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Quantifying egg attendance behaviours of wild Asian houbara can improve artificial incubation outcomes

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ABSTRACT: Artificial incubation is fundamental in *ex situ* avian conservation interventions for an increasing range of threatened species, but incubation parameters can differ between species. Both egg-turning angle and frequency are critical for successful embryonic development, but measuring these parameters in wild birds has historically been difficult. Using data-loggers inside artificial eggs that accurately record parental egg attendance behaviours, we quantified turning angles and rates throughout the incubation of 6 nesting wild Asian houbaras *Chlamydotis macqueenii*, a species bred in captivity in response to human over-exploitation. Wild females turned their eggs 0.6 times h^{-1} with a mean angle of 40.9° $turn^{-1}$. Mimicking the patterns of wild birds ('wild' treatment), we applied a mean angular change of 40–50° turn once h^{-1} to artificially incubated eggs and compared this to eggs treated with an existing protocol ('control') of 120–130° per turn. Mean hatchability for the wild treatment (78.8%, n = 766) was similar to the control (76.6%, n = 1196). The wild treatment (n = 591) produced significantly heavier hatchlings (2.7%) than the control treatment (n = 893). However, chick growth rates (g d^{-1} and daily % mass change) and survival to Day 10 were not different between groups. The wild treatment demonstrates that turning angles affect embryo development and, perhaps, hatchability. Investigating natural incubation behaviours using egg-loggers and applying these patterns to artificial incubation appears efficacious to achieve optimal incubation protocols and reduce the need for *ex situ* trial and error refinement in conservation interventions of captive-bred threatened bird species.

KEY WORDS: Biologging · *Chlamydotis macqueenii* · Conservation breeding · Egg-turning

1. INTRODUCTION

Ex situ conservation interventions, such as captive-breeding and head-starting, are becoming increasingly important for managing threatened bird species and preventing their extinction (Collar & Butchart 2014). For example, artificial incubation is a critical part of avian conservation interventions (Deeming & Jarrett 2015, Dolman et al. 2018). However, to attain the greatest hatchability and highest chick quality possible, artificial incubation should replicate natural incubation processes, using a mechanised incubator to regulate 4 key parameters: temperature, mass loss (water loss regulated by humidity), ventilation and egg-turning. Incubation equipment and settings for these parameters have been largely developed and optimised for commercial poultry and other galliform species; however, incubation parameters vary across

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taxa (Deeming & Jarrett 2015, Shaffer et al. 2021). Aviculturists adaptively modify incubation parameters for a given species based on experience, analogy and trial and error. However, if a species is threatened, a sound understanding of its specific evolutionarily optimised incubation behaviour in the wild can be used to enhance or maximize hatching success (Deeming & Jarrett 2015).

Of the 4 incubation parameters, egg turning behaviour has been studied the least in wild birds because of difficulties in observing it accurately. Turning rates, which can be inferred from the repositioning of a bird on eggs, have been shown to vary across bird orders ranging from <1 to 12 times h^{-1} (Deeming & Jarrett 2015). Individual egg turning events can range from 1–180° per turn (Shaffer et al. 2014) but available evidence suggests the mean turning angle can differ (ca. 30–75° per turn) between species (Graul 1975, Beaulieu et al. 2010, Shaffer et al. 2014, Clatterbuck et al. 2017). Only recently with the advent of data-loggers has it been possible to accurately document turning angles in the wild (Shaffer et al. 2014).

Egg-turning is fundamental for developing avian embryos, where unturned eggs have significantly reduced hatching success compared to turned eggs (Tullett & Deeming 1987). In unturned eggs, chicks have decreased oxygen consumption, lower heart rates (Pearson et al. 1996), longer incubation periods and reduced mass (Tullett & Deeming 1987). The periodic movement of an egg during early embryonic development is important for vascularisation, development of extraembryonic membranes, gaseous exchange and the elimination of diffusion gradients (Deeming 1989b). In addition, egg turning prevents the stratification of albumen, thereby promoting greater uptake of protein and water by the embryo while also improving redistribution of heat around the egg (Deeming 2016). In late incubation, movement aids the correct orientation prior to hatching, with low turning rates increasing the number of embryonic malpositions in chickens (Robertson 1961b, Elibol & Brake 2006a). However, turning too much can also decrease hatchability, although this is less detrimental than turning too little (Robertson 1961a, Elibol & Brake 2006a, Oliveira et al. 2020). The interaction between turning angle and turning frequency is likely important but has rarely been explored (Elibol & Brake 2006a).

Studies on egg-turning in artificial incubation have primarily focused on hatchability (Deeming 1989b, Elibol & Brake 2006a,b, Deeming & Jarrett 2015, Oliveira et al. 2020), and we are unaware of any published studies examining its influence on chick quality. It is, however, well demonstrated that abnormalities in incubation temperature and humidity can affect hatchling quality (Webb 1987), including their hatching mass and post-hatch growth rates (Hepp et al. 2015). Therefore, it seems logical that abnormal turning rates, though still acceptable enough to allow hatching, could also affect hatchling quality in some manner. Chick quality is of interest to breeding programmes, as early life experiences have the potential to impact the long-term fitness of individuals (Metcalfe & Monaghan 2001).

