Trading off hatching success and cost in the captive breeding of Whooping Cranes

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Abstract

Captive breeding is an increasingly used conservation strategy for species with a high risk of extinction in the wild, but managing a captive breeding programme can be challenging if there is a deficiency in knowledge about the species’ breeding biology. A knowledge gap can make it difficult to evaluate different management options. For avian species, egg hatching success is a key demographic parameter, and data-logging egg technology can provide important information on optimal species-specific incubation conditions, which can help inform captive breeding practises and identify efficient captive management options. In the context of a captive breeding programme for endangered Whooping Cranes Grus americana, we investigated associations between hatching success and incubation conditions, including environmental parameters (temperature, relative humidity and egg turning rate), and incubation type (artificial incubation; foster incubation by Sandhill Cranes, Grus canadensis; and Whooping Crane incubation). Finally, we considered both cost and breeding output in an analysis of incubation practises. We found that daily mean temperatures were negatively associated with hatching success, and that hatching success was highest with incubation under Sandhill Cranes. However, incubation by artificial incubators, rather than Sandhill Cranes, provided a trade-off between cost and breeding output that is likely to be acceptable to many captive programme managers. We encourage other captive breeding programmes to use innovations that help to increase potential release numbers for conservation translocations by considering biological and financial constraints.

Introduction

Captive breeding is a conservation strategy used to maintain captive populations as genetic reservoirs, to supplement declining populations, and to establish new populations, and is increasingly used for species with a high risk of extinction in the wild (IUCN/SSC 2013; Brichieri-Colombi et al. 2019). However, achieving desired reproductive rates in captive populations has proven difficult for many imperilled species (Snyder et al. 1996). The failure to achieve target vital rates in captivity is likely due to a number of factors, including poor adaptation to novel environments and practises (McDougall et al. 2006; Araki et al. 2007), inbreeding among close relatives (Ralls et al. 1988), and inadequate information with which to inform management practises (Smith et al. 2011). For captive breeding to constitute a successful conservation strategy, captive managers must identify the most effective captive management techniques while accounting for financial constraints. However, trade-offs between monetary costs and captive production are rarely assessed in a rigorous manner (Smith et al. 2011).

Among birds, hatch failure in captive breeding programmes can hinder captive-to-wild translocation efforts by lowering the number of available release candidates (Burnham 1983; van Heezik et al. 2005; Smith et al. 2011), and is often indicative of a poor understanding of the species reproductive biology (Smith et al. 2012). For instance, artificial incubation protocols developed for domestic fowl (Gallus gallus) are frequently used to incubate eggs of different species (Deeming 2002a), and can result in high hatching failure because key incubation parameters differ between species (e.g., temperature, humidity and rate of egg turning rate). A sub-optimal thermal environment during incubation can affect developmental trajectories and hatching outcomes (Romanoff 1972; Hepp et al. 2006; Olson et al. 2008; DuRant et al. 2010; Nord and Nilsson 2011), and have long-term fitness consequences (Hepp and Kenmamer 2012; DuRant et al. 2013; Bernts and Bech 2016; Nord and Nilsson 2019).
Deviations from the optimum humidity can result in swollen tissue preventing development, or conversely, desiccation of tissue (Ar and Rahn 1980). Also, if the egg is not turned sufficiently, oxygen consumption, heart rate and the amount of protein in the albumen are reduced (Ar and Sidis 2002; Deeming 2002b).

The captive breeding programme for Whooping Cranes (Grus americana) has struggled to achieve reproductive rates comparable to those exhibited in the free-ranging Aransas-Wood Buffalo population (fertility = 73–95% and hatch success = 95%, Black and Swan, 2019; Brown et al. 2016; Erickson and Derrickson 1981). In captivity, poor fertility (40–94%, Black and Swan, 2019) and hatch success (57–89%, Smith et al. 2011) limit the number of eggs and chicks available for ongoing reintroduction efforts, presently in the Eastern United States (Eastern migratory population, EMP, established 2001) and Louisiana (Louisiana non-migratory population, LNMP, established 2011; French et al. 2019). This limitation is particularly problematic for the recovery of a long-lived, late-maturing species with slow intrinsic population growth rates.

To counteract poor productivity in the captive Whooping Crane population, eggs are removed to stimulate re-clutching (Mirande et al. 1996). These surplus eggs can be incubated artificially or by foster incubators (e.g., Sandhill Crane Grus canadensis or Whooping Crane pairs, Gabel and Mahan 1996). Artificial incubation can also help to avoid egg death due to egg abandonment and potential egg breaking by adult cranes (Smith et al. 2011). Although artificial incubation is a substantially cheaper captive rearing practise for dealing with surplus eggs than foster incubation, it is not as effective as crane incubation (Converse et al. 2010; Smith et al. 2011, 2012). Smith et al. (2011) found a positive correlation between hatch success and the number of days a Whooping Crane egg was incubated by a crane (as opposed to an artificial incubator). In a further study, Smith et al. (2012) found that the variance and mean of temperature, humidity and egg turning rate were significantly higher and less variable during artificial incubation than crane incubation. Notably, changes in these environmental parameters are known to influence hatching outcomes in other avian species (Gabel and Mahan 1996; Deeming 2002b; Deeming 2002c; Hepp and Kennamer 2012; DuRant et al. 2013; Berntsen and Bech 2016; Nord and Nilsson 2016), yet no study has investigated this association to inform the captive breeding practises of Whooping Cranes.

