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REPRODUCTIVE HEALTH AND PERFORMANCE OF THE FLORIDA FLOCK OF INTRODUCED WHOOPING CRANES

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Abstract: We retrospectively examined the reproductive parameters of 122 breeding-age whooping cranes (*Grus americana*) in a reintroduced flock in central Florida from 1992 to 2007. The flock performed poorly when compared with an existing wild flock for all reproductive parameters when controlled for age. Pairs first formed in 1995, nested in 1999, and the first chick fledged in 2002. By 2007, 19 of 63 clutches produced 25 chicks, 9 of which fledged. Drought conditions were ruled out as the sole cause of failure when the drought lessened and productivity increased, but not in all years. We examined adult health, mortality, gonad size and function, pair formation and duration, egg laying, hatching success, egg size, clutch size, fertility, and microorganisms cultured from eggs. Annual mortality was high (13%). The tendency for males to be killed when hitting power lines when females survived may be sufficient to explain the lack of males older than 10 years in this small population. As much as 65% of birds were delayed or non-reproductive due to morphologic abnormalities of the reproductive tract, pairing with sandhill cranes, or more commonly, due to unidentified causes. Pair duration was short (2 years). Extreme annual variability in fertility and hatchability (0-62%) suggest a disease or environmental influence. Captive parent pairs differed in the average reproductive value of their offspring and in the number of second generation wild offspring produced. The remaining small flock is at risk of extinction unless changes are made to improve contiguous wetland availability and reduce the hazards of power lines in these areas. Identification of innate reproductive qualities (behavioral, genetic, and morphologic) and improving survival behaviors may enhance the quality, and thus performance, of birds released.

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Key words: egg size, *Grus americana*, hatchability, mortality, nest success, parentage, power lines, precipitation, reproduction, water level, whooping crane.

A plan to recover the endangered whooping crane (*Grus americana*) to sustainable numbers included the establishment of 3 independent flocks of 25 breeding pairs each. Central Florida was chosen to reintroduce a non-migratory population because it had resident Florida sandhill cranes (*G. canadensis pratensis)*, was isolated from the existing wild flock, and because mortality associated with migration could be avoided (CWS and USFWS 2007). Introductions of captive-reared birds began in 1993 but were discontinued in 2006 when there were 18 pairs, when it became apparent that reproduction was not as expected (Folk et al. 2005). Initially drought was thought to be the cause of poor reproduction, but inconsistent nesting after drought reversal indicated additional problems. Here we retrospectively evaluate reproductive health parameters and examine the possible causes of poor reproduction. In a second paper (Spalding et al. 2009), we explore the association between environmental conditions and successful reproduction.

METHODS

Whooping cranes destined for release were raised in captivity to 6-10 months of age and released into Osceola, Polk, and Lake Counties in central Florida (see Fig. 2 in Folk et al. 2005) by the soft release method (Nesbitt and Carpenter 1993) into suitable crane habitat near other cranes. Released birds were reared by the isolation technique of costumed humans or by captive parents at 1 of 4 institutions: (U.S. Geological Survey Patuxent Wildlife Research Center (PWRC), Laurel, Maryland, USA; International Crane Foundation, Baraboo, Wisconsin, USA; Calgary Zoo, Alberta, Canada; and San Antonio Zoo, San Antonio, Texas, USA). Six- to 10-month-old chicks were penned for 2 weeks in Florida for acclimation, fitted with radio-transmitters (Nesbitt et al. 1997), and released. The release sites were large cattle ranches (>400 ha) with wetland and upland habitat and low human

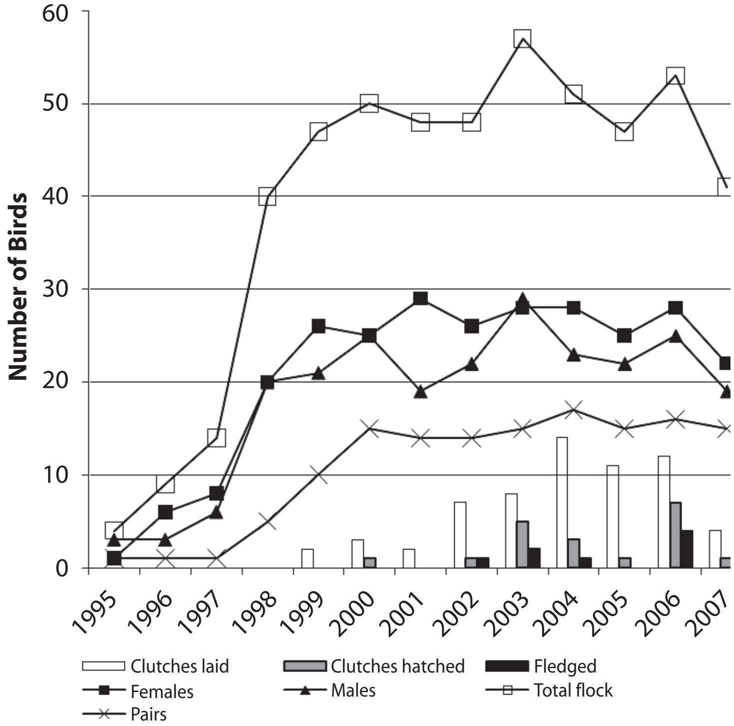

Figure 1. The number of whooping crane males, females, and pairs of reproductive age in the Florida population, 1995-2007. The number of clutches laid, clutches hatched, and clutches to fledge a chick are indicated by bars for each year.

