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A NONLETHAL MICROSAMPLING TECHNIQUE TO MONITOR THE EFFECTS OF MERCURY ON WILD BIRD EGGS

KATHERINE R. STEBBINS,*† JON D. KLIMSTRA,† COLLIN A. EAGLES-SMITH,‡ JOSHUA T. ACKERMAN,‡ and GARY H. HEINZ†

†Patuxent Wildlife Research Center, U.S. Geological Survey, BARC-East, Building 308, 10300 Baltimore Avenue, Beltsville, Maryland 20705

‡Western Ecological Research Center, U.S. Geological Survey, Davis Field Station, One Shields Avenue, Davis, California 95616

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Abstract—Methylmercury is the predominant chemical form of mercury reported in the eggs of wild birds, and the embryo is the most sensitive life stage to methylmercury toxicity. Protective guidelines have been based mainly on captive-breeding studies with chickens (*Gallus gallus*), mallards (*Anas platyrhynchos*), and ring-necked pheasants (*Phasianus colchicus*) or on field studies where whole eggs were collected and analyzed and the effects of the mercury were measured based on the reproductive success of the remaining eggs. However, both of these methods have limitations. As an alternative, we developed a technique that involves extracting a small sample of albumen from a live egg, sealing the egg, returning the egg to its nest to be naturally incubated by the parents, and then relating the hatching success of this microsampled egg to its mercury concentration. After first developing this technique in the laboratory using chicken and mallard eggs, we selected the laughing gull (*Larus atricilla*) and black-necked stilt (*Himantopus mexicanus*) as test subjects in the field. We found that 92% of the microsampled laughing gull eggs met our reproductive endpoint of survival to the beginning of hatching compared to 100% for the paired control eggs within the same nests. Microsampled black-necked stilt eggs exhibited 100% hatching success compared to 93% for the paired control eggs. Our results indicate that microsampling is an effective tool for nonlethally sampling mercury concentrations in eggs and, as such, can be used for monitoring sensitive species, as well as for improving studies that examine the effects of mercury on avian reproduction.

Keywords—Albumen Mercury Nonlethal microsample Reproductive success Wild bird eggs

INTRODUCTION

Mercury is one of the most globally widespread and harmful contaminants to birds. Methylmercury is the most toxic form of mercury in the environment, and its toxicity can be manifested through numerous endpoints that can impair adult survival, behavior, reproductive success, and cellular development, as well as cause teratogenic effects [1]. Methylmercury is readily accumulated by organisms through their diet and is biomagnified through food chains [2]. Birds may also depurate mercury into their eggs [3], which is problematic because the embryo is the most sensitive life stage for birds [1,4–6].

Researchers have conducted studies to determine the toxic effects of mercury on avian reproduction by using both laboratory and field studies. Controlled laboratory breeding studies have demonstrated reduced reproductive success due to elevated mercury levels in avian eggs [7–12]. However, the difficulty and expense of conducting captive breeding studies limits the broad application of this approach. An alternative laboratory approach is to use egg injections to measure the effects of mercury on avian embryos [13]. Egg injections are particularly useful for comparing the relative sensitivity to mercury among various species of birds. However, mercury is more toxic when injected into the egg compared to when it is maternally deposited into the egg; therefore, egg injections alone cannot be used to determine a threshold concentration of mercury that would begin to cause harm [13]. An alternative to laboratory studies is the use of field studies that examine the effects of mercury on bird reproduction in the wild.

Two methods are generally implemented in the field when using eggs to assess the effects of mercury on reproductive success. The first method involves comparing the average mercury concentrations in failed eggs to the overall reproductive success of the remaining eggs at the breeding site [14,15]. This method is especially useful with a threatened or endangered species for which collection of a viable egg for analysis is not an option. A disadvantage of this method is the possibility of sampling bias toward collecting eggs with higher mercury. The second and more refined method is called the sample egg technique, in which a viable egg is taken from a nest and analyzed and then the fate of the remaining eggs in the nest are monitored and related to the mercury concentration in the sampled egg [16–18]. Implicit in this method is the assumption that mercury concentrations in the sampled eggs are appropriate proxies for those in the sibling eggs. Unfortunately, this is not always the case, as mercury concentrations can vary substantially within a clutch. For example, Evers et al. [19] found a mean difference in mercury concentrations of 25% among 86 common loon (*Gavia immer*) nests where two eggs were analyzed for total mercury. This variation among eggs can be attributed to laying order, nest parasitism, or changes in feeding habits. Intraclutch variability due to laying order was demonstrated among three-egg clutches of herring gulls (*Larus argentatus*) and common terns (*Sterna hirundo*), with the mercury concentration in the first egg laid measuring 39 and 37% higher than in the last egg, respectively [20]. Evers et al. [21] recorded variations in mercury of up to one order of magnitude in common merganser (*Mergus merganser*) eggs from clutches

