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Pattern of Mercury Allocation into Egg Components is Independent of Dietary Exposure in Gentoo Penguins

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Abstract Avian eggs have become one of the most common means of evaluating mercury contamination in aquatic and marine environments and can serve as reliable indicators of dietary mercury exposure. We investigated patterns of mercury deposition into the major components of penguin eggs (shell, membrane, albumen, and yolk) using the Gentoo penguin (*Pygoscelis papua*) as a model species. Eggs were collected from both wild and captive populations of Gentoo penguins to compare the allocation of mercury into individual egg components of birds feeding at disparate trophic positions as inferred by stable isotope analysis. Mercury concentrations in captive penguins were an order of magnitude higher than in wild birds, presumably because the former were fed only fish at a higher trophic position relative to wild penguins that fed on a diet of 72–93% krill (*Euphausia* spp.). Similar to previous studies, we found the majority of total egg mercury sequestered in the albumen (92%) followed by the yolk (6.7%) with the lowest amounts in the shell (0.9%) and membrane (0.4%). Regardless of dietary exposure, mercury concentrations in yolk and membrane, and to a lesser degree shell, increased with increasing albumen mercury (used as a proxy for whole-egg mercury), indicating that any component, in the absence of others, may be suitable for monitoring changes in dietary mercury. Because accessibility of egg tissues in the wild varies, the establishment of consistent relationships among egg components will facilitate comparisons with any other study using eggs to assess dietary exposure to mercury.

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Due to the noninvasive nature and relative ease of collection, bird eggs have become one of the most widely employed means of evaluating mercury availability in aquatic and marine ecosystems (Evers et al. 2003; Ikemoto et al. 2005; Kennamer et al. 2005; Morrissey et al. 2010). Egg laying is a major elimination route in female birds through which mercury is deposited into the egg yolk, albumen, membrane, and shell (Kennamer et al. 2005). Egg mercury concentrations are significantly correlated with female blood mercury concentrations, as well as prey mercury concentrations, and are therefore equally informative of contamination levels (Evers et al. 2003; Brasso et al. 2010).

Although most studies use homogenized albumen and yolk to determine the mercury concentration of an egg, the shell and membrane can also be used document mercury concentrations (Morera et al. 1997; Evers et al. 2003; Akearok et al. 2010). It is imperative to understand how mercury is allocated into the major components of bird eggs (shell, membrane, albumen, and yolk) to make comparisons across species and studies using different components. These relationships have previously been investigated in wood ducks (*Aix sponsa*) and Audouin's gulls (*Lchthyaetus audouinii*). Kennamer et al. (2005) analyzed mercury concentrations among egg components for wood ducks and found approximately 86% of the mercury in an egg to be concentrated in the albumen, 11% in the yolk, and 3% in the shell (including the membrane). Furthermore, mercury concentrations among components were found to differ significantly. Kennamer et al. (2005) also found a strong positive relationship between albumen

and whole-egg mercury ($r^2 = 0.99$) as well as between albumen and eggshell ($r^2 = 0.61$). Similarly, Morera et al. (1997) found a significant relationship between the shell and homogenized albumen and yolk mercury concentrations in Audouin's gulls. Because migratory patterns, foraging strategies, the toxicodynamics of mercury in eggs, and the process of egg formation can vary among species, it is important to establish species-specific tissue ratios to ensure accurate assessment of the mercury signal from any one tissue (Evers et al. 2003; Kennamer et al. 2005; Akearok et al. 2010; Morrissey et al. 2010). These well-established intertissue comparisons can allow for sampling in remote areas, such as the Antarctic, where access to multiple tissue types may not be practical.

The accessibility of egg components, such as albumen and yolk, at active or abandoned penguin colonies in the Antarctic is limited to chance discovery of abandoned or infertile eggs. Furthermore, the storage and transport of whole, frozen penguin eggs is often logistically impractical. However, predated and hatched eggshell and membrane are abundant in most penguin colonies. In addition, due to their structural integrity and storage in constant, subfreezing conditions, eggshells and membrane are also found in great abundance in ornithogenic soils [bird-formed soils distinct to abandoned penguin colonies in Antarctica (Emslie and Patterson 2007)]. Therefore these two egg components may represent a practical means of evaluating mercury availability across space and time in this remote ecosystem. Egg membrane is primarily composed of protein, and mercury tends to accumulate in protein-rich tissues in the egg (Blundell and Jenkins 1977). Because the nutrients in egg membrane are derived from the recent maternal diet (Burley and Vadehra 1989; Oppel et al. 2009), membrane may provide an effective means for determining maternal dietary exposure. As the use of the shell and/or membrane, exclusive of the egg contents, to monitor environmental mercury is fairly uncommon, it is important to understand the relationship of the mercury deposited into these components relative to the yolk and albumen.

