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Methylmercury Exposure Affects Risk-Taking Behaviors in Zebra Finches: Tradeoffs between Eating and Being Eaten

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**Methylmercury Exposure Affects Risk-Taking Behaviors in Zebra Finches:
Tradeoffs between Eating and Being Eaten**

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Virginia Beach, Virginia

Bachelor of Science, University of Virginia, 2010

**A Thesis presented to the Graduate Faculty
of the College of William and Mary in Candidacy for the Degree of
Master of Science**

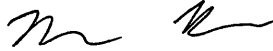
Department of Biology

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May 2013**

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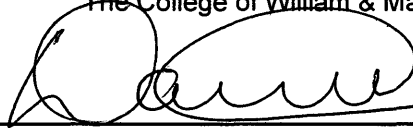


Megan Elizabeth Kobiela

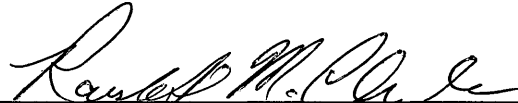
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COMPLIANCE PAGE

Research approved by

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ABSTRACT

Methylmercury (MeHg) is a ubiquitous neurotoxin that is associated with reproductive failure, reduced cognitive ability, and increased mortality in aquatic ecosystems. It was recently discovered that MeHg can enter terrestrial food webs and affect passerine birds. Research on behavioral effects of environmentally-relevant doses of MeHg in songbirds is a conservation priority as this pollutant is widespread, still poorly regulated, and little is known about sub-lethal effects that could still have devastating effects for populations. To help close this knowledge gap, I examined how MeHg affects captive zebra finches' (*Taeniopygia guttata*) tradeoff between starvation and predation risk using a sub-lethal dose likely to be found at a contaminated site. Managing this tradeoff is essential to fitness because a bird that is too occupied with foraging is likely to be eaten whereas a bird avoiding all predation risk will likely starve. Because many physiological abilities and cognitive assessments of risk are involved, a neurotoxin like MeHg may cause suboptimal tradeoffs. I quantified the birds' response to risk by measuring regulation of body mass, vigilance, time spent away from protective cover, and latency to forage after a disturbance. Dosed and undosed birds were placed in an experimental arena and were video-recorded on each of three consecutive mornings. Perceived level of predation risk was elevated by increasing the distance between food and cover and by the addition of a taxidermic hawk mount. I found that MeHg-exposed birds, compared to control birds 1) lost significantly more mass and 2) waited significantly longer to forage in the highest predation risk setting. Both of these results indicate that MeHg-exposed birds may react more strongly to the predation threat and increase their starvation risk. This is the first mechanistic study of how this pervasive pollutant may alter optimal decision making and survival in wild songbirds.

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This M.S. is dedicated to my family, both at home and in the lab

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Chapter 1: Introduction

Mercury is a naturally occurring element that is found in the Earth's crust and is released when volcanoes erupt (Boening 2000). However, mercury is increasingly being released worldwide due to coal burning plants (Wang et al. 2000) and artisanal gold mining (van Straaten 2000). When elemental mercury enters water, it is methylated by sulfur-reducing bacteria (Boening 2000) and forms methylmercury. This organic form of mercury is much more dangerous to organisms because of its ability to cross the blood-brain barrier, making it a potent neurotoxin with pronounced effects throughout the central nervous system (Scheuhammer 1987; Wolfe et al. 1998).

Because mercury methylation occurs in aquatic ecosystems, a lot of attention has been given to aquatic organisms, especially large predatory fish and top predators that eat fish (e.g. mink, otter, seals; Scheuhammer et al. 2007). This emphasis on top predators is due to the fact that methylmercury biomagnifies up the food chain, such that primary producers and consumers have relatively low amounts of methylmercury while secondary and tertiary consumers accumulate methylmercury at a much higher rate in their bodies (Gardner et al. 1978). In high trophic level birds, such as bald eagles *Haliaeetus leucocephalus*, belted kingfishers *Megaceryle alcyon*, and common loons *Gavia immer*, methylmercury exposure has been linked with various neurological and reproductive effects (Evers et al. 2005).

It has only recently been discovered, however, that methylmercury can enter terrestrial food webs and accumulate in terrestrial passerine birds. This finding was made in 2008 by Cristol et al. after surveying birds near a point source contamination of mercury on the South River in Waynesboro, Virginia (Carter 1977). Some passerine species (e.g. “songbirds” such as red-eyed vireo *Vireo olivaceus*, Carolina wren *Thryothorus ludovicianus*) downstream of the point source were found to have total blood mercury levels as high as or even higher than the levels found in aquatic bird species on the same site. These findings have been corroborated at an unrelated site in Vermont (Rimmer et al. 2010). Similar to studies in aquatic taxa, field studies have shown the sublethal effects of methylmercury on passerine reproduction (Hallinger and Cristol 2011; Bouland et al. 2012) and immune competence (Hawley et al. 2009). However, despite the fact that passerines represent over half of all birds (Sibley and Monroe 1990), songbirds have received much less attention in ecotoxicology studies in the field and especially in the laboratory. Captive dosing studies are essential for determining the causal link between a certain level of toxin in the diet and any adverse effects, whereas field studies can only establish a correlation. Of the few captive dosing studies conducted with songbirds, most have focused on lethal effects of acute methylmercury exposure (e.g. Scheuhammer 1988), but experiments with more environmentally relevant, chronic, and sublethal levels are increasing in number (e.g. Lewis et al. 2013).

Behavior is conspicuously absent among traits studied that might be affected by methylmercury (and several other neurotoxins, but see Walker 2003;

Blocker and Ophir 2012). As a neurotoxin, methylmercury especially affects the cerebrum and cerebellum (Scheuhammer 1987), two parts of the brain that are essential for proper sensory processing, learning, and locomotion (Kolb and Whishaw 2009). Two problems exist with the current literature on how methylmercury affects behavior: 1) it has been focused on aquatic, non-passerine bird species (e.g. Nocera and Taylor 1998; Bouton et al. 1999; Frederick and Jayasena 2011) and 2) observations have often been done in the field, leading some authors to conclude that toxins have minimal negative effects on behavior (Peakall 1996). Therefore, research in passerine behavior is a priority to help elucidate how mercury contamination may negatively impact birds and wildlife in general (Seewagen 2009).