* To whom correspondence may be addressed (stebbins.katherine@epamail.epa.gov).
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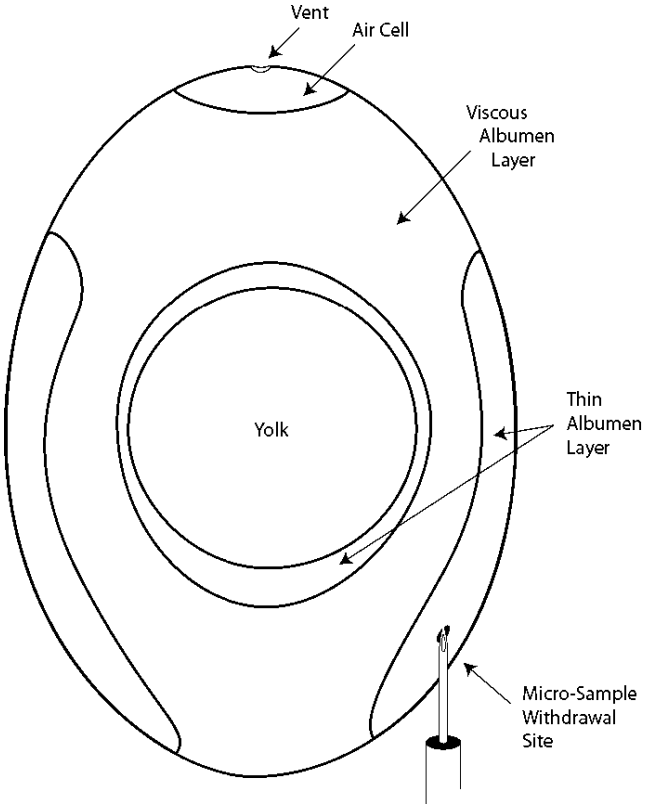

Fig. 1. Location of the vent and microsample withdrawal site on a chicken egg.

containing more than 10 eggs, with nest parasitism being a possible cause of these large differences. Daily changes in diet have also been suggested to cause variability in mercury deposited into sequentially laid tree swallow (*Tachycineta bicolor*) eggs [22].

While valuable information has been gained using the current field methods, it would be ideal to measure the mercury concentration in an egg and be able to report the fate of that same egg. The objective of the present study was to develop a nondestructive method that would allow researchers to sample the mercury concentration in wild bird eggs, return the live egg to the nest to be naturally incubated by the parent or parents, and relate the hatching success of the microsampled eggs to their mercury concentrations.

MATERIALS AND METHODS

Experiment 1

We conducted an initial study in the laboratory using fertile chicken (*Gallus gallus*) eggs (CBT Farms, Chestertown, MD, USA) to determine how and when to withdraw a small sample of albumen without harming the developing embryo. Before incubation, we randomly assigned 10 to 11 eggs to be microsampled on days 0, 5, and 9 of development. In addition, we had a set of 45 eggs that served as controls and from which no albumen samples were taken. We dipped all of the eggs in a dilute (~1%) Betadine® solution (Purdue Frederick, Norwalk, CT, USA) followed by a tap water rinse. We oriented the eggs with the blunt (cap) end facing up and swabbed the cap and albumen withdrawal site with an isopropyl alcohol swab. The albumen withdrawal site was located approximately one-fifth of the way above the pointed (apex) end of the egg (Fig. 1). We then used a Dremel® rotary tool (Dremel, Racine, WI, USA) with a 3/32-inch diamond bit to drill a hole over the air cell, with the hole functioning as a vent to relieve pressure when we took the albumen sample. Next, we used the rotary tool to drill through nearly all of the outer shell at the withdrawal site, being careful not to drill through the shell membranes. After making these two holes, we held the egg at a 45° angle to allow the yolk to float away from the withdrawal site. We used a 20-gauge needle to gently penetrate through the shell membranes until the bevel of the needle had just barely entered the egg. Once inside the egg, the bevel of the needle remained facing the shell to target the thin albumen layer. We extracted approximately 200 μl of albumen, which is less than 1% of the total egg mass. After the albumen sample was taken, we removed the needle and quickly applied a drop of hot glue over the withdrawal site. Finally, the vent hole in the cap end of the egg was sealed with hot glue. The eggs were then returned to a Kuhl incubator (Flemington, NJ, USA) set at 37.6°C and 35 to 40% relative humidity with 180° rotation of the eggs every hour. After 19 d of incubation, we transferred the eggs to a Kuhl hatcher set at 37.1°C and 70% relative humidity for hatching. Hatchlings were euthanized according to the Patuxent Wildlife Research Center's Animal Care and Use Committee guidelines.

