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EMBRYO MALPOSITION AS A POTENTIAL MECHANISM FOR MERCURY-INDUCED HATCHING FAILURE IN BIRD EGGS

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Abstract—We examined the prevalence of embryo malpositions and deformities in relation to total mercury (THg) and selenium (Se) concentrations in American avocet (*Recurvirostra americana*), black-necked stilt (*Himantopus mexicanus*), and Forster's tern (*Sterna forsteri*) eggs in San Francisco Bay (CA, USA) during 2005 to 2007. Overall, 11% of embryos were malpositioned in eggs ≥18 d of age ($n = 282$) and 2% of embryos were deformed in eggs ≥13 d of age ($n = 470$). Considering only those eggs that failed to hatch ($n = 62$), malpositions occurred in 24% of eggs ≥18 d of age and deformities occurred in 7% of eggs ≥13 d of age. The probability of an embryo being malpositioned increased with egg THg concentrations in Forster's terns, but not in avocets or stilts. The probability of embryo deformity was not related to egg THg concentrations in any species. Using a reduced dataset with both Se and THg concentrations measured in eggs ($n = 87$), we found no interaction between Se and THg on the probability of an embryo being malpositioned or deformed. Results of the present study indicate that embryo malpositions were prevalent in waterbird eggs that failed to hatch and the likelihood of an embryo being malpositioned increased with egg THg concentrations in Forster's terns. We hypothesize that malpositioning of avian embryos may be one reason for mercury-related hatching failure that occurs late in incubation, but further research is needed to elucidate this potential mechanism. Environ. Toxicol. Chem. 2010;29:1788–1794. © 2010 SETAC

Keywords—Embryo deformity Embryo malposition Mercury San Francisco Bay Selenium

INTRODUCTION

Avian reproduction is among the most sensitive endpoints for toxicity to contaminants, especially for persistent and bioaccumulative contaminants [1–4]. The mechanisms causing egg hatching failure are well documented for many contaminants, such as selenium (Se)-induced teratogenicity of the embryo [1,2]. Methylmercury (MeHg) is well known for its ability to reduce hatching success of avian eggs [3,5–7]. Much of the foundational work that linked impaired hatching success with Hg exposure was developed via laboratory experiments, where egg Hg concentrations might exceed those observed in wild populations [5–7]. Therefore, the symptoms of exposures often focused on overt outcomes, such as embryo deformities [8–10] or mortalities [3]. However, other, less obvious effects can occur at more environmentally relevant concentrations that could result in Hg-induced egg hatching failure.

One potential mechanism for Hg to exert its toxicity is through embryo malpositions. Embryo malpositions occur when chicks cannot orient themselves into the correct hatching position, preventing chicks from being able to break out of their shell [8–10]. Poultry science studies have shown that 50 to 85% of eggs that fail to hatch during late incubation have malpositioned embryos [10–12]. Embryo malpositions can result from eggs not being rotated sufficiently during incubation [8–11], or because of variability in incubation temperature [13–16]. Elevated Hg concentrations in adult birds often have been associated with impaired nesting behaviors that are necessary for proper maintenance and incubation of eggs [17–19]. Hence, embryo malpositions may have an important influence on egg hatchability, but few studies have examined the relationship between avian egg Hg concentrations and the occurrence of embryo malpositions.

We evaluated the influence of Hg and Se on embryo malpositions and deformities in three species of waterbirds breeding in San Francisco Bay (CA, USA). San Francisco Bay is polluted with legacy Hg contamination [20,21] and is currently listed under section 303(d) of the Federal Clean Water Act as being impaired by both Hg and Se ([22]; http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs). Recent studies have found elevated concentrations of Hg [23–25] and Se [26] in waterbirds breeding in San Francisco Bay. We examined the prevalence of embryo malpositions and deformities in eggs of American avocets (*Recurvirostra americana*, hereafter ''avocet''), black-necked stilts (*Himantopus mexicanus*, hereafter ''stilt''), and Forster's terns (*Sterna forsteri*, hereafter ''tern''), and then assessed whether egg Hg and Se concentrations were related to the probability of an embryo being malpositioned or deformed.

