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Dietary mercury exposure causes decreased escape takeoff flight performance and increased molt rate in European starlings (*Sturnus vulgaris*)

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Abstract Mercury is a widespread and persistent environmental contaminant that occurs in aquatic and terrestrial habitats. Recently, songbirds that forage from primarily terrestrial sources have shown evidence of bioaccumulation of mercury, but little research has assessed the effects of mercury on their health and fitness. There are many indications that mercury negatively affects neurological functioning, bioenergetics, and behavior through a variety of mechanisms and in a wide array of avian taxa. Effective flight is crucial to avian fitness and feather molt is an energetically expensive life history trait. Therefore, we investigated whether mercury exposure influenced flight performance and molt in a common songbird, the European starling (*Sturnus vulgaris*). Specifically, we dosed the diet of captive starlings with methylmercury cysteine at 0.0, 0.75, or 1.5 μg/g wet weight and recorded changes in flight performance after 1 year of dietary mercury exposure. We also recorded the annual molt of wing feathers. We found that individuals dosed with mercury exhibited decreased escape takeoff flight performance compared with controls and blood mercury was also correlated with an increased rate of molt, which can reduce flight performance and thermoregulatory ability. This study reveals two novel endpoints, flight performance and molt, that may be affected by dietary mercury exposure. These findings suggest a potential impact on wild songbirds exposed to mercury levels comparable to the high dosage levels in the present study. Any decrease in flight efficiency could reduce fitness due to a direct impact on survival during predation events or by decreased efficiency in other critical activities (such as foraging or migration) that require efficient flight.

Keywords Flight · Mercury · Molt · Sub-lethal effects · European starling

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Introduction

Methylmercury (MeHg) bioaccumulates and biomagnifies in aquatic (Wolfe and Norman 1998; Boening 2000) and terrestrial (Driscoll et al. 2007; Cristol et al. 2008; Jackson et al. 2011) ecosystems. Many studies have documented the effects of mercury (Hg) on the health and fitness of aquatic bird species, but studies focusing on terrestrially foraging birds are fewer (Seewagen 2010). Recent research suggests that songbirds can suffer reduced reproductive success (Brasso and Cristol 2008; Hallinger and Cristol 2011; Varian-Ramos et al. 2014), altered stress hormone profiles (Franceschini et al. 2009; Wada et al. 2009), and disrupted immune functioning (Hawley et al. 2009; Lewis et al. 2013) in response to Hg exposure, but more research is needed to fully understand the potential impact of MeHg on the health and fitness of terrestrially foraging species.

Hg can disturb a myriad of neurological and behavioral endpoints across taxa. Changes in locomotion, coordination, and sensory response have been associated with Hg exposure in birds (Laties and Evans 1980; Bouton et al. 1999; Spalding et al. 2000), fish (Alvarez et al. 2006; Jakka et al. 2007), and amphibians (Burke et al. 2010). Behavioral alterations in parent/offspring interactions (Nocera and Taylor 1998; Evers et al. 2008), courtship (Frederick and Jayasena 2011), foraging (Olsen et al. 2000) and singing (Hallinger et al. 2010) have also been documented

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in a variety of bird species that were environmentally or experimentally exposed to Hg. The mechanisms underlying these changes have not been fully elucidated, but bioenergetic disruptions such as increased oxidative stress (Hoffman and Heinz 1998; Aschner et al. 2007; Glaser et al. 2010) and inhibited production of adenosine triphosphate (ATP) (Cambier et al. 2009) may contribute to overall changes in neurological functioning and behavior. Neurological and behavioral effects of Hg are likely interrelated. For example, Hg is related to decreased γ-aminobutyric acid (GABAergic) and glutamatergic neurotransmission in birds, both of which are critical neurological pathways involved in vertebrate behavior (Nam et al. 2012).

Because Hg has been demonstrated to affect several aspects of neurophysiology and health, it has the potential to impact a complex and demanding task such as flight. Some flight mechanisms that could likely be disrupted include efficient muscle energetics, motor sensory nerve conduction, feather quality maintenance, and appropriate predator response behavior—all of which are essential for proficient flight (Metcalfe and Ure 1995; Swaddle et al. 1996; Tobalske et al. 2005). Decreased bioenergetic capability in relation to elevated Hg could also affect flight performance by causing inefficient muscular output or inability to maintain energy-expensive activities for long durations of time. Disruptions of any of these non-mutually-exclusive mechanisms may affect distinctive parameters of flight performance.

