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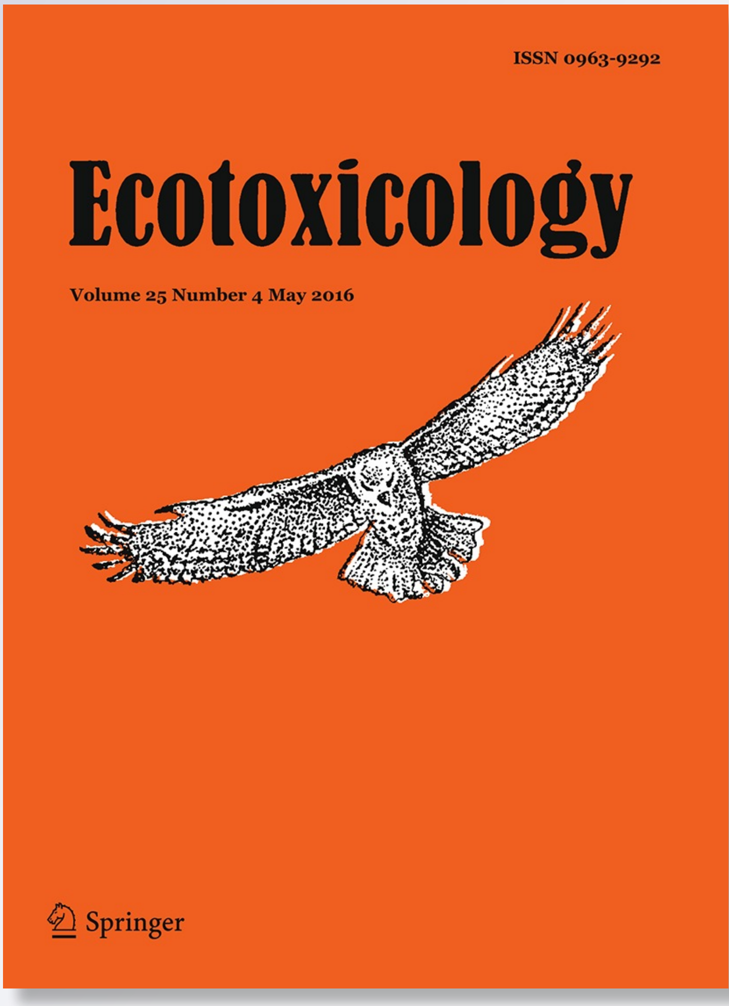

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It's what's inside that counts: egg contaminant concentrations are influenced by estimates of egg density, egg volume, and fresh egg mass

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Abstract In egg contaminant studies, it is necessary to calculate egg contaminant concentrations on a fresh wet weight basis and this requires accurate estimates of egg density and egg volume. We show that the inclusion or exclusion of the eggshell can influence egg contaminant concentrations, and we provide estimates of egg density (both with and without the eggshell) and egg-shape coefficients (used to estimate egg volume from egg morphometrics) for American avocet (*Recurvirostra americana*), black-necked stilt (*Himantopus mexicanus*), and Forster's tern (*Sterna forsteri*). Egg densities (g/cm^3) estimated for whole eggs (1.056 $\pm$ 0.003) were higher than egg densities estimated for egg contents (1.024 $\pm$ 0.001), and were 1.059 $\pm$ 0.001 and 1.025 $\pm$ 0.001 for avocets, 1.056 $\pm$ 0.001 and 1.023 $\pm$ 0.001 for stilts, and 1.053 $\pm$ 0.002 and 1.025 $\pm$ 0.002 for terns. The egg-shape coefficients for egg volume (K_v) and egg mass (K_w) also differed depending on whether the eggshell was included ($K_v = 0.491 \pm 0.001$; $K_w = 0.518 \pm 0.001$) or excluded ($K_v = 0.493 \pm 0.001$; $K_w = 0.505 \pm 0.001$), and varied among species. Although egg contaminant concentrations are rarely meant to include the eggshell, we show that the typical inclusion of the eggshell in egg density and egg volume estimates results in egg contaminant concentrations being underestimated by 6–13 %. Our results demonstrate that the inclusion of the eggshell significantly influences estimates of egg density, egg volume, and fresh egg mass, which leads to egg contaminant concentrations that are biased low. We suggest that egg contaminant concentrations be calculated on a fresh wet weight basis using only internal egg-content densities, volumes, and masses appropriate for the species. For the three waterbirds in our study, these corrected coefficients are 1.024 $\pm$ 0.001 for egg density, 0.493 $\pm$ 0.001 for K_v, and 0.505 $\pm$ 0.001 for K_w.