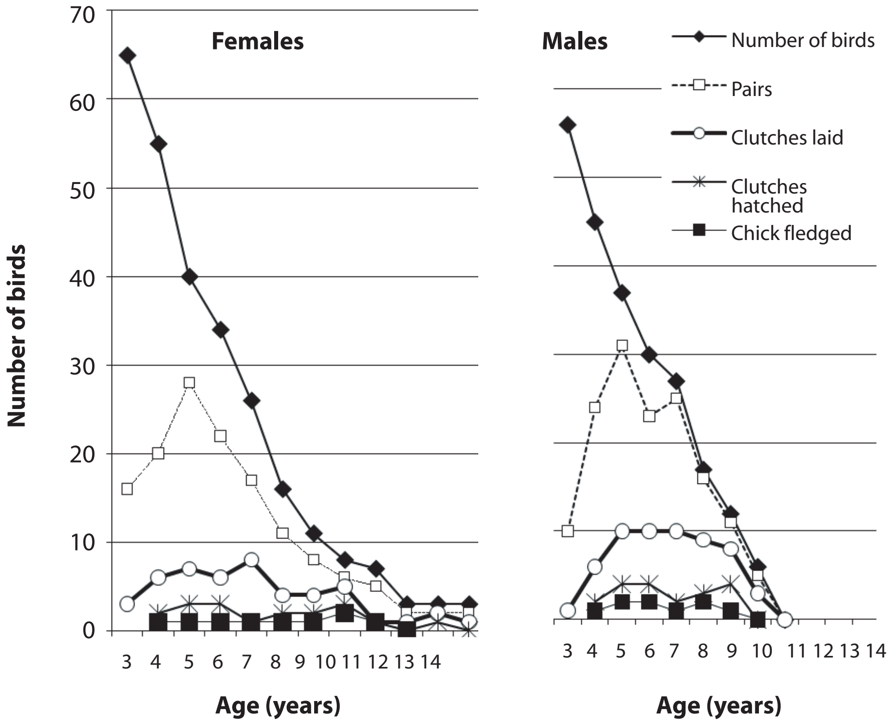

Figure 2. Adult female (left) and male (right) whooping crane survival and reproductive performance based on age. Numbers of birds surviving, paired, laying eggs, hatching chicks, and fledging chicks for each age are illustrated.

density. Primary habitats used by cranes included shallow marshes with emergent vegetation and adjacent grasslands where vegetation was kept low by grazing, burning, and mowing. Peninsular Florida is occupied by about 5,000 Florida sandhill cranes, listed as threatened by the State of Florida (FFWCC 2008) and serves as wintering grounds for approximately 20,000 greater sandhill cranes (*G. canadensis tabida*).

The population examined was limited to the 122 (57 males and 65 females; 119 captive-reared, 3 wild-fledged) whooping cranes that survived through their first possible breeding season at 3 years of age. We also examined reproductive tracts from younger birds for developmental problems. Health data, including a physical examination, weight, and collection of blood and feces, were obtained prior to and upon arrival in Florida, prior to release, and when cranes were recaptured for changing radio-transmitters or were injured or sick. They were monitored 3 to 7 times weekly from the ground or air to determine when incubation began and hatching occurred. Birds that died were examined as soon as possible to determine cause of death and reproductive condition. Those that went missing and were not found for 1 year were presumed to have died at the time that they went missing. Tissues were fixed in 10% neutral buffered formalin, and hematoxylin and eosin-stained tissue sections were prepared and examined. The largest follicle in the ovary was measured using a micrometer or assigned a value of 200 µm for grossly observed minute follicles. The diameter of the testicle was measured to the nearest mm, and the degree of spermatogenesis was ranked from 1 to 5 (1 = inactive, no spermatogonia [sperm precursor cell], 2 = few spermatogonia, 3 = abundant spermatogonia with no mature sperm, 4 = few mature sperm, 5 = abundant mature sperm with sperm in the vas deferens).

Birds were considered delayed or unproductive if they failed to hatch a chick during their lifetime and each was placed in subcategories depending on their highest achievement (Table 1). Those that died or were less than 6 years in 2007 and failed to demonstrate pairing activity were considered too young to evaluate and were assumed to perform in similar proportions as older birds. Thus the percentages obtained for the older birds were applied to the flock as a whole.

Reproductive performance for pairs was based on the highest achievement per season. We recorded the first observed date of incubation and clutch size if visible. Nests either: 1) hatched, 2) failed prior to expected hatch date (pre-hatch failure), or 3) failed to hatch after 34 days of incubation (incubation-failure). Hatching of an egg or presence of a visible embryo was used to indicate the "fertility" of the pair, the clutch, and the eggs.

Table 1. Numbers of reintroduced whooping cranes in Florida that were productive (fledged or hatched chicks) or that were delayed or unproductive, 1992-2007.

