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COMMENTARY

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ON THE ALLOMETRIC RELATIONSHIP BETWEEN SIZE AND COMPOSITION OF AVIAN EGGS: A REASSESSMENT

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Abstract. Numerous investigators have used allometric regression to characterize the relationship between proportional egg composition and egg size, which is a potentially important characterization for assessing maternal investment in reproduction. Herein, we document two important shortcomings of this approach. First, regressing log component mass against log egg mass involves regressing Y on itself, since each component (Y) is necessarily a part of the whole egg (X). This creates correlated errors, which leads to biased estimates of the regression slope. To circumvent this problem, we recommend regressing egg component masses on a relatively inert component like total water mass. Secondly, investigators routinely use ordinary least squares regression to estimate the slope of allometric relationships, which assumes that all error resides in Y. We demonstrate that this assumption is false, but so are the underlying error assumptions of commonly used alternatives such as reduced major axis and major axis regression. Because each egg is unique and determining composition involves destructive sampling, there is no obvious way to assess measurement error in Y versus X. As a solution, we recommend that investigators analyze multiple eggs per clutch whenever possible and fit a reduced major axis based on the among-female component of variability.

Key words: *allometry, egg composition, egg size, ordinary least squares regression, reduced major axis regression.*

Sobre la Relación Alométrica entre el Tamaño y la Composición de los Huevos de las Aves: Una Reevaluación

Resumen. Numerosos investigaciones han usado regresiones alométricas para caracterizar la relación entre la proporción de la composición del huevo y el tamaño del huevo, lo cual representa una caracterización potencialmente importante para evaluar la inversión materna en la reproducción. Aquí documentamos dos limitaciones importantes de este enfoque. Primero, la regresión del logaritmo de los componentes sobre el logaritmo de la masa del huevo representa la regresión de Y sobre sí misma, ya que cada componente (Y) es necesariamente una parte del huevo completo (X). Esto genera errores correlacionados, lo cual lleva a estimados sesgados de la pendiente de la regresión. Para evitar este problema, recomendamos hacer una regresión de los componentes de la masa del huevo sobre un componente relativamente inerte, como la masa total de agua. Segundo, los investigadores usan de forma rutinaria regresiones comunes de mínimos cuadrados para estimar la pendiente de las relaciones alométricas, lo cual supone que todo el error reside en Y. Demostramos que esta suposición es falsa, al igual que las suposiciones del error subyacente de las alternativas comúnmente usadas como la reducción del eje mayor y la regresión del eje mayor. Debido a que cada huevo es único y a que la determinación de la composición requiere la destrucción de muestras, no existe un modo obvio para determinar el error de medición de Y versus X. Como solución, recomendamos que los investigadores analicen múltiples huevos por nidada cuando sea posible y que ajusten un eje mayor reducido basado en el componente de variabilidad entre hembras.

Large eggs often confer survival advantages upon the offspring that hatch from them (Williams 1994). Presumably this is not a function of egg size per se, but rather a function of the greater amount of essential nutrients contained within large eggs (Ricklefs et al. 1978). If large eggs are proportionally similar to small eggs, then they will contain higher absolute amounts of essential nutrients than small eggs (i.e., more protein and lipid), rather than simply containing more water (Ankney 1980). Moreover, large eggs might have even greater advantages if they contain proportionally more nutrients than small eggs. For example, if a 30 g egg contains 5% yolk lipid (1.5 g) whereas a 40 g egg contains 7.5% yolk lipid (3.0 g), this represents a 100% increase in yolk lipid that far exceeds either the 33% increase in egg size or the 50% increase in relative lipid content. Consequently, measuring egg size is not sufficient to

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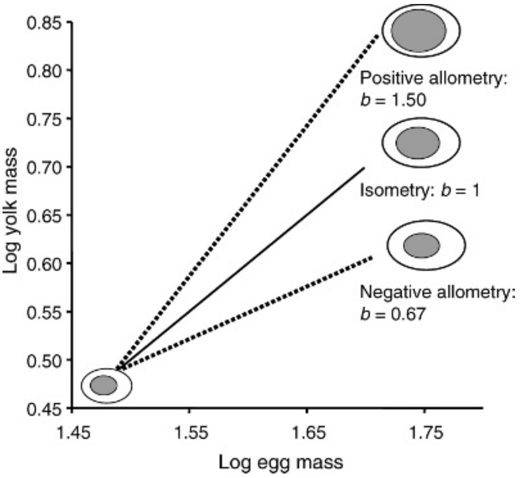

FIGURE 1. Allometric relationships between yolk mass and egg mass. Diagrams are illustrative only and are not drawn to scale.

document egg quality if proportional composition of essential nutrients varies with egg size.

To explore this issue, investigators have used allometric (i.e., log-log) regression to examine how proportions of egg components vary with total egg mass (Fig. 1). In a log-log regression, if the regression slope (*b*) is greater than one, then Y (e.g., yolk mass) is presumed to increase at a greater rate than X (total egg mass), and the relationship is said to be positively allometric. A slope equal to one suggests that the component changes in direct proportion to total egg mass, which is referred to as isometry. Finally, a slope less than one indicates negative allometry, which implies that component mass does not keep pace with total egg mass, even though the component would nevertheless be increasing in absolute mass provided that *b* is greater than zero. In practice, eggs have been assumed to display positive or negative allometry at $P = \alpha$ if the $(1 - \alpha)$ confidence interval for *b* excludes one.