Keywords Egg density · Egg-shape coefficients · Egg volume · Fresh wet weight · Eggshell · Egg contaminants

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Introduction

The contents of avian eggs represent one the most ideal matrices for understanding the toxicological risk of environmental contaminants (Ackerman et al. 2013; Hartman et al. 2013). In avian biology and contaminant ecology, eggs are often collected at various stages of incubation, and egg mass and egg density change throughout incubation as eggs respire and moisture is lost (Romanoff 1932; Drent 1970; Brown 1976). For example, studies have shown that egg mass changes by at least 15 % over the incubation period (Westerskov 1950; Drent 1970; Brown 1976). Because eggs are rarely sampled in the field at the time of egg laying, resulting egg contaminant concentrations are substantially influenced by the mass of the egg at the time of collection. Thus, it is necessary to estimate the mass of an egg at the time it was laid (fresh egg mass) because contaminant concentrations are determined on a per-unit mass basis and fresh egg mass is the only comparable metric for egg mass across egg contaminant studies.

Specifically, estimates of an egg's contaminant concentration is significantly biased high relative to the egg contaminant concentration at the time the egg was laid, and requires an adjustment for the change in an egg's mass as it develops (Stickel et al. 1973). The most common way to determine the egg mass at the time of laying is to estimate the original mass based on the density of a freshly laid egg and external egg morphometrics. Following an approach developed by Pearl and Surface (1914), Stickel et al. (1973) suggested estimating fresh egg mass using estimated egg volume and assuming an egg density of 1.0 g/ml. Hoyt (1979) used the same approach to estimate egg volume, but also incorporated a more specific estimate of egg density in order to provide additional accuracy in the estimation of fresh egg mass at laying. This approach to estimating the fresh wet weight of eggs that were collected at various times during incubation has proven to be useful and has been widely implemented in egg contaminant studies (Ackerman et al. 2013).

Although Hoyt (1979) demonstrated the value of incorporating the density of a freshly laid egg into calculations of the mass of a freshly laid egg, egg density can vary by taxa and specific estimates of egg density for many species are lacking. Moreover, estimates of egg density and egg volume can incorporate the entire egg (contents plus the eggshell) or only the egg contents (without the eggshell). Eggshell density is significantly higher than the density of egg contents, and likewise the volume of the eggshell is not insignificant relative to total volume of the egg. Thus, the inclusion or exclusion of the eggshell can be important issue for egg contaminant studies. Researchers are rarely interested in the contaminant concentration found within the eggshell and typically exclude the eggshell during chemical determination. However, if the eggshell is included when estimating egg volume or egg density, then the final estimates of egg contaminant concentrations will be inaccurate.

Herein, we estimate egg densities for three species of waterbirds, and provide estimates for egg density of the entire egg (including the eggshell) as well as egg density of only the egg contents (without the eggshell). We also provide egg-shape coefficients both including and excluding the eggshell which can be used to accurately estimate egg volume and fresh egg mass from external egg morphometrics for three species. Finally, we use mercury concentrations in eggs as an example to show the consequences for estimating egg contaminant concentrations when using egg densities and egg volumes that are based only on egg contents versus those that also include the eggshell. We offer recommendations for estimating egg contaminant concentrations and provide three new species-specific egg densities and egg volume coefficients for calculating fresh egg masses.