The purpose of the present study was to investigate the pattern of mercury deposition into the albumen, yolk, membrane, and shell of penguin eggs using the Gentoo penguin (*Pygoscelis papua*) as a model for the three species of Antarctic brush-tailed penguins. Gentoo penguins are the northern-most species in this genus and are typically nonmigratory, being found year-round on or near their breeding colonies on sub-Antarctic islands south to the Antarctic Peninsula (Davis and Renner 2003; Tanton et al. 2004). Gentoo penguins feed on a diet of fish and krill almost continually up to egg laying, only fasting for approximately 5 days before laying the first egg of their two-egg clutch [average laying interval is 3.4 days (Trivelpiece and Trivelpiece 1990; Davis and Renner 2003)]. Therefore, based on their nonmigratory behavior, egg mercury concentrations in Gentoo penguin eggs are likely representative of local mercury availability.

Through analysis of eggs from both wild and captive populations of the same species, our goal was to compare the allocation of mercury into eggs of wild birds with those of captive birds fed known diets at an elevated trophic position. Because the toxicodynamics of mercury in avian tissues can vary with differences in certain factors, such as dietary composition and foraging strategies (Anderson et al. 2009; Akearok et al. 2010), it was important to the present study to investigate whether patterns of mercury elimination detected were robust at different trophic positions and dietary mercury exposure (as determined by differences in nitrogen stable isotope [δ^{15}N] values and mercury concentrations in eggs components and prey). δ^{15}N can be used to infer trophic position because the isotopic abundance in a consumer's tissues is related to the isotopic composition of its prey (Rau et al. 1991; Anderson et al. 2009). In the Antarctic marine food web, Antarctic krill (*Euphausia superba*) are primary consumers and occupy a lower trophic level compared with most penguin prey fish species as reflected by relatively lower δ^{15}N values in Antarctic krill compared with these fish (Rau et al. 1991, 1992). Due to the high biomagnification capability of mercury, higher trophic-level prey should have a higher mercury concentration than lower trophic-level prey (Thompson 1996; Anderson et al. 2009). Subsequently, penguins feeding proportionally more on fish than krill should have higher mercury levels. Our main objectives were to (1) determine the pattern in which Gentoo penguins allocate mercury into individual egg components, (2) investigate whether patterns are consistent at different levels of dietary mercury exposure (captive vs. wild populations), and (3) investigate intertissue relationships among egg components to evaluate the suitability of egg membrane for assessing mercury availability in the Antarctic marine food web.

Methods

Captive Penguin Tissue Collection

The Gentoo penguins used in this study were from a captive breeding population maintained at the Henry Doorly Zoo in Omaha, NE. Penguins that were part of this study were fed a consistent diet of Atlantic herring (*Clupea harengus*) for 8 months before egg laying and subsequent tissue collection (Polito et al. 2009). Random sampling of individual herring during the 2 months before the mean date of egg laying in the population provided 10 tissue samples for isotopic and mercury analysis.

Because intraclutch variability has previously been reported in marine birds in which the first egg in a clutch has higher mercury than subsequent eggs (Becker 1992; Morera et al. 1997; Akearok et al. 2010), eggs were marked with a permanent marker as they were laid to establish laying order and investigate this trend in penguins. Approximately 1 week after laying, eggs were candled to determine if they were fertile; a subsample of 5 whole infertile eggs were collected and frozen for isotopic and mercury analysis. An additional 13 eggshells with intact membranes were collected from nests after the chicks had hatched or the eggs were determined to be addled. Overall, we were able to collect 18 eggshells with intact membranes from six 2-egg clutches and two 3-egg clutches from 8 female penguins.