MATERIALS AND METHODS

Egg collections

Avocet, stilt, and tern eggs were collected as part of a larger study examining MeHg and Se bioaccumulation in waterbirds and their effects on avian reproductive success [23–28]. Avocet, stilt, and tern nests were monitored throughout the breeding season (March to August) during 2005 to 2007 at 12 estuarine wetlands in South San Francisco Bay. Eggs were collected in the following categories: randomly sampled eggs collected at mid-to-late incubation, failed to hatch eggs in otherwise successful nests (≥1 egg hatched from clutch), abandoned eggs,

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and eggs that died in nests that were subsequently destroyed or abandoned (hereafter, dead eggs). Upon collection, whole eggs were placed in labeled whirlpaks and stored in egg cartons with the air cell up. Egg cartons were placed on ice packs in portable coolers until their return to the laboratory, where they were stored at 4°C until processing. Eggs were collected from colonies that represented a range of Hg exposure for breeding adults; details of Hg concentrations associated with adults and chicks at these colonies are provided in Ackerman et al. [23–25,27,28]. All eggs were collected under U.S. Fish and Wildlife Service and California Department of Fish and Game scientific collecting and salvage permits as well as the Animal Care and Use Committee, Western Ecological Research Center, U.S. Geological Survey.

Egg dissection

Prior to dissection the length and breadth of each egg was measured to the nearest 0.01 mm using digital calipers and total egg weight to the nearest 0.01 g using a digital balance. Using stainless steel scissors rinsed with nitric acid and distilled water, we cut a small hole (≈2 cm diameter) on the large end of the egg, staying within the air cell. Following Hutt [10], chick embryo positions were classified using six classifications of malposition: head between thighs, head in narrow end of shell, head under left wing, head under right wing, but embryo rotated so bill pointed away from air cell, one or both feet positioned above the head, and bill lying over right wing. These six malpositions are commonly associated with difficulty in hatching and increased incidence of embryo mortality during the hatching stage [11]. Embryonic chicks with bills tucked under the right wing were classified as normal [10].

Embryo and entire egg contents were removed into a glass Petri dish using clean, stainless steel forceps. We then examined the embryonic chick for signs of deformities and weighed the egg contents to the nearest 0.01 g. Embryo age was classified for terns [29] and avocets and stilts (J.T. Ackerman, unpublished data). Incubation length for birds studied averaged 24 d [30–32]. After examination the embryo and remaining egg contents were transferred to a clean polypropylene jar and stored at −20°C until processing. Any infertile, cracked, or damaged eggs were excluded from analysis.

To estimate the frequency of embryo malpositions, any eggs with embryos that were <18 d of age were excluded since embryos commonly reposition themselves prior to this age [9,33], and we only wanted to include embryos that were considered to be in their final, prehatching position. For embryo deformities, any eggs that were <13 d old were excluded, which was the earliest age at which a deformity was found (see *Results*). Some deformities may have been detectable at younger ages by using advanced optical techniques (see Hoffman et al. [2]), but this approach may have biased low the estimate of the frequency of embryo deformities.

Mercury and selenium determination

Egg THg concentrations were determined at the U.S. Geological Survey, Davis Field Station Environmental Mercury Lab (Davis, CA) and Se concentrations were determined by Laboratory and Environmental Testing (Colombia, MO, USA). Methods are described in detail in Ackerman and Eagles-Smith [25] and Eagles-Smith et al. [34]. Briefly, egg contents were thawed to room temperature, each wet sample was homogenized with a steel-bladed tissue homogenizer, all egg contents at 50–60°C were then dried for approximately 48 h, or until they reached a stable mass. Dried egg contents were then ground to a uniform consistency using a Wiley mill and porcelain mortar and pestle. We analyzed each egg homogenate for total mercury on a Milestone DMA-80 Direct Mercury Analyzer following U.S. Environmental Protection Agency method 7473 [35]. Recoveries of certified reference materials, calibration checks, and matrix spikes, respectively averaged (±standard error [SE]) 103.5% ± 0.5 ($n = 223$), 101.6% ± 0.4 ($n = 333$), and 102.6% ± 0.3 ($n = 204$). Relative percent difference for all duplicates and matrix spike duplicates respectively averaged (±SE) 5.7% ± 0.4 ($n = 196$) and 3.5% ± 0.3 ($n = 104$) for eggs.

