IBIS The International Journal of Avian Science
BOU
Ibis (2012), doi: 10.1111/j.1474-919X.2012.01244.x

A comparison of indices and measured values of eggshell thickness of different shell regions using museum eggs of 230 European bird species

GOLO MAURER,[1,2]* STEVEN J. PORTUGAL[1,3] & PHILLIP CASSEY[1,2]
[1]*Centre for Ornithology, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[2]*School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia*
[3]*Structure and Motion Lab, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK*

The thickness of avian eggshells is used to assess shell quality in wild and domestic species, as an indicator of environmental pollution and as an adaptive explanation for shell maculation. Both direct measurements and calculated eggshell thickness indices (ETI) are used in such research, yet this is the first study to quantify, across a large spectrum of bird families (and thus egg shapes), the correlation between measured thicknesses and ETI. Furthermore, few studies have quantified thickness variation across the entire length of the shell, although this variation may influence both gas transfer and embryonic development. We measured the thickness of 942 eggshells of 230 European bird species from the Class II material at the Natural History Museum, Tring, UK, both in the conventional manner, at the equator through the blowhole and, uniquely, after a single longitudinal, cut at its equator at the blunt and pointed ends. Over half of the samples revealed shell defects, cautioning against the indiscriminate use of museum specimens. Strong positive associations were found between species-specific means of shell thickness with each other and also with ETI, especially those derived from Schönwetter's *Handbuch der Oologie* method, validating the interspecific comparative use of ETI. Thickness measurements and ETI factors are provided for all 230 species. Eggshells were usually thinner at the blunt end (the location of the air sac) than at the equator, but of equal thickness in passerine eggs. This difference was greatest in species producing elongate eggs and suggests that there is a functional significance of shell thickness variation among species that requires further investigation.

Keywords: Biomonitor, DDT, embryonic development, micrometer, Schonwetter.

Eggshells form the physical interface between the avian embryo and its environment. This crystallized maternal effect mediates a multitude of functions that affect a species reproductive success. Some of these functions depend directly on shell thickness, for example the structural integrity of the egg during incubation or hatching (Porter & Wiemeyer 1969, Ar *et al.* 1979, Honza *et al.* 2001, Birchard & Deeming 2009), gas conductance (Ar *et al.* 1974, Paganelli 1980), light transmission through the shell (Maurer *et al.* 2011a) or protecting the embryo from infection (Messens *et al.* 2005). Additional functions, such as the supply of calcium to the embryo for bone development (Johnston & Comar 1955, Abdel-Salam *et al.* 2006, Karlsson & Lilja 2008), can be monitored using shell thickness as a proxy. Eggshell thickness serves as an accurate and influential bio-monitor, and a decrease in shell thickness can indicate environmental pollution, especially by organochlorine pesticides such as DDT (Porter & Wiemeyer 1969, Ratcliffe 1970, Jagannath *et al.* 2008) or a shortage of dietary calcium (Graveland *et al.* 1994, Nybø *et al.* 1997). Studies of variation in shell thickness have also increased our

*Corresponding author.
Email: golo.maurer@gmx.net

understanding of the evolution of eggshell pigmentation (Gosler *et al.* 2005, Jagannath *et al.* 2008) and the arms race between brood parasites and their hosts (Blankespoor *et al.* 1982, Picman & Pribil 1997, Spottiswoode & Colebrook-Robjent 2007, Spottiswoode 2010). Shell thickness data thus have an important role to play both in applied ecology and for research into fundamental processes in biology.

The efficient and reliable collection of eggshell thickness data, either through direct measurements or the use of calculated eggshell thickness indices (ETI), is of key concern and has received considerable attention (Schönwetter 1961, Klaas *et al.* 1974, Moriarty *et al.* 1986, Sooncharenying & Edwards 1989, Nybø *et al.* 1997, Nygård 1999, Green 2000). Three common approaches have been used to gather thickness data, but all incur limitations and their relationship with each other is currently unclear. Non-destructively, eggshell thickness can be determined by calculating an ETI (Schönwetter 1961, Ratcliffe 1967, Moriarty *et al.* 1986, Nybø *et al.* 1997) from shell mass and dimensions. However, egg shape will influence the accuracy of the calculation (Green 2000). Alternatively, direct eggshell thickness measurements can be made through the blowhole, but blowhole size and location as well as egg size restrict the choice of samples. Eggshell thickness can be measured after eggs have been cut longitudinally, but few eggshell collections will be available for this destructive approach.

Species averages for eggshell thickness can also be obtained from Schönwetter's (1960–1992) *Handbuch der Oologie* (Handbuch hereafter). These volumes provide data on calculated ETI for *c.* 10 000 species and subspecies and have formed the basis for large comparative studies (e.g. Ar *et al.* 1979, Hoyt 1979, Birchard & Deeming 2009), although their mathematical basis and relation to measured thickness value remain unclear (Maurer *et al.* 2010).

Studies using museum eggshell samples (summarized in Green & Scharlemann 2003) are based on the assumption that a shell's inner surfaces are free from contaminations or defects, which could influence both direct measurements and ETI. Remnant albumen, for instance, can increase shell thickness measurements and the shell mass used to calculate the ETI. Mechanical or chemical removal of the inner shell membrane during the blowing process can systematically reduce shell thickness measurements (Maurer *et al.* 2011b). Here we use the unique Class II collection of eggshells available for destruction at the Natural History Museum (NHM) at Tring, UK (Russell *et al.* 2010), to test this assumption and assess the internal quality of museum samples. We hypothesize that the internal quality of eggshells (in our sample) will be highly variable, with a number of specimens suffering from more serious defects or contamination.