We used the captive Sandhill and Whooping Crane populations at the Calgary Zoo’s Devonian Wildlife Conservation Centre in Calgary, Alberta, Canada (DWCC), the International Crane Foundation breeding facility in Baraboo, Wisconsin, USA (ICF) and the U. S. Geological Survey (USGS) GS Patuxent Wildlife Research Center in Laurel, Maryland, USA (PWRC) to evaluate the association between environmental parameters during incubation – temperature, relative humidity (hereafter humidity) and egg turning rate – and hatching outcomes using data-logger egg technology. Crane incubation is associated with higher variability in temperature and humidity, lower mean temperatures and humidity, and a rate of egg turning that allows for an even representation of egg positions, as well as greater hatch success compared to artificial incubators (Smith et al. 2011; Smith et al. 2012). We therefore hypothesized that low variability in temperature and humidity, high mean temperature and humidity, and a low number of egg turns would lead to greater hatching failure of Sandhill/Whooping Crane eggs. Because artificial incubation protocols are often not species-specific (Deeming 2002a), we hypothesized that artificial incubators would also lead to greater hatching failure of Sandhill/Whooping Crane eggs. To confirm that the variance and mean of temperature, humidity and egg turning rate were significantly higher and less variable during artificial incubation compared to natural incubation. Finally, to inform captive breeding management, we developed a stochastic simulation to predict the expected number of hatched eggs when using Whooping Crane incubation, Sandhill Crane incubation and an artificial incubator, and discuss trade-offs between cost and breeding output in the selection of captive breeding practises.

Materials and methods

Data-logger eggs

Data-logger eggs were collaboratively developed by the Calgary Zoo’s Centre for Conservation Research and Advanced Telemetry Systems. The eggs were equivalent in size and fresh weight to an average Whooping Crane egg (length = 98 mm; diameter = 63 mm; weight = 200 g). They had two temperature–humidity sensors (temp range = 8–48°C; resolution = 0.16°C; accuracy = 0.4°C, and humidity range = 0%–100%; resolution = 0.05%; accuracy = 3%). A third temperature sensor was located on the wide end of the egg (temp range = 0–62.5°C, resolution = 0.25°C; accuracy = 0.5°C). They also contained a position sensor that measured 360° of positional rotation. Additional details on the design and function of these data-logger eggs can be found in Smith et al. (2012).

Data collection

We collected data between 2014 and 2019 at the DWCC and ICF, and between 2014 and 2018 at the PWRC. Both Sandhill and Whooping Crane eggs were obtained from pens of captive breeding pairs as soon after laying as feasible. We paired the eggs with a data-logger egg under different incubation types until hatching, or until development had stopped. Data on each of the three environmental parameters (temperature, humidity and position) were collected each minute by the data-logger eggs. The incubation type included: a Whooping Crane pair; a Sandhill Crane pair (for both species this included incubation either by the pair that produced the egg or by a surrogate pair); a George Quail Farm 1527 Sportsman incubator (GQF1; forced air,
180° rotation) and George Quail Farm 1500 Professional/1502 Sportsman incubator (GQF2; forced air incubator, 45° rotation); a Brinsea incubator (X8, contact incubator, 360° rotation); and a Petersime incubator (forced air incubator, 47° rotation). Artificial incubator models vary between each facility due to accessibility, affordability and keeper familiarity. Eggs that had stopped developing were necropsied to determine whether the egg was infertile or if embryo death had occurred. If embryo death had occurred, then the timing of death was estimated using embryo size/stage of growth and video of activity around the nest. When monitoring on a given egg was terminated, we downloaded the data from the data-logger egg using ATS Archive Tag Reader v 1.1.0.6. While all eggs determined to be infertile were excluded from the dataset, the use of macroscopic inspection to determine the fertility status of an egg does have the potential to misclassify early embryo death as infertility (Brown et al. 2019). It is therefore possible that some very early embryo deaths were mistakenly excluded. Table S1 shows the sample sizes for each egg type and incubation type combination.

Data analysis

We analysed the data in a Bayesian framework. Our conceptual model of the system was that hatching success is influenced by incubation conditions, which in turn are influenced by incubation type. Given this, we conducted three analyses to assess (1) the effect of environmental conditions during incubation on hatch success, (2) the effect of incubation type on hatch success and (3) environmental conditions associated with each incubation type. Analyses were conducted using JAGS 4.3.0 (Plummer 2003) in R Version 4.0.3 (R Core Team 2019) with the jagsUI library (Plummer et al. 2018). We assessed convergence based on the Gelman-Rubin statistic (R < 1.1; Gelman et al. 2013) and plots of the MCMC chains.