Experiment 2

We randomly assigned fertile chicken eggs to be microsampled on day 0 of incubation (n = 30), to be microsampled on day 4 (n = 30), or to serve as unsampled controls (n = 40). The control eggs were further subdivided into two sets of 20, one set to accompany each sampling day. For the set of eggs that was incubated for 4 d before microsampling, we candled all of the eggs and their respective controls and replaced any eggs that were infertile or that incidentally died before the microsampling day.

Our sampling procedure for experiment 2 was the same as in experiment 1, except that a second person began to apply the hot glue around the needle while the albumen was being removed. This allows for an expedited seal at the extraction site, because once the needle clears the threshold of the egg, the hot glue can be smoothed over the withdrawal site in one fluid motion.

Experiment 3

To determine the distribution of mercury in various parts of the egg, we purchased mallard (*Anas platyrhynchos*) breeding pairs (Schrader's game farm, Henderson, MD, USA) and randomly assigned them to treatment diets of 1, 2, 4, or 8 μg/g of mercury as methylmercury chloride. Each pair was placed in an outdoor breeding pen that contained fresh running water, a swimming bowl, a nest box, and feed. We mixed diets by dissolving methylmercury chloride in corn oil, which was then mixed with a commercial game bird breeder diet (Purina Mills, St. Louis, MO, USA). After 5 d of treatment, and every 5 d thereafter through day 35, we began collecting fresh, unincubated eggs for albumen sampling. We carefully cracked open each egg and poured the contents into a weigh boat. Using a 1-ml syringe and a 20-gauge needle, we sampled the albumen from two locations: one sample from the viscous albumen that is closer to the yolk and the other from the thin albumen that forms the outer albumen layer of the egg. We weighed the albumen samples to the nearest 0.01 g and stored them in cryovials. We also weighed the remaining egg contents and transferred them to 2-oz plastic jars. All samples were

immediately frozen and sent to the U.S. Geological Survey Western Ecological Research Center, Davis Field Station's Mercury Lab (Davis, CA, USA), for total mercury analysis following U.S. Environmental Protection Agency method 7473 [23], using a DMA 80 direct mercury analyzer (Milestone, Monroe, CT, USA).

We log-transformed the thin albumen and the homogenized whole egg (mathematically reconstituted with its thin and viscous albumen components) mercury concentrations and used linear regression of the mercury concentrations in the thin albumen versus the mercury concentrations of the homogenized whole egg. Because mercury concentrations in bird eggs rarely exceed 4 μg/g when found in nature [24], we performed a second regression that excluded any eggs with reconstituted whole egg mercury concentrations greater than 4 μg/g.

We also compared the concentration of the mercury in the thin albumen versus the viscous albumen (excluding samples with >4 μg/g in the whole egg) using a paired *t* test.

Experiment 4

On May 23, 2007, we used our microsampling technique to sample laughing gull (*L. atricilla*) eggs from a colony at Chincoteague Island, Virginia, USA. Since laughing gulls typically lay a clutch of three eggs and we wanted to sample eggs that were as close to fresh as possible, we timed our visit to coincide with laying so that most nests contained only two eggs. We randomly selected 25 of these two-egg nests from throughout the colony using the criteria that all eggs in the nest were no more than 3 d through incubation. When a nest was found with two eggs, we placed the eggs in a dilute (~1%) Betadine solution to determine the incubation stage of each egg according to flotation [25,26]. In most cases, both of the eggs appeared to have undergone no incubation; however, in a few cases, we allowed the eggs to pass if the air cell end of the egg was just starting to tilt upward as long as the other egg in the nest remained flat. To be conservative, we assigned this slightly incubated egg as the control egg; if both eggs floated flat, we randomly selected which egg would be the control egg. Using an indelible marker, we marked eggs from the same nest with the nest identification number and an M or C to identify microsample and control eggs, respectively. We swabbed the cap and the (potential) extraction site of the control egg with an alcohol pad and placed it back in the nest. Next, following the procedure we developed in the laboratory, we prepared the shell of the microsample egg and withdrew approximately 200 μl of albumen. We used a cordless glue gun to seal each egg in the field, and once the glue had dried we returned the microsampled egg to the nest.