	Males	Females	Males and Females	% of total flock	Estimated % of older flock[a]
6 YEARS OR OLDER					
Productive					
Fledged	6	5	11	9	16
Hatched chicks	6	6	12	10	19
Unproductive					
Never hatched	11	9	20	16	30
Never laid	5	8	13	11	19
Never paired	1	5	6	5	9
SHC associated	3	2	5	4	7
< 6 YEARS OLD					
Alive	6	5	11	9	-
Dead	19	25	44	36	-
TOTAL	57	65	122	100	100

[a] Birds not surviving to their sixth year were considered too young to evaluate. Assuming that they were likely to behave similarly to older birds, the percentages listed for the older birds would be equivalent to those of the entire breeding flock.

Unhatched eggs were collected. The fertility of eggs containing no visible embryo was unknown since a small embryo could not be detected in an autolyzed egg. We used the term "no embryo" rather than infertile, realizing that calculations of fertility may be underestimated. Eggs were measured using calipers at the widest length and width and water displacement of intact eggs was used to measure volume. Egg contents and embryo tissues were cultured for aerobic and anaerobic bacteria (University of Florida, College of Veterinary Medicine, Gainesville, FL) and histologic slides prepared as described above.

Reproductive achievements of 8-year-old females were compared between 13 females in the Florida flock and 23 females in the wild Aransas National Wildlife Refuge/Wood Buffalo National Park population

(AWBP, banded 1977-1988, Brian Johns and Lea Craig-Moore, personal communication). Productivity was calculated as an average of the number of fledged young-of-the-year per 100 adults in the flock.

In order to evaluate the contribution of the captive pairs (G1 = first generation) we devised a scoring system to designate the reproductive value (RV) of each released crane (G2 = second generation). Only those 9 G1 pairs that had 4 or more G2 offspring surviving to breeding age were evaluated. Beginning in their third year, G2 offspring were given 2 points for each stage attained: unpaired = 2, paired = 4, embryo produced = 6, hatched = 8, and fledged chick = 10 (G3). If a bird failed to move to the next stage within 2 years or regressed to an earlier stage, then 1 point was removed from the score for each year until 2007 or the year the bird died. For example, a bird surviving to 7 years and pairing after 5 years received a 3 (unpaired at 3 years = 2, paired at 5 years = 4, no eggs at 7 years = 3). For each G1 pair the G2's final RVs were averaged. The calculated RVs were also used to compare performance of isolation-reared with parent-reared techniques.

Analysis of variance was used to model mean egg volume per clutch as a response to clutch order across years (first vs. later) and incubation initiation date of failed vs. hatched nests. Logistic regression was used to relate egg volume with hatch/fail and fertile/no embryo. Fisher's exact test was used for the clutch size contingency table analysis. A Wilcoxon nonparametric 1-way analysis of ranks was used to compare the RV of isolation-reared to parent-reared released birds. Because this was an exploratory post-hoc examination of existing data, we present P values, but consider trends even when P values are > 0.05. Statistical analyses were performed using SAS/STAT(r) procedures (SAS version 9.1.3, SAS Institute Inc., Cary, NC).

Table 2. Comparison of reproductive performance (8-year-old females), productivity (mean no. of fledged young per 100 breeding age adults), age of first hatch, and survival of the Aransas-Wood Buffalo population (AWBP) and the reintroduced Florida population. Eight-year-old females were used for the reproductive performance. Dashes indicate no data available.

	Florida		AWBP	
	Number *n* = 13	%	Number *n* = 28	%
8-yr-old females[a]				
Paired	12	92	28	100
Clutch laid	7	54	28	100
Hatch	4	31	27	96
Fledge	2	15	21	75
Fertile	6	46	27	96
Mean productivity	-	3.1	-	13.1[b]
Mean age at first hatch (yr)	5.7	-	5[c]	-
Annual adult mortality (%)	-	13	-	1.4[d]

[a] Achievement of females in the population by the time they reach 8 years of age. Data from AWBP, banded 1977-1988 (Brian Johns and Lea Craig-Moore, personal communication).

[b] Averaged over 55 years (Drewien et al. 1995).

[c] From Kuyt and Goossen (1987).

[d] 1950-1986 (Lewis 1995).

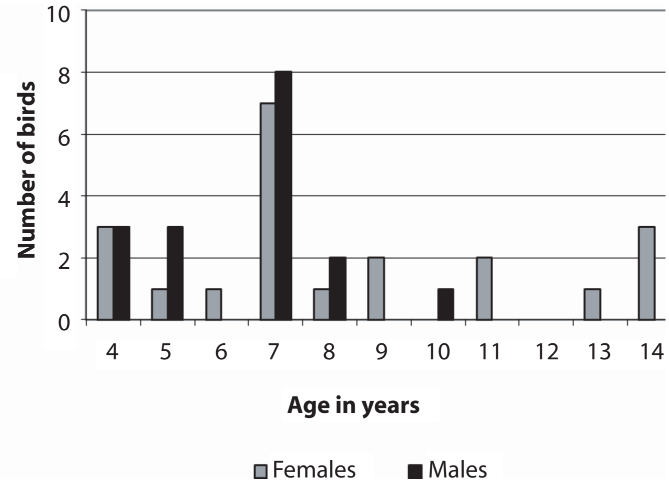

Figure 3. The age structure of the reproductive flock of whooping cranes in Florida in 2007.

