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# EVALUATING PROPAGATION METHOD PERFORMANCE OVER TIME WITH BAYESIAN UPDATING: AN APPLICATION TO INCUBATOR TESTING

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***Abstract:*** : In captive-rearing programs, small sample sizes can limit the quality of information on performance of propagation methods. Bayesian updating can be used to increase information on method performance over time. We demonstrate an application to incubator testing at USGS Patuxent Wildlife Research Center. A new type of incubator was purchased for use in the whooping crane (*Grus americana*) propagation program, which produces birds for release. We tested the new incubator for reliability, using sandhill crane (*Grus canadensis*) eggs as surrogates. We determined that the new incubator should result in hatching rates no more than 5% lower than the available incubators, with 95% confidence, before it would be used to incubate whooping crane eggs. In 2007, 5 healthy chicks hatched from 12 eggs in the new incubator, and 2 hatched from 5 in an available incubator, for a median posterior difference of <1%, but with a large 95% credible interval (-41%, 43%). In 2008, we implemented a double-blind evaluation method, where a veterinarian determined whether eggs produced chicks that, at hatching, had no apparent health problems that would impede future release. We used the 2007 estimates as priors in the 2008 analysis. In 2008, 7 normal chicks hatched from 15 eggs in the new incubator, and 11 hatched from 15 in an available incubator, for a median posterior difference of 19%, with 95% credible interval (-8%, 44%). The increased sample size has increased our understanding of incubator performance. While additional data will be collected, at this time the new incubator does not appear adequate for use with whooping crane eggs.

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**Key words:** Bayesian updating, exposed yolk sac, hatching success, incubator, sandhill crane, whooping crane.

Whooping cranes (*Grus americana*) are listed as federally endangered under the U.S. Endangered Species Act, and as of September 2008 there were fewer than 400 of the birds in the wild, with approximately 150 more in captivity (T. Stehn, U.S. Fish and Wildlife Service, unpublished data). Free-roaming whooping cranes exist in 3 distinct populations, including the naturally-occurring Aransas-Wood Buffalo population (AWBP), which breeds at Wood Buffalo National Park, Canada, and winters at Aransas National Wildlife Refuge on the Texas coast. The 2 additional populations, including the Florida non-migratory population in central Florida (FNMP), and the eastern migratory population (EMP), which migrates between Wisconsin and Florida, are the product of reintroductions using chicks hatched in captivity.

Captive whooping cranes are located at 5 breeding centers: USGS Patuxent Wildlife Research Center, Laurel, Maryland (PWRC); the International Crane Foundation, Baraboo, Wisconsin; the Calgary Zoo, Calgary, Alberta; the Audubon Species Survival Center, Belle Chase, Louisiana; and the San Antonio Zoo, San Antonio, Texas (CWS and USFWS 2007). The largest population is located at PWRC, with 64 birds >1 year old as of July 2008. PWRC has produced whooping crane chicks for release both to the FNMP from 1993-2004, and to the EMP from 2001-present. PWRC's annual productivity is a large determinant of the total number of birds available for release in any year. It is critical for meeting release goals that PWRC develop methods to produce the greatest possible number of healthy chicks for release.

To ensure that the methods employed in the captive breeding of whooping cranes are most likely to produce the largest numbers of healthy chicks, it is necessary to regularly evaluate breeding and rearing methods. However, it is often difficult to achieve rigorous evaluations (i.e., make rigorous predictions of management outcomes) for 2 reasons: 1) among managers there is often interest in avoiding experimentation with endangered species, especially, as in this case, with chicks that are needed in a release program, and 2) sample sizes in a captive setting are often limited. To address the first issue, PWRC maintains a flock of sandhill cranes for use as experimental

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surrogates (as well as surrogate egg incubators). To address the second issue, Bayesian statistical methods can be used to facilitate learning and decision-making over time, through Bayesian updating.