Considering the potentially comprehensive effects of Hg on neurological function and behavior, we hypothesized that birds consuming Hg would suffer deficiencies in flight performance. Decreased flight performance could directly influence individual fitness through reduced proficiency at escaping predators (Lima 1993; Witter et al. 1994), or indirectly by potential deficiencies in any number of behaviors that require efficient flight, such as provisioning offspring, migrating seasonally, or competing for food. To test our hypothesis, we measured within-individual changes in flight performance of captive European starlings (*Sturnus vulgaris*) that we dosed with chronic, sub-lethal levels of Hg over the course of 1 year. We measured flight performance with two assays: (1) Escape takeoff in response to a predator stimulus; and (2) turning ability during level flapping flight. We chose these two modes of flight because of their relevance to predator escape in starlings, which are a model organism for avian flight performance (Rayner 1985; Fryday et al. 1995; Dial et al. 2008).

In addition to our flight performance tests, we conducted an assessment of molt patterns. Molt (the process by which feathers are replaced annually) directly influences flight performance (Swaddle and Witter 1997; Swaddle et al. 1999) and is also an avenue for Hg excretion from the body (Condon and Cristol 2009; Lodenius and Solonen 2013). Specifically, the rate of molt affects feather quality (Dawson et al. 2000), which can influence flight performance in starlings (Swaddle et al. 1996). The timing of feather loss is also important for ensuring birds suffer the least possible reduction in flight ability by fully replacing a lost feather before losing another (Dawson 2003). The interaction between thyroid and sex-steroid hormones can affect regulation of molt (Hahn et al. 1992), hence changes in any of these hormones could lead to changes in the timing and pattern of molt. Hormonal disruptions have been previously linked to Hg exposure in birds (Giesy et al. 2003; Heath and Frederick 2005; Frederick and Jayasena 2011). We therefore hypothesized that Hg exposure would cause changes in molt patterns, though we did not make any directional predictions.

Methods

Dosing

We used 60 wild-caught, juvenile European starlings. All birds were captured between May and July 2010 using baited walk-in traps. Birds were housed in groups of ten in large outdoor cages (2.5W × 2.5H × 3.5L m) with plentiful perches and given poultry starter crumbs and fresh drinking and bathing water ad libitum. Each cage of birds was randomly assigned a treatment group. Specifically, each of the three treatment groups contained two cages of birds for a total of 20 birds per treatment group. We re-assorted the birds within their treatment groups and rotated the groups through the cages every 6 weeks to minimize the possibility of pseudoreplication. Beginning in March 2011, we began feeding the birds poultry starter crumbs that we dosed with aqueous MeHg–cysteine at concentrations of 0.0 μg/g (ppm), 1.5 or 3.0 ppm wet weight (equivalent to 0.0, 1.83 or 3.66 ppm dry weight). Within 6 weeks on this treatment, three birds on the highest dose had died. The determined cause of death was kidney failure, which was based on glomerular filtration rate tests. Because we were interested in sub-lethal effects, we halved the doses to 0.75 and 1.5 ppm (wet weight, equivalent to 0.92 and 1.83 ppm dry weight), respectively. There were no further deaths or obvious detrimental effects on general health due to Hg in either group throughout the study. The flight tests on dosed birds were performed 36 weeks after the switch to lower doses. By this time birds had long since reached an asymptote of blood Hg concentration.

We collected blood samples from the birds immediately before dosing began to ensure that none of the birds in the experiment contained appreciable blood Hg prior to dosing,

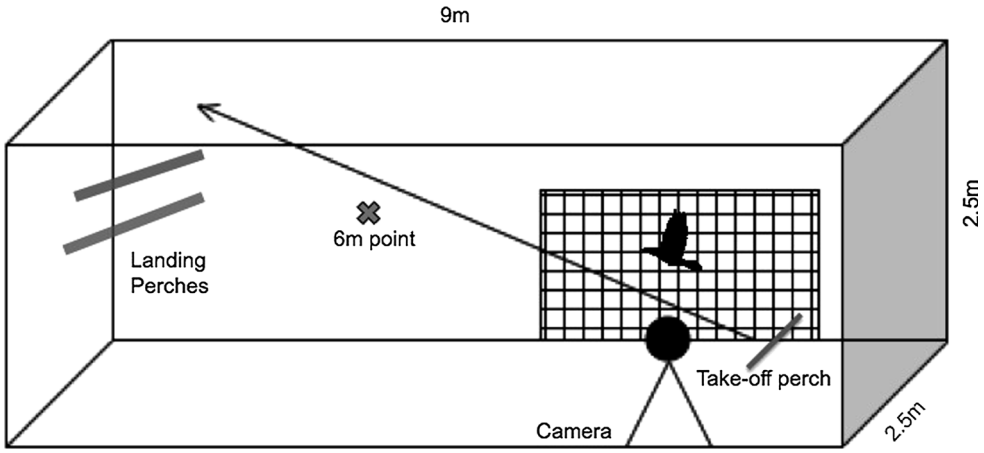

Fig. 1 3-D view of takeoff experimental arena. Birds were released by hand from the takeoff perch, with simultaneous presentation of a loud whistle startle stimulus, and filmed at a perpendicular angle as they flew past the 6 m point to the landing perches

and continued collecting samples weekly for the first 8 weeks to detect when blood levels had reached an asymptote. After 8 weeks of dosing, we began collecting blood samples monthly—but never prior to a flight test. All blood samples were collected in capillary tubes by puncturing the brachial vein with a 34-gauge needle. After the flight tests began, collection of blood samples always occurred immediately after birds were tested to prevent any effects of bleeding stress on flight performance.