The reliability of selected ETI compared with direct measurements has been tested for a very limited number of species with little variation in egg shape (Green 2000). Furthermore, these assessments were based on measurements of eggshell thickness through the blowhole. Here we use our measurements of high-quality eggshells to evaluate the accuracy of different ETI, including those published by Schönwetter (1960–1992), which have often been mistaken as actual measurements and have not previously been assessed (Maurer *et al.* 2010). We predict minor differences between the accuracy of ETI in general but more pronounced differences for eggshells that diverge markedly from the classical ovoid shape.

A limitation of both equatorial blowhole eggshell thickness measurements and ETI is that they produce a single value for shell thickness only. This prevents the assessment of interspecific latitudinal variation in shell thickness along the long-axis of the egg. Shell thickness is not uniform across the eggshell and also changes during the course of embryonic development (e.g. Finnlund *et al.* 1985, Ancel & Girard 1992, Balkan *et al.* 2006, Castilla *et al.* 2007, 2010). Latitudinal variation in shell thickness is potentially biologically relevant as it could determine areas of greatest gas conductance and shell breaking strength or the major source of calcium for embryonic growth and development. Currently it is unknown whether the latitudinal distribution of shell thickness varies taxonomically or with egg shape. We hypothesize that shell thickness variation across the egg is uniform within species and varies both with taxonomic relatedness and egg shape.

Shell thickness data and analyses for a broad range of species are combined to provide an assessment and validation of the use of ETI and museum eggs in environmental and ecological studies. Our analyses also explore the potential role of shell thickness variation in understanding species-specific differences in the interaction of eggshells with their environment and the developing embryo. Our standardized shell thickness measurements for 230 European breeding bird species are made available (Supporting Information Appendix S1) to facilitate further comparative analyses.

METHODS

Eggshell samples

We measured the shell thickness of 942 eggshell samples of 230 British breeding bird species (Dudley *et al.* 2006) incorporating 44 families and 143 genera (Sibley & Monroe 1990). On average we sampled four (between one and six samples; standard deviation = 1.1) eggs per species. The samples were borrowed from the NHM Class II collection, Section for Ornithology, Tring, UK (Russell *et al.* 2010). This collection consists of intact eggs identified unambiguously to species level but lacking exact provenance (date and location) information and hence are unsuitable for inclusion in the museum main collection. None of these eggs was collected by professional collectors; however, many of the contributing collectors had significant levels of experience with egg preparation, as witnessed by the volume of their collection. Eggshell samples varied in quality and thus not all samples were suitable for all of the analyses conducted in this study. Appendix S1 lists all samples used with species name, the museum set number, our measurements and the specific analyses for which each sample was included. No clutch information is available for any of the Class II collection eggs and samples should therefore be treated as independent. Wherever possible we further ensured independence of samples by choosing eggs collected by different collectors, at different times or of different appearance. Furthermore, Class II eggs generally lack exact collection location and date information, so these could not be included as covariates in the statistical models.

Sample quality

The full set of 942 samples was used to assess the suitability of the eggshell samples for thickness analyses by checking for the defects described below. Further analyses used subsamples of the full dataset as defects allowed (Table 1).

Unreliable dimensions

Shell samples with blowholes at the poles of the egg or paper labels covering most of the widest part of the shell were excluded if those defects interfered with the measurements of length and breadth.

Unreliable shell mass

We considered the shell mass of the sample as unreliable if the egg showed any of the following

Table 1. Overview of the quality, number and taxonomic composition of samples used in the assessment of eggshell thickness measures taken through the blowhole or on eggshell segments with indices of eggshell thickness (ETI, see text for details). Details of the defects that led to exclusion of a sample from an analysis are given in the text. Defects marked with a tick ✓ did not lead to exclusion from the assessment. The last column provides the frequency of each defect.

	Assessment					
Defect	Blowhole vs. ETI	Blowhole vs. Segment	Segment vs. ETI	Segment vs. Moriarty	Within-shell thickness	Samples with defect: *n* (%)
Unreliable dimensions	Excluded	✓	Excluded	Excluded	✓	10 (1.1)
Unreliable mass	Excluded	✓	Excluded	Excluded	✓	471 (50.0)
Incomplete segment measures	✓	Excluded	Excluded	Excluded	Excluded	45 (4.8)
Inadequate blowhole	Excluded	Excluded	✓	✓	✓	605 (64.2)
No blowhole diameter	✓	✓	✓	Excluded	✓	98 (10.4)
Inner membrane deteriorated	Excluded	Excluded	✓	✓	✓	188 (20.0)
Heavy material inside shell	Excluded	✓	Excluded	Excluded	✓	281 (29.8)
Samples, *n* = 942	190	277	451	434	877	756 (79.8)
Families, *n* = 44	31	32	42	42	44	43 (97.7)
Genera, *n* = 143	65	76	127	127	142	142 (99.3)
Species, *n* = 230	97	118	191	191	228	227 (98.7)

defects: (1) glued on paper label, (2) irremovable nest material or dirt stuck to the outside, (3) foreign material or remnant egg contents inside the egg, (4) the combined blowholes affected a shell mass reduction > 5% of the mean mass provided in Schönwetter (1960–1992).

