Environmental Pollution 246 (2019) 790–796

Contents lists available at ScienceDirect

Environmental Pollution

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

Environmentally relevant methylmercury exposure reduces the metabolic scope of a model songbird☆

Alexander R. Gerson [a, *], Daniel A. Cristol [b], Chad L. Seewagen [c, d]

[a] Department of Biology, University of Massachusetts, Amherst, MA, 01003, USA
[b] Biology Department, College of William & Mary, Williamsburg, VA, 23187, USA
[c] Great Hollow Nature Preserve & Ecological Research Center, New Fairfield, CT, 06812, USA
[d] AKRF Inc., White Plains, NY, 10601, USA

ARTICLE INFO

Article history:
Received 17 October 2018
Received in revised form
21 December 2018
Accepted 22 December 2018
Available online 29 December 2018

Keywords:
Basal metabolic rate
Peak metabolic rate
Mercury exposure
Ornithology
Eco-toxicology

ABSTRACT

For most birds, energy efficiency and conservation are paramount to balancing the competing demands of self-maintenance, reproduction, and other demanding life history stages. Yet the ability to maximize energy output for behaviors like predator escape and migration is often also critical. Environmental perturbations that affect energy metabolism may therefore have important consequences for fitness and survival. Methylmercury (MeHg) is a global pollutant that has wide-ranging impacts on physiological systems, but its effects on the metabolism of birds and other vertebrates are poorly understood. We investigated dose-dependent effects of dietary MeHg on the body composition, basal and peak metabolic rates (BMR, PMR), and respiratory quotients (RQ) of zebra finches (*Taeniopygia guttata*). Dietary exposure levels (0.0, 0.1, or 0.6 ppm wet weight) were intended to reflect a range of mercury concentrations found in invertebrate prey of songbirds in areas contaminated by atmospheric deposition or point-source pollution. We found adiposity increased with MeHg exposure. BMR also increased with exposure while PMR decreased, together resulting in reduced metabolic scope in both MeHg-exposed treatments. There were differences in RQ among treatments that suggested a compromised ability of exposed birds to rapidly metabolize carbohydrates during exercise in a hop-hover wheel. The elevated BMR of exposed birds may have been due to energetic costs of depurating MeHg, whereas the reduced PMR could have been due to reduced oxygen carrying capacity and/or reduced glycolytic capacity. Our results suggest that environmentally relevant mercury exposure is capable of compromising the ability of songbirds to both budget and rapidly exert energy.

1. Introduction

Mercury has dramatically increased in the environment since pre-industrial times and is now a global pollutant, largely due to the deposition of atmospheric mercury far from coal-fired power plants and other emissions sources over the past two centuries (Travnikov et al., 2013; UNEP Global Mercury Assessment, 2013). Reduced emissions in many industrialized nations have resulted in a steady global decline in circulating atmospheric mercury in recent decades (Pacnya et al., 2016; Zhang et al., 2016). However, uncertainty about future emissions from artisanal gold mining, currently the biggest global emission source (Beal et al., 2013), along with potential climate-change mediated processes, such as increased emissions from wildfires (Kasischke and Turetsky, 2006), mean that anthropogenic mercury contamination will be a continuing global threat. Environmental mercury contamination is readily converted by bacteria into a highly toxic and bioavailable form, methylmercury (MeHg), which can then become prevalent in both aquatic and terrestrial food webs and have wide-ranging detrimental effects on many aspects of organismal biology (Evers, 2018). Animals at high trophic levels and associated with aquatic systems typically accumulate the most MeHg and are therefore potentially at greatest risk for health effects, but animals, including songbirds, that are in lower positions within food webs or occupy terrestrial habitats may also be vulnerable to harmful levels of MeHg exposure (Rimmer et al., 2005; Whitney and Cristol, 2017).

In several taxonomic groups of birds, MeHg has been shown to cause histological and biochemical changes that in turn affect

☆ This paper has been recommended for acceptance by Prof. Wen-Xiong Wang.
* Corresponding author.
E-mail address: argerson@bio.umass.edu (A.R. Gerson).

https://doi.org/10.1016/j.envpol.2018.12.072
0269-7491/

numerous physiological systems and endpoints. This includes endocrine function, immunocompetence, oxidative balance, feather symmetry, flight performance, and cognition, among many others (Evers, 2018; Whitney and Cristol, 2017). Several such effects have been observed in free-living wild birds and/or captive birds exposed to environmentally relevant concentrations of MeHg, indicating that current levels of mercury pollution in many parts of the world are capable of impacting bird health and fitness.

In fish and rats, MeHg has been shown to limit mitochondrial oxidation and ATP production (Cambier et al., 2009; Gonzalez et al., 2005; Stohs and Bagchi, 1995), but the effects of MeHg on whole animal peak metabolic rate do not appear to have been investigated in any vertebrate taxa, including humans. It has been suggested that the binding of MeHg to the sulfhydryl groups of proteins could compromise the functionality of catabolic enzymes and fatty acid transporters (Seewagen, 2018). These proteins play key roles in the rapid mobilization, uptake, and oxidation of fuel that is needed to sustain high intensity exercise by birds, such as long-distance flight (Guglielmo, 2010). MeHg inhibits glycolysis, which fuels shorter-term peak metabolic output, such as burst flight, also by inhibiting enzyme activity (Ramírez-Bajo et al., 2014). Maximum energy production might be further limited by the suppressive effects of MeHg on hemoglobin production and oxygen carrying capacity (Seewagen, 2010; Seewagen, 2018). Simultaneously, MeHg, like other contaminants, is likely to increase a bird's resting metabolism due to the energetic costs of depurating toxicants from the body (Calow, 1991; Hopkins et al., 1999; Rowe et al., 1998). Such effects would together reduce the metabolic scope (i.e., the difference between basal and peak metabolic rate) of a bird and hinder its ability to both budget and rapidly expend energy.