Here, we sought to quantify and experimentally test if measuring and subsequently mimicking the egg-turning behaviours of wild Asian houbara *Chlamydotis macqueenii* could improve hatchability and chick quality of artificially incubated eggs. The 2 houbara bustards (*C. macqueenii* and *C. undulata*) occur in the semi-deserts of Arabia and central Asia, and North Africa, respectively. Both species have declined historically in the wild owing to overexploitation from hunting along with a reduction of suitable habitat (Allinson 2014). These threats remain current, with both species classified as Vulnerable to extinction and in need of urgent conservation action (Dolman et al. 2021a). The primary conservation response has been captive-breeding to reinforce exploited populations (Dolman et al. 2021b). This process started with a small-scale houbara captive-breeding program in the early 1990s and has since developed into a commercial industry based on artificial insemination, incubation and release (Dolman et al. 2018, IFHC 2019). With continual technical refinements, captive hatching rates have increased and were recently estimated to be 67 % (Dolman et al. 2015). However, hatching rates of wild birds are considerably higher (at 88 %; Dolman et al. 2018). Whilst incubation temperature and mass loss across incubation (18 %) have been well studied in captivity (Saint Jalme & Van Heezik 1996), there is no information on egg attendance behaviour of wild incubating houbaras, as this process is difficult to observe (Deeming et al. 2001).

Our approach to improve hatchability of artificially incubated houbara eggs was to use novel egg data-loggers with triaxial sensors placed in wild houbara nests to characterize the turning rates and angle changes through the entire incubation period. We then aimed to experimentally apply these wild turning behaviours to mechanically incubated eggs laid by artificially inseminated females to compare fertile hatchability (the proportion of eggs hatching from fertile eggs, with infertile eggs excluded) of the wild treatment against existing egg-turning protocols (control treatment). For the fertile eggs that failed to hatch, we compared the rates of early, mid and late embryo death and the probability of malposition between treatments. Last, we compared chick quality in terms of hatch mass, growth rate and survival to 10 d of age between treatments.

2. MATERIALS AND METHODS

2.1. Study area and species

The wild breeding population under study was located in the Bukhara district of Uzbekistan (39.34–40.56° N, 62.21–65.20° E). This region is semi-arid (Kyzyl-kum desert) with a continental climate characterised by cold winters and hot summers. The Emirates Bird Breeding Centre for Conservation (EBBCC) is an Asian houbara breeding centre housing ca. 800 breeding houbaras, located at the core of the study area in Bukhara, Uzbekistan, and was established in 2007 using eggs collected under licence from the local wild population. Therefore, captive-bred and wild birds under study all come from the same geographical and genetic population.

The Asian houbara is a bustard that breeds in the semi-arid belt from east of the Nile in Egypt through the Middle East and Central Asia to Mongolia; in the north-eastern portion of its range including Uzbekistan, this population is migratory, and breeding occurs from March to June. Houbaras are polygamous and sexually dimorphic; smaller females weigh on average (±SD) 1.28 ± 0.1 kg (range: 1.11–1.59 kg) during the breeding season (Burnside et al. 2019) and take sole responsibility for incubation and chick-rearing. In free-ranging houbaras, the average clutch size is 3.2 ± 0.6 eggs (range: 2–5) and mean egg dimensions are 60.9 (±2.6) × 44.1 (±1.7) mm; mean fresh mass is 65.4 ± 6.4 g (Koshkin et al. 2016, Burnside et al. 2019). Females lay an egg every 1.5 d until the clutch is complete and then fully commence incubating the clutch on the third or penultimate egg (whichever comes first; authors' unpubl. data). The total incubation period lasts 22–23 d, with the internal pip occurring on Day 21 and external pip on Day 22. Nest attentiveness has been estimated to be 87.1–90% (Deeming et al. 2001). Females nest on the ground, typically amidst low shrubs (30 cm tall) that provide concealment and some microclimatic benefit or protection from winds (Guilherme et al. 2018). Chicks are precocial and leave the nest site with their mother as soon as their down is dry.

2.2. Quantifying egg-turning in the wild

Battery-powered data-loggers encapsulated inside artificial eggs (hereafter egg-loggers) were used to accurately record egg orientation and turning frequency in the nest. Egg-loggers recorded data at 1 Hz intervals for 8 d with a 60 mAh lithium-polymer battery. Egg-loggers contained a triaxial accelerometer and triaxial magnetometer capable of sensing 1–2° angular changes in roll, pitch and yaw (*x*-, *y*- and *z*-axis); for further design details see Shaffer et al. (2014). These devices allowed us to calculate the number of turning events as well as the magnitude (angular change) and direction of each turning event. Artificial egg shells were 3D-printed in plastic to be the same size as an average houbara egg and painted to mimic a real egg (Fig. 1). An inert material (soap shavings) was sealed inside the egg-logger to replicate the approximate average wild egg mass (ca. 60 g) and reduce possible interference of different mass with rotation. Trials, using the internal temperature sensors within the egg-logger, in the artificial incubators prior to deployment in real nests

Fig. 1. Egg logger housed inside a 3D-printed and painted artificial egg (uppermost egg) in an Asian houbara *Chlamydotis macqueenii* nest scrape with 2 real eggs

confirmed that the artificial eggs maintain temperature similar to the incubation temperature, therefore reducing the likelihood of artificial eggs being turned at different rates due to being a different temperature than the real eggs.