Examination of allometric relationships among egg components has been a secondary objective of numerous papers on intraspecific variation in egg composition (Table 1). Here, we document two important shortcomings of this approach that we believe render most of these published relationships unreliable: 1) Regressing log component mass on log total egg mass involves regressing Y on itself (or more correctly, regressing Y on X + Y). Since the component is necessarily a part of the whole, any error in Y will also be present in X + Y, resulting in correlated errors; and 2) Using ordinary least squares to regress log Y on log X assumes that all error resides in Y and none in X. Clearly this is not the case when Y is part of X, but it is likely false even if the first problem can be resolved.

It is important to recognize that the "error" we refer to above includes many different sources. Dividing small eggs into yolk, albumen, and shell components is delicate work, and so is separation of these fractions into lipid, protein, and ash subcomponents, which leads to classical measurement errors that are likely not trivial. Due to the destructive nature of determining egg composition, these measurement errors cannot be calculated by conventional repeated-measures approaches (Lougheed et al. 1991). Egg composition studies are also subject to classical sampling error: making inferences about an entire species or population from a small sample of eggs that was probably not randomly selected. More than half of the 22 egg composition studies summarized in Table 1 are based on samples of fewer than 50 eggs, which were often obtained from even smaller samples of nesting females. Even if measurement and sampling errors are nil, egg size is not fixed by the investigator, and is therefore subject to natural sources of variation or "equation error" (McArdle 2003, Warton et al. 2006). For example, if the same bird could independently produce the same egg 100 times, there would be natural variation in both yolk dry mass and total egg mass, over and above any sources of measurement error.

Our general approach in this paper is threefold. First, we document both of these identified shortcomings using simulated data with known error distributions and known functional relationships. We believe this is the only reliable way to illustrate the potential magnitude of methodological problems, given that we can never know the true nature of the underlying relationship and error structure when using empirical data (McArdle 1988). Next, we compare results from our simulations with previously published data to determine whether documented patterns of variation in egg composition can be explained more parsimoniously by a biased null model that assumes simple isometry. Finally, we propose analytical solutions to both of these problems and use simulations to evaluate their efficacy.

SHORTCOMING 1: REGRESSING Y ON X + Y

Linear regression describes bivariate data by fitting a model of the form: $Y = \alpha + \beta X$. In practice, the true values of Y and X are unknown, so they are replaced by their observed values, which inevitably include errors; e.g., $y = Y + \varepsilon$ and $x = X + \delta$. Ordinary least squares (OLS) regression assumes that X is measured without error (i.e., $\delta = 0$), whereas other specialized forms of the structural relationship model such as reduced major axis (RMA) or major axis (MA) regression make their own restrictive assumptions about the relative values of ε and δ (McArdle 1988). Importantly, all of these models assume that ε and δ are uncorrelated, but when a component is regressed on the whole, this assumption of uncorrelated errors is necessarily violated; if an egg yolk is 20% larger than expected, the entire egg is probably also going to be somewhat larger than expected. This concern about correlated errors has been addressed in basic statistical texts (Sokal and Rohlf 1981:578), and Gebhardt-Henrich (2000) provides an example from the ornithological literature illustrating how correlated errors between variables can lead to spurious relationships.

TABLE 1. Coefficients of variation for masses of dry yolk, dry albumen, dry shell, total water content, and total egg mass from 22 published studies that have examined allometric variation in egg composition. Dashes indicate no data.