Bayesian data analysis differs most fundamentally from traditional, frequentist methods in the interpretation of parameter estimates obtained using these methods (Link et al. 2002). Through the application of Bayes formula,

$$f(\theta|Y) = \frac{f(Y|\theta)\pi(\theta)}{\int f(Y|\theta)\pi(\theta)d\theta}$$

and prior information, $\pi(\theta)$, the posterior distribution of the parameter, $f(\theta|Y)$, can be estimated and interpreted as a random variable, rather than as a fixed, unknown quantity as in the likelihood estimates, $f(Y|\theta)$, familiar to frequentists. The value of this property is that probability statements can be made about the parameter itself without requiring the somewhat logically troubling statements that frequentists must use in their interpretation of parameter estimates (e.g., correct interpretation of a frequentist confidence interval relies on invoking many similarly-calculated intervals; see Link et al. 2002 for elaboration on this point). In addition, while many Bayesian analysts invoke noninformative, or flat, priors and thus obtain posterior estimates that are similar to frequentist estimates (an approach known as Objective Bayes), the prior does allow for explicit inclusion of existing knowledge, and updating of that knowledge with subsequent data collection. This property is particularly useful in an iterative management setting, where periodic management decisions are made based on the current state of knowledge. As such, Bayes formula comprises a critical tool in formal adaptive management (Williams et al. 2002).

At PWRC, artificial incubators are critical for incubating crane eggs. For example, eggs that will produce chicks that are to be released into the EMP are incubated artificially during the final portion of incubation so that they imprint on costumed handlers and ultralight aircraft (ultralights have been used to teach chicks the migratory route, Urbanek et al. 2005). Additionally, incubators can be used to increase productivity; eggs can be removed from whooping crane nests and placed in incubators, which encourages whooping crane pairs to produce an additional clutch. In 2005, PWRC purchased a new model of incubator as a potential replacement for existing incubators that, while reliable at the time, were of an age that replacement parts were no longer available when and if repairs were needed. Before using the incubator to incubate whooping crane eggs, it was necessary to evaluate its efficacy. Here, we demonstrate the use of Bayesian updating to inform annual decision-making about whether a newly purchased incubator should be adopted for use in the whooping crane breeding program.

## STUDY SUBJECTS

PWRC maintains a colony of endangered whooping cranes and a colony of non-endangered captive sandhill cranes to use as experimental subjects and surrogate incubators. The sandhill cranes have been used to test medications (Carpenter et al. 1992) and vaccines (Clark et al. 1987), equipment (Olsen et al. 1992), husbandry techniques (Chen et al. 2001) and release techniques (Ellis et al. 1997). They also serve as surrogate incubators of whooping crane eggs, encouraging whooping cranes to lay multiple clutches annually (Mirande et al. 1996). Experimental protocols for this work were approved by the USGS Patuxent Wildlife Research Center Animal Care and Use Committee.

The PWRC crane colony is located on lands managed by the U.S. Fish and Wildlife Service near Laurel, Maryland. The captive crane colony is located in an area secluded from public use, with breeding pairs of cranes housed in chain link pens, in complexes surrounded by electric perimeter fences. Mechanical incubators and hatchers are housed in a concrete block, climate-controlled building within 1 km of the crane pens.

## METHODS

Based on discussions, the crane flock manager (JNC) developed a decision rule that, if the success rate of the new incubator was no more than 5% poorer than the existing incubator, with 95% confidence, the new incubator would be adopted for use in the whooping crane breeding program. Success was identified as hatching of a chick with no health problems that would preclude it from future release. Until the 5% criterion was met, additional data collection would be conducted and, if indications were that the incubator was not performing well, exploration of additional alternatives

would be undertaken.

## Incubators

The study incubators consisted of an aging Petersime incubator (Model 4, Petersime Incubator Company, Gettysburg, OH) and a new Kuhl forced air incubator (Model AZYSS-600-110, Kuhl Corporation, Flemington, NJ). Both incubators were set at a dry bulb temperature of 37.5°C and wet bulb temperature of 30.6°C, and maintained within 0.3°C (dry bulb) and 1°C (wet bulb). Incubators were housed side by side in a room where the temperature and humidity averaged 26.1°C and 52%, respectively. An emergency generator automatically provided power to the incubators in the event of power failure. Once a week, incubators were cleaned with a Nolvasan solution (29 mL Nolvasan/3.8 L water) and fumigated using 17.5g of potassium permanganate and 35 mL of 40% formalin per 2.83 $m^3$ of incubator volume for 20 minutes. Eggs that were 5 days or younger and eggs with a chick in the air cell were temporarily moved to a different incubator during fumigation.

