On the behaviour and ecology of the Black-tailed Godwit
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7 Variation in egg size of Black-tailed Godwits

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ABSTRACT

As is the case for most avian species, there is considerable variation in the egg size of Continental Black-tailed Godwits *Limosa l. limosa* breeding in The Netherlands. It is interesting that egg size has costs and benefits yet varies considerably at the population level. To better understand this variation in egg size, we tested its relationship to a suite of individual and environmental factors. We found that egg size can decrease up to 2.8% throughout a breeding season and that egg size increases with clutch size by 1.4% with each additional egg in the clutch. Female body mass and body size explained 5% of the total variation in egg size observed across the population. Furthermore, females wintering south of the Sahara laid 3% smaller eggs than those wintering north of the Sahara. We also found that egg size increases with age, which may indicate age-related differences in the endogenous and/or exogenous conditions of females. The variation in egg size was, however, mostly the result of consistent differences among individuals across years (repeatability = 0.60). A comparison of daughters with mothers suggested that most of this individual repeatability reflects heritable variation (heritability = 0.64). The actual individual traits that underlie this heritable variation among individuals remain mostly undetermined. Smaller eggs did have a slightly lower chance of hatching, but we found no relationship between egg size and chick survival. Finally, nest and chick survival were strongly correlated with lay date. Thus, in Black-tailed Godwits, lay date may actually reflect a female's endogenous and/or exogenous condition at the moment of egg-laying. This finding may be general across birds, since food supplementation experiments usually result in advanced laying and larger clutch sizes rather than in larger eggs.

INTRODUCTION

Egg sizes of birds vary considerably at the population level; the largest eggs are generally at least 50% larger than the smallest eggs within a population (Christians 2002). Such variation could result from consistent differences in egg size among individuals. These consistent differences among individuals, in turn, could be the result of consistent differences in environmental context (e.g. habitat quality before egg laying) or the result of genetic and ontogenetic factors that help determine egg size and differ among individuals (e.g. female size). On the other hand, it is also possible that the observed variation in egg size is the result of differences within individuals, in response to annual variation in exogenous conditions (e.g. food, precipitation or temperature) or changes in endogenous condition (e.g. female age or body condition). To understand the causes of variation in egg size, it is therefore necessary to first partition the variation in egg size into among- and within-individual differences.

Among- and within-individual differences are usually partitioned by calculating the repeatability of egg size (Christians 2002). In previous studies, the egg size of individual birds has been found to be significantly repeatable (mean repeatability = 0.68; 23 studies of 18 species reviewed by Christians 2002). In other words, in most species, individuals consistently differ from one another in egg size and show only relatively minor within-individual variation in egg size. However, in his review, Christians (2002) also concluded that only 10–20% of the variation in egg size was explained by environmental and among-individual factors such as food availability, temperature, body size, age and mass.

Pick *et al.* (2016a) discovered that Japanese Quails *Coturnix japonica* laying larger eggs invested more energy into their reproductive organs and also had a

higher resting metabolic rate. Larger eggs also generally contain higher absolute levels of egg components and produce larger and heavier chicks that survive better (reviewed in Krist 2011 and Williams 2012). These results suggest that egg size has costs and benefits, and that individuals might balance these costs and benefits when allocating resources to egg size. Interestingly, since there is large between-individual variation in egg size and only minor within-individual variation, the outcome of this potential trade-off must differ both considerably and consistently among individuals. That the factors determining this variation among individuals remain largely undiscovered makes it all the more intriguing (Christians 2002, Williams 2012). To further examine the considerable variation in the egg sizes of birds, we explore the determinants of egg size in Continental Black-tailed Godwits (hereafter Godwits).

The egg size of Godwits may be particularly interesting to study because this species differs in two ways compared with most of the 18 species reviewed by Christians (2002). First, Godwits have a relatively invariant clutch size of four eggs (87% of all clutches, $n = 6396$), and second, the chicks forage for themselves (Schroeder et al. 2009, 2012). Thus, most Godwits do not vary their total parental effort by varying their clutch size and might instead vary their egg size more than would species with a variable clutch size. Furthermore, since they have chicks that forage for themselves, Godwits are relieved of the energetically demanding task of food provisioning (but not of supplemental heating; see Schekkerman et al. 2003). This means that in terms of allocating parental effort to reproduction, the effort put into eggs plays a proportionally greater role in Godwits than it does in altricial bird species (Williams 1994, Starck & Ricklefs 1998). It is worth noting that the effects of egg size on offspring phenotype have been found to differ between altricial and precocial species; in the altricial Blue Tit *Cyanistes caeruleus* the effects of egg size dissipate early in ontogeny (Hadfield et al. 2013), whereas in the precocial Japanese Quail they remain until adulthood (Pick et al. 2016b).