RESULTS

The Florida population of re-introduced non-migratory whooping cranes performed poorly in all stages of reproduction when compared with age-matched females in the AWBP; the greatest differences were in hatching and fledging (Table 2). Productivity was also considerably lower (3.1) than the more mature AWBP. The reproductive flock size peaked at 56 birds in 2003, and declined to 41 by 2007 (Fig. 1). The first egg was laid in 1999, the first chick hatched in 2000, and the first chick fledged in 2002; 9 chicks were fledged by 2007. The mean age of females in the flock was still increasing (mean = 7.9 years) in 2007, but males peaked at a mean of 6.3 years old in 2002.

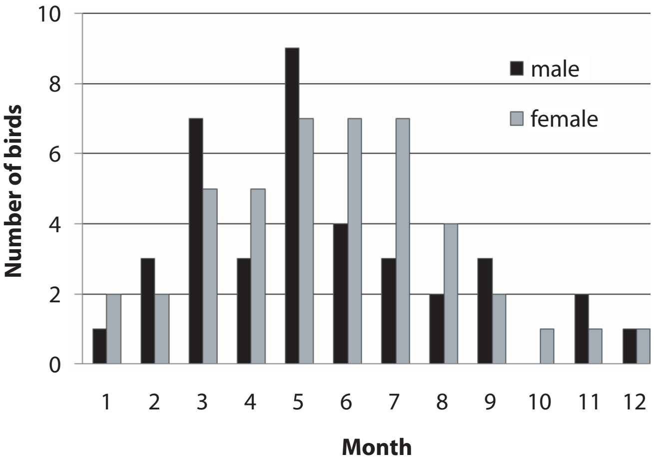

Figure 4. The month of mortality for breeding age male and female whooping cranes in Florida, 1995-2007.

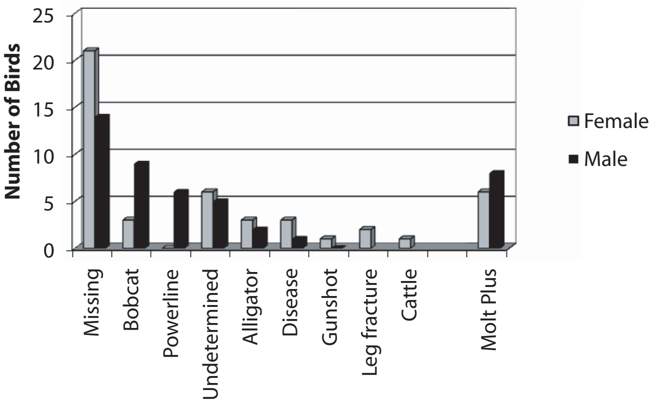

Figure 5. The cause of mortality for breeding age whooping cranes in Florida, 1995-2007. The last category includes birds that also appear in other categories that were known to be molting.

Mortality

Of the 122 birds reaching breeding age 71% (46/65) of females and 79% (45/57) of males died by 2007. Mean age of mortality was similar between sexes (males = 5.95 years, females = 5.24 years). Mean annual mortality after 1999 was 13% for all birds 3-10 years old, but declined to 1.9% for females 10-14 years old. No males survived beyond 10 years of age (Figs. 2 and 3).

Mortality was highest during the nesting season (Fig. 4). In most cases birds went missing, especially females (Fig. 5). Of 12 cranes killed by bobcats, 9 (75%) were males. Fourteen birds presumably died from predation while flightless during molt in the summer. Based on injuries, location, and loss of transmitters, 25 whooping cranes were believed to have struck power lines; none of the 12 females died, whereas 7 of the 13 males died directly or from sustained injuries.

Unproductive Birds

Sixty-five percent of the breeding age birds were estimated to be delayed or unproductive (no chicks produced). In most cases the reason was not known, except for birds associating with sandhill cranes, and those with poorly developed reproductive tracts (estimated at 4% and 10%, respectively). Five birds (4%) associated with sandhill cranes during the nesting season, but only 2 males actually nested with them. One hybrid clutch failed to hatch; another hybrid pair hatched twins, but did not fledge them. One male that initially associated with sandhill cranes later produced a fledging with a whooping crane.

Carcasses were in sufficient condition to evaluate gonad size for 23 females and 35 males, but only 3 females and 10 males were of breeding age (Fig. 6). Gonads from 12 females and 26 males (3 females and 8 males >3 years old) were also examined microscopically. Follicles >1 mm occurred only in breeding-age females in September, December, and January. Enlarged testes (>5 mm diameter) were only found in males older than 1 year and during December to May. The testes diameter of males <3 years old did not increase over 4 mm, but the appearance of sperm in the testes and vas deferens occurred from December to May even in males without increased testes diameter and as young as 8 months old (February).

Six of 58 birds examined (10%) had small gonads for their age or season. They included 2 young females (16 and 20 months), 2 young males (10 and 11 months), a 14-month-old bird DNA-sexed female that lacked gender-specific gross and microscopic characteristics, and a 9-year-old molting post-nesting male (June) with immature-appearing testes lacking spermatogenesis that failed to hatch a clutch of no-embryo eggs and also failed 2 previous seasons. None of the birds shared the same parents. Additionally, the testes of an 8-month-old male that died from wasting syndrome (mentioned above) in February were seasonally small, but there was evidence of