Species	Yolk	Albumen	Shell	Water	Egg	Reference
Wood Duck (*Aix sponsa*)	10.2	9.2	9.7	–	8.0	Hepp et al. (1987)
Blue-winged Teal (*Anas discors*)	9.2	13.1	11.9	–	9.3	Rohwer (1986)
Greater Scaup (*Aythya marila*)	6.9	8.7	5.7	–	–	Flint and Grand (1999)
Japanese Quail (*Coturnix japonica*)	–	–	13.2	9.3	9.0	Martin and Arnold (1991)
Pied-billed Grebe (*Podilymbus podiceps*)	8.1	9.6	4.6	7.9	6.5	Arnold (1989)
Horned Grebe (*Podiceps auritus*)	12.5	11.2	8.8	5.7	5.3	Arnold (1989)
Eared Grebe (*Podiceps nigricollis*)	10.4	11.7	9.4	7.9	7.3	Hill et al. (1995)
Western Grebe (*Aechmophorus occidentalis*)	10.4	14.2	13.5	9.3	8.4	Hill et al. (1995)
American Coot (*Fulica americana*)	11.3	8.5	7.9	–	6.5	Alisauskas (1986)
American Coot (*Fulica americana*)	–	12.3	9.9	8.3	8.1	Arnold et al. (1991)
Ring-billed Gull (*Larus delawarensis*)	8.1	10.7	8.2	–	7.0	Meathrel and Ryder (1987)
Herring Gull (*Larus argentatus*)	7.4	9.9	9.6	8.0	7.2	Meathrel et al. (1987)
Common Murre (*Uria aalge*)	15.9	9.4	8.4	–	12.4	Birkhead and Nettleship (1984)
Razorbill (*Alca torda*)	11.0	18.3	9.6	–	9.4	Birkhead and Nettleship (1984)
Ancient Murrelet (*Synthiliboramphus antiquus*)	9.8	20.0	7.9	–	7.3	Birkhead and Gaston (1988)
Atlantic Puffin (*Fratercula arctica*)	8.2	17.9	9.2	–	7.9	Birkhead and Nettleship (1984)
Eastern Kingbird (*Tyrannus tyrannus*)	9.3	11.1	10.3	–	10.6	Murphy (1986)
Black-billed Magpie (*Pica hudsonia*)	–	9.5	7.6	–	7.5	Hochachka (1988)
European Starling (*Sturnus vulgaris*)	–	–	–	–	7.5	Ricklefs (1984)
Red-winged Blackbird (*Agelaius phoeniceus*)	9.6	10.3	9.1	8.3	8.1	Muma and Ankney (1987)
Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)	12.7	12.2	9.3	7.4	7.3	Arnold (1992)
Brown-headed Cowbird (*Molothrus ater*)	11.1	15.5	14.6	13.3	12.6	Ankney and Johnson (1985)
Mean	10.3	12.4	9.6	8.5	8.2	
SD	2.1	3.4	2.3	2.0	1.8	

SIMULATION

To assess the importance of dependence of errors in allometric regressions, we conducted a simulation in which we assumed that eggs were comprised of four components: dry yolk, dry albumen, dry shell, and total water. Total egg mass was equal to the summed masses of these four components. Each component was assumed to have isometric variation with a coefficient of variation (CV) of 5%, so if yolk was 5% bigger (1 SD) than average, so were albumen, shell, and water, and hence total egg mass. However, we also assumed that each component had random error with a CV of 8%, which varied independently among the four components. So, in a given egg, the yolk might be a further 4% larger (+0.5 SD), whereas the albumen might be an additional 12% smaller (−1.5 SD). Our use of 5% CV for an isometric egg-size effect and 8% CV for an error effect are admittedly arbitrary, but they result in overall CVs that are consistent with empirical data (Table 1). Moreover, we obtained similar results with other levels of error, provided there was some nontrivial component of random error.

If regressing Y on X + Y produces bias, we expected this bias to vary with the proportion: $p = Y \cdot (X + Y)^{-1}$. If Y is a trivially small component of the total egg (e.g., lean dry yolk at 2%–6%), then whether Y is relatively large or small is not going to have much influence on total egg mass (X + Y). Conversely, if Y is a huge component of the total egg (e.g., total water at 65%–80%), then regressing Y on (X + Y) will resemble regressing Y on itself. Hence, we altered proportional mass of the component of interest and regressed it on total egg mass to determine how slope estimates varied with p. Each simulation involved 1000 eggs with a mean expected mass of 100 g. We conducted 10 simulations for each value of p and estimated the regression slopes using both OLS and RMA regression.

Because our data were simulated to be isometric, all slope estimates should have been approximately 1, but this was only the case when p approached 1, or when p approached 0 in the case of RMA regression (Fig. 2). For OLS regressions, slopes were biased anywhere from 72% too low when p was approximately 0, to 21% too high when p was approximately

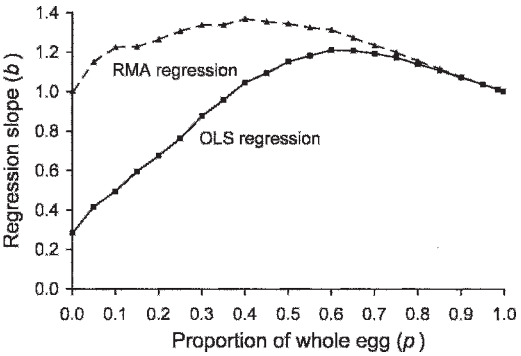

FIGURE 2. Simulation results illustrating the bias in regression slopes (b) from regressing egg component mass (Y) on total egg mass (Y + X) for ordinary least squares (OLS) and reduced major axis (RMA) regression. Each data point represents the mean from 10 Monte Carlo simulations involving 1000 eggs with known error structure and a true underlying slope of $\beta = 1.0$.

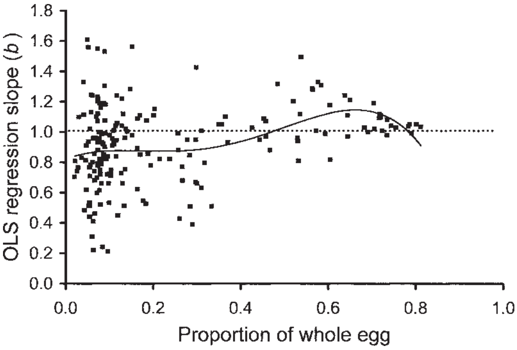

FIGURE 3. Ordinary least squares (OLS) slope (b) estimates from 22 published studies (Table 1) that regressed log egg component mass (Y) on log total egg mass (Y + X). The trend line is from a fourth-order polynomial regression. The tendency for most slopes to be less than 1 for components comprising less than half the entire egg ($p < 0.5$) and greater than 1 for components comprising more than half the egg ($p > 0.5$) is consistent with the biased null model shown in Figure 2.

