyrhynchos*, ducklings exposed to mercury, where dosed birds ran farther away from a novel, frightening apparatus (involving a rotating axel and flashing pattern) than controls in an avoidance test (Heinz, 1979). We feel that the hypothesis encompassing mercury-induced hypersensitivity to risk, combined with a potentially reduced flight performance, most clearly explains the body mass and behavioural differences found between control and mercury-dosed birds.

While more experiments that measure predation directly are needed to corroborate these results with free-living birds on contaminated sites, we speculate that mercury-exposed birds, by waiting longer to start foraging, may not increase their exposure to avian predators compared to controls, which is potentially beneficial for food chains affected by mercury. Biomagnification, or the concentration of contaminants in top predators, has long been a concern (Scheuhammer et al., 2007), but if contamination indeed increases starvation risk, then mercury exposure might move down to decomposers or scavengers rather than biomagnifying to top predators first. However, increase in starvation risk will still affect bird populations, especially in areas with particularly high predation risk or harsh winters, where finding food is difficult and fat reserves are of the utmost importance. Furthermore, several songbird species of conservation concern may have high MeHg loads, such as the saltmarsh sparrow, *Ammodramus caudacutus* (Lane et al., 2011; Scoville & Lane, 2013) and rusty blackbird, *Euphagus carolinus* (Edmonds et al., 2010), and any sublethal negative effects of this toxin may put yet another burden on struggling populations.

A possible increase in the risk of starvation is certainly worrying in terms of increased mortality and potentially reduced fecundity in populations affected by mercury contamination. Perhaps more troublesome, however, are the long-term consequences of selection acting on birds that may be hypersensitive to predation risk and at an increased risk for starvation. While selection for reduced sensitivity to predation risk would be adaptive in birds that spend their whole lives on mercury-contaminated sites (so that they avoid starvation), any dispersal by the less sensitive individuals to sites not affected by this neurotoxin could cause even more problems for populations.

Furthermore, where mercury contamination is combined with other human disturbances, or even other natural stressors, birds that are hypersensitive will be at an even greater disadvantage when trying to forage. Similar detrimental synergies between contaminants and other stressors have been shown in other taxa, including amphibians (as reviewed in Sih, Bell, & Kerby, 2004) and insects (Campero, Slos, Ollevier, & Stoks, 2007). Therefore, we emphasize the importance of using behaviour to illuminate the sublethal effects of contaminants, which are often much more complex and pervasive than might be expected from typical LD50 studies, and could have drastic implications for animal populations. On a more practical note, our result, that a complex behaviour is affected in subtle ways by sublethal exposure to a widespread pollutant, underscores the common-sense need for researchers to be aware of the pollutant exposure at their study sites, especially when studying species high on food chains, or in areas prone to concentrate pollutants, such as agricultural areas, wetlands and high latitudes.

Acknowledgments

Research was completed with oversight from the South River Science Team, which is a collaboration of state and federal agencies, academic institutions and environmental interests. This study benefited greatly by conversation with Claire Varian-Ramos, Randy Chambers and Emilie Snell-Rood, animal care and assistance with methylmercury analysis from Peggy Whitney, and building of the experimental arena by Steve Kobiela. We also thank Pierre-Olivier Montiglio, Alex Ophir and one anonymous referee for their thoughtful comments to improve the manuscript. This study was funded by an E. I. DuPont de Nemours and Company grant to D.A.C, and by National Science Foundation grant IOS-1257590 to J.P.S. and D.A.C. Additional funding to M.E.K. was provided by the Williamsburg Bird Club, College of William & Mary Graduate School of Arts and Sciences and College of William & Mary Charles Center. The funders had no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript. All procedures were approved by the College of William & Mary's Institutional Animal Care and Use Committee (protocol number IACUC-IBC-2012-05-23-7982).

References

Ackerman, J. T., Overton, C. T., Casazza, M. L., Takekawa, J. Y., Eagles-Smith, C. A., Keister, R. A., et al. (2012). Does mercury contamination reduce body condition of endangered California clapper rails? *Environmental Pollution, 162*, 439–448. http://dx.doi.org/10.1016/j.envpol.2011.12.004.

Adams, E. M., & Frederick, P. C. (2008). Effects of methylmercury and spatial complexity on foraging behavior and foraging efficiency in juvenile white ibises (*Eudocimus albus*). *Environmental Toxicology and Chemistry, 27*(8), 1708–1712.