Prior to analyses using data from data-logger eggs, we sampled every 40th datapoint during each 24-hour period, to minimize autocorrelation in the time series (informed by autocorrelation function plots). The 24-hour period began when the data-logger egg was paired with a crane egg. From the thinned data, we calculated summary statistics including the daily mean temperature (average across the three temperature sensors and across all datapoints within a 24-hour period), the variance of daily temperature (variance across all datapoints within a 24-hour period of the mean value from the three temperature sensors), the daily mean humidity (average across the two humidity sensors and across all datapoints within a 24-hour period), the variance of daily humidity (variance across all datapoints within a 24-hour period of the mean from the two humidity sensors). We also calculated the difference between each pair of consecutive position measurements, in degrees, assuming the minimum measure was applicable (e.g., an egg with consecutive position measurements of 10° and 15° was assumed to have made a 5° rotation rather than a 355° rotation). We then calculated the average and variance within a 24-hour period to get the daily mean absolute change in egg position, and the variance of daily absolute change in egg position.

Once we had summarized environmental parameters at the daily scale, we evaluated the effect of these parameters on daily egg survival. To avoid overly large models, given our sample size, we built models containing only a single environmental parameter. We considered two models for each environmental parameter: a linear relationship and a quadratic relationship. The environmental parameters were normalized, \((X - \bar{X}) / SD\), prior to analysis (Gelman and Hill 2006). The daily egg survival model was specified as:

\[
y_{t,i} \sim \text{Bernoulli}(S_{i,t} \cdot y_{t,i-1}),
\]

\[
\logit(S_{i,t}) = \beta X_{i,t} + \alpha_{\langle i \rangle} + \varepsilon_{t,i},
\]

where \(y_{t,i}\) is data on whether egg \(i\) was alive on day \(t\), \(S_{i,t}\) is the daily egg survival probability, \(\beta X_{i,t}\) is a general form for the modeled relationship between the environmental parameter and daily egg survival, \(\alpha_{\langle i \rangle}\) are fixed effects of facility (ICF, DWCC or PWRC) and \(\varepsilon_{t,i}\) is a normally distributed random deviate for egg type (Sandhill or Whooping Crane egg) crossed with incubation type (Brinsea, Petersime, GQF1, GQF2, Sandhill Crane or Whooping Crane; see Table S1 for sample sizes). The random effect was designed to account for residual variance not accounted for by the environmental parameters. As is typical in nest survival models (e.g., Jehle et al. 2004), \(y_{t,i}\) is sometimes missing because the exact date of egg death was not known; these were estimated through the Markov Chain Monte Carlo (MCMC) algorithm (Converse et al. 2013).

A challenge of the daily egg survival analysis was that some values of environmental parameters were missing because data-logger eggs were sometimes removed before the end of incubation. Therefore, we also included a model of the \(X_{i,t}\) of the form:

\[
X_{i,t} \sim \text{Normal}(\theta_{i,t}, \sigma_{T_{i,t}}),
\]

\[
\theta_{i,t} = \mu + \rho(X_{i,t} - \mu).
\]

The prior distribution for the mean of the environmental parameter, \(\mu\), was normal with mean = 0 and precision = 0.01; the prior for the autoregressive term in the environmental parameter model, \(\rho\), was uniform (0,1); and the priors for the SDs of the environmental parameter, \(\sigma_{T_{i,t}}\) – with a separate SD specific to each egg type crossed with incubation type – were uniform (0, 5). In addition, the priors for the \(\alpha_{\langle i \rangle}\) and \(\beta\) fixed effects in the survival model were normal with mean = 0 and precision = 0.01, and the priors for the SD of the \(\varepsilon_{t,i}\) egg type within incubation type random deviates was uniform (0, 25). For all egg survival models, we ran four Markov chains for 300 000 iterations, with a burn-in period of 220 000 iterations. For model selection purposes, we calculated the efficient approximate leave-one-out (LOO) cross-validation statistic using LOO 2.3.1 (Vehtari et al. 2020) and compared the LOO values for the two models – quadratic and linear – for a given environmental parameter.
In our second analysis, we evaluated the effect of incubation type on overall hatching outcome. For this, we built a generalized linear model evaluating the effects of the incubation types on the hatching outcomes (hatch/fail). The model contained only incubation type (Brinsea, Petersime, GQF1, GQF2, Sandhill Crane, Whooping Crane) as a categorical fixed effect. We assumed the response variable, hatching outcome, was Bernoulli-distributed and we used a logit link function. For the intercept we set our priors to uniform (−10,10), and for the model coefficients we used normal priors with mean 0 and precision 0.01. For all models we ran four Markov chains for 3,000,000 iterations, with a burn-in period of 2,000,000 iterations.

Finally, we modelled the effect of incubation type on the average daily mean and variance of each environmental parameter observed during incubation using linear mixed models. The model contained incubation type (Brinsea, Petersime, GQF1, GQF2, Sandhill Crane, Whooping Crane), facility (ICF, DWCC, PWRC) and egg type (Sandhill Crane or Whooping Crane) as fixed effects. We assumed the environmental parameters were normally distributed with mean, \( \mu \), and SD, \( \sigma \). Each mean, \( \mu \), was modelled as a function of treatment, facility and species. We used a uniform (0,100) prior on the SD, \( \sigma \) and for the other parameters we used normal priors with mean 0 and precision 0.01. For all models we ran four Markov chains for 300,000 iterations, with a burn-in period of 220,000 iterations.

