**Female scramble competition, not senescence, shapes egg size variation in a long-lived polyandrous shorebird**

*Abstract (X words):*

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*Main text:*

INTRODUCTION (X words)

A key theory for the evolution of reduced male gamete size is sexual selection under male-male competition: sperm quality is traded off with sperm quantity to maximize fertilization success. Likewise, enlarged female gamete size is the evolutionary response to disruptive selection under reduced male gamete quality. The divergent gamete sizes of males and females (anisogamy) is a fundamental principle in sex role evolution, however, the effect of female-female competition on egg size variation in sex role reversed species remains unclear. Comparative analysis of birds has shown that a lineage’s egg size tends to decrease following the evolution of polyandry – supposedly due to the selective advantages that laying smaller eggs has on minimizing remating time and, hence, maximizing a female’s reproductive out given temporal constraints of her breeding schedule.

A female’s breeding schedule depends in part on her local resource availability to commence a breeding attempt – resources that include vacant space to conduct reproductive activities (e.g., courting, nesting, etc.), obtainable food to launch the physiological investments of egg production, and accessible males to seek copulations from and form pair bonds. In sequentially polyandrous species, females scramble competitively over these forms of breeding resources to maximize their opportunity for multiple breeding attempts, often resulting in high inter-female variation in reproductive success and breeding schedule. Females able to secure early nesting sites have more time to renest following failure of the first attempt and perhaps even enough time to pursue a sequential attempt. However, breeding early may come with the cost of physiological stress or increased likelihood of failure due to the higher environmental stochasticity associated with the early season, such as low food availability, inclement weather, or frequency-dependent predation risk. Taken together, a seasonal trade-off between quality and quantity exists for females: early nesting females may compromise the quality of an early investment to maximize time for prospective future investments.

In many organisms, reproductive productivity is age-dependent (Bouwhuis et al., 2009; Hammers et al., 2012; Lemaître et al., 2015; Zhang et al., 2015; Salguero-Gómez et al., 2016; Jankowiak et al., 2018; Dingemanse et al., 2020): an individual’s performance increases over early life to a maximum and is followed by a decline in older ages. The increase in breeding performance in early life is thought to be an indication of physiological or competitive inferiority of inexperienced breeders compared to their older conspecifics {Curio:1983fy}, whereas the decline in reproductive performance at old age is considered a sign of senescence – a within-individual age-specific decline in reproductive performance paired with an increase in the probability of death caused by the loss of physiological and cellular function (Medawar, 1952; Williams, 1957). A prominent hypothesis explaining the occurrence of senescence is the disposable soma theory (*sensu* Kirkwood, 1977), which states that individuals are constrained by the amount of resources they have to invest in survival and reproduction (Kirkwood & Austad, 2000). Therefore, senescence is the consequence of age-dependent trade-offs between energy investments in reproduction at the expense of somatic repair (Kirkwood & Rose, 1991; Kirkwood & Austad, 2000; Drenos & Kirkwood, 2005). Thus, an individual’s investment in current reproduction may exacerbate physiological and cellular damage, which might have knock-on effects on their capability for future reproductive investments at older ages – ultimately impairing performance.

A key issue for studying senescence in wild populations is that stochastic extrinsic mortality reduces the frequency of observable individuals in older age classes, hence making it challenging to disentangle among- vs. within-individual age-dependent variation – a phenomenon known as “selective disappearance” (Nussey et al., 2008; van de Pol & Wright, 2009). Investigations using longitudinal data to test for senescence are particularly powerful as they can quantitatively control for the confounding effects of selective disappearance through repeated measures of individuals as they age (Nussey et al., 2008; van de Pol & Wright, 2009; Dingemanse et al., 2020).

In oviparous organisms, egg size represents a fundamental measure of female reproductive investment (Kaplan, 1980; Fox, 1994; Williams, 1994, 2012; Starck & Ricklefs, 1998; Moran & Emlet, 2001; Xu et al., 2019) and is shown to be related to inter- and intra-specific variation in several life history traits. For example, egg size is associated with developmental mode with precocial species typically producing larger eggs than altricial species (Deeming & Reynolds, 2015). Within individuals, variation in egg size is shown to be highly repeatable (Christians, 2002), and age-dependent changes in egg or clutch size are often consistent with senescence although complicated by considerable among-individual variation (Beamonte‐Barrientos et al., 2010; Dingemanse et al., 2020).

Among oviparous animals, shorebirds (part of the order Charadriiformes) produce some of the largest eggs in relation to body mass due to the needs of their precocial nidifugous young (Lack, 1968; Rahn et al., 1975). As a clade, shorebirds also exhibit a disproportionately high prevalence of polyandry (Oring, 1986; Colwell, 2010). Sexual and natural selection presumably act divergently on egg size with natural selection on offspring viability favouring larger eggs with more nutrients to enhance offspring survival (Blomqvist et al., 1997; Starck & Ricklefs, 1998; Williams, 2012) whereas sexual selection on polyandry favours smaller eggs that can be produced prior to peak seasonal food availability in order to maximize multiple nesting attempts (Liker et al., 2001; Andersson, 2004).

Here, we investigate seasonal- and age-dependent egg size variation using a 15-year longitudinal mark-recapture dataset of snowy plovers (*Charadrius nivosus*) breeding at Bahía de Ceuta – a wild sub-tropical population in western Mexico. The snowy plover is a long-lived shorebird (Colwell et al., 2017) exhibiting a rare breeding behaviour characterized by sex-role reversal including facultative sequential polyandry (Eberhart-Phillips et al., 2017; Kupán et al., 2019). The prominent variation of reproductive investment within the same sex provides a highly suitable study system to investigate how scramble competition shapes female reproductive output and scheduling in a species with non-conventional sex roles. Our aims were to 1) examine whether age-related dynamics in egg size and breeding schedule follow a pattern typical for senescence and 2) assess the seasonal relationship between egg size and polyandry potential in regard to reproductive trade-offs associated with female-female scramble competition. Following others (i.e., Bouwhuis et al., 2009 and Jankowiak et al., 2008), we hypothesized that a pattern of early-life increase in egg size and advance in lay date, followed by a peak maximum, and a decline thereafter, would be indicative of age-related changes associated with senescence. Furthermore, we hypothesized that the likelihood of polyandry and egg size would be inversely associated with lay date: early breeding females would have the highest rates of seasonal polyandry and would also lay the smallest eggs.

MATERIALS AND METHODS (X words)

*Data collection*

We studied the reproductive effort and breeding schedules of female snowy plovers (Figure 1a) at Bahía de Ceuta – an important breeding site located on the coast of Sinaloa, northwest Mexico (23o54’N, 106o57’W). Details on the study site and population are provided elsewhere (e.g., Cruz-López et al., 2017; Eberhart-Phillips et al., 2020a). In brief, we annually monitored breeding birds from mid-April until early July, and collected mark-recapture data following the methods described in Székely et al. (2008). We exhaustively searched for nests using telescopes and mobile hides to minimize disturbance. Upon finding a nest, we measured each eggs’ length and width to the nearest tenth of a mm to determine egg size (Figures 1b and 1c). Using these egg dimensions, we calculated egg volume (Figure 1d) following Hoyt (1979) as:

Eq. 1 ,

where *K* is 0.486, a volume-index constant for snowy plovers determined by Székely et al. (1994) through the use of an egg volumeter (Hanson, 1954). The modal clutch size of snowy plovers is three (87%) and is the maximum number of eggs we have observed in this population (Eberhart-Phillips et al., 2020b). We regularly checked incomplete nests until the clutch was completed and assigned the age of these nests according to the lay date of the last egg laid (Plaschke et al., 2019). If the clutch was complete upon discovery and had not been incubated longer than 10 days, we determined its lay date by floating the egg and estimating the stage of embryonic development {Nosaly:1993uz}. For successful clutches found after 10 days we assumed an incubation period of 25 days and back-calculated the laying date based on the hatching date (Plaschke et al. 2019). In the rare case that the nest did not hatch and we discovered it after day 10 of embryonic development, we simply used the nest’s found date as a crude approximation for lay date.