Energy efficiency and conservation are often paramount to balancing the competing demands of self-maintenance, reproduction, and other demanding life history stages such as migration, over-wintering, molt, or provisioning food to offspring, while the ability of birds to maximize energy output for episodic behaviors like predator escape and migratory flight is often also important. Environmental change that affects the energy metabolism of birds may therefore have important consequences for their fitness and survival. To better understand the potential impact of mercury pollution on the energetics of birds, we used respirometry and an exercise wheel to measure dose-dependent effects of dietary MeHg on the basal and peak metabolic rates (BMR, PMR), and metabolic scope of domestic zebra finches (*Taeniopygia guttata*). We also compared respiratory quotients (RQ, i.e., CO_2 evolved divided by O_2 consumed) and fat and lean body mass among treatments to investigate the influence that mercury exposure may have on metabolic fuel selection and the body composition of birds.

2. Materials and methods

2.1. Mercury exposure and analysis

Adult domestic zebra finches from lineages not previously exposed to experimental MeHg were maintained on a diet of Zupreem Fruit Blend® dosed with either 0.0 (N = 12), 0.1 (N = 20), or 0.6 (N = 20) ppm (μg MeHg-cysteine g^{-1} food on a wet weight basis) for approximately 8 wk, beginning in April of 2015. Birds were housed together in 3 × 2.5 × 2 m outdoor aviaries without artificial lighting and provided with *ad libitum* food, water, oyster shell grit and cuttlefish (for beak conditioning and calcium). Birds were held in three adjacent aviaries, one for each diet treatment. The food was prepared as in Varian-Ramos et al. (2014), tested, and rejected if it was >7.5% different from the nominal concentration. The group receiving 0.0 ppm of MeHg was used as a control, and the exposure levels of 0.1 and 0.6 ppm were intended to span the middle of the range of mercury concentrations recently reported for songbird prey items in North America at sites contaminated by atmospheric deposition or point-source pollution (e.g., black flies at remote wetlands in Ontario (Harding et al., 2006); various arthropods at a site in Virginia 50 years after cessation of industrial contamination (Cristol et al., 2008); spiders in high-elevation forests of Vermont (Rimmer et al., 2010); or spiders in remote wetlands of the Canadian Maritimes (Edmonds et al., 2012)).

After approximately 8 wk on the three diet treatments and immediately before the measurement of BMR, we collected up to 75 μL of blood by brachial venipuncture to determine the birds' blood total mercury (THg) levels. We measured THg (ppm wet weight), which is representative of MeHg in birds (Fournier et al., 2002; Rimmer et al., 2005), using cold vapor atomic absorption spectroscopy as described by (Varian-Ramos et al., 2014). Mean percent recoveries of standard reference materials were 95.1% (DOLT-4) and 99.0% (BCR463-tuna). The detection limit of the mercury analyzer was checked regularly and averaged 0.005 ng, which was below even the trace quantities of THg detected in the blood samples of the birds in the 0.0 ppm control group.

2.2. Body composition analysis and respirometry

Food was removed 2 h prior to all measurements to ensure the birds were post absorptive. After collecting blood, the birds were weighed to the nearest 0.01 g on a digital balance (Ohaus Scout Pro, NJ, USA), measured (wing and tarsus length to 1 mm and 0.5 mm, respectively), and then scanned in duplicate in a quantitative magnetic resonance body composition analyzer (QMR; Echo MRI, TX, USA) to measure fat mass and lean body mass to 0.001 g (Guglielmo et al., 2011) before being placed into dark respirometry chambers (1.3 L; 135 × 102 × 185 mm) that were maintained at 30 °C for the night (~12 h) (n = 26). During this period, flow rate, O_2 consumption, and the rates of carbon dioxide and water production were measured using a standard respirometry setup (Sable Systems International, NV, USA) to determine BMR of 5–7 individuals each night. Flow rates were approximately 500 ml min^{-1} through each chamber. Birds were removed from their chambers at sunrise (approximately 0600), weighed and scanned in the QMR again, and then transferred to indoor holding cages to complete a fast as part of a previously published study (Seewagen et al., 2016), after which they were returned to their aviary. We determined PMR in a subset of the birds (N = 18) 48–72 h after the determination of BMR by using a hop-hover wheel (12.7 cm × 30 cm (Chappell et al., 1999; Pierce et al., 2005; Price and Guglielmo, 2009); receiving 3–5 LPM of dry air. Once the bird was inside, the wheel was covered for 5–10 min to allow habituation to surroundings and to determine resting levels of oxygen consumption. After this waiting period, the cover was removed so the bird could be observed, and the wheel was turned manually by the investigator to encourage the bird to continually hop and hover, resulting in a rapid increase in metabolic rate. This was continued until metabolic rate peaked, usually less than 3 min, after which it usually dropped to a steady but still elevated plateau. Birds were then removed from the wheel and returned to their outdoor aviary with food and water.