The Petersime is a forced-air incubator which contains a rotating drum, houses 6 egg-storage drawers and has a total interior volume of 0.91 $m^3$. Eggs were rotated every 2 hours, at 47° above and below the horizontal. Temperature was controlled by a mercury contact thermometer system and humidity was supplied by water trays. The Petersime incubator is the primary incubator used in the whooping crane propagation program, so it contained whooping crane eggs, in addition to the sandhill crane eggs, during the experiment.

The Kuhl, also a forced-air incubator, has 5 egg trays and an interior volume of 0.68 $m^3$. The top tray of the Kuhl allowed the eggs to roll slightly during rotation. These eggs effectively rotated 34° above and below the horizontal every hour. All other eggs were held stationary in the trays. Eggs in the other 4 trays were rotated every hour at 25° above and below the horizontal. Temperature and humidity were controlled electronically by a Zytron controller (Humi-Temp Series 516, Zytron Control Products Inc., Trenton, NJ). Humidity was supplied by an automatic humidifier (Model HUM.RK, Lyon Technologies Inc., Chula Vista, CA).

## Experimental Design

In all of the trials, eggs were taken from nests of sandhill crane pairs located in pens at the PWRC crane facility and placed in the incubators. Sandhill cranes begin producing eggs in late February and finish in late June, and typically produce 2-egg clutches. Because cranes will produce multiple clutches if their eggs are removed, in many cases more than 2 eggs were contributed to the experiment by a pair each year. Eggs in the incubators were periodically evaluated through candling to determine fertility and viability. If the egg was not alive, it was dissected and determinations were made regarding: 1) whether the egg was infertile, and if not 2) the age of the embryo at death. Any eggs that were either infertile or died before they were placed in the incubators were eliminated from analyses.

Sandhill crane eggs typically hatch between 28 and 34 days of age (Gabel and Mahan 1996). The hatching process takes about 48 hours, beginning when the chick breaks into the air cell and begins audible vocalizing (peeping), continues to pipping (when the chick begins making a hole in the shell, ~24 hours later) and is completed when the chick emerges (Hartman et al. 1987). Once an egg pips at PWRC, it is transferred to a hatcher, where it is checked frequently throughout the day.

In 2006, sandhill crane eggs were placed in the incubators between 0 and 8 days of age. Two-egg clutches ($n = 9$) were divided, with 1 egg of each clutch going into the Kuhl incubator and 1 egg into the Petersime incubator; eggs were assigned systematically, with the older of the pair of eggs assigned first to the Petersime, then to the Kuhl, etc. Single eggs ($n = 6$) were placed in the Kuhl. Eggs remained in the incubators until the embryos broke into the air cell. At this point, the majority of the eggs were euthanized. However, a few eggs from each incubator were allowed to hatch before euthanasia, and it was noticed that some of the chicks from the Kuhl incubator had exposed yolk sacs, a potentially critical health problem. Therefore, it was determined that the appropriate end-point in additional trials would be after hatching of the eggs, and the 2006 data were not used further in these analyses.

In 2007, sandhill crane eggs were placed in the incubators between 0 and 8 days of age. Two egg clutches ($n = 7$) were divided and assigned as in 2006

and all single eggs ($n$ = 7) were again placed in the Kuhl. After hatching, chicks were examined by staff members for any physical abnormalities and then were euthanized.

In 2008, sandhill crane eggs were placed in the incubators between 1 and 11 days of age. Again, 2-egg clutches ($n$ = 13) were divided and assigned systematically, with 1 egg from each clutch being placed in each incubator. However, in 2008 both incubators also received equal numbers of single eggs ($n$ = 5 per incubator), with the first available egg randomly assigned, and additional eggs then systematically assigned alternately to each incubator. Again, chicks were allowed to hatch and were examined before they were euthanized. Another change in 2008 was that the health evaluation was conducted by a staff veterinarian (GHO).