Wild Penguin Tissue Collection

During the 2008–2009 austral summer, eggs were opportunistically collected from wild Gentoo penguins at two adjacent breeding colonies: Danco Island (64°44′S 62°37′W) and Cuverville Island (64°41′S 62°38′W) in the Errera Channel along the western Antarctic Peninsula. One egg from each of 10 abandoned Gentoo penguin nests at each colony was collected on December 12, 2008. Eggs from these 20 nests were frozen at the time of collection due to the nests being built on snow during a period of persistent and heavy snowfall across the breeding site during this season; eggs were subsequently kept frozen during transport and before isotopic and mercury analysis. Representative samples of the major dietary components of Gentoo penguins in this region (homogenized Antarctic krill [*E. superba*: $n = 6$, homogenized 10-g samples containing approximately 20 individuals/sample]) and a representative prey fish [*Lepidonotothen squamifrons*: $n = 10$ (Karnovsky 1997)] were collected during the 2008–2009 austral summer during trawls conducted in the vicinity of the Antarctic Peninsula and South Shetland Islands by the United States Antarctic Marine Living Resource Program.

Sample Preparation

Whole eggs were removed from the freezer and allowed to thaw slightly to remove the shell; the yolk was separated from the albumen while still frozen to prevent cross-contamination between these components. When these eggs were processed, no signs of embryonic development were detected. After thawing, the whole yolk, albumen, and shell (with attached membrane) were weighed separately to obtain the whole mass (to the nearest 0.001 g) of each component. An aliquot sample (approximately 1.0–2.0 g) each of the yolk and albumen were collected in separate glass vials and weighed to the nearest 0.0001 g before and after and being freeze dried. For each sample, an approximately 0.20-g piece of membrane was separated from the shell by soaking a shell fragment in deionized water; a stainless steel scalpel was then used to remove as much of the membrane as possible. Membranes were thoroughly cleaned with a toothbrush and rinsed in deionized water to remove any remnants of albumen or yolk to ensure an accurate mercury signal from this tissue. A Dremel tool with a sanding attachment was used to remove any remaining membrane from the shell; clean shells were ground into a fine powder using an analytical mill. Egg membrane and shell were allowed to air dry before mercury analysis. Krill and fish samples were homogenized separately and dried in an oven at 60°C for 48 h before analysis.

Mercury Analysis

Because nearly all mercury in avian eggs (Bond and Diamond 2009) and in fish and crustacean tissues (Bloom 1992) is present in the form of methylmercury, a measurement of total mercury concentration was used as a proxy for this highly bioavailable form. Total mercury concentration in individual egg components and prey items were analyzed by way of atomic absorption spectrophotometry on a Milestone 80 Direct Mercury Analyzer at The College of William and Mary (Williamsburg, VA). Each set of 20 samples analyzed was preceded and followed by two method blanks, a sample blank, and two samples each of standard reference material (DORM-3, DOLT-3, fish protein, and dogfish liver certified reference materials, respectively, provided by National Research Council Canada). All mercury concentrations are reported as parts per million (ppm) dry weight (dw). Mean percent recoveries for standard reference materials were 98.5 ± 1.1% (DORM-3) and 99.2 ± 1.1% (DOLT-3). The relative percent difference between 20 pairs of duplicate samples was 1.1 ± 0.8%; spike recoveries ($n = 12$) were 100.6 ± 2.8%. Detection limit of the assay was 0.005 ng mercury.

In addition to determining mercury concentrations for each component, total mercury (ng) also was calculated separately for albumen, yolk, shell, and membrane. Dry mass (g) of the whole yolk and albumen was determined by multiplying the average moisture loss in each component after freeze-drying (yolk = 0.548 ± 0.06; albumen = 0.874 ± 0.01) by the total wet mass of each component and then subtracting this product from the original wet mass. The shell (WS in following equations) was originally weighed with the membrane intact to preserve the integrity of both components. Therefore, to estimate the whole mass (g) of the shell (S) and membrane (M) separately, we calculated the percent difference (PD) in weight of a 1.0- to 2.0-g piece of shell before and after removal of the attached

membrane. This difference represented the component of the shell mass that was membrane (mean 0.05 ± 0.01). The mass (g) of the membrane and shell were calculated as follows: [M = PD × WS] and [S = WS – M], respectively. Total mercury (ng) for each component was calculated as the product of its dry mass (g) and the component concentration of mercury (ng/g). Whole-egg mercury (ng) was thus the sum of the mercury in all components. These calculated values were used for the determination of the percentage of total egg mercury in each component and to estimate whole-egg mercury.