2.3. Respirometry calculations

Rates of oxygen consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), and water production ($\dot{V}H_2O$) were calculated using standard equations for push mode respirometry (Lighton, 2008). After calculation of $\dot{V}O_2$ (ml O_2 consumed min^{-1}), BMR was determined as the lowest continuous 60 s during the night. The corresponding $\dot{V}CO_2$ and $\dot{V}H_2O$, and RQ values were also selected from the same time window. PMR was determined as the mean of the highest 30 s of $\dot{V}O_2$

recorded, and the corresponding $\dot{V}CO_2$, $\dot{V}H_2O$, and RQ from the same selection were also recorded.

2.4. Statistical analyses

Differences in body composition and structural size were compared among treatments using analysis of variance (ANOVA). Differences in BMR, PMR, and metabolic scope among treatments were tested using general linear models that included wing or tarsus as measures of structural size and lean mass as covariates to account for differences in metabolic tissue among groups. Differences in RQ were evaluated using ANOVA. BMR and PMR were natural log transformed to meet the assumption of normality. All statistical analyses were performed in R (R core team 2018). For each test, non-significant terms were removed from the model using a standard backwards stepwise approach until only significant terms remained ($\alpha = 0.05$). All post-hoc comparisons were made with Tukey HSD tests.

3. Results

3.1. Exposure

Blood THg concentrations increased significantly with increasing exposure concentration ($F_{2,46} = 300.18$, $P < 0.001$) and averaged 0.968 (±0.243 SD) and 5.694 (±1.09 SD) ppm in the 0.1 and 0.6 ppm groups, respectively, upon completion of the approximately 8 wk exposure period. Control birds had only traces of blood THg (mean: 0.0062 ppm ± 0.0027 SD).

3.2. Body composition

The birds for which we measured BMR did not differ significantly in structural size among treatment groups (tarsus: $F_{2,23} = 0.598$, $P = 0.56$; wing: $F_{2,24} = 0.16$, $P = 0.85$), but total body mass was significantly different ($F_{2,24} = 6.91$, $P = 0.004$) and increased with exposure concentration (Table 1). This was largely driven by differences in fat mass, which differed significantly among treatments ($F_{2,24} = 3.99$, $P = 0.032$; Table 1), while lean body mass did not ($F_{2,24} = 2.01$, $P = 0.15$; Table 1). Because fat is not metabolically active and lean body mass did not differ, both were dropped from the model in comparisons of BMR among treatments (see BMR below).

Among the subset of birds for which we measured PMR, there were no significant differences in structural size (tarsus: $F_{2,14} = 0.57$, $P = 0.575$; wing: $F_{2,15} = 0.33$, $P = 0.724$), total body mass ($F_{2,14} = 2.29$, $P = 0.138$), fat mass ($F_{2,15} = 0.052$, $P = 0.949$), or lean body mass ($F_{2,15} = 2.33$, $P = 0.137$).

Table 1
Total body mass, fat mass, and lean mass (g) of zebra finches exposed to 0.0 (control), 0.1, or 0.6 ppm dietary methylmercury cysteine and used to measure basal and peak metabolic rates. Values are means±SD and test statistics are from general linear models. *P < 0.05, **P < 0.01, different superscript lowercase letters denote significant differences within a row.

		Treatment		
		Control	0.1 ppm	0.6 ppm
BMR	Mass (g)**	14.60 ± 0.93 [a]	15.63 ± 0.72 [ab]	16.57 ± 1.40 [b]
	Fat Mass (g)*	0.63 ± 0.20 [a]	0.92 ± 0.38 [ab]	1.17 ± 0.45 [b]
	Lean Mass (g)	12.45 ± 0.92	12.83 ± 0.66	13.39 ± 1.25
PMR	Mass (g)	14.66 ± 0.97	14.23 ± 0.52	15.43 ± 1.32
	Fat Mass (g)	0.62 ± 0.22	0.59 ± 0.39	0.64 ± 0.24
	Lean Mass (g)	12.48 ± 0.97	11.65 ± 0.20	12.73 ± 1.21

3.3. Basal metabolic rate

BMR was significantly different among treatment groups ($F_{2,23} = 4.58$, $P = 0.021$), the difference increasing with exposure concentration (Fig. 1a). Initial models comparing BMR among treatments included tarsus and lean mass as covariates, but neither was significant and thus they were removed from the final model. Post-hoc tests showed that BMR was 19% higher among the 0.6 ppm group than the control group ($P = 0.015$); differences in BMR were smaller and statistically non-significant between the 0.1 ppm group and either the control ($P = 0.22$) or 0.6 ppm group ($P = 0.33$).