Selenium concentrations were determined using hydride generation followed by atomic fluorescence spectroscopy. Certified reference materials for Se in tissues (National Institute of Standards and Technology 1566b; NRCC DOLT-3, and NRCC TORT-2), matrix spikes, duplicate samples, and blanks were analyzed for quality control purposes. Recoveries for certified reference materials and matrix spikes averaged (±SE) 98.5% ± 0.5 ($n = 49$), and 99.2% ± 0.37 ($n = 98$), respectively. Absolute relative percent difference for duplicates averaged (±SE) 3.4% ± 0.3 ($n = 98$). Mean percent moisture in eggs was 74.0% ± 0.0 SE.

Contaminant concentrations in all eggs were determined on a dry weight basis and then adjusted to fresh wet weight following Evers et al. [36].

Statistical analyses

We determined the frequency of occurrence for embryo malpositions and deformities in three ways (refer to Table 1): embryo malpositions and deformities as a percentage of all eggs by species examined (≥18 d for malpositions and ≥13 d

Table 1. Occurrence of embryo malpositions and deformities in Forster's tern, American avocet, and black-necked stilt eggs in South San Francisco Bay, (CA, USA) during 2005–2007

	Forster's tern				American avocet				Black-necked stilt			
	Malpositioned		Deformed		Malpositioned		Deformed		Malpositioned		Deformed	
Egg type	Total eggs	*n* (%)	Total eggs	*n* (%)	Total eggs	*n* (%)	Total eggs	*n* (%)	Total eggs	*n* (%)	Total eggs	*n* (%)
Random	53	1 (2%)	98	0 (0%)	69	2 (3%)	132	1 (1%)	21	1 (5%)	42	0 (0%)
Failed to hatch	22	6 (27%)	27	1 (4%)	30	6 (21%)	35	5 (14%)	10	3 (30%)	19	0 (0%)
Abandoned	16	3 (19%)	33	1 (3%)	29	6 (21%)	36	0 (0%)	9	0 (0%)	12	0 (0%)
Dead: nest destroyed	12	2 (17%)	15	0 (0%)	10	0 (0%)	16	0 (0%)	1	0 (0%)	5	0 (0%)
Total	103	12 (12%)	173	2 (1%)	138	14 (10%)	219	6 (3%)	41	4 (10%)	78	0 (0%)

Only eggs = 18 d old were included in the estimate of embryo malpositions, whereas eggs = 13 d old were included in the estimate of embryo deformities.

for deformities); embryo malpositions (all eggs ≥18 d) and deformities (all eggs ≥13 d) by egg collection type (random, failed to hatch, abandoned, or dead); and gross bill deformities (all eggs ≥13 d). Bill deformities were examined separately to make comparisons to previous studies that focused primarily on bill deformities. G-tests with William's correction were used [37] to determine if the frequency of normal and malpositioned embryos differed among random (viable) and failed to hatch or abandoned eggs for all species pooled.

The relationship between egg THg or Se concentrations and the occurrence of either embryo malpositions or deformities was then examined using logistic regression models [37] separately for each species and each embryo abnormality type (malpositioned or deformed). Embryo state (normal or malpositioned, and normal or deformed) were the nominal dependent variables and embryo age, THg, or Se concentrations, and all two-way interactions were independent variables. All eggs ≥18 d were included for the embryo malposition analysis and all eggs ≥13 d in the embryo deformity analysis.

Separate models for THg and Se were employed because only a subset of eggs were analyzed for Se concentrations, whereas all eggs were analyzed for THg. In the Se model, egg THg concentration was included and the THg × Se interaction as covariates. Nonsignificant ($p > 0.10$) interaction terms were dropped from the final models. Box-Tidwell tests were used to determine if independent variables had a linear relationship with the dependent variables and log transformations were used to meet this assumption [38]. Because the purpose of the present study was to examine the relationship between THg or Se and the occurrence of embryo malpositions or deformities, regardless of when or where the egg was collected, terms for egg collection type, site, or year were not included in the models.