0.65. For RMA regression, the bias was always positive and peaked at about +35% when p was approximately 0.50.

REVIEW OF EMPIRICAL DATA

We believe that the bias created by regressing a part on the whole is widely prevalent in published accounts of allometric variation in egg components. When we plotted published OLS regression slopes of log component mass versus log egg mass against the proportion of the whole egg that each component comprised (Fig. 3), we observed a noisier version of the same general pattern that we observed when using simulated data. On average, regression slopes were <1 for values of $p < 0.45$, but >1 for values of $p > 0.45$. Rather than reflecting true departures from isometry, we argue that this pattern is most parsimoniously explained as an artifact of regressing Y on itself.

This statistical bias may explain many previously identified "phylogenetic patterns" in egg composition. For example, Hill (1995) showed that when wet albumen was regressed on total egg mass, altricial families had significantly greater slopes than did precocial families (the opposite pattern existed for wet yolk mass). However, we suspect that this has more to do with wet albumen mass averaging 55%–70% of altricial eggs (high potential bias) versus 25%–60% of precocial eggs (lower potential bias) than it does to any underlying difference in how altricial versus precocial birds allocate nutrients to eggs of different sizes. Likewise, when regressing albumen mass on total egg mass among precocial families (Hill 1995:fig. 1), families with a relatively small albumen content (kiwis, waterfowl) had lower slopes than did families with a relatively large albumen content (grebes, larids). Many studies have reported greater slope estimates for wet masses of yolk and albumen than for dry masses (Alisauskas 1986, Meathrel et al. 1987, Arnold 1989, 1992) and we suspect that this is due to greater positive bias for the larger wet components.

PROPOSED ANALYTICAL SOLUTION

Our recommended solution to this problem of correlated errors is to regress components of interest (e.g., dry yolk, albumen, and shell, or subcomponents thereof) on an independent component of little interest, and we suggest that total water content represents the least interesting and most inert component of avian eggs. With the possible exceptions of oceanic or desert-dwelling birds, we suspect that fresh water neither limits egg production nor influences chick survival. One possible drawback to using water content for X is that it is not directly measurable (at least not without substantial measurement error; Ricklefs 1982). However, if fresh egg mass can be determined before substantial water loss occurs, total water content can be determined by subtracting dry component masses from fresh egg mass. This approach has the advantage of allowing different components to be compared against a common frame of reference, thus allowing direct comparisons among components, which would not be possible if each component was regressed against total egg mass minus that particular component.

When we adopted this approach with our original simulation, we found that average RMA regression slopes of component mass on total water mass differed from 1 by less than 1.5% for all reasonable values of p and beyond (range: 0.001–0.400). This approach also produced OLS slopes that were independent of p, but they averaged approximately 0.28, far below their true value of 1. This result conveniently introduces our second source of bias in allometric studies of eggs: nontrivial error variance in X.

SHORTCOMING 2: ASSUMING NO ERROR IN X

Ordinary least squares regression is a special form of the structural relationship model that assumes all error variation resides in Y; X is assumed to be error free. Hence, the ratio of $(\sigma^2_\varepsilon)\cdot(\sigma^2_\delta)^{-1}$ (also known as λ; McArdle 1988) is presumed to be infinity, which greatly simplifies the maximum-likelihood equation for estimating β. In simplistic terms, the size of every egg is assumed to be "fixed" and any lack of fit in the regression of log yolk on log egg mass is attributed to a bigger or smaller than expected yolk (ε_Y). Likewise, when regressing albumen, shell, or water content on total egg mass, all lack of fit is presumed to reside in ε_A, ε_S, and ε_W, respectively. Clearly, the four major components of eggs cannot have random error when they are dependent variables, but have no random error when they are added together to create the independent variable, a whole egg. Likewise, total water content cannot have random error when it is regressed on total egg mass, but have no random error when it is used as the regressor to estimate dry yolk mass. Thus, the assumption of no random error in X is clearly false, whether X is represented by total egg mass or total water mass. But the more germane question of how much error variation occurs in X, relative to Y, is not so readily answered.

Reduced major axis (RMA) and major axis (MA) regression are two less commonly used forms of the structural relationship model that recognize random error in X (McArdle 1988). RMA regression assumes that error variances of Y and X are proportional to the overall variation in Y and X: $\lambda = (\sigma^2_\varepsilon)\cdot(\sigma^2_\delta)^{-1} = (\sigma^2_Y)\cdot(\sigma^2_X)^{-1}$, whereas MA regression assumes that $\varepsilon = \delta$, hence $\lambda = (\sigma^2_\varepsilon)\cdot(\sigma^2_\delta)^{-1} = 1$. The RMA regression is calculated as the first principle component of the correlation matrix of Y and X, whereas the MA regression represents the first principle component from the covariance matrix. Both RMA and MA slopes can be easily derived from summary statistics obtained from a standard OLS regression (Warton et al. 2006). McArdle (1988) provided a comprehensive overview of RMA and MA regression models and suggested that RMA almost always outperformed MA, except in cases when OLS regression was the more appropriate choice. McArdle (1988:2332) provided a simple recommendation: "If the error rate in X exceeds one-third of the error rate in Y then RMA should be used [otherwise use OLS]." However, RMA should not be used unless the Pearson correlation coefficient between X and Y is significantly different from zero; in such a case MA regression is a suitable alternative (Legendre and Legendre 1998).