3.4. Peak metabolic rate

There appeared to be a difference in PMR among treatment groups, although it was non-significant ($F_{2,14} = 3.43$, $P = 0.06$), with PMR declining with increasing exposure concentration (Fig. 1b). Neither log body mass nor log lean body mass were significant

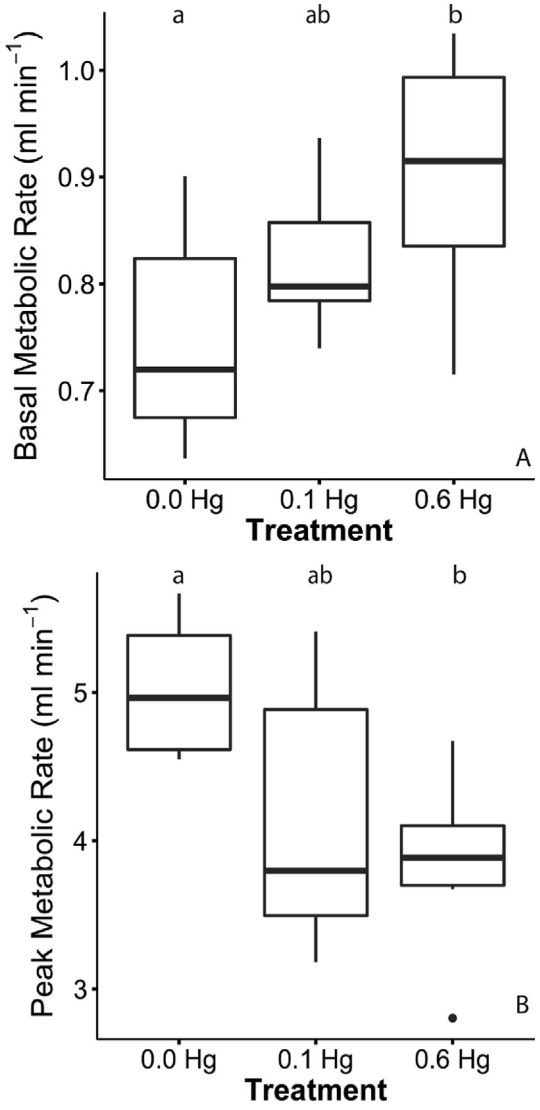

Fig. 1. Box plots of basal (A) and peak (B) metabolic rates (BMR, PMR) of zebra finches exposed to 0.0, 0.1, or 0.6 ppm dietary methylmercury cysteine (BMR: $F_{2,23} = 4.58$, $P = 0.021$; PMR: $F_{2,14} = 3.43$, $P = 0.06$). Significant differences denoted by different lowercase letters ($P < 0.05$).

covariates (log Mass: $F_{1,14} = 0.001$, $P = 0.97$; log lean body mass $F_{1,14} = 0.03$, $P = 0.86$). In post-hoc pairwise comparisons, the PMR of birds on the higher mercury diet was 23% lower than that of control birds ($P = 0.051$). PMR in the 0.1 ppm group averaged 18% lower than in the control birds, but this apparent difference was not significant ($P = 0.162$). There was no significant difference in PMR between the 0.1 and 0.6 ppm treatments ($P = 0.815$).

3.5. Metabolic scope

There was a significant reduction in metabolic scope with increasing dietary mercury concentration ($F_{2,14} = 4.18$, $P = 0.038$; Fig. 2); neither body mass ($F_{1,13} = 0.068$, $P = 0.80$) nor lean mass ($F_{1,13} = 0.07$, $P = 0.79$) were significant covariates. Post-hoc tests showed the metabolic scope of birds in the 0.6 ppm group to be significantly lower by 31% than that of control birds ($P = 0.030$). The metabolic scope of birds in the 0.1 ppm group averaged 24% lower than that of the control birds, but the difference was not significant ($P = 0.125$). Metabolic scope also did not differ between the 0.1 and 0.6 ppm treatments ($P = 0.669$).

3.6. Respiratory quotient

There were near-significant differences in RQ among treatment groups during the measurement of BMR ($F_{2,23} = 3.14$, $P = 0.06$; Fig. 3a), but all values during BMR were near the theoretical low-limit of 0.72. Post-hoc tests showed the RQ during BMR in the 0.6 ppm group to be higher than the control group ($P = 0.051$). The RQ of birds in the 0.1 group were not significantly different from control birds ($P = 0.261$) and the RQ of 0.6 ppm group were not significantly different from the 0.1 ppm group ($P = 0.578$).

Mercury exposure appeared to reduce RQ during the measurement of PMR (Fig. 3b), although the differences among groups only approached significance ($F_{2,15} = 2.83$, $P = 0.09$). In post-hoc tests, there was a near-significant difference between the 0.1 ppm and control groups ($P = 0.076$) while the RQ of birds in the 0.6 group were not significantly different from control birds ($P = 0.386$) and the RQ of 0.6 ppm group were not significantly different from the 0.1 ppm group ($P = 0.421$).

4. Discussion

Depurating toxicants like MeHg from the body increases the energetic demands of self-maintenance (Calow, 1991). Simultaneously, MeHg may also interfere with several of the biochemical processes that enable large amounts of stored energy to be drawn upon and rapidly exerted for either short or prolonged periods of time (Ramírez-Bajo et al., 2014; Seewagen, 2018). Yet we are unaware of any previous study of the effects of mercury on the metabolic rates of birds. Here, at environmentally relevant dietary and blood concentrations, MeHg substantially reduced the metabolic scope of songbirds by up to 31%, by increasing resting metabolic costs while also limiting their peak metabolic output. Given the significance of both energy conservation and the ability to quickly maximize energy production for behaviors involving flight, such as predator avoidance and migration, the impacts of mercury on the metabolism of birds could have important consequences for their fitness and survival.