We identified nesting adults that had been previously marked based on their unique colour ring combination. We captured unmarked adults on their nests during incubation using funnel traps and assigned a unique colour combination for subsequent recognition (Hall & Cavitt, 2012). Because snowy plovers have circadian sex roles during incubation (Vincze et al., 2017), we generally targeted females for captures during the day and males during the night. In the rare circumstance when we were unable to identify parents before hatching, we attempted capturing parents whilst they tended chicks. As snowy plovers only show a small degree of sexual dimorphism (Küpper et al., 2009), we determined sex of all captured plovers in the field through a combination of plumage characteristics (Argüelles-Ticó et al., 2016), time of capture, and other behavioural cues (e.g., sex-specific brood care; Kupán et al., 2019). Furthermore, we confirmed sex molecularly from DNA extracted from blood samples through PCR amplification of Z and W specific DNA regions with two sex-typing markers: P2/P8 and Calex-31 (Griffiths et al., 1998; Küpper et al., 2007; Dos Remedios et al., 2010).

We visited known active nests every four or five days to determine the status of the nest (e.g., active, abandoned, predated) until the 20th day and thereafter daily until the eggs hatched or the nest failed. We weighed chicks as soon as possible after hatching and marked them with an alphanumeric metal and a single colour ring for subsequent identification in the chance that these individuals recruited into the breeding population as adults in future years.

For the years 2006 to 2016 all longitudinal data collected has been compiled as part of the *CeutaOPEN* project – an open-access database for individual-based field studies in evolutionary ecology and conservation biology (Eberhart-Phillips et al., 2020a). We accessed these data directly from the open source repository (Eberhart-Phillips et al., 2020b) and supplemented them with data from four additional field seasons: 2017, 2018, 2019, and 2020. The *CeutaOPEN* database is composed of five tables that correspond to our routine data collection in the field. Here we used the “Captures”, “Resights”, and “Nests” tables. The “Captures” and “Resights” tables contain information about all the individuals captured and observed, whereas the “Nests” table contains the morphometric and spatiotemporal information related to each nest monitored. Please refer to our RMarkdown vignette that connects to *CeutaOPEN* and reproduces all analytical methods and results discussed below (Supplementary File 1).

*Statistical Analyses*

*Age estimation of individuals with unknown origin*—Investigating age-dependent processes of marked populations in the wild is challenging as they are often composed of a mix of individuals that are of known or unknown age (Colchero et al., 2012) – with the former being individuals initially marked at birth (i.e., ‘uncensored’), and the latter being immigrants of unknown age or those that were born before the study’s first marking occasion (i.e., ‘left-truncated’). To estimate the ages of unknown individuals in our marked population we employed a capture-mark-recapture analysis using the ‘Bayesian Survival Trajectory Analysis’ (BaSTA) package in R (v1.9.4, Colchero et al., 2012), which uses a Bayesian hierarchical framework to fit parametric survival functions of the marked population while accounting for imperfect detection. BaSTA derives birth year estimates of left-truncated individuals from the population mean of the projected survival function. As snowy plovers show prominent sex differences in survival (Eberhart-Phillips et al., 2017, 2018), we estimated female-specific survival functions for this study. Due to high natal dispersal we could not confidently determine the fate of juveniles marked in our population. To acknowledge this uncertainty, our capture-mark-recapture sample only included individuals that survived to their first breeding season, i.e. we constrained first-year survival probability to 1.

In total, our capture-mark-recapture data comprised of 450 uniquely marked females, of which 45 hatched locally and subsequently recruited into the adult population as known-age individuals (Fig. 1a), and the remaining 405 individuals were adults of unknown age and origin. Over the 14-year study period we monitored the presence or absence of marked individuals annually by recapturing or observing them in the field, amounting to a total of 916 post-birth detections of the 450 females in our sample (median detections per adult = 2; mean = 2.04 *±* 1.45 SD). In short, BaSTA determined that a logistic bathtub-shaped mortality model best fit our data – revealing that female mortality rate increased until age 5 years, after which it became constant (Fig. 1a; see Appendix S1 for detailed methods). Using this model, we extracted the birth year estimate posteriors for each unknown-age individual in the capture-mark-recapture sample. Note that three individuals (one first encountered as an adult [CA1579] and two local recruits [CA2036 and CA1526]; Fig. 2) had been already marked two years prior to the start of our monitoring period (i.e., pre-2006) and were thus added to our sample after running BaSTA on the 2006–2020 capture-mark-recapture data.

*Modelling seasonal variation in polyandry potential (“Polyandry model”)*—Our sample for studying seasonal polyandry dynamics included 425 females for which the identity of their mates had been verified through observation of unique leg-ring colour combinations. We defined observed polyandry as a binomial variable that scored an individual as being monogamous or polyandrous each year based on our observations of them having one or multiple breeding partners, respectively (see Fig. 2 for an example of the sampling distribution). Following this, our sample included a mixture of females that were observed breeding once or multiply within the season – by definition, all polyandrous cases bred at least twice, but also 12.4% of monogamous cases were observed breeding multiply (i.e., remained with the same partner between breeding attempts within the same season). To assess the relationship between the likelihood of polyandry and lay date, we fitted a binomial linear mixed effects model that tested the likelihood of polyandry predicted by the fixed effect of lay date (i.e., of an individual’s first nest of the season), with individual and year included as random effects.

*Modelling individual variation in egg volume (“Egg volume model”)*—Our sample for studying egg volume dynamics included 2391 eggs from 840 nests of 425 females. 34 (13.2%) females had three or more years of repeated measures, 83 (19.5%) had two years of repeated measures, and 286 (67.3%) were measured in a single year. Furthermore, 43 (10.1%) individuals in our sample were marked as hatchlings but later recruited as breeding adults in subsequent years (i.e., known age; Fig. 2a), with the remaining 382 (89.9%) individuals being initially marked as adults (i.e., unknown age; Fig. 2b). We followed common statistical approaches to investigate senescence in birds (e.g., Bouwhuis et al., 2009, 2010; Schroeder et al., 2012; Herborn et al., 2016; Graham et al., 2019; Dingemanse et al., 2020) by fitting a quadratic function of age to model age-specific trends in egg volume. This model controlled for selective appearance and disappearance of females differing in average egg volume by fitting ‘first observed age’ and ‘last observed age’ as fixed effects – a method that estimates between-individual age effects introduced by selective disappearance and appearance (van de Pol & Verhulst, 2006; Dingemanse et al., 2020). We modelled within-individual age effects on egg volume by fitting a univariate mixed-effect model, that included linear and quadratic forms of a within-group deviation score for age (henceforth ‘age-deviance’), calculated for individual *i* at age *j* as: *ageij* – (*first observed age*)*I* ({Snijders:2011wt, vandePol:2015eh}). Tarsus length was also included as a fixed effect to control for female structural size, and was averaged over an individual’s lifetime of measurements (i.e., our *a priori* expectation was that tarsus length is static over life and that any variation in this trait was due to measurement error) – grand average 24.5 mm (±0.96 SD), grand average within-individual standard deviation 0.66 mm (±1.14 SD). In addition to these fixed covariates, we included a quadratic function of lay date to assess seasonal variation in egg volume as several shorebird studies report a seasonal increases ({Skrade:2013bu, Kwon:2018hl} and decreases (Dittmann & Hötker, 2001; Skrade & Dinsmore, 2013; Kwon et al., 2018; Kubelka et al., 2020; Verhoeven et al., 2020) in egg volume. We included random intercepts for nest, individual, and year, and assumed a Gaussian error distribution of egg volume.

We verified the relationship between egg volume and chick weight using the egg dataset described above but reduced observations to the nest level and filtered to only include nests that had chicks measured within one day of hatching, resulting in 456 nests from 276 females. As it was unclear which chick came from which egg, each datum represented the nest-level average of chick weights and egg volumes. We included random intercepts for mother identity and year, and assumed a Gaussian error distribution of egg volume.

*Modelling individual variation in lay dat*e *(“Laydate Model”)*—Our sample for studying lay date dynamics used the same nest-level sample as the model of polyandry potential above, however, as we were interested in how the recruitment status of an individual influenced breeding phenology, we excluded 2006 as this was the first year of our study when all birds were first individually marked – resulting in 567 nests from 375 females. Modelling the age effects of first nest lay date followed the same logic as the above egg model, with a univariate mixed-effect structure that included age-deviance, age-deviance-squared, first observed age, last observed age, and average tarsus length as fixed covariates, and individual and year as random intercepts. Furthermore, recruitment status was also fitted as a two-level fixed effect describing if a breeding female hatched locally (“local recruit”) or was first encountered as an adult (“immigrant”). We visualized the distribution of lay dates to confirm normality and to assess the population-level variance in breeding schedule – an indication of inter-female breeding asynchrony and the intensity of scramble competition {Andersson:2004bz}.

