Endocrine disruption in white ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury

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ABSTRACT

Methylmercury is a globally distributed pollutant and upper trophic level aquatic fauna are at particularly high risk of exposure. Although methylmercury is known to have a number of neurological and developmental effects, relatively little is known about effects on endocrine disruption and reproduction in aquatic fauna, particularly in response to chronic exposure at low concentrations. We experimentally exposed captive white ibises for 3.5 years (2005–2008) to dietary methylmercury at three environmentally relevant concentrations (0.05, 0.1 and 0.3 ppm wet weight in diet). We measured fecal concentrations of estradiol and testosterone metabolites in two consecutive breeding seasons (2007 and 2008). When effects were controlled for stage of breeding, this resulted in altered estradiol and testosterone concentrations in adult breeders of both sexes. Changes in endocrine expression were not consistent over both years, and a clear dose–response relationship was not always present. Endocrine changes were, however, associated at all dose levels with changes in reproductive behavior, reduced reproductive success and altered mate choice in males. Male–male pairing and altered courtship behavior in males were related both to dose treatment and, in 2008, to a demasculinized pattern of endocrine expression. Changes in hormone concentrations of dosed homosexually paired males, when present, were in the same direction but at a higher magnitude than those in heterosexual dosed males. Dosed homosexual males showed decreased testosterone during nest-building and elevated testosterone during incubation when compared with their dosed heterosexual counterparts during the 2008 breeding season. In the same year, exposed males had elevated estradiol during courtship, but had decreased estradiol during other stages in comparison with controls. Dosed females generally showed decreased estradiol and testosterone concentrations compared to controls, albeit not with a clear dose–response effect. Our findings suggest that endocrine disruption due to chronic exposure to even low concentrations of dietary methylmercury may be a widespread mechanism by which reproduction is impaired in wild bird populations.

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1. Introduction

Mercury (Hg), a globally distributed pollutant, is known to have adverse effects on the health of many wildlife species (Scheuhammer et al., 2009; Tan et al., 2009; Wolfe et al., 1998). Wetlands and other aquatic environments often display conditions conducive for the conversion of Hg to methylmercury (CH_3Hg, MeHg hereafter; Gilmour et al., 1992; Zillioux et al., 1993) which is the more potent and bioaccumulative form of mercury. Complex food webs typical of aquatic environments also lead to high bioaccumulative potential, such that higher trophic level organisms

are typically at higher risk for MeHg exposure in these ecosystems (Scheuhammer et al., 2009). Though less well documented than its neurotoxic and embryotoxic effects, Hg and MeHg are also known to have endocrine effects (Drevnick and Sandheinrich, 2003; Franceschini et al., 2009; Tan et al., 2009; Wada et al., 2009). Since Hg can affect the hypothalamo-pituitary-gonadal (HPG) hormonal system (Tan et al., 2009) disruption of this axis could adversely affect reproduction (Ottinger et al., 2009).

Although there are relatively few studies on MeHg-induced endocrine disruption in aquatic biota, studies in fathead minnows (*Pimephales promales*), have demonstrated adverse effects on both reproductive success and sex hormones (Drevnick and Sandheinrich, 2003). Spawning was absent in the group exposed to MeHg at 3.93 ppm dry weight (dw) via diet and significantly lowered in the group dosed at 0.87 ppm dw. Importantly, growth and survival of minnows were not affected in this study although testosterone and estradiol concentrations were markedly lowered at both

exposure levels. Endocrine and reproductive endpoints therefore may be more sensitive indicators of adverse effects of MeHg than survival and growth.

Several studies of birds show MeHg effects on aspects of reproduction known to be mediated via endocrine pathways (Nelson, 2005). Increased nest abandonment, poor nest attendance, and reduced fledgling production have been recorded in wild common loons (*Gavia immer*; Barr, 1986; Evers et al., 2008; Nocera and Taylor, 1998). In mallards (*Anas platyrhynchos*) fed MeHg at 0.5 ppm dw over three generations, a greater percentage of dosed females laid eggs outside the nest (Heinz, 1979). However, there is also some evidence that low doses of methylmercury may have a positive effect upon hatching rate (Heinz et al., 2010).