Methods

Egg collection and field measurements

We monitored nests of American avocet (*Recurvirostra americana*; hereafter avocet), black-necked stilt (*Himantopus mexicanus*; hereafter stilt), and Forster's tern (*Sterna forsteri*; hereafter tern) in South San Francisco Bay between April and July 2009. During weekly visits to the nesting colonies, we marked all eggs in every nest in the colony with a permanent marker. Using egg flotation to determine embryo age (Westerskov 1950; Ackerman and Eagles-Smith 2010), we identified nests containing freshly laid eggs (i.e., day 0 of incubation) in clutches that had not yet been completed. We returned within 24 h to visit these nests, and determined if a new egg had been laid. If our field observations confirmed that a new egg had been laid within the past 24 h, then we collected it. We collected only 1 egg per clutch.

Collected eggs were immediately placed in egg cartons and stored in small coolers with wet ice during transport back to the laboratory. Immediately upon returning from the field, we cleaned the egg with deionized (DI) water and allowed the egg to dry. We then measured egg length (±0.01 mm) and egg width (±0.01 mm) with digital calipers (Fisherbrand©), and fresh whole egg mass (±0.0001 g) with a digital balance (Precisa XB220A). We then stored eggs in a refrigerator (2 °C) until egg dissection and processing.

Estimating morphometry: egg volume and egg mass

We estimated the internal volume of each egg, by measuring the amount of water each egg could hold. The contents of each egg were emptied in a manner that maintained the integrity of the eggshell. First, a very small hole, <3 mm in diameter, was made in the center of the wide end of the egg using a Dremel© tool. Then, using a polyethylene transfer pipet (Fisherbrand©), we removed the contents of the egg.

The inside of each emptied egg was rinsed with deionized water repeatedly until completely clean, and then placed in a drying oven hole-side down, and allowed to dry. Then, when cooled, we inverted the egg upright, and, using a 5 ml needle-tipped syringe, we injected deionized water into the egg until it was completely full, ensuring that there were no trapped air bubbles, and recorded the mass (±0.0001 g; including eggshell mass). The internal egg volume was estimated as the difference in egg mass before and after filling the eggshell with deionized water, because 1.0 ml of deionized water weighs approximately 1.0 g.

After estimating the internal egg volume, we removed all the water from inside the eggshell and then the empty eggshell was placed within a drying oven at 50 °C for 72 h in order to completely dry the eggshell. We then removed the eggshell from the drying oven, allowed the egg to return to room temperature, and measured the dried weight of the eggshell with a digital balance. Fresh egg-content mass was calculated as the difference between fresh whole egg mass (measured immediately after collection in the field) and dried eggshell mass (measured after drying in a drying oven).

In order to estimate the whole egg (egg contents plus shell) volume, it was necessary to estimate eggshell volume in addition to the internal egg volume described above. We estimated the eggshell volume of each egg by dividing the dried eggshell mass by the estimated eggshell density. Eggshell density was estimated using an allometric relationship between eggshell density and fresh whole egg mass developed by Paganelli et al. (1974):

$$Density_{eggshell} = 1.945\left(Mass_{whole\_egg\_fww}\right)^{0.014} \quad (1)$$

Whole egg volume was then estimated as the sum of the eggshell volume and internal egg volume.