We are curious about what the variation in egg size of Godwits reflects and how this might differ from other bird species. Therefore, we investigated whether Godwits show variable egg size and related their egg size to a suite of environmental and individual factors. First, we estimated the repeatability and heritability of egg size. Second, we searched for annual and seasonal patterns in egg size, and then turned to individual factors such as female size, mass, age, lay date, and wintering location to explain the variation. Finally, to understand why variation in egg size persists in Godwits, we explored how egg size and lay date are associated with nest and chick survival. Our goal is to better understand the variation in egg size for Godwits and birds in general.

METHODS

We studied Godwits in southwestern Friesland, The Netherlands, from 2004 until 2017. In 2004, our study area was a single 250 ha polder (52°59'55"N, 5°24'31"E). Polders are historical and hydrological entities characterized by fields with similar water levels (Groen et al. 2012). In 2007, the study area was expanded to 8970 ha in order to include multiple polders; in 2012 the study area was expanded again to 11,415 ha (see Groen et al. 2012 and Senner et al. 2015 for a detailed description of the study area). Each year, we searched the study area for nests and measured the width and length of the eggs when a nest was found. We found most nests during the incubation phase and therefore measured their egg flotation angles to estimate the lay date of each nest (van Paassen et al. 1984). For Godwits, more than 80% of all estimates are accurate within two days and 95% of all estimates are accurate within three days (van Paassen et al. 1984). In most cases we floated two eggs and used the average to better estimate lay date, but even with this approach some measurement error persists. To put this measurement error into context, lay dates vary from 32–55 days within a breeding season. Following Schroeder et al. (2009), we calculated egg volume using the formula (length × width^2 × 0.52); we refer to this as egg size. In our analyses we use clutch size as a fixed effect, but we cannot distinguish between partial predation and a clutch that naturally contained fewer than four eggs. To ensure that we did not include any clutches that were still in the laying phase, we included clutches of fewer than four eggs only when upon initial discovery the clutch had already been incubated for two or more days, or when two or more days after its initial discovery in the laying phase the clutch was being incubated and contained the same number of eggs (thus excluding any noticeable predation that might have occurred in the meantime). In this way we excluded 351 clutches that failed before reaching what might have been their final clutch size. However, our sample still unavoidably includes clutches that were partially depredated.

On a subset of the found nests, we caught incubating adults to individually mark them with colour rings

and take blood for molecular sexing (e.g. Schroeder *et al.* 2009). From 2008 onwards, we colour-ringed recently hatched juveniles as well as juvenile Godwits that we captured in the study area after they had already left the nest. For the latter group of juveniles, we know the year in which they were born but often not their maternal nest. Our analyses that include juveniles or birds with known age are based on data from 2008 onwards. In the years after capture, we tried to link individual adults to specific nests through visual observation or, coincidentally, by recapturing them. However, either because we failed to link the marked female or because the female was unmarked, our dataset includes many clutches with unknown females. It is important to realize that this group of unknown females also includes marked females that were successfully linked to nests in other years. To avoid pseudoreplication we have therefore used in our analyses only those nests to which a marked female was linked, meaning that we excluded 4596 clutches for which the female was unknown in that year. The sample size of clutches we could include in our analyses was limited not only by not knowing the identity of the female, but also by not knowing traits specific to that female (such as body mass, age and wintering location). This means that the numbers of covariates and clutches vary considerably among analyses and that we were hardly ever able to estimate multiple parameters in the same model. To keep track of what is included in each analysis, we have numbered every analysis and corresponding result and included Tables 1 and 2 for a complete overview.

Analysis

All mixed models in this paper use the package 'lme4' (Bates *et al.* 2015) in the R programming environment (R Core Team 2018). We obtained chi-squared values for the significance of the fixed effects from likelihood ratio tests of nested models with and without the variable of interest. We used the function 'rpt' which is part of the R package 'rptR' (Stoffel *et al.* 2017) to estimate the proportion of variance explained by the fixed effects of these models and assessed the uncertainty of these estimates with 1000 parametric bootstraps. We used a range of models for the analyses of egg size, which are described next.

(1) Global model: In this linear mixed effect model (LMM) we related an individual's egg size to its lay date (continuous covariate), clutch size (continuous covariate) and nesting attempt (three-level factor). The random intercepts were clutch, individual, year and polder. In 96% of the cases we were unaware of whether a nest was a first or second attempt and we therefore added 'unknown' as a third factor level. We used this model and the function 'rpt' to estimate the adjusted repeatabilities of the different groups specified as random effects, and assessed the uncertainty of these estimates with 1000 parametric bootstraps (Nakagawa & Schielzeth 2010).