RESULTS

Frequency of malpositioned and deformed embryos

In all, 282 eggs were examined for malpositions (138 avocets, 41 stilts, and 103 terns) and 470 eggs for deformities (219 avocets, 78 stilts, and 173 terns; Table 1). Malpositions were observed in 11% of all eggs ≥18 d of age and embryo deformities in 2% of all eggs ≥13 d of age (Table 1). Considering all eggs ≥18 d, embryo malpositions occurred in 10% of all avocets eggs, 10% of all stilts eggs, and 12% of all tern eggs (Table 1). The most common malpositions were head between thighs (47%), bill under right wing, but embryo rotated so bill pointed away from air cell (24%), bill lying over right wing (10%), and head under left wing (10%). Head in narrow end of shell and one or both feet positioned above over the head accounted for 7% and 3% of the remaining malpositions, respectively. One of the embryos with its head in the narrow end of shell also had one foot positioned above the head.

Considering all eggs ≥13 d, 3% of avocet, no stilt, and 1% of tern embryos were deformed (Table 1). The proportion of all embryos ≥13 d of age with bill deformities was 1%. The eight deformities consisted of embryos with: gastroschisis (exposed organs); two upper mandibles, lower mandible fused, and three eyes; upper mandible twisted to the left and undeveloped eyes; crossbill; lower mandible irregularly shaped; no feet; curved mandible, legs twisted, and short forelimbs; and lower mandible curved up.

Many of the eggs collected were either failed to hatch, abandoned, or otherwise dead, and therefore may have had a higher likelihood of also having embryos being malpositioned or deformed if those abnormalities increased the likelihood of embryo mortality. Thus, the frequency of embryo malpositions and deformities for each egg collection type was also estimated. In order of importance, both embryo malpositions and deformities occurred most often in failed to hatch, abandoned, and dead eggs, and were least common in randomly collected (viable) eggs (Table 1). Malpositioned embryos occurred more frequently in failed to hatch eggs ($G_w = 16.92$, *degrees of freedom* [*df*]= 1, $p < 0.001$) and abandoned eggs ($G_w = 8.70$, $df = 1$, $p = 0.003$) than in randomly collected eggs. Using our entire egg dataset, 70% of all eggs that failed to hatch were ≥18 d old, which is the stage when embryos would be in the final prehatch position.

Malpositioned and deformed embryos and contaminant concentrations

In terns, the probability of an embryo being malpositioned increased with egg THg concentrations ($\chi^2_1 = 10.75$, $p = 0.001$, $n = 81$) but not embryo age ($\chi^2_1 = 1.99$, $p = 0.15$, $n = 81$). Conversely, the probability of an embryo being malpositioned in avocet and stilt eggs was not related to egg THg concentrations (avocets: $\chi^2_1 = 2.05$, $p = 0.15$ $n = 84$; stilts: $\chi^2_1 = 2.36$, $p = 0.12$, $n = 36$), but was inversely related to embryo age for both species (avocets: $\chi^2_1 = 5.04$, $p = 0.02$, $n = 84$; stilts: $\chi^2_1 = 4.31$, $p = 0.03$, $n = 36$). Embryo deformities in avocet and tern eggs were not related to THg concentrations ($\chi^2_1 = 1.05$, $p = 0.30$, $n = 162$, $\chi^2_1 = 0.99$, $p = 0.31$, $n = 151$, respectively) or embryo age ($\chi^2_1 = 0.35$, $p = 0.54$, $n = 162$, $\chi^2_1 = 0.97$, $p = 0.32$, $n = 151$, respectively).

To facilitate the interpretation of the relationship between the probability of a tern embryo being malpositioned and egg THg concentrations, we used the model-generated logistic equation:

$$p(\text{abnormality}) = 1 - (1/1 + \exp(-3.1630 + (5.2463 \times THg)))$$ (Fig. 1).