Bean, T. G., Boxall, A. B. A., Lane, J., Herborn, K. A., Pietravalle, S., & Arnold, K. E. (2014). Behavioural and physiological responses of birds to environmentally relevant concentrations of an antidepressant. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1656), 20130575. http://dx.doi.org/10.1098/rstb.2013.0575.

Blem, C. R. (1990). Avian energy storage. *Current Ornithology, 7*, 59–113.

Blocker, T. D., & Ophir, A. G. (2013). Cryptic confounding compounds: a brief consideration of the influences of anthropogenic contaminants on courtship and mating behavior. *Acta Ethologica, 16*, 105–125.

Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: a general review. *Chemosphere, 40*(12), 1335–1351. http://dx.doi.org/10.1016/S0045-6535(99)00283-0.

Bouland, A. J., White, A. E., Lonabaugh, K. P., Varian-Ramos, C. W., & Cristol, D. A. (2012). Female-biased offspring sex ratios in birds at a mercury-contaminated river. *Journal of Avian Biology, 43*(3), 244–251. http://dx.doi.org/10.1111/j.1600-048X.2012.05612.x.

Bouton, S. N., Frederick, P. C., Spalding, M. G., & McGill, H. (1999). Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environmental Toxicology and Chemistry, 18*(9), 1934–1939.

Burbacher, T. M., Grant, K. S., Mayfield, D. B., Gilbert, S. G., & Rice, D. C. (2005). Prenatal methylmercury exposure affects spatial vision in adult monkeys. *Toxicology and Applied Pharmacology, 208*(1), 21–28. http://dx.doi.org/10.1016/j.taap.2005.01.011.

Campero, M., Slos, S., Ollevier, F., & Stoks, R. (2007). Sublethal pesticide concentrations and predation jointly shape life history: behavioral and physiological mechanisms. *Ecological Applications, 17*(7), 2111–2122.

Carlson, J. R., Cristol, D. A., & Swaddle, J. P. (2014). Dietary mercury exposure causes decreased escape takeoff flight performance and increased molt rate in European starlings (*Sturnus vulgaris*). *Ecotoxicology, 23*(8), 1464–1473.

Clotfelter, E. D., Bell, A. M., & Levering, K. R. (2004). The role of animal behaviour in the study of endocrine-disrupting chemicals. *Animal Behaviour, 68*, 665–676.

Cresswell, W., Quinn, J. L., Whittingham, M. J., & Butler, S. (2003). Good foragers can also be good at detecting predators. *Proceedings of the Royal Society B: Biological Sciences, 270*(1519), 1069–1076. http://dx.doi.org/10.1098/rspb.2003.2353.

Cristol, D. A., Brasso, R. L., Condon, A. M., Fovargue, R. E., Friedman, S. L., Hallinger, K. K., et al. (2008). The movement of aquatic mercury through terrestrial food webs. *Science, 320*(5874), 335.

Edmonds, S. T., Evers, D. C., Cristol, D. A., Mettke-Hofmann, C., Powell, L. L., McGann, A. J., et al. (2010). Geographic and seasonal variation in mercury exposure of the declining rusty blackbird. *Condor, 112*(4), 789–799. http://dx.doi.org/10.1525/cond.2010.100145.

Gentle, L. K., & Gosler, A. G. (2001). Fat reserves and perceived predation risk in the great tit, *Parus major*. *Proceedings of the Royal Society B: Biological Sciences, 268*(1466), 487–491.

Hallinger, K. K., & Cristol, D. A. (2011). The role of weather in mediating the effect of mercury exposure on reproductive success in tree swallows. *Ecotoxicology, 20*(6), 1368–1377. http://dx.doi.org/10.1007/s10646-011-0694-1.

Harada, M. (1978). Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology, 18*(2), 285–288. http://dx.doi.org/10.1002/tera.1420180216.

Hart, A., & Lendrem, D. W. (1984). Vigilance and scanning patterns in birds. *Animal Behaviour, 32*, 1216–1224. http://dx.doi.org/10.1016/S0003-3472(84)80239-0.

Hawley, D. M., Hallinger, K. K., & Cristol, D. A. (2009). Compromised immune competence in free-living tree swallows exposed to mercury. *Ecotoxicology, 18*(5), 499–503. http://dx.doi.org/10.1007/s10646-009-0307-4.

Heinz, G. (1979). Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. *Journal of Wildlife Management, 43*(2), 394–401.