(2) Condition Model: In this LMM we related an individual's egg size to its lay date, clutch size, body mass and structural size (all continuous covariates). The random intercepts were clutch, individual, year and polder. Body mass was measured during incubation of the clutch. For ease of comparison to the other models, we scaled the body mass of individuals by subtracting the mean and dividing by the standard deviation. The first principal component was derived from five linear dimensions: bill length (exposed culmen; ±0.1 mm), total-head length (±0.1 mm), wing length (flattened and straightened; ±1 mm), tarsus length (±0.1 mm) and tarsus-toe length (tarsus plus mid-toe without claw; ±1 mm), and explained 55% of the variation. We multiplied the first principal component value by –1 such that it was positively related to the linear dimensions (i.e. a higher value = a larger female) and used it as a measure of overall structural size.

(3) Age Model: In this LMM we related an individual's egg size to its lay date, clutch size, body mass, structural size and age (all continuous covariates). We checked whether the model improved when a quadratic term was added to age, but it did not (+4.3 AICc). The random intercepts were clutch, individual, year and polder. For this analysis we could only use individuals that were ringed as chicks (i.e. of known age) and recaptured as adults (i.e. of known size and mass). This limited our sample size to 53 clutches of 40 females. In order to include an additional 81 clutches of 40 females for which the age but not the size was known, we also performed this analysis without body mass and structural size included as covariates (Age Model 3B). We did add a quadratic term to age in this analysis because it improved the model (–11.3 AICc). Although this model uses a larger dataset, the results could be misleading because the effects of body condition and age cannot be disentangled.

(4) Daughter-mother relation: We could correlate the average egg volume of 49 daughters to that of their mother. Because egg volume increases with age (see Results and Discussion), we plotted the relationship between the egg volumes of daughters and their mothers for every age of the daughters (Figure 7.3A). This suggested that the relationship between daughters and

mothers might only become apparent when the daughters are three years old or older. To test for this possibility, we included an interaction with age as a two-level factor (≤2 years old and ≥3 years old) in a linear model in which we related the average volume of daughters to the average volume of their mothers (continuous covariate). This interaction was not significant (see Results) and we therefore averaged the egg size of daughters across all their ages and related this to the average egg size of their mother in a linear model. We estimated the heritability of egg size as two times the slope of this linear regression (Becker 1984). Such parent-offspring regressions with small sample sizes ($n < 100$ pairs) are imprecise and may overestimate heritability (de Villemereuil *et al.* 2013). However, we lack the sample size and pedigree information needed for a more precise analysis.

(5) Wintering Model: In this LMM we related an individual's egg size to its lay date, clutch size, body mass, structural size (continuous covariates) and wintering location (two-level factor). The random intercepts were clutch, individual, year and polder. Individual Godwits consistently winter either north or south of the Sahara (Verhoeven *et al.* 2019). To determine whether this consistent migratory behaviour results in consistent differences in egg size, we correlated egg size with wintering location. We obtained wintering location in two ways. First, like Kentie *et al.* (2017), we used resightings to classify individuals as wintering south of the Sahara if they were seen in West Africa at least once during the nonbreeding period (July–March) in the years 2004–2017; we classified an individual as wintering north of the Sahara if it was resighted on the Iberian Peninsula in October during the years 2007–2017. Second, we used geolocators (Migrate Technology Ltd, Cambridge, U.K.) to determine whether females wintered north or south of the Sahara. Using package 'FLightR' (Rakhimberdiev *et al.* 2017), we analysed the light-level data obtained from 37 females carrying geolocators (2012–2017). A detailed example of this analytical method in Godwits can be found in Rakhimberdiev *et al.* (2016). Eighteen of these geolocator-carrying females were also assigned a wintering location on the basis of resightings, and in 17 cases the two different methods led to the same result. However, one individual was incorrectly assigned a wintering location north of the Sahara based on the resighting method; the geolocator data showed that at the time of the faulty resighting, the bird was actually south of the Sahara. We have also observed that of 36 geolocator-carrying females that crossed the Sahara in 2012–2018, two returned north to the Iberian

Peninsula before the end of October (unpubl. data). As such, some birds resighted on the Iberian Peninsula in October and classified as wintering north of the Sahara may in fact have migrated all the way to wintering grounds south of the Sahara and already travelled north again. In the present study, eight individuals were resighted both north and south of the Sahara either in the same winter ($n = 4$) or in different winters ($n = 4$) and were thus assigned both wintering locations by the resighting method. However, we are confident that these cases were the result of the errors and limitations of the resighting method described above, and we have therefore excluded these eight individuals from our analysis. It is highly likely that our sample still includes some unidentified resighting errors of this type, and these results should therefore be interpreted with care.