Stable Isotope Analysis

Approximately 10 mg of eggshell and 0.5 mg of other egg and prey tissues were flash-combusted (Costech ECS4010 elemental analyzer) and analyzed for δ^{15}N through an interfaced Thermo Delta V Plus continuous-flow stable isotope ratio mass spectrometer. Eggshells were acidified to remove carbonates before analysis (Polito et al. 2009). Egg yolk and prey tissues were not lipid extracted before analysis. Raw δ values were normalized on a two-point scale using depleted and enriched glutamic acid standard reference materials United States Geological Survey (USGS)-40 (δ^{13}C: −26.389 ± 0.042; δ^{15}N: −4.5 ± 0.1) and USGS-41 (δ^{13}C: 37.626 ± 0.049; δ^{15}N: 47.6 ± 0.2). Sample precision based on duplicate standard and sample materials was 0.2‰ for both δ^{13}C and δ^{15}N. Stable isotope abundances are expressed using a δ notation in per-milliliter units (‰) based on the following equation:

$$\delta X = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] * 1000,$$

where X is ^{15}N, and R is the corresponding ratio of ^{15}N/^{14}N. The R_{standard} value was based on the atmospheric N_2 for ^{15}N.

We quantified the relative importance of krill (*E. superba*) *versus* fish (*L. squamifrons*) to the diets of wild Gentoo penguins by using a single-isotope, two-source linear mixing model (Phillips and Gregg 2001) based on the δ^{15}N values of the membrane, albumen, and yolk. Model results provide SEs and confidence intervals for source proportion estimates that account for the observed variability in the isotopic signatures for the sources as well as the mixture. Before incorporation into the isotopic model, we corrected egg tissue values for dietary isotopic fractionation using species- and tissue-specific discrimination factors (Polito et al. 2009).

Statistical Analysis

SPSS software (version 16.0; Chicago, IL) was used to perform all statistical analyses. Mercury values were not normally distributed for all egg components, as determined by Kolmogorov–Smirnov tests, and therefore all values were log-transformed to normalize these data. One-way analyses of variance (ANOVAs) with Tukey's HSD post hoc comparisons were used to determine whether differences existed in mercury concentrations and δ^{15}N values among egg components in the wild and captive populations, which were analyzed separately unless otherwise noted. Mercury concentrations in captive (herring) and wild (Antarctic krill and *L. squamifrons*) prey items were also log-transformed and compared using one-way ANOVAs with Tukey's HSD *post hoc* comparisons. To assess relationships among egg components, the data from wild and captive birds were combined. The combined data were not distributed normally, as determined by Kolmogorov–Smirnov tests, and thus more conservative nonparametric Spearman correlations were used to test for relationships among components. One-way ANOVA was used to assess interclutch variation of mercury among clutches from the captive population; paired Student t test was used to investigate intraclutch variation. All means are presented ±SDs, except for wild penguin diet composition estimates resulting from our linear mixing model, which are presented ±SEs. In all cases, statistical significance was defined as $P < 0.05$.

Results

Mercury concentrations in all components of eggs from captive Gentoo penguins were significantly higher than in wild Gentoo penguins (Table 1, $P < 0.001$ in all cases). In both populations, there was a significant effect of egg component on mercury concentration (captive: $F_{3,45} = 182.13$, $P < 0.001$; wild: $F_{3,78} = 370.15$, $P < 0.001$). *Post hoc* comparisons indicated that in both wild and captive populations, mercury concentrations in the albumen were significantly higher than in the other three components ($P < 0.001$ in all cases). Mercury concentrations in shell were significantly lower than in the other three components ($P < 0.001$), but mercury did not differ significantly between the yolk and membrane ($P > 0.07$ in both cases). Despite disparities in mercury concentrations between the captive and wild populations, the pattern of mercury allocation into each egg component was similar across all eggs (albumen > yolk = membrane > shell; Fig. 1).