We used white ibises (*Eudocimus albus*) as a model for understanding endocrine effects of MeHg exposure. In wild ibises, feather Hg levels were negatively correlated with estradiol in females and positively correlated with testosterone in males (Heath and Frederick, 2005). The same study showed a negative correlation between inter-annual differences in mercury exposure and numbers of white ibises nesting, suggesting the possibility that MeHg exposure may contribute to poor nesting success or increased abandonment. However, many variables including hydrology may have contributed to nesting success and nesting numbers during these years. We hypothesized that chronic MeHg exposure at environmentally relevant levels results in reproductive impairment of ibises via endocrine disruption. Elsewhere we have shown experimentally that MeHg exposure in white ibises at environmentally relevant levels resulted in high levels of male–male pairing (to 55% of males) that resulted in a 13–15% average reduction in egg production (Frederick and Jayasena, 2011). In this study, we report on experimental effects of MeHg on sex steroids, estradiol and testosterone, and on the relationship between reproductive behavior and endocrine expression in dosed birds.

2. Materials and methods

2.1. Dietary methylmercury exposure of captive white ibises

This study used the same experimental procedures and birds as reported in Frederick and Jayasena (2011). White ibis nestlings collected from a breeding colony in the Florida Everglades in spring 2005 were randomly assigned to one of four groups of 20 males and 20 females. Birds were individually identifiable by leg-bands, genetically sexed (Avian Biotech International, Tallahassee, Florida), and housed in a $1200\,m^2$ circular free-flight aviary with the four exposure groups separated by net-walls. Dosing was started at 90 days of age and continued throughout the experiment (2005–2008). Birds received 0.00 (control/C), 0.05 (low/L), 0.1 (medium/M) or 0.3 (high/H) ppm ww MeHg in food, introduced to pelletized food via spraying in a corn oil vehicle.

2.2. Behavioral sampling during the breeding season

Reproductive behavior and success was observed in February to August of 2006, 2007 and 2008. Nesting was encouraged by providing nesting structures in the perches (total of 48 platforms per experimental group, eight nests per perch) and an *ad libitum* supply of nesting material in the form of twigs (*Quercus* spp.) and fresh cattail (*Typha* sp.). Identities of birds displaying, nest-building, laying, incubating or chick-rearing were recorded daily shortly after sunrise throughout the breeding season. Each nest platform was inspected for presence/absence of a nest and if present, the status of the nest (i.e. few sticks/partial nest/full nest). The number of eggs and/or chicks and the pair associated with the nest were recorded, individually marked and tracked till hatching, fledging or death.

Observers and caretakers were blinded to the identity of treatment groups.

2.3. Collection of fecal samples

We measured hormone metabolite concentrations of estradiol and testosterone in individually identified fecal samples collected during breeding seasons in 2007 and 2008. Fecal samples were identified by feeding individual birds with bait fish stuffed with glass beads of different colors (sizes 18/0 or 15/0). Baits were fed between 09:00 and 11:00 h, and samples collected 3–4 h after feeding to control for diurnal variability of hormone concentrations. Fresh feces were collected from clean floor areas or from 0.5 to $2\,m^2$ framed panels of clean plastic sheeting placed beneath perches. Samples were collected into 2 ml polypropylene cryotubes (Fisher Scientific), placed on ice immediately, stored temporarily for up to 8 h in a $-4\,°C$ freezer, and transferred to a $-20\,°C$ freezer for longer term storage.

The reproductive cycle was divided into six stages: pre-breeding (only in 2007), display, nest-building, egg-laying, incubation and chick-rearing and birds were repeatedly sampled in each stage. Homosexually paired males (Frederick and Jayasena, 2011) had no eggs to incubate or young to raise, thus, the period following completion of nest-building was defined as the incubation stage for these individuals. There were no female–female pairs.

2.4. Radioimmunoassays for steroid hormone metabolites in fecal extracts

Fecal samples were freeze-dried, homogenized, and 0.05 g of each sample used for extraction (by 80% ethanol) of steroid hormone metabolites (Adams et al., 2009). Fecal extracts were stored in a $-20\,°C$ freezer until analysis for steroid hormones by radioimmunoassay (RIA).