(6) Sexual size dimorphism: Adult female Godwits are 10–20% bigger than male Godwits (Schroeder *et al.* 2009) and Godwits have sexually specific growth rates (Loonstra *et al.* 2018). We hypothesized that this sexual size dimorphism might already be present in eggs, with larger eggs containing females and storing more nutrients than smaller eggs containing males. We therefore related the genetic sex of the chick that hatched from an egg to the size of that egg (continuous covariate) using a generalised linear mixed model (GLMM) with a binomial error distribution, a logistic link function, and nest included as random intercept.

(7) Hatching success: We used a GLMM with a binomial error distribution and a logistic link function to analyse whether the hatching success of a clutch depended on its average egg volume, lay date, clutch size or the age of the nest in days when it was found (all continuous covariates). To make the intercept of this model more meaningful, we scaled all these continuous covariates by subtracting the mean and dividing by the standard deviation. The random intercepts were year and polder. The hatching success of a clutch is determined by whether or not at least one egg hatches. We were not able to model the number of hatched eggs as a proportion of the total because we often arrived at nests when only some of the eggs had hatched or after the chicks had already left the nest. Because the chance that a nest hatches is larger when a nest is found closer to hatching, we included the age of the nest when it was found, i.e. the number of days the nest was already incubated when it was first discovered, as a covariate.

(8) Fledging success: We used a GLMM with a binomial error distribution and a logistic link function to analyse whether the fledging success of a clutch – the

number of chicks that survived in relation to the number that were marked – depended on its average egg volume or lay date (both scaled continuous covariates). The random intercepts were year and polder. A chick was considered to have fledged if it was resighted 35 or more days after being marked in the nest.

RESULTS

(1) There is considerable variation in the egg size of our Godwit population (Figure 7.1). The global model – the model with the largest sample size (Table 7.1) – showed that egg size decreases with lay date and increases with clutch size, but does not relate to nesting attempt (Table 7.2). In this analysis, the time
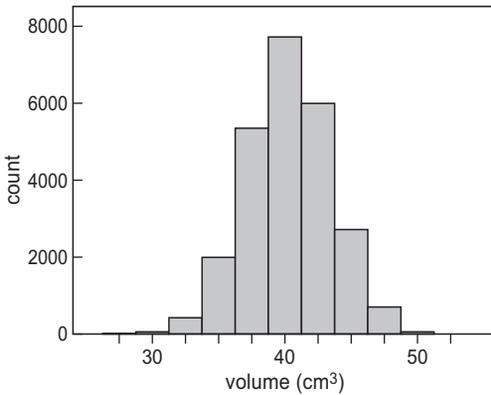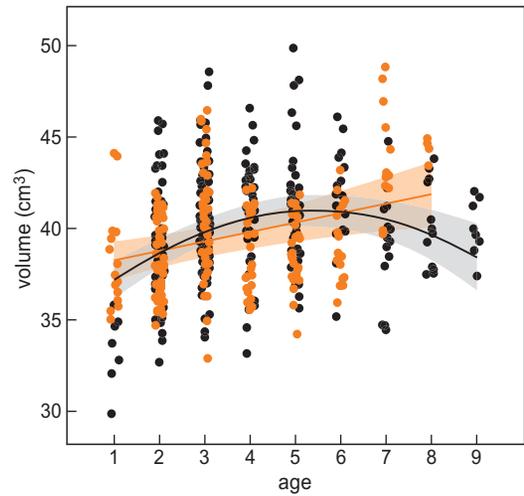
Figure 7.2. The relationship between egg volume and age for Godwits in southwest Friesland. This graph shows the raw data used in the analysis with body condition included (Model 3, red dots) and without body condition included (Model 3B, red + black dots combined). It also shows the modelled estimates and standard errors from Model 3 (red line, linear relationship) and Model 3B (black line, quadratic relationship).