For my thesis, I wanted to determine if methylmercury contamination could affect two essential suites of behaviors in animals: avoiding predation and finding food. Both are obviously critical for survival and overall fitness, so anything that negatively alters these behaviors could have significant effects on individuals and populations. Foraging has been shown to be negatively impacted by methylmercury exposure in non-passerines, such as reduced motivation to forage in great egrets *Ardea albus* (Bouton et al. 1999) and decreased foraging efficiency in white ibis *Eudocimus albus* (Adams and Frederick 2008). Due to the differences in foraging and overall life histories between these Ciconiiformes and passerines, however, it is important to test the effect of methylmercury in passerines.

I am unaware of any study linking mercury to increased risk of predation in any avian taxa. However, exposure to other neurotoxic environmental contaminants can increase the likelihood of birds being taken by predators. Two notable studies both involved pesticides that act as cholinesterase inhibitors, which are known to affect neuromuscular activity (Moser 1995). House sparrows *Passer domesticus* dosed with fenthion, an organophosphate pesticide, were captured in twelve out of fifteen trials by an American kestrel *Falco sparverius* over undosed birds (Hunt et al. 1992). Another organophosphate pesticide, parathion, was associated with bobwhite quail *Colinus virginianus* being captured more frequently by a domestic cat (Galindo et al. 1985). Outside of birds, a study of golden shiner fish *Notemigonus crysoleucas* showed that important anti-predator shoaling behaviors were negatively impacted by environmentally relevant levels of methylmercury (Webber and Haines 2003). Furthermore, vision and hearing, the two senses most essential for predator detection in birds, are compromised in monkeys exposed to chronic low levels of methylmercury (Rice and Gilbert 1992; Burbacher et al. 2005).

Rather than examining foraging and predator avoidance behaviors separately, I conducted one experiment encompassing both of these key determinants of survival at the same time. Foraging and anti-predator vigilance are often mutually exclusive activities because looking for food takes away attention from looking for potential predators and vice-versa (but see Cresswell et al. 2003). Because these behaviors are usually incompatible, most animals experience a tradeoff between the risk of starvation and the risk of predation

(Lima and Dill 1990; Houston et al. 1993). By examining foraging and predator avoidance together, I expected to gain a better picture of how methylmercury might affect more complex traits and better emulate the tasks that birds must accomplish to survive in the wild.

This tradeoff between starvation and predation is essential to the vast majority of all animals' survival. While it has been studied in mammals and reptiles (e.g. Pérez-Tris et al. 2004; MacLeod et al. 2007), it is very important and extensively examined in small birds because of their high metabolism, energy constraints, and costs of fat storage (Blem 1990; Witter and Cuthill 1993). Birds that are too fat have a harder time escaping from predators (Witter et al. 1994), so, in response to increased predation risk, birds are able to adaptively lower their body mass (e.g. Lilliendahl 1997; Gentle and Gosler 2001). If a bird does not eat enough, however, it can easily use up its fat reserves and starve in less than 24 to 36 hours (Ketterson and King 1977). Carrying an "optimal" amount of fat is very important, although the precise mechanism for mass loss or the decisions going into mass regulation are not known.

On the behavioral side of the tradeoff are three things a bird can do to minimize its risk of predation.

- 1) How vigilant a bird is, or how often it lifts its head from foraging to scan for potential predators, greatly impacts how successful a bird will be at detecting an incoming threat (Hart and Lendrem 1984).
- 2) How long a bird spends in or near protective cover, such as dense brush, will determine how easily accessible they are to potential

predators. Birds are often forced to forage away from protective cover because food is more readily depleted close to cover (Lima and Dill 1990).

- 3) How long a bird is willing to wait to resume foraging after being disturbed by a potential predator, or latency to forage, indicates how willing a bird is to expose itself to predation risk in order to eat (Seress et al. 2011). This one metric neatly represents a bird's tradeoff between starvation and predation risks under the direct threat of predation because it must decide when to expose itself to eat and when it will hide and use its fat reserves for energy.

Under increased threat of predation, birds are predicted to reduce their mass, increase their time spent vigilant, increase their time in protective cover, and increase their latency to forage. These established predictions are represented graphically in Figure 1.1.

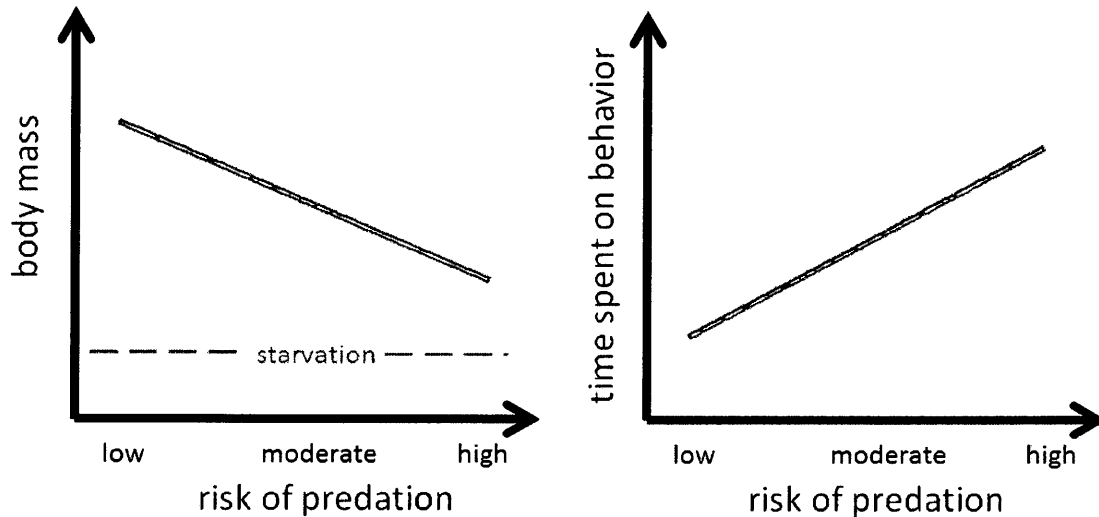


Figure 1.1 Predicted responses to increased predation risk

On the right, “time spent on behavior” can refer to vigilance, time spent in cover, or latency to resume foraging after a disturbance.