**Simulation study**

We developed a stochastic simulation to predict the expected number of hatched eggs for each incubation type. We simulated the number of hatched eggs that would result from a captive Whooping Crane flock of 10 breeding pairs if the eggs were incubated solely by Whooping Cranes, by 5, 10 or 15 Sandhill Crane pairs, or by the best performing artificial incubator (GQF1) at one breeding centre. We used the MCMC samples from the posterior distributions of predicted hatching outcome by incubation type, thereby accounting for uncertainty. The simulation progressed by looping through each pair, wherein each pair would lay eggs, eggs would either be incubated by the pair or diverted to another incubation method, and eggs would then be subjected to a stochastic survival process. Incubation method decisions and survival of eggs determined when Whooping Cranes could lay additional eggs, or whether Sandhill Cranes were available to foster incubate eggs. We assumed in our simulation the annual laying period was 60 days, the capacity in the GQF1 incubator was 25 eggs and the maximum number of eggs a Whooping Crane pair could lay was 6. The minimum number of days a Whooping Crane would lay an egg after laying an egg or finishing incubation of an egg was assumed to be 10 days (and that the pair would lay within one of the following 10 days with a multinomial probability of 0.1 each day), and an egg would not be diverted to another incubation type if the Whooping Crane pair laid an egg within 10 days of the end of the breeding season (i.e., at the end of the season, Whooping Cranes would be allowed to incubate their own egg). We further assumed Whooping Crane and Sandhill Crane pairs incubated single eggs and that a hand rearing method would be used to rear chicks, leaving Whooping Cranes available to incubate throughout the season.

Additionally, we calculated the monetary cost per year for each incubation type, based on expert judgements of the animal care staff at the DWCC, ICF and PWRC (See Table S4 for cost details). For all incubation types we included the monetary cost per year of feeding, housing and caring for a flock of 10 Whooping Crane pairs to produce the eggs for incubation. The monetary cost for housing included the capital cost of a pen divided by a predicted lifespan of 25 years, while the care cost included keeper and veterinarian time. For the artificial incubators, we calculated the monetary cost per year as the purchase cost of the incubator plus a lifetime upkeep cost divided by a predicted lifespan of 25 years. This monetary cost was calculated for the artificial incubator that had the highest predicted probability of hatching an egg from our prior analyses. For Sandhill Crane incubation, we included the same feeding, housing and care cost parameters as for Whooping Crane incubation and calculated this for 5, 10 or 15 pairs. Due to the inclusion of the monetary cost per year for 10 Whooping Crane pairs into all of the incubation-type scenarios, no additional costs were associated with the Whooping Crane incubation type. We plotted the expected number of hatched eggs from the simulation against the yearly monetary cost to visualize the trade-off between the number of hatched eggs and monetary cost.

**Results**

For all the environmental parameters, a linear relationship between the environmental parameter and daily egg survival was a better fit than a quadratic model (Table S2). Daily mean temperatures were negatively associated with daily egg survival probability, with 70% of eggs successfully hatching when daily mean temperature was <33.64°C (Fig. 1 and Table S5). Variance in daily temperature was not associated with hatching outcome (Table S6) based on the 95% credible interval for the coefficient including 0. The daily mean humidity and the variance of daily humidity were also not associated with hatching outcome (Table S7 and S8). The daily mean absolute change in egg position per day and the variance of daily absolute change were both positively associated with hatching outcome (Table S9 and S10). Additionally, we calculated the monetary cost per year for each incubation type, based on expert judgements of the animal care staff at the DWCC, ICF and PWRC (See Table S4 for cost details). For all incubation types we included the monetary cost per year of feeding, housing and caring for a flock of 10 Whooping Crane pairs to produce the eggs for incubation. The monetary cost for housing included the capital cost of a pen divided by a predicted lifespan of 25 years, while the care cost included keeper and veterinarian time. For the artificial incubators, we calculated the monetary cost per year as the purchase cost of the incubator plus a lifetime upkeep cost divided by a predicted lifespan of 25 years. This monetary cost was calculated for the artificial incubator that had the highest predicted probability of hatching an egg from our prior analyses. For Sandhill Crane incubation, we included the same feeding, housing and care cost parameters as for Whooping Crane incubation and calculated this for 5, 10 or 15 pairs. Due to the inclusion of the monetary cost per year for 10 Whooping Crane pairs into all of the incubation-type scenarios, no additional costs were associated with the Whooping Crane incubation type. We plotted the expected number of hatched eggs from the simulation against the yearly monetary cost to visualize the trade-off between the number of hatched eggs and monetary cost.
All artificial incubators had a higher mean temperature and a lower variation in temperature than Sandhill Crane incubation (Table S12 and S13). Whooping Crane incubation had a higher variation in temperature than Sandhill Crane incubation (Table S13). The Petersime incubator and Whooping Crane incubation had a higher humidity mean, and the Brinsea incubator a lower humidity mean than Sandhill incubation (Table S14). The variation in humidity was lower for the Petersime, GQF1 and GQF2 incubators, and higher for Whooping Crane incubation than Sandhill Crane incubation (Table S15). Finally, the mean absolute change in egg position was lower for the Brinsea incubators, and higher for the GQF1 incubator than Sandhill Crane incubation (Table S16). The variance of absolute change in egg position was lower for the Petersime, GQF1 and GQF2 incubators, and higher for Whooping Crane incubation than Sandhill Crane incubation (Table S17).