All Hg analyses were performed using a DMA-80 Direct Mercury Analyzer (Milestone, Shelton, CT). The instrument was calibrated every 1–2 months or as needed. The calculated minimum detectable concentration during the period including these analyses was between 0.0042 and 0.0088 ppm (3.14 × SD of seven egg samples spiked near the expected lower limit of the instrument). Each batch of 20 blood samples was accompanied by a duplicate, two standard reference samples (DORM-3 and DOLT-4, National Research Council Canada), two blanks, and two empty sample containers. Relative percent difference of duplicate starling blood was 3.73 ± 2.93 % and standard recovery was 95.64 ± 7.80 % for DORM-3 and 97.76 ± 2.40 % for DOLT-4. When we spiked ten samples of starling blood with standard reference material our recovery was 96 ± 0.79 %. Approximately 95 % of Hg in avian blood and feathers is MeHg (Rimmer et al. 2005), so we made the assumption that the Hg concentrations we measured were a good approximation of MeHg levels.

Escape takeoff flight performance

We measured takeoff flight during escape in a large outdoor flight cage (3W × 2.5H × 9L m) by placing individual birds on a perch 10 cm from the ground and sounding a whistle as the bird was released (Fig. 1). We filmed flight from a perpendicular angle as the birds flew away from the perch in front of a visible grid (following Swaddle et al. 1999). Criteria for a successful flight test was that birds fly directly away from the predator stimulus for at least 6 m in a plane perpendicular to the camera lens. In cases where these criteria were not met during the initial release, the bird was given two additional attempts. Takeoff flight performance tends to be repeatable within individuals (Veasey et al. 1998; Criscuolo et al. 2011) and successive releases can cause fatigue, which results in increasingly lower performance (Renner 2006). Thus, birds were put in a small container for a 5-min resting period between each release and were returned to their cages once a single successful flight was recorded.

We used a Sony Handycam wide-angle HDR-CX350 camera (30 frames per second at 7.1 mega pixels) to record all flight tests and analyzed video using the public domain software program ImageJ version 1.440 (written by Wayne Rasband at the U.S. National Institutes of Health) to digitize flight trajectories on a Macintosh OS X 10.6.8. All videos were digitized and analyzed blind to treatment groups.

We assessed two parameters of takeoff flight: (1) Joules of energy exerted per unit mass over the first ten frames of flight; and (2) angle of takeoff (in degrees). To obtain a measure of Joules exerted by each bird during escape takeoff, we employed an equation for instantaneous mechanical energy (Swaddle et al. 1999) where V_x and V_y are the horizontal and vertical components of flight speed, g is acceleration due to gravity, and z is height. We then multiplied change in instantaneous energy by mass (kg) to obtain a measure of Joules.

$$E = 1/2\left[V_x^2 + V_y^2\right] + gz \quad (1)$$

We attempted to minimize effects of physical variation between individuals and changes of body mass within individuals by (a) using each individual as its own point of reference in a repeated-measures design; (b) explicitly including body mass in the calculation of instantaneous

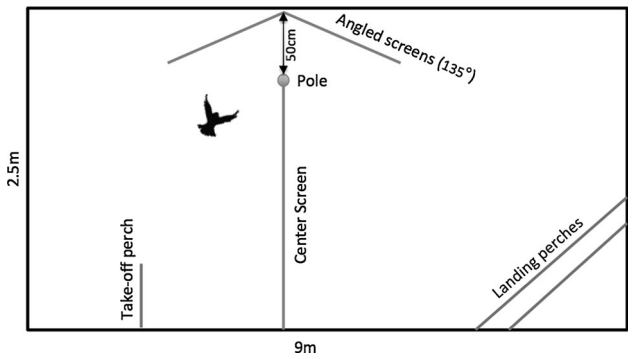

Fig. 2 View from above of the maneuverability flight cage. Birds were released by hand from the takeoff perch and filmed from below as they navigated around the center screen against a grid suspended from the ceiling to landing perches on the other side of the aviary

energy; and (c) examining effects of feather wear on energy expenditure. We classified feather wear based on criteria from the Monitoring Avian Productivity and Survivorship program (Ralph et al. 1993) during the pre-dosing flight test and found that feather wear score was not related to Joules of energy exerted during takeoff (linear regression, $F_{1,56} = 0.086$, $r^2 = 0.002$, $P = 0.756$) or angle of takeoff ($F_{1,56} = 0.171$, $r^2 = 0.003$, $P = 0.680$). Thus, we did not analyze differences in individual feather wear score throughout the experiment.