Although global concentrations of MeHg have been declining in many parts of North America since the implementation of air and water pollution regulations in the 1970s, mercury contamination continues to be a serious threat to wildlife in North America and around the world. Trends in tissue level mercury in fish have

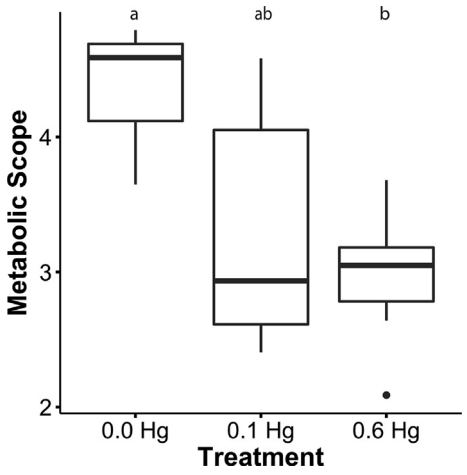

Fig. 2. Box plot of differences in metabolic scope among zebra finches exposed to 0.0, 0.1, or 0.6 ppm dietary methylmercury cysteine ($F_{2,14} = 4.18$, $P = 0.038$). Significant differences denoted by different lowercase letters ($P < 0.05$).

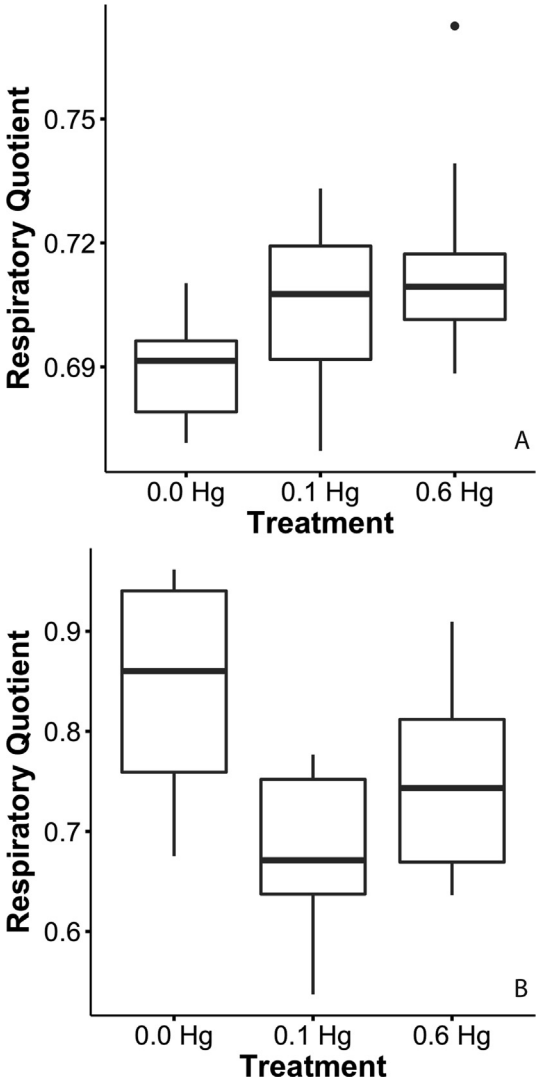

Fig. 3. Box plots of differences in respiratory quotient (RQ) among zebra finches exposed to 0.0, 0.1, or 0.6 ppm methylmercury cysteine. RQ derived during measurement of basal metabolic rate shown in panel A ($F_{2,23} = 3.14$, $P = 0.06$); RQ derived during measurement of peak metabolic rate shown in panel B ($F_{2,15} = 2.83$, $P = 0.09$).

declined significantly between 1969 and 2005 in the United States overall (Chalmers et al., 2011), but there are areas where MeHg has not declined and recovery or remediation can take many decades (Santschi et al., 2017). Concentrations of MeHg in invertebrates, the primary prey item for most songbirds, can still be substantial. The blood mercury concentrations we report for the birds in the 0.6 ppm treatment in this study fall well within the range detected recently in birds consuming insects from legacy contaminated sites (Cristol, 2008; Kopec et al., 2018). Our low-dose treatment (0.1 ppm) resulted in blood mercury levels of approximately 1.0 ppm, a value that has been exceeded by various species of songbirds, even at sites without point-source contamination (e.g., marsh-inhabiting sparrows, Winder and Emslie, 2012).

Basal metabolic rate represents the standard maintenance cost of sustaining basic metabolic processes, and exposure to a contaminant typically raises that cost due to the energy required to eliminate the toxicant from the body (Calow, 1991). This depuration cost most likely explains the positive relationship that we observed between MeHg-exposure concentration and BMR in the zebra finches. Exposing birds to MeHg at 0.6 ppm substantially elevated their BMR by 19% above that of the controls. Such an increase would be expected to have adverse effects on several important physiological processes and behaviors that are fundamental to the fecundity and survival of wild birds. For example, it might be particularly problematic for birds wintering in cold climates, where food availability is often low and the energy demands of thermoregulation are great, or in migrating birds attempting to maximize fuel efficiency in flight and rapidly store energy during stopovers, or birds that fast for prolonged periods while nesting (e.g., seabirds). It could also limit reproduction in species whose reproductive potential is largely influenced by the amount of energy they are able to consume and store in advance (e.g., Smith and Moore, 2003). Unless birds are able to compensate by increasing their foraging activity and caloric intake, which itself can have negative effects (e.g., increased predation risk, oxidative stress (Krebs, 1980; Yap et al., 2017), energy that could otherwise be allocated towards beneficial processes like reproduction, migration, immune defense, and molt, or put into storage, is instead lost to the elimination of MeHg from the body.