Of the simulated incubation scenarios, 15 Sandhill Cranes produced the most hatched eggs on average (mean = 34.26, 95% quantiles = 26.43–40.53). The GQF1 incubator was close behind, with an average of 34.07 eggs hatched (95% quantiles = 21–42). However, the cost of 15 Sandhill Cranes was CA$253699.83 per year while the cost of the GQF1 was CA$104364.39 per year (Figure 3). The cheapest incubation option was Whooping Cranes (CA$104268.72 per year) but production declined substantially (mean = 16.52; 95% quantiles = 9.48–20) with this option, partly because birds do not produce eggs while incubating, and partly because Whooping Crane incubation resulted in lower overall hatching success than Sandhill Crane incubation and the GQF1 incubator. Other options we evaluated included five pairs of Sandhill Cranes (production = 28.46, 95% quantiles = 22.48–35; cost = CA$162804.65 per year) and 10 pairs of Sandhill Cranes (production = 33.28, 95% quantiles = 24.48–39; cost = CA$208252.24 per year). Both of these latter two options are dominated alternatives (Converse 2020), that is, they result in lower production while costing more than the GQF1, thus a logical decision-maker would not choose these options.

Discussion

A poor understanding of breeding biology can contribute to hatch failure in avian captive breeding programmes and subsequently lower the number of individuals available to maintain the captive population or to release as part of a conservation translocation programme. In this study, we found that the daily mean temperature during the incubation period is an important factor in predicting hatching outcome, and that Whooping Crane and Sandhill Crane eggs had a higher probability of hatching when incubated by Sandhill Cranes than when incubated by Whooping Cranes or artificial incubators. However, to maximize the number of hatched eggs and minimize monetary cost, artificial incubation by a GQF1 incubator was identified as the incubation type that achieved the best trade-off between breeding output and monetary cost.

Eggs incubated by a Sandhill Crane had the highest probability of hatching, slightly higher than incubation in the GQF1 artificial incubator, thus our results support Smith et al.’s (2011) finding that eggs subjected to crane incubation (Whooping or Sandhill) were more likely to hatch than eggs subjected to artificial incubation. Similar relationships have
been observed in other avian species including Peregrine Falcons (Falco peregrinus, Burnham 1983), Brown Kiwi (Apteryx mantelli, Robertson et al. 2006) and Hawaiian Crows (Corvus hawaiiensis, Hoeck et al. 2015). This association is likely due to the selection of parental incubation behaviours that optimize environmental conditions and thus embryo development (discussed belowRomanoff 1972; Ar and Rahn 1980; Ar and Sidis 2002; Deeming 2002c), and suggests that captive Sandhill Cranes exhibit better incubation behaviour (high nest and egg attendance) to optimize these environmental parameters than captive Whooping Cranes. Furthermore, natural incubation may facilitate embryo development via parental effects (when the offspring’s phenotype is influenced by its parents, independent of the direct effects of the genes contributed by them), such as prenatal acoustic communication between the embryo and parent (Mariette and Buchanan 2016).

When investigating the association between the environmental parameters experienced during incubation and hatching outcome, we found that daily incubation temperatures were negatively associated with hatching outcome. We should note that our model only looked at acute effects, not cumulative effects of the environmental parameters on survival. Extreme temperatures during avian incubation can reduce hatching success (Hepp et al. 2006; DuRant et al. 2010; Nord and Nilsson 2011; Carroll et al. 2018) and even have negative long-term fitness consequences (e.g., DuRant et al. 2013). For example, variation in temperature during incubation are associated with differences in embryonic development (Olson et al. 2008; DuRant et al. 2011), and differences in immunocompetence (DuRant et al. 2012b), thermoregulation (DuRant et al. 2012a), metabolic rate (Nord and Nilsson 2011), locomotor performance (Hopkins et al. 2011), growth (Nord and Nilsson 2011), early life behaviour (Hope et al. 2019), reproduction (Hepp and Kennamer 2012) and survival (Hepp and Kennamer 2012; Berntsen and Bech 2016; Nord and Nilsson 2016) in young birds. This highlights the importance of investigating optimal incubation parameters with technology such as data-loggers and with the continued improvement of this technology, application in captive breeding programmes for other avian species will be possible and valuable. Our results suggest that temperatures >33.64 °C are not beneficial for Sandhill and Whooping Crane eggs. An open, and important, question is how the incubation environment affects fitness of hatchlings, as this may have consequences for the success of conservation translocation programmes.

Smith et al. (2012) suggested that a more successful artificial incubator would incubate at a lower temperature, would have greater variance in temperature, and have a rate of egg turning that allowed for a more even representation of egg positions relative to current models. We have now demonstrated an association between high daily temperatures and decreased daily egg turning during incubation and low hatch success. Therefore, in addition to Smith et al’s (2012) recommendations for Whooping Crane artificial incubation protocols to include a dry-bulb temperature of 33–34°C and machine turning of the eggs, to maximize hatching success our results indicate that that daily mean temperatures remain below 33.64°C (the temperature at which 70% of eggs successfully hatch).