SIMULATION

Although the ratio of error variances $\lambda = (\sigma^2_\varepsilon)\cdot(\sigma^2_\delta)^{-1}$ is not directly measurable, it can be approximated as $(CV_Y)^2\cdot(CV_X)^{-2}$ under the RMA model. We used $(CV_Y)^2\cdot(CV_X)^{-2}$ as calculated from the arithmetic data, rather than $(\sigma^2_Y)\cdot(\sigma^2_X)^{-1}$ from the log-transformed data, because summary statistics for published data sets have been reported only for the arithmetic data. We used CVs, rather than SDs, because this allowed us to at least partially standardize the data across variables (as would log transformation).

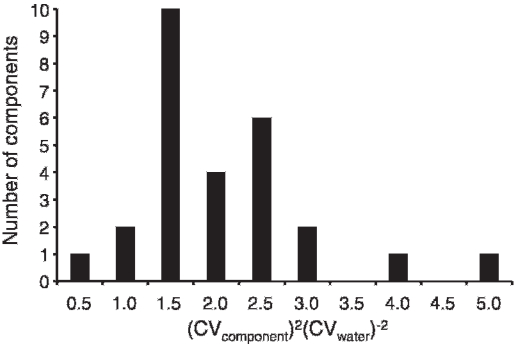

FIGURE 4. Frequency distribution of the ratio of squared coefficients of variation in egg components (e.g., dry yolk, dry albumen, or dry shell) versus total water content from published egg composition studies in Table 1. For major axis regression, the distribution should be centered on 1, whereas for ordinary least squares regression the distribution should be greater than 3. The sample of 27 data points has a mean of 1.82 ± 0.18 SE and falls primarily between 1 and 3, suggesting that reduced major axis regression is most appropriate.

For the 22 studies summarized in Figure 3, CVs averaged 8%–12% for most major components, including water and total egg mass (Table 1). Nevertheless, dry component masses had average CVs of 9.6%–12.4%, versus 8.5% for total water content and 8.2% for whole egg mass, and the ratio $(CV_Y)^2\cdot(CV_X)^{-2}$ averaged significantly greater than a 1:1 ratio, but was usually less than 3:1 (Fig. 4). Hence, neither the MA assumption of $\lambda = 1$ nor the OLS rule-of-thumb that λ should be greater than 3 was met by most existing data sets. Thus, based on McArdle's (1988) simulations, RMA regression should perform better than either MA or OLS regression.

We therefore examined the performance of RMA regression in relation to different error structures for Y (dry yolk mass) and X (total water mass). As in our first simulation, we assumed that both components had a CV of 5% associated with egg size per se (i.e., an isometric size gradient). We further assumed that water content had an additional random error (δ) of 4% (low), 6% (medium), or 8% (high), and that yolk content had additional random error (ε) ranging from 2% to 14% (in 1% increments). Although arbitrary, these values produced levels of overall variation and covariation that bracketed the actual ranges reported in various egg-composition studies (Table 1). Each simulation involved 1000 eggs, and we conducted 10 replicates for each combination of ε and δ. Our measure of effectiveness was the average mean square error of the regression slope (MSE =

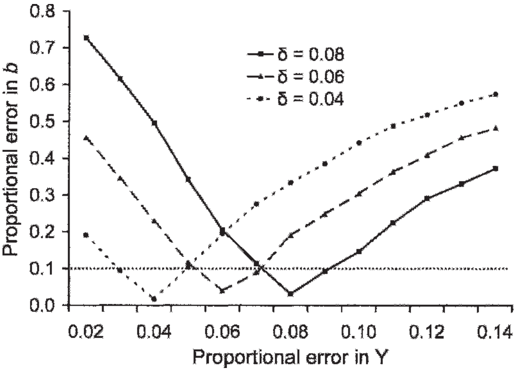

FIGURE 5. Proportional mean squared error (MSE) in reduced major axis (RMA) slope (b) estimates for different percentages of error variation in ε (error in log egg component mass) and δ (error in log total water mass; $\beta = 1$, $n = 1000$, with 10 replicates for each combination of ε and δ). Proportional error on the slope estimate is defined as $[\mathrm{MSE}^{0.5}]\cdot(\beta')^{-1}$ and measures both bias and variance; all combinations having proportional errors >0.095 (the dotted horizontal line) would be incorrectly identified as differing from 1 at $P < 0.05$. For all combinations listed, ordinary least squares regressions performed worse than the RMA regressions.