We performed the first escape takeoff flight tests in March 2011, before any Hg dosing began and before the birds had undergone their first "pre-basic molt" (i.e. annual molt occurring after the breeding season). The pre-dosing tests served as baseline measurements for each bird. We ran escape takeoff flight tests on five occasions between March 2011 and February 2012, with the middle three tests occurring during molt, which is known to affect both blood Hg levels (Condon and Cristol 2009) and individual flight performance (Swaddle and Witter 1997). Here we report results from only the pre-dosing and final tests, both of which occurred outside the molt period. The post-dosing flight test occurred in February 2012, after the birds had been consuming MeHg for 42 weeks (11 months) and approximately 20 weeks after they had completed molt. Comparison of the pre-dosing and final post-dosing tests allows us to examine the effects of long-term exposure to Hg on flight performance.

Turning ability during level flight

To measure turning ability during level flight, we filmed birds from below as they navigated around a semi-translucent screen that formed an obstacle across the width of a flight cage (Fig. 2). Two additional screens formed a 135° angle around the center barrier to help encourage the birds to fly around the obstacle. Birds were released by hand from a perch 1.2 m from the ground and filmed from below against a grid on the ceiling to allow measurement of location. It was necessary for the birds to learn to fly around the center barrier successfully; hence, before each session the birds were exposed to the experimental arena in groups of three until they had each flown around the screen four times. Successful criteria during a flight test was that the birds flew directly around the barrier to the perches on the other side of the aviary, at a height of at least 1.2 m (i.e. close to the grid on the ceiling and far from the camera below so that we could record the birds' location in each frame of video with sufficient precision).

We measured three parameters of flight performance in this test of turning ability: (1) Total velocity (m s^{-1}) while navigating around the barrier; (2) turn angle (in degrees) while navigating around the barrier; and (3) minimum distance (cm) from the barrier as the bird turned the corner (Fig. 2). Similar to the escape takeoff experiment, we completed pre- and post-dosing turning ability flight tests. The pre-dosing flight test took place before dosing and molt had begun (March 2011) and the post-dosing turning ability flight test occurred after molt was complete and the birds had been consuming MeHg treated food for approximately 40 weeks (January 2012). The purpose of this flight test was to evaluate whether aspects of turning during level flight differed in response to Hg dosing.

Molt assessment

We quantified molt using the Ginn and Melville (1983) method, whereby each of the nine primary feathers on the right wing was assigned a score from 0 to 5 depending on the stage of regrowth. Therefore birds had a cumulative molt score of zero before molt commenced and a score of 45 at completion. We scored molt eight times, every 8–12 days, throughout the approximately 100 day molting period (June–September 2011) to assess whether Hg exposure influenced the progression of primary feather molt.

Statistical analyses

To test whether flight parameters varied by Hg treatment group, we used one-way ANOVAs to compare within-individual changes in flight performance (energy exerted in takeoff, angle of takeoff, velocity around the obstacle during level flight, angle around the obstacle, and distance from the barrier during a level flight turn) across treatment groups, using data from each bird's pre- and post-dosing tests. As there were differences in takeoff performance by

treatment, we further analyzed within-individual changes in takeoff by individual blood Hg levels (which varied notably within but did not overlap among treatment groups) using linear regression. Specifically, we used the mean blood Hg level for each bird throughout the post molt period (October 2011–February 2012) as we had no a priori way of knowing whether the putative effects of Hg on flight would be the result of recent or longer term levels.

To examine how Hg affected the rate of molt, we analyzed within-individual change in molt scores using a repeated measures ANOVA test and further explored differences among the groups with Tukey's post hoc tests. The within-subjects independent variable was time and the among-subjects independent variable was treatment group. We also employed linear regression analyses to examine relationships between individual blood Hg level before molt (taken in May 2011) and molt scores. We used individual blood Hg level before molt as blood Hg changes substantially throughout molt and, therefore, it did not seem biologically relevant to use an average of these dynamic levels in this analysis. There were limitations in using the complete data set (i.e. molt scores from the entire course of molt) in our analyses because, by definition, all birds converged on the same molt score (45) upon completion, potentially masking among-treatment differences at other stages of molt. Thus, we compared molt scores taken only during the period of molt when the fastest rate of change in molt score occurred (June–August 2011), which included eight total molt scores per individual.

All statistical analyses were performed in IBM SPSS 19 (SPSS Inc. 2010) employing two-tailed tests of probability. Data are reported as mean ± SE unless otherwise noted.