Relationships Among Egg Components

On average, 92.0% of the total egg mercury was allocated into the albumen, 6.7% in the yolk, 0.4% in the membrane, and 0.9% in the shell of Gentoo penguin eggs. Total albumen mercury was highly positively correlated with whole-egg mercury (Spearman's $\rho = 0.99$, $P < 0.001$). Using albumen mercury concentration as a proxy for

Table 1 Mercury concentrations of individual egg components (ppm, dw; ±SDs)

Penguin type	Albumen	Yolk	Membrane	Eggshell
Captive				
Mean	3.792 ± 0.379	0.362 ± 0.076	0.266 ± 0.329	0.004 ± 0.001
Range	3.232 − 4.162	0.250 − 0.425	0.034 − 1.409	0.003 − 0.008
Wild				
Mean	0.162 ± 0.029	0.009 ± 0.001	0.010 ± 0.003	0.001 ± 0.001
Range	0.108 − 0.218	0.006 − 0.011	0.006 − 0.021	0.001 − 0.002

Wild eggs: $n = 20$ for all components; captive eggs: $n = 5$ for albumen and yolk; $n = 18$ for membrane and shell. Although reported here, eggshell mercury concentrations often approached or were just lower than the minimum detection limit of the assay

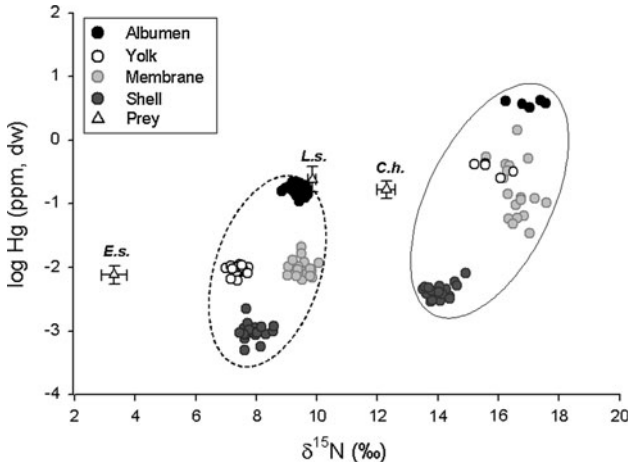

Fig. 1 Mercury was allocated in similar proportions into each egg component regardless of dietary mercury exposure (albumen > membrane = yolk > eggshell). Eggs from the captive population are encircled with a *solid black line*; eggs from the wild population are encircled by a *dashed line*. Prey items are represented by *triangle symbols*; error bars indicate SDs; *E.s.* = *E. superba* (krill, $n = 6$); *L.s.* = *L. squamifrons* (wild-prey fish, $n = 10$); C.h. = *C. harengus* (herring, $n = 10$). Egg component δ^{15}N values are presented uncorrected for isotopic discrimination

whole-egg mercury, mercury concentrations in the yolk and membrane also were found to increase with increasing whole-egg mercury (Fig. 2). Although there was tendency for eggs with higher mercury in the albumen to have higher mercury in the shell, the correlation was not significant ($P = 0.12$). Mercury concentrations in membrane did not differ among clutches in the captive population ($P = 0.33$). Furthermore, there was no effect of laying sequence on mercury concentration in the captive Gentoo population; no differences were detected in membrane mercury concentration between the first and second eggs across all clutches ($n = 8$, paired Student t test, $P = 0.50$).

Dietary Mercury Exposure

Mercury concentrations differed significantly among the prey items sampled ($F_{2,25} = 15.18$, $P < 0.001$). *Post hoc* comparisons indicated that the mercury concentration in *L. squamifrons* (0.265 ± 0.133 ppm) was similar to herring (0.175 ± 0.056 ppm) and that mercury concentrations in both fish species were higher than in Antarctic krill (0.008 ± 0.002 ppm; $P < 0.004$ in both cases). Although captive Gentoo penguins consumed only fish (herring), our isotopic models estimated that Antarctic krill dominated the prelaying diets of wild female Gentoo penguins. Separate calculations based on membrane, albumen, and yolk estimated that Antarctic krill comprised approximately 72.3 ± 2.4%, 78.5 ± 2.4%, and 93.8 ± 2.5% of wild penguin diets compared with fish (*L. squamifrons*), respectively. The disparity in diet composition between captive and wild penguins is reflected in the higher δ^{15}N values found in the captive population across all four egg components ($P < 0.0001$ in all cases). Overall, higher concentrations of mercury in egg components from captive Gentoo penguins corresponded with the dominance of fish in the diet (herring) and thus the increased δ^{15}N values in this population (Fig. 1).