*Evaluating effect sizes and uncertainty*—We employed the “lme4” (Bates et al., 2015), “rptR” {Stoffel:2017dp} and “partR2” {Stoffel:2020fn} packages in R (R Core Team, 2013) to conduct our statistical modelling and assessed homoscedasticity by visually examining the residuals (see Fig. S3). For each of the three mixed-effect models above, we evaluated uncertainty in our parameter estimates by simulating 1000 parametric bootstraps via the “partR2::partR2” function {Stoffel:2020fn}. Likewise we derived nest-, individual-, and year-level repeatabilities (i.e., intra-class correlation) by simulating 1000 parametric bootstraps of the three mixed-effect models using “rptR::rpt”. We report fixed effects as standardized regression coefficients (i.e., beta weights) and repeatability as the ‘adjusted repeatability’ – interpreted as the repeatability of a given hierarchical group after controlling for fixed effects {Nakagawa:2010ta}. For the “Egg Model” and “Date Model” we ran an additional simulation that acknowledged uncertainty in the BaSTA age estimate of a given individual: we bootstrapped each model 1000 times, with every iteration randomly drawing an age estimate for an individual from their posterior distribution provided by BaSTA. For both simulations, we made interpretations of the influence of age estimate uncertainty by examining the effect size distribution of the 1000 bootstraps in relation to the 95% confidence interval for effect sizes of the original model. To ensure that intercepts of our age-dependent models represented the reproductive performance for the earliest age at reproduction (i.e., age 1 in snowy plovers, Page et al., 2009), we fitted age as ‘*age* – 1’ – otherwise it would represent reproduction as age 0, which is an empirically meaningless estimate. Despite the recommendation of {Schielzeth:2009da} to allow individuals to differ in the slopes of their responses, a random slope form of the model (i.e., incorporate a quadratic function of age-deviance as a random slope within individuals) failed to converge due to limitations of individual-based repeated measures – as such we present random intercept models.

RESULTS(XX words)

We collected measurements from 2391 eggs, originating from 840 clutches of 425 females over a 14-year period. Modal clutch size was 3 eggs (724 nests, 86%; 2-eggs: 103 nests, 12.3%, 1-egg: 13 nests, 1.5%). Average egg length was 3.09 cm (±0.10 cm SD, Fig. 3a) and width was 2.24 cm (±0.05 cm SD, Fig. 3b), which translated into an average egg volume of 7.58 cm3 (± 0.46 cm3 SD). The average egg volume of a clutch strongly predicted the average hatch weight of the subsequent brood (*β* [95% CIs]: 0.628 [0.552–0.704]; R2*marginal* = 0.370 [0.310–0.436]; Fig. 3c, Table SX). Based on BaSTA’s estimated birth year, 184 of the 382 unknown-age females in our sample were first observed nesting at age 1 (48.1%), 120 at age two (31.4%), 72 at age three (18.8%; Fig. 2), five at age 4 (1.3%), and one at age 5 (0.3%). Of the 42 locally hatched females in our sample, 29 first nested at age one (67.4%), six were first observed nesting at age two (14.0%), two at age 3 (4.7%), three at age 4 (7.0%), three at ages 5, 7, and 8, respectively (6.9%; Fig. 2). The average tenure of all females in the sample was 1.57 years (± 2.16 SD) with an average age span of 3.12 years (±2.03 SD, median: 3, range: 1 to 14 years) and an average of 1.56 years of observed ages per female (±1.04 SD, median: 1, range: 1 to 8 age-specific observations; Fig. 2). Females in our sample were typically observed nesting every consecutive year since their first observation, however, some individuals skipped years (average yearly interval between nesting attempts = 1.07 ±0.29 SD).

*Seasonal variation in polyandry potential*

A female’s likelihood of being polyandrous was strongly dependent on the lay date of their first nest (*β* [95% CIs]: -2.24 [-3.09, -1.77]; R2*marginal* = 0.367 [0.244, 0.493]; Fig. 4c, Table S2, Fig. S2). On average, females made 1.43 (±0.56 SD) nesting attempts per season (median = 1, range 1 to 3) and, as expected, polyandrous females laid more nests per year than their monogamous conspecifics (Fig. S5). The lay date distribution of polyandrous females was bimodal, with peaks in the first and second nests occurring 11.7 days before and 29.2 days after the unimodal seasonal peak for monogamous females (Fig. 4b). Females had low repeatability in polyandry among years (adjusted individual cross-year repeatability (*r*) [95% CIs] = 0.011 [0, 127]; Table S2, Fig. S2). In at least one breeding season, 76 (17.9%) females were polyandrous and 127 (30.0%) females laid multiple clutches throughout the observation period.

*Individual variation in egg volume*

Overall, mixed effects accounted for 71.4% of variation in egg volume, with fixed effects explaining 6% of this variation (Table S3). Females were highly repeatable in their egg volumes between clutches: *r* = 0.50 ([0.44, 0.56] 95%CI; Fig. 6, Table S3). Furthermore, eggs within the same clutch were moderately repeatable in volume (*r* = 0.16 [0.12, 0.20]; Fig. 6, Table S3). We detected no evidence for senescence in egg volume (*βage* [95% CIs]: 0.08 [-0.05, 0.21], *βage2*: -0.11 [-0.21, 0.00]; semi-partial R2 of quadratic senescence function = 0.005 [0, 0.05]; Fig. 6, Table S3). Furthermore, we found no support for selective (dis)appearance of individuals according to egg volume, as the effects of first and last observed ages of reproduction were not strongly supported (Fig. 6, Table S3). These results remained consistent when we ran a bootstrap that incorporated the individual birth-year posteriors estimated from BaSTA (Fig. S5). The strongest fixed effect explaining egg volume variation was the between-individual quadratic season function: eggs were smallest at the start of the season (model prediction: 6.95 *cm*3 [6.78, 7.13] 95%CI, Fig. 4c) and largest shortly after the middle of the season (model prediction: 7.65 *cm*3 [7.58, 7.72] 95%CI, Fig. 4c). Average egg volume also increased between sequential clutches within individuals but with smaller magnitude than the population-level trend (*βwithin*: -0.11 [-0.21, 0.00]). As expected, females with larger tarsi laid larger eggs (*βtarsus* [95% CI]: 0.14 [0.07, 0.21]; semi-partial R2 of female tarsus = 0.02 [0, 0.06]; Fig. 6, Figure S4).

*Individual variation in lay date*

Females had low repeatability in the lay date of their first nest among years (*r* = 0.07 [0, 0.18] 95%CI; Fig. 7, Table S4). We found moderate support for the linear term of the age function predicting the lay date of a female’s first nest in the season: young individuals laid later nests compared to their older conspecifics with lay date advancing by 2.98 days per year of age (95% CI: [0.62, 5.24]), however our uncertainty in this trend became unwieldly in the oldest age classes of our sample (Fig. 5a). The strongest effects explaining first nest lay date were the age at first reproduction (i.e., ‘first age’) and female structural size (i.e., ‘tarsus’ length): individuals that first bred in the population at older age classes tended to start nesting later in the season and smaller-bodied females initiated the earliest nests.

DISCUSSION(X words)

Identifying trade-offs between reproductive effort and survival in wild organisms is central for understanding of the evolutionary mechanisms of senescence (Lemaître et al., 2015). Here we show that egg size variation in snowy plovers is not a senescent trait – but is rather a seasonally dynamic trait driven by female-female scramble competition to breed early and increase polyandry potential. Consistent with previous work (Christians, 2002), we found that egg size was highly repeatable for individual females, even after controlling for their structural size. The distribution of lay dates in this snowy plover population extended over a *ca*. 110-day period, indicating high phenological asynchrony within the breeding population, a pre-cursor for intra-sexual competition ({Andersson:2004bz}). Early nesting females had a much higher likelihood of being sequentially polyandrous than late nesters, likely due to the generous time budget early breeders have for engaging in multiple nesting attempts. At the population-level, early season eggs tended to be smaller than those laid in the latter half of the season and this was mimicked by the within-individual effect: females generally increased egg volume between sequential nesting attempts (albeit the effect size was small). A seasonal increase in egg volume at both between- and within-individual levels indicates that maternal investment during early breeding attempts is likely constrained by poor local food availability, whereas late breeders can take advantage of peak food availability. Taken together, our results reveal a trade-off between current maternal investment and future breeding potential.