I examined the effect of chronic sublethal dietary methylmercury exposure on the tradeoff between starvation and predation risk in a model songbird, the zebra finch *Taeniopygia guttata*. I predicted that mercury exposed birds would exhibit poorer decision making compared to control birds and have suboptimal risk tradeoffs. Based on the body of literature, discussed previously, indicating that 1) neurotoxin exposure increases a songbird’s chances of being eaten by a predator and that 2) methylmercury impairs hearing and vision in mammals, I predicted that methylmercury dosed birds would be unable to properly assess increased risk of predation. Compared to the controls, I expected that dosed birds would not spend as much time vigilant or lose as much mass under risk of predation. Also, I anticipated that dosed birds would not wait as long as controls to resume foraging after a disturbance or spend as much time in protective cover, especially because their foraging efficiency might also be reduced by

methylmercury exposure. These predictions are depicted graphically in Figure 1.2.

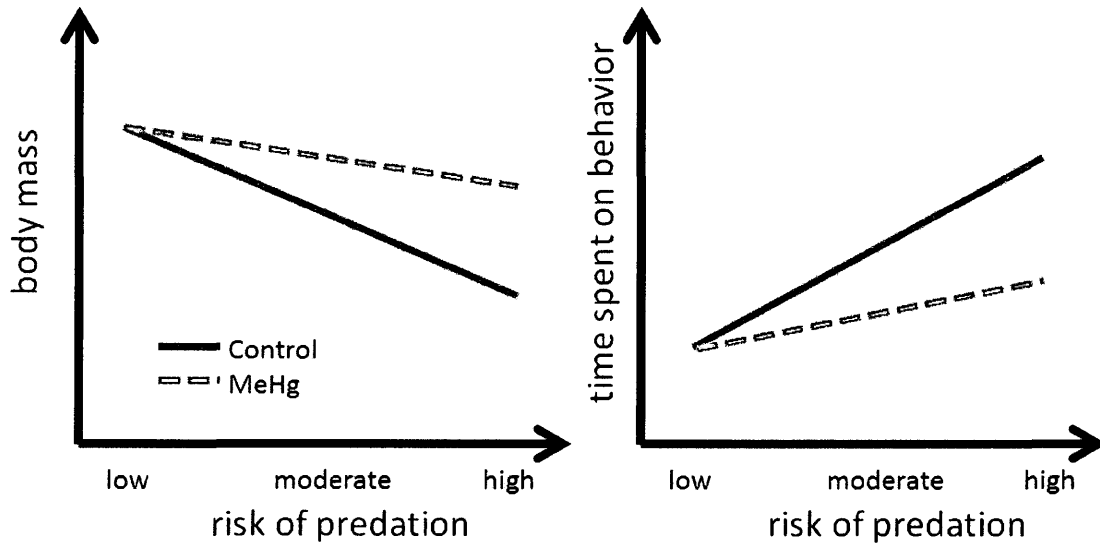


Figure 1.2 Predicted responses to increased predation risk with MeHg
I predict that birds exposed to methylmercury will lose less body mass (left) compared to the controls under elevated predation risk. I also expect that methylmercury birds will spend less time on anti-predator behaviors (right) than control birds.

Chapter 2: Methods

I conducted this experiment in an aviary with captive born zebra finches developmentally exposed to chronic sublethal, dietary methylmercury. Control birds were hatched and raised by parents receiving no methylmercury, while the methylmercury treated subjects were hatched and raised by parents receiving a diet of 1.2 ppm methylmercury cysteine. This methylmercury level simulates exposure of wild songbirds at a highly mercury-contaminated site (Cristol et al. 2008). At such a site, methylmercury exposure would begin as an embryo because mothers deposit methylmercury into their eggs (Wolfe et al. 1998) and continue as a nestling when parents bring back contaminated food. Developmental exposure to neurotoxins typically has more impact than exposure late in life (Harada 1978). To achieve proper methylmercury concentration in the diet, a methylmercury cysteine solution was added to commercial zebra finch food (ZuPreem FruitBlend) and homogenized in a rock tumbler (see Lewis et al. 2013). Food was analyzed on a direct mercury analyzer (DMA-80; Milestone, Shelton, CT) to ensure that mercury concentrations were within 10% of 1.2 ppm (or contained no detectable mercury in the case of control food).

I used young adult females between 100 and 200 days old that had been maintained on the same diet as their parents ($n = 20$ in the control group and $n = 20$ in the treatment). These birds were housed in 75 x 45 x 45 cm wire enclosures (“home cages”) with *ad libitum* food and water for their entire lives before entering my experimental trials. Because zebra finches are highly social

(Zann 1996), they cannot be tested individually, and thus I conducted each trial with one focal and one non-focal companion bird. To reduce animal use as much as possible while maintaining independent samples, I used each bird in two trials (described below), once as a focal and once as a non-focal individual. At least two weeks passed between each bird's two trials and I assigned trials such that no bird was in the arena twice with the same companion.

Experimental arena

I created two identical arenas in two 4.3 x 4.3 x 2.7 m rooms (Figure 2.1). I constructed an observation blind (1.2 x 1.5 x 2.7 m) around the entrance door and delineated two experimental patches (84 x 84 cm), each 1.5 m from the blind and 3 m apart. These patches contained the protective cover (provided by artificial evergreen trees), water dishes, and food dishes where the birds foraged. The food dishes were pie pans situated within larger 35 x 25 x 6 cm aluminum 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 to 1.2 ppm methylmercury, and mixed with inedible dried black beans to increase difficulty of foraging. I 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 recorded the birds' behaviors, with one pointing at each experimental patch (Figure 2.2). The birds were housed and tested on a 14:10 hour light:dark cycle, with the lights turning on at 8AM each morning.