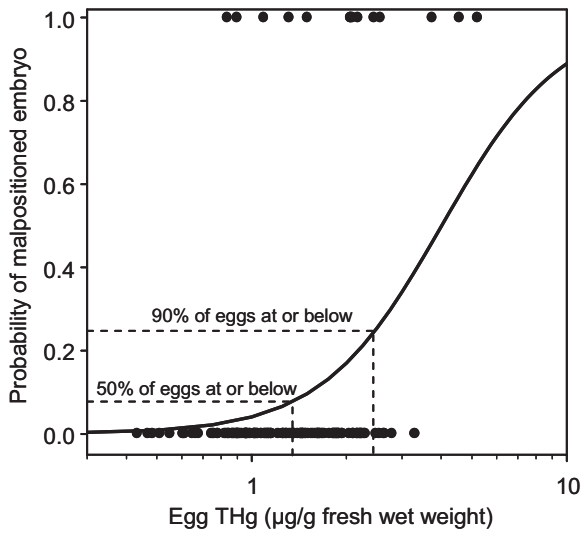

Fig. 1. The probability that Forster's tern embryos were malpositioned increased with egg total mercury (THg) concentrations in San Francisco Bay (CA, USA) during 2005 to 2007. Data points represent eggs with either a malpositioned embryo (value of 1) or a normal embryo position required for successful hatching (value of 0). The curved line indicates the logistic regression between the probability of an embryo being malpositioned and THg concentrations in eggs based on the equation: p (malpositioned) $= 1 - (1/1 + \exp(-3.1630 + (5.2463 \times \text{THg})))$. The 8 and 24% probabilities of an egg having a malpositioned embryo represent the 50th and 90th percentiles, respectively, of the observed egg THg concentrations for Forster's terns in San Francisco Bay.

To illustrate, the 50th and 90th percentiles of the observed tern egg THg data in San Francisco Bay are used. Based on the model, a tern embryo with an egg THg concentration of 1.36 μg/g fresh wet weight (50th percentile) had an 8% (95% confidence interval [CI] = 5–12%) probability of being malpositioned, whereas an embryo with an egg THg concentration of 2.41 μg/g fresh wet weight (90th percentile) had a 24% probability (95% CI = 20–27%) of being malpositioned (Fig. 1).

The effect of Se was then evaluated on embryo abnormalities in avocet and tern eggs using a reduced dataset where we measured both Se and THg concentrations. No relationship was found between the probability of an embryo being malpositioned and the combination of THg and Se in tern eggs (embryo age: $\chi^2_1 = 3.41$, $p = 0.07$; Se = $\chi^2_1 = 0.74$, $p = 0.38$; THg: $\chi^2_1 = 0.44$, $p = 0.50$, $n = 26$), and no relationship between the probability of an embryo being deformed and the combination of THg and Se in avocet (embryo age: $\chi^2_1 = 0.007$, $p = 0.93$; Se = $\chi^2_1 = 0.28$, $p = 0.59$; THg: $\chi^2_1 = 1.09$, $p = 0.29$, $n = 32$) or tern eggs (embryo age: $\chi^2_1 = 0.72$, $p = 0.39$; Se = $\chi^2_1 = 0.34$, $p = 0.55$; THg: $\chi^2_1 = 0.33$, $p = 0.56$, $n = 55$).

DISCUSSION

Although MeHg is known to impair egg hatchability, the specific mechanisms involved in embryo mortality are still largely unknown. To our knowledge, this is the first field study to find a relationship between malpositioned embryos and THg concentrations in eggs. Tern eggs, but not avocet or stilt eggs, with higher THg concentrations had an increased probability of containing a malpositioned embryo. Recent studies in San Francisco Bay have found Hg concentrations in breeding terns to be substantially higher than in any other species studied, including stilts or avocets [39]. Thus, it is logical that a relationship between egg THg concentrations and the occurrence of embryo malpositions in terns compared to shorebirds was detected. However, it is unclear why tern eggs did not have a greater incidence of embryo malpositions in failed to hatch eggs than did avocets or stilts, but may be related to differences in species sensitivities. We also found that 70% of the failed to hatch eggs died after 18 d in incubation, the stage at which embryos are in the final prehatch position. These data indicate that effects occurring during late incubation, such as embryo malpositions, are responsible for many egg failures. Accordingly, 24% of all failed to hatch eggs sampled in San Francisco Bay had malpositioned embryos, suggesting that embryo malpositioning could be an important mechanism reducing hatching success in many contaminated eggs. We emphasize that these results are correlative and urge caution in interpreting causality without further detailed studies, such as those conducted in a controlled laboratory setting.