Despite being long-lived and investing substantially in reproduction year-after-year, we found no evidence of age-dependent trade-offs in egg size in this snowy plover population. However, older females tended to initiate nesting earlier in the season compared to their younger conspecifics – indicating age-dependent competitive abilities that likely reflect experience and local knowledge. This age-dependent variation in lay date followed a non-linear pattern indicative of senescence in competitive ability: lay date advanced with each year of age until a peak at age 6, however limited sampling in older age classes makes this non-linear trend hard to robustly interpret. Nonetheless, the pre-peak effect is well supported. Moreover, locally recruited females (i.e., hatched locally) bred earlier than immigrant females, further suggesting a competitive advantage for individuals with prior experience at the breeding site. Importantly, polyandry was not repeatable within individuals – likely due to stochastic socio-ecological dynamics, such as local mate availability and breeding success, which are known to influence mating tactics in plovers.

Several studies of oviparous organisms have observed age-dependent variation in egg size, with some studies finding a positive relationship (Cooch et al., 1992; Flint & Sedinger, 1992; Robertson et al., 1994; Warner et al., 2016; Verhoeven et al., 2020) and others observing a negative relationship (Reid, 1988; Potti, 1993; Ito, 1997). However, several of these studies failed to account for selective disappearance (e.g., Cooch et al., 1992; Flint & Sedinger, 1992; Potti, 1993) and thus complicate the interpretation of individual- vs. population-level effects. Recent longitudinal studies document an increase in egg size in early life, followed by peak and then a late-life decline (Bouwhuis et al., 2009; Jankowiak et al., 2018) – the early-life increase in egg size may indicate individual improvement through more efficient foraging abilities, better mate choice, or improved predator avoidance (Forslund & Pärt, 1995). Although we did not find statistical support for a relationship between age and egg volume, we did find evidence that females tended to nest earlier as they aged. We suspect that this early-life delay in lay date is related to the inferior competitive abilities and lack of experience that young females have when faced with the seasonal scramble for early breeding opportunities.

Furthermore, under the polyandrous breeding system of the species, females may be engaging in reproduction before their physiological development is completed. We recorded females first breeding as early as 10 months after hatching (Eberhart-Phillips et al., 2020b) – an age at which females might not be fully mature and consequently unable to invest heavily into reproduction.

Given that we have observed females still breeding at an age of 14 years (Fig. 2), it is remarkable that we find no evidence of senescence. Such within-individual consistency over life becomes relevant when considering the developmental mode of snowy plovers. For plovers with their nidifugous chicks, small egg volume differences can have significant ramifications for chick survival (Starck & Ricklefs, 1998), as chicks are not fed by the parents but rather must forage for themselves immediately after hatching. Moreover, chicks of polyandrous females are typically cared for solely by their father, forcing them to rely more on their intrinsic reserves than the added benefits of biparental care. Consequently, comparatively small differences in egg size could have large knock-on effects for chick survival (Williams, 1994; Starck & Ricklefs, 1998), hence maintaining stable egg volume production over life. Yet, females need to trade-off the survival benefits of increased egg size for their offspring with their own resources required for maintenance and future reproductive investments, particularly when they attempt to maximize their reproductive output through polyandry. In snowy plovers, a completed clutch equals approximately 60% of a female’s body mass (Page et al., 2009): representing a substantial investment. Furthermore, snowy plover females incubate the clutch jointly with their male partner but desert soon after hatching to seek a sequential breeding attempt with a different male (Warriner et al., 1986; Eberhart-Phillips, 2019). Consistent with theoretical assumptions and previous empirical findings, we found early breeding females had a higher likelihood of being polyandrous but with smaller eggs due to a mismatch with local peak resource availability – suggesting a trade-off with between maternal investment and future breeding opportunities.

Most studies conducted on temperate or high latitude breeding shorebirds have found a negative association between time of the season and egg size (Byrkjedal & Kalas, 1985; Sandercock et al., 1999; Kubelka et al., 2020) although in polyandrous red-necked phalaropes (*Phalaropus lobatus*)egg size increased across the breeding season (Kwon et al., 2018). However, most investigations of seasonal egg size dynamics did not disentangle whether the observed changes were due to within- or among-individual effects. For example, laying schedules associated with female quality and/or age could be responsible for much of the observed seasonal variation in egg size. A study including 15 arctic shorebirds suggested that indeed among-individual variation may account for more of the seasonal variation in egg size than within-individual variation (Weiser et al., 2018). We observed a between-individual quadratic effect of time of season on egg size: early and late season clutches had smaller eggs than those nesting at the middle of the season. The within-individual effect complemented the population-level trend, with eggs of sequential nests being larger than those of first clutches.

Our observed seasonal variation in egg size might be explained by a combination of mating strategy and environmental constraints. Polyandrous females generally produced early- and late-season clutches (Fig. 5). This indicates that polyandrous females trade-off egg quality with mating multiply. The seasonal variation could represent a trade-off between a female’s capacity to build up adequate energy reserves for egg volume investment while also attempting to breed early enough in order to allow time for sequential mating opportunities later in the season. At the end of the season, late nesting females are under a tight schedule for producing sequential clutches rapidly before impending high tides and precipitation flood the breeding grounds (Plaschke et al., 2019). Notably, chick survival is especially high for nests laid at the beginning of the season due to the peak resource availability at hatching ~30 days after clutch completion (Cruz-López et al., 2017; Kupán et al., 2019), which would support the notion that females able to compete for early breeding opportunities may avoid the consequences of small eggs on chick condition at hatch.

Past studies have linked polyandry and sex-role reversal to reduced female gamete size (Slotow, 1996; Andersson, 2004). Smaller eggs would permit females to lay several clutches rapidly (Liker et al., 2001). Since Snowy Plover females are sequentially polyandrous (Warriner et al. 1986), early breeding females are more likely to have a second breeding attempt with a different male. Indeed, brood desertion by females early in the breeding season is very often followed by re-mating locally (Kupán et al. 2019). Chick survival also decreases with the season because environmental conditions deteriorate (Cruz-López et al. 2017). Although it is tempting to interpret our results as evidence that early laying polyandrous females adjust their reproductive investment into the first clutch and produce small eggs to enable them to quickly produce a second clutch, we believe there is no selective opportunity for this because polyandry is highly unpredictable and not guaranteed – selection would rather favor females that maximize their current investment due to uncertain future breeding opportunities.

One limitation of our study is that some snowy plover females show high breeding dispersal and can produce sequential nests hundreds of kilometres apart (Stenzel et al., 1994; D’Urban Jackson et al., 2020). As our population is open to immigration and emigration, we have likely missed documenting polyandrous breeding attempts at unmonitored neighbouring nesting sites – meaning that we underestimate the true extent of polyandry. Yet, our observed clutch laying distribution (Fig. 5) suggests that this unavoidable limitation is not a major concern because we would have otherwise expected a larger share of seemingly monogamous females breeding at the beginning and end of the breeding season – instead we see that monogamous breeders tend to nest in the middle of the season. Furthermore, we acknowledge that our relatively small sample of known-age individuals presents a limitation to our study, however this simply reflects the challenge of studying an open population in the wild that exhibits high natal dispersal (jackdaw *Corvus monedula*, *N* = 30, Boonekamp et al., 2014; white-throated sparrow *Zonotrichia albicollis*, *N* = 59, Grunst et al., 2018). Nonetheless, we believe our study provides important insights that are robust to our sample size.

In conclusion, we show that egg size variation in snowy plovers is highly repeatable within individuals and remains stable over life despite a substantial cumulative maternal investment. Rather, egg size variation is driven by seasonal fluctuations in resource availability which, in combination with female-female scramble competition over early nesting opportunities, creates a trade-off between current and future reproductive investments. Our results suggest that senescence is not a major driver of age-dependent dynamics of egg size – a surprising result that is inconsistent with the disposable soma theory. Yet, we do show that the prior experience gives older and local a competitive advantage over younger and naïve conspecifics scrambling for early nesting opportunities. We suggest that future research should explore whether and how polygamy and senescence interact to affect the reproductive output and what consequences these factors have on offspring survival. Studying patterns of senescence in wild populations with flexible mating systems may help shed light on how variation in mating strategies shape individual life history trajectories and lifetime reproductive success.