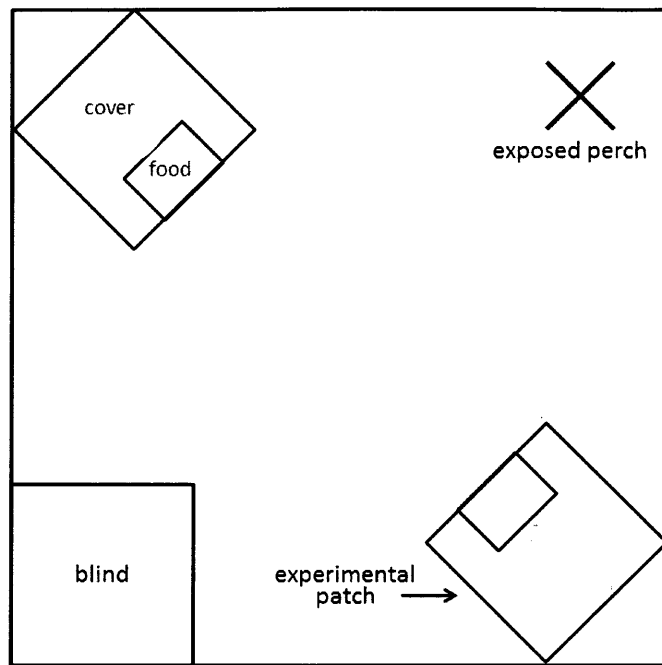


Figure 2.1 Experimental arena layout

This shows a top-down view of the initial experimental arena layout, including the experimental patches with food pans and artificial evergreen trees for cover.

I ran each pair of birds through an experimental trial that lasted five days, and changed the arena conditions each day to increase the birds' perception of predation risk. On the first day of each trial, I removed the birds from their smaller home cages, outfitted them with colored leg bands for identification, and weighed them before 8AM to obtain their pre-dawn body mass. I placed the birds in the arena with both patches containing protective cover, food dishes, and water dishes. The birds were allowed to acclimate to the arena for the first day. On the morning of the second day, I captured the birds pre-dawn to measure body mass and replaced them in the arena in protective cover. I then video recorded their behaviors from 8AM to 11AM, providing a record of behavior during a "low risk" situation. Between 11AM and 3PM on the same day, I entered the arena and altered the patch composition: I removed the artificial cover from one patch and

removed the food dish from the other patch. This created a distance of three meters between the food and the protective cover, which produced a “moderate risk” situation (Figure 2.2). On the third morning, I again measured the birds’ pre-dawn mass and video recorded their behaviors in the moderate-risk situation from 8AM to 11AM. Because the birds were always weighed pre-dawn, their mass on one morning reflects their response to the previous day’s treatment (e.g. the mass of the birds on the morning of the moderate-risk day actually reflects their decisions on the previous, low risk, day).

On the fourth morning, the birds were captured, weighed, and returned to the arena. At approximately 8:05AM, I brought a red-tail hawk *Buteo jamaicensis* mounted in a flight position into the arena and hung it from the ceiling 2.5 m from both patches (Figure 2.2). I played calls of two zebra finch natural predators, black kite *Milvus migrans* and pied butcherbird *Cracticus nigrogularis* (Zann 1996), acquired from the Macaulay Library of Natural Sounds (ML #1520 and #57224; Cornell University, Ithaca, New York), while the hawk mount was in the room. I removed the hawk mount from the arena at approximately 9:05AM. The video recorded from 8AM to 12PM showed the behaviors exhibited in this “high risk” situation; I recorded the behaviors for four hours in the high-risk situation instead of three (as for the low and moderate) to encompass both the hour of the hawk present and the three hours following its removal. I kept the birds in the arena until the morning of the fifth, “post-predator,” day to acquire a final pre-dawn mass (again, to quantify their mass response to the predator on the

previous day), and then returned them to their home cages. A summary of the data collected appears in Table 2.1.

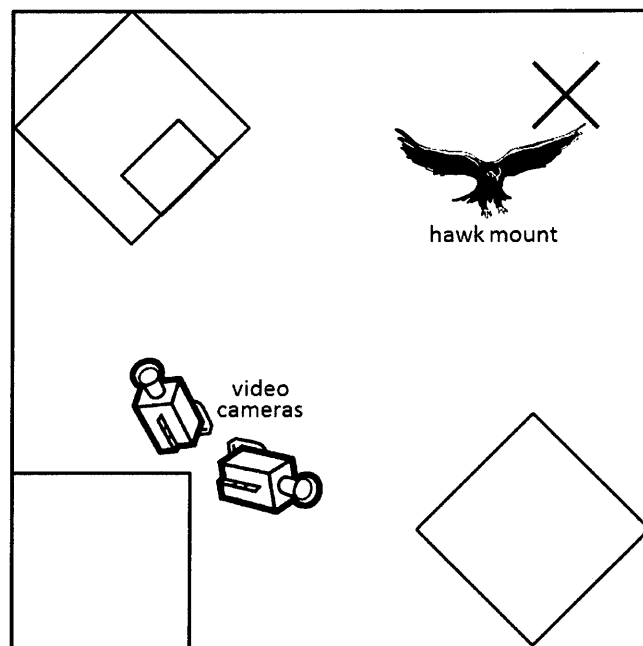


Figure 2.2 Experimental arena layout for moderate and high-risk days
This shows a top-down view of the experimental arena layout modified for the moderate and high-risk treatment, along with the positioning of the video cameras. The experimental patch composition was altered as described above after the low-risk situation was recorded. The hawk mount was only present in the high-risk situation.

Video analysis

I analyzed videos for three behaviors: proportion of time spent in each experimental patch, proportion of time spent vigilant while not in protective cover, and latency to forage. I determined proportion of time spent in each experimental patch by using KMPlayer media software to advance each video at 30 second intervals and I recorded whether the focal bird was in the patch or not. If the focal bird was in the food dish during that 30-second snapshot, I recorded if the bird's head was up (vigilant) or down (not vigilant). I determined latency to forage by noting how long the focal bird took to begin eating after 8AM on the low and

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

Analysis of mercury levels

I took blood samples from each bird at the end of the 5-day trial and analyzed them on the direct mercury analyzer (DMA-80; Milestone, Shelton, CT) at the College of William & Mary for total mercury concentration, following protocols described in Cristol et al. (2008). Control birds had blood mercury concentrations of 0.072 ± 0.016 ppm (range 0.011-0.364 ppm) and methylmercury dosed birds averaged 14.074 ± 0.626 ppm (range 7.3-24.8 ppm). These blood mercury concentrations are associated with reduced reproductive success in zebra finches from the same colony (Varian-Ramos unpub. data), but not with any outwardly aberrant behavior in the birds' home cages.