Figure 7.1. Frequency distribution of 25,148 egg sizes measured in southwest Friesland between 2004–2018. Three eggs with a volume larger than 55 cm^3 (55.04 cm^3, 55.11 cm^3 and 62.68 cm^3, respectively), are omitted from this figure for graphical purposes.

between the first and the last egg in a year varied from 32–55 days, which means that over the entire season egg size declined between 1.6–2.8%. The proportion of variance explained by the fixed effects in this model was 0.004 (CI = 0.002–0.01). The adjusted repeatabilities from this model were for individual: 0.60 (CI = 0.57–0.63), clutch: 0.12 (CI = 0.10–0.14), year: 0.008 (CI = 0.001–0.02) and polder: 0.003 (CI = 0–0.01). As a result, the residual variance – the proportion of variance attributed to undiscovered differences within individuals – was 0.27 (CI = 0.25–0.29).

Table 7.1. The number, mean size and standard deviation of eggs included in the different models. Also shown are the sample sizes for the different grouping factors in each analysis. When the number of eggs is not given, the average egg size of the clutch was used in the analysis. [a]In these cases, 'Individuals' was not included as a grouping factor, but the entry still shows how many individuals were part of the analysis.

| Model	Eggs	Volume	Clutches	Individuals	Years	Polders
(1) Global Model	5866	40.46 ± 3.23	1554	840	14	58
(2) Condition Model	3486	40.45 ± 3.24	925	756	14	57
(3) Age Model with	203	39.48 ± 2.81	53	40	9	21
(3B) Age Model without	504	39.84 ± 2.97	134	80	9	35
(4) Daughter ~ Mother	–	39.13 ± 2.12	49	98[a]	–	–
(5) Wintering Model	1099	40.07 ± 3.24	286	207	14	48
(6) Sexual size dimorphism	148	40.09 ± 3.21	99	–	–	–
(7) Hatching success	–	40.28 ± 2.88	6328	–	14	61
(8) Fledging success	–	40.36 ± 2.80	1823	5265[a]	9	61
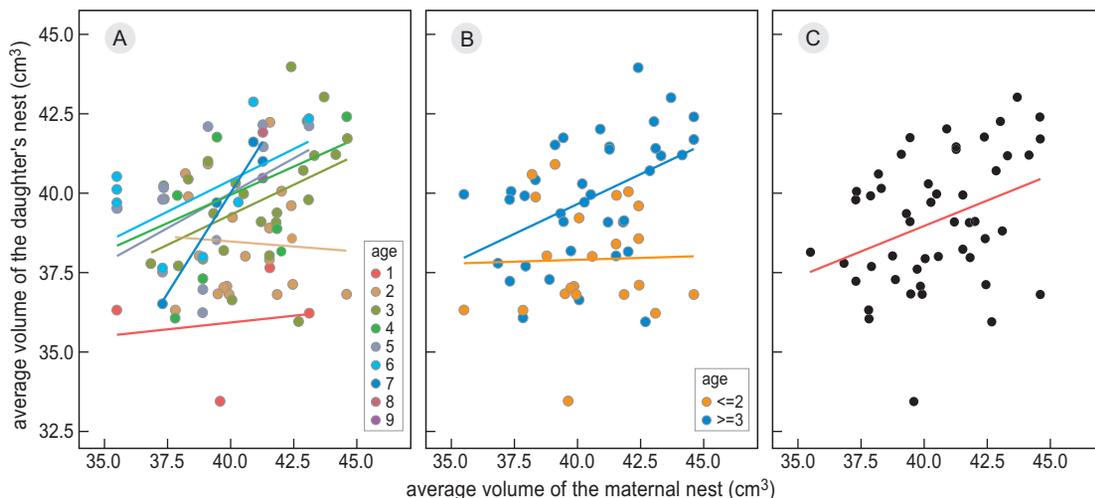
Figure 7.3. The relationship between average egg volume of the maternal nest and average egg volume of the daughter's nest, with average egg volume of the daughters (A) given at each age for every daughter, (B) averaged into two different age categories for every daughter, and (C) averaged for each daughter across her attempts at all ages. Heritability is estimated from relationship (C): $Y = 26.07 + 0.32 \times X$, $R^2 = 0.11$, $n = 49$.

(2) Heavier and larger females lay larger eggs; egg size was positively correlated with both body mass during incubation and the first principal component of the linear measurements (Table 7.2). The proportion of variance explained by the fixed effects in this model was 0.06 (CI = 0.04–0.09).

(3) In the age model that included body mass and structural size, the average egg size increased with age ($\beta = 0.52 \pm 0.63$, $P = 0.002$; Table 7.2, Figure 7.2) and body mass was again a significant predictor of the variation in egg size (Table 7.2). The proportion of variance explained by the fixed effects in this model was 0.18 (CI = 0.08–0.35). (3B) In the age model with a larger dataset that did not exclude birds for which body mass and structural size were missing, the average egg size also increased with age ($\beta = 1.99 \pm 0.37$, $P < 0.001$; Table 7.2), but the slope of this increase decreased with every additional year of age as identified by the significant quadratic term ($\beta = -0.18 \pm 0.04$, $P < 0.001$; Table 7.2, Figure 7.2). The proportion of variance explained by the fixed effects in this model was 0.12 (CI = 0.06–0.20).