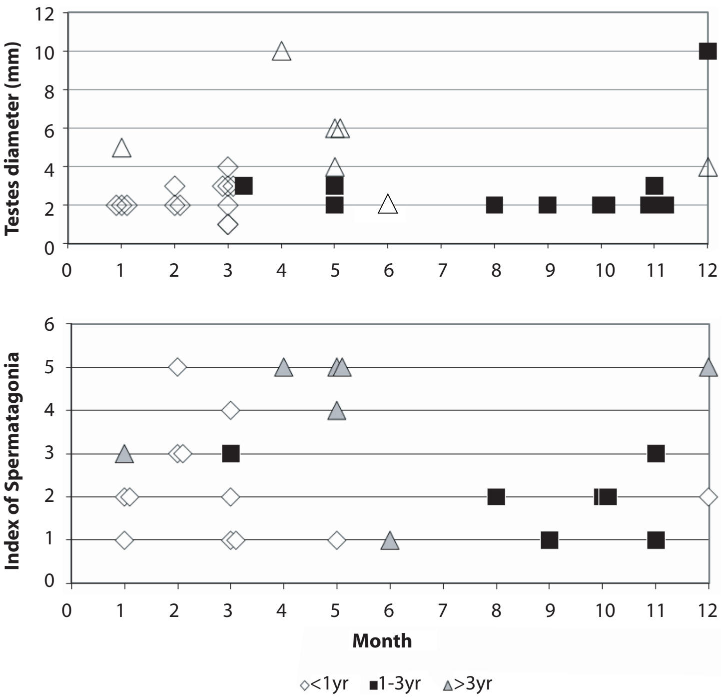

Figure 6. The diameter of testes (top) and index of spermatogenesis (bottom) from 3 age groups of whooping cranes in Florida. The index of spermatogenesis was ranked from 1 to 5 (1 = inactive with no spermatogonia (sperm precursor cell), 2 = a few spermatogonia, 3 = abundant spermatogonia with no mature sperm, 4 = few mature sperm, 5 = abundant mature sperm with sperm in the vas deferens in males).

early spermatogenesis.

Disease was never identified as a cause of clutch failure. A 10-year-old male, the parent of 2 fledged chicks from previous seasons, died acutely from eastern equine encephalitis shortly after 2 failed nesting attempts in May 2005. Twenty-three birds (most HY2001) had clinical signs of wasting syndrome shortly after their release that was likely associated with infectious bursal disease virus (Spalding et al. 2008). Only 5 survived to enter the breeding age flock; none of them demonstrated fertility.

Pairing and Nesting

The number of pairs in a season peaked at 18 in 2004, the same year that the proportion of pairs to nest (80%) was highest (Fig. 1). The ratio of males to females was initially near 1:1, but shifted to a female bias (1:1.27) by 2007. Mean age of first pairing was 4.26 years (range 2-8 years) for males and 4.11 years (range 3-10 years) for females. By 2007, 77 pairs had formed (including 2 males paired with sandhill cranes). Individuals paired as often as 4 times. Sixty-one pairs separated (34 due to the death of 1 or both of the birds (5 during active nesting or chick rearing), 24 due to 1 or both members of the pair switching mates, 1 dissolution of the pair, and 16 pairs were still active. Of 77 pairings, 18 (23%) demonstrated evidence of fertility. Four unsuccessful birds became successful (hatched a clutch) by changing mates, 1 remained unsuccessful with 4 different mates. Pairs lasted an average of 2.03 years (*n* = 77, range 1-6 years), non-laying pairs 1.48 years (*n* = 39, range 1-5 years), egg-producing pairs 2.69 years (*n* = 16, range 1-5 years), pairs hatching a chick 3.17 years (*n* = 6, range 1-6 years), and pairs fledging chicks 2.60 years (*n* = 6, range 1-9 years). Intraspecific and conspecific aggression disrupted 4 clutches; there were 2 cases of a new male displacing a paired male and the existing eggs were broken.

Sixteen males (28%) and 15 females (25%) demonstrated fertility. Evidence of fertility in nesting females increased from 66% for first time nesters to 88% for nesting females 10 years of age or older. The mean age that fertility was first demonstrated was 6.17

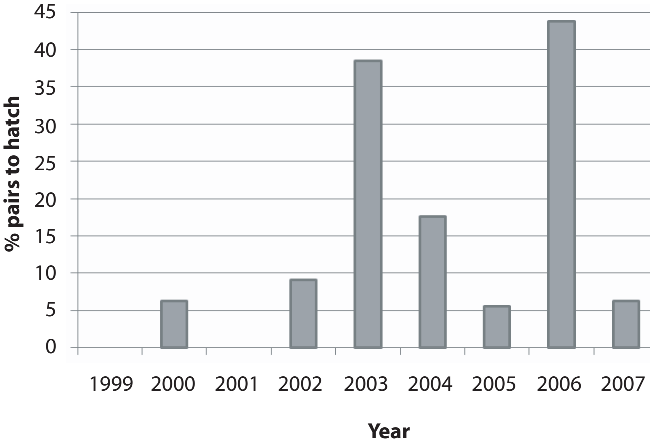

Figure 7. Percent of whooping crane pairs in Florida that hatched a clutch, 1999-2007.