Day in Arena	Predation Treatment	Data Collected
1	low – first day	mass
2	low	mass, video recording (3 hours)
3	moderate	mass, video recording (3 hours)
4	high	mass, video recording (4 hours)
5	none – post-predator	mass, blood sample

Table 2.1 Summary of data collected for each trial
 Predation treatment and type of data collected are summarized for each of the five days in each trial. I weighed all birds before dawn. I removed birds from the arena before 8AM on the last day and weighed them before obtaining a blood sample.

Chapter 3: Results

All analyses were conducted in SPSS for Windows v20 (SPSS Inc., Chicago, Illinois, USA) and averages are reported as average \pm standard error. All graphs were drawn in Minitab v16 (Minitab Inc., State College, Pennsylvania, USA). I will present the overall results with control and methylmercury-treated birds combined in the analyses first, then show the comparisons between treatments for each metric. I collected mass data for every bird each time they were in the arena, regardless of if they were the focal or companion bird, so mass data are described for both first and second trials. There was no effect of trial number, or whether it was the bird's first or second time in the experimental arena, on any of the focal birds' behavioral metrics.

3.1 – General outcome of trials

Mass during the first trial

Before entering the arena the first time, the birds weighed 15.06 ± 0.19 g on average. On the morning of the post-predator day, they weighed 13.95 ± 0.14 g on average, so the birds lost 1.11 g, or 7.4%, of their body mass over the course of their first trial. The overall trajectory of mass loss, given as a percentage of each bird's starting mass, is shown in Figure 3.1.1. The effect of day in the arena on percentage of body mass lost was significant (Greenhouse-Geisser corrected repeated measures ANOVA, $F_{1,833,71.477} = 6.878$, $p = 0.002$). Sample size for first trial masses was $n = 40$ birds.

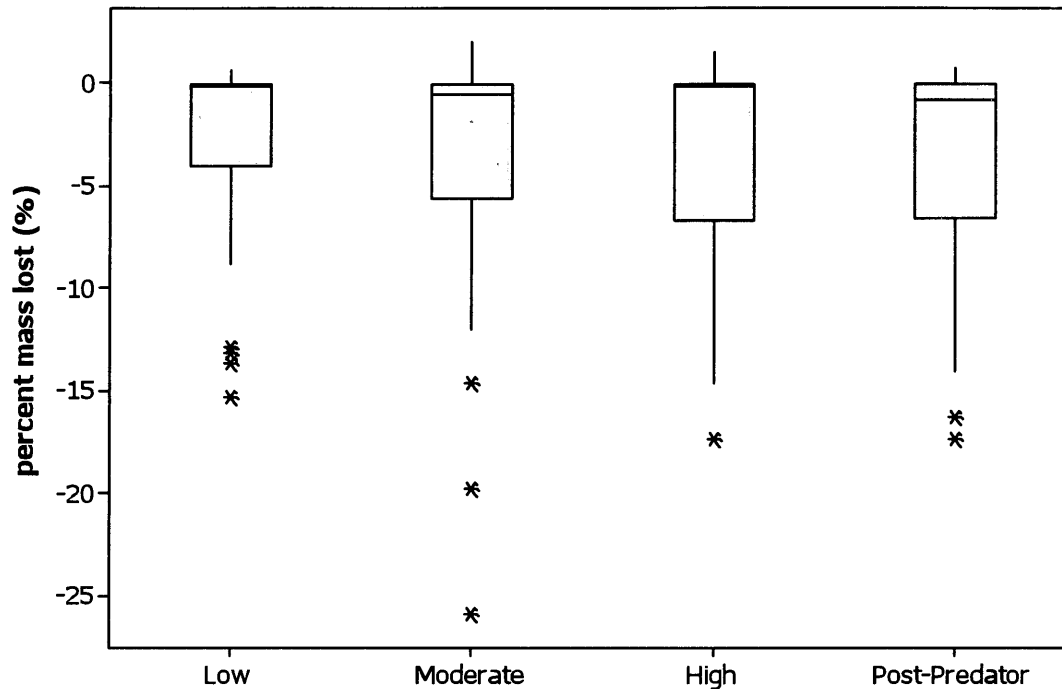


Figure 3.1.1 Percent mass lost over the course of all first trials
 Percent mass lost is shown for all birds during their first trial in the experimental arena. All pre-dawn masses are relative to initial mass on first day of the trial.

Mass during the second trial

Before entering the arena the second time, the birds weighed 15.14 ± 0.17 g on average. On the morning of the post-predator day, they weighed 14.23 ± 0.12 g on average, so the birds lost 0.91 g, or 6.0%, of their body mass over the course of their second trial. The effect of day in the arena on percentage of body mass lost was significant (Greenhouse-Geisser corrected repeated measures ANOVA, $F_{2,280,86.652} = 9.622$, $p < 0.001$). Since the overall trajectory of mass loss was so similar between the first and second trials, no graph is presented. Sample size for second trial masses was $n = 39$ birds because one original focal bird died in her home cage before her trial as a companion bird, so she was replaced with an alternate companion bird that was not scored.

Latency to forage

Focal birds ($n = 39$ for behaviors – see methods) waited on average 20.3 ± 5.3 min after dawn to forage on the low-risk day and 22.3 ± 3.5 min on the moderate-risk day. After the addition of the hawk into the experimental arena, focal birds waited 37.4 ± 3.8 min to forage on average. Overall, latency to forage significantly increased during the experimental trials (repeated measures ANOVA, $F_{2,76} = 6.112$, $p = 0.003$), shown graphically in Figure 3.1.2.

