**Mercury Correlates With Altered Corticosterone but not Testosterone
or Estradiol Concentrations in Common Loons**

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28 Abstract

29

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

51

52 Keywords: Corticosterone, Mercury, Loon, Piscivorous bird, Stress

53 Introduction

54

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

63 There is increasing interest on the impact of Hg on the endocrine system.
64 High Hg levels decrease breeding performance in many avian species (e.g.
65 Goutte et al., 2015; Goutte et al., 2014a; Goutte et al., 2014b; Tartu et al.,
66 2015a), but not in all (Bustamante et al., 2016). One potential mechanism is that
67 Hg can decrease testosterone and estradiol concentrations (Jayasena et al.,
68 2011), making these reproductive hormones attractive targets for study. Hg has
69 also been shown to affect corticosterone concentrations. However, the impact of
70 HG is not consistent across studies. Some studies show a positive correlation of
71 Hg with corticosterone (e.g. Franceschini et al., 2009; Meillère et al., 2016), some
72 show a negative correlation (e.g. Franceschini et al., 2009; Wada et al., 2009),
73 and some show no correlation (e.g. Pollock and Machin, 2009; Tartu et al.,
74 2015b). The reason for this difference in results is currently unclear.

75 Piscivorous birds like the common loon (*Gavia immer*) are especially
76 sensitive to Hg toxicity because they are susceptible to bioaccumulation over
77 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; Evers et al., 2003; Kenow et al., 2003; Kenow et al., 2007b). Loons are a good study species for this purpose because they occupy a high trophic level, are long-lived, and 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; Evers et al., 1998; Evers et al., 2003; Meyer et al., 1995; Meyer et al., 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; Kenow et al., 2003; Kenow et al., 2010; Kenow et al., 2008; Kenow et al., 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

103 be dangerous impacts on body functions, health and survival. Within minutes
104 after exposure to a stressful stimulus, the adrenocortical component of the stress
105 response is activated and glucocorticoids (GCs, e.g. corticosterone in birds,
106 cortisol in humans) are released into the bloodstream via the hypothalamic-
107 pituitary-adrenal (HPA) or -interrenal (HPI) axis. Analysis of relative stress levels
108 between individual animals and animal populations within a species can be
109 evaluated by measuring GC concentrations (Cockrem, 2005; Cockrem and
110 Silverin, 2002). GCs are involved in many vital body processes including glucose
111 metabolism, reproduction, growth, tissue repair, musculoskeletal health and
112 immune, cardiovascular and neurologic function (Romero and Wingfield, 2016;
113 Sapolsky et al., 2000). Therefore, GC concentrations can provide information
114 about the health of an animal and potentially the relative health of animal
115 populations. Abnormal alteration of GC synthesis or regulation can have a
116 negative impact on multiple physiological processes affecting health, survival and
117 fitness (e.g. Romero et al., 2009).

118 Only a limited number of studies in birds have explored the impact of Hg
119 on the adrenocortical stress response or the reproductive hormones, and their
120 results have not been consistent. Consequently, we tested the hypothesis that
121 Hg would correlate with changes in these hormones using two different tissue
122 levels of Hg – plasma and feathers. We further tested whether the correlation of
123 plasma Hg with stress-induced corticosterone was potentially causal by feeding
124 Hg directly to loon chicks. Identifiable alterations in the adrenocortical stress
125 response or testosterone or estradiol concentrations associated with subacute
126 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.

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 ($\mu\text{g/g}$) wet weight (ww) and $\mu\text{g/g}$ 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.

152 At the U.S. Geological Survey Upper Midwest Environmental Sciences
153 Center in La Crosse, Wisconsin, blood samples for corticosterone analysis were
154 collected from captive loon chicks as part of a larger study investigating the
155 health effects of chronic consumption of dietary Hg (e.g. Kenow et al., 2007a;
156 Kenow et al., 2003; Kenow et al., 2010; Kenow et al., 2008; Kenow et al., 2007b).
157 Common loon eggs were collected from northern Wisconsin in 2003 at about 22-
158 24 days incubation from low (<6.3) pH lakes where loon Hg exposure is known to
159 be high and neutral (>6.3) pH lakes where exposure is known to be low. One
160 egg was randomly collected from each nest as authorized in U.S. Fish and
161 Wildlife Service Scientific Collecting Permit MB030466-0 and Wisconsin
162 Department of Natural Resources Permit SCP-WCR-15-D-03. The collection site
163 in northern Wisconsin has been a research site for loons and Hg since 1992
164 (Meyer et al., 1995; Meyer et al., 1998). Details concerning the source, collection,
165 and incubation of eggs along with chick husbandry have been provided
166 previously by Kenow et al. (2003).

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

5 samples; $\pm$ 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 minutes 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 hours 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 hr 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 variability and intra-assay variability were <8% and <12% respectively.