2.3. Egg-logger deployment

During the 2019 breeding season, using satellite-tracked wild females from which we received multiple GPS locations per day, it was possible to identify and locate nests at the laying stage (details in Burnside et al. 2019). Once a clutch was complete and incubation began, an egg-logger was placed into the nest. To keep the clutch size the same, one houbara egg was removed under licence from the Uzbekistan State Committee for Nature (2019) for subsequent artificial incubation, hand-rearing and release. All nests were fitted with cameras (detailed in Koshkin et al. 2016) to monitor final outcomes, which confirmed that females accepted the egg-logger without problem (see the video in the Supplement at www.int-res.com/articles/suppl/n046p193_supp/). Nests were visited every 6 d until the end of incubation following a standard monitoring protocol (detailed in Koshkin et al. 2016). During each visit, the *in situ* egg-logger was removed for downloading data and replaced with a new logger. Outcomes and the exact end of incubation were determined from nest cameras. Six wild nests were monitored, 3 of which hatched and 3 were depredated (see Table 1). The predators, desert monitor *Varanus griseus* and wild cat *Felis lybica*, did not attempt to eat the dummy eggs and left them in the nest scrape.

2.4. Data processing

Following methods in Shaffer et al. (2014), we quantified the mean hourly turning rates and the mean magnitude of hourly angular changes and whether these 2 parameters changed over the course of incubation and between day (05:00–21:00 h) and night. Raw logger data were processed using custom routines in MATLAB (The Mathworks) following the supplementary methods in Shaffer et al. (2014). In brief, accelerometer and magnetometer data were converted to 3-2-1 Euler angles from which the egg orientation and angular changes were calculated. Each turning event and corresponding angular change were counted when turning events exceeded 10°, as smaller angle changes were considered to be sensor noise (Shaffer et al. 2014). Overall mean number of hourly turns was calculated as the total number of turning events per nest divided by the total number of hours that nest was monitored, and then averaged across all nests. Overall mean angle change per egg turning event was calculated as the mean of all angular changes observed in a nest, and then averaged across all nests. To assess how the turning rate and turn angle were influenced by the stage of incubation, separately for each incubation day (2–22 d), we calculated the mean number of hourly turns per nest (daily total, divided by 24) and the mean angle change per turn per nest per incubation day. Days that were monitored incompletely at the beginning or end of incubation were excluded from analysis of daily turning rate.

2.5. Artificial incubation protocols

In the wild, females would raise one brood per season, but if nests fail, they can relay up to 2 times (Dolman et al. 2018). In captivity, eggs are collected and females will continue to lay and be inseminated for the duration of the season. An individual female can lay multiple clutches, sometimes up to 25 eggs, in a season. Female houbaras in the EBBCC breeding facility are housed separately in outdoor cages that were inspected each morning and evening for eggs. After collection, eggs were measured, weighed (fresh mass) and cleaned using a STERILOVO UVC steriliser for 10 min. Eggs were batched together based on the day of collection so all batches were of the same age. Batches were artificially incubated in Grumbach BSS 300 incubators (Interhatch) that accommodate up to 210 houbara eggs. All incubators were set to run at a constant 37.6°C (Saint Jalme & Van Heezik 1996) and 40% humidity (based on EBBCC protocols). Eggs were placed horizontally in a tray between rows of rollers. In the incubator, egg rotation angle was determined by the duration of rolling (minutes per hour) and eggs only rotated about the x-axis (long axis of egg). Eggs were weighed every 7 d using digital scales accurate to 0.01 g to assess and manage the rate of mass loss; shell surface temperatures were checked daily, using a laser infrared handheld thermometer accurate to 0.1°C, to ensure standard operating conditions. Eggs remained in the incubator and continued to be rotated until the morning of Day 21, when they were transferred to a Grumbach BSS 300 (8202/MP; Interhatch) hatcher set at 37.0°C and 70% humidity. The chicks are hatched in compartments. Successfully hatched chicks were ringed, weighed and transferred to a chick area, where they were hand-reared with minimal human contact. A subset of chicks were then weighed daily from Day 3 to Day 10 to evaluate growth rates. Eggs that failed to hatch were opened to assess fertility and age (days) of embryonic development.

We were interested in fertile hatching rate, so infertile eggs were excluded from further analysis. Embryo deaths were classed as early (age 1–7 d), mid (8–17 d) and late (18–22 d). All failed eggs were swabbed and samples cultured on agar plates to identify any bacterial infections. Malpositions of embryos (Wilson et al. 2003) were classified as follows: head under yolk sac, head under left wing, head under left leg, head under right leg, head on left side, head between legs, upside down (following the existing monitoring protocols of the breeding programme; K. M. Scotland unpub. data). Embryos with congenital defects were also recorded.

2.6. Experimental application

Experiments were conducted during the 2019 and 2020 breeding seasons (March–June) and involved comparing the hatching success of eggs in the wild treatment (natural incubation turning regime) and captive control (artificial incubation turning regime). Essentially, we modified the magnitude of turns to mimic those of wild incubating females established with the egg-logger data. A control treatment group of incubators were set to turn eggs once per hour (24 times per day) for 30 min, with 30 min rotation of the eggs on the rollers equating to 120–130° on the *x*-axis, consistently from Day 1 to Day 21 of incubation. The wild treatment group had adjustments in mean angle during the course of incubation. In the wild, a gradual decrease in mean angle change was observed as incubation progressed but the magnitude of the daily decrease was small in comparison to the accuracy of the angular change the incubator could achieve (ca. 10°). Based on visual examination of angular change data through incubation, Day 18 was chosen as a point to make a second adjustment of the angular change for the wild treatment to replicate the naturally observed decline as closely as possible given the limitation of the incubation equipment.

Our original aim was to also replicate wild turning rates in the experiment; however, we observed that in the wild the turning rate on average was less than once per hour. Due to a constraint with the incubator, it was not possible to reduce the turning rate to less than once per hour and so this parameter had to remain constant at once per hour for the duration of the experimental treatments in both the control and treatment.