## Health Assessments

In 2008 we implemented a blind assessment technique: the veterinarian did not know in which incubator an egg had been incubated. All hatched chicks were examined as soon after hatching as was practical and within 24 hours. Chicks were weighed, then examined starting at the bill, for any visible abnormalities. Areas examined included the bill, mouth, eyes, head, neck, wings, legs and feet, thorax, and abdomen. The umbilical area was examined to check for an exposed yolk sac or other pathological conditions. All chicks were ausculated to assess cardiac and respiratory function. Chicks or embryos that were found dead were necropsied to determine the time during incubation of death and the cause of death, if this could be determined. The position of the embryo within the egg was recorded. All dead embryos were cultured for pathogens, usually cultures were taken of the yolk sac if no obvious infection was present. For each live-hatched bird, the veterinarian produced an assessment of whether the bird had any health problems that would have rendered it unreleasable (i.e., as if it were part of a cohort that would be released to the wild).

## Data Analysis

The number of healthy chicks hatching from fertile eggs is distributed binomially ($n$, $p$) where $n$ is the number of live fertile eggs placed in the incubator, and $p$ is the probability of success (i.e., of producing a healthy, normal chick). We were interested in the difference in $p$ between the 2 incubators. We begin by computing $p$ for each incubator. The Bayesian posterior distribution for the probability of success $\Pr(p \mid n, y)$ is proportional to

$$\Pr(p \mid n, y) \propto \Pr(p) \bullet \max(£)$$

where $\Pr(p)$ is the prior distribution on p, and where the maximum log likelihood is the maximum over $p$ proportional to

$$\ln £(p \mid n,y) \propto y \bullet \ln(p)+(n - y) \bullet \ln(1 - p).$$

We began by analyzing the 2007 data with a flat conjugate prior, distributed as beta(1,1); equivalent to a uniform distribution on the interval 0-1. A flat or "uninformative" prior is one that assumes essentially no knowledge of the value of the estimated parameter at the outset of the analysis; a flat prior will then produce a parameter estimate that is very similar to the estimate produced by traditional likelihood methods. The primary benefit of a conjugate prior is that the prior and the posterior are of the same distribution (e.g., beta), which facilitates Bayesian updating, as the posterior result from year $t$ can be used as the analysis prior in year $t$+1. The posterior from a beta(1,1) prior and a binomial likelihood is distributed beta(1+$y$,1+$n$-$y$); this was the form of the posterior probability of success for each incubator after 2007. We then used the 2007 posterior as the prior probability for the 2008 data, producing a posterior probability of success for each incubator distributed as beta(1+$y_{2007}$+$y_{2008}$,1+$n_{2007}$-$y_{2007}$+$n_{2008}$-$y_{2008}$), where 2007 and 2008 indicate the year.

In this case, the mean and the variance of the posterior distributions of $p$ can be computed by hand. However, the distribution of derived parameters describing the difference between the probabilities of success for each incubator are not of known form, and so Markov Chain Monte Carlo methods were used to approximate these distributions. In particular, we calculated the absolute difference $p_{\text{Petersime}} - p_{\text{Kuhl}}$, as this was the form in which the flock manager's decision rule was stated (i.e., Kuhl success probability within 5% of the Petersime). We also calculated the odds ratio

$$\frac{p_{\text{Kuhl}}/(1 - p_{\text{Kuhl}})}{p_{\text{Petersime}}/(1 - p_{\text{Petersime}})},$$

which is frequently reported for data of this type. The odds

ratio is the relative increase in the odds of successful hatching (if greater than 1) or decrease in the odds of success (if less than 1) that the Kuhl incubator would provide over the Petersime incubator. An odds ratio of 1 would indicate that the incubators offered essentially the same odds of success.

Analyses were conducted and results were plotted using the computational software WinBUGS (Gilks et al. 1996) and the R programming language (Ihaka and Gentleman 1996). The posterior distributions of parameters from both 2007 and 2008 data were based on 3 independent Markov chains, with a thinning interval of 2. A total of 7,500 samples from the posterior distributions were used for inference. Convergence of the chains was good, with $\hat{R} \approx 1.001$ for each of the variables in the model (Gelman et al. 2004 recommend a value < 1.2 to demonstrate convergence).