Henry, K. A., Cristol, D. A., Varian-Ramos, C. W., & Bradley, E. L. (2015). Oxidative stress in songbirds exposed to dietary methylmercury. *Ecotoxicology, 24*(3), 520–526. http://dx.doi.org/10.1007/s10646-014-1400-x.

Hooper, M. J., Ankley, G., Cristol, D. A., Maryoung, L., Noyes, P., & Pinkerton, K. (2013). Interactions between chemical and climate stressors: a role for mechanistic toxicology in assessing climate change risks. *Environmental Toxicology and Chemistry, 32*, 32–48.

Houston, A. I., McNamara, J. M., & Hutchinson, J. M. C. (1993). General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions of the Royal Society B: Biological Sciences, 341*(1298), 375–397. http://dx.doi.org/10.1098/rstb.1993.0123.

Jinadasa, B. K. K. K., Edirisinghe, E. M. R. K. B., & Wickramasinghe, I. (2013). Total mercury content, weight and length relationship in swordfish (*Xiphias gladius*) in Sri Lanka. *Food Additives and Contaminants, 6*(4), 244–248. http://dx.doi.org/10.1080/19393210.2013.807521.

Jones, K. A., Krebs, J. R., & Whittingham, M. J. (2007). Vigilance in the third dimension: head movement not scan duration varies in response to different predator models. *Animal Behaviour, 74*(5), 1181–1187. http://dx.doi.org/10.1016/j.anbehav.2006.09.029.

Ketterson, E. D., & King, J. R. (1977). Metabolic and behavioral responses to fasting in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Physiological Zoology, 50*, 115–129.

Lane, O. P., O'Brien, K. M., Evers, D. C., Hodgman, T. P., Major, A., Pau, N., et al. (2011). Mercury in breeding saltmarsh sparrows (*Ammodramus caudacutus caudacutus*). *Ecotoxicology, 20*(8), 1984–1991. http://dx.doi.org/10.1007/s10646-011-0740-z.

Lewis, C. A. (2012). *Sublethal effects of methylmercury on the songbird immune response: An experimental study*. Williamsburg, VA: College of William & Mary (Master's thesis).

Lewis, C. A., Cristol, D. A., Swaddle, J. P., Varian-Ramos, C. W., & Zwollo, P. (2013). Decreased immune response in zebra finches exposed to sublethal doses of mercury. *Archives of Environmental Contamination and Toxicology, 64*(2), 327–336.

Lilliendahl, K. (1997). The effect of predator presence on body mass in captive greenfinches. *Animal Behaviour, 53*, 75–81. http://dx.doi.org/10.1006/anbe.1996.0279.

Lima, S. L., & Bednekoff, P. A. (1999). Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? *Animal Behaviour, 58*, 537–543.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology, 68*(4), 619–640. http://dx.doi.org/10.1139/z90-092.

McGraw, K. J., Lee, K., & Lewin, A. (2011). The effect of capture-and-handling stress on carotenoid-based beak coloration in zebra finches. *Journal of Comparative Physiology A, 197*(6), 683–691.

Mineau, P., & Whiteside, M. (2013). Pesticide acute toxicity is a better correlate of US grassland bird declines than agricultural intensification. *PLoS One, 8*(2), e57457.

Montiglio, P.-O., & Royauté, R. (2014). Contaminants as a neglected source of behavioural variation. *Animal Behaviour, 88*, 29–35. http://dx.doi.org/10.1016/j.anbehav.2013.11.018.

Moore, C. S., Cristol, D. A., Maddux, S. L., Varian-Ramos, C. W., & Bradley, E. L. (2014). Lifelong exposure to methylmercury disrupts stress-induced corticosterone response in zebra finches (*Taeniopygia guttata*). *Environmental Toxicology and Chemistry, 33*(5), 1072–1076. http://dx.doi.org/10.1002/etc.2512.

Rashotte, M. E., Sedunova, E. V., Johnson, F., & Pastukhov, I. (2001). Influence of food and water availability on undirected singing and energetic status in adult male zebra finches (*Taeniopygia guttata*). *Physiology & Behavior, 74*(4–5), 533–541. http://dx.doi.org/10.1016/S0031-9384(01)00600-X.

Rice, D. C., & Gilbert, S. G. (1992). Exposure to methyl mercury from birth to adulthood impairs high-frequency hearing in monkeys. *Toxicology and Applied Pharmacology, 115*(1), 6–10. http://dx.doi.org/10.1016/0041-008X(92)90361-U.