**FIGURES**

Chart, histogram

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**Figure 1.** Logistic-bathtub mortality function for snowy plover females: a) frequency distribution of age-specific observations of 41 known-aged females and b) age-dependent mortality hazard.

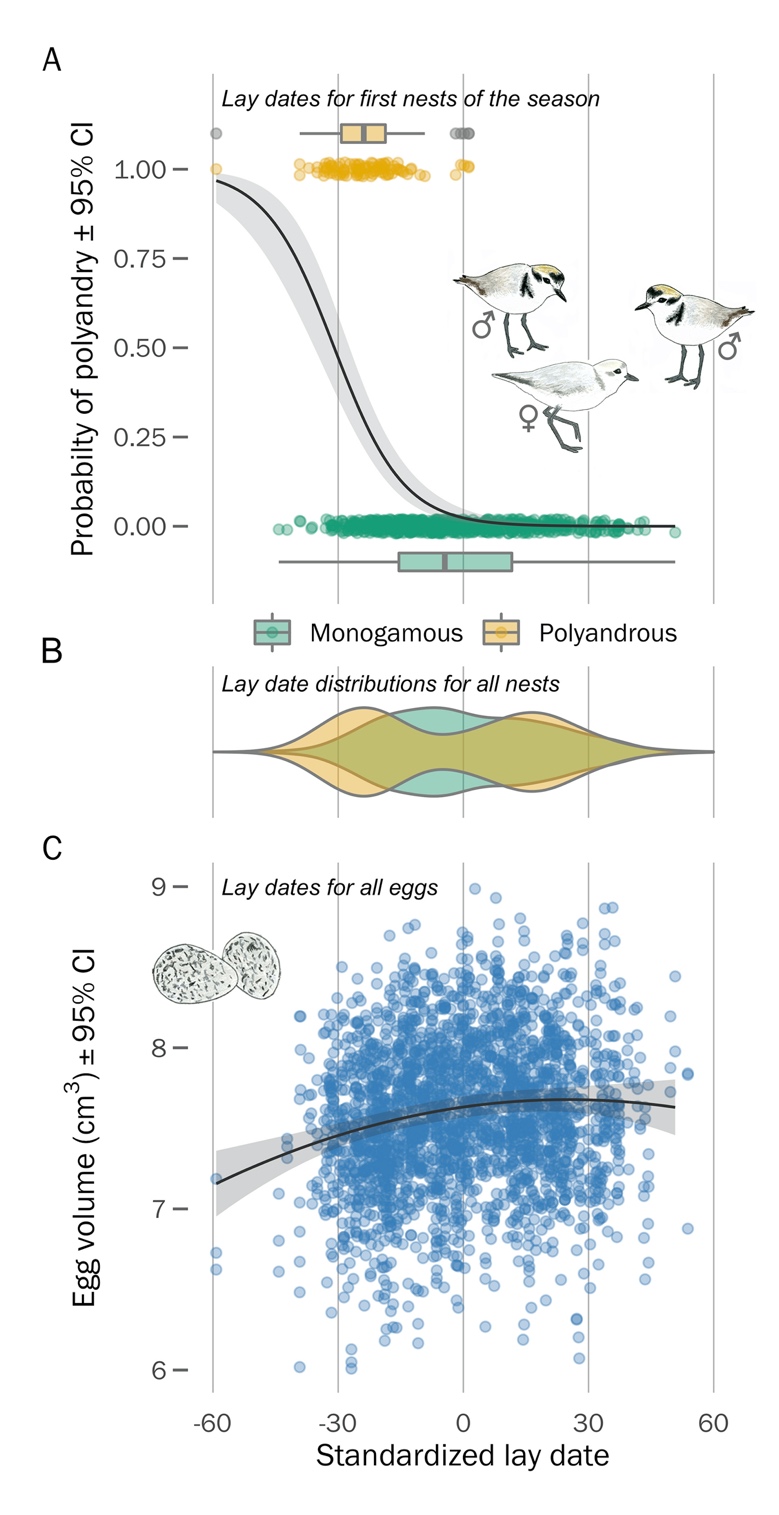
Graphical user interface, table

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**Figure 2 (previous page).** Mating strategy and clutch number of female snowy plovers according to age. Each row shows an individual female in the population for which we have at least three years of observations (note that our analysis also includes females with one or two years of observation, but for visual purposes only individuals with a minimum of three years are plotted). Panel a) shows known-aged females which were born locally, whereas b) shows females that were initially captured as adults and are therefore of unknown age. Points illustrate the age at which we collected observations of egg volume, with the size of the point corresponding to the number of clutches measured at a given age, and the colour indicating if we observed the female mating with one or two distinct males (i.e., in case of multiple clutches at a given age). The light grey buffer around unknown-age females indicates the 95% credible interval of the ages for an individuals’ observed period (i.e., lower limit indicates the minimum age the individual could have entered the population and the upper limit indicates the maximum age of an individual’s last observation). The dark grey lines indicate the period for which an individual was observed alive (i.e., in some cases we encountered an individual in the field and confirmed its survival, but we did not observe its nest to be able to measure the eggs. Note also that the age at first encounter of all known-aged individuals is 0).

Diagram

Description automatically generated**Figure 3.** Egg size variation for snowy plovers breeding in Bahía de Ceuta, Mexico between 2006 and 2020. Egg volume was estimated using an allometric equation combining length (A) and width (B) measurements collected in the field (see *Methods*). C) Average egg volume of a clutch was highly correlated with the clutch’s average chick weight at hatch (*β* [95% CIs]: 0.628 [0.552–0.704]; R2*marginal* = 0.370 [0.310–0.436]). Ribbon shows the 95% CI of the model prediction, vertical and horizontal error bars visualize within clutch variation in egg volume and chick hatch weight (1 SD).

**Figure 4.** Female scramble competition over polyandrous matings and its consequences for seasonal egg volume dynamics. A) Relationship between polyandry potential and lay date of a female’s first nest of the season. Each datum is the lay date of individual’s first nest and their observed local mating behaviour of each year. C) Lay date distributions of all nests for females that were polyandrous (yellow) or monogamous (green). B) Seasonal variation in egg volume – trend shows the between individual polynomial function of the model prediction. Each datum is an egg’s volume (cm3) and lay date. Lay date is standardized for each year.

Chart

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**Figure 5.** Age- and origin-dependent breeding phenology of female snowy plovers. A) Within-individual variation in age-specific nest initiation date – as females gained more experience in the local population, they started nesting earlier, however this trend reversed at older ages. Each datum represents an individual’s ‘age-deviance’ (i.e., a within-group centered measure of the number of years since the individual’s first observed local breeding attempt, see *Methods* for more details) and the lay date of its first nest each year. B) Origin-specific variation in nest initiation date – females that hatched locally and recruited into the breeding population tended to nest earlier than birds originating from elsewhere. Inner-most distributions show the model estimates and 95% CI, outer-most box plots show the inter-quartile ranges of the raw data (point-cloud).