## RESULTS

In 2006, of 15 eggs incubated in the Kuhl incubator, 4 were determined to be infertile and were removed. Of the remaining 11, all of the embryos successfully broke into the air cell toward the end of incubation. At this point, 8 were euthanized, while the remaining 3 were allowed to hatch. Two of these chicks hatched with exposed yolk sacs. Six of the 9 eggs incubated in the Petersime were fertile; all of the embryos broke into the air cell. Five were euthanized and 1 hatched successfully. Based on the abnormal development in the Kuhl chicks, the decision was then made not to euthanize embryos before hatching in future trials. Because it was impossible to accurately assess the desired outcome (i.e., whether a healthy chick was produced) in all of the 2006 study eggs, these data were not used in further analyses.

In 2007, 14 eggs were placed in the Kuhl incubator, and 2 were later determined to be infertile. Of the remaining 12 eggs, 4 failed to hatch (Table 1). Of the 8 eggs that hatched, 5 chicks were normal, while 2 had abnormal development of the feet and 1 had an exposed yolk sac. Seven eggs were placed in the Petersime incubator, 5 of which were determined to be fertile. One of these eggs did not hatch, and of the remaining 4, 2 chicks were normal, 1 had deformed feet, and 1 had an exposed yolk sac. The estimated median of the posterior distribution of the probability of success, $\hat{p}_{\text{Kuhl}}$, was 0.43, with a 95% credible interval (0.20, 0.68). The posterior median of $\hat{p}_{\text{Petersime}}$ was 0.42 (0.11, 0.77). The posterior median of the absolute difference was -0.006 (-0.41, 0.43; Fig. 1, solid distribution). The posterior median of the odds ratio was 1.02 (0.15, 7.51). The large credible intervals underscored the importance of obtaining more data.

In 2008, we placed 18 eggs in the Kuhl incubator. Two

**Table 1. Egg-specific results from the 2007 incubator trial in each of 2 incubators - the new Kuhl incubator and the established Petersime incubator - at the USGS Patuxent Wildlife Research Center crane facility.**

| Kuhl | | | | Petersime | | | |
|---|---|---|---|---|---|---|---|
| Lay date | Date in | S/T[a] | Fate | Lay date | Date in | S/T | Fate |
| 1 Apr | 9 Apr | S | Abnormal[b] | 11 Apr | 16 Apr | T | Normal |
| 10 Apr | 11 Apr | S | Abnormal[c] | 19 Apr | 24 Apr | T | Failed[e] |
| 13 Apr | 16 Apr | S | Normal | 22 Apr | 25 Apr | T | Normal |
| 14 Apr | 16 Apr | T | Normal | 24 Apr | 24 Apr | T | Abnormal[c] |
| 15 Apr | 18 Apr | S | Failed[d] | 7 May | 13 May | T | Abnormal[b] |
| 16 Apr | 16 Apr | S | Failed[d] | | | | |
| 17 Apr | 29 Apr | S | Abnormal[b] | | | | |
| 17 Apr | 24 Apr | T | Normal | | | | |
| 18 Apr | 21 Apr | S | Failed[e] | | | | |
| 21 Apr | 24 Apr | T | Failed[e] | | | | |
| 24 Apr | 25 Apr | T | Normal | | | | |
| 9 May | 13 May | T | Normal | | | | |

[a] S = egg from single-egg clutch, T = egg from 2-egg clutch.
[b] Deformed legs and/or feet.
[c] Exposed yolk sac.
[d] Unknown.
[e] Malpositioned in egg, and/or difficulty in hatching.