We returned to the colony on June 14, 2007, which would have been day 23 of incubation, and relocated each nest. We recorded any observations of hatching or external pipping. On June 19, 2007, we returned to the colony to record any further observations of hatching and collected any remaining unhatched eggs to age in the laboratory. For the present experiment, we are limited to reporting the survival up through day 23 of incubation, which marks the beginning of a 28- to 46-h hatching interval for the laughing gull (bna.birds.cornell.edu/bna/species/225). One reason for this limitation is that on June 13 (day 22) there had been a severe storm at our site and the associated high water levels had swept away or damaged many of our sampled nests and others at the colony. In addition, when we visited on day 28, many of the chicks had moved off the nests and their shells had been removed; therefore, we combined any further usable information we were able to gather with what we had learned from our observations on day 23 to come up with our survival endpoint. Based on our observations during these two postsampling visits, we assigned a fate to each egg by observing a hatched chick, observing an egg that had externally pipped, or opening and aging any unhatched, dead eggs. For the present study, a successful egg is defined as having hatched, pipped, or survived to day 22 to 23 of incubation.

Experiment 5

We monitored black-necked stilt (*Himantopus mexicanus*) nests from April 9, 2007, to July 9, 2007, at the Don Edwards National Wildlife Refuge in San Francisco Bay (CA, USA). We selected 45 stilt nests for microsampling using the criteria that all eggs in the clutch were less than 3 d in incubation. Each nest was assigned a unique identification number, and we recorded the nest coordinates with a handheld global positioning unit. We also recorded the total number of eggs in each nest, numbered each egg sequentially with an indelible marker, and determined the incubation stage of each egg through flotation [25]. We randomly selected one of the eggs from a clutch for microsampling and another egg from the same nest to treat as a control. Before microsampling, we dipped both the control and the microsample eggs in a dilute (~1%) Betadine solution. We swabbed the cap and the (potential) extraction site of the control egg with isopropyl alcohol and placed it back in the nest. Next, we prepared the shell of the microsample egg and withdrew approximately 200 μl of albumen, following the procedure we developed in the laboratory except for three minor changes. First, due to the shape of the stilt eggs, the sampling site was located higher up on the egg (about two-fifths above the apex of the egg rather than one-fifth). Second, after the albumen sample was drawn, we plugged the top vent hole with a finger, slowly removed the needle, rotated the egg so that the extraction site now faced upward (to avoid having albumen leak out), and quickly applied a drop of hot glue over the site. Finally, we applied a layer of cyanoacrylate glue over the hot glue to ensure a strong seal. Once the glue had dried, we returned the eggs to their nests. We revisited each nest every 7 d and recorded the incubation stage of each egg via floating and the status of each egg until a fate could be assigned (hatched, depredated, or abandoned).

RESULTS

Experiment 1

The eggs sampled on day 0 had 64% hatching success as compared to 80% for the control eggs. Of the nine eggs sampled on day 5 of incubation, only 22% hatched. On day 9, we could tell by candling that the vasculature in the egg was too developed to realistically attempt microsampling. We did attempt to sample one 9-d-old egg, and because of changes in the albumen composition or the heavy network of vessels, no albumen could be extracted. The present experiment demonstrates the difficulties and negative effects of using the microsampling technique on chicken eggs that have progressed to day 5 of development and (potentially) beyond that. In addition, we discovered we still needed to improve on the sealing process, which is something we addressed with the following experiments.