Overall fertile hatchability was calculated using 2198 eggs from 102 females. Individual females in captivity can have different mean egg hatchability attributed to intrinsic differences (under investigation). Therefore, rather than comparing batches of eggs between incubators, we compared individual female changes in hatching probability between control and treatment groups. To minimize bias, we removed eggs from the analysis that had no chance of hatching because their development was compromised due to congenital defects, contamination or infection. Furthermore, the data set was restricted to females that had a minimum of 6 eggs (an average of 8.5 eggs) in each experimental group, yielding a total of 1962 eggs from 78 females, with 766 in the wild treatment versus 1196 in the control. Lastly, egg fresh mass can affect hatching probability, so fresh egg mass was included as a factor in hatchability models. Examination of the data prior to analysis showed a 2-factor categorical variable (with eggs in the lower quartile of masses [<55 g] versus all other egg masses [>55 g]) fitted the data better than either a quartile factor or incorporating fresh mass as a continuous variable.

To examine chick quality, we used all eggs hatched from the original 102 females included in the experiment and compared hatching mass between treatment and control while controlling for fresh egg mass. Growth rates (beta coefficient for the slope of a regression of the log-transformed masses) of 89 (wild treatment) and 34 (control treatment) chicks from Days 3–10 were compared. Finally, we compared the proportion of chicks alive at 10 d old from each treatment in the whole hatched sample.

2.7. Analysis

For all wild incubation parameters, using generalised linear mixed models (GLMMs) with Gaussian errors and nest ID as a random factor, the effects of incubation day and the diurnal period (explanatory variables) on turning rate and turning angle (response variables) were tested by examining whether the model deviance increased significantly (analysis of deviance using a chi-squared test) on removing the explanatory variable from the full model (Hastie & Pregibon 1992). For the outcomes of the artificial incubation experiment between the wild treatment and the control group (2-factor categorical explanatory variable), GLMMs with female ID as a random factor were used to test fertile hatching probability (binomial errors), probability of malposition (binomial errors), probability of embryo survival during early, mid and late incubation (binomial errors), chick survival to Day 10 (binomial errors), and fresh egg mass, chick mass at hatching and chick growth rates (all Gaussian errors). Egg fresh mass was included as a categorical 2 factor fixed effect in analyses of hatchability, malposition probability, embryo survival, chick mass at hatching and a 4-factor (4 egg mass quartiles) categorical variable for the proportion of fresh egg mass lost between laying and hatch. Whether outcomes differed between wild treatment and control groups was again tested using the analysis of deviance on removal of the explanatory variable (Hastie & Pregibon 1992). The correlation of hatching mass with Day 10 mass was tested using a generalised linear model (GLM) and an analysis of variance with an *F*-test was used to test for an increase in variance upon removal of the predictor variable (Hastie & Pregibon 1992). All means and standard errors were estimated from the selected model for the variable under investigation, and GLMM residuals were examined for conforming to assumptions of normality and homoscedasticity. All analyses were conducted in the software R v.3.6.1 (R Core Team 2019).

3. RESULTS

Six wild nests were monitored using egg-loggers (Table 1) over an average (±SD) deployment of 14 ± 6.3 d (range: 4–21 d) spanning the entire incubation period (Days 2–22).

3.1. Wild incubation

In the 6 wild houbara nests, females turned their eggs throughout incubation on average 0.58 times h^{-1} (95% CI = 0.125–1.08) with a mean angular change of 40.9° $turn^{-1}$ (95% CI = 8.0–146.8°). The number of turning events per day (χ^2 = 7.50, df = 1, p = 0.006; Fig. 2a) and the mean angle per turn (χ^2 = 6.79, df = 1, p = 0.009; Fig. 2b) decreased as incubation progressed to hatching.

Hourly turning rate (Fig. 3a) and angle change per turn (Fig. 3c) varied over 24 h periods, with a decrease in turning rate (mean ± SE: −0.19 ± 0.05 h^{-1}, χ^2 = 12.3, df = 1, p = 0.001; Fig. 3b) but with larger angle per turn (6.04 ± 2.61°, χ^2 = 6.18, df = 1, p = 0.013; Fig. 3d) at night than by day (controlling for incubation day). However, the overall relationship between turning rate h^{-1} and mean angular change h^{-1} (controlling for incubation day) was not significant (Beta = 0.013 ± 0.108, χ^2 = 0.01, df = 1, p = 0.942).

3.2. Artificial incubation hatchability

Based on parameters of egg attendance behaviours in wild houbaras, incubators turned eggs for 6 min h^{-1}, equivalent to 40–50°. At Day 18 of incubation,

Table 1. Details of wild Asian houbara *Chlamydotis macqueenii* nests in which egg data loggers were deployed to monitor incubation turning behaviours. PTT.ID: Platform Transmitter Terminal ID; dates are dd/mm/yy

Nest ID	PTT.ID	Outcome	Incubation start date	First egg hatch date	Egg logger deployment Start End	Incubation days covered	Logger deployment (no. of days)
Nst_02	167472a	Hatched	24/03/19	15/04/19, 01:49 h	25/03/19, 18:31 h 15/04/19, 01:49 h	2–22	21
Nst_05	145069a	Depredated by monitor lizard	29/03/19	Failed	30/03/19, 10:29 h 19/04/19, 14:08 h	2–21	20
Nst_06	No PTT	Hatched	28/03/19	19/04/19, 02:00 h	15/04/19, 18:52 h 19/04/19, 02:00 h	18–22	4
Nst_07	167471a	Hatched	24/03/19	15/04/19, 18:28 h	01/04/19, 11:31 h 15/04/19, 18:28 h	8–22	14
Nst_16	167477a	Depredated by wild cat	05/04/19	Failed	09/04/19, 23:23 h 23/04/19, 14:04 h	2–17	15
Nst_19	145061a	Depredated by monitor lizard	05/04/19	Failed	08/04/19, 19:22 h 18/04/19, 12:11 h	4–14	10