Our stochastic simulation results predicted that 15 Sandhill Cranes would produce the most hatched eggs, closely followed by a GQF1 incubator. The cheapest incubation option, Whooping Cranes, was also the option associated with the lowest production of eggs. Each of these three incubators represent possible trade-offs between cost and production that may appeal to a given decision-maker, depending on how the decision-maker weighs costs versus production. Moving from Whooping Crane incubation to GQF1 incubation required a small increase in cost (CAS$95.67 per year, or <0.1% of the cost of supporting a flock of 10 Whooping Crane pairs) but results in a substantial increase in production (17.6 hatched eggs, or >106% increase in hatched eggs over Whooping Crane incubation). Moving from a GQF1 to 15 Sandhill Crane pairs required a substantial increase in cost (CAS$253699.83 per year, or >143% increase in cost compared to GQF1 incubation) while only giving a small benefit in production (0.19 hatched eggs, or <0.6% increase over GQF1 incubation). Identifying the optimal incubation decision via a formal multi-criteria decision analysis would require that we know the relative importance of production versus cost for a particular decision-maker (Converse 2020); however, in practice, the GQF1 incubator represents a

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**Figure 3** The number of eggs hatch under each incubation type versus the monetary cost per year (CAD). The incubation types were 5, 10 and 15 pairs of Sandhill Cranes, a GQF1 incubator and 10 pairs of Whooping Cranes.
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practically dominating alternative (Fig 4), such that few or no decision-makers are likely to favour either of the other options. Consequently, additional improvements might be made by making the conditions in the GQF1 artificial incubator more similar to those under a Sandhill Crane, though these improvements would be small. By using this cost-benefit approach in avian captive rearing practises, captive managers can make informed decisions and identify their preferred trade-off between cost and production of individuals available for conservation translocation programmes.

There are some considerations that should be noted about the cost benefit analysis. Egg production is costly in terms of time, energy and nutrients and so a trade-off exists between maximising the number of hatched eggs and parental fitness (Monaghan and Nager 1997). A further consideration is the potentially important biological consequences of using Whooping Crane incubation for the release candidate. Avian embryos respond to acoustic stimuli (Colombelli-Négrel et al. 2012; Colombelli-Négrel and Kleindorfer 2017) and this exposure to conspecific vocalizations is associated with development and postnatal behavior (Colombelli-Négrel et al. 2012; Mariette and Buchanan 2016; Katsis et al. 2018), and can have consequences for later reproductive success (Mariette and Buchanan 2016). We should note, however, that a limiting factor on Whooping Crane incubation is the number of reliable incubating pairs in the captive flock. Whooping Cranes cannot lay as many eggs if they are rearing their own chicks, suggesting that the gains seen in production when moving from Whooping Crane to artificial incubation might in practice be smaller than our simulations predict. This limitation highlights the potential trade-offs for the captive breeding release programme between producing high numbers of release candidates and increasing their long-term fitness prospects. It may be of benefit to consider these trade-offs when identifying the most effective captive management option for this species and other avian species.

To conclude, we have shown that low daily temperatures (<33.64°C) during the incubation period is an important factor in predicting hatching outcome for Whooping Crane. We have also shown that eggs incubated by Sandhill Cranes have the highest probability of hatching relative to incubation by Whooping Cranes or artificial incubators. However, artificial incubation may represent, in practice, a favourable trade-off between production and cost. Overall, we encourage other captive breeding programmes to not only use innovations that help to increase potential release numbers for conservation translocations, but also conduct cost-benefit trade-off analyses to identify optimal captive breeding practises.

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Data Availability Statement

Code is available on GitHub [https://github.com/Quantitative-Conservation-Lab/Edwards_etal_2021_AnimalConservation] and both data and code are available on Figshare [https://doi.org/10.6084/m9.figshare.14640267.v1].

References

Ar, A. & Rahn, H. (1980). Water in the avian egg: overall budget of incubation. Am. Zool. 20, 373–384.

Ar, A. & Sidis, Y. (2002). Nest microclimate during incubation. In Deeming, D.C. (Ed) Avian incubation: behaviour, environment and evolution (pp 143–160). Oxford: Oxford University Press.

Araki, H., Cooper, B. & Blouin, M.S. (2007). Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318, 100–103.

Bemtsen, H.H. & Bech, C. (2016). Incubation temperature influences survival in a small passerine bird. J. Avian Biol. 47, 141–145.

Black, S.R. & Swan, K.D. (2019). Advances in conservation breeding and management of whooping cranes (Grus Americana). In French, J.B., Converse, S.C. & Austin, J.E. (Eds) Whooping Cranes: Biology and Conservation. Biodiversity of the World: Conservation from Genes to Landscapes (pp 355–371). London, UK: Academic Press.

Brichteri-Colombi, T.A., Lloyd, N.A., McPherson, J.M. & Moehrensclager, A. (2019). Limited contributions of released animals from zoos to north American conservation translocations. Conserv. Biol. 33, 33–39.
Brown, M.E., Converse, S.J., Chandler, J.N., Brown, C., Keefer, C.L. & Songsasen, N. (2016). Female gonadal hormones and reproductive behaviours as key determinants of successful reproductive output of breeding whooping cranes (Grus americana). *Gen. Comp. Endocrinol.* **230**, 158–165.