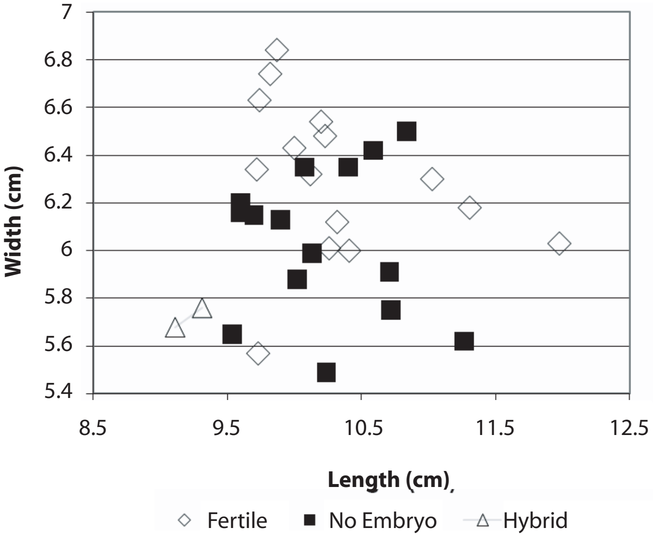

Figure 8. Dimensions of eggs with and without embryos collected from whooping cranes in Florida, 1999-2007. Eggs did not hatch or were collected. Also shown are 2 hybrid eggs (male whooping crane × female Florida sandhill crane).

years (*n* = 17, SE = 0.39, range 4-9 years) for males and 7.14 years for females (*n* = 14, SE = 0.58, range 4-10 years).

Clutches were laid in 63 nests by 26 females (39% of breeding age females). Five were second and 2 were third clutches of the season. The most productive female laid 10 clutches in 7 years. The mean age for laying the first clutch was similar for males (mean = 5.72 years, range 3-10 years) and females (mean = 5.71 years, range 3-11 years). The percentage of pairs to hatch a clutch was highest in 2006 (43%, Fig. 7). The year that the most females laid (82%, 2004) was also the year that significant incubation-failure (73% of nests) occurred. Individual females laid as often as 7 consecutive years. Clutch size averaged 1.68 eggs (*n* = 44, range 1.33-2.00 eggs). The mean date for initiation of incubation was 15 March (range = 27 January-27 May, renest range = 11 February-9 May). Clutches that hatched were laid on the average 20 days earlier (mean = 10 March, *n* = 19, range = 29 January-15 May) than clutches that failed (mean = 31 March, *n* = 40, range = 27 January-27 June; ANOVA, $F = 5.54$, df = 1,57, $P < 0.03$). When the nesting season was divided into three equal portions, 56% (24 January-6 March), 45% (7 March -17 April) and 13% (18 April-27 May) of the clutches hatched.

Examined Eggs

Information was obtained from 41 eggs from 27 clutches (3 were incubated and hatched, 2 were hybrid eggs, 2 were examined in the field). Evidence of fertility was found in 20/41 eggs (14/27 clutches, 52%). Three of 7 eggs from clutches that hatched contained embryos. Of 14 pairs of eggs examined, 13 were either both with, or both without, embryos. Nine eggs had embryos that were fully developed (4 hatched in captivity), 9 were mid-development, and 2 in early stages of development. One near-term embryo was malpositioned with the head on the wrong side such that it could not pip the shell. Another near-term embryo in a cracked egg had bacterial pneumonia and enteritis, likely due to the cracked shell.

Eggs (excluding hybrids) averaged 10.27 × 6.17 cm (*n* = 30, range 9.54-11.98 × 5.49-6.84 cm) (Fig. 8). Mean egg volume (excluding hybrids) for all eggs was 204.3 ml (*n* = 31 from 20 clutches, range 151-250 ml). The two hybrid eggs were smaller (9.3 × 5.8 and 9.1× 5.7 cm, 150 and 145 ml). The mean length, width, or length/width ratio did not differ significantly between eggs with and without embryos, but volume tended to be greater in eggs containing embryos (216.2 vs. 199.6 ml, Table 3). Mean volume of eggs in the first clutch laid (naive female) were smaller than eggs laid in subsequent clutches. Bacteria were cultured from 19 eggs, 9 with an embryo and 10 without an embryo. There was no indication that any of the species cultured

Table 3. Whooping crane egg volume and clutch size based on fertility, nesting experience, and nest outcome.

	Egg volume (ml)				Clutch size (frequency)			
Nest category	Mean	SE	*n*	*P* value[a]	1 egg	2 eggs	*n*	*P* value[a]
Fertile nest	216.2	6.4	10	0.12	5	20	25	0.41
Nest no embryo	199.6	6.6	10	-	4	7	11	-
First clutch	182.1	4.1	5	0.0003	5	14	19	0.75
Later clutches	216.5	4.5	15	-	9	17	26	-
Hatched[b]	215.5	5.9	4	0.44	3	12	15	0.70
Failed	206.0	5.9	16	-	6	15	21	-

[a] *P* values based on logistic regression for egg volume and on Fisher exact test for clutch size.

[b] Hatched eggs were all collected eggs for management purposes.

were the cause of failure. Apparent fertility was significantly greater in pre-hatch failure nests (8/11, 72%) than in incubation-failure nests (4/12, 33%) (Fisher's exact test, *P* = 0.01). Incubation-failure clutches were incubated earlier (mean = 22 March) than pre-hatch-failure clutches (mean = 4 April).