(4) The relationship between egg volumes of daughters and mothers was different between the two age groups ($\beta_{\leq 2\,years} = 0.02 \pm 0.19$ vs. $\beta_{\geq 3\,years} = 0.38 \pm 0.23$; Figure 7.3B), but not significantly so ($F_{1,55} = 2.47$, $P = 0.12$). We therefore averaged the egg volumes of daughters across all their ages; this average was significantly related to the average egg volume of their mothers ($F_{1,47} = 6.83$, $P = 0.012$; Figure 7.3C). The slope of this relationship was 0.32 ± 0.12, and the

heritability of egg size is therefore estimated to be 0.64 ± 0.24.

(5) The egg size of females that crossed the Sahara was on average 3% smaller than the egg size of females that did not cross the Sahara. Body mass was also a significant predictor of the variation in the egg size of this sample (Table 7.2). The proportion of variance explained by the fixed effects in this model was 0.15 (CI = 0.09–0.22).

(6, 7 and 8) The genetic sex of a chick was not correlated with the size of the egg from which it hatched (Table 7.2). Our exploratory analysis of the fitness consequences of egg size indicated that the chance of hatching increased with larger average egg volume and declined with lay date (Table 7.2). The chance that a marked hatchling survived to fledging was not dependent on the average egg volume of the clutch, but declined with lay date (Table 7.2).

DISCUSSION

We observed considerable variation in the egg size of Continental Black-tailed Godwits and investigated which individual and environmental factors might contribute to this variation. We also explored whether this variation in egg size was associated with reproductive success. Approximately 60% of the observed variation in egg size was due to different individual Godwits laying differently sized eggs, and only 27% of the observed variation was the result of variation within

Table 8.2. The fixed effect coefficients and random effect variances of all models. The numbers and names correspond to those used throughout the manuscript. We obtained chi-squared values for the significance of the fixed effects from likelihood ratio tests of nested models with and without the variable of interest. Model 1 also shows the estimated adjusted repeatabilities.

(1) Global Model

Fixed effects	Estimate	SE	χ^2	P
Intercept	39.41	0.62		
Lay date	−0.02	0.01	10.00	0.002
Clutch size	0.34	0.13	6.43	0.011
Attempt (2nd)	0.38	0.40	0.97	0.617
Attempt (unknown)	0.09	0.33	–	–

Random effects	Variance	R	SE
Clutch	1.23	0.12	0.01
Individual	6.32	0.60	0.02
Year	0.08	0.01	0.01
Polder	0.04	0.00	0.01
Residual	2.85	0.27	0.01

(2) Condition Model

Fixed effects	Estimate	SE	χ^2	P
Intercept	38.23	0.83		
Lay date	−0.02	0.01	3.18	0.074
Clutch size	0.65	0.21	9.81	0.002
Body Mass (scaled)	0.57	0.09	38.68	0.000
PC1	0.15	0.06	5.09	0.024

Random effects	Variance
Clutch	0.79
Individual	6.16
Year	0.00
Polder	0.02
Residual	2.78

(3) Age Model

Fixed effects	Estimate	SE	χ^2	P
Intercept	36.17	2.55		
Lay date	−0.03	0.03	1.02	0.313
Clutch size	0.52	0.63	0.84	0.359
Body Mass (scaled)	0.73	0.34	4.87	0.027
PC1	−0.16	0.24	0.41	0.523
Age	0.52	0.17	9.36	0.002

Random effects	Variance
Clutch	0.05
Individual	3.67
Year	0.36
Polder	0.00
Residual	2.51

(3B) Age Model without condition

Fixed effects	Estimate	SE	χ^2	P
Intercept	34.23	1.52		
Lay date	0.003	0.02	0.02	0.882
Clutch size	0.24	0.33	0.55	0.460
Age	2.15	0.37	27.91	0.000
Age^2	−0.20	0.04	21.66	0.000

Random effects	Variance
Clutch	0.93
Individual	3.28
Year	0.11
Polder	1.28
Residual	2.74

females across years. Consequently, we found that individual factors such as female size, mass, age and wintering location explained more of the variation in egg size than did environmental factors such as year, polder, or lay date. Although we found egg size to be heritable ($h^2 = 0.64 \pm 0.24$), the actual individual traits that underlie this heritable variation among individuals remain mostly undetermined. Finally, clutches with larger eggs did, on average, have a slightly higher chance of hatching, but chicks that hatched from larger eggs were not more likely to fledge than chicks that hatched from smaller eggs.