Estimating egg density and egg-shape coefficients

We calculated the fresh egg density at laying of the entire egg ($Density_{whole\_egg}$; egg contents and eggshell) as well as for the egg contents only ($Density_{egg\_content}$). For $Density_{whole\_egg}$, fresh whole egg mass (measured at the time of egg collection soon after laying) was divided by whole egg volume.

$$Density_{whole\_egg} = \frac{Mass_{whole\_egg\_fww}}{Volume_{whole\_egg}} \quad (2)$$

For density of only the egg contents (without the eggshell), the fresh egg-content mass was divided by the internal egg volume (excluding the eggshell's volume).

$$Density_{egg\_content} = \frac{Mass_{egg\_content\_fww}}{Volume_{egg\_content}} \quad (3)$$

Egg-shape coefficients for egg volume (K_v) were estimated as the ratio of egg volume to the product of egg length (L) and egg width (W) squared, and, likewise, the egg-shape coefficients for fresh egg mass (K_w) were estimated as the ratio of fresh egg mass to the product of egg length and egg width squared following equations of Hoyt (1979). We estimated K_v and K_w separately for whole egg ($K_{v\_whole\_egg}$ and $K_{w\_whole\_egg}$) and internal egg contents only (excluding the eggshell; $K_{v\_egg\_content}$ and $K_{w\_egg\_content}$). The internal egg-shape coefficients required egg length and egg width measurements that did not include the thickness of the eggshell and were estimated as the externally measured egg length and egg width minus twice the average eggshell thickness (representing the eggshell surrounding each side of the egg). Mean eggshell thickness was estimated for each species using data from the literature (Schönwetter 1960–1992; Roberts 1997; Pyle et al. 1999; Ainley et al. 2002; Henny et al. 2008).

$$K_{v\_whole\_egg} = \frac{Volume_{whole\_egg}}{LW^2} \quad (4)$$

$$K_{v\_egg\_content} = \frac{Volume_{egg\_content}}{(L - 2 \times Egg\,Shell\,Thickness)(W - 2 \times Egg\,Shell\,Thickness)^2} \quad (5)$$

$$K_{w\_whole\_egg} = \frac{Mass_{whole\_egg\_fww}}{LW^2} \quad (6)$$

$$K_{w\_egg\_content} = \frac{Mass_{egg\_content\_fww}}{(L - 2 \times Egg\,Shell\,Thickness)(W - 2 \times Egg\,Shell\,Thickness)^2} \quad (7)$$

Results and discussion

A total of 78 freshly-laid (less than 24 h old) eggs were collected, including 22 avocet eggs, 24 stilt eggs, and 32 Forster's tern eggs (Table 1). We provide the mean ± SE of egg morphometrics for each species (Table 1) and the egg density and egg-shape coefficients for whole eggs (including the eggshell) and egg contents only (excluding the eggshell; Table 2).

The egg-shape coefficients used to estimate egg volume (K_v) in these 3 waterbird species were lower than the average egg-shape coefficient ($K_v = 0.509$) derived by Hoyt (1979) for 26 species and is likely a result of the more asymmetrical shape of the eggs of avocet, stilt, and tern. In fact, Hoyt (1979) specified that this outcome is expected for species, such as shorebirds, that produce more asymmetrically shaped eggs and recommends empirically estimating K_v for individual species when possible.

Rahn et al. (1982) summarized densities of whole eggs and for egg contents for 23 species of birds. For the nine species of birds in the order Charadriiformes (the taxonomic order of avocets, stilts, and terns), egg-content density varied from 1.025 to 1.034 (mean = 1.030) and whole egg density ranged from 1.055 to 1.100 (mean = 1.065; Rahn et al. 1982). Our estimates of egg density for avocets, stilts, and terns were on the lower end of these ranges (mean egg-content density = 1.024; mean whole egg density = 1.056; Table 2). These results were expected because egg density generally increases with egg mass (Rahn and Paganelli 1989), and the mean fresh whole

Table 1 Egg morphometrics for American avocets, black-necked stilts, and Forster's terns nesting in San Francisco Bay, California