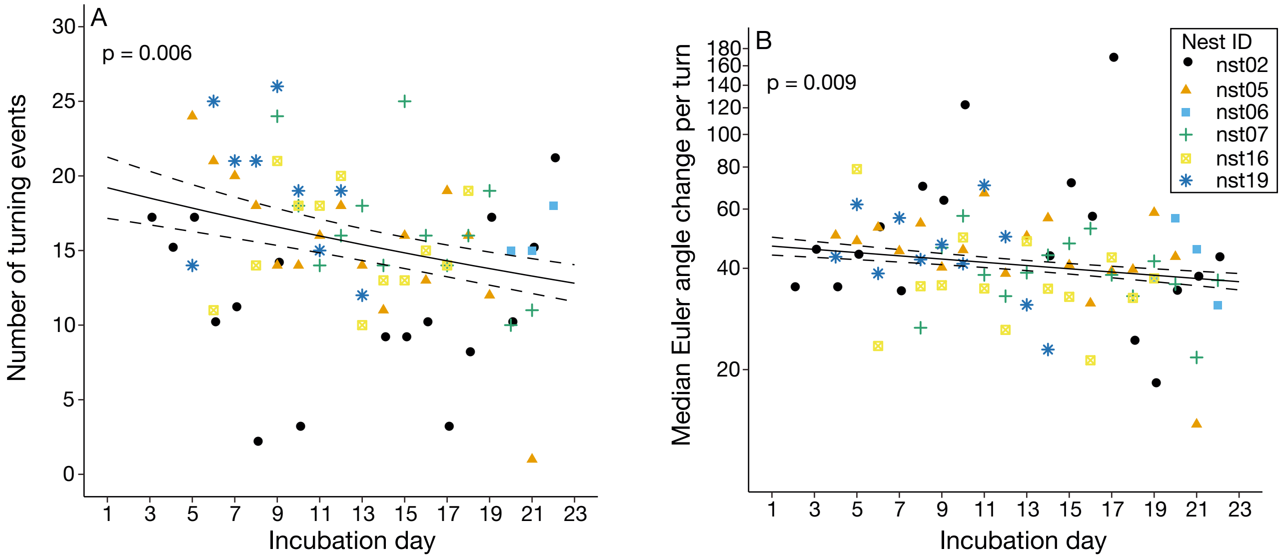

Fig. 2. Egg turning events recorded by egg loggers during 21 d of the 23 d incubation period of 6 Asian houbara *Chlamydotis macqueenii* females. (A) Number of turning events logged during each day of incubation for each nest; (B) median angular change of turning events per day of incubation. Black solid lines: fitted models; dashed lines: SE

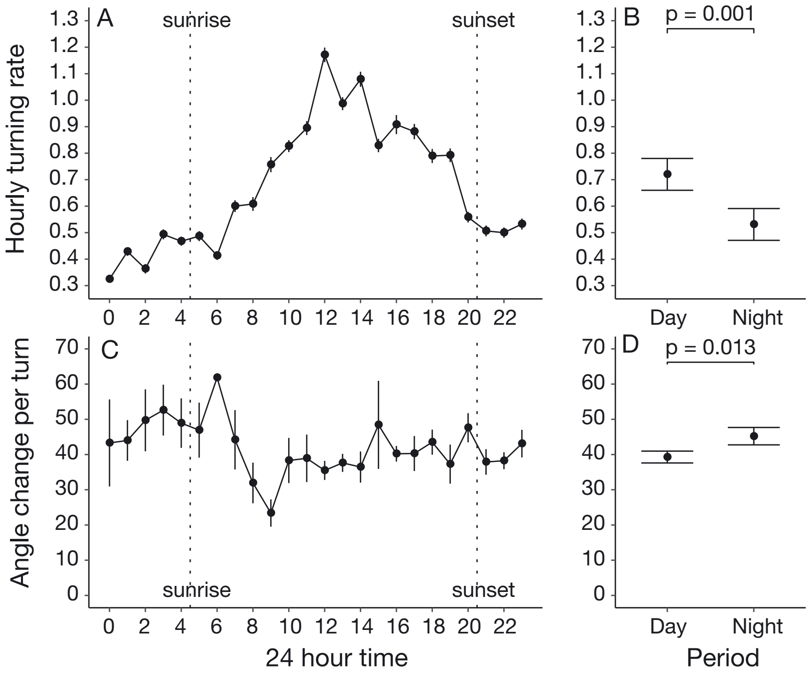

Fig. 3. Asian houbara *Chlamydotis macqueenii* incubation behaviour showing (A) hourly turning rates and (C) angle change per turn, averaged (±SE) for each hour of the day across the entire incubation period; and the model estimated means (±SE), controlling for nest identity and incubation period, for the diurnal period for (B) hourly turning rates and (D) angle change per turn

turning rate was reduced to 3.6 min h^{-1}, 25–35° until Day 21, and then eggs were transferred to the hatcher.