Avian flight is among the most expensive modes of transport for vertebrates, and most volant birds cannot survive if flight ability is compromised. Flight requires an approximately 10-fold increase in metabolism above basal levels, whether brief or for more sustained periods (Butler, 1991). Carbohydrates may be the primary substrate used for short duration, high-intensity activity or during the early stages of long-distance flight, while fatty acids increasingly become the predominant fuel source as long-distance flight progresses (Guglielmo, 2010; McClelland et al., 1995; Vaillancourt and Weber, 2007). Birds have evolved unique mechanisms that allow rapid flux through aerobic metabolic pathways in order to fuel and sustain such high intensity activity. For example, migratory birds prepare for the heightened energy demands of migration by substantially upregulating enzymes and transport proteins that enable rapid mobilization, transport, uptake, and oxidation of fuel (Guglielmo et al., 2002; McFarlan et al., 2009). Mercury has the potential to inhibit the production and function of enzymes and other proteins involved in the catabolism of carbohydrates and fatty acids that allow for peak metabolic output by birds (Ramírez-Bajo et al., 2014; Seewagen, 2018). Mercury is expected to further limit a bird's metabolic capacity by reducing blood hemoglobin concentrations and the availability of oxygen for aerobic reactions (Seewagen, 2010; Seewagen, 2018). These effects of mercury on substrate delivery, uptake, and/or oxidation are likely responsible for the substantial reduction in zebra finch PMR that we observed in relation to MeHg exposure concentration.

At rest, all treatment groups had RQs that were indicative of primarily fat and/or uricotelic protein metabolism (Schmidt-Nielsen, 1997). During exercise, however, there were apparent differences in fuel use between the control and mercury-exposed birds. The RQ of the control group (0.89) shows that a mixture of fuels was used and there was a large contribution from carbohydrates, as expected for short-duration, high-intensity exercise. In contrast, the RQs of birds in the two exposure treatments (0.68 and 0.75) showed a complete or nearly complete reliance on fat and/or protein. Mercury preferentially binds to the exposed cysteine-SH residues on the enzymes hexokinase and phosphofructokinase, which catalyze the first and third step in glycolysis, reducing their activity, and reducing overall flux through glycolysis (Ramírez-Bajo et al., 2014). We therefore hypothesize that mercury impacted the carbohydrate metabolism of birds in the two exposure treatments, which in turn, reduced their PMR. Although birds are renowned for their unique ability to fuel long-duration aerobic exercise with primarily fat, the mobilization and uptake of fatty acids may not occur early and rapidly enough to fuel short-term, burst-type activity like that of the zebra finches in the hop-hover wheel (Jenni-Eiermann, 2017; Price and Guglielmo, 2009; Tucker, 2005). Nevertheless, there is still some contribution from fat to short-term high-intensity exercise, and it is possible that the PMR of the exposed birds was also partly limited by the impacts of mercury on lipid metabolism pathways (Seewagen, 2018). Given what has been shown for enzymes associated with carbohydrate metabolism (Ramírez-Bajo et al., 2014), it seems that enzymes involved in fatty acid oxidation with a greater number of exposed cysteine-SH residues would also be more susceptible to covalent modification by mercury. If these exposed cysteine-SH residues occur in high proportion near the binding site, as they do for hexokinase, then we can predict disproportionate impacts on rates of fatty acid oxidation. Further analysis of the structure of enzymes involved in the mobilization and catabolism of fat in birds would yield strong predictions regarding the potential impact of mercury on fat metabolism.

A reduced ability of exposed birds to metabolize carbohydrates might also explain the differences in body composition that we observed among treatment groups. There was a significant and dose-dependent increase in fat mass with increasing dietary mercury concentration, and all birds were fed a high-carbohydrate grain-based diet. If carbohydrate metabolism were impaired by the mercury exposure, carbohydrates consumed by the exposed birds would have been alternatively routed to fatty acid synthesis pathways and stored as fat rather than immediately metabolized. It is also possible that the effects of MeHg on risk-taking and social dominance behaviors in zebra finches (Kobiela et al., 2015; Swaddle et al., 2017) influenced their body composition, as small birds can strategically regulate the size of their fat stores (Rogers, 2015). It is possible, for example, that a perceived increase in the risk of starvation due to social exclusion from food caused mercury-exposed birds to carry larger fat stores as insurance.

The reduction in metabolic scope demonstrated here at environmentally relevant exposure and blood mercury levels could have dramatic consequences for survival and reproduction. Due to their complex life cycles, many species of birds are at high risk for exposure to pollutants, and even low levels of exposure could impede performance during critical life history stages. For example, a recent study of songbirds in Canada found that autumn, southbound migrants had higher feather mercury levels than conspecifics during return northward migration through the same area the following spring, suggesting that the individuals that migrated south with the greatest exposure to mercury were at some point lost from the population (Ma et al., 2018). Although here we investigated the impact of mercury on a model songbird species

that is non-migratory, our results further underscore the need for concern about whether declines in migratory birds are being exacerbated by their widespread exposure to sub-lethal amounts of mercury. Mercury exposure, even for just part of the life cycle, strongly affects reproduction in songbirds (Paris et al., 2018) and could affect several aspects of migratory ability, such as orientation and navigation (Seewagen, 2018). Thus, our finding that exposure to environmentally relevant concentrations of MeHg reduces metabolic scope in songbirds illuminates an additional pathway through which this global pollutant may be harming songbird populations.