Species	Number of eggs	Egg length (mm)	Egg width (mm)	Fresh whole egg mass (g)	Dry eggshell mass (g)	Internal egg volume (ml)	Eggshell volume (ml)	Total egg volume (ml)
American avocet	22	49.87 ± 0.37	34.62 ± 0.18	30.62 ± 0.40	1.98 ± 0.02	27.93 ± 0.37	0.97 ± 0.01	28.91 ± 0.38
Black-necked stilt	24	43.70 ± 0.31	31.27 ± 0.15	22.05 ± 0.26	1.40 ± 0.02	20.19 ± 0.24	0.68 ± 0.01	20.88 ± 0.25
Forster's tern	32	42.90 ± 0.24	30.24 ± 0.16	20.70 ± 0.26	1.12 ± 0.02	19.11 ± 0.24	0.55 ± 0.01	19.65 ± 0.24

Table 2 Egg density (g/cm^3) and egg-shape coefficients (K_v and K_w) for American avocets, black-necked stilts, and Forster's terns nesting in San Francisco Bay, California

Species	Number of eggs	Whole egg (includes eggshell)			Egg contents (does not include eggshell)		
		Density ± SE	K_v ± SE	K_w ± SE	Density ± SE	K_v ± SE	K_w ± SE
American avocet	22	1.059 ± 0.001	0.483 ± 0.001	0.512 ± 0.002	1.025 ± 0.001	0.483 ± 0.001	0.495 ± 0.002
Black-necked stilt	24	1.056 ± 0.001	0.488 ± 0.001	0.516 ± 0.001	1.023 ± 0.001	0.490 ± 0.001	0.501 ± 0.001
Forster's tern	32	1.053 ± 0.002	0.500 ± 0.001	0.527 ± 0.001	1.025 ± 0.002	0.506 ± 0.001	0.519 ± 0.001
Species mean	78	1.056 ± 0.003	0.491 ± 0.001	0.518 ± 0.001	1.024 ± 0.001	0.493 ± 0.001	0.505 ± 0.001

egg mass for the Charadriiformes (mean = 69.8 g) in Rhan et al.'s (1982) study was more than double the mean fresh whole egg masses for the species of Charadriiformes we used in our study (mean = 24.5 g; Table 1).

Within a species, our results indicate that the estimated egg-shape coefficients (K_v) for egg-content volume and whole egg volume were essentially the same, because the shape of the egg contents is ultimately derived from the shape of the entire egg that the contents are confined within. Thus, we believe the egg shape coefficients (K_v) available in the literature, regardless of whether they include eggshell or not in the estimation of the egg volume shape coefficient, are acceptable to use in calculations of egg volume. We have provided additional egg-shape coefficients for American avocets, black-necked stilts, and Forster's terns that were not previously available (Table 2).

On the other hand, the egg-shape coefficients for egg mass (K_w) differed depending on whether or not the eggshell was included in the estimate. Egg-shape coefficients for egg mass (K_w) represent the combination of egg density and the egg shape coefficient for volume. Because egg densities differed between whole egg density and egg-content density, egg-shape coefficients that were derived to estimate the mass of only the egg contents (excluding eggshell) were smaller than those egg-shape coefficients derived to estimate the mass of the whole egg (including eggshell). As a result, egg-shape coefficients for egg mass (K_w) should be used only when it can be determined whether the egg-shape coefficient (K_w) was estimated using the whole egg or only the egg contents.

It is important to note, that our estimate of eggshell density was not measured directly, but instead derived based on an allometric relationship with whole egg mass (see Paganelli et al. 1974). This could result in some measure of error associated with our eggshell density and, in turn our estimate of eggshell volume. However, the results of Paganelli et al. (1974) indicate that there is very little variation in eggshell density across avian taxa. Paganelli et al. (1974) showed that even when comparing two species whose mean egg mass were 1 and 1000 g, the difference in predicted eggshell density was only 10 % (1.95–2.14 g/cm^3), and is significantly less than that for eggs with more similar egg mass. Therefore, Paganelli et al.'s (1974) allometric relationship between eggshell density and egg size should adequately predict eggshell density for most species except those, for example, that have evolved specific life history strategies related to eggshell strength.