Incomplete segment thickness measures
The loss of internal membrane in part or all of the shell segments prevented the measurement of shell thickness. Such samples were excluded from comparative analyses of measurements but were still part of the ETI calculations as membrane loss has only a negligible effect on shell mass (Schönwetter 1960–1992).

Inadequate blowhole
Thickness measurements through the blowhole were possible only for samples with: (1) an equatorial blowhole, (2) shell width < 35 mm, (3) a blowhole diameter > 1.35 mm (see methods), (4) no labels glued over the blowhole, (5) shells heavier than 0.1 g to ensure safe measurements and (6) intact inner shell membrane.

No blowhole diameter
Calculating the Moriarty–Nygård (Moriarty *et al.* 1986) index of shell thickness requires a reliable blowhole diameter measurement.

Shell measurements

All shells in our sample were weighed to an accuracy of 0.001 g on a Mettler PC 440 digital scale. Length and width of the eggshell and blowhole diameters were measured to 0.1 mm using Mitutoyo ABS Digimatic Callipers CD-6″C. We measured shell thickness of our samples in two different ways but always with the inner membrane intact: (1) if the shell had an adequate, equatorial blowhole ($n = 337$) we measured the thickness of the shell in the opposite equatorial section of the egg through the blowhole (blowhole thickness hereafter) to 1 μm, using the same modified Mitutoyo Digimatic Micrometer 2-3″ described and assessed for accuracy in Green (1998). A single measurement only was taken to minimize the risk of damaging the shell. (2) All shells including those for which blowhole thickness had been measured were then cut in half along their long axis using a diamond-tipped dentist drill (Milnes Bros., Surrey, UK). The shell thickness of one of the egg halves was measured to 1 μm three times each for the regions at the blunt end, the equator and the pointed end of the egg using a modified Mitutoyo micrometer, Series 227-203, Absolute Digimatic, at its 1.5-N constant pressure setting. Both anvils of the micrometer had been capped with an aluminium pin with a diameter of 1.35 mm (radius 0.35 mm).

The repeatability (Lessells & Boag 1987) of thickness measurements with the Series 207 micrometer was assessed on a sample of 20 Helmeted Guineafowl *Numida meleagris* eggs measured 10 times at the same location of the equator. Measurements showed a highly consistent repeatability ($r > 0.99$).

Eggshell indices

We compared our blowhole thickness and measurements of shell thickness of segments with six established ETI listed below. The ETI compared in this study fall into two categories: those that provide an approximation of the actual thickness of the eggshell (all of Schönwetter's indices and the Nybø–Green index) and those that do not (Ratcliffe and Moriarty–Nygård indices). The sample sizes and samples available for these comparisons are given in Table 1 and Appendix S1, respectively.

Eggshell thickness values provided in the Handbuch
The published shell thickness values in the Handbuch are not based on thickness measurements but constitute an ETI that represents exact shell thickness accounting for shape and density of the shell, but the specific calculation of each species value is unclear (Maurer *et al.* 2010).

Explicit ETI calculation following Schönwetter (1960–1992)
In the Handbuch, Schönwetter specifies a detailed formula (1) to calculate shell thickness d taking into account egg shape and shell density (explicit Schönwetter in short).

$$d = \left(\frac{g}{AB}\right)\left(\frac{k}{Kc\gamma}\right) \quad (1)$$

where g = the shell mass, A and B are length and width of the eggshell, $k = A/B$, K = a reduction factor, c = a shape factor, determined for each egg based on table 3 in Schönwetter (Schönwetter 1961) and γ = the specific weight of the shell, determined at family level following table 20 in Schönwetter (Schönwetter 1961). K is defined as

$$K = 0.914 + 2.228k. \quad (2)$$

The values for K, c and γ used for each sample are listed in Appendix S1.

Simple ETI calculation followed Schönwetter (1960–1992)

The Handbuch also provides a simpler equation (3) sufficient to approximate shell thickness for most species with typical ovoid eggshells (simple Schönwetter in short):

$$d = \frac{0.175g}{AB} \quad (3)$$

Ratcliffe index (Ratcliffe 1967)

This index (4) is the same as (3) but without the constant and provides a relative index of shell thickness rather than an actual shell thickness value (Ratcliffe in short):

$$d = \frac{g}{AB} \quad (4)$$

Nybø and Green index (Nybø et al. *1997)*

The Nybø–Green index (5) uses the shell mass relative to the surface area to calculate an ETI (NG in short):

$$d = \frac{g}{4.83(0.5\pi AB^2)^{2/3}} \quad (5)$$

Moriarty and Nygård index (Moriarty et al. *1986)*

This index (6) controls for the effects of egg shape and the reduction of shell mass lost when drilling the blowhole (MN in short):

$$d = \frac{g}{AB}\left(1.25 - \frac{0.32B}{A} + \frac{0.338D^2}{AB} + \frac{0.0867D^2}{A^2}\right) \quad (6)$$

where D is the diameter of the blowhole.

Statistical analysis

We compared the relationship between mean species values of the different ETI and our own measured thicknesses values by producing individual linear regressions for all the ETI and measurements against a single ETI or measurement across all samples suitable for each of the comparisons (Table 1). In addition, we compared separately the ETI with the equatorial shell thicknesses and the overall shell mean for three species groups with distinct egg shapes (Schönwetter 1960–1992): (1) the owls, Sibley and Monroe (1990) families Tytonidae and Strigidae, known for their near-spherical eggs; (2) the waders, families Scolopacidae, Burhinidae, Charadriidae and Laridae, all with pyriform eggs; and (3) the passerines, all British breeding families as a group with typical egg shapes.