Environmental factors: annual, seasonal and local variation

Both year and lay date explained a small amount of the variation in egg size. These annual and seasonal changes in egg size could be the result of differences in both environmental and individual factors, such as the temperature during egg laying and the age of females (Robertson 1995, Hipfner *et al.* 1997). The seasonal decline in egg size could also be an adaptive response to the environment if laying smaller eggs earlier is more beneficial than laying larger eggs later (Winkler & Allen 1996). Because the seasonal decline in egg size remains present (though non-significant) when body mass, structural size and age are included in the model (Table 7.2, models 2 and 3), we can infer that these factors are likely not the cause of the seasonal decline. In any case, since we did not find that second attempts were smaller than first attempts nor that polders differed consistently in egg size, we have no real indication that egg size is influenced by environmental factors. This idea is consistent with the outcome of food

Table 8.2. Continued.

(4) Daughter-mother relation

Fixed effects	Estimate	SE	t	P
Intercept	26.07	5.01		
Egg volume mother	0.32	0.12	2.61	0.012

(6) Sexual size dimorphism

Fixed effects	Estimate	SE	χ^2	P
Intercept	−0.05	0.16		
Egg volume (scaled)	0.06	0.17	0.14	0.705

Random effects	Variance
Clutch	0.00

(7) Hatching success

Fixed effects	Estimate	SE	χ^2	P
Intercept	0.21	0.14		
Lay date	−0.35	0.03	144.58	0.000
Clutch size	0.24	0.03	67.98	0.000
Average volume	0.06	0.03	4.84	0.028
Nest Age	0.59	0.03	373.41	0.000

Random effects	Variance
Year	0.13
Polder	0.47

(5) Wintering Model

Fixed effects	Estimate	SE	χ^2	P
Intercept	38.50	1.53		
Lay date	−0.01	0.01	0.54	0.461
Clutch size	0.64	0.38	2.89	0.089
Body Mass (scaled)	0.86	0.16	25.73	0.000
PC1	0.18	0.12	2.27	0.132
Sahara (South)	−1.13	0.36	9.49	0.002

Random effects	Variance
Clutch	0.74
Individual	4.86
Year	0.32
Polder	0.47
Residual	2.44

(8) Fledging success

Fixed effects	Estimate	SE	χ^2	P
Intercept	−2.01	0.21		
Lay date	−0.37	0.05	67.49	0.000
Average volume	0.01	0.04	0.03	0.855

Random effects	Variance
Year	0.34
Polder	0.06

supplementation experiments, which usually result in advanced lay dates and larger clutch sizes but not in larger eggs (see Christians 2002 for a review, and Ruffino *et al.* 2014 for a meta-analysis).

Individual factors: wintering location, size, mass, age, identity and reproductive performance
On the basis of resightings and geolocator data, individual females wintering north of the Sahara laid on average 3% larger eggs, which was also found by Kentie *et al.* (2017). Body mass, structural size and lay date were included in this analysis and are therefore not the factors that explain the difference between wintering locations. If disproportionally more young birds were found to winter south of the Sahara than north of the Sahara, we would speculate that the difference between wintering locations is the result of differences in female ages, but currently we are at a loss to explain this difference in egg size between wintering

locations. Perhaps the longer migration itself results in a decrease in egg size. If this were the case, individuals from the same breeding location would differ in migratory distance, and that difference in migratory distance itself – independent of body condition – would be related to differences in egg size. We have not found any examples in birds to support this idea, but in the migratory Chinook Salmon *Oncorhynchus tshawytscha*, individuals migrating longer distances laid smaller eggs than did individuals from the same breeding location that migrated shorter distances (Kinnison *et al.* 2001).