Consequences for estimating egg density: an example with egg contaminant concentrations

Although these results have wide applicability in avian biology anywhere egg density and egg volume are used, they are particularly important for ecotoxicological research for egg contaminant concentrations. Estimates of egg contaminant concentrations represent the mass of contaminants within the egg divided by the mass of the egg. As such, error in either the numerator (contaminant amount) or denominator (fresh egg-content mass) is reflected directly in the estimate of the final egg contaminant concentration.

If the egg density estimate used to calculate fresh egg mass includes the eggshell and eggshell is not included in

Table 3 Examples of egg morphometrics that include and exclude eggshell volume as part of egg-content volume and the consequences using either approach will have on final contaminant concentrations

	American avocet*	Black-necked stilt*	Forster's tern*	Common murre**	Source
Egg Morphometrics					
Mean Egg Length (L; mm)	49.75	43.70	42.95	82.52	This paper; Ainley et al. 2002
Mean Egg Width (W; mm)	34.81	31.27	30.16	50.51	This paper; Ainley et al. 2002
$K_{v\text{-}egg\_content}$	0.483	0.488	0.501	0.458	This paper; Hoyt 1979
Eggshell Thickness (EST; mm)	0.21	0.21	0.22	0.70	Schönwetter 1960–1992; Roberts 1997; Henny et al. 2008; Pyle et al. 1999
Whole Egg Volume (V_{whole_egg}; cm^3)	29.12	20.85	19.57	96.42	
Volume of Egg Contents Only ($V_{egg\_content}$; cm^3)	28.18	20.1	18.81	89.61	$K_v(L - 2 * EST)(W - 2 * EST)^2$
Egg-Content Density (g/cm^3)	1.025	1.023	1.025	1.032	This paper; Rahn et al. 1982
Whole Egg Density, including eggshell (g/cm^3)	1.059	1.056	1.053	1.100	This paper; Rahn et al. 1982
Amount of Contaminant within Egg Contents (μg)	30	30	30	30	Arbitrary value used for this example
Recommended approach A: Egg volume and egg density includes only egg contents (excludes eggshell)					
Estimated fresh egg-content mass (g)	28.88	20.56	19.28	92.48	$\text{Density}_{\text{egg\_content}} \times V_{egg\_content}$
Egg contaminant concentration (μg/g)	1.039	1.459	1.556	0.324	
Approach B: Egg volume includes eggshell, but egg density is based on egg contents only					
Estimated fresh egg-content mass (g)	29.85	21.33	20.06	99.51	$\text{Density}_{\text{egg\_content}} \times V_{whole\_egg}$
Egg contaminant concentration (μg/g)	1.005	1.406	1.496	0.301	
Approach C: Egg volume includes only egg contents, but egg density is based on whole egg (includes eggshell)					
Estimated fresh egg-content mass (g)	29.84	21.23	19.81	98.57	$\text{Density}_{\text{wholegg}} \times V_{egg\_content}$
Egg contaminant concentration (μg/g)	1.005	1.413	1.515	0.304	
Approach D: Egg volume and density includes egg shell					
Estimated fresh egg-content mass (g)	30.84	22.02	20.61	106.06	$\text{Density}_{\text{wholegg}} \times V_{whole\_egg}$
Egg contaminant concentration (μg/g)	0.973	1.363	1.456	0.283	
Calculated errors for each approach (relative to Recommended Approach A)					
Approach B versus approach A	−3.2 %	−3.6 %	−3.9 %	−7.1 %	
Approach C versus approach A	−3.2 %	−3.1 %	−2.7 %	−6.2 %	
Approach D versus approach A	−6.3 %	−6.6 %	−6.4 %	−12.8 %	