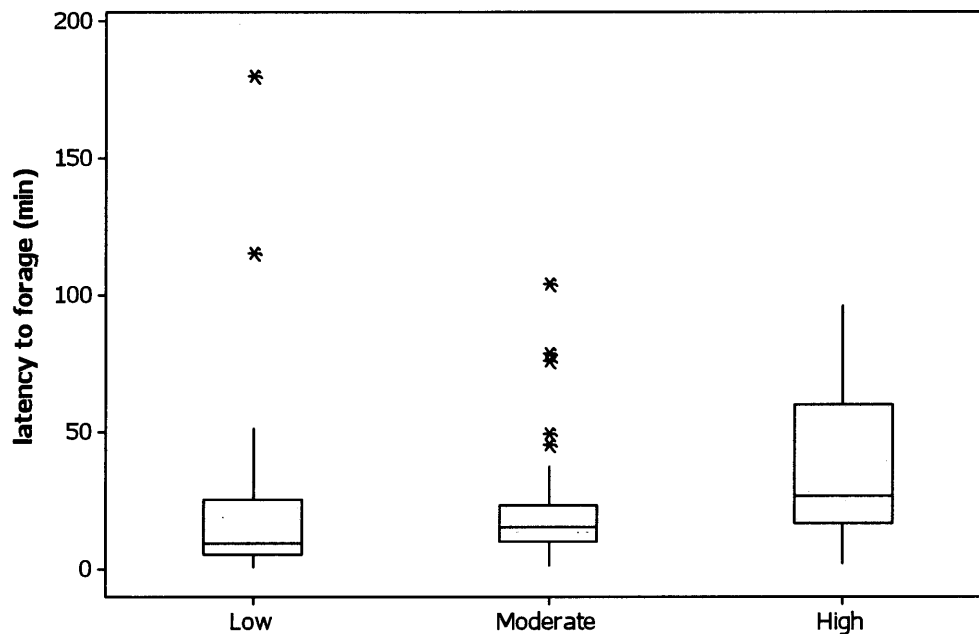


Figure 3.1.2 Latency to forage in each risk situation

Focal birds' latency to forage, in minutes, is given for each of the three risk situations.

Time spent in protective cover

With an artificial tree in both experimental patches on the low-risk day, focal birds spent an average of $93.2 \pm 0.02\%$ of their time in protective cover. Birds spent significantly more time in cover during the high predation risk situation ($61.7 \pm 4.3\%$) than during moderate risk ($53.5 \pm 5.3\%$; paired t-test, $t =$

3.21, $df = 36$, $p = 0.003$; Figure 3.1.3). 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, thus time in cover could not be scored accurately.

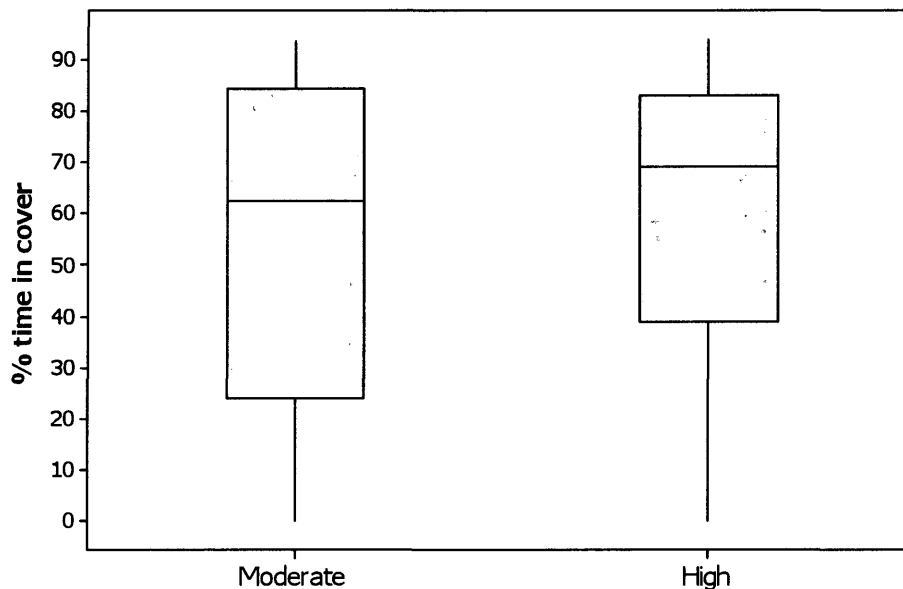


Figure 3.1.3 Time spent in protective cover under moderate and high risk
Percent of focal birds' time spent in protective cover is shown for the moderate and high predation risk situations.

Vigilance

Focal birds ($n = 39$) spent $65.5 \pm 0.03\%$ of their time vigilant in the low-risk situation and $69.1 \pm 0.02\%$ in the moderate-risk situation, both during the three hour video recording period. On the high-risk day, birds were vigilant $65.5\% \pm 0.02$ over the entire 4 hour video recording period. Post-hoc analysis indicates that focal birds spent a slightly larger proportion of time vigilant in the hour with the hawk present ($77.9 \pm 0.04\%$) than the first hour of the moderate-risk situation ($71.5 \pm 0.03\%$; paired t-test, $t = 1.885$, $df = 29$, $p = 0.069$). In this situation, $n = 30$

focal birds because birds who did not forage at all in the presence of the hawk could not be scored for vigilance.

3.2 – Comparisons between methylmercury and control birds

There were no significant correlations found between any of the tested variables and the birds' individual blood mercury concentrations, so reported comparisons are for the two treatments - control and methylmercury – rather than blood mercury level.

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. There was no effect of treatment (methylmercury or control) on the pattern of mass loss over the first trial.

However, post-hoc analysis shows that there was a significant difference in mass lost between the high-risk day and last (post-predator) day in the arena:

methylmercury-dosed birds lost, on average, 0.85% of their mass in one day while control birds remained at the same weight (one-way ANOVA, $F_{1,38} = 5.549$, $p = 0.024$; Figure 3.2.1).