**Figure 6.** Sources of egg volume variation.

Diagram

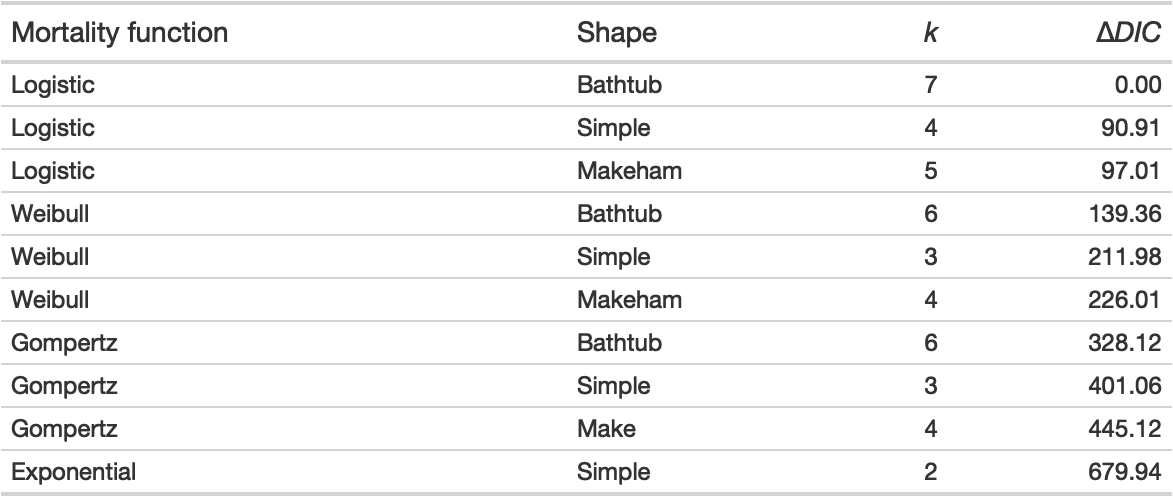
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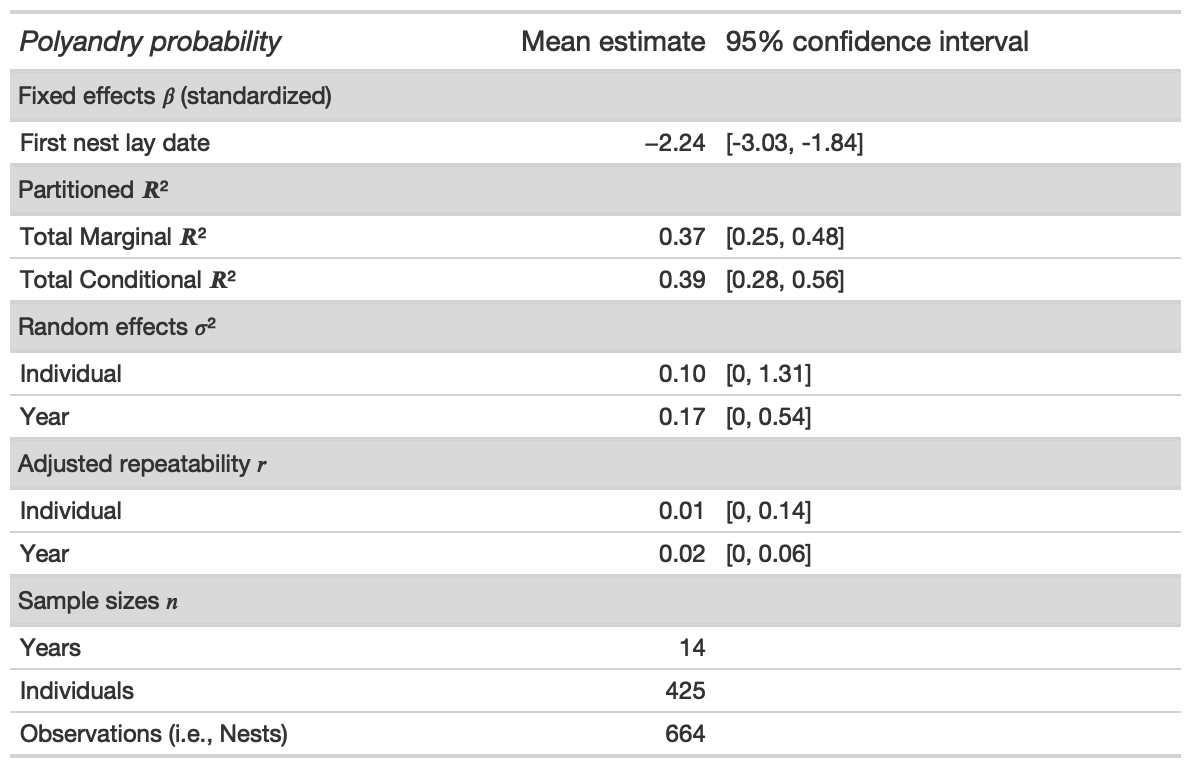
**Figure 7.** Sources of lay date variation.