Results

Blood Hg levels

The treatment group originally consuming 3.0 ppm MeHg reached a mean blood Hg peak of 14.4 ± 1.4 ppm (95 % CI 11.4–17.4 ppm). Once this group was switched to a 1.5 ppm MeHg diet and began molting, their mean blood Hg level dropped to 2.4 ± 0.3 ppm (95 % CI 3.2–6.6 ppm) during molt, when Hg was being deposited into newly growing feathers. This 1.5 ppm treatment group then plateaued at a mean blood Hg of 9.8 ± 2.8 ppm (95 % CI 8.2–11.3 ppm) following molt. The group of birds that were originally being fed a diet of 1.5 ppm MeHg had a mean blood Hg concentration of 7.9 ± 0.5 ppm (95 % CI 6.8–9.0 ppm), which dropped to 1.3 ± 0.1 ppm (95 % CI 1.3–1.4 ppm) when they were switched to a lower dietary exposure of 0.75 ppm MeHg and molt commenced. Following molt, this 0.75 ppm group had a mean blood Hg level of 4.9 ± 1.4 ppm (95 % CI 4.2–5.7 ppm). The mean blood Hg level of the control group during monthly samples remained close to 0.05 ppm throughout the duration of the study (Fig. 3).

Takeoff flight

Hg dosing influenced how birds expended energy during takeoff flight ($F_{2,40} = 3.80$, $P = 0.031$). Specifically, birds from the 1.5 ppm MeHg treatment group exerted less energy during the post-dosing test compared with the pre-dosing test; whereas birds from the 0.75 ppm group exerted a similar amount of energy in both tests, and control (0.0 ppm MeHg) birds demonstrated an increase in energy

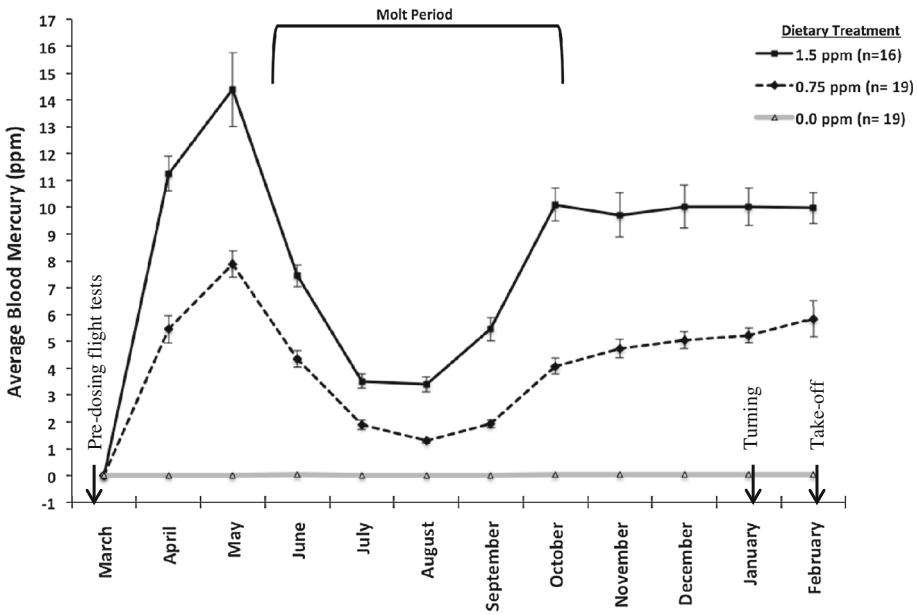

Fig. 3 Mean blood mercury concentrations of birds in each treatment group throughout the experiment. *Error bars* indicate standard deviation. Pre-dosing flight tests were run in March 2011, before any mercury dosing began and before the birds had undergone molt. The post-dosing tests for escape takeoff and turning ability were run in January and February 2012 respectively and are indicated by *arrows*

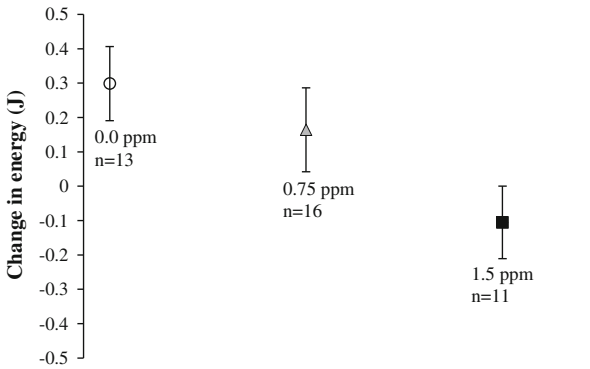

Fig. 4 Mean (±SE) change in energy from the pre-dosing test (March 2011) to the post-dosing test (February 2012, 42 weeks after dosing; 20 weeks after molt) across treatment groups

expenditure from pre- to post-dosing periods (Fig. 4). Therefore, dietary Hg dosing appears to be associated with decreased energy expenditure during an escape takeoff.