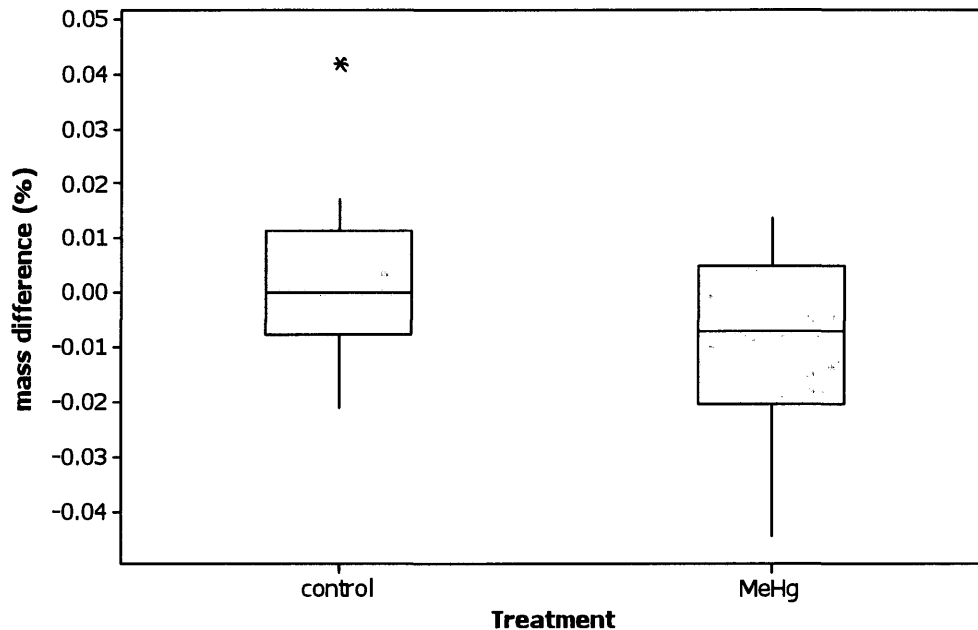


Figure 3.2.1 Percent mass lost in response to the high-risk situation
Methylmercury exposed birds lost more of their body mass in response to the high-risk situation, given as a percent difference between the high risk and post-predator pre-dawn masses during their first trial in the experimental arena.

Mass during the second trial

Control birds (n = 19, see Section 3.1) began their second trials averaging 14.93 ± 0.26 g, which was slightly higher than their beginning mass for their first trials (one-sided paired t-test, $t = 1.428$, $df = 38$, $p = 0.085$). Treatment birds (n = 20) did not change mass on average between trials, weighing 15.34 ± 0.20 g at the beginning of their second trials. However, control birds lost more mass ($0.64\% \pm 0.4$) between the high risk and post-predator measurements on their second trials. Methylmercury birds lost a similar amount to their first trials ($0.79\% \pm 0.4$). Thus, there was no difference in mass loss due to the high-risk situation between the control and methylmercury groups in their second trials.

Latency to forage

Table 3.2.1 summarizes the control and treatment bird's latency to forage in each of the three risk situations. There was no overall effect of the interaction between level of predation risk and the birds' methylmercury exposure on latency to forage (repeated measures ANOVA, $F_{2,74} = 1.554$, $p = 0.22$) However, post-hoc, there was a significant effect of treatment on latency to forage in the high-risk situation (one-way ANOVA, $F_{1,37} = 6.381$, $p = 0.016$; Figure 3.2.2), with methylmercury birds waiting 18 minutes longer than control birds on average. The 8-minute difference in the moderate-risk situation was not statistically significant (one-way ANOVA, $F_{1,37} = 1.281$, $p = 0.27$).

Focal Bird	n	Low (min)	Moderate (min)	High (min)
Control	20	20.6 ± 8.7	18.4 ± 3.8	28.6 ± 3.8
MeHg-dosed	19	20.0 ± 6.1	26.4 ± 6.1	46.6 ± 6.2

Table 3.2.1 Summary of focal birds' latencies to forage in each risk situation
This table summarizes the control and treatment birds' average latency to forage. The low and moderate predation risk columns give time (in minutes) for the focal bird to forage post-dawn, while the high predation risk shows latency to forage measured from the time the hawk entered the room (approximately 5 minutes post-dawn).

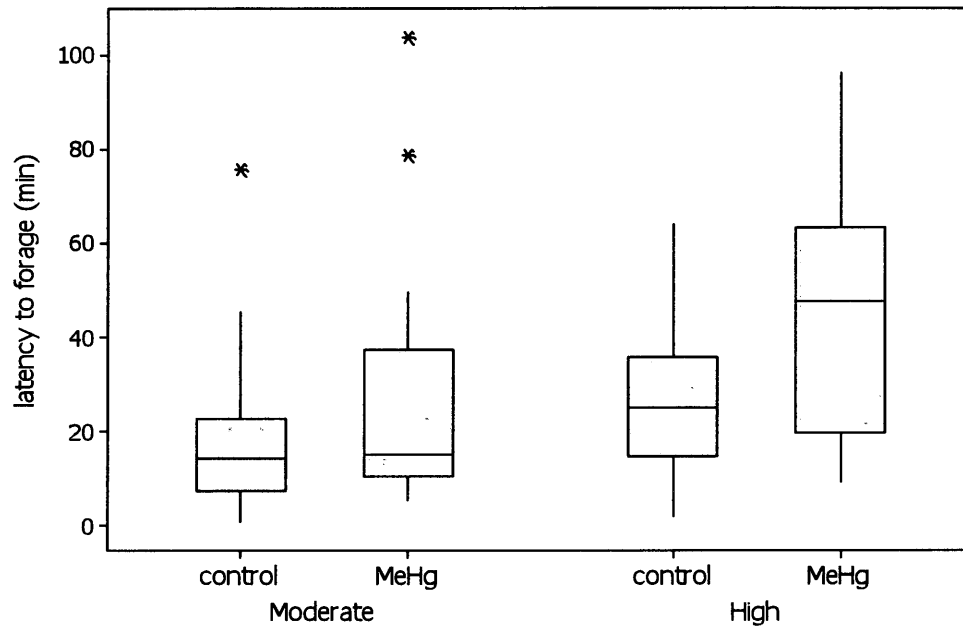


Figure 3.2.2 Latency to forage under moderate and high risk by treatment
Methylmercury-exposed birds waited longer to forage than controls in both the moderate and the high-risk situations, but only significantly so in the presence of the hawk.