Each sample extract was assayed for estradiol and testosterone metabolites following RIA methods previously validated for these hormones in white ibis fecal extracts (Adams et al., 2009). Estradiol ^{125}I Coat-a-Count RIA kits (Diagnostic Products, Los Angeles, CA, USA) were used for measuring estradiol metabolites. Testosterone ^{125}I double-antibody RIA kits (MP Biomedicals, Solon, OH, USA) were used to measure testosterone metabolites. Samples were analyzed in duplicate, and those with a coefficient of variation more than 15% were reanalyzed (if there was sufficient sample for reanalysis) or discarded. Hormone concentrations from RIA assays were corrected for mean extraction efficiency in 80% ethanol (estradiol – 77%; testosterone – 64%; Adams et al., 2009). The intra-assay coefficients of variation (CV) for estradiol assays ranged between 1.9% and 4.4% in 2007 and between 2.6% and 4.0% in 2008. The inter-assay CV for estradiol was 16.9% in 2007 and 8.9% in 2008. The intra-assay CV for testosterone assays ranged between 2.1% and 5.6% in 2007 and between 4.2% and 6.7% in 2008. The inter-assay CV for testosterone was 11.7% in 2007 and 12.9% in 2008.

2.5. Statistical analyses of hormone concentrations

R version 2.11.1 (R Development Core Team, 2010) was used for statistical analyses. Natural logarithms of hormone concentrations (testosterone as nanograms per gram feces dw and estradiol as picograms per gram feces dw) were analyzed separately by sex and year. Heterogeneous variances (Bartlett's test; $P < 0.05$) by breeding stage, treatment, and type of pairing behavior (for males: heterosexual/homosexual) when present, were accounted for by a variance function (Pinheiro and Bates, 2000). We used extended linear models with a correlation structure to account for repeated samples of individuals within a breeding season (Pinheiro and Bates, 2000). Models were estimated using generalized least

squares methods with maximum likelihood using the nlme package in R (Pinheiro et al., 2009).

All predictor variables were determined *a priori* (breeding stage, MeHg treatment group, type of pairing behavior for males) and all possible combinations of interactions were used in the full models to test hypotheses. In females and heterosexual males we tested the effects of MeHg treatment and breeding stage on hormone concentrations. In dosed males, we tested type of pairing behavior (homosexual/heterosexual) as well as MeHg treatment and breeding stage on hormone concentrations. In all models non-significant interaction terms ($P > 0.05$; conditional F-test) were removed to simplify interpretation of parameters (Pinheiro and Bates, 2000). Model fit was not significantly decreased by this simplification as indicated by likelihood ratio tests. We report results of conditional F-tests (F-tests) which test the significance of explanatory variables that could include several coefficients (e.g. the explanatory variable for MeHg effects has coefficients for each dose group) and of conditional t-tests (t-tests) which test the marginal significance of each separate coefficient when all other coefficients are present in the model (Pinheiro and Bates, 2000).

Residual plots and quantile–quantile plots of the models were examined to see whether data conformed to the assumption of normality.

3. Results

3.1. Effects of methylmercury on estradiol in females

Mean feather and blood mercury levels in each treatment group are shown in Table 1. Breeding stage was a significant predictor of estradiol concentrations in both years (Table 2). Treatment (MeHg) effects on estradiol concentrations of females were only seen in 2007, when the medium dose group had lower estradiol than control females ($t = -2.8$, $P = 0.006$). There were no significant differences between control and low ($t = 0.7$, $P = 0.47$) or control and high ($t = -1.3$, $P = 0.18$) dose groups.