To investigate the relation between Hg exposure (blood and feather) and stress-induced (capture and handling) corticosterone concentrations in wild adult loons, we performed separate analysis of variances (ANOVAs) for blood and feather Hg using sex, site of capture, and date of capture as factors. There were no significant main effects or interactions of site and date of capture, so these factors were removed and the ANOVA run again. Although there was no main effect of sex or sex-by-corticosterone interaction, there was a sex-by-Hg interaction, so males and females were analyzed separately. For each sex, we then ran a linear regression. Although this provided an overall estimate of the correlation between corticosterone and Hg exposure, it could not test whether there was a threshold of exposure. To test for the presence of a threshold, corticosterone concentrations were grouped based on binned Hg levels and compared by ANOVA with post-hoc Tukey tests for pair-wise comparisons. Sex steroid concentrations (testosterone and estradiol) were compared to Hg exposure in the same way, except that the analyses were conducted on a subgroup of 242 adult loons due to insufficient plasma from all animals.

For captive juvenile loons, repeated measures ANOVA was used to compare baseline and stress induced concentrations to Hg treatment groups and source of eggs (low vs. neutral pH lakes). Sample sizes were too small to include sex in the model. All statistical analyses were conducted using JMP 6.

Results

227 For wild adult loons, there was no overall difference in mean stress
228 induced (capture and handling) corticosterone concentrations between males
229 and females (males: 37.02 ± 1.19 ng/ml, $n=222$; females: 34.14 ± 1.29 ng/ml,
230 $n=189$; $F_{1,409}=2.68$, $p=0.10$). Mean blood Hg was significantly higher in males
231 than in females (males: 2.24 ± 0.1 ww $\mu\text{g/g}$, $n=222$; females: 1.79 ± 0.10 ww $\mu\text{g/g}$,
232 $n=189$; $F_{1,409}=10.04$, $p=0.0016$). Mean feather Hg was also significantly higher in
233 males than in females (males: 15.00 ± 0.45 , $n=221$; females: 9.89 ± 0.49 , $n=190$;
234 $F_{1,406}=58.40$, $p<0.0001$).

235 In adult males there was a positive correlation between blood Hg and
236 corticosterone ($F_{1,221}=5.75$, $p<0.02$, $r^2=0.02$) and there appeared to be a
237 threshold ($F_{4,217}=3.47$, $p=0.009$; Fig.1). The group with the lowest Hg
238 concentrations (0-1 ww $\mu\text{g/g}$) had the lowest corticosterone concentrations
239 compared to all but the next highest Hg group (1-2 ww $\mu\text{g/g}$). The group with the
240 highest corticosterone concentrations was the second highest Hg group (3-4 ww
241 $\mu\text{g/g}$) and was not significantly different from the next lowest (2-3 ww $\mu\text{g/g}$) or
242 highest (>4 ww $\mu\text{g/g}$) Hg groups. There were no significant differences detected
243 in corticosterone concentrations in females ($F_{1,188}=1.69$, $p=0.20$, $r^2=0.004$) and
244 there was no indication of a threshold ($F_{4,184}=0.95$, $p=0.44$; Fig. 1).

245 There was no correlation between feather Hg and stress induced
246 corticosterone in adult males ($F_{1,221}=1.92$, $p=0.17$, $r^2=0.009$) and there was no
247 evidence of a threshold ($F_{3,216}=0.17$, $p=0.92$; Fig. 2). There was a slight correlation
248 between feather Hg and stress induced corticosterone in females that explained
249 very little of the variation ($F_{1,188}=4.22$, $p<0.42$, $r^2=0.022$), and there was no
250 evidence of a threshold ($F_{3,184}=2.2$, $p=0.09$; Fig. 2).

For captive juveniles, there was an overall effect of Hg treatment on corticosterone ($F_{2,12}=5.21$, $p=0.02$, Fig. 3), an overall effect of the isolation stressor ($F_{1,12}=28.86$, $p=0.0002$, Fig. 3) and a significant interaction between Hg treatment and the increase in corticosterone between baseline and 30 min ($F_{2,12}=6.22$, $p=0.014$, Fig. 3). There was no significant effect of source lake pH where the eggs were collected or interactions between the stress response or Hg treatment and lake pH ($p>0.05$ for all comparisons). Blood Hg concentrations of the captive-reared loon chicks by dose group are summarized in Kenow et al. (2007).

Neither testosterone nor estradiol concentrations correlated with Hg levels ($p>0.05$ for all comparisons).