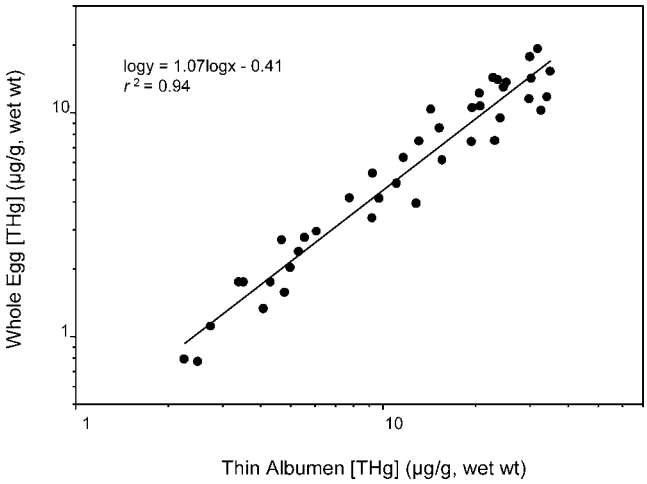

Fig. 2. Total mercury (THg) concentrations in the thin albumen versus THg concentrations in the whole egg. THg concentrations are log ($\log_{10}$)–transformed.

Experiment 2

Eggs sampled on day 0 exhibited 77% hatching success compared to 65% for their control eggs, and eggs sampled on day 4 exhibited 70% hatching success compared to 60% for their control eggs. A hatching success of only 65 and 60% for untreated control chicken eggs is unusually low, and we have no explanation for the poor hatching success of these eggs.

Experiment 3

The log of mercury concentrations in thin albumen was highly correlated with the log of mercury concentrations in the whole egg ($n = 41$, $r^2 = 0.94$, $p < 0.0001$), indicating that microsampling an egg is a practical way to predict the mercury level in the whole egg (Fig. 2). When we excluded eggs with whole-egg mercury levels above 4 µg/g, which are levels rarely seen in wild bird eggs, the correlation still indicated a strong relationship ($n = 15$, $r^2 = 0.83$, $p < 0.0001$) between the microsample and the whole egg contents.

When comparing the two types of albumen, we found that the mean ± standard error for the concentration of mercury in the viscous albumen was 4.0 ± 0.5 µg/g, which was significantly lower than the 5.1 ± 0.7 µg/g in the thin albumen ($n = 15$, $p = 0.002$, paired t test).

Experiment 4

Of the 25 gull nests initially sampled, only 12 nests provided usable survival data from both the control and the microsampled eggs due to a rain and flooding event. Control and microsampled eggs had 100% (12 of 12) and 92% (11 of 12) success as defined as hatched, pipped, or survival up to the beginning of the hatching stage at days 22 to 23 of incubation.

Experiment 5

From the 45 black-necked stilt nests we initially microsampled, 31 nests were subsequently depredated, abandoned, or destroyed, leaving 14 nests with both the control and the microsampled eggs. Of those 14 nests that survived, 100% (14 of 14) of the microsampled eggs and 93% (13 of 14) of the control eggs hatched.

DISCUSSION

Our results indicate that our final microsampling procedure is a viable technique that can be used to monitor mercury exposure in avian eggs. Microsampling lends itself well to monitoring methylmercury because 85 to 95% of the methylmercury in the avian egg is in the albumen [1,7]. Several experiments on albumen withdrawal have been performed on avian eggs in the past [27–29]. However, to our knowledge, this method has not been used for monitoring environmental contaminants. The following discussion sections highlight some considerations we feel are important to include when planning to use this technique as a method for measuring the effects of mercury or possibly other contaminants on wild bird eggs.

Amount of albumen to withdraw

Albumen provides water, mineral ions, and proteins for the developing embryo [30], which raises the question of how much albumen is safe to remove from an egg. Using a method similar to our final technique, Ferrari et al. [29] demonstrated that albumen withdrawal of up to approximately 3.4% of the total egg mass from barn swallow (*Hirundo rustica*) eggs did not cause reduced hatchability but did reduce nestling survival. In our experiments with the chicken, mallard, laughing gull, and black-necked stilt eggs, the microsample volume was 200 µl, which was 1% or less of the total egg mass for all four species. As demonstrated by our laboratory and field experiments, removing up to 1% of the total egg mass (in the form of albumen) did not affect the hatchability of the eggs we tested. However, it is important to set limits when selecting a sample volume, with the size of the egg dictating the amount of sample that can safely be taken. Thus, for some species with very small eggs, detection limit issues may arise due to the limited sample amount.