Brown, M.E., Keefer, C.L. & Songsasen, N. (2019). Factors affecting Whooping Crane egg fertility: a retrospective analysis. *J. Wildl. Manag.* **83**, 1377–1386.

Burnham, W. (1983). Artificial incubation of falcon eggs. *J. Wildl. Manag.* **47**, 158.

Carroll, R.L., Davis, C.A., Fuhlendorf, S.D., Elmore, R.D., DuRant, S.E. & Carroll, J.M. (2018). Avian parental behavior and nest success influenced by temperature fluctuations. *J. Therm. Biol.* **74**, 140–148.

Colombelli-Négrel, D., Hauber, M.E., Robertson, J., Sulloway, F.J., Hoi, H., Griggio, M. & Kleindorfer, S. (2012). Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Curr. Biol.* **22**, 2155–2160.

Colombelli-Négrel, D. & Kleindorfer, S. (2017). Prenatal environment affects embryonic response to song. *Biol. Lett.* **13**, 20170302.

Converse, S.J. (2020). Introduction to multi-criteria decision analysis. In Runge, M.C., Converse, S.J., Lyons, J.E. & Smith, D.R. (Eds) *Structured decision making: Case studies in natural resource management* (pp 51–61). Baltimore, MD: John Hopkins University Press.

Converse, S.J., Chandler, J.N., Olsen, G.H. & Shafer, C.C. (2010). Evaluating propagation method performance over time with Bayesian updating: an application to incubator testing. Hartup, B.K. & Urbanek, R.P. (Eds). Baraboo, WI: International Crane Foundation.

Converse, S.J., Royle, J.A., Adler, P.H., Urbanek, R.P. & Barzen, J.A. (2013). A hierarchical nest survival model integrating incomplete temporally-varying covariates. *Ecol. Evol.* **3**, 4439–4447.

Deeming, D.C. (2002a). Importance of evolution and incubation in avian reproduction. In Demming, D.C. (Ed) *Avian incubation: behaviour, environment and evolution* (pp 1–7). Oxford: Oxford University Press.

Deeming, D.C. (2002b). Patterns and significance of egg turning. In Demming, D.C. (Ed) *Avian incubation: behaviour, environment and evolution* (pp 161–178). Oxford: Oxford University Press.

Deeming, D.C. (2002c). Embryonic development and utilisation of egg components. In Demming, D.C. (Ed) *Avian incubation: behaviour, environment and evolution* (pp 43–53). Oxford: Oxford University Press.

DuRant, S.E., Hepp, G.R., Moore, I.T., Hopkins, B.C. & Hopkins, W.A. (2010). Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (Aix sponsa) ducklings. *J. Exp. Biol.* **213**, 45–51.

DuRant, S.E., Hopkins, W.A., Hawley, D.M. & Hepp, G.R. (2012b). Incubation temperature affects multiple measures of immunocompetence in young wood ducks (Aix sponsa). *Biol. Lett.* **8**, 108–111.

DuRant, S.E., Hopkins, W.A. & Hepp, G.R. (2011). Embryonic developmental patterns and energy expenditure are affected by incubation temperature in wood ducks (Aix sponsa). *Physiol. Biochem. Zool.* **84**, 451–457.

DuRant, S.E., Hopkins, W.A., Hepp, G.R. & Walters, J.R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol. Rev. Camb. Philos. Soc.* **88**, 499–509.

DuRant, S.E., Hopkins, W.A., Wilson, A.F. & Hepp, G.R. (2012a). Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird. *Func. Ecol.* **26**, 416–422.

Erickson, D.H. & Derrickson, S. (1981). The whooping crane. In Lewis, J.C. (Ed) *Crane Research around the World* (pp 103–134). Baraboo, WI: International Crane Foundation.

French, J.B. Jr, Converse, S.J. & Austin, J.E. (Eds) *Whooping cranes: biology and conservation*. Biodiversity of the world: conservation from genes to landscapes (pp 3–16). London, UK: Academic Press.

Gabel, G.F. & Mahan, T.A. (1996). Incubation and hatching. In Ellis, D.H., Gee, G.F. & Miranda, C.M. (Eds) *Cranes: their biology, husbandry and conservation*. Washington, DC: US Government Printing Office.

Gabel, G.F. & Mahan, T.A. (1996). Incubation and hatching. In Ellis, D.H., Gee, G.F. & Miranda, C.M. (Eds) *Cranes: their biology, husbandry and conservation*. Washington, DC: US Government Printing Office.

Gelman, A. & Hill, J. (2006). *Data analysis using regression and multilevel/hierarchical models*. Cambridge: Cambridge University Press.

Hepp, G.R. & Kennamer, R.A. (2012). Warm is better: Incubation temperature influences apparent survival and recruitment of wood ducks (Aix sponsa). *PLoS One* **7**, e47777.

Hepp, G.R., Kennamer, R.A. & Johnson, M.H (2006). Maternal effects in Wood Ducks: incubation temperature influences incubation period and neonate phenotype. *Func. Ecol.* **20**, 308–314.