Discussion

Similar to previous studies investigating the allocation of mercury into the individual components of bird eggs (Morera et al. 1997; Kennamer et al. 2005), we found the highest concentration of mercury sequestered in the albumen followed by the yolk, with the lowest concentrations in the membrane and shell. To our knowledge, the present study is the first to document mercury concentrations of the shell and membrane independently. Overall, mercury concentrations in each egg component increased with increasing albumen mercury concentrations (used as a proxy for whole-egg mercury), indicating that any one component, in the absence of others, may be suitable for tracking changes in dietary mercury exposure. However, the lack of a statistically significant correlation between shell and albumen mercury suggests that shell mercury

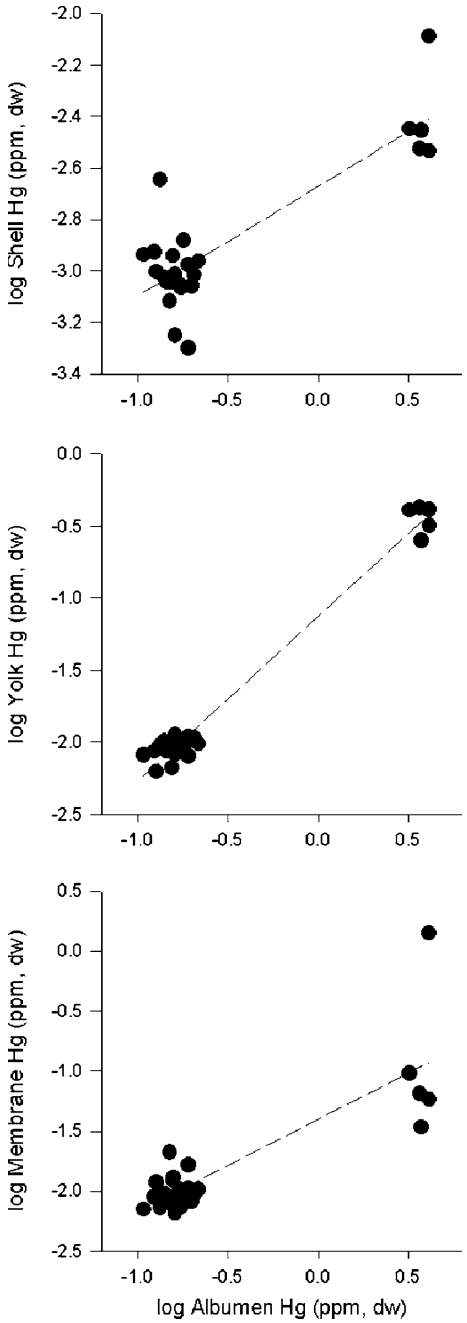

Fig. 2 An increase in albumen mercury concentration (used as a proxy for whole-egg mercury) was positively correlated with increases in mercury concentrations in the yolk ($\rho = 0.71$, $P < 0.001$) and membrane ($\rho = 0.55$, $P < 0.01$); there was no significant correlation between shell and albumen mercury concentrations ($\rho = 0.32$, $P = 0.12$). Linear regression analysis was not applied to these data; the dashed line is for graphical display of intercomponent relationships only

may not be as informative of egg mercury as the other components, especially at low egg mercury concentrations.

Mercury concentrations in eggs of captive Gentoo penguins were an order of magnitude higher than in wild penguins, and this difference was most likely due to differences in diet composition. The captive population was fed a consistent diet of herring for 8 months before and during the egg-laying period, whereas the wild population was feeding on a mixture of fish and krill. In the Antarctic ecosystem, Antarctic krill are primary consumers and occupy a lower trophic level compared with most penguin prey fish species, which are commonly secondary consumers (Rau et al. 1992). These trophic differences are reflected in the relatively lower δ^{15}N values and mercury concentrations found in Antarctic krill relative to Antarctic fish species found in this and other studies (Rau et al. 1991; Becker et al. 2002; Anderson et al. 2009). Similarly, we found that the δ^{15}N values and mercury concentrations in Antarctic krill were significantly lower than those found in *L. squamifrons* and the Atlantic herring used in our captive study. It is important to note that herring and the wild prey items examined in our study are not naturally found in the same food web; thus, comparisons of δ^{15}N values between these groups may not be an absolute reflection of trophic levels per se. Even so, in the context of our study, the low δ^{15}N values and mercury concentrations found in Antarctic krill, as well as the similar δ^{15}N values and mercury concentrations found in herring and *L. squamifrons*, allow us to infer the mercury exposure of Gentoo penguins feeding at two different trophic levels (krill *vs*. fish). Subsequently, captive penguins feeding at a higher trophic level (solely fish) had higher mercury concentrations in their eggs relative to wild penguins feeding at a relatively lower trophic level (approximately 72–93% Antarctic krill). Furthermore, these findings are likely applicable to wild studies and suggest that the trophic biomagnification of mercury in the Antarctic food web can allow researchers to track the relative dietary importance of prey items occupying different tropic levels by examining the mercury concentration of egg components.