When to microsample

Microsamples should be taken early in incubation because once incubation has begun changes occur in the composition of the albumen that could cause changes in the mercury concentration. In the domestic chicken, there are two periods of change. Starting from day 2, a measurable amount of subembryonic fluid is formed around the embryo [31]. This buildup of fluid continues for several days, moving the water reservoir to the yolk, where it is easily accessible for the later formation of the allantoic and amniotic fluids and hydration of embryonic tissues [30]. The second change occurs on day 12 in the chicken, and for the next 3 to 4 d albumen proteins transfer into the amniotic fluid [30]. Another reason for microsampling only young eggs is related to the growth of the embryo and its supporting vasculature. As we found with experiment 1, by day 5, our ability to get an adequate sample was reduced and the hatching success was poor among those eggs we did sample. Although researchers could attempt to candle the eggs to make sure the extraction site was not close to any vasculature, it would be difficult and slow in the field, especially with eggs that are heavily mottled. The mistake of hitting a blood vessel not only could cause harm to the embryo but also could render the microsample useless if it became contaminated with blood.

Given the preceding reasons and our experience both in the lab and in the field, we concluded that it is best to use this technique on fresh eggs; however, we realize that this may be difficult to achieve in the field. In experiment 3, we only tested the mercury concentration in fresh eggs, so we do not know if the formation of the subembryonic fluid starting on day 2 would cause the mercury concentration in the albumen to change. While we feel it is likely that the mercury concentra-

tion in the thin albumen located around the microsampling site would remain the same for several days into incubation, further research is needed to determine whether and when it changes throughout development.

Other considerations

We found that one of the most important elements of successfully using this technique is to practice the drilling and sealing process. Drilling through the hard outer shell at the extraction site is an important part of this technique and involves training and considerable practice before its application on live eggs. When performed correctly, the needle can be gently pressed through the shell membranes with little force, thus eliminating stress cracks that can weaken the shell and introduce bacteria. Also, the intact shell membranes create a tighter fit around the needle, which prevents albumen from leaking out of the egg. Preventing leakage of albumen is important because the presence of albumen on the surface of the outer shell makes it difficult to get a good seal with the hot glue; this was a challenge we faced in experiment 1, and we improved our technique in experiments 2, 4, and 5. In these three later experiments, we used two strategies to improve sealing the egg, both of which were designed to reduce the risk of albumen leaking from the egg. The Maryland team of researchers (K.R. Stebbins and J.D. Klimstra) used the sealing process in experiments 2 and 4 that involved applying the hot glue around the needle before removal and found that it was especially helpful because it allowed them to begin sealing the egg as the needle was being removed. This early application of glue works well, but it does require two people to execute. The second sealing technique that was used by the California team (C.A. Eagles-Smith and J.T. Ackerman) in experiment 5 involved covering the top vent hole with a finger after the albumen sample was drawn, slowly removing the needle, rotating the egg so the extraction site faced upward, and quickly applying a drop of hot glue over the site. When using this technique, an additional drop of cyanoacrylate glue was applied over the hot glue to ensure a strong seal. This alternative technique of covering the vent hole and rotating the egg can be implemented by one person. Both of these sealing techniques seem to work well; therefore, the choice comes down to what is comfortable for those performing the work and perhaps the type of egg being sampled.

The size, shape, and hardiness of the egg are all factors to be considered when preparing to use this microsampling technique. For example, when we designed this technique, we used chicken and mallard eggs, which generally have similar characteristics. However, appropriate modifications will sometimes be necessary for certain target species. For example, some may find that using the Dremel drill is inappropriate for a species with a thinner, more fragile shell or that the sampling site is too high or low given the shape of the egg. We suggest practicing with fresh chicken eggs first to get a feel for the drilling and sealing process and then using a few eggs from the species of interest (perhaps those used to make the predictive equation) to refine the method accordingly.

Albumen microsample versus whole egg mercury

In experiment 3, we compared the mercury concentrations in the two types of albumen to see whether they were similar in the event that we inadvertently collected a microsample that was a mixture of the thin and viscous albumen. We feel that, when using the sampling technique we recommend, it is unlikely that any viscous albumen would be inadvertently collected with the intended thin albumen. It is also quite easy to distinguish when extracting viscous albumen, because it makes the extraction difficult and requires more backpressure on the syringe. In these cases, researchers can choose to discard the samples. Nonetheless, we tested whether thin and viscous albumen mercury concentrations within the same egg differed. While we found there were statistically significant differences in mercury concentrations, the concentration was only slightly lower in the viscous albumen. Therefore, we feel that collecting a sample that was not purely thin albumen would make little difference in predicting the mercury level in the whole egg.