In 2008, overall MeHg effects on estradiol in females were non-significant (Table 2). However, low and medium groups showed non-significant trends towards reduced estradiol compared to control (Low: $t = -1.81$, $P = 0.07$; Medium: $t = -1.76$, $P = 0.08$). The
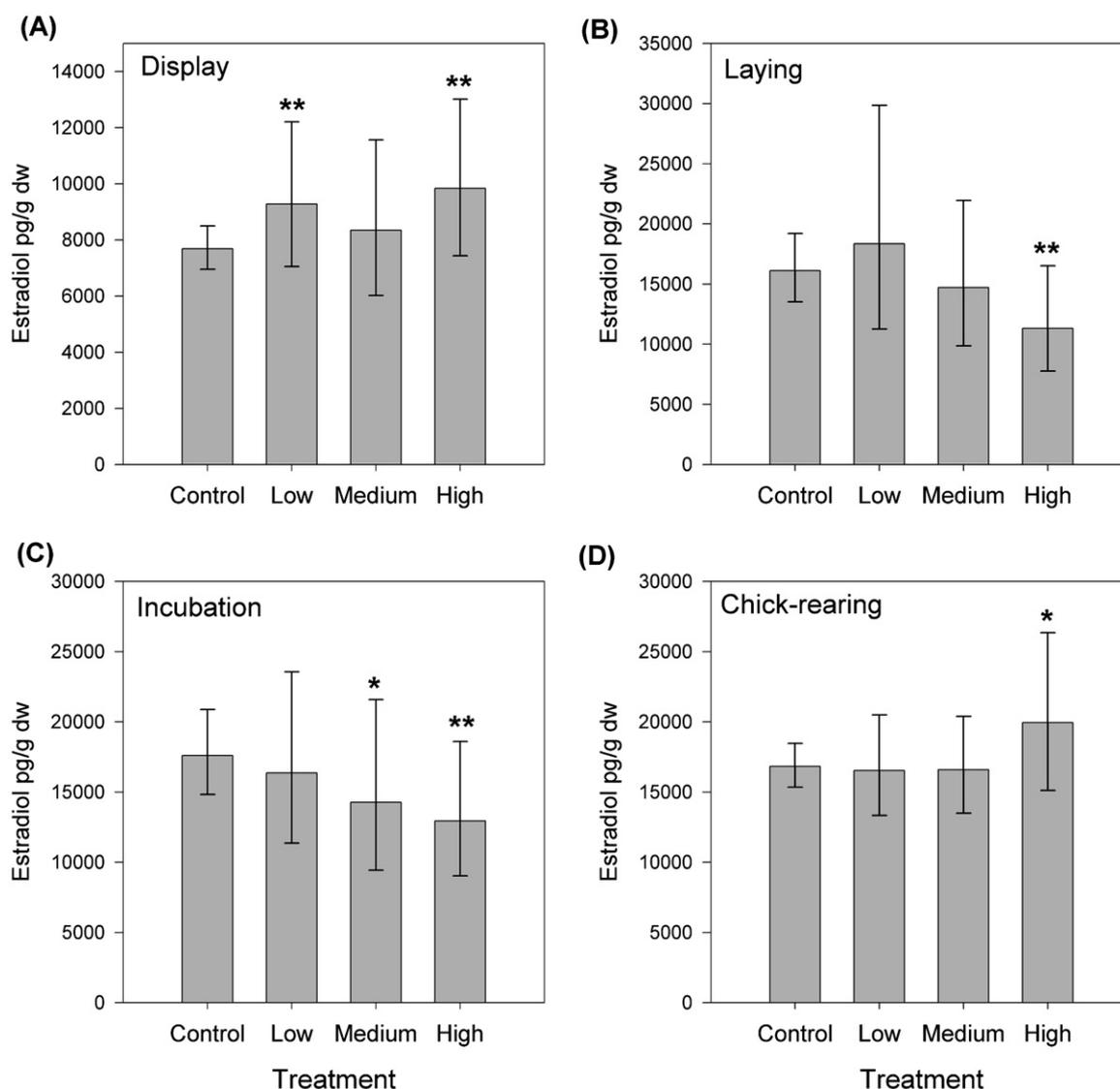
Fig. 1. Parameter estimates (±95% CI) of the extended linear model explaining fecal estradiol concentrations (pg/g dw) of heterosexual male white ibises in each methylmercury treatment group during various breeding stages in 2008. Display (A); laying (B); incubation (C); and chick-rearing (D). Two asterisks (**) indicate significant ($P < 0.05$) differences in heterosexual dosed males in comparison to heterosexual control males. A single asterisk (*) indicates non-significant trends ($P \leq 0.1$) in the same comparison.

Table 1
Total mercury concentrations in feather and blood samples of white ibises exposed to different levels of dietary methylmercury.