Several studies of marine and aquatic birds have found significant effects of laying order on mercury concentration in eggs in which mercury concentration in the first egg laid is on average >25% higher than in the second or third egg (Becker 1992; Morera et al. 1997; Evers et al. 2003; Akearok et al. 2010). For this reason, knowledge of the laying sequence can have significant implications regarding egg collection in the field; nonrandom sampling could lead to overestimates or underestimates of mercury concentrations if only early or late eggs, respectively, are collected (Evers et al. 2003; Akeraok et al. 2010). Because laying sequence was known in the captive population in the present study, we were able to investigate this potentially confounding factor that has not previously been investigated in penguins. In addition to little interclutch variation, we did not detect intraclutch variation in mercury concentrations between the first and second eggs laid. In the

two clutches of three eggs each, mercury concentrations tended to be lower in the third egg compared with the first; however, the small sample size of the third eggs precluded more detailed analysis.

Although we did not detect intraclutch variation in the captive population, care should be taken in extrapolation of this result to wild birds because captive penguins in the present study were being hand fed a controlled diet of herring, *ad libitum*, for 8 months before egg laying. Intraclutch variation in penguins must be investigated in the field to make a more sound determination for this species; however, the small amount of variation detected in the 20 wild eggs collected at random (Table 1) suggests that random sampling and a large sample size may be sufficient to eliminate noise from this potentially confounding factor. The nonmigratory behavior of Gentoo penguins could lead to decreased local-scale variation in dietary mercury; it would be interesting to address intraclutch variation in the Adélie penguin (*P. adeliae*) because this species spends the winter at sea, away from the breeding colonies, and fasts for $\leq$2 weeks before egg laying, thus relying significantly on stored energy reserves for egg formation (Astheimer and Grau 1985).

Because most previous studies using avian eggs to monitor environmental contaminants have analyzed the albumen and yolk exclusive of the shell and membrane, it was important to document the relationships among these components to allow for comparison among studies using different components. Previous studies have found a significant positive relationship between the egg contents (homogenized yolk and albumen) and eggshell (including the membrane) and suggest the suitability of eggshell to trace historical trends in mercury availability given large-scale differences in the absence of these other tissues. Because of their long-term persistence in the environment, the use of eggshells has become increasingly common in documenting historical changes in diet and contaminant availability (Emslie and Patterson 2007; Xu et al. 2011). Using a 700 year environmental archive of seabird eggshells from the Xisha Archipelago in the South China Sea, Xu et al. (2011) were able to document historical changes in mercury deposition in the marine environment reflective of industrial anthropogenic activities. Furthermore, Xu et al. (2011) found no evidence of changes in the ultrastructure of eggshells (as determined by scanning electron microscopy analysis). Ancient eggshells had similar ultrastructure as modern shells, suggesting little, if any, diagenetic changes and thus further documenting the robustness of this hard avian tissue and its suitability in the monitoring of long-term environmental change (see also Emslie and Patterson 2007).

Because membrane from penguin eggs is often found separated from the shell in the Antarctic, it was necessary to understand how mercury is allocated to this egg component compared with the others to reliably use this tissue. Although albumen may provide the most robust measure of whole-egg mercury, the present study has shown that mercury is allocated in a small proportion into the membrane (<0.5%) and can be used to document trophic-level changes in dietary mercury uptake. Because differences in dietary composition between the wild and captive populations in the present study were reflected by a corresponding change in mercury concentration in all four egg components, our findings further demonstrate the suitability of any one of these components for tracking changes in dietary mercury exposure. These findings add to the body of literature to strongly suggest that the pattern in which mercury is allocated to individual egg components is independent of dietary mercury exposure and appears to be consistent among taxa.

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