Rimmer, C. C., Miller, E. K., McFarland, K. P., Taylor, R. J., & Faccio, S. D. (2010). Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. *Ecotoxicology, 19*(4), 697–709.

Scheuhammer, A. M. (1987). The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. *Environmental Pollution, 46*(4), 263–295. http://dx.doi.org/10.1016/0269-7491(87)90173-4.

Scheuhammer, A. M., Meyer, M. W., Sandheinrich, M. B., & Murray, M. W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio, 36*(1), 12–18.

Scoville, S. A., & Lane, O. P. (2013). Cerebellar abnormalities typical of methylmercury poisoning in a fledged saltmarsh sparrow, *Ammodramus caudacutus*. *Bulletin of Environmental Contamination and Toxicology, 90*, 616–620.

Seress, G., Bókony, V., Heszberger, J., & Liker, A. (2011). Response to predation risk in urban and rural house sparrows. *Ethology, 117*(10), 896–907. http://dx.doi.org/10.1111/j.1439-0310.2011.01944.x.

Sih, A., Bell, A. M., & Kerby, J. L. (2004). Two stressors are far deadlier than one. *Trends in Ecology & Evolution, 19*(6), 274–276.

Spalding, M. G., Frederick, P. C., McGill, H. C., Bouton, S. N., & McDowell, L. R. (2000). Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. *Journal of Wildlife Diseases, 36*(3), 411–422.

Stuebe, M. M., & Ketterson, E. D. (1982). A study of fasting in tree sparrows (*Spizella arborea*) and dark-eyed juncos (*Junco hyemalis*): ecological implications. *Auk, 99*, 299–308.

Takekawa, J. Y., Wainwright-De La Cruz, S. E., Hothem, R. L., & Yee, J. (2002). Relating body condition to inorganic contaminant concentrations of diving ducks wintering in coastal California. *Archives of Environmental Contamination and Toxicology, 42*(1), 60–70.

Van Straaten, P. (2000). Mercury contamination associated with small-scale gold mining in Tanzania and Zimbabwe. *Science of the Total Environment, 259*(1–3), 105–113. http://dx.doi.org/10.1016/S0048-9697(00)00553-2.

Varian-Ramos, C. W., Swaddle, J. P., & Cristol, D. A. (2013). Familial differences in the effects of mercury on reproduction in zebra finches. *Environmental Pollution, 182*, 316–323.

Varian-Ramos, C. W., Swaddle, J. P., & Cristol, D. A. (2014). Mercury reduces avian reproductive success and imposes selection: an experimental study with adult- or lifetime-exposure in zebra finch. *PLoS One, 9*(4), e95674. http://dx.doi.org/10.1371/journal.pone.0095674.

Wada, H., Cristol, D. A., McNabb, F. A., & Hopkins, W. A. (2009). Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. *Environmental Science & Technology, 43*(15), 6031–6038.

Walker, C. H. (2003). Neurotoxic pesticides and behavioural effects upon birds. *Ecotoxicology, 12*(1), 307–316.

Wang, Q., Shen, W., & Ma, Z. (2000). Estimation of mercury emission from coal combustion in China. *Environmental Science & Technology, 34*(13), 2711–2713. http://dx.doi.org/10.1021/es990774j.

Webber, H. M., & Haines, T. A. (2003). Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). *Environmental Toxicology and Chemistry, 22*(7), 1556–1561.

Witter, M. S., & Cuthill, I. C. (1993). The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society B: Biological Sciences, 340*(1291), 73–92. http://dx.doi.org/10.1098/rstb.1993.0050.

Witter, M. S., Cuthill, I. C., & Bonser, R. H. (1994). Experimental investigations of mass-dependent predation risk in the European starling, *Sturnus vulgaris*. *Animal Behaviour, 48*, 201–222.

Witter, M. S., Swaddle, J. P., & Cuthill, I. C. (1995). Periodic food availability and strategic regulation of body mass in the European starling, *Sturnus vulgaris*. *Functional Ecology, 9*, 568–574.

Wolfe, M. F., Schwarzbach, S., & Sulaiman, R. A. (1998). Effects of mercury on wildlife: a comprehensive review. *Environmental Toxicology and Chemistry, 17*(2), 146–160.

Zann, R. A. (1996). *The zebra finch: A synthesis of field and laboratory studies*. Oxford, U.K.: Oxford University Press.