Furthermore, we assessed the differences in thickness between the different sections of the shell: blunt end (B), equator (E) and pointed end (P). For all families for which more than three eggshell samples were available, we calculated the differences between each shell region and determined whether these differences differed from zero in a one-sample t-test ($\alpha \leq 0.01$). The relative difference between regions (μm) was calculated as: (blunt end thickness–equator thickness)/equator thickness.

Finally, we assessed whether this relative difference in thickness between the blunt end and the equator was related to egg shape as described by Schönwetter's K value (Equation 2) using a phylogenetic generalized linear model (Freckleton *et al.* 2002) to account for relatedness among species. The phylogenetic model (function pgls) was implemented in R version 2.14.0 (http://www.R-project.org/) package caper (Comparative Analyses of Phylogenetics and Evolution version 0.4; http://CRAN.R-project.org/package=caper) and used a phylogeny of breeding British birds (Thomas 2008).

RESULTS

Of the 942 samples, only 190 (20.2%) were entirely without shell defect (Table 1). Defects of 390 eggs (41.4%) were discovered after the shell was cut in half. These eggs had either lost a section of the inner membrane opposite the blowhole, thus affecting blowhole thickness measurements, or were contaminated with foreign material. Of the eggs with internal defects, 267 (58.4%) had no faults visible from the outside. The most common defect of shell samples was to contain foreign material affecting the shell mass (281 eggs, 29.8%) and an adhesive label (266 eggs, 28.2%).

For the most rigorous comparison between blowhole thickness and ETI, the shell thickness means of 97 species containing 190 samples without any shell defects could be used (Table 1 and Appendix S1). All regressions between the ETI and blowhole thickness measurements were highly significant (all $r^2 > 0.94$; Table 2) and crossed the axes close to the origin (intercept < |0.02|). The slopes indicate that Schönwetter's explicit ETI provides the closest fit (blowhole: $r^2 = 0.966$, slope = 1.003, $P < 0.001$, $n = 97$ species) to the measured thickness, but other ETI only slightly underestimated the measured thickness (Table 2).

A total of 118 species' shell thickness means (277 samples) could be used to compare blowhole thickness and shell segment thickness. Blowhole thicknesses were strongly related to measures of shell thickness in all egg regions (Table 2); however, shell segment thickness measurements resulted in slightly lower values than those obtained by measurements through the blowhole, as indicated by the fact that the regressions of segment on blowhole measurements had slopes $\leq$ 0.943. The relationship was weakest for measures taken at the pointed end ($r^2 = 0.963$) and this measurement was also the smallest when compared with that through the blowhole (slope = 0.88).

All the ETI were strongly related to the thickness measured at either the equator or the overall average calculated as the mean thickness of all three shell regions ($r^2 > 0.96$). Only a single ETI, Schönwetter's explicit (Equation 1), produced greater values than the equator measurement (slope = 1.017). The MN index had the weakest association ($r^2 = 0.966$).

The calculated thickness values of the *Handbuch der Oologie* could be compared with the mean blowhole thickness, equator and overall average shell thickness for 118 species (277 samples) (Table 2). All three measurements were strongly associated with the Handbuch values ($r^2 \geq 0.94$) and had an intercept < 0.02. Measurements taken at the equator fit the calculated values best (Fig. 1).

Table 2. Linear regressions comparing species means of measurements and indices of eggshell thickness (ETI, see text for details). All relationships were significant at $t > 41.4$ and $P < 0.001$; r = Pearson's correlation coefficient. For each set of regressions the header line provides first the independent variable and the number of species (n) and samples (M) used and the mean number of samples per species (μ).

Comparison	r	Slope	Intercept
Blowhole thickness vs. ETI, n = 97, M = 190, μ = 2.0			
Explicit Schönwetter	0.983	1.003	−0.002
Simple Schönwetter	0.985	0.984	< 0.0001
Ratcliffe	0.985	0.172	< 0.0001
Nybø	0.985	0.945	0.001
Moriarty	0.980	0.206	0.003
Blowhole vs. segment thickness, n = 118, M = 277, μ = 2.3			
Blunt end	0.986	0.943	0.006
Equator	0.988	0.934	0.001
Pointed end	0.981	0.88	0.008
Equator thickness vs. ETI, n = 191, M = 451, μ = 2.4			
Explicit Schönwetter	0.995	1.017	0.002
Simple Schönwetter	0.991	0.977	0.006
Ratcliffe	0.991	0.171	0.006
Nybø	0.992	0.89	0.012
Moriarty (n = 191, K = 434, ì = 2.3)	0.981	0.217	0.003
Overall shell mean thickness vs. ETI, n = 191, M = 451, μ = 2.4			
Explicit Schönwetter	0.996	0.988	0.004
Simple Schönwetter	0.991	0.948	0.008
Ratcliffe	0.991	0.166	0.008
Nybø	0.987	0.864	0.014
Moriarty (n = 191, K = 434, ì = 2.3)	0.982	0.212	0.004
Handbuch vs. measured thickness, n = 118, M = 277, μ = 2.3			
Blowhole	0.968	0.965	0.002
Equator	0.969	1.029	0.004
Cross-shell mean	0.972	1.040	0.006