Clutch Outcome

Twenty-four (41%) of 59 clutches (4 of the total 63 clutches were excluded because they were collected) failed prior to the full 34 days of incubation (pre-hatch failure). Ten clutches were abandoned (2 with eggs remaining in the nest), 4 clutches were thought to be disturbed (2 by airboats), 4 flooded, 4 were disturbed by cranes (2 by whooping cranes, 2 by sandhill cranes), 1 was abandoned when the marsh dried, and 1 was abandoned in a drying marsh when the female was killed by a cow. Fifteen of 59 clutches (25%) were found with the eggs missing or shell on the nest; some may have been due to egg predation.

Sixteen of 59 (27%) clutches failed after full incubation (incubation-failure). Of the 8 clutches with no embryo (of 12 clutches examined), 3 were from first-time nesting pairs, 1 was a sandhill/whooping crane hybrid pair, and 4 were experienced nesters. Incubation-failure clutches were not more frequently first-time clutches when compared with other clutches (Fisher's exact test, *P* = 0.77).

Hatched Clutches

Twenty-six chicks hatched from 19 clutches (30%, 19/63 clutches). The clutches that hatched were laid by 11 females (17% of females). No association between clutch size and fertility of the egg was found. The proportion of pairs that hatched a clutch varied among years (Fig. 7). The mean age of first hatching a chick was slightly greater for females than males (6.91 and 6.21 years, respectively, range 4-10 years). Twins hatched on 7 occasions. The oldest surviving sibling chick died by 12 days of age. Mean age at death for the chicks that did not fledge was 24 days (range 1-77 days). Among the 16 pairs nesting more than 1 time during this study, 7 of 8 that hatched clutches, and all of those that fledged chicks, did so on their first attempt. Only 1 pair first failed and then later hatched a clutch. No significant association was found between experience level and clutch size.

Nine of the 19 (47%) clutches that hatched resulted in a fledged chick (14% of all clutches), from 6 pairs (5 females and 6 males). The mean age for first fledging a chick was slightly older for adult females (7.4 years, range 4-10 years) than males (6.0 years, range 4-8 years). None of the manipulated clutches resulted in fledged chicks. Two male and 7 female chicks fledged. Eight of 9 fledglings survived and 3 entered the reproductive flock by 2007.

Parentage and Captive Rearing Method

The 4 G1 (captive first generation) pairs with the largest proportion of high scoring G2 offspring produced 5 of the 9 wild-hatched G3 chicks, but also produced a similar number of low-scoring G2 chicks (Table 4). The G1 grandparents of the other 4 G3 chicks

Table 4. The contribution of 9 captive pairs (G1 = first generation) to the G2 offspring released in Florida that became members of the breeding flock. The calculated reproductive value (RV) for each G2 was averaged for each G1 pair. Also, for each G1 pair the number of high and low scoring G2 offspring, number of fertile G2 offspring, and number of G3 wild fledged chicks are listed.

Captive pair (G1)	Number of offspring (G2)	Mean RV of offspring (G2)	Number of high score RV>5 (G2)	Number of low score RV< 2 (G2)	Number of fertile offspring (G2)	Number of wild-fledged chicks (G3)
A	5	4.40	2	2	4	7[a]
B	5	3.60	1	2	5	1
C	4	3.50	2	1	3	0
D	15	3.27	3	5	8	1
E	19	2.53	4	6	4	3[a]
F	8	2.50	2	4	0	0
G	7	1.86	1	4	1	0
H	5	1.00	0	3	0	0
I	7	1.00	0	4	0	0

[a] 3 and 1 chicks, respectively, were counted twice because they were counted as the offspring of both the male and female of brother/sister pairs.

were not evaluated because they produced fewer than 4 G2 offspring. The 4 G1 pairs with the lowest scoring G2 offspring did not produce any high score G2 offspring, nor did their G2 offspring fledge any G3 chicks. The proportion of G2 offspring exhibiting fertility exceeded 50% for the top 4 G1 pairs, but for the lowest 4 G1 pairs, only 1 of their 27 G2 offspring demonstrated fertility. Among the G2 offspring of low scoring G1pairs were 2 females that switched partners annually or biannually with 7 males over the course of 3 and 6 years, never producing eggs. Of the 7 males involved in these short term pairings, 5 subsequently sired chicks with other females.

Mean reproductive performance was similar for parent-reared (RV = 2.74, n = 19, SE = 0.89, range = -9-8), and isolation reared (RV = 2.48, n = 103, SE = 0.31, range = -6-10, Wilcoxon's 2-sample non-parametric, Z = 0.88, P = 0.38).

DISCUSSION

Reintroduced whooping cranes in Florida performed poorly across several reproductive measures, especially hatching and fledging chicks in a comparison of similar-aged females from the AWBP. The combined high mortality of breeding age birds, large proportion of delayed or non-productive birds, flock immaturity, and poor hatching and fledging rates in most years explain the low productivity (3.1%, number of chicks/100 adults per year, 2002-2007). Other reports of productivity vary due to different methodologies and are poorly comparable: AWBP (13.9%, over 55 years and based on fall/winter post-migration observation, Drewien et al. 1995), Florida sandhill cranes in north central Florida (35%, observations at independence in winter/early spring, 1977-1989, Nesbitt 1992), Florida sandhill cranes in south-central Florida (27.7%, before independence, July-December, 1973-1979, Layne 1983).