$\Sigma(b_i - \beta)^2\cdot 10^{-1}$; McArdle 1988), which measures both bias and imprecision.

We found that unless $|\varepsilon - \delta| < 1\%$, RMA regression slopes were always significantly different from 1 and the MSE of the slope estimate was $>10\%$ (Fig. 5). Given that our data were simulated to be isometric ($b = 1$), this implies that simulated slope estimates were almost always "precisely wrong." OLS regression performed even worse, differing from isometry by a larger relative margin for every combination of ε and δ that we examined. The true slope was always underestimated using OLS regression, whereas RMA regression underestimated the slope when $\varepsilon < \delta$ and overestimated it when $\varepsilon > \delta$.

REVIEW OF EMPIRICAL DATA

When we compared RMA slope estimates from the literature (in most cases calculated from the original OLS slopes by dividing by r) with the relative error in Y versus X, approximated as $\lambda \approx (\mathrm{CV_Y})^2\cdot(\mathrm{CV_X})^{-2}$, we found that 84% of the variation in observed slope estimates could be explained by λ. However, 124 of 139 slope estimates (89%) were >1, implying that almost every component increased at a greater rate than all the other components combined (i.e., the collective parts were seemingly greater than the whole), which is clearly nonsensical. When we limited our analysis to components comprising less than 15% of total egg mass to minimize problems with correlated errors in ε and δ, we found that 91% of the variation in b could be explained by λ and 89% of slope estimates were >1 (Fig. 6). Of the 32 slope estimates that exceeded 1.5, 75% were based on correlations with $r^2 < 0.50$, suggesting structurally weak relationships (Fig. 7). Our simulation and review demonstrate that the RMA assumption of $\lambda \approx (\mathrm{CV_Y})^2\cdot(\mathrm{CV_X})^{-2}$ is unlikely to be true, and hence most of the apparent variation in b will reflect bias and imprecision in our estimate of λ, rather than true structural variation in β. We conclude that although RMA regression is much better than OLS regression, it too provides badly biased results and therefore does not offer an acceptable solution to the dilemma of error variance in X.

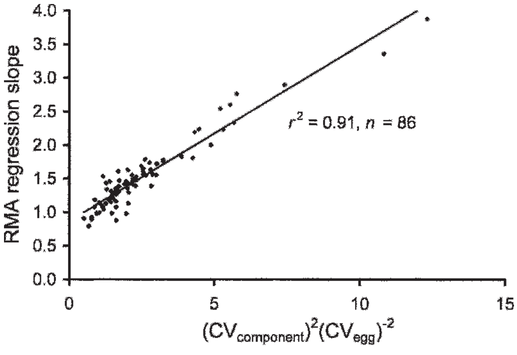

FIGURE 6. Relationship between reduced major axis (RMA) slope estimates and measures of the ratio of egg component versus egg size variability $(\mathrm{CV_Y})^2\cdot(\mathrm{CV_X})^{-2}$ based on previously published studies (Table 1). Only components comprising less than 15% of the total egg were utilized to minimize bias from regressing Y on X + Y (see Fig. 2). Differences in relative variance between component masses and total egg mass explained 91% of the variation in RMA slope estimates.

PROPOSED ANALYTICAL SOLUTIONS

Sampling multiple eggs from the same clutch provides a means to estimate among- and within-clutch variation in egg composition (i.e., repeatability; Lessells and Boag 1987). If the primary objective is to assess among-female variation in allocation of nutrients to eggs, and if individual females tend to produce similar eggs within clutches, then sampling multiple eggs per clutch to assess within-clutch variance might provide a reasonable proxy for measurement (i.e., analytical) plus sampling error (i.e., which egg happened to be sampled from the clutch). Using previously published data on Horned (*Podiceps auritus*) and Pied-billed Grebes (*Podilymbus podiceps*; Arnold 1989) and American Coots (*Fulica americana*; Alisauskas 1986), we used the NESTED procedure in SAS (SAS Institute 1990) to calculate among (σ^2_{a}) and within (σ^2_{w}) clutch components of variance for dry shell, dry albumen, dry yolk, and total water (Table 2).

These results could be used in several different ways. If λ can be estimated, investigators could use McArdle's (1988) "rules of thumb" to choose the most appropriate method from among OLS, RMA, and MA regression. Assuming $\lambda \approx (\sigma^2_{\mathrm{w\text{-}compo\text{-}}}$