The effect of egg shape on ETI was assessed using eggs from families known for their distinct egg shapes. The regressions between spherical owl-type eggs (Strigidae and Tytonidae) were weaker than those observed for the full sample across all ETI ($r^2 \leq 0.95$, slopes $\leq$ 0.788; Table 3). Alternately, the measurements of the pyriform wader-type eggs (Scolopacidae, Burhinidae, Charadriidae, Laridae) showed a much stronger association with the ETI (r^2 values $\geq$ 0.977). Schönwetter's ETI (Equations 1 and 3) both had slopes closest to one, indicating that they represent the best fit to the measured thickness values. The average shell thickness across all regions for samples of typical egg-shaped passerine samples was strongly associated with all the ETI

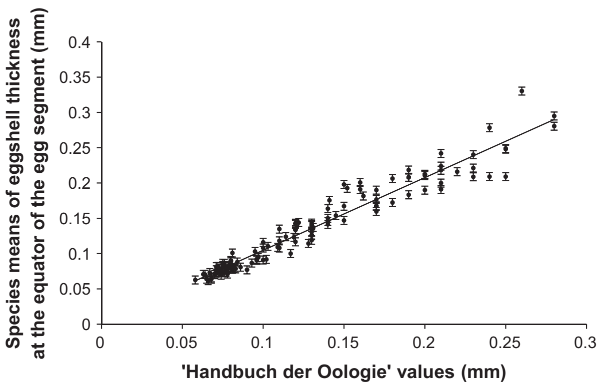

Figure 1. Relationship between the mean measured thickness at the equator of eggshell segments of 118 species (277 samples) of British breeding birds with three or more suitable samples and the calculated value provided in Schönwetter's 'Handbuch der Oologie' (1962–1990) for eggshell thickness: $y = 1.029x + 0.002$; $r^2 = 0.94$.

($r^2 \geq 0.96$; Table 3). The NG ETI with a slope of 1.0 matched the actual thickness measurements best whereas all Schönwetter ETI produced a slight overestimate (Table 3).

In 16 of the 40 families containing at least four samples, shell thickness measured at the equator exceeded that at the blunt end, and in 13 of those families the pointed end also exceeded the blunt end in thickness (Supporting Information Appendix S2). The difference between the shell thickness at the equator and the pointed end was more variable. There was no significant difference in thickness between any of the eggshell regions in 21 families (Appendix S2).

Across species, in a phylogenetic generalized linear model controlling for relatedness, species whose eggs show increased average elongation (Schönwetter's K value; Equation 2) had a greater relative difference in shell thickness between the blunt end and the equator of the egg (Schönwetter's $K \pm$ se = -0.21 ± 0.04, $t_{210} = -5.99$, adj-R^2 = 0.24, $P < 0.001$). Specifically, as the egg shape of species increased in average elongation the shell thickness at the equator compared with the blunt end also increased (Fig. 2).

Table 3. Results of linear regressions comparing the cross-shell mean of egg segments to indices of eggshell thickness (ETI, see text for details); r = Pearson's correlation coefficient. All relationships were significant at $t > 41.4$ and $P < 0.001$; r = Pearson's correlation coefficient. For each set of regressions the header line provides the group of species used, the characteristic egg shape according to Schönwetter (1960–1992) and the number of samples available.

Species group	r	Slope	Intercept
'Owls', Spherical, n = 15			
Explicit Schönwetter	0.975	0.782	0.046
Simple Schönwetter	0.974	0.788	0.048
Ratcliffe	0.974	0.138	0.048
NG	0.974	0.774	0.048
MN	0.967	0.157	0.054
'Waders', Pyriform, n = 71			
Explicit Schönwetter	0.995	0.944	0.019
Simple Schönwetter	0.995	1.023	0.014
Ratcliffe	0.995	0.179	0.014
NG	0.995	0.896	0.026
MN	0.989	0.25	−0.006
Passerines, Typical, n = 214			
Explicit Schönwetter	0.980	1.017	0.000
Simple Schönwetter	0.987	1.081	−0.005
Ratcliffe	0.987	0.189	−0.005
NG	0.987	1.000	0.000
MN (n = 199)	0.961	0.241	−0.009

DISCUSSION

Eggshell thickness measurements taken through the blowhole (blowhole thickness, see above) and on eggshells cut in half correlated strongly with each other and with established ETI. Notably, these measures are all equally suitable to address most comparative questions. Of all the indices assessed here, Schönwetter's explicit ETI (Equation 1), which accounts for both egg shape and shell density, was the most consistent at providing the strongest relationship and the closest fit to the direct shell measurements. The shape and reduction factors needed to calculate this, the most accurate index, are provided for the majority of British breeding birds in Appendix S1.

The egg shell thickness values in the *Handbuch der Oologie* have been used regularly in comparative studies; however, their exact derivation is unclear, which puts their reliability in doubt (for a review see Maurer *et al.* 2010). It is therefore noteworthy that our study revealed both a strong correlation and a tight fit between Handbuch values and measured thickness, making these data a valid alternative to measured or calculated thicknesses.