We explored these differences further by regressing individual blood Hg (averaged throughout the post-molt period) against the change in energy between the two flight tests. Consistent with the statistical analysis based on dose, there was a negative relationship between individual blood Hg and change in energy exerted during takeoff ($y = -0.025x + 0.249$, $F_{1,40} = 5.097$, $r^2 = 0.631$, $P = 0.030$; Fig. 5). Both analyses are informative because one indicates whether the different treatments produced different responses, whereas the other includes the unplanned variation of individual blood Hg levels within treatments.

Hg dosing did not influence the within-individual change in takeoff angle over the course of the experiment ($F_{2,40} = 0.350$, $P = 0.707$).

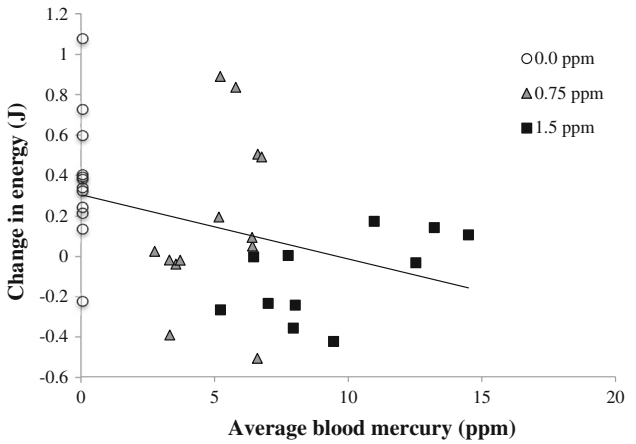

Fig. 5 Relationship between average individual blood mercury (during the post-molt period) and within-individual change in energy exerted from the pre- to post-dosing test

Turning around an obstacle during level flight

Hg exposure did not influence the velocity (m s^{-1}) or angle of turn during level flight ($F_{2,41} = 0.446$, $P = 0.644$; $F_{2,41} = 1.33$, $P = 0.277$, respectively). There were also no differences between the groups in minimum distance from the barrier ($F_{2,41} = 2.43$, $P = 0.119$).

Molt assessment

There was a tendency for birds from the highest Hg treatment to proceed more quickly through molt than the control group ($F_{2,54} = 2.46$, $P = 0.096$; *Tukey's posthoc test*, $P = 0.090$; Fig. 6). This pattern was more marked when we regressed individual blood Hg on change in individual molt score ($y = 0.035x + 5.884$, $F_{2,55} = 10.1$, $r^2 = 0.160$, $P = 0.003$; Fig. 7).

As expected, all 19 control birds replaced their flight feathers from the most proximal primary feather (P1) outward to the most distal flight feather (P9) (Pyle 1997). In contrast, three birds in the 1.5 ppm MeHg group (of 16 birds) and one bird in the 0.75 ppm MeHg group (of 18 birds) exhibited abnormal molt sequences (Fig. 8). For example, in a bird from the 1.5 ppm MeHg treatment group, P7 was shed and regrown before both P5 and P6, resulting in molt proceeding from two separate places across the wing. These four birds did not exhibit identical molt aberrations, but each shed and regrew feathers out of the usual order for European starlings. This unexpected observation must be considered anecdotal because its frequency did not differ significantly among treatments (Fisher's exact test, $P = 0.158$), however researchers in our lab have casually observed molt in hundreds of captive European starlings over two decades and have never previously noticed this molt pattern aberration.

Discussion

Flight performance

Chronic exposure to dietary Hg at a level comparable to what might be encountered at the most contaminated industrial sites resulted in a general decrease in escape takeoff flight performance. Between their pre- and post-dosing tests, control birds exhibited an approximate 40 % increase in energy expenditure, while birds dosed with 1.5 ppm MeHg exhibited a mean decrease of approximately 30 %, relative to control birds. Birds on the lower Hg dose (0.75 ppm MeHg) demonstrated an intermediate decrease of approximately 17 %, relative to controls. Across all treatments, individual blood Hg was negatively related to change in energy expenditure (Fig. 5), which

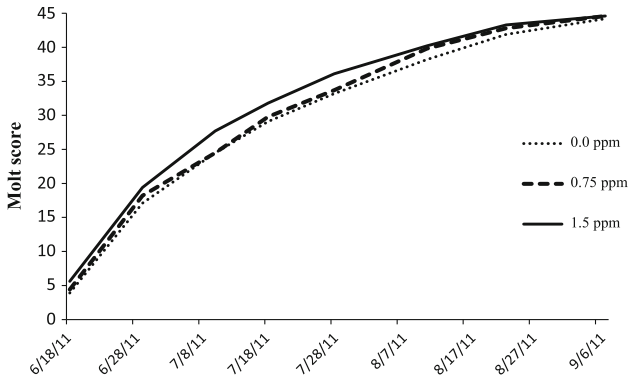

Fig. 6 Average molt score across treatment groups from shortly after the onset of molt through to the end of molt