Total mercury (mg/kg fw)	Year	Control		Low		Medium		High	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Feathers	2006	0.74	0.25	7.15	2.60	15.24	8.65	23.86	8.77
	2007	0.47	0.11	8.20	1.53	14.13	5.92	51.32	12.33
	2008	0.62	0.21	4.31	1.28	17.96	9.15	35.04	16.94
Blood	2008	0.07	0.01	0.73	0.09	1.60	0.32	3.95	0.68

Reproduced from Frederick and Jayasena (2011).
fw: fresh-weight; and S.D.: standard deviation.

high dose group did not show any significant difference from control birds ($t = -0.44$, $P = 0.66$).

3.2. Effects of methylmercury on estradiol in males

In 2007, there were significant effects of breeding stage but no effect of MeHg on estradiol in heterosexual males (Table 3). There was no significant difference in estradiol concentrations between MeHg-dosed heterosexual and homosexual males in 2007 (Table 3).
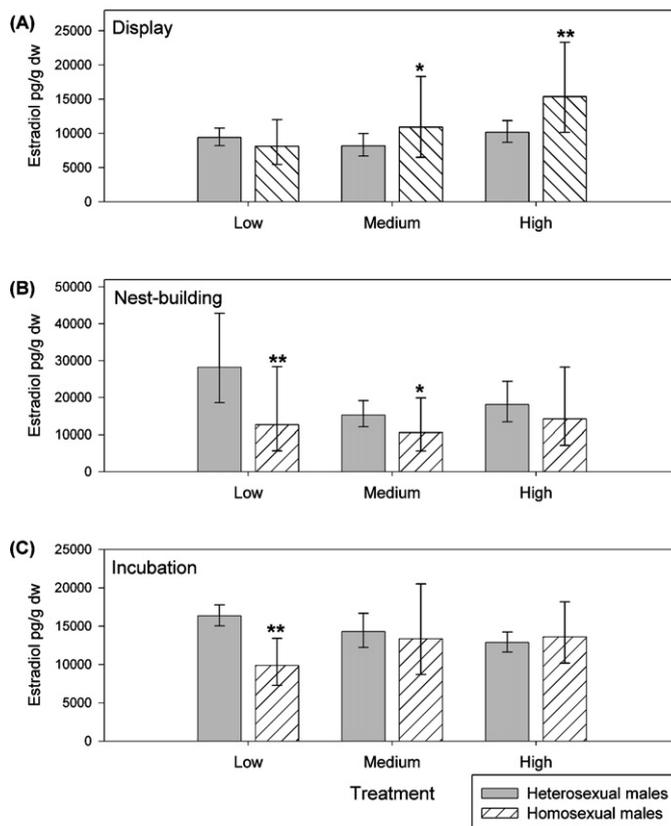
In 2008, there were significant effects of breeding stage and stage × treatment interaction on estradiol concentrations of heterosexual males (Table 3). Low and high groups showed elevated estradiol compared to control males during display (Low: $t = 2.1$, $P = 0.03$; High: $t = 2.7$, $P = 0.007$, Fig. 1A). The high group showed significantly decreased estradiol compared with control males during laying ($t = -3.4$, $P = 0.0007$, Fig. 1B) and incubation ($t = -3.2$, $P = 0.002$, Fig. 1C). Compared with control males, the medium group showed a non-significant trend towards decreased estradiol during incubation ($t = -1.7$, $P = 0.09$). The high group showed a non-significant trend towards increased estradiol during chick-rearing ($t = 1.8$, $P = 0.07$, Fig. 1D).

There were significant effects of breeding stage × treatment, treatment × pairing behavior, and stage × pairing behavior on estradiol concentrations of dosed males in 2008 (Table 3). Individual coefficients showing significant differences or marginal trends between heterosexual and homosexual males within each MeHg group are as follows (Fig. 2A–C). During display, high dose homosexual males had significantly elevated estradiol compared with heterosexual males in the same group ($t = 3.1$, $P = 0.002$) while medium group homosexual males showed marginally higher concentrations ($t = 1.8$, $P = 0.07$). During nest building, low dose homosexual males had lower estradiol concentrations ($t = -4.1$, $P = 0.0001$), and medium dose homosexual males showed a similar but non-significant trend ($t = -1.8$, $P = 0.07$). During incubation, only low dose homosexual males showed significant differences from their heterosexual counterparts with depressed estradiol concentrations ($t = -4.5$, $P < 0.0001$).