Our survey of eggshell quality revealed a high incidence (> 50%) of eggshell defects. Even amongst samples that appeared faultless from the outside, almost 60% revealed defects that precluded them from use in an analysis of the corre-

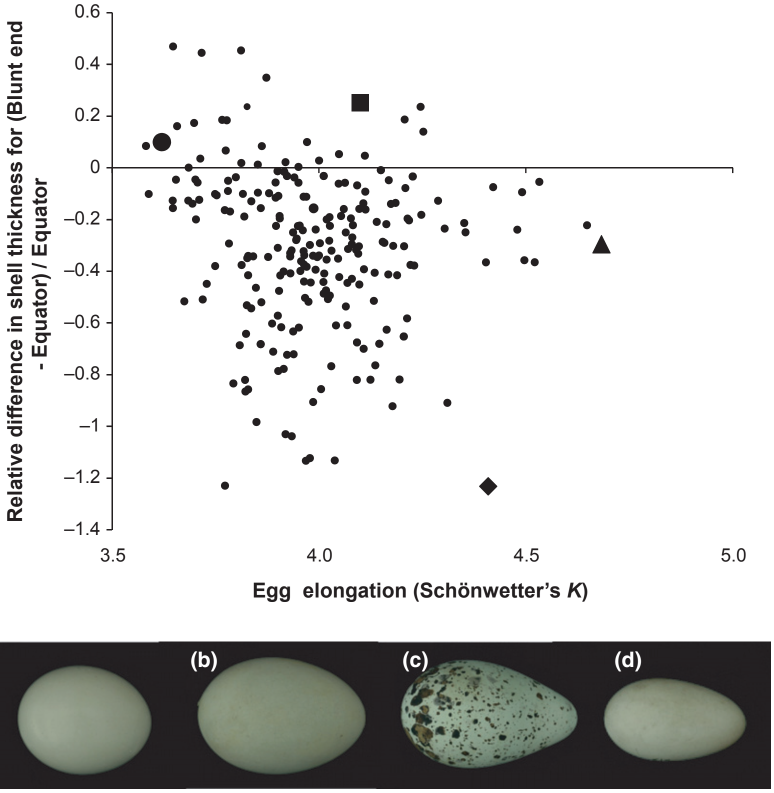

Figure 2. The species average of the percentage difference in measured shell thickness between the blunt end and the equator of eggs decreases (i.e. the equator is increasingly thicker than the blunt end) with an increasing value for Schönwetter's (1960–1992) *K* and thus with eggshell elongation. In a phylogenetic generalized linear model this relationship is highly significant (see Results). The eggs pictured are for: (a) the spherical Tawny Owl *Strix aluco*, circle; (b) the ellipsoid Red-necked Grebe *Podiceps grisegena*, square; (c) the pyriform Common Murre *Uria aalge*, triangle; and (d) the elongate Common Swift *Apus apus*, diamond.

lation between comparisons of ETI and blowhole thicknesses. The samples of the NHM Class II collection used here, by definition, are of lower quality with respect to their associated data than samples in the main collection of most museums. Main collections, which are typically prepared by more experienced collectors, are likely to display a lower incidence of shell defects than those reported here. Nonetheless, our findings highlight the frequency and the broad spectrum of shell defects which may complicate the use of shell masses and blowhole thicknesses of museum samples of unknown provenance. Experience with egg preparation is likely to affect the frequency of some of the defects we found, such as remnant egg contents. In addition, the unique storage conditions of each collection may affect the quality of eggshell samples (e.g. insect infestation, Bynes disease). We therefore recommend that analyses of large eggshell series use collector identity as a random variable to account for potential systematic differences in shell quality between collections.

To our knowledge, this study represents the most comprehensive comparison of within-egg variation of shell thickness to date. In accordance with previous studies, we found that the eggshell is commonly thinner at the blunt end than at the equator (and often the pointed end) but we never observed the reverse pattern in any of the 40 families studied. Importantly, however, shell thinning at the blunt end does not appear to be universal. Namely, many passerine families showed no thickness gradient between the different shell sections, suggesting a structural difference between passerine and non-passerine eggshells or a different mechanism of maternal shell deposition. The distinctive, less ordered composition of the eggshell palisade layer of passerines compared with that of non-passerines may explain the more homogeneous shell thickness in this group (Mikhailov

1997). All passerines show altricial development, whereas some non-passerine young hatch at various levels of precociality. Shell thickness could play a role in facilitating this diversity in developmental strategies by moderating gas exchange, although our own further research does not support this possibility (S.J. Portugal *et al.* unpubl. data). The high correlation between the ETI and equatorial thickness measurements also suggests that thickness at the equator serves as a good index of average thickness across the shell for a broad range of species.

The difference in shell thickness between the blunt end and equator is not independent of the egg size and shape, which themselves can be correlated (Olsen *et al.* 1994). In our study, average length and shape were weakly related ($r^2 = 0.51$; $P < 0.01$), and controlling for phylogenetic relatedness, larger, more elongate-shaped eggs showed a much thinner blunt end compared with their equator than shorter, more spherical eggs. This could be due to the need to reinforce the structurally weaker elongate eggs (Picman 1989) by increasing the thickness at the equator, where most of the mass of the incubating parent falls (Birchard & Deeming 2009). Our data seem to fit this idea and some of the most distinctive examples of egg shape variation illustrate this (Fig. 2). The extremely pyriform eggs of Common Murre *Uria aalge* have markedly thinner shells at the blunt ends than at the equator. By contrast, the near spherical eggs of Tawny Owls *Strix aluco* show no significant difference in shell thickness between egg regions. Interestingly, in the elongate eggs of grebes (Podicipedidae), the equator is similar in thickness to the blunt end but thinner than the pointed end, perhaps to improve water vapour conductance of the shell in their unusually wet and hot nests (Davis *et al.* 1984). Alternatively, increased equator thickness of larger eggs could facilitate the use of shell calcium by the developing embryo (Johnston & Comar 1955, Blom & Lilja 2004, Karlsson & Lilja 2008) without compromising breaking strength. Larger eggs have a smaller ratio of surface to volume than smaller eggs, and embryonic calcium reabsorption should thus reduce shell thickness more in larger than in smaller eggs. The reinforcement we found in the equatorial areas of larger eggs may serve to compensate for this effect.