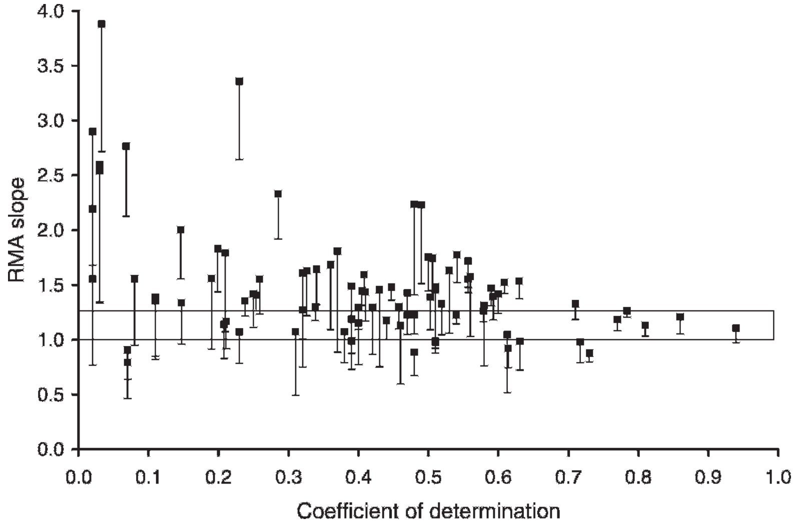

FIGURE 7. Relationship between reduced major axis (RMA) slope estimates of log egg component mass regressed against log total egg mass plotted against the coefficient of determination (R^2) for each relationship, based on previously published studies of allometric variation in egg composition (Table 1). Error bars are based on 2 SE from the published ordinary least squares regressions. The rectangular area between slope = 1.00 and 1.25 includes the range of expected values given isometry plus some positive bias due to correlated errors for components comprising <15% of the total egg (based on Fig. 2). Most of the slope estimates are well above this region, indicating the nonsensical observation that almost all of the separate components of eggs increase at rates faster than the whole.

$_{\text{nent}})\cdot(\sigma^2_{\text{w-water}})^{-1}$, we obtained estimates of λ that ranged from 0.6 to 14.3 (Table 2). In four of nine comparisons, λ was greater than 3, suggesting that OLS regression would be most appropriate (Table 2). In two cases λ was approximately equal to the ratio of among-clutch variances, suggesting that RMA would be most appropriate. And in three cases λ was close to 1, suggesting that MA regression would be most appropriate. This lack of consistency suggests that λ varies widely among species, and among

TABLE 2. Estimates of among- and within-clutch variation in logarithmically transformed egg component masses from three previously published datasets on egg composition. The proportion of error variance that can be attributed to within-clutch versus among-clutch components (1 – within-clutch error) is highly variable among species and components. Moreover, there is little concordance between among-clutch and within-clutch ratios of error variances for shell, albumen, and yolk versus total water, suggesting that the within-clutch error variances would make poor estimators of $\lambda = (\sigma^2_{\varepsilon})\cdot(\sigma^2_{\delta})^{-1}$.

Species	Variance component	Dry shell	Dry albumen	Dry yolk	Total water
Horned Grebe	Among-clutch σ^2_{a}	0.0063	0.0066	0.0183	0.0028
	Within-clutch σ^2_{w}	0.0017	0.0077	0.0004	0.0007
	Within-clutch error (%)	21	54	2	20
	$\sigma^2_{\text{a-component}}/\sigma^2_{\text{a-water}}$	2.3	2.4	6.6	
	$\sigma^2_{\text{w-component}}/\sigma^2_{\text{w-water}}$	2.4	10.9	0.6	
Pied-billed Grebe	Among-clutch σ^2_{a}	0.0016	0.0031	0.0015	0.0055
	Within-clutch σ^2_{w}	0.0004	0.0065	0.0029	0.0005
	Within-clutch error (%)	19	68	66	8
	$\sigma^2_{\text{a-component}}/\sigma^2_{\text{a-water}}$	0.3	0.6	0.3	
	$\sigma^2_{\text{w-component}}/\sigma^2_{\text{w-water}}$	0.8	14.3	6.5	
American Coot	Among-clutch σ^2_{a}	0.0038	0.0025	0.0025	0.0024
	Within-clutch σ^2_{w}	0.0035	0.0053	0.0177	0.0024
	Within-clutch error (%)	47	68	88	50
	$\sigma^2_{\text{a-component}}/\sigma^2_{\text{a-water}}$	1.6	1.0	1.0	
	$\sigma^2_{\text{w-component}}/\sigma^2_{\text{w-water}}$	1.5	2.3	7.5	

TABLE 3. Comparison of allometric relationships derived from reduced major axis (RMA) and ordinary least squares (OLS) regressions of log egg component mass on log egg water mass estimated among clutches of three species using the methods-of-moments (MM) method to control for within-clutch variation (Warton et al. 2006). For comparison we also calculated the OLS regression of log egg component mass on log total egg mass for all individual eggs. Samples sizes are number of clutches, number of eggs.

Species	Component	RMA-MM slope (95% CI); R^2	OLS-MM slope (95% CI)[a]	OLS slope (95% CI); R^2
Horned Grebe	Dry shell	1.51 (0.67, 3.39); 0.38	0.93 (−0.43, 2.29)	1.19 (0.62, 1.76); 0.53
(*n* = 7, 19)	Dry albumen	1.54 (0.88, 2.70); 0.74	1.32 (0.41, 2.24)	1.55 (0.72, 2.37); 0.48
	Dry yolk	−2.57 (−6.81, −0.96); 0.02	−0.37 (−3.29, 2.55)	0.43 (−0.81, 1.67); 0.03
Pied-billed Grebe	Dry shell	0.54 (0.26, 1.11); 0.08	0.16 (−0.27, 0.58)	0.27 (0.00, 0.53); 0.15
(*n* = 10, 26)	Dry albumen	0.75 (0.52, 1.09); 0.79	0.67 (0.38, 0.95)	0.98 (0.48, 1.47); 0.41
	Dry yolk	0.52 (0.43, 0.64); 0.94	0.51 (0.40, 0.61)	0.65 (0.31, 0.99); 0.40
American Coot	Dry shell	1.26 (0.91, 1.73); 0.38	0.78 (0.37, 1.18)	0.85 (0.66, 1.04); 0.45
(*n* = 27, 101)	Dry albumen	1.01 (0.81, 1.26); 0.71	0.85 (0.63, 1.08)	0.92 (0.73, 1.11); 0.48
	Dry yolk	1.02 (0.77, 1.34); 0.54	0.75 (0.47, 1.03)	1.36 (1.02, 1.69); 0.40