We are aware of only two previous field studies that have examined the occurrence of embryo malpositions simultaneously with contaminant concentrations. Conover and Vest [40] observed no embryo malpositions in California gull eggs (*Larus californicus*) at the Great Salt Lake, Utah; however, egg Hg concentrations were low. Conversely, Schwarzbach et al. [41] found that 21% of failed to hatch California clapper rail (*Rallus longirostris obsoletus*) eggs contained malpositioned embryos in San Francisco Bay, with egg MeHg concentrations ranging from 0.12 to 2.51 μg/g fresh wet weight. In both studies the sample sizes were limited and the prevalence of embryo malpositions were derived from salvaged eggs in Schwarzbach et al. [41]. The prevalence of embryo malpositions in the current study was 3% in randomly sampled, apparently viable eggs and 24% in failed to hatch eggs, suggesting that embryo malpositions may reduce egg hatchability, as has been found in poultry [10–12].

Although the gross occurrence of embryo deformities (2%) in this study was higher than in recent studies of chick deformities associated with contaminants [42,43], no relationship was found between Hg or Se concentrations and deformities in any of the three species studied. Substantially higher deformity rates (16–20%) were reported from highly Se contaminated sites such as Kesterson Reservoir in the Central Valley of California (see Ohlendorf et al. [1] and Hoffman et al. [2]). Ludwig et al. [44] conducted a comprehensive review of embryo and chick deformities in the Great Lakes for data from 1986 to 1991 and found that estimates of deformities for double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) were as high as 8 and 10%, respectively. Embryo deformities are generally associated with polychlorinated biphenyls [42–45] and Se exposure [1,2], and we are not aware of environmentally relevant Hg concentrations being related to embryo deformities in the wild. Thus, results of the present study are consistent with these prior studies since polychlorinated biphenyls and Se in waterbird eggs from San Francisco Bay are generally below concentrations found to induce deformities [41,46].

Assessments of contaminant effects on egg hatching success in the field are often limited in interpretation to in ovo effects. However, egg Hg concentrations often are related to parental Hg exposure and bioaccumulation [36,47–49]. Thus, egg failure associated with high egg THg concentrations may be partially caused by altered parental care, rather than due to a direct effect of in ovo egg Hg concentrations. For example, Evers et al. [17] recently showed that incubation bout lengths were negatively influenced by elevated blood Hg concentrations in nesting common loons (*Gavia immer*). It follows that if elevated Hg concentrations in adults lead to altered nesting behavior, then Hg-induced hatching failure might be caused by impaired parental behavior [50–52]. In other words, Hg concentrations in eggs may not be the direct cause of embryo malpositions; rather, malpositions may be an artifact of poor parental behavior by females with elevated Hg concentrations that lay eggs also with elevated Hg concentrations. Support for this hypothesis is provided by recent research that has shown that environmentally relevant Hg exposure can result in endocrine disruption [53–58], and sex hormones (e.g., progesterone, testosterone) are important in regulating nesting behavior [59–64]. Not only did we find that failed to hatch eggs (24%) had a higher occurrence of malpositioned embryos than random eggs (3%), but eggs abandoned by parents also had a higher frequency of malpositioned embryos (17%). These data suggest that parental behavior may play an important role in the effect of Hg on egg hatchability.

Parental behavior during incubation is important for maintaining constant egg temperature and humidity for successful embryo development and egg hatching. In addition to parental coordination of incubation activities for species with biparental care (such as the three species studied), manual turning of eggs by parents is necessary for successful embryo development [8–11]. Failure to properly turn eggs during incubation can result in a higher incidence of embryo malposition [8,11,65,66] and improper egg temperatures can lead to embryo deformities [15,67,68] and malpositions [13–16]. Failure of embryos to move into the correct pipping position also can result in hatching failure [8,9,11]. Additionally, improper parental care of eggs during embryogenesis can result in the chorioallantois