Conflicts of interest

The authors declare no competing interests.

Author contributions

C.S. and A.G. designed and conducted the experiment, analyzed data, and drafted the manuscript; D.C. contributed to experimental design, provided experimental subjects, laboratory and aviary facilities, and commented on the manuscript.

Funding

Funding for this study was provided by the University of Massachusetts (A.R.G.), the Great Hollow Nature Preserve and Ecological Research Center and AKRF Inc. (C.L.S.), and NSF IOS grant 1257590 (D.A.C.).

Data availability

All data are available upon request.

Acknowledgements

T. Diehl provided valuable assistance with data collection and we thank R. Ellick for analyzing blood samples. The original manuscript was improved by feedback from two anonymous reviewers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.072.

References

Beal, S.A., Jackson, B.P., Kelly, M.A., Stroup, J.S., Landis, J.D., 2013. Effects of historical and modern mining on mercury deposition in southeastern Peru. Environ. Sci. Technol. 47, 12715–12720.
Butler, P.J., 1991. Exercise in birds. J. Exp. Biol. 160, 233–262.
Calow, P., 1991. Physiological costs of combating chemical toxicants - ecological implications. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. 100, 3–6.
Cambier, S., Bénard, G., Mesmer-Dudons, N., Gonzalez, P., Rossignol, R., Brèthes, D., Bourdineaud, J.P., 2009. At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (*Danio rerio*). Int. J. Biochem. Cell Biol. 41, 791–799.
Chalmers, A.T., Argue, D.M., Gay, D.A., Brigham, M.E., Schmitt, C.J., Lorenz, D.L., 2011. Mercury trends in fish from rivers and lakes in the United States, 1969- 2005. Environ. Monit. Assess. 175, 175–191.
Chappell, M., Bech, C., Buttemer, W., 1999. The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. J. Exp. Biol. 202, 2269.
Cristol, D.A., Brasso, R.L., Condon, A.M., Fovargue, R.E., Friedman, S.L., Hallinger, K.K., Monroe, A.P., White, A.E., 2008. The movement of aquatic mercury through terrestrial food webs. Science 320, 335.
Edmonds, S.T., O'Driscoll, N.J., Hillier, N.K., Atwood, J.L., Evers, D.C., 2012. Factors regulating the bioavailability of methylmercury to breeding rusty blackbirds in northeastern wetlands. Environ. Pollut. 171, 148–154.
Evers, D., 2018. The effects of methylmercury on wildlife: a comprehensive review and approach for interpretation, approach for interpretation. In: Encyclopedia of the Anthropocene. Elsevier, Oxford, pp. 181–194.
Fournier, F., Karasov, W.H., Kenow, K.P., Meyer, M.W., Hines, R.K., 2002. The oral bioavailability and toxicokinetics of methylmercury in common loon (*Gavia immer*) chicks. Comp. Biochem. Physiol. A 133, 703–714.
Gonzalez, P., Dominique, Y., Massabuau, J.C., Boudou, A., Bourdineaud, J.P., 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). Environ. Sci. Technol. 39, 3972–3980.
Guglielmo, C.G., 2010. Move that fatty acid: fuel selection and transport in migratory birds and bats. Integr. Comp. Biol. 50, 336–345.
Guglielmo, C.G., Haunerland, N., Hochachka, P., Williams, T.D., 2002. Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. Am. J. Phys. 282, R1405–R1413.
Guglielmo, C.G., McGuire, L.P., Gerson, A.R., Seewagen, C.L., 2011. Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. J. Ornithol. 152, 75–85.
Harding, K.M., Gowland, J.A., Dillon, P.J., 2006. Mercury concentration in black flies Simulium spp. (Diptera, Simuliidae) from soft-water streams in Ontario, Canada. Environ. Pollut. 143, 529–535.
Hopkins, W.A., Rowe, C.L., Congdon, J.D., 1999. Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. Environ. Toxicol. Chem. 18, 1258–1263.
Jenni-Eiermann, S., 2017. Energy metabolism during endurance flight and the post-flight recovery phase. J. Comp. Physiol. 203, 431–438.
Kasischke, E.S., Turetsky, M.R., 2006. Recent changes in the fire regime across the North American boreal region—spatial and temporal patterns of burning across Canada and Alaska. Geophys. Res. Lett. 33, L09703.
Kobiela, M.E., Cristol, D.A., Swaddle, J.P., 2015. Risk-taking behaviours in zebra finches affected by mercury exposure. Anim. Behav. 103, 153–160.
Kopec, A.D., Bodaly, R.A., Lane, O.P., Evers, D.C., Leppold, A.J., Mittelhauser, G.H., 2018. Elevated mercury in blood and feathers of breeding marsh birds along the contaminated lower Penobscot River, Maine, USA. Sci. Total Environ. 634, 1563–1579.
Krebs, J.R., 1980. Optimal foraging, predation risk and territory defence. Ardea 68, 83–90.
Lighton, J.R.B., 2008. Measuring Metabolic Rates. Oxford Univ Press, New York.
Ma, Y., Branfireun, B.A., Hobson, K.A., Guglielmo, C.G., 2018. Evidence of negative seasonal carry-over effects of breeding ground mercury exposure on survival of migratory songbirds. J. Avian Biol. 49 jav-01656.
McClelland, G., Zwingelstein, G., Taylor, C.R., Weber, J.-M., 1995. Effect of exercise on the plasma nonesterified fatty acid composition of dogs and goats: species with different aerobic capacities and diets. Lipids 30, 147–153.
McFarlan, J.T., Bonen, A., Guglielmo, C.G., 2009. Seasonal upregulation of fatty acid transporters in flight muscles of migratory white-throated sparrows (*Zonotrichia albicollis*). J. Exp. Biol. 212, 2934–2940.
Pacyna, J.M., Travnikov, O., Simone, F.D., Hedgecock, I.M., Sundseth, K., Pacyna, E.G., Steenhuisen, F., Pirrone, N., Munthe, J., Kindbom, K., 2016. Current and future levels of mercury atmospheric pollution on a global scale. Atmos. Chem. Phys. 16, 12495–12511.
Paris, O.J., Swaddle, J.P., Cristol, D.A., 2018. Exposure to dietary methyl-mercury solely during embryonic and juvenile development halves subsequent reproductive success in adult zebra finches. Environ. Sci. Technol. 52, 3117–3124.
Pierce, B.J., McWilliams, S.R., O'Connor, T., Place, A., 2005. Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. J. Exp. Biol. 208, 1277–1285.
Price, E.R., Guglielmo, C.G., 2009. The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis*). Am. J. Physiol. 297, R775–R782.
Ramírez-Bajo, M.J., de Atauri, P., Ortega, F., Westerhoff, H.V., Gelpí, J.L., Centelles, J.J., Cascante, M., 2014. Effects of cadmium and mercury on the upper part of skeletal muscle glycolysis in mice. PLoS One 9 e80018–7.
Rimmer, C.C., McFarland, K.P., Evers, D.C., Miller, E.K., Aubry, Y., Busby, D., Taylor, R.J., 2005. Mercury concentrations in Bicknell's thrush and other insectivorous passerines in montane forests of northeastern North America. Ecotoxicology 14, 223–240.
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, 697–709.
Rogers, C.M., 2015. Testing optimal body mass theory: evidence for cost of fat in wintering birds. Ecosphere 6, 1–12.
Rowe, C.L., Kinney, O.M., Nagle, R.D., Congdon, J.D., 1998. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. Physiol. Zool. 71, 27–35.
Santschi, P.H., Yeager, K.M., Schwehr, K.A., Schindler, K.J., 2017. Estimates of recovery of the Penobscot River and estuarine system from mercury contamination in the 1960's. Sci. Total Environ. 596, 351–359.
Schmidt-Nielsen, K., 1997. Animal Physiology, 5 ed. Cambridge, New York.
Seewagen, C.L., 2010. Threats of environmental mercury to birds: knowledge gaps and priorities for future research. Bird Cons. Int. 20, 112–123.
Seewagen, C.L., 2018. The threat of global mercury pollution to bird migration: potential mechanisms and current evidence. Ecotoxicology (published online 29 August 2018). https://doi.org/10.1007/s10646-018-1971-z.
Seewagen, C.L., Cristol, D.A., Gerson, A.R., 2016. Mobilization of mercury from lean