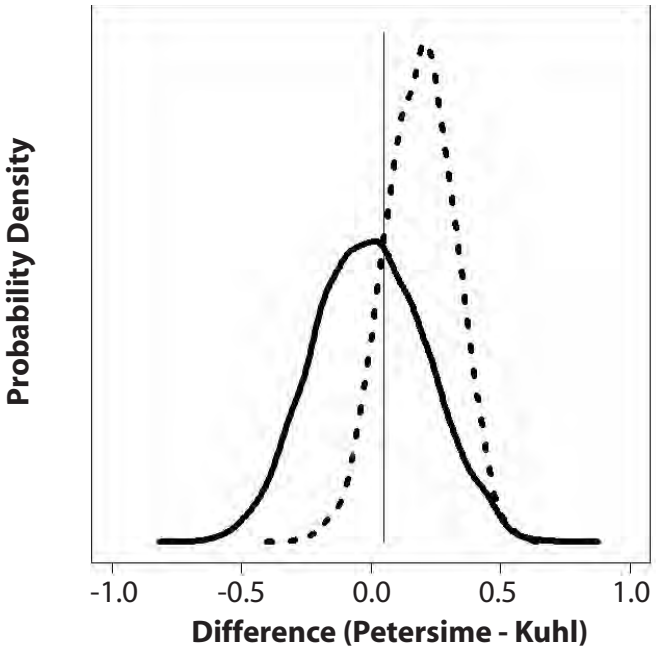


**Figure 1. The posterior probability density of the absolute difference between probabilities of success (p) for the Petersime and the Kuhl incubators after the 2007 trial (dashed distribution) and after the 2008 trial (dotted distribution). The management objective was to have a difference less than 5% (vertical line) with 95% confidence.**

of these eggs were later determined to be infertile, and 1 was determined to have died before being placed in the incubator. Of the remaining 15 eggs, 7 produced normal chicks; 5 failed to hatch and 3 had significant health problems post-hatching that would have rendered the chick unreleaseable (Table 2). Eighteen eggs were also placed in the Petersime incubator. Two of these were determined to be infertile, and 1 had died before being placed in the incubator. Of the remaining 15, 11 hatched normally, 2 failed to hatched, and 2 had significant health problems. The posterior median of $\hat{p}_{\text{Kuhl}}$ was 0.45 (0.28, 0.63), and the posterior median of $\hat{p}_{\text{Petersime}}$ was 0.64 (0.43, 0.82). The posterior median of the absolute difference was 0.19 (-0.08, 0.44; Fig. 1, dotted distribution). The posterior median of the odds ratio was 0.45 (0.14, 1.40).

## DISCUSSION

Based on current results, the Kuhl incubator does not perform adequately for use with whooping crane eggs. While the uncertainty is still large, based on the 2008 data it appears that use of the Kuhl incubator may result in substantially lower hatching success than use of the Petersime incubator.

At PWRC, using a combination of surrogate cranes and artificial incubators is essential to maximizing egg production because by removing eggs from nests, flock managers encourage whooping crane pairs to produce multiple clutches. Typically, eggs are removed from a whooping crane pair's nest as soon as the clutch is complete, although for pairs prone to egg-breaking, eggs are removed immediately after oviposition. When an egg is removed from a nest, it is disinfected in an iodine solution, examined for abnormalities, weighed and measured, and then placed in either an artificial incubator or the nest of a surrogate. The whooping crane incubation period is approximately 30 days (Gabel and Mahan 1996), and ideally whooping crane eggs are incubated by a crane for about 20 of those days, then placed in an incubator for the last 10 days, although eggs may spend more time in an incubator if a surrogate nest is not available. At approximately 10 and 20 days of age, eggs are examined and weighed to determine viability and rate of weight loss. After the last examination, eggs are moved permanently to the artificial incubator, where they are examined and weighed periodically. If an egg is losing weight too rapidly, it can be moved to an incubator with higher humidity to slow the rate of loss. Eggs are removed from the incubator and placed in a hatcher after pipping.

In our experiment, eggs were placed in artificial incubators at an average of 5 days, much sooner than whooping crane eggs are usually transferred. This was done for 2 reasons. First, in some cases, it is necessary to keep eggs in the incubators for longer periods, either because a surrogate nest is not available or occasionally because an egg has a shell abnormality (thin or cracked shells) and is not sturdy enough to be safely incubated in a nest. Second, by increasing the artificial incubation time, any differences in performance between the 2 incubators would likely be accentuated, thus increasing the power of the experiment to detect these differences.