After noticing that more control birds foraged when the hawk was present in the arena than methylmercury-dosed birds, a post-hoc chi-square test (Table 3.2.2) shows that this observed difference was significant ($\chi^2 = 6.72$, $n = 39$, $df = 1$, $p = 0.01$).

Treatment	Foraged?	
	yes	no
control	18	2
MeHg	10	9

Table 3.2.2 Chi-squared table for foraging in the presence of the hawk
This table gives the number of birds in each treatment group that foraged when the hawk was present in the high-risk situation.

Time Spent in Protective Cover

There was no effect of the interaction between level of predation risk and the birds' methylmercury exposure on time spent in protective cover over the course of the experiment (Greenhouse-Geisser corrected repeated measures ANOVA, $F_{1.453,50.847} = 0.954$, $p = 0.37$). Because of the corruption of some video footage (see Section 3.1), control $n = 20$ and MeHg $n = 17$.

Vigilance

There was no effect of the interaction between level of predation risk and the birds' methylmercury exposure on time spent vigilant over the course of the experiment (Greenhouse-Geisser corrected repeated measures ANOVA, $F_{1.560,57.705} = 0.197$, $p = 0.767$).

Chapter 4: Discussion

The results were consistent with my predictions that increasing the birds' perceived level of predation risk would cause both treatment groups to significantly 1) reduce their mass 2) increase their latency to forage and 3) increase their time in protective cover. Overall, percent mass loss was about 7%, which is a biologically significant amount: zebra finch males (Rashotte et al. 2001) and three species of sparrow (Ketterson and King 1977; Stuebe and Ketterson 1982) can only lose approximately 20% of their body mass before they die of starvation. All of the birds waited on average 15 minutes longer to forage in the presence of the hawk than they had on the previous (moderate risk) day, 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 I predicted that proportion of time spent vigilant would change among the risk situations, it did not, so perhaps my method of quantifying vigilance through 30-second snapshots did not account for variation in rate of vigilance (Cresswell et al. 2003) or amount of side-to-side head movement (Jones et al. 2007) that can be important in how birds assess their surroundings. In addition, because the focal birds spent on average 78% of their time vigilant when the hawk was in the arena, lack of differences may be due to a ceiling effect. Other studies of vigilance have found similar proportions of time spent vigilant while foraging (e.g. 80% in brown-headed cowbirds *Molothrus ater*, Fernández-Juricic et al. 2007; 70% in European

starlings *Sturnus vulgaris*, Fernández-Juricic et al. 2005) so these birds may not have been able to increase their vigilance further.

In examining the effect of methylmercury exposure, I found that the methylmercury group 1) reduced their mass more in response to the high-risk situation and 2) waited longer to forage in the presence of a predator compared to the control group. These were contrary to my expectations that methylmercury exposure would lead birds to have a higher risk of predation than controls. My predictions were informed by the literature showing that birds exposed to neurotoxins were more likely to be taken by predators, however, I did not explicitly test escape behavior in this experiment. In addition, control and methylmercury birds had very similar patterns of vigilance and time spent in cover. If the methylmercury birds were trading off starvation risk with predation risk, I would expect them to spend significantly more time in cover than control birds, but they did not. Therefore, methylmercury birds appear to be at a higher risk of starvation than controls.

I propose two mechanisms to explain this pattern of increased starvation risk in methylmercury exposed birds. First, methylmercury may make birds hypersensitive to risk, as shown by Heinz (1979) in a startle response experiment with mercury exposed mallard ducklings *Anas platyrhynchos*. Second, they may have had reduced motivation to forage. Great egrets dosed with methylmercury had reduced appetites (Spalding et al. 2000) and reduced motivation to forage (Bouton et al. 1999). While my experiment was not designed to explicitly test these two hypotheses, I did observe anecdotally that two dosed

birds failed to forage at all on the first day of their first trial, leading to one starving to death on the second morning despite cessation of the trial and return to the home cage. Because nothing was in the arena to startle the birds, they might have lacked motivation to forage in the new environment. A study is currently underway to examine the effect of methylmercury on behavioral syndromes, specifically neophobia, which will help shed light on this still unanswered question.

A confounding factor is that the methylmercury-exposed birds may be in overall poor condition and lethargic (Scheuhammer et al. 2007), and may have been adaptively reducing their predation risk because their escape responses were compromised. Reduction in body condition has been associated with mercury exposure on naturally contaminated sites in Gruiformes (Ackerman et al. 2012) and Anseriformes (Takekawa et al. 2002; Wayland et al. 2003), but to my knowledge there are no data on Passeriformes. However, because there was no difference between the control and methylmercury birds in vigilance behaviors and amount of time spent away from protective cover, I conclude that birds exposed to methylmercury were not adaptively reducing predation risk, and thus I favor the explanation that they are more prone to starvation risk due to decreased motivation to feed.

I speculate that the differences in control birds' initial mass between the first and second trials in the arena might be explained by risk aversion (Lima 1986). After exposure to four days of risky foraging conditions, the control birds may have stored more fat once they were returned to their home cages. This

addition of fat when foraging conditions are uncertain has been shown to be adaptive (Ekman and Hake 1990). There was no such change exhibited by the dosed birds, further indicating that their risk perception might have been altered by methylmercury.

While more experiments are needed to validate these findings with free-living birds on contaminated sites, the fact that mercury-exposed birds did not increase their exposure to predation compared to controls is potentially good news 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 will move down to decomposers or potentially scavengers. However, increase in starvation risk will still affect bird populations, especially in areas with particularly high predation risk or especially harsh winters, where finding food is difficult and fat reserves are of the utmost importance. Furthermore, several species of conservation concern may have high methylmercury loads, such as the saltmarsh sparrow *Ammodramus caudacutus* (Lane et al. 2011; Scoville and 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. This study highlights the importance of using complex behavioral assays to better determine the effects of neurotoxins in situations that more closely mimic nature, and will help inform risk assessments on contaminated sites.

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