Discussion

The two major results from our study are a positive correlation between blood Hg and corticosterone in wild male loons (Fig. 1) and lower stress-induced corticosterone concentrations in chicks fed MeHg (Fig. 3). We did not find any relation between corticosterone and feather Hg. Feather Hg reflects Hg exposure levels at the time of feather growth in the winter so does not reflect Hg exposure at the time of sampling for plasma corticosterone during breeding. This likely explains the different findings for blood versus feather Hg. The significant findings for adult males but not females may be due to the higher mercury levels found in the males.

A number of studies in a variety of species show a relation between changes in glucocorticoid concentrations and chronic exposure to environmental

contaminants, both organic pollutants and heavy metals, including Hg. Most studies are in fish and amphibians, where there seems to be a common predictable pattern of alteration. Generally, chronic contaminant exposure in fish and amphibians results in an inhibited stress response to both capture stress and ACTH administration (e.g. Gendron et al., 1997; Hontela, 1998; Hontela et al., 1997; Hontela et al., 1995; Hontela et al., 1992; Hopkins et al., 1999; Norris et al., 1999). This is opposite the effect we found in male adult loons, but similar to the effect we found in chicks.

Unlike the fish and amphibian literature, studies conducted in birds do not point to a consistent pattern between contaminant exposure and short-term glucocorticoids. For example, some studies on the relationship between PCBs and corticosterone in tree swallow (*Tachycineta bicolor*) nestlings found no correlations (e.g. Martinovic et al., 2003) whereas others report elevated stress induced plasma corticosterone at the more contaminated sites and an inverted-U association with PCBs in adult females (e.g. Franceschini et al., 2008). On the other hand, controlled studies in nestlings of other species show decreased corticosterone in response to crude oil exposure (e.g. Gorsline and Holmes, 1982; Holmes et al., 1979; Lattin et al., 2014; Miller et al., 1978).

There are a number of investigations of the impact of Hg on circulating GC concentrations in birds and, as with the other contaminants, there is not a consistent pattern. Decreased baseline corticosterone, but not stress induced, was associated with blood Hg in adult and nestling tree swallows (Franceschini et al., 2009) and Forster's tern chicks (*Sterna forsteri*) (Herring et al., 2012), but another study showed increased baseline with decreased stress-induced corticosterone in nestling tree swallows (Wada et al., 2009). In contrast, zebra

finches (*Taeniopygia guttata*) fed Hg showed no change in baseline but a reduction in stress-induced corticosterone (Moore et al., 2014). Research investigating the relation between Hg, other trace metal concentrations and corticosterone in northern common eiders (*Somateria mollissima borealis*) show inconsistent results for selenium and cadmium but no relation with Hg (Wayland et al., 2002; Wayland et al., 2003), although there was a nearly significant decrease in baseline corticosterone with higher Hg in females in another study (Provencher et al., 2016). This is similar to the results in females in our study. Furthermore, no relation was seen between corticosterone and blood Hg in bald eagle (Bowerman et al., 2002), great egret (*Ardea alba*) (Herring et al., 2014) or white ibis (*Eudocimus albus*) (Herring et al., 2014) nestlings, nor in black-legged kittiwake (*Rissa tridactyla*) (Tartu et al., 2015b) or snow petrel (*Pagodroma nivea*) (Tartu et al., 2015a) adults. There was also no correlation between corticosterone and feather Hg in adult white ibises (*Eudocimus albus*) (Heath and Frederick, 2005), but feather Hg and feather corticosterone were positively correlated in blackbirds (*Turdus merula*) (Meillère et al., 2016). These results are all consistent with the lack of significant findings for adult female loons in the present study but not for the adult males or juveniles.

Based on the available literature and our data, Hg appears to impact corticosterone concentrations in birds, but without a predictable and consistent pattern. The difference we found between the adult male and female wild loons, and the further difference between captive chicks, emphasizes some of the variation seen in contaminant studies investigating the impact on corticosterone. One explanation for the variety of reported results is that it is essentially impossible to find individual contaminants in isolation in the environment. For

example, it has been estimated that 30,000 different chemicals, including some believed to be toxic to birds, enter the Great Lakes (Fox and Weseloh, 1987). Polluted sites generally contain mixtures of numerous contaminants which may interact in ways not predictable based on individual chemical concentrations (Becker, 2003). These chemicals are likely to have different individual and combined effects on corticosterone synthesis and regulation. For example, the relationship between Hg and corticosterone was found to vary with cadmium concentrations in greater and lesser scaup (*Aythya marila* and *A. affinis*) (Pollock and Machin, 2009). Furthermore, these mixtures of contaminants might impact corticosterone regulation and/or synthesis stronger in one sex (e.g. males in our study) than the other.