Of a total of 27 eggs incubated in the Kuhl incubator, 9 (33%) died before hatching; in the Petersime, 3 of 20 (15%) died before hatching. Of chicks successfully hatched, post-hatching abnormalities were present in 6 of 18 (33%) hatched from the Kuhl and 4 of 17 (24%) hatched from the Petersime. Therefore, the difference in performance between the 2 incubators appears to be a

**Table 2. Egg-specific results from the 2008 incubator trial in each of 2 incubators - the new Kuhl incubator and the established Petersime incubator - at the USGS Patuxent Wildlife Research Center crane facility.**

| Kuhl | | | | Petersime | | | |
|---|---|---|---|---|---|---|---|
| Lay date | Date in | S/T[a] | Fate | Lay date | Date in | S/T | Fate |
| 1 Apr | 7 Apr | S | Normal | 22 Mar | 1 Apr | S | Normal |
| 10 Apr | 14 Apr | T | Abnormal[b] | 5 Apr | 10 Apr | T | Normal |
| 12 Apr | 18 Apr | S | Failed[c] | 9 Apr | 16 Apr | S | Failed[f] |
| 12 Apr | 22 Apr | T | Normal | 11 Apr | 16 Apr | T | Normal |
| 14 Apr | 16 Apr | T | Abnormal[b,d,e] | 12 Apr | 14 Apr | T | Normal |
| 19 Apr | 25 Apr | T | Failedf | 15 Apr | 22 Apr | T | Normal |
| 19 Apr | 25 Apr | T | Normal | 16 Apr | 22 Apr | S | Normal |
| 19 Apr | 29 Apr | S | Normal | 17 Apr | 25 Apr | T | Normal |
| 20 Apr | 25 Apr | S | Failed[g] | 20 Apr | 25 Apr | T | Normal |
| 25 Apr | 28 Apr | T | Abnormal[b,d] | 22 Apr | 28 Apr | T | Normal |
| 28 Apr | 2 May | S | Failed[h] | 26 Apr | 29 Apr | S | Abnormal[d] |
| 28 Apr | 2 May | T | Normal | 26 Apr | 2 May | T | Normal |
| 29 Apr | 7 May | T | Normal | 2 May | 7 May | T | Failed[h] |
| 6 May | 14 May | T | Normal | 4 May | 8 May | T | Abnormal[d] |
| 7 May | 8 May | T | Failed[h] | 8 May | 14 May | T | Normal |

[a] S = egg from single-egg clutch, T = egg from 2-egg clutch.
[b] Exposed yolk sac.
[c] Hemorrhage around heart.
[d] Deformed legs and/or feet.
[e] Respiratory difficulty.
[f] Malpositioned in egg, and/or difficulty in hatching.
[g] Bacterial infection.
[h] Unknown.

function of both higher pre-hatching mortality and higher post-hatching abnormality in the Kuhl. Egg mortality can be due to several causes specific to artificial incubation, including high or low incubation temperature, improper humidity, improper egg turning, excessive vibration (i.e., from incubator motor), poor ventilation of the incubator leading to a carbon dioxide build-up, and build-up of chemical fumes around the incubator (Olsen and Clubb 1997, Olsen 2000). In addition, mixing eggs from various females in the warm, humid environment of the artificial incubator can result in the spread of certain diseases. The two common post-hatching problems we observed were: 1) deformed legs and curled toes, and 2) an exposed yolk sac, either of which also may be due to problems with artificial incubation (Olsen 2000).

The factors responsible for reduced hatching success in the Kuhl incubator are not clear, but additional experimentation will be designed to determine whether modifications to the Kuhl could increase its hatching success. In the near future, flock managers will also be investigating the availability of other incubator brands on the market.

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## LITERATURE CITED

Canadian Wildlife Service [CWS], and U.S. Fish and Wildlife Service [USFWS]. 2007. International recovery plan for the whooping crane. Recovery of Nationally Endangered Wildlife (RENEW), Ottawa, Ontario, Canada, and U.S. Fish and Wildlife Service, Albuquerque, New Mexico, USA.