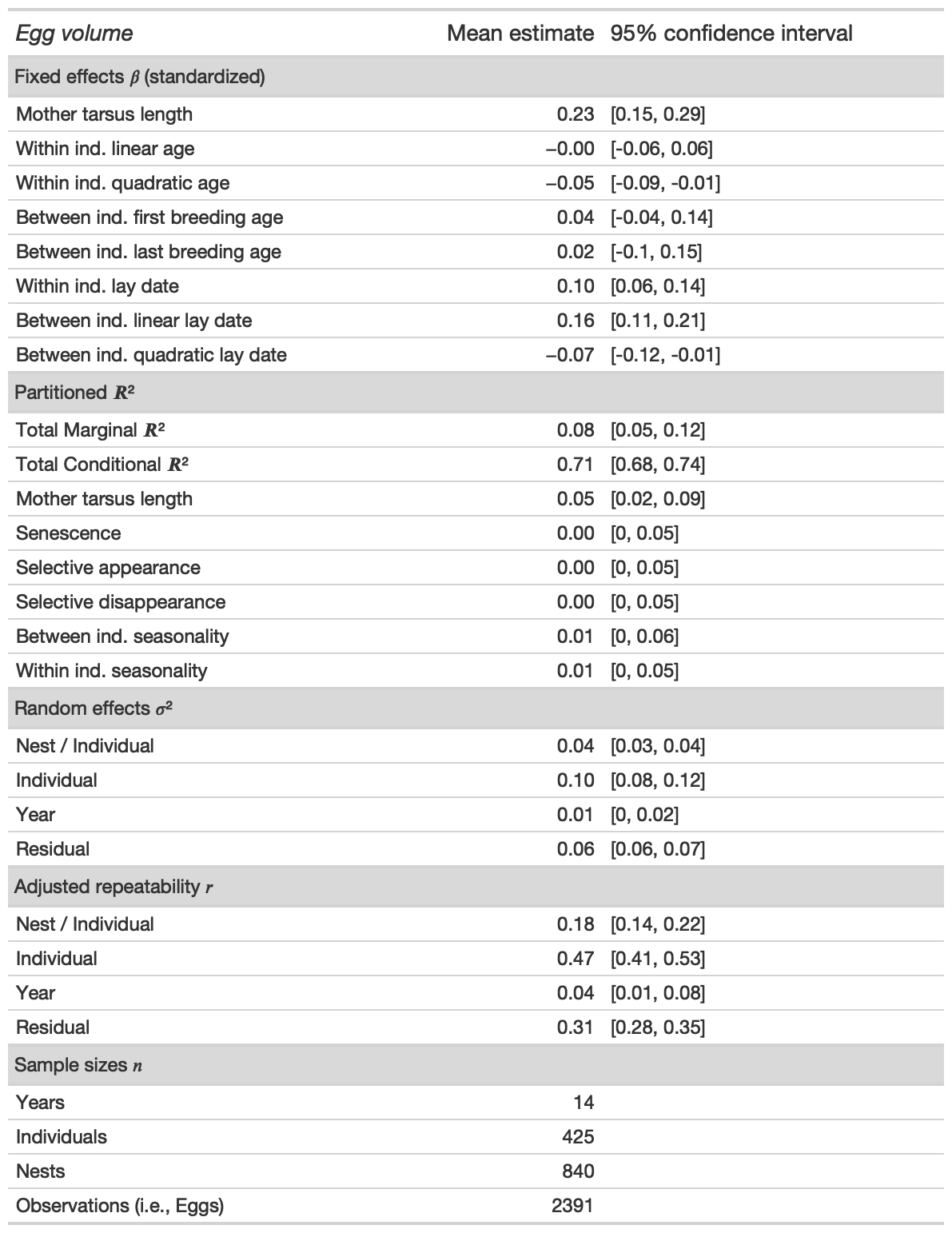
**Diagram

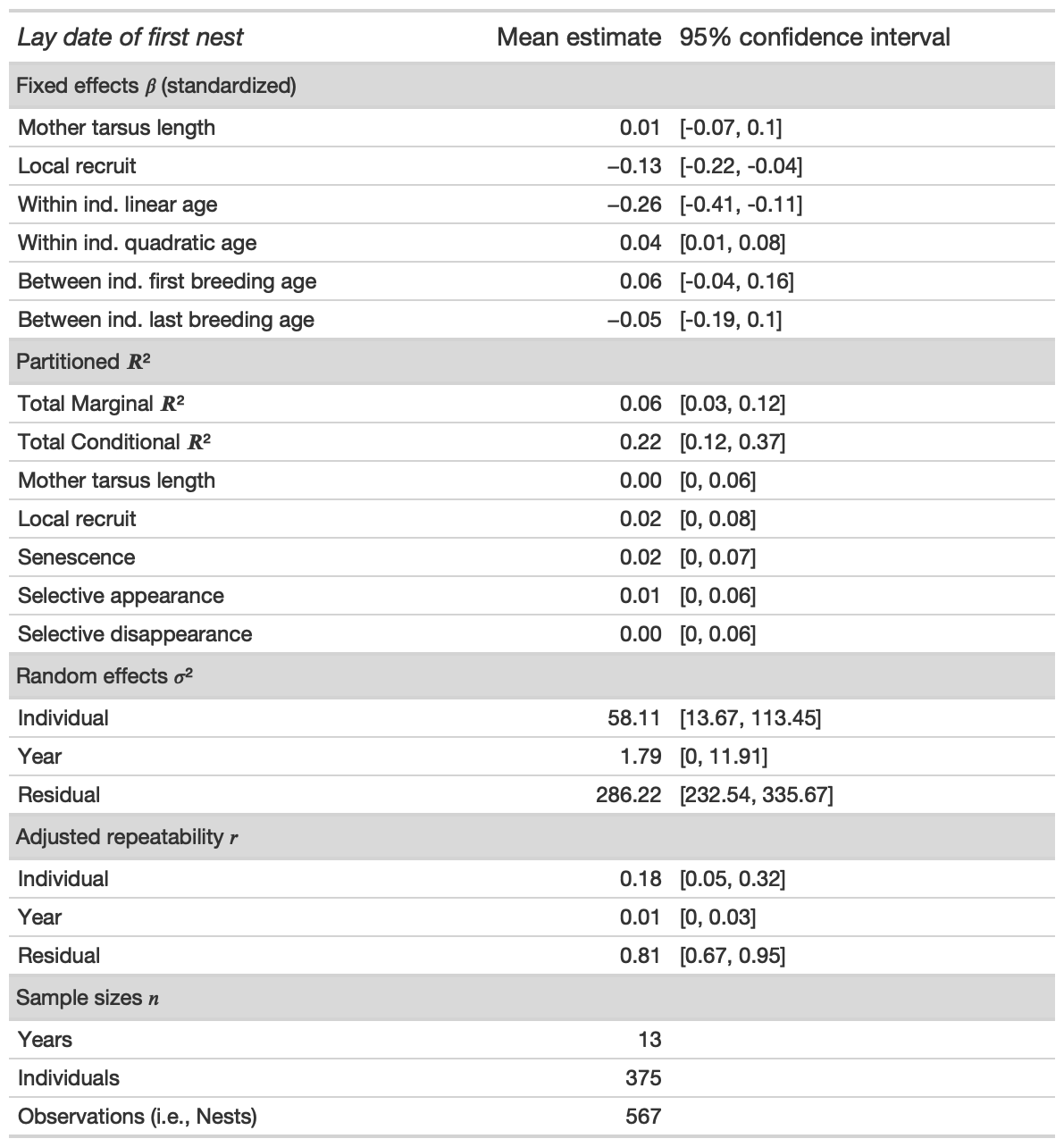
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**SUPPLEMENTARY MATERIAL**

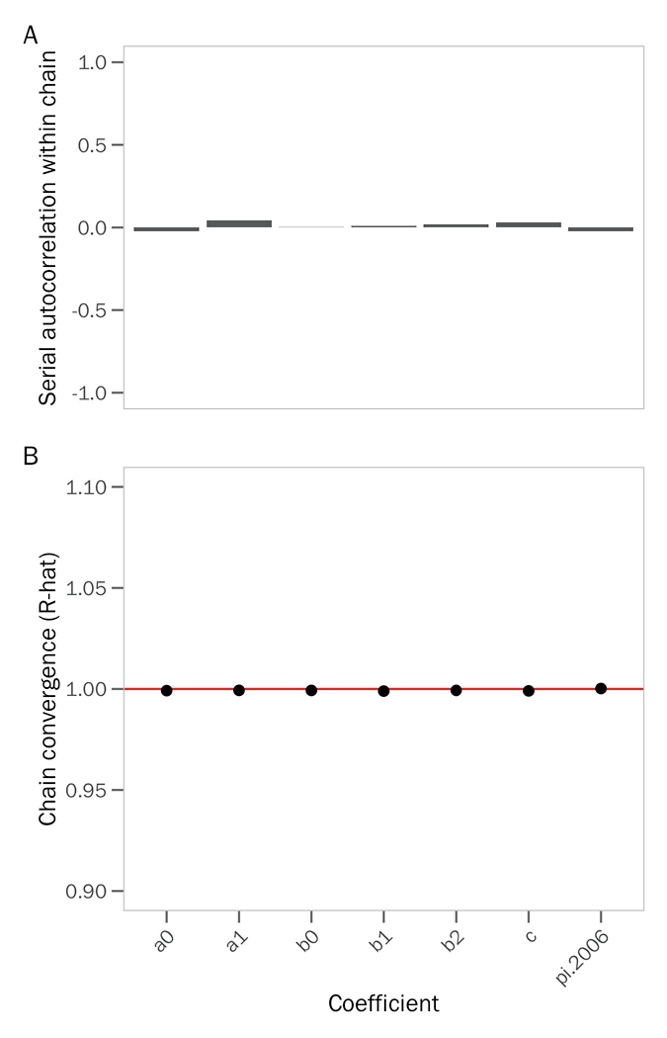
**Table S1.** Hierarchical capture-mark-recapture models used to describe mortality patterns of snowy plovers at Bahía de Ceuta, Mexico, between 2006 and 2020. *k:* number of modelled parameters; Δ*DIC:* difference in deviance information criterion (*DIC*) between a given model and the top model. *DIC* value of top model was 5279.1.

**Table S2.** Relationship between the lay date of an individual’s first nest and their likelihood of polyandry each year. Fixed effect size of ‘first nest lay date’ is the standardized estimate on the logit scale.

**Table S3.** Sources of egg size variation.

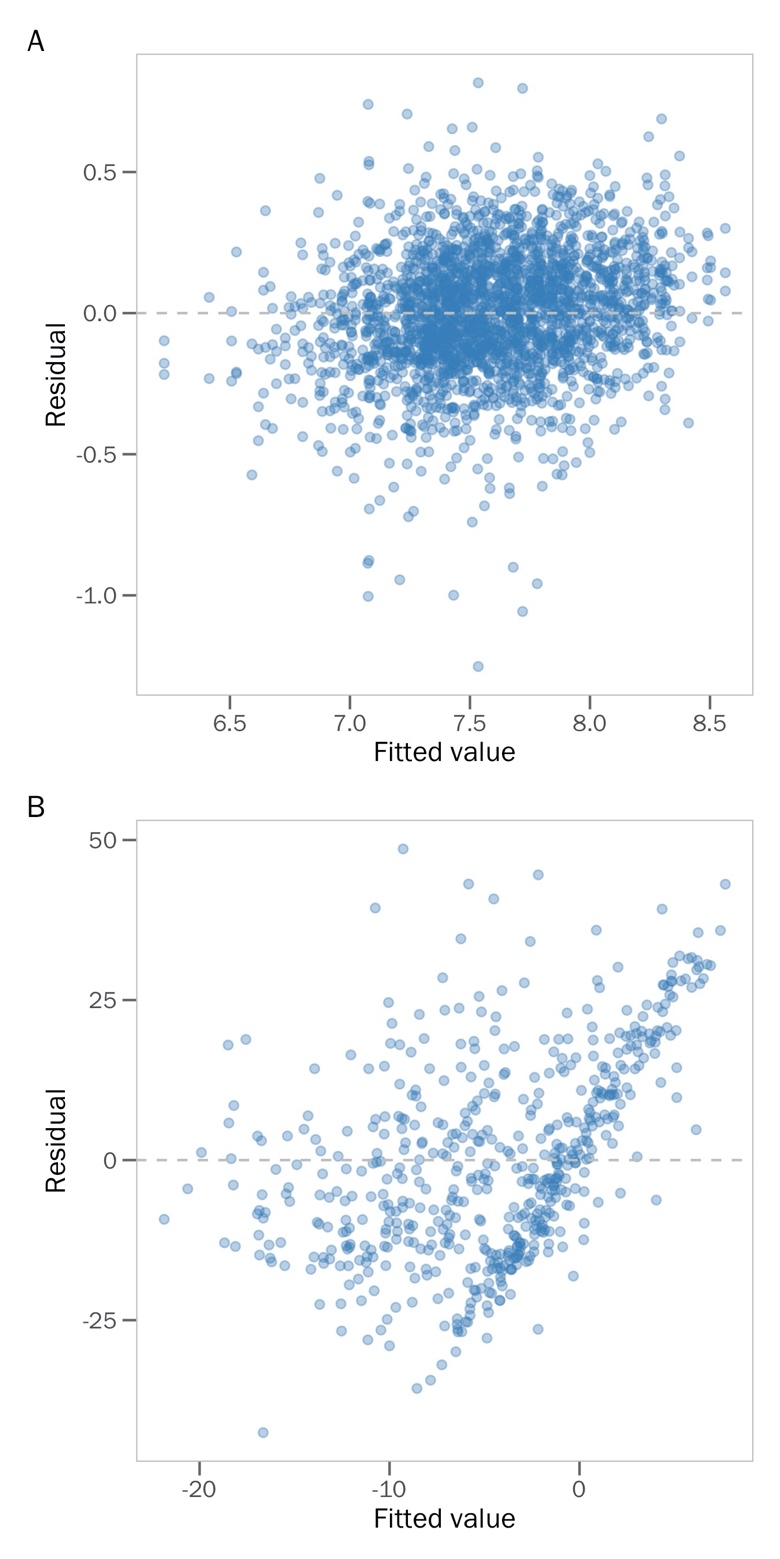
**Table S4.** Sources of lay date variation.