Fig. 2. Parameter estimates (±95% CI) of the extended linear model explaining fecal estradiol concentrations (pg/g dw) of dosed male white ibises in methylmercury-dosed groups during various breeding stages in 2008. Display (A); nest-building (B); and incubation (C). Two asterisks (**) indicate significant ($P < 0.05$) differences between heterosexual and homosexual dosed males within the same treatment group. A single asterisk (*) indicates non-significant trends ($P \leq 0.1$) in the same comparison.

Table 2
Results of generalized least square models for methylmercury effects on estradiol and testosterone levels in female white ibises.

Year	Hormone	Degrees of freedom		Explanatory variables (df)	Conditional F test	
		Total	Denominator		F value	P value
2007	Estradiol	272	263	Breeding stage (5)	8.52	<0.0001
				Treatment (3)	4.09	0.0074
2008	Estradiol	301	293	Breeding stage (4)	41.63	<0.0001
				Treatment (3)	1.68	0.1721
2007	Testosterone	273	264	Breeding stage (5)	5.95	<0.0001
				Treatment (3)	1.92	0.1274
2008	Testosterone	286	278	Breeding stage (4)	21.55	<0.0001
				Treatment (3)	1.29	0.2775

df: degrees of freedom.

Table 3
Results of generalized least square models for methylmercury effects on estradiol and testosterone levels in male white ibises.

Year	Hormone	Model	Degrees of freedom		Explanatory variables (df)	Conditional *F* test	
			Total	Denominator		*F* value	*P* value
2007	Estradiol	Heterosexual	306	207	Breeding stage (5)	4.49	0.0007
					Treatment (3)	0.52	0.6694
2007	Estradiol	Dosed	120	114	Breeding stage (2)	3.00	0.0540
					Treatment (2)	0.32	0.7276
					Pairing behavior (1)	0.60	0.4409
2008	Estradiol	Heterosexual	299	279	Breeding stage (4)	75.7	<0.0001
					Treatment (3)	2.3	0.0757
					Breeding stage × Treatment (12)	3.8	<0.0001
2008	Estradiol	Dosed	176	162	Breeding stage (2)	51.96	<0.0001
					Treatment (2)	0.62	0.5372
					Pairing behavior (1)	0.65	0.4216
					Breeding stage × Treatment (4)	4.61	0.0015
					Treatment × Pairing behavior (2)	6.39	0.0021
					Breeding stage × Pairing behavior (2)	10.41	0.0001
2007	Testosterone	Heterosexual	216	207	Breeding stage (5)	2.94	0.0137
					Treatment (3)	0.36	0.7814
2007	Testosterone	Dosed	120	114	Breeding stage (2)	3.383	0.0374
					Treatment (2)	1.086	0.3409
					Pairing behavior (1)	0.171	0.6797
2008	Testosterone	Heterosexual	284	276	Breeding stage (4)	31.26	<0.0001
					Treatment (3)	0.83	0.4775
2008	Testosterone	Dosed	166	158	Breeding stage (2)	24.38	<0.0001
					Treatment (2)	0.74	0.4778
					Pairing behavior (1)	16.83	0.0001
					Breeding stage × Pairing behavior (2)	10.51	0.0001

df: degrees of freedom.

3.3. Effects of methylmercury on testosterone in females

In 2007, we observed significant effects of breeding stage but not of MeHg on testosterone concentrations in females (Table 2). Individual coefficients for high group females showed significantly decreased testosterone concentrations during all breeding stages ($t = -2.3$, $P = 0.02$) and a similar non-significant trend was seen in medium group females in all stages ($t = -1.7$, $P = 0.097$), but there were no changes in low group females ($t = -0.97$, $P = 0.33$). In 2008, there were significant effects of breeding stage but no MeHg effects (Table 2).

3.4. Effects of methylmercury on testosterone in males

There were effects of breeding stage but not of MeHg on testosterone concentrations of heterosexual males in 2007 (Table 3). We also found no significant effects of pairing behavior in dosed males in 2007.