Egg morphometric data are mean values from this paper (*) or sourced from the literature for common murres which we chose as an example of a species with a much thicker eggshell

the contaminant analysis, as is often the case, then the estimate of fresh egg-content mass will be overestimated due to the fact that eggshell densities are more than twice that of egg-content densities (Rahn and Paganelli 1989). Furthermore, if egg volume is derived from the external measurements of the egg, then egg volume will be overestimated by the percentage of volume that is associated with the thickness of the eggshell. In both situations, metrics associated with the denominator of the egg contaminant concentration equation will be overestimated, and, thus, the resulting estimate of egg contaminant concentration will be underestimated. Therefore, it is important to understand the impact of these potential biases on final egg contaminant concentrations, and to specifically use the correct egg density and egg volume, that either includes or excludes the eggshell depending on what was actually analyzed for contaminants.

If the egg contaminant determination is performed on the egg contents only (excluding the eggshell), as is typically done, then the egg density and egg volume used in estimating fresh egg mass should also be based on the egg contents without the eggshell. At first glance, ignoring the eggshell volume may seem inconsequential, however, for the species presented within this paper, the eggshell represents 3–4 % of the total egg volume (Table 3). Consequently, for these 3 waterbird species, including eggshell volume in egg volume calculations will bias egg contaminant concentrations low by 3–4 %. This is an issue that is likely unaccounted for in most current and past egg contaminant studies. We illustrate this potential error for four species of birds in Table 3. In species with larger eggs or relatively thicker shells, where eggshell volume is an even higher percentage of total egg volume, this underestimate in egg contaminant concentrations is even greater. For example, in common murre (*Uria aalge*) eggs, where eggshell volume is 7 % of the whole egg volume, egg contaminant concentrations would be underestimated by 7 % if external egg measurements were used to estimate the internal volume of the egg contents (Table 3; Approach B).

Similarly, using the wrong egg density also will result in error in the egg contaminant concentration. Using an estimate of egg density that includes the eggshell with an accurate estimate of egg-content volume, will ultimately underestimate egg contaminant concentrations by 3–6 % (Table 3; Approach C). Finally, combining these errors of using whole egg density and entire egg volume when estimating contaminant concentrations only in the egg contents would result in egg contaminant concentrations that were biased low by 6–13 % (Table 3; Approach D). Both issues create substantial impediments to accurately estimating egg contaminant concentrations and possibly limit the ability to compare results among studies.

It could be argued that this error is small relative to other potential errors, such as common analytical error in chemical determination. However, analytical error would generally be random and not result in a consistent bias, whereas including the eggshell in egg volume and density estimates when calculating fresh egg mass will result in a consistent negative bias. Given the potential errors in underestimating egg contaminant concentrations by 3–10 %, we recommend that egg contaminant concentrations be presented on a fresh wet weight basis by using species-specific density estimates of the egg contents only (excluding the eggshell) and egg-content volume estimates that account for eggshell thickness. When species-specific density estimates are unavailable, researchers could employ a density value from a closely related species, a combined estimate derived from a group of closely related species, or a more general egg density estimate provided from synthesis papers such as Hoyt (1979).

In summary, the suggested equation for estimating fresh egg content mass is:

$$Mass_{egg\_content\_fww} = Density_{egg-content} \times K_v(EggLength - 2 \times EggShellThickness) \times (EggWidth - 2 \times EggShellThickness)^2$$

where, $EggLength$ and $EggWidth$, and $EggShellThickness$ (when feasible to measure) are measured for each egg, and $Density_{egg-content}$, K_v, and $EggShellThickness$ (when not feasible to measure) are obtained from the literature. Estimates of $Density_{egg-content}$ and $EggShellThickness$ are available for many species within the literature. The egg database compiled by Schönwetter (1960–1992) contains eggshell thickness data on hundreds of bird species (Maurer et al. 2010, 2012) and egg content densities can be obtained from a number of studies including Hoyt (1979), Manning (1979), Rahn et al. (1982), and Rahn and Paganelli (1989).

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