[a] R^2 values for the RMA-MM regression also apply to the OLS-MM regression.

different egg components within species, and thus there is no reliable "rule of thumb" to follow.

Another alternative would be to take the estimated value of λ for each bivariate relationship and substitute it into the maximum likelihood equation (McArdle 1988):

$$\beta_{\mathrm{ML}} = \left[\sigma^2_y - \lambda\sigma^2_x + \left(\left(\sigma^2_y - \lambda\sigma^2_x\right)^2 + 4\lambda\sigma^2_{xy}\right)^{1/2}\right]\cdot\left(2\sigma_{xy}\right)^{-1}. \quad (1)$$

Aside from being computationally difficult, this method assumes that there is a proportional relationship between measurement error $[(\sigma^2_{\text{w-component}})\cdot(\sigma^2_{\text{w-water}})^{-1}]$ and equation error $[(\sigma^2_{\text{a-component}})\cdot(\sigma^2_{\text{a-water}})^{-1}]$, but examination of the nine different relationships in Table 2 shows that these errors were only comparable in two cases (dry shell of Horned Grebes and American Coots).

We think that a better alternative is to use methods-of-moments estimators, as described by Warton et al. (2006). In effect, this allows investigators to remove measurement error in Y and X, but it does not remove (or provide any insight into) equation error remaining in each variable, so investigators must still choose among OLS, RMA, MA, or maximum-likelihood techniques. In Table 3, we provide methods-of-moments estimators for RMA and OLS slopes of log egg component mass regressed against log egg water mass, and we compare them to the original OLS slopes of log egg component mass versus log total egg mass calculated across all eggs. In all nine cases, accounting for measurement error resulted in attenuation of the OLS slope toward zero (Table 3; compare columns 2 and 3). Owing to the smaller sample sizes of clutches versus eggs, confidence intervals were generally wider for the methods-of-moments estimators. Warton et al. (2006) provide guidelines for comparing methods-of-moments regression lines (i.e., analogous to analysis of covariance), and their methods could be used to compare relative component masses within species (e.g., yolk vs. albumen in Horned Grebes) or particular components across species (e.g., yolk mass among the three species).

RECOMMENDATIONS FOR FUTURE STUDIES

Most egg composition studies have had numerous objectives, the evaluation of allometric relationships being only one of them. Our simplest recommendation is to recognize the enormous difficulties inherent in trying to estimate allometric relationships, and simply eliminate this objective from future analyses. If the intent is to determine whether larger eggs are better by virtue of greater nutrient content, simply correlating the nutrient(s) of interest (e.g., dry yolk mass) with an independent measure of egg size (e.g. egg volume index, or total mass less dry yolk mass) ought to suffice. As Ankney and Bisset (1976:731) succinctly declared: "it is not necessary for large eggs to contain *proportionately* more yolk, only that they contain *absolutely* more yolk, which they do."

Our criticisms apply to identifying structural relationships amongst variables, but are less relevant to predictive relationships. If an investigator is regressing yolk lipid content on fresh egg mass to predict lipid content in another sample of eggs that will not be subjected to destructive analysis, then OLS regression will provide the least biased estimator of Y given X. However, the regression slope will not provide any reliable information about allometry and, because of correlated errors, the coefficient of determination (R^2) will be unreasonably high. To prevent further errors of interpretation of such predictive analyses, we recommend that they be done on the arithmetic data (unless log-transformation is necessary to reduce heteroscedasticity of variance).

If investigators are interested in examining allometric relationships among egg components, they should first avoid the problem of regressing Y on itself. This can be accomplished by choosing an appropriate X variable (e.g., total water). For species with multiple-egg clutches, we recommend trying to estimate "measurement error" by analyzing at least two eggs per clutch. We suggest using sequentially laid midsequence eggs, because first- and last-laid eggs are more likely to be "atypical" (Arnold 1991). Using methods-of-moments estimators (Warton et al. 2006), investigators can estimate allometric relationships based on only among-female variation. The choice of regression method is still unresolved, but we believe RMA has the greatest merit when regressing other egg components on total egg water, because they are likely to have similar amounts of equation error after variables have been standardized. Finally, we would encourage investigators to sample numerous clutches ($n > 50$), because the more typical sample sizes included in our reanalysis (n = 7–27) resulted in large confidence intervals for slope estimates.

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