In 2008, there were significant effects of breeding stage but not of MeHg on testosterone in heterosexual males (Table 3). Among dosed males there was a significant effect of pairing behavior on testosterone and a significant interaction of breeding stage and pairing behavior on testosterone (Table 3). In all MeHg-dosed groups, homosexual males showed significantly decreased testosterone during nest-building ($t = -2.4$, $P = 0.02$) and significantly elevated testosterone during incubation ($t = 5.8$, $P < 0.0001$), in comparison to heterosexual males (Fig. 3A and B).

4. Discussion

We found evidence that MeHg exposure was associated with altered expression of estradiol and testosterone in both sexes during breeding, albeit not in a consistent pattern. This finding is noteworthy as this is the first avian experimental study, to our knowledge, showing evidence of endocrine disruption at low levels of exposure (0.05–0.3 ppm ww) of MeHg. Endocrine changes documented were not always consistent across sexes, years, breeding stages or MeHg groups but there were some broadly discernible patterns. All differences in estradiol concentrations in females, when present, were towards lower concentrations by comparison with control birds, and agreed in direction of change with the field results of Heath and Frederick (2005) at similar exposure levels. Dosed homosexual males showed decreased testosterone in comparison to dosed heterosexual males, in all treatment groups, during nest-building and increased concentrations during incubation. Heath and Frederick (2005) also describe increased testosterone in males during incubation.

Male estradiol expression was related to an interaction of breeding stage and dose levels in 2008. It is not surprising that stage should be a powerful explanatory variable since sex hormones typically vary with breeding stage in birds and are known to do so in this species particularly (Heath et al., 2003). A prominent change in 2008 was increased estradiol in dosed heterosexual and homosexual males compared to controls during display, with this change reflected at a higher magnitude in homosexual males (medium and high groups). Compared with controls, high dose males in 2008 showed differences in estradiol concentrations during display, laying, incubation and chick-rearing. The high dose group also had significantly reduced fledging success in the 2008 breeding season (Frederick and Jayasena, 2011), which may be related to endocrine disruption (McCarthy and Ball, 2008). Except during display, all other significant changes in estradiol of dosed heterosexual and homosexual males showed a pattern of decreased concentrations in comparison to control and/or heterosexual birds.

Changes in endocrine expression during display may have played a key role in the significantly reduced frequencies of courtship display behaviors that we recorded in dosed males in the same year (Frederick and Jayasena, 2011). It is possible that higher concentrations of estradiol in males (and especially in
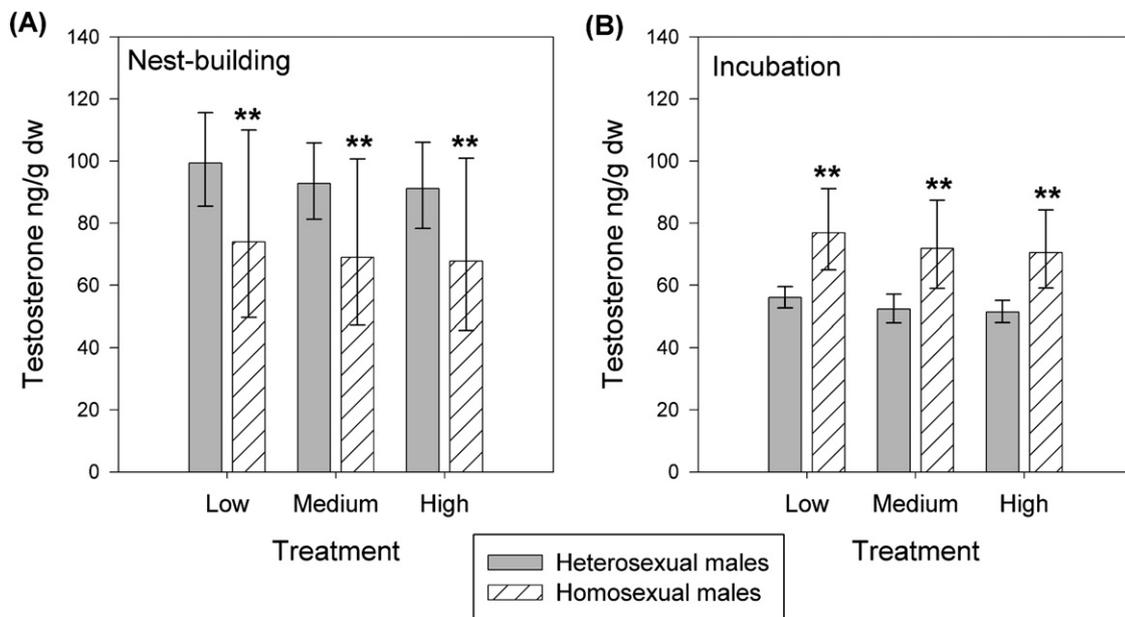
Fig. 3. Parameter estimates (±95% CI) of the extended linear model explaining testosterone concentrations (ng/g dw) of dosed male white ibises in methylmercury-dosed groups during various breeding stages in 2008. Nest-building (A); and incubation (B). Two asterisks (**) indicate significant ($P < 0.05$) differences between heterosexual and homosexual dosed males within the same treatment group.