tissues during simulated migratory fasting in a model songbird. Sci. Rep. 6, 25762.
Smith, R.J., Moore, F.R., 2003. Arrival fat and reproductive performance in a long-distance passerine migrant. Oecologia 134, 325–331.
Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Rad. Biol. Med. 18, 321–336.
Swaddle, J.P., Diehl, T.R., Taylor, C.E., Fanaee, A.S., Benson, J.L., Huckstep, N.R., Cristol, D.A., 2017. Exposure to dietary mercury alters cognition and behavior of zebra finches. Curr. Zool. 63, 213–219.
Travnikov, O., Dastoor, A., Friedman, C., Ryzhkov, A., Selin, N., Song, S., 2013. Global mercury modelling: update of modelling results in the global mercury assessment 2013. In: Arctic Monitoring and Assessment Programme.
Tucker, V., 2005. Respiratory exchange and evaporative water loss in the flying budgerigar. J. Exp. Biol. 48, 67–87.
UNEP Global Mercury Assessment, 2013. UNEP Global Mercury Assessment. UNEP Chemicals Branch, Geneva, Switzerland.
Vaillancourt, E., Weber, J.-M., 2007. Lipid mobilization of long-distance migrant birds in vivo: the high lipolytic rate of ruff sandpipers is not stimulated during shivering. J. Exp. Biol. 210, 1161–1169.
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, e95674.
Whitney, M.C., Cristol, D.A., 2017. Impacts of sublethal mercury exposure on birds: a detailed review. Rev. Environ. Contam. Toxicol. 244, 113–163.
Winder, V.L., Emslie, S.D., 2012. Mercury in Nelson's sparrow subspecies at breeding sites, sites. PLoS One 7, e32257.
Yap, K.N., Kim, O.R., Harris, K.C., Williams, T.D., 2017. Physiological effects of increased foraging effort in a small passerine. J. Exp. Biol. 220, 4282–4291.
Zhang, Y., Jacob, D.J., Horowitz, H.M., Chen, L., Amos, H.M., Krabbenhoft, D.P., Slemr, F., Louis, V.L.S., Sunderland, E.M., 2016. Observed decrease in atmospheric mercury explained by global decline in anthropogenic emissions. Proc. Nat. Acad. Sci, USA 113, 526–531.