Previous research on Godwits has hypothesized that female Godwits vary the size of their eggs between years to adjust to their endogenous condition (Schroeder *et al.* 2009, 2012, Lourenço *et al.* 2011). We found that approximately 5% of the total variation in egg size observed across the population was explained by differences in female body mass and

structural size, which indicates that in Godwits only minor adjustments in egg size are associated with body mass. However, we measured differences in body mass at the end of incubation, which might not necessarily reflect the differences in body mass during egg laying. Pick *et al.* (2016a) found in an artificial selection experiment with Japanese quail that the difference in egg size between the study's selection lines was mainly driven by a differential increase in reproductive organ mass, which was independent of body size. We can draw three important conclusions from these findings: (1) some variation in egg size is explained by the size of the reproductive organs, (2) this is mostly independent of female size, just as has been found in the present study and other correlative studies on body size, and (3) a change in body mass before or during the laying period is potentially a good predictor of egg size, whereas body mass during incubation – which is what we measured – is likely less reliable. Regardless, our finding that body mass and size explain only a small amount of the variation in egg size is similar to the results previously published on other species (reviewed in Christians 2002). It is also consistent with the high repeatability of egg size in other avian species with invariant clutch size, such as other shorebirds (e.g. Väisänen *et al.* 1972, Nol *et al.* 1997, Dittmann & Hötker 2001) and species with one-egg clutches (Christians 2002).

We found that the egg size of Godwits increases with age and that there is a significant relationship between the average egg size of a mother and that of her daughter. Both the increase in egg size with age and the heritability of egg size are commonly found in studies of avian egg size (reviewed in Christians 2002 and Williams 2012). Using the larger dataset of known-age females we found a quadratic relationship between egg size and age (Figure 7.2), which suggests that a female's egg size increases relatively more during her first attempts and remains more stable later on. In addition, the relationship between daughters and mothers becomes most apparent when the daughters are three years old or older (Figure 7.3A, 7.3B). Both of these patterns, as well as the high individual repeatability of egg size, suggest that Godwits mostly increase their egg size during their first two years of breeding and attain their final egg size in the third year of breeding. However, given the knowledge that some Godwits breed in their first year while others defer breeding by one or maybe even two years (Kentie *et al.* 2014), an alternative explanation is that Godwits lay smaller eggs only during their first attempt. The increase in egg size during the first three years could simply reflect that the proportion of females laying for the first time decreases every year during that period, because of deferred breeding. In any case, these results suggest that the factors determining avian egg size partly develop with age and are inherited.

The absence of a relationship between egg size and the genetic sex of a chick might not be very surprising. As the meta-analysis of egg sexual size dimorphism in birds by Rutkowska *et al.* (2013) shows, there is very little evidence for such a relationship across avian species. We did find that smaller eggs had a slightly lower chance of hatching. A potential reason for this is that younger females tend to lay smaller eggs, and younger females might also be less experienced and therefore of lower quality in their execution of reproductive activities such as choosing a nest site or defending their nests against predators. It is also possible that larger eggs inherently have a higher chance of hatching. However, in the period from 2015–2017, we collected 216 eggs from 40 clutches within our study area and incubated them in an incubator (Loonstra *et al.* unpubl. data); the hatching success of these eggs was independent of egg size (binomial GLMM: $\beta = 0.16 \pm 0.23$, $\chi^2_1 = 0.49$, $P = 0.49$). We therefore believe that there is no inherent relationship between hatching success and egg size. This same conclusion was drawn from a cross-fostering experiment in the ecologically similar Northern Lapwing *Vanellus vanellus* (Blomqvist *et al.* 1997).

Egg size did not correlate with chick survival. This corresponds to the lack of support for this relationship in reviews by Williams (1994, 2005, 2012), but contrasts with the more formal meta-analysis done by Krist (2011); in the latter, egg size was positively related to most studied offspring traits and the survival of the chicks. One possibility for the lack of an effect of egg size on chick survival in our study is that chick survival is presently so low for Godwits that the original benefit of larger eggs can no longer be observed (Loonstra *et al.* 2019).

Lay date did influence both nest and chick survival: earlier clutches had a higher chance of hatching and fledging a chick. Previous studies have frequently connected this decline in hatching success with the seasonality of agricultural activities and predation pressure (Kleijn *et al.* 2010, Schroeder *et al.* 2012, Kentie *et al.* 2015). The seasonal decrease in fledging success was also observed by Kentie *et al.* (2018) and might be related to the seasonal decrease in body condition of chicks (Loonstra *et al.* 2018). In any case, the mechanisms underlying these survival patterns remain poorly understood.

Conclusion

Even though Godwits have precocial chicks and an invariant clutch size, our findings are strikingly similar to results previously published on other avian species (reviewed in Christians 2002). This is true of the amount of variation in egg size, the degree of repeatability and our inability to explain more than a small fraction of the observed variation with either individual or environmental factors. The high individual repeatability of egg size across all of these studies indicates that most birds do not adjust their total parental effort to exogenous or endogenous conditions by varying their egg size. Both nest and chick survival are negatively correlated with lay date (Kentie *et al.* 2018, this study) and the differences in lay dates within and among females might therefore be a better indicator of female condition.

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