**Figure S1.** Bayesian model diagnostics of the Gompertz bathtub shaped survival model. a) Serial autocorrelation of iterations within chains, b) chain convergence (*R-hat*) of model, and c) trace plots of chain dynamics.



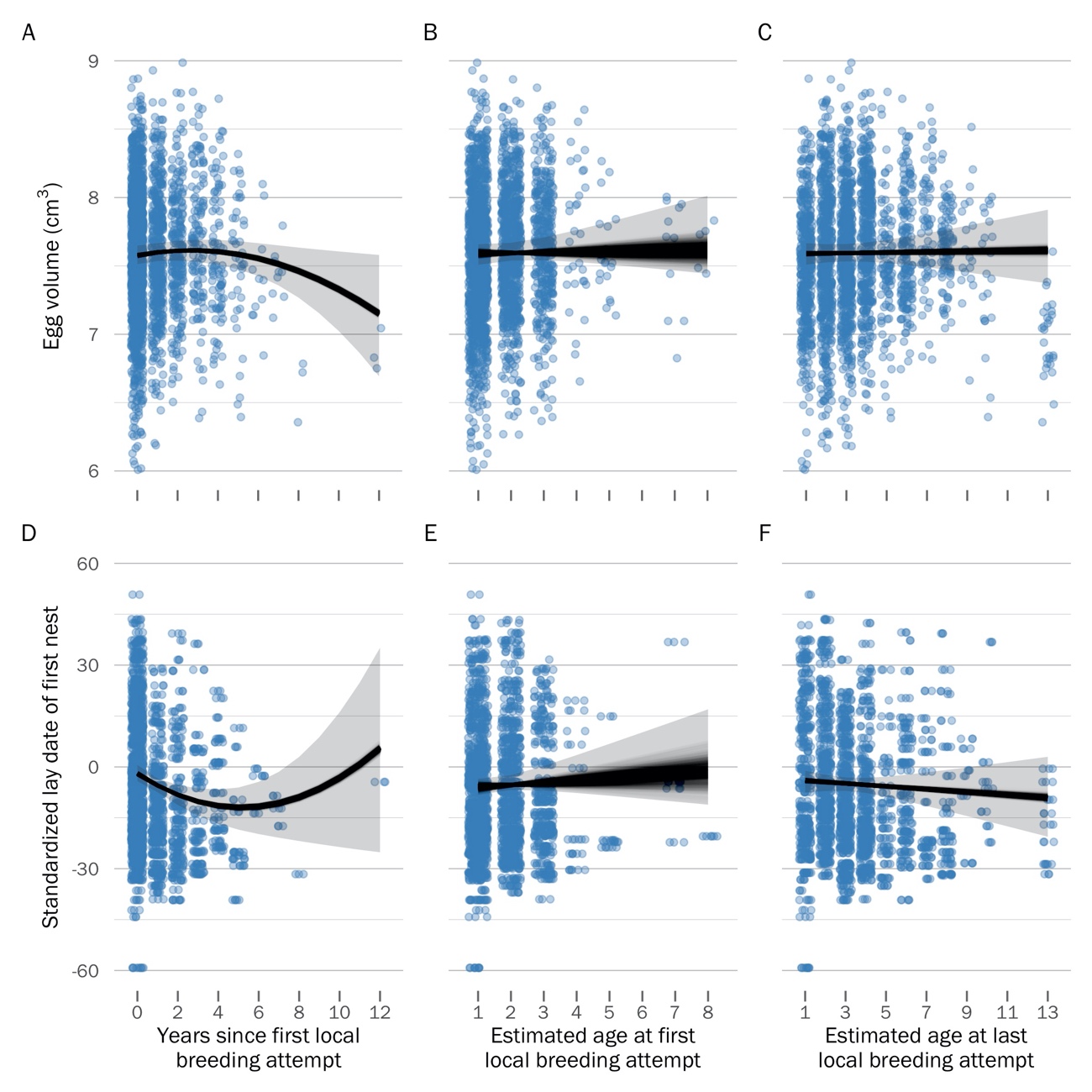
Diagram

Description automatically generated**Figure S2.** Sources of variation in a female’s probability of being polyandrous each year.

**Figure** **S3.** Homoscedastic spread of residuals from the A) ‘egg volume’ and B) ‘lay date’ models.

**Figure S4.** Chart, scatter chart

Description automatically generatedRelationship between a female’s structural size (i.e., her tarsus length) and A) the lay date of her first nest of the season, and B) the volume of her eggs.

**Figure S5.** Visualization of the effect of uncertainty in the age estimate provided by BaSTA on age-dependent trends in egg volume (top row) and lay date (bottom row) dynamics. Black trends show the model predictions for the 1000 bootstraps of the BaSTA age estimate posteriors (see *Methods*).Panels A and D show the within-individual trends of the ‘age-deviation’ score – as expected, these measures are not impacted by uncertainty in the BaSTA age estimate because they are centered for each individual (i.e., the absolute age is irrelevant). Panels B and E show the between-individual trend of the ‘age at first breeding’, and panels C and F show the between-individual trend of the ‘age at last breeding’. Ribbons visualize the 95% CI of the model predictions when using the mean age estimate provided by BaSTA (i.e., the effect sizes of the ‘egg volume’ and ‘lay date’ models shown in figs. 6 and 7).

**Appendix S1.**

To explain how mortality risk is associated with age, we compared four commonly used survival functions: exponential, Gompertz, logistic, and Weibull models. The exponential function keeps survival constant across age (Cox & Oakes, 1984), whereas the other functions allow for age-dependent variation in survival: the Gompertz model is an exponential function in which age-specific mortality is scaled by baseline mortality (Gompertz, 1825; Pletcher, 1999) and the Weibull model is a power function which assumes that baseline and age-specific mortality rates are independent (Pinder III et al., 1978). In addition to the stand-alone versions of these functions, we considered two alternative forms of the Gompertz, logistic, and Weibull functions – “Makeham” and “bathtub” shapes. The Makeham shape constrains the survival function to converge to a constant, rather than zero, as age increases (Pletcher, 1999), whereas the bathtub shape enables concavity in the function, such that mortality could decrease at early ages, but increase later in life (Siler, 1979).

We used four parallel simulations to run the Markov chain Monte Carlo (MCMC) optimization procedure in BaSTA with 800,000 iterations, a 100,000 burn-in period, and a thinning interval of 2000 to minimize serial autocorrelation in the chain (see Fig. S1 for simulation diagnostics). We ranked the survival models according to their deviance information criterion (*DIC*) and determined that the logistic model with a bathtub shape fitted our data best (i.e., lowest *DIC*, Spiegelhalter et al., 2002; Table S1). This logistic model revealed that female mortality rate increased until age 5 years, after which it became constant (Fig. 1b). Using the top model, we extracted the point estimate and 95% credible interval birth year for each individual in the capture-mark-recapture sample.

**File S1.** Commented RMarkdown vignette detailing the analytical steps needed to reproduce all our models and results. Click here to view on your internet browser: <https://lorenf95.github.io/Polyandry-and-senescence-shape-egg-size-variation-in-a-precocial-bird/>