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Mercury correlates with altered corticosterone but not testosterone or estradiol concentrations in common loons

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ABSTRACT

We investigated the relation between environmental mercury exposure and corticosterone concentrations in free-living adult common loons (*Gavia immer*). We determined blood and feather mercury concentrations and compared them to testosterone, estradiol, and stress-induced plasma corticosterone concentrations. Although neither testosterone nor estradiol correlated with Hg levels, there was a robust positive relation between blood Hg and stress-induced corticosterone concentrations in males, but not in females. The lack of an effect in females may have been due to overall less contamination in females. There were no significant correlations between feather Hg and stress-induced corticosterone in either sex. To help determine whether Hg had a causal effect on corticosterone, we investigated the impact of experimental Hg intake on the corticosterone stress response in captive juvenile loons. Juveniles were subjected to three different feeding regimes: 0, 0.4 and 1.2 μg Hg (as MeHgCL)/g wet weight (ww) fish. We then measured baseline and 30 min post-solitary confinement stressor corticosterone concentrations. The Hg fed chicks exhibited a decreased ability to mount a stress response. From these data, we conclude that Hg contamination does appear to alter the corticosterone response to stress, but not in a consistent predictable pattern. Regardless of the direction of change, however, exposure to mercury contamination and the resulting impact on the corticosterone stress response in common loons may substantially impact health, fitness and survival.

1. Introduction

Mercury (Hg) contamination of aquatic ecosystems is an important environmental concern and there is an ever-expanding amount of scientific investigation assessing the biological impacts. Mercury is found in aquatic environments both as inorganic Hg and as the more toxic form, organic methylated mercury (MeHg) (Wiener et al., 2003). High trophic level species are at an increased risk for Hg toxicity through bioaccumulation by ingesting contaminated prey. Many of the toxic effects of Hg exposure in birds and other wildlife are well documented (e.g. Wolfe et al., 1998).

There is increasing interest on the impact of Hg on the endocrine system. High Hg levels decrease breeding performance in many avian species (e.g. Goutte et al., 2015, 2014a, 2014b; Tartu et al., 2015a), but not in all (Bustamante et al., 2016). One potential mechanism is that Hg can decrease testosterone and estradiol concentrations (Jayasena et al., 2011), making these reproductive hormones attractive targets for study. Hg has also been shown to affect corticosterone concentrations. However, the impact of HG is not consistent across studies. Some studies show a positive correlation of Hg with corticosterone (e.g. Franceschini et al., 2009; Meillère et al., 2016), some show a negative correlation (e.g. Franceschini et al., 2009; Wada et al., 2009), and some show no correlation (e.g. Pollock and Machin, 2009; Tartu et al., 2015b). The reason for this difference in results is currently unclear.

Piscivorous birds like the common loon (*Gavia immer*) are especially sensitive to Hg toxicity because they are susceptible to bioaccumulation over time and biomagnification through the food chain. Loons are frequently used as a bioindicator species for assessing MeHg availability and risk in freshwater ecosystems (e.g. Barr, 1986; Burgess et al., 2005; Burgess and Meyer, 2008; Depew et al., 2012; Evers et al., 2005, 2003; Kenow et al., 2003, 2007b). Loons are a good study species for this purpose because they occupy a high trophic level, are long-lived, and

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have complex social, reproductive and behavioral requirements that could be affected by contaminant exposure. For example, reproductive problems associated with Hg contamination have been documented in loons (Barr, 1986; Fimreite, 1974; Meyer et al., 1998). One potential mechanism for these reproductive problems is disruption of the reproductive hormones, testosterone and estradiol (Wingfield and Mukai, 2009), which regulate many of the behavioral and physiological pathways necessary for successful reproduction (Wingfield and Silverin, 2009).

Measurement of Hg concentrations in tissues is necessary for confirming exposure to environmental Hg. Numerous studies have focused on the measurement of Hg concentrations in loon tissues and there is an extensive body of literature on Hg exposure in loons (e.g. Burger et al., 1994; Burgess et al., 2005; Evers et al., 1996, 1998, 2003; Meyer et al., 1995, 1998; Scheuhammer et al., 1998). However, in order to demonstrate the impact on wildlife health and for management decision making, it is important to not only determine exposure but also to identify and measure suitable indicators of sub-lethal effects (e.g. Kenow et al., 2007a, 2003, 2010, 2008, 2007b).

The stress response is an essential mechanism for coping with acute adverse conditions (e.g. Blas, 2015; Wingfield and Romero, 2001). If the stress response is chronically activated or its normal functioning altered, then there can be dangerous impacts on body functions, health and survival. Within minutes after exposure to a stressful stimulus, the adrenocortical component of the stress response is activated and glucocorticoids (GCs, e.g. corticosterone in birds, cortisol in humans) are released into the bloodstream via the hypothalamic-pituitary-adrenal (HPA) or -interrenal (HPI) axis. Analysis of relative stress levels between individual animals and animal populations within a species can be evaluated by measuring GC concentrations (Cockrem, 2005; Cockrem and Silverin, 2002). GCs are involved in many vital body processes including glucose metabolism, reproduction, growth, tissue repair, musculoskeletal health and immune, cardiovascular and neurologic function (Romero and Wingfield, 2016; Sapolsky et al., 2000). Therefore, GC concentrations can provide information about the health of an animal and potentially the relative health of animal populations. Abnormal alteration of GC synthesis or regulation can have a negative impact on multiple physiological processes affecting health, survival and fitness (e.g. Romero et al., 2009).