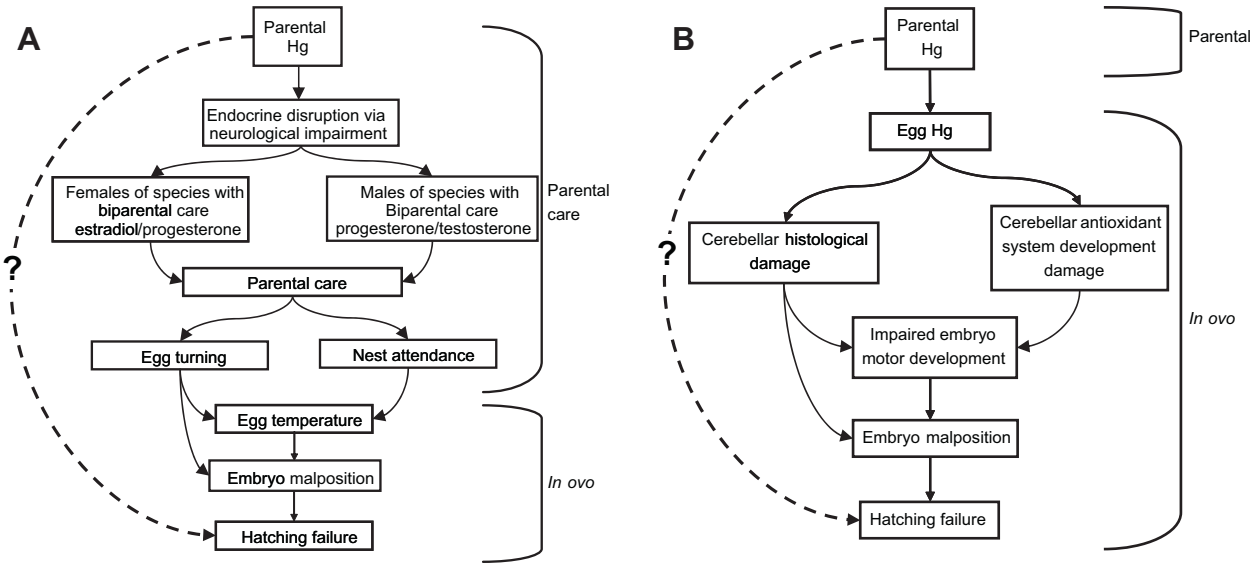

Fig. 2. Conceptual models of potential mechanisms for avian egg hatching failure due to (**A**) parental dietary mercury (Hg) exposure during reproduction and (**B**) parental transfer of dietary Hg to the embryo. Solid lines indicate the cascading direct effects of Hg exposure, whereas the dotted line indicates the indirect effect of parental Hg exposure on egg hatching failure. Although Hg within the avian egg in model (A) might not directly cause an embryo to be malpositioned, adult Hg exposure may reduce parental care of eggs and maintenance of nests and thereby increase the likelihood of embryos within eggs to be malpositioned. Elevated mercury in the embryo in model (B) may result in cerebellar damage and impaired embryo motor development and thereby increase the likelihood of embryos within eggs to be malpositioned. Malpositioned embryos often have a reduced ability to successfully hatch from an egg.

adhering to the inner shell membrane [69], which interferes with albumen uptake and obstructs the embryo's ability to attain the correct hatching position [8]. Therefore, one hypothetical mechanism of egg failure may be Hg-induced impairment of parental nest attendance behaviors (Fig. 2A).

Alternatively, malpositioned avian embryos may be caused by Hg-induced impairment of embryo neurological function (Fig. 2B). Carvalho et al. [70] experimentally demonstrated that in ovo exposure to MeHg resulted in increased cerebellar glutathione (GSH) levels and the enzymes GSH reductase and GSH peroxidase in chick cerebellums. Collectively, cellular damage and increased antioxidants were correlated with decreased exploratory and increased irregular movements in 1-d-old chicks [70]. We hypothesize that an alternative mechanism causing failure of eggs with elevated Hg concentrations may be the direct neurological impairment of embryos, resulting in an increased likelihood of an embryo being malpositioned and reducing the chances of successfully hatching from an egg (Fig. 2B).

Results of the present study do not directly identify a mechanism linking Hg and malpositioned embryos, but they do suggest that Hg contamination can increase the likelihood of embryo malpositions in some species. The relative novelty of this finding suggests future studies of the effects of Hg on embryos should include an assessment of the occurrence of embryo malpositions. Further, controlled experimental studies are needed to better understand whether Hg contamination in eggs or parents causes embryos to become malpositioned, and whether embryo malpositions due to Hg contamination reduces the likelihood of successfully hatching from an egg. Finally, we caution that our results should be considered correlative until experimental studies have been undertaken to identify the specific mechanism associated with toxicity-induced embryo malpositioning in avian eggs.

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