Other unknown environmental and physiologic variables that influence the corticosterone stress response (e.g. food availability, weather, predators, illness) may have a combined effect with contaminants, influencing corticosterone concentrations. These unknown variables may have an additive effect with Hg on the corticosterone stress response and may also differentially influence males. This additive effect of stressors is known to occur under laboratory conditions and is hypothesized to occur under field conditions (McEwen and Wingfield, 2003; Romero, 2004; Romero et al., 2009). Consequently, the inability to control for all these variables makes comparisons between field studies very difficult. Other possible confounding factors are the variation in capture and holding times prior to blood collection and the lack in this study of detailed information of breeding stage, although neither factor is likely to have a significant impact in the present study due to the robust sample sizes. Finally, altered stress-induced glucocorticoid concentrations are thought to impact health

and survival, but this is still an area of active research (Romero and Wingfield, 2016).

Captive studies, like the one we conducted with juvenile loons, attempt to eliminate this problem by controlling exposure to multiple contaminants, but do not mimic the reality of wild birds living in polluted sites. In our captive study, stress induced corticosterone concentrations in controls were higher than in Hg fed chicks, suggestive of an impaired corticosterone response to stress (Fig. 3). Since the corticosterone concentrations from the wild loons are stress-induced, the captive and wild results are inconsistent. The difference may be partly due to age. Contaminant exposure during growth and development may have a greater and/or different impact on the corticosterone stress response, the HPA axis and survival than during adulthood. It is difficult to determine specifically how the impact may differ in adults and young, especially since other contributing environmental and physiologic factors may affect age groups differently. However, similar results were found in zebra finches fed Hg, with older adults showing a decrease in stress induced corticosterone (Moore et al., 2014) but younger adults showing no changes (Maddux et al., 2015).

Interestingly, the inhibited stress response seen in the captive loons fed Hg is consistent with the pattern seen in fish and amphibians (e.g. Gendron et al., 1997; Hontela, 1998; Hontela et al., 1997; Hontela et al., 1995; Hontela et al., 1992; Hopkins et al., 1999; Norris et al., 1999). It is possible that this pattern emerges when other environmental and physiologic variables are controlled for, preventing an additive influence on glucocorticoid synthesis and regulation. However, similar to our results in males, contaminant-induced increases in the glucocorticoid stress response have been documented in other species. For

example, corticosterone concentrations were elevated in marine iguanas (*Amblyrhynchus cristatus*) exposed to an oil spill (Wikelski et al., 2001) and in tree swallows (*Tachycineta bicolor*) living in PCB contaminated habitats (Franceschini et al., 2008).

In conclusion, these results suggest that chronic Hg exposure is impacting corticosterone synthesis and/or regulation in common loons. Additional research should focus on trying to identify the mechanism of Hg influence on corticosterone synthesis, secretion and regulation, which may shed light on why the field and captive studies showed such different results. However, an altered stress response, regardless of whether it is increased or decreased, is likely to have a physiological impact that substantially affects survival by altering the animal's ability to cope with stressful situations. For example, Hg contamination can alter escape performances and disrupt molt (Carlson et al., 2014), change singing behavior (Hallinger et al., 2010), inhibit immune function (Hawley et al., 2009), and decrease fledging success (Hallinger and Cristol, 2011). The next step should be to focus on the nature and extent of the impact on overall health and fitness. Integrative field studies combining Hg levels, corticosterone concentrations and definitive indicators of survival and fitness are needed to better assess the usefulness of corticosterone as a biomarker and the extent of impact of Hg pollution.

Compliance with Ethical Standards

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Conflict of Interest: The authors declare that they have no conflict of interest

402 Ethical Approval: All applicable international, national, and institutional guidelines
403 for the care and use of animals were followed. All procedures involving
404 animals were in accordance with the ethical standards of the institution at
405 which the studies were conducted.

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Figure Legends:

Figure 1. Comparison of blood Hg concentrations with plasma stress-induced corticosterone concentrations in (A) male and (B) female adult wild common loons. Upper panels are linear regressions and lower panels are data binned by blood Hg concentrations to test for a threshold. Each bar represents the mean $\pm$ se corticosterone concentration for the sample sizes indicated inside each bar. Different letters represent significantly different concentrations using Tukey's post-hoc test.

Figure 2. Comparison of feather Hg concentrations with plasma stress-induced corticosterone concentrations in (A) male and (B) female adult wild common loons. Upper panels are linear regressions and lower panels are data binned by feather Hg concentrations to test for a threshold. Each bar represents the mean $\pm$ se corticosterone concentration for the sample sizes indicated inside each bar.

Figure 3. Common loon chicks subjected to three different feeding regimes: control, 0.4, and 1.2 Hg (delivered as MeHgCl)/g wet weight (ww) fish. At 42 days old, baseline samples were collected within 3 min of handling and stress-induced samples were collected following 30 min of solitary confinement. Each bar represents the mean $\pm$ se corticosterone concentration for the sample sizes indicated inside each bar.

* indicates $p < 0.05$ compared to stress-induced corticosterone from other treatments.

Fig 1

Fig 2

Fig 3