Hoeck, P.E.A., Wolak, M.E., Switzer, R.A., Kuehler, C.M. & Lieberman, A.A. (2015). Effects of inbreeding and parental incubation on captive breeding success in Hawaiian crows. *Biol. Cons.* **184**, 357–364.

Hope, S.F., Kennamer, R.A., van Montfrans, S.G. & Hopkins, W.A. (2019). Incubation temperature and social context affect the nest exodus of precocial ducklings. *Behav. Ecol.* **30**, 518–527.

Hopkins, B.C., DuRant, S.E., Hepp, G.R. & Hopkins, W.A. (2011). Incubation temperature influences locomotor performance in young wood ducks (Aix sponsa). *Journal of...*
Experimental Zoology Part A: Ecological Genetics and
Physiology 315A, 274–279.
IUCN/SSC. (2013) Guidelines for Reintroductions and Other
Conservation Translocations. Version 1.0. Gland,
Switzerland: IUCN Species Survival Commission, viii + 57
pp.
Jehle, G., Yackel Adams, A.A., Savidge, J.A. & Skagen, S.K.
(2004). Nest survival estimation: a review of alternatives to
the Mayfield estimator. Condor 106, 472–484.
Katsis, A.C., Davies, M.H., Buchanan, K.L., Kleindorfer, S.,
Hauber, M.E. & Mariette, M.M. (2018). Prenatal exposure
to incubation calls affects song learning in the zebra finch.
Sci. Rep. 8, 15232.
Mariette, M.M. & Buchanan, K.L. (2016). Prenatal acoustic
communication programs offspring for high posthatching
temperatures in a songbird. Science 353, 812–814.
McDougall, P.T., Réale, D., Sol, D. & Reader, S.M. (2006).
Wildlife conservation and animal temperament: causes and
consequences of evolutionary change for captive,
reintroduced, and wild populations. Anim. Conserv. 9, 39–48.
Mirande, C.M., Gee, G.F., Swengel, S.R. & Whitlock, P.
(1996). Egg and semen production. In: Ellis, D.H., Gee,
G.F. & Mirande, C.M. (Eds.), Cranes: their biology,
husbandry and conservation. department of the interior (pp
175–183). Washington, DC, Baraboo, WI: National
Biological Service, International Crane Foundation.
Monaghan, P. & Nager, R.G. (1997). Why don’t birds lay
more eggs? Trends Ecol. Evol. 12, 270–274.
Nord, A. &Nilsson, J.-Å. (2011). Incubation temperature
affects growth and energy metabolism in blue tit nestlings.
Am. Nat. 178, 639–651.
Nord, A. & Nilsson, J.-Å. (2016). Long-term consequences of
high incubation temperature in a wild bird population. Biol.
Let. 12, 20160087.
Olson, C.R., Vleck, C.M. & Adams, D.C. (2008). Decoupling
morphological development from growth in periodically
cooled zebra finch embryos. J. Morphol. 269, 875–883.
Plummer, M. (2003). JAGS. A program for analysis of
Bayesian graphical models using Gibbs sampling. Working
Papers 8.
Plummer, M. (2018). rjags: Bayesian Graphical Models using
MCMC. R package version 4–8. http://CRAN.R-project.org/
package=rjags.
R Core Team (2019). R: A language and environment for
statistical computing. Vienna, Austria: R Foundation for
Statistical Computing https://www.R-project.org/.
Ralls, K., Ballou, J.D. & Templeton, A. (1988). Estimates of
lethal equivalents and the cost of inbreeding in mammals.
Conserv. Biol. 2, 185–193.
Robertson, H., Colbourne, R., Nelson, A. & Westbrooke, I.M.
(2006). At what age should brown kiwi (Apteryx mantelli) eggs
be collected for artificial incubation? Notornis 53, 231–234.
Romanoff, A. (1972). Pathogenesis of the avian embryo: a
quantitative analysis of causes of malformations and
prenatal death. New York, NY: John Wiley and Sons.
Smith, D.H.V., Converse, S.J., Gibson, K.W.,
Moehrensclager, A., Link, W.A., Olsen, G.H. & Maguire,
K. (2011). Decision analysis for conservation breeding:
maximizing production for reintroduction of whooping
cranes. J. Wildl. Manag. 75, 501–508.
Smith, D.H.V., Moehrensclager, A., Christensen, N., Knapik,
D., Gibson, K. & Converse, S.J. (2012). Archive eggs: a
research and management tool for avian conservation
breeding. Wildl. Soc. Bull. 36, 342–349.
Snyder, N.F.R., Derrickson, S.R., Beissinger, S.R., Wiley,
J.W., Smith, T.B., Toone, W.D. & Miller, B. (1996).
Limitations of captive breeding in endangered species
recovery. Conserv. Biol. 10, 338–348.
van Heezik, Y., Lei, P., Maloney, R. & Sancha, E. (2005).
Captive breeding for reintroduction: influence of
management practices and biological factors on survival of
captive kaki (black stilt). Zoo Biology 24, 459–474.
Vehtari, A., Gabry, J., Magnusson, M., Yao, Y., Bürkner, P.,
Paananen, T. & Gelman, A. (2020). loo: Efficient leave-one-
out cross-validation and WAIC for Bayesian models. R
package version 2.3.1.

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