Our finding confirms and expands a previous comparison of the accuracy of three eggshell indices, the Ratcliffe, Moriarty–Nygård and Nybø–Green ETI for four British thrush species. Green (2000) concluded that these ETI can be substituted for each other without losing the ability to detect real differences in shell thickness. The results presented here suggest that this conclusion holds for a much wider taxonomic sample. Our study also conclusively demonstrates the accuracy of the shell thickness values published in Schönwetter's *Handbuch der Oologie* (1960–1992), and thus justifies their previous use (e.g. Ar *et al.* 1974, Rahn & Paganelli 1989, Birchard & Deeming 2009) as well as the possibility of future comparisons of shell thickness. Finally, our discovery of a widespread, although not universal, increase of shell thickness from the blunt end towards the equator, especially in elongate eggs, indicates the need for further research into the functional and ecological significance of shell thickness variation among species.

We would like to thank Tony Rothin for providing the modification of the micrometer. The work was funded by an HFSP young investigators grant, a NESTA project grant, and a Leverhulme Trust project grant to P.C. P.C. is an ARC Future Fellow.

REFERENCES

Abdel-Salam, Z.A., Abdou, A.M. & Harith, M.A. 2006. Elemental and ultrastructural analysis of the eggshell: Ca, Mg and Na distribution during embryonic development via LIBS and SEM techniques. *Int. J. Poult. Sci.* **5**: 35–42.

Ancel, A. & Girard, H. 1992. Eggshell of the domestic guinea fowl. *Bri. Poult. Sci.* **33**: 993–1001.

Ar, A., Paganelli, C., Reeves, R.B., Greene, D.G. & Rahn, H. 1974. The avian egg: water-vapor conductance, shell thickness, and functional pore area. *Condor* **76**: 153–158.

Ar, A., Rahn, H. & Paganelli, C.V. 1979. The avian egg mass and strength. *Condor* **81**: 331–337.

Balkan, M., Karakas, R. & Biricik, M. 2006. Changes in eggshell thickness, shell conductance and pore density during incubation in the Peking Duck (*Anas platyrhynchos* f. *dom.*). *Ornis Fenn.* **83**: 117–123.

Birchard, G.F. & Deeming, D.C. 2009. Avian eggshell thickness: scaling and maximum body mass in birds. *J. Zool. Lond.* **279**: 95–101.

Blankespoor, G.W., Oolmann, J. & Uthe, C. 1982. Eggshell strength and cowbird parasitism of red-winged blackbird. *Auk* **99**: 363–365.

Blom, J. & Lilja, C. 2004. A comparative study of growth, skeletal development and eggshell composition in some species of birds. *J. Zool. Lond.* **262**: 361–369.

Castilla, A.M., Herrel, A., Díaz, G. & Francesch, A. 2007. Developmental stage affects eggshell-breaking strength in two ground-nesting birds: the partridge (*Alectoris rufa*) and