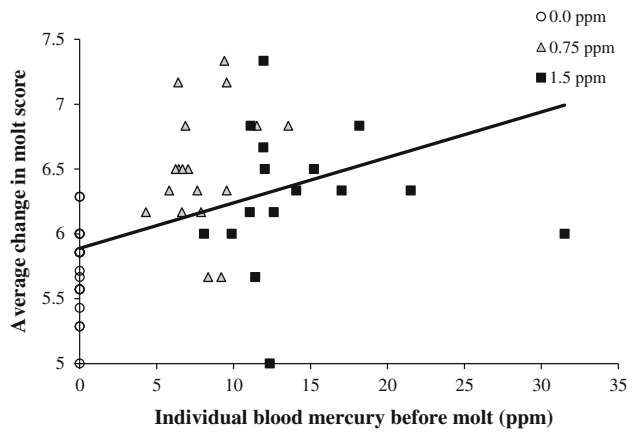

Fig. 7 Relationship between individual blood mercury at the onset of molt (May 2011) and average change in molt score per scoring occasion

suggests that increasing blood Hg resulted in decreased energy expenditure during escape takeoff. In summary, Hg dosing led to weaker escape takeoff flight performance in a captive songbird. Weaker escape takeoff flight may increase the risk of predation and likely lower individual fitness (Lima 1993; Metcalfe and Ure 1995; Dawson et al. 2000).

The consistent increase in energy expenditure demonstrated by controls indicates that there may have been changes within this group that allowed for better performance during the post-dosing test compared with the pre-dosing test. One possibility is that control birds were able to adapt to captivity, which led to better escape takeoff flight during the post-dosing test, which occurred about a year after capture from the wild. Captive starlings adjust to captivity by regulating their energy balance (Wiersma et al. 2005) and typically reestablish normal production of stress hormones only after 10 months in captivity (Dickens and Romero 2009). Energy budget is directly related to escape takeoff flight performance (Swaddle and Witter 1997), thus the control birds may have had stronger takeoff performance during the post-dosing flight test due to physiological adjustment to captivity. We did not test whether bleeding itself affects flight performance or molt, however all birds were exposed to equal bleeding frequency (once per month) and were allowed an equal recovery period between bleeding and flight tests (1 month).

The specific cause underlying the observed effects of Hg on flight performance is probably complex considering the interconnectivity between physiological and neurological flight mechanisms and the numerous known effects of Hg on these systems (Seewagen 2010; Cai et al. 2011). While many possible hypotheses exist, immediate effects of Hg on cellular activity (such as oxidative stress and lower ATP production) seem likely since the birds were not exposed to the toxin during development. Reduced feather quality is also possible and seems probable given the hastier molt in the dosed birds (see below). Although body mass can influence take-off flight speeds in some birds this is generally not the case with small songbirds when they are alarmed (Witter et al. 1994; Metcalfe and Ure 1995) as these small birds tend to defend flight speed at the cost of the angle of ascent (Veasey et al. 1998). Nevertheless, we explicitly included body mass in our calculations of take-off flight performance (Eq. 1) and thus accounted for any differences in mass between individuals and within-

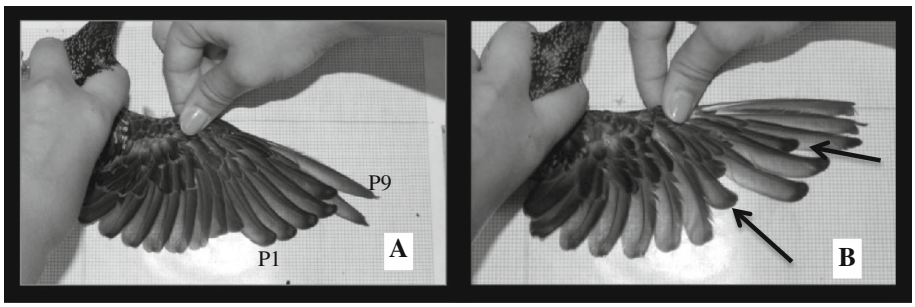

Fig. 8 Photographs of right wings of a bird displaying a normal molt sequence from the control group (**a**) and a bird from the 1.5 ppm MeHg group showing abnormal molt sequence (**b**). Four birds on mercury, but none of the control birds, showed this disruption in flight feather molt sequence. *Arrows* indicate points along the wing where feather regrowth has started

individual changes in mass between flight measurements. In addition, individuals' mass did not fluctuate differently between treatment groups from the pre- to the post-dosing test ($F_{2,49} = 0.058$, $P = 0.944$). Hence, we conclude that body mass is not driving the reduction in take-off performance associated with increasing MeHg exposure. Further mechanistic research is needed to identify the specific effect(s) on bioenergetics, neurological, physiological, and/or behavioral systems that may have reduced performance.