Carpenter, J., M. Novilla, and J. Hatfield. 1992. The safety and physiological effects of the anticoccidial drugs Monensin and Clazuril in sandhill cranes (*Grus canadensis*). Journal of Zoo and Wildlife Medicine 23:214-221.

Chen, G., G. F. Gee, J. M. Nicolich, and J. A. Taylor. 2001. The effects of semen collection on fertility in captive, naturally fertile, sandhill cranes. Proceedings of the North American

Crane Workshop 8:185-194.

Clark, G. G., J. F. Dein, C. Crabbs, J. W. Carpenter, and D. M. Watts. 1987. Antibody response of sandhill and whooping cranes to an eastern equine encephalitis virus vaccine. Journal of Wildlife Diseases 23:539-544.

Ellis, D. H., B. Clauss, T. Watanabe, R. C. Mykut, M. Kinloch, and C. H. Ellis. 1997. Results of an experiment to lead cranes on migration behind motorized ground vehicles. Proceedings of the North American Crane Workshop 7:114-122.

Gabel, R. R., and T. A. Mahan. 1996. Incubation and Hatching. Pages 59-76 *in* D. H. Ellis, G. F. Gee, and C. M. Mirande, editors. Cranes: their biology, husbandry, and conservation. National Biological Service, Washington, D.C., and International Crane Foundation, Baraboo, Wisconsin, USA.

Gelman, A., J. B. Carlin, H. S. Stern, and D. B. Rubin. 2004. Bayesian data analysis. Second edition. Chapman and Hall/CRC, Boca Raton, Florida, USA.

Gilks, W., A. Thomas, and D. Spiegelhalter. 1996. A language and program for complex Bayesian modelling. The Statistician 43:169-178.

Hartman, L., S. Duncan, and G. Archibald. 1987. The hatching process in cranes with recommendations for assisting abnormal chicks. Pages 387-397 *in* J. Lewis, editor. Proceedings of the 1985 crane workshop. Platte River Whooping Crane Habitat Maintenance Trust, Grand Island, Nebraska, USA.

Ihaka, R., and R. Gentleman. 1996. R: A language for data analysis and graphics. Journal of Computational and Graphical Statistics 5:299-314.

Link, W. A., E. Cam, J. D. Nichols, and E. G. Cooch. 2002. Of *BUGS* and birds: Markov chain Monte Carlo for hierarchical modeling in wildlife research. Journal of Wildlife Management 66:277-291.

Mirande, C. M., G. F. Gee, A. Burke, and P. Whitlock. 1996. Egg and semen production. Pages 45-58 *in* D. H. Ellis, G. F. Gee, and C. M. Mirande, editors. Cranes: their biology, husbandry, and conservation. National Biological Service, Washington, D.C., and International Crane Foundation, Baraboo, Wisconsin, USA.

Olsen, G. 2000. Embryological considerations. Pages 189-212 *in* G. Olsen, and S. Orosz, editors. Manual of Avian Medicine. Mosby Inc., St. Louis, Missouri, USA.

Olsen, G., and S. Clubb. 1997. Embryology, incubation and hatching. Pages 54-71 *in* R. Altman, S. Clubb, G. Dorrestein, and K. Quesenberry, editors. Avian Medicine and Surgery. W. B. Saunders Company, Philadelphia, Pennsylvania, USA.

Olsen, G. H., D. H. Ellis, S. E. Landfried, L. J. Miller, S. S. Klugman, M. R. Fuller, and C. H. Vermillion. 1992. Behavior of sandhill cranes harnessed with different satellite transmitters. Proceedings of the North American Crane Workshop 6:50-56.

Urbanek, R. P., L. E. A. Fondow, C. D. Satyshur, A. E. Lacy, S. E. Zimorski, and M. Wellington. 2005. First cohort of migratory whooping cranes reintroduced to eastern North America: the first year after release. Proceedings of the North American Crane Workshop 9:213-223.

Williams, B. K., J. D. Nichols, and M. J. Conroy. 2002. Analysis and management of animal populations. Academic Press, San Diego, California, USA.