Overall mean fertile hatchability in the programme, including all causes of embryo mortality, was 71.3 ± 1.4%. Of the 1962 eggs from 78 females included in the hatchability analysis, and controlling for female identity and fresh egg mass, mean fertile hatchability for the wild treatment group (78.8 ± 1.8% for 766 eggs; Fig. 4a) was not significantly different (χ^2 = 1.20, df = 1, p = 0.274) from that of the control group (76.6 ± 1.6% for 1196 eggs; Fig. 4a). Fresh egg mass from the captive population (mean mass = 58.7 g, interquartile range = 55.1–62.2 g, range = 44.5–78.2 g) significantly influenced hatchability (χ^2 = 15.6, df = 1, p < 0.001), with egg masses in the lower quartile (<55.1 g) having reduced hatchability (mean = 72.7 ± 2.4%) compared to the upper 3 quartiles (82.1± 1.2%) across both treatments (Fig. 4a).

Across the incubation period, there was no difference in embryo survival between the treatments (early: χ^2 = 1.77, df = 1, p = 0.183; middle: χ^2 = 0.84, df = 1, p = 0.360; late: χ^2 = 0.73, df = 1, p = 0.393; Fig. 4b). Embryo deaths mainly occurred in the early and late stages (Fig. 4c), with the probability of embryo survival being lower dur-

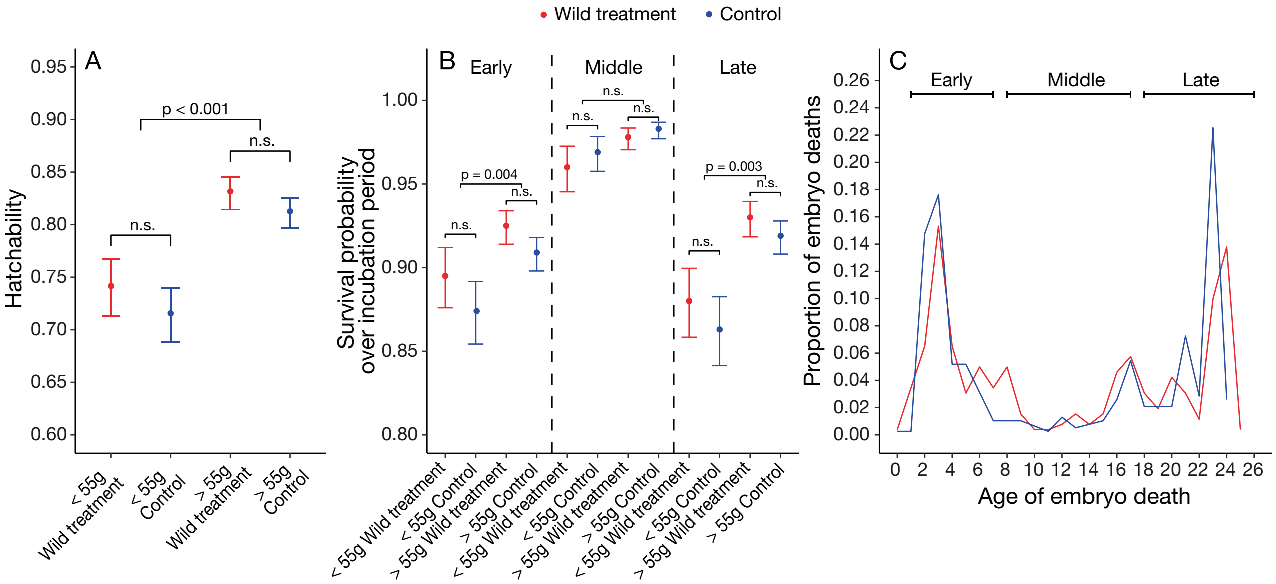

Fig. 4. Captive-bred Asian houbara *Chlamydotis macqueenii* eggs artificially incubated under a wild treatment turning protocol (40–50° turn h^{-1} reduced to 35° turn h^{-1} on Day 18 of incubation) compared to the control protocol (120–130° turn h^{-1} throughout incubation). Differences were considered significant at $p \leq 0.05$; n.s.: not significant. (A) 78 individual females' mean hatchability rates (n = 1962 eggs) as coloured dots for the model estimated mean (±SE) across treatments controlling for female identity and fresh egg mass. (B) Embryo survival probabilities (±SE) during early, middle and late incubation periods (see C for delimitation of periods) controlling for fresh egg mass. (C) Comparison of the proportional contribution of embryo deaths occurring at different ages across the incubation period for eggs produced through artificial insemination across the 2 treatments (red: wild treatment, blue: control)

ing early (90.2 ± 1.0%) and late incubation (90.0 ± 1.0%) than the middle (97.3 ± 1.0%). Fresh egg mass was only an important factor in the early- ($\chi^2 = 4.08$, df = 1, p = 0.004; Fig. 4b) and late-stage survival probability ($\chi^2 = 9.09$, df = 1, p = 0.003; Fig. 4b), with eggs in the lower quantile having lower survival (Fig. 4b). Egg mass was not an important factor in the middle period of incubation ($\chi^2 = 3.31$, df = 1, p = 0.069; Fig. 4b).

Controlling for female identity and fresh egg mass, the probability of a malposition within a post-mortem egg that died during the late stage of incubation was significantly lower ($\chi^2 = 9.78$, df = 1, p = 0.002) in the wild treatment (0.262 ± 0.064) than in the control group (0.529 ± 0.055).