Only a limited number of studies in birds have explored the impact of Hg on the adrenocortical stress response or the reproductive hormones, and their results have not been consistent. Consequently, we tested the hypothesis that Hg would correlate with changes in these hormones using two different tissue levels of Hg – plasma and feathers. We further tested whether the correlation of plasma Hg with stress-induced corticosterone was potentially causal by feeding Hg directly to loon chicks. Identifiable alterations in the adrenocortical stress response or testosterone or estradiol concentrations associated with subacute Hg exposure have the potential to serve as biomarkers of Hg impact on wildlife health. For the reasons described above, loons are an excellent model species for studying Hg effects.

2. Materials and methods

Adult breeding loons (n = 410, 189 females and 222 males) were captured on their breeding grounds from lakes and ponds throughout northern New England, northern New York, parts of the Midwest U.S., and southern Canada, using night-lighting coupled with vocalizations, between 1996 and 2001. Mercury concentrations were measured in blood and feathers. Secondary flight feathers were collected for Hg analysis by cutting the calamus (i.e. below the base of the vane). Blood was drawn from the metatarsal vein for circulating plasma corticosterone, estradiol and testosterone, as well as whole blood Hg concentrations. Laboratory procedures for analysis of Hg in blood and feathers are described in Evers et al. (1998). All blood Hg concentrations are in (μg/g) wet weight (ww) and μg/g fresh weight for feather Hg.

Following capture, blood for corticosterone analysis was collected just prior to release which was between 20 and 30 min post-capture in > 96% of the individuals sampled. Therefore, corticosterone concentrations are considered stress-induced levels and are reflective of the animal's ability to mount a physiological stress response in response to capture and handling. The variability in time of blood collection for corticosterone analysis is unlikely to have a significant impact on the results due to the large sample sizes (n = 410) and lack of directional bias. Baseline samples (collected within 3 min of capture) were not collected for wild loons.

At the U.S. Geological Survey Upper Midwest Environmental Sciences Center in La Crosse, Wisconsin, blood samples for corticosterone analysis were collected from captive loon chicks as part of a larger study investigating the health effects of chronic consumption of dietary Hg (e.g. Kenow et al., 2007a, 2003, 2010, 2008, 2007b). Common loon eggs were collected from northern Wisconsin in 2003 at about 22–24 days incubation from low (< 6.3) pH lakes where loon Hg exposure is known to be high and neutral (> 6.3) pH lakes where exposure is known to be low. One egg was randomly collected from each nest as authorized in U.S. Fish and Wildlife Service Scientific Collecting Permit MB030466-0 and Wisconsin Department of Natural Resources Permit SCP-WCR-15-D-03. The collection site in northern Wisconsin has been a research site for loons and Hg since 1992 (Meyer et al., 1995, 1998). Details concerning the source, collection, and incubation of eggs along with chick husbandry have been provided previously by Kenow et al. (2003).

Chicks were assigned to one of three dietary treatment groups and fed rainbow trout (*Oncorhynchus mykiss*) containing a daily dose of methylmercury chloride (MeHgCl) (Crescent Chemical Co., Inc., Islanida, New York) in a gelatin capsule beginning at one day of age and continuing through to the completion of the experiment. Daily food intake was documented for each chick, and the daily MeHgCl dose was based on total food consumption from the previous day. One group served as a control and was fed a diet containing no added Hg, and treatment groups received a fish diet containing either 0.4 or 1.2 μg Hg (delivered as MeHgCl)/g wet-weight fish. Background levels of Hg in representative samples of forage fish was determined to be 0.0206 ± 0.005 μg/g wet weight (n = 5 samples; ± standard error) and was assumed to be the Hg concentration in the diet of control chicks.

Samples for corticosterone analysis were collected when chicks were 42 days old (see Kenow et al., 2007b). The chicks were well habituated to humans so baseline samples were collected within 3 min of handling but not within 3 min of entering the enclosure. Following baseline sample collection, each chick was placed in solitary confinement as a stressor and re-sampled after 30 min to reflect stress induced corticosterone levels. Blood samples were stored on ice and plasma separated within 2 h of collection. Plasma samples were then stored at −70 °C until shipped to Tufts University for radioimmunoassay (RIA) analysis.

For free-living adult loons, blood samples were stored on ice in the field, centrifuged within 12 h and the plasma separated and frozen at −4 °C until assayed. For corticosterone analysis, plasma samples from wild and captive loons were assayed by RIA following the methods of Wingfield et al. (1992). Briefly, tritiated corticosterone was added to all plasma samples for determination of recoveries. Following equilibration, samples were extracted with redistilled dichloromethane. The supernatant extracts were dried and resuspended in phosphate buffer for assay. Dextran-coated charcoal was used to separate bound from unbound steroid. All samples were assayed in duplicate and compared to a standard curve, with concentrations adjusted by the recovery and reported as ng/ml of plasma.

We also determined plasma testosterone and estradiol concentrations in the adult loons. Radioimmunoassays followed the same basic protocol described for corticosterone, substituting the appropriate tritiated hormone and antibody. For all hormone assays, inter-assay