In the second part of experiment 3, we used the mercury concentration in the thin albumen sample and the whole egg contents to develop a regression equation from which we could predict mercury in the whole egg from mercury in the microsample. This predictive equation could be specific to the mallard and may not work for other species of birds. Egg composition varies in birds, especially between altricial and precocial species. For example, on average, up to 46% more yolk is found in precocial bird eggs compared with altricial species [32]. Using the microsampling technique for a species in which a predictive equation is not available would require conducting a small pilot study to derive an equation.

Cases may exist for which a predictive equation cannot be determined, for example, when dealing with a threatened or endangered species. In this case, the mercury concentration in the whole egg may be estimated based on the fraction of yolk versus albumen contents (either from the literature or from a surrogate species) and the measured mercury concentrations from the microsample. Alternatively, microsampling could be used as a screening tool without knowing the mercury concentration in the whole egg because the measured mercury concentrations in the albumen could still be used to make associations between exposure and possible effects on reproductive endpoints such as hatching success.

Microsampling other contaminants

At this time, we have only used this method to sample mercury found in the albumen of the egg. The potential exists for this method to be used with other contaminants found in the egg. It is not unreasonable to expect that this method would work for a contaminant for which the respective concentrations of the contaminant in the yolk and albumen are known, such as selenium. Unlike mercury, where the overwhelming majority is found in the albumen of an egg, a smaller majority of selenium is found in the albumen [33]. Once the ratio of a contaminant in the yolk versus albumen for a particular species is known, a microsample of albumen could be taken and used to determine the contaminant concentration in the whole egg. For a given species, however, it would be important that this ratio remain relatively constant during the sampling period.

While it seems that a logical next step might be to microsample the yolk for certain lipophilic contaminants, we feel this could put the egg in unnecessary jeopardy. It is difficult to sample from the yolk given the delicate structure of the vitelline membrane. The vitelline membrane around the yolk is also the area where the blastodisc is cradled in a newly laid egg [34]. Others have tried taking a sample of yolk with mixed results on hatching [28,35]. From a logistical standpoint, sampling the yolk would be more difficult than sampling the albumen because researchers would need a candling device to

locate the yolk and a device to hold the egg steady to sample the yolk. All of this could be difficult in the field. While we do not rule out microsampling the yolk, it would have to be shown to be practical and safe in the field.

CONCLUSION

We envision microsampling to be an important tool that wildlife researchers and managers can use at mercury-contaminated sites to measure the effects of mercury on avian reproduction. By sampling the mercury concentration and monitoring the hatching success of the same egg, the microsampling technique can eliminate a source of error, namely, intraclutch variability, that has complicated field studies in the past. It can also provide valuable information on a range of levels: from the individual, to the nest, to the population. In addition, it is unlikely to reduce reproduction and recruitment in the population and thus could potentially be used to monitor sensitive species. The potential also exists for the microsampling technique to be used in studying avian diseases, which would reduce the costs and exposure associated with capturing and handling birds.