3.3. Artificial incubation chick quality

For hatchlings with recorded hatching masses, there was no difference ($\chi^2 = 2.92$, df = 1, p = 0.087) in mean fresh egg mass between the wild treatment (59.3 ± 0.38 g, n = 591) and control (59.0 ± 0.380 g, n = 893). In contrast, the mass of chicks at hatching was significantly different ($\chi^2 = 38.0$, df = 1, p < 0.001), where wild treatment chicks were 2.7% heavier (41.5 ± 0.22 g) than control treatment chicks (40.4 ± 0.21 g). Moreover, examination of the proportion of fresh egg mass lost between laying and hatch (% mass loss) showed the difference between treatments was greatest for eggs <55 g in the lower quartile of masses (mean difference in % mass loss: 4.41 ± 0.51%, Tukey's post hoc test, $z = -8.59$, p < 0.001; Fig. 5a); less but still significantly different between treatments for the middle 2 quartiles (mean difference in % mass loss for 55–58 g: 1.66 ± 0.47%, $z = -3.50$, p = 0.011; mean difference in % mass loss for 58–62 g: 1.32 ± 0.41%, $z = -3.21$, p = 0.029; Fig. 5a) and not different between treatments for eggs in the upper quartile (0.63 ± 0.42%, $z = 1.59$, p = 0.809; Fig. 5a).

Masses of 89 (wild treatment) and 34 (control) chicks were recorded daily from Days 3–10. Day 10 chick mass was positively correlated with hatch mass (Beta = 1.17 ± 0.47, $F = 936$, df = 1, p = 0.013), indicating that heavier chicks at hatching were also heavier at Day 10 (Fig. 5b). Rates of growth from Days 3–10 (Fig. 5b) were the same between treatment and control groups ($\chi^2 = 2.92$, df = 1, p = 0.087).

There was no difference ($\chi^2 = 0.01$, df = 1, p = 0.945) in the proportion of chicks surviving to Day 10 between the wild treatment (99.0 ± 0.1%) and the control (98.9 ± 0.5%).

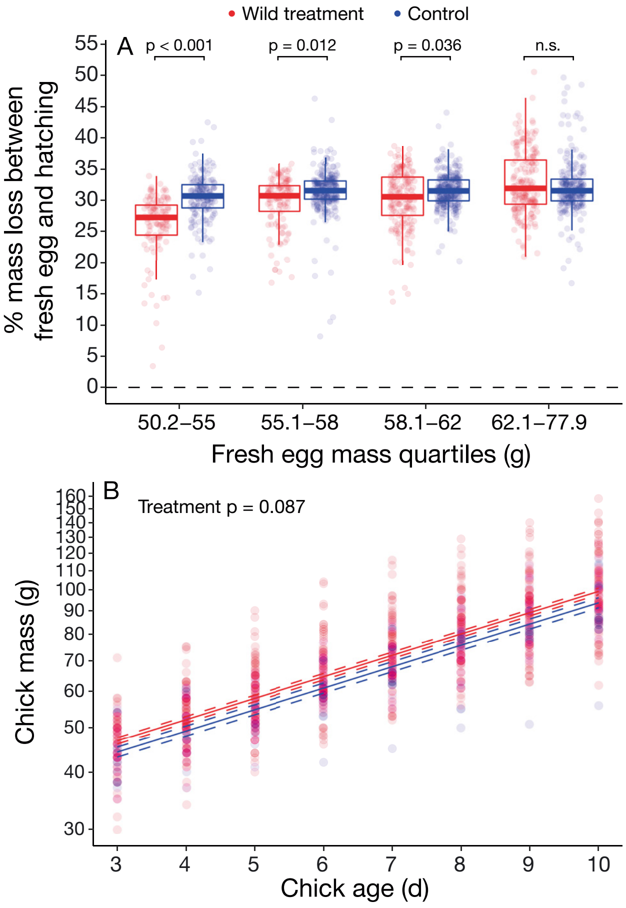

Fig. 5. Relation of (A) percentage mass loss at hatching relative to fresh egg mass and (B) chick mass and age for captive-bred Asian houbara *Chlamydotis macqueenii* chicks produced from artificial incubation under a wild treatment turning protocol (40–50° turn h^{-1} reduced to 35° turn h^{-1} on Day 18 of incubation) compared to the control protocol (120–130° turn h^{-1} throughout incubation). In (A) and (B), model (generalized linear mixed models)-estimated relationships are shown as solid lines ±SE (dashed lines)

4. DISCUSSION

Although artificial incubation practices used in the conservation-breeding of Asian houbaras have been refined by extensive experience over 30 yr, we found that applying the incubation turning parameters measured in wild houbaras (during one season) to artificial incubators was able to yield similar results to the existing (control) artificial incubation protocols whilst important mass loss management was favourably altered to produce heavier hatchlings. This supports the argument that egg-loggers can provide a short-cut to developing suitable artificial incubation protocols for threatened species in situations where trial and error refinement is not possible.