homosexual males) during display contributed to demasculinization of courtship behavior (McCarthy and Ball, 2008). Reduced rates of courtship behavior may have made dosed males unattractive to females, and therefore, male–male pairing may have been due to female mate choice and hence an indirect effect of endocrine disruption. It is also possible that the changes in endocrine profiles were a result of behavioral changes which in turn were affected directly by MeHg. Expression of sexual behavior is an interaction of environmental, hormonal, and social cues with individual contributions from each of these factors being unquantified (Crews and Moore, 1986). Thus, we are unable to state whether the hormonal changes we recorded are a result of, or because of the changes in mating behavior in ibises. However, both endocrine and behavioral changes were clearly related to experimental exposure to MeHg.

While our study cannot provide mechanistic evidence for a relationship between sexual partner preference and endocrine disruption, it is known that hormones play an important role in the development of sexual partner preferences in animals, including birds (Adkins-Regan, 1998, 2009; Adkins-Regan and Leung, 2006; Adkins-Regan et al., 1997; Adkins-Regan and Wade, 2001). Hormonal effects of sex-steroids can be either organizational (acting during development of organs and organ systems) or activational (Adkins-Regan, 2007). In our study, MeHg exposure started at 90 days of age, at a time when the birds were still developing into juveniles. Since sexual maturity occurred during the period of exposure it is possible that MeHg influenced organizational mechanisms of sexual partner preference.

The sex-related differences that we found in the patterns of MeHg-induced endocrine disruption were not unexpected, since susceptibility to MeHg as well as pathways for metabolism and excretion differ strongly between the sexes in birds (Lewis et al., 1993; Monteiro and Furness, 2001; Tan et al., 2009). Further, the white ibis has been found to be sexually dimorphic with regard to sex-steroid concentrations in the breeding season (Heath et al., 2003) which could lead to different pathways being affected by MeHg.

It may not be surprising that effects of MeHg on sex steroids in ibises did not always show a linear dose–response relationship, since responses to EDCs can be non-monotonic even with monoton-

ically increasing doses, resulting in U-shaped or inverted U-shaped response curves (Clotfelter et al., 2004; Welshons et al., 2003). Time of exposure may also have influenced the nature of responses, since MeHg can accumulate over time in the hypothalamic–pituitary axis, the main regulator for sex steroid pathways (Tan et al., 2009). Indeed, we found more effects in 2008 when birds had been exposed the longest.

The most important implication of our study is that we observed endocrine effects even at the lowest dietary exposure level of MeHg (0.05 ppm ww), a level not tested in previous experimental studies. The implications of these results are that chronic exposure to even very low concentrations of MeHg can result in measurable endocrine changes. This pattern of exposure (low, chronic dose) is typical of wildlife in environments contaminated at low levels. Furthermore, these endocrine effects were coupled both with altered mating strategies and poor parenting, leading to loss of fitness (up to 35% reduction in fledging) even at the lowest levels of MeHg exposure in this study (Frederick and Jayasena, 2011). Endocrine disruption of sex steroids is a plausible and likely mechanism for the altered sexual preference, altered courtship behavior and reduced reproductive success observed in dosed birds. The net effects on reproduction observed in our study were of large enough magnitude to affect demographics of wild populations (Frederick and Jayasena, 2011), underscoring the importance of contaminant-induced endocrine disruption in populations of wild animals.

Conflict of interest statement

None of the authors have any conflict of interest to declare.

Environmental Protection, US Fish and Wildlife Service and US Geological Survey.

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