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REFERENCES

1. Wiener JG, Krabbenhoft DP, Heinz GH, Scheuhammer AM. 2003. Ecotoxicology of mercury. In Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr, eds, *Handbook of Ecotoxicology,* 2nd ed. CRC, Boca Raton, FL, USA, pp 409–463.
2. Scheuhammer AM, Perrault JA, Bond DE. 2001. Mercury, methylmercury, and selenium concentrations in eggs of common loons (*Gavia immer*) from Canada. *Environ Monit Assess* 72:79–94.
3. Lewis SA, Becker PH, Furness RW. 1993. Mercury levels in eggs, tissues, and feathers of herring gulls (*Larus argentatus*) from the German Wadden Sea coast. *Environ Pollut* 80:293–299.
4. Heinz G. 1974. Effects of low dietary levels of methylmercury on mallard reproduction. *Bull Environ Contam Toxicol* 11:386–392.
5. Scheuhammer AM. 1987. The chronic toxicity of aluminum, cadmium, mercury and lead in birds: A review. *Environ Pollut* 46: 263–295.
6. Thompson DR. 1996. Mercury in birds and terrestrial mammals. In Beyer WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations.* CRC, Boca Raton, FL, USA, pp 341–356.
7. Tejning S. 1967. Biological effects of methyl mercury dicyandiamide–treated grain in the domestic fowl *Gallus gallus* L. *Oikos Suppl* 8:1–116.
8. Borg K, Wanntorp H, Erne K, Hanko E. 1969. Alkyl mercury poisoning in terrestrial Swedish wildlife. *Viltrevy* 6:301–379.
9. Fimreite N. 1971. Effects of dietary methylmercury on ring-necked pheasants. Occasional Paper 9. Canadian Wildlife Service, Ottawa, ON.
10. Finley MT, Stendell RC. 1978. Survival and reproductive success of black ducks fed methyl mercury. *Environ Pollut* 16:51–64.
11. Heinz GH. 1979. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. *J Wildl Manag* 43: 394–401.
12. Albers PH, Koterba MT, Rossman R, Link WA, French JB, Bennett RS, Bauer WC. 2007. Effects of methylmercury on reproduction in American kestrels. *Environ Toxicol Chem* 26:1856–1866.
13. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Konrad SL, Erwin CA. 2009. Species differences in the sensitivity of avian embryos to methylmercury. *Arch Environ Contam Toxicol* 56: 129–138.
14. Fimreite N. 1974. Mercury contamination of aquatic birds in northwestern Ontario. *J Wildl Manag* 38:120–131.
15. Schwarzbach SE, Albertson JD, Thomas CM. 2006. Effects of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris obsoletus*) in San Francisco Bay. *Auk* 123:45–60.
16. Blus LJ. 1984. DDE in bird's eggs: Comparison of two methods for estimating critical levels. *Wilson Bull* 96:268–276.
17. Henny CJ, Herron GB. 1989. DDE, selenium, mercury, and white-faced ibis reproduction at Carson Lake, Nevada. *J Wildl Manag* 53:1032–1045.
18. Henny CJ, Grove RA, Kaiser JL. 2008. Osprey distribution, abundance, reproductive success, and contamination burdens along Lower Columbia River, 1997/1998 versus 2004. *Arch Environ Contam Toxicol* 54:525–534.
19. Evers DC, Taylor KM, Major A, Taylor RJ, Poppenga RH, Scheuhammer AM. 2003. Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* 12:69–81.
20. Becker PH. 1992. Egg mercury levels decline with the laying sequence in charadriiformes. *Bull Environ Contam Toxicol* 48: 762–767.
21. Evers DC, Burgess NM, Champoux L, Hoskins B, Major A, Goodale WM, Taylor RJ, Poppenga R, Daigle T. 2005. Patterns and interpretations of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology* 14:193–221.
22. Longcore JR, Haines TA, Halteman WA. 2007. Mercury in tree swallow food, eggs, bodies, and feathers at Acadia National Park, Maine, and an EPA superfund site, Ayer, Massachusetts. *Environ Monit Assess* 126:129–143.
23. U.S. Environmental Protection Agency. 2000. Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry: Test methods for evaluating solid waste, physical/chemical methods. Method 7473, SW 846, Update IVA. U.S. Government Printing Office, Washington, DC.
24. Eisler R. 2000. *Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals,* Vol 1. CRC, Boca Raton, FL, USA.
25. Hays H, LeCroy H. 1971. Field criteria for determining incubation stage in eggs of the common tern. *Wilson Bull* 83:425–429.
26. Dinsmore JJ, Schreiber RW. 1974. Breeding and annual cycle of laughing gulls in Tampa Bay, Florida. *Wilson Bull* 86:419–427.
27. Hill WL. 1993. Importance of prenatal nutrition to the development of a precocial chick. *Developmental Psychobiology* 26:237–249.
28. Finkler MS, Van Orman JB, Sotherland PR. 1998. Experimental manipulation of egg quality in chickens: Influence of albumen and yolk on the size and body composition or near-term embryos in a precocial bird. *J Comp Physiol Behav* 168:17–24.
29. Ferrari RP, Martinelli R, Saino N. 2006. Differential effects of egg albumen content on barn swallow nestlings in relation to hatch order. *J Evol Biol* 19:981–993.
30. Deeming DC. 2002. *Avian Incubation.* Oxford University, New York, NY, USA.
31. Romanoff AL. 1967. *Biochemistry of the Avian Embryo.* John Wiley, New York, NY, USA.
32. Sotherland PR, Rahn H. 1987. On the composition of bird eggs. *Condor* 89:48–65.
33. Heinz GH. 1993. Selenium accumulation and loss in mallard eggs. *Environ Toxicol Chem* 12:775–778.
34. Romanoff AL, Romanoff AJ. 1949. *The Avian Egg.* John Wiley, New York, NY, USA.
35. Cassey P, Ewen JG, Boulton RL, Karada F, Moller AP, Blackburn TM. 2007. A nondestructive method for extracting maternally derived egg carotenoids. *J Field Ornithol* 78:314–321.