4.1. Wild incubation turning behaviours

The egg turning patterns of Asian houbaras with small-angle changes per turn (40.9°) and low turning rate (0.6 h^{-1}) are consistent with predictions for birds with low albumen content eggs (Asian houbara wet albumen = 63.5%; Anderson & Deeming 2002) and precocial hatchlings. Conversely, birds with altricial hatchlings and high albumen content eggs exhibit greater angle changes at higher turning rates (Deeming 2002). Furthermore, our data add to the few species studied that show how turning rates and angles changes vary as incubation progresses. Asian houbara decreased their turning angles and rates over the incubation period, which differs from observations of western gulls *Larus occidentalis* and Laysan albatrosses *Phoebastria immutabilis*, where turning frequency and angles remained unchanged throughout the course of incubation (Clatterbuck et al. 2017). In contrast, Adelie penguins *Pygoscelis adeliae* decreased their turning frequency but not turn angles (Beaulieu et al. 2010). Some species also exhibit diurnal variation in incubation behaviours. Asian houbaras decreased turning frequency and increased turn angles during night time periods, which has also been observed in western gulls but not in Cassin's auklets *Ptychoramphus aleuticus* or Laysan albatrosses (Shaffer et al. 2014, Clatterbuck et al. 2017). Asian houbaras are largely diurnal and undertake several feeding bouts during the day, but tend to remain on the nest at night (Deeming et al. 2001), so daytime leads to more egg-shifting events when a female resettles onto the clutch. Interestingly, the reduction in turning events at night was apparently compensated for through a larger degree of turning. This suggests that the cumulative turning interaction between frequency and angle is important for embryonic development, as experimentally shown in poultry (Elibol & Brake 2006a).

4.2. Artificial incubation

Using the data obtained for the wild houbara, we applied changes in the captive-breeding programme by modifying the mean turning angle per turning event from 120° to 40° and a subsequent reduction to 30° by Day 18. The turning frequency of once per hour for artificially incubated eggs was higher than we found for wild houbaras but could not be replicated in the treatment. Overall, the modification of turning angle did not significantly alter hatchability for eggs in the wild treatment group. Experimental studies have determined that reductions in hatchability are often associated with unturned eggs (Tullett & Deeming 1987, Deeming 1989a) or reductions in turning angle (Elibol & Brake 2006a), whereas turning more than the optimum in poultry only has marginally negative effects (Robertson 1961a). As the existing protocols were already turning at a higher frequency than that of wild houbaras, eggs were likely meeting their turning requirements without a detrimental effect on hatchability. It should be noted, however, that 'over-turning' eggs can be detrimental to hatchability in some cases, such as observed with the Adelie penguin (Beaulieu et al. 2010).

The modified turning protocol (i.e. wild treatment) significantly reduced the number of embryo malpositions observed in post-mortem eggs, but this did not result in a proportionate increase in hatching success. A similar relationship of hatchability and incidence of malpositions was found in poultry eggs after adjusting turning frequencies (Elibol & Brake 2006a). That study concluded that while malpositions can occur, they may not be the primary cause of hatching failure. Many sources of variation may contribute to and influence hatchability more than egg turning per se, like individual female condition, egg size, genetics, infection and egg handling.

We found that hatching mass was influenced in eggs turned using the wild treatment, especially in smaller eggs, whereby chicks were heavier than chicks incubated using the control treatment. Egg size and resulting chick sizes are highly predictive of chick quality (Krist 2011), so hatching heavier chicks for a given egg size is desirable. Eggs lose mass via water loss through pores in the shell surface, with the rate of water loss being influenced by the thickness of the cuticle around the shell and the managed levels of humidity in the artificial incubator (Snyder & Birchard 1982). Humidity was the same across treatments, so a greater percentage mass loss in the control group may be a result of increased turning eroding the cuticle at a faster rate (Beaulieu et al. 2010), thus increasing water loss. The observed decreased percentage mass loss in heavier eggs compared to lighter eggs appears to support this inference, as shell surface area relative to egg volume is inversely related to egg size. Therefore, larger eggs have lower surface area per unit volume from which to lose water (and likely less shell cuticle) as a result of being in contact with the incubator turning mechanism, so cuticle erosion should have less influence on the rate of water loss. Nonetheless, further investigation is needed to determine if this mechanism is responsible.

The growth rates of chicks between Days 1 and 10 were similar between the 2 treatments, with heavier chicks at hatching maintaining their advantage at Day 10 over lighter individuals. The rates of survival to Day 10 were exceptionally high and similar between the groups.

This study attempted to measure average incubation turning behaviour in the wild and apply it to mechanically incubated eggs. However, it must be acknowledged that artificial incubation procedures in general are a simplified model of what happens in nature and do not replicate natural incubation in many aspects, such as eggs being air heated rather than contact heated with a brood patch. There were some limitations in the ability of artificial incubators to match the exact turning behaviours observed in the wild, but the conditions were matched as closely as feasibly possible here. In particular, we could not reduce the turning rates to less than once per hour during this experiment to match the natural rate, as turning angles were achieved through eggs rotating slowly on rollers rather than instantaneous turns that a female may do. Further work could be done to more closely replicate these natural conditions.

4.3. Implications for captive breeding

Chlamydotis macqueenii is globally threatened with extinction (BirdLife International 2019). Large-scale captive-breeding and release has been the primary conservation measure in response to population declines (Dolman et al. 2021a). Successful releases are equally reliant on the quantity and quality of chicks being produced (Dolman et al. 2021b). Given the large number of breeding individuals in captivity, marginal gains in any breeding parameter can have important effects on breeding output when scaled up to an entire breeding population consisting of thousands of individuals. By implementing the knowledge of egg attendance patterns from wild houbaras, the *ex situ* breeding programmes should be able to contribute to a meaningful improvement in both chick quantity and quality.

More broadly, the application of biologging technology here is transferable to any *ex situ* avian captive-breeding programme, and will be beneficial for species that are less well known, have not been bred in captivity before, or are so threatened that the opportunity for trial and error incubation development are not an option. Our evidence-based approach provides a protocol based on monitoring wild incubation that can inform decision making in the artificial incubation of captive-laid eggs and reduces the time needed to develop incubation protocols *ex situ*.

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