We did not find any differences between the groups in their ability to fly around an obstacle during level flight. It is possible that tradeoffs between speed and angle rendered any effects of Hg on maneuverability undetectable. It is also worth noting that this test was a novel approach to measuring turning flight performance and the measurements taken were less comprehensive than those taken in the takeoff test because they did not include a measure of energy expenditure.

Molt

The rate of primary feather molt increased with blood Hg across treatments, which indicates that Hg caused birds to molt faster. Further investigation is needed to elucidate whether Hg dosed birds also experienced an earlier date of onset.

An increased rate of molt is of general ecological significance because it can result in poor-quality feathers, which negatively affect thermoregulatory ability and flight (Dawson et al. 2000). The increased rate of molt among Hg-exposed birds could therefore have played a role in the observed decrease in takeoff flight performance. Specifically, the dosed birds may have grown poor-quality feathers as a result of accelerated molt and/or high levels of Hg deposition into the feathers. If newly grown feathers of Hg-dosed birds were of poor initial quality, they would have been more susceptible to degradation (Vagasi et al. 2011). Low-quality feathers in combination with increased feather degradation could play a role in explaining the decrease in escape takeoff performance by Hg-dosed birds during the post- versus the pre-dosing takeoff flight test. However, we did not directly assess the change in quality of feathers within individuals throughout the experiment, thus our hypothesis is speculative. More testing is needed to determine whether Hg affects feather quality, potentially leading to decreased flight performance.

During the molt assessment, we observed out of sequence feather loss in four starlings dosed with Hg (Fig. 8). During normal molt in starlings, primary feather loss is staggered so that birds do not have large gaps in their flight feathers at any one time; this helps to ameliorate the reduction in flight performance caused by molt (Swaddle et al. 1999). Out of sequence feather loss and the simultaneous re-growth of feathers creates abnormally large gaps and could affect flight and other functioning of the wing. The number of birds exhibiting this abnormality was not significantly different between treatments, yet to our knowledge this kind of alteration has never been recorded despite abundant research on molt in starlings (J. P. Swaddle, unpublished data). Hence, our lack of ability to note a statistically significant occurrence of these altered molt patterns may simply be a result of their infrequent expression in relation to Hg exposure. We hypothesize that Hg can cause aberrant molt patterns and encourage others to investigate this further in lab and field tests.

Changes in hormonal fluxes are thought to drive timing and rate of molt, and have also been associated with Hg in a variety of taxa (Hontela et al. 1992; Friedman et al. 1998; Leblond and Hontela 1999), including songbirds (Franceschini et al. 2009; Wada et al. 2009). Thus, disturbances in steroidogenesis may have resulted in differential molt between the dosed and control birds. One specific hypothesis is that Hg depressed testosterone (Frederick and Jayasena 2011) and that alteration of this hormone influenced the onset and rate of molt in starlings (Nolan et al. 1992). Tracking the progress of molt in wild birds exposed to environmental Hg would be necessary to determine whether the differences we observed in captivity are repeatable in wild populations experiencing normal hormonal fluxes.

Conclusion

In summary, we found that starlings chronically exposed to dietary Hg for approximately 1 year expended less energy during escape takeoff and molted differently. In general, control birds improved in flight performance over time while Hg-dosed birds worsened. Dosed birds also experienced a faster rate of molt compared with controls. Further testing is needed to understand how the underlying mechanisms of escape takeoff flight and molt are affected by Hg exposure. A limited number of studies have measured blood Hg in passerine birds, particularly those that forage in terrestrial ecosystems. Typically, blood Hg levels from environmentally exposed songbirds are well below those induced by the dosing in this study, however, the dietary treatments used are close to reported levels in potential bird prey items at highly contaminated sites (Cristol et al. 2008, Varian-Ramos et al. 2014). Songbirds experiencing similar Hg exposure are likely to suffer direct fitness consequences as a result of decreased ability to escape predators during escape takeoff (Lima 1993; Witter et al. 1994; Metcalfe and Ure 1995) and reduced survival due to faster and possibly abnormal molt (Dawson et al. 2000). Because our study was performed in an aviary with unlimited food and no predators or strenuous

locomotory requirements, it might be expected that the effects of the treatments would have been more severe in free-living birds. There is high potential for indirect fitness consequences resulting from deficiencies in foraging success, courtship, provisioning of offspring, migratory journeys, or any number of vital behaviors that require efficient flight. This study revealed two additional endpoints, flight performance and molt, that may be affected by dietary Hg exposure. Understanding sub-lethal effects of Hg will allow us to better determine whether there is an environmentally "safe" level of Hg for wildlife. However, finding an environmental threshold that can accurately be applied across species is challenging considering the amount of variation of Hg exposure and sensitivity within and across species, including the relatively high levels of Hg that some species, particularly marine predators, encounter.

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Conflict of interest The authors declare that they have no conflict of interest.

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