the quail (*Coturnix japonica*). *J. Exp. Zool. Part A* **307A**: 471–477.
Castilla, A., Van Dongen, S., Herrel, A., Francesch, A., Martínez de Aragón, J., Malone, J. & José Negro, J. 2010. Increase in membrane thickness during development compensates for eggshell thinning due to calcium uptake by the embryo in falcons. *Naturwissenschaften* **97**: 143–151.
Davis, T.A., Platterreiger, M.F. & Ackerman, R.A. 1984. Incubation water-loss by Pied-billed Grebe eggs – adaptation to a hot, wet nest. *Physiol. Zool.* **57**: 384–391.
Dudley, S.P., Gee, M., Kehoe, C., Melling, T. & Committee, T.B.O.U.R. 2006. The British List: A Checklist of Birds of Britain (7th edition). *Ibis* **148**: 526–563.
Finnlund, M., Hissa, R., Koivusaari, J., Merilä, E. & Nuuja, I. 1985. Eggshells of arctic terns from Finland – effects of incubation and geography. *Condor* **87**: 79–86.
Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.
Gosler, A.G., Higham, J. & Reynolds, S.J. 2005. Why are birds' eggs speckled? *Ecol. Lett.* **8**: 1105–1113.
Graveland, J., Vanderwal, R., Vanbalen, J.H. & Vannoordwijk, A.J. 1994. Poor reproduction in forest passerines from decline of snail abundance on acidified soils. *Nature* **368**: 446–448.
Green, R.E. 1998. Long-term decline in the thickness of eggshells of thrushes, *Turdus* spp., in Britain. *Proc. Roy. Soc. B* **265**: 679–684.
Green, R.E. 2000. An evaluation of three indices of eggshell thickness. *Ibis* **142**: 676–679.
Green, R.E. & Scharlemann, J.P.W. 2003. Egg and skin collections as a resource for long-term ecological studies. *Bull. Bri. Orn. Club* **123A**: 165–176.
Honza, M., Picman, J., Grim, T., Novak, V.T., Capek, J.M. & Mrlik, V. 2001. How to hatch from an egg of great structural strength. A study of the Common Cuckoo. *J. Avian Biol.* **32**: 249–255.
Hoyt, D.F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **96**: 73–77.
Jagannath, A., Shore, R.F., Walker, L.A., Ferns, P.N. & Gosler, A.G. 2008. Eggshell pigmentation indicates pesticide contamination. *J. Appl. Ecol.* **45**: 133–140.
Johnston, P.M. & Comar, C.L. 1955. Distribution and contribution of calcium from the albumen, yolk and shell to the developing chick embryo. *Am. J. Physiol.* **183**: 365–370.
Karlsson, O. & Lilja, C. 2008. Eggshell structure, mode of development and growth rate in birds. *Zoology* **111**: 494–502.
Klaas, E.E., Ohlendorf, H.M. & Heath, R.G. 1974. Avian eggshell thickness: variability and sampling. *Wilson Bull.* **86**: 156–164.
Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116–121.
Maurer, G., Russell, D.G.D. & Cassey, P. 2010. Interpreting the lists and equations of egg dimensions in Schönwetter's 'Handbuch der Oologie'. *Auk* **127**: 940–947.
Maurer, G., Portugal, S.J. & Cassey, P. 2011a. Review: an embryo's eye view of avian eggshell pigmentation. *J. Avian Biol.* **42**: 494–504.
Maurer, G., Portugal, S.J. & Cassey, P. 2011b. Speckles of cryptic Black-headed Gull eggs show no mechanical or conductance structural function. *J. Zool. Lond.* **285**: 194–204.
Messens, W., Grijspeerdt, K. & Herman, L. 2005. Eggshell penetration by *Salmonella*: a review. *World's Poult. Sci. J.* **61**: 71–86.
Mikhailov, K.E. 1997. *Avian Eggshells: An Atlas of Scanning Electron Micrographs*. London: British Ornithologists' Club.
Moriarty, F., Bell, A.A. & Hanson, H. 1986. Does p,p'DDE thin eggshells? *Environ. Pollut. (Ser. A)* **40**: 257–286.
Nybø, S., Staurnes, M. & Jerstad, K. 1997. Thinner eggshells of Dipper (*Cinclus cinclus*) eggs from an acidified area compared to a non-acidified area in Norway. *Water Air Soil Pollut.* **93**: 255–266.
Nygård, T. 1999. Correcting eggshell indices at raptor eggs for hole size and eccentricity. *Ibis* **141**: 85–90.
Olsen, P.D., Cunningham, R.B. & Donnelly, C.F. 1994. Avian egg morphometrics – allometric models of egg volume, clutch volume and shape. *Aust. J. Zool.* **42**: 307–321.
Paganelli, C.V. 1980. The physics of gas-exchange across the avian eggshell. *Am. Zool.* **20**: 329–338.
Picman, J. 1989. Mechanism of increased puncture resistance of eggs of Brown-headed Cowbirds. *Auk* **106**: 577–583.
Picman, J. & Pribil, S. 1997. Is greater eggshell density an alternative mechanism by which parasitic cuckoos increase the strength of their eggs? *J. Ornithol.* **138**: 531–541.
Porter, R.D. & Wiemeyer, S.N. 1969. Dieldrin and DDT – effects on Sparrowhawk eggshells and reproduction. *Science* **165**: 199–200.
Rahn, H. & Paganelli, C.V. 1989. Shell mass, thickness and density of avian eggs derived from the tables of Schönwetter. *J. Ornithol.* **130**: 59–68.
Ratcliffe, D.A. 1967. Decrease in eggshell weight in certain birds of prey. *Nature* **215**: 208–210.
Ratcliffe, D.A. 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *J. Appl. Ecol.* **17**: 67–107.
Russell, D.G.D., White, J., Maurer, G. & Cassey, P. 2010. Data-poor egg collections: cracking an important research resource. *J. Afrotrop. Zool.* Special Issue: 77–82.
Schönwetter, M. (Meise, W. ed.) 1960–1992. *Handbuch der Oologie*. Berlin: Akademie Verlag.
Schönwetter, M. 1961. Mathematischer Teil B. In Schonwetter, M. & Meise, W. (eds) *Handbuch der Oologie*. Vol. 3: 11–178. Berlin: Akademie Verlag.
Sibley, C.G. & Monroe, B.L. Jr.1990. *Distribution and Taxonomy of Birds of the World*. New Haven: Yale University Press.
Sooncharenying, S. & Edwards, H.M. 1989. Modelling the relationships of egg weight, specific-gravity, shell calcium and shell thickness. *Br. Poult. Sci.* **30**: 623–631.
Spottiswoode, C.N. 2010. The evolution of host-specific variation in cuckoo eggshell strength. *J. Evol. Biol.* **23**: 1792–1799.
Spottiswoode, C.N. & Colebrook-Robjent, J.F.R. 2007. Egg puncturing by the brood parasitic Greater Honeyguide and potential host counter adaptations. *Behav. Ecol.* **18**: 792–799.
Thomas, G.H. 2008. Phylogenetic distributions of British birds of conservation concern. *Proc. R. Soc. B* **275**: 2077–2083.

Received 20 July 2010;
revision accepted 30 April 2012.
Associate Editor: Pamela Rasmussen.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Measurements, quality assessment and register numbers for specimens of eggshells of 230 European breeding bird species housed at the Natural History Museum, Tring, UK.

Appendix S2. Mean differences between thickness of shell regions compared for each family using a one-sample *t*-test.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.