We identified 3 characteristics that best explain the poor productivity: 1) adult mortality, especially of older males, 2) a high proportion of delayed and non-productive birds, and 3) poor hatching in some years. At the time of this review, the reintroduced flock had begun a slow decline in size and there were fewer males than females. Although reproduction could improve as the flock matures and wild-hatched chicks come of age, a model suggests that the population is unlikely to sustain adequate numbers to survive (Moore et al. 2008).

Mortality

Annual mortality of the AWBP was highest during migration (Lewis 1995); in Florida most occurred during the nesting season. Female mortality frequently involved disappearance, whereas males more often died from power line collision and bobcat (*Lynx rufus*) predation. Female “disappearance” may indicate a different cause of death than for males. Hiding birds (injury, sick, or molt), dispersing, or birds killed or scavenged by alligators (*Alligator mississippiensis*) are less likely to be found than those that die from bobcat predation or power line strikes where the carcass is left on dry land. Flight feather molt of both genders occurs every 2-4 years from early April to late June, and results in about 44 days of flightlessness (Folk et al. 2008) during which birds may be more vulnerable to predation. Immaturity, lack of predator avoidance training, and inappropriate habitat selection may put captive-raised cranes at greater risk for predation than wild-raised birds.

Power line strikes were recorded for both genders, and although females were injured hitting power lines, death was only recorded for males (Miller et al. 2010). Males may fly ahead of females, hitting power lines first, but we have no observations to test this hypothesis. The power line mortalities of males are conservatively estimated at 11%, a number sufficient to explain the loss of older males.

Annual mortality declined in older females, but this was not the case for males, which continued to decline at the same rate resulting in a complete absence of males older than 10 years. The magnitude of this phenomenon seems to be unique to the resident Florida flock. Annual survival of resident Florida sandhill crane females was also higher than males (females/males = 0.918/0.884, Tacha et al. 1992), whereas for both migratory whooping cranes (0.78/0.86, Lewis 1995) and migratory greater sandhill cranes (0.78/0.87, Tacha et al. 1992), males had higher annual survival. Further research is needed to explain why male whooping crane mortality would be higher in Florida, or in resident populations.

Unproductive Birds and Fertility

We estimated that 65% of birds released were either reproductively delayed, or, in the case of some, incapable of reproducing due to unknown causes, morphologic or functional disorders of the reproductive tract, or pairing with sandhill cranes. Captive raised chicks lack experience with bonding, nesting, and chick rearing behaviors. Behavioral dysfunction, genetic or endocrine disorders, environmental toxins, hormonally active toxins (Fry and Toone 1981, Berg et al. 1999), poor adaptation to climate/habitat, and inappropriate and limited mate choice could all be contributing factors. Unproductive pairs may also occur when 1 member of the pair is infertile, masking the potential fertility of the mate and resulting in an underestimation of fertile birds. Some may later pair with fertile birds and produce young.

Overall infertility did not appear to be a significant problem in any of the flocks, with the exception of the cluster of incubation-failure clutches in Florida whooping cranes during 2004 and 2005 (see Spalding et al. 2009). The apparent fertility of first-time nesters was slightly lower than older nesters.

Little is known about the reproductive physiology of wild whooping cranes. The season for semen collection at PWRC is 16 March to 14 May with a peak at 30 March to 26 April (Mirande et al. 1996). Sperm were present in the testes of released Florida whooping cranes from December to May in our limited sample with earliest appearance as young as 8 months of age. Sperm production was also noted in some birds prior to enlargement of the testes.

Pairing and Nesting

Pairing age was delayed by 1 to 2 years when compared to the AWBP (first egg at 5 years and the first fertile egg at 5.4 years, Kuyt and Goossen 1987) and Florida sandhill cranes (modal 5 years, Nesbitt 1992). Kuyt (1981) estimated that about 80% of AWBP pairs nested each year and that 60% of adults produce nestlings. In 2004, 82% of pairs nested in Florida; however, fledging never exceeded 25% of pairs.

Pairings of whooping cranes in Florida were of shorter duration than in the AWBP, and infrequently productive. Factors influencing pair duration included mortality of mates, limited mate choices due to the skewed sex ratio, lack of older experienced males, limited encounters with potential mates due to population size and distribution, disrupted pairing due to intraspecific (and interspecific) competition for mates, and pairing with sandhill cranes. Speculative factors include inexperience with pairing behavior due to rearing method, and unproductive pairs may be less likely to remain together. Nesbitt and Wenner (1987) reported frequent short-term pairings in sub-adult Florida sandhill cranes prior to establishment of a long-term bond and successful nesting. Contrary to these findings, all whooping crane pairs in Florida that fledged chicks did so on their first attempt. This suggests that reproductive capability may be innate rather than learned.

Whooping cranes that nested earlier in the season were more likely to hatch a chick than those nesting later. Nesting dates for whooping cranes were nearly identical to the range of the Florida sandhill crane (late January to 22 May, Nesbitt 1988). Extremes of incubation, December to August, are mentioned in some older papers for Florida sandhill cranes (Bent 1926, Walkinshaw 1973). Early nesters may avoid the higher temperatures and dryer marshes that can occur later in the season and the potential for flooding from early tropical storms. Nesbitt (1988) noted that Florida sandhill cranes, however, had greater success in hatching clutches later in the season. This difference in timing needs further investigation.

The size of eggs did not differ significantly from previously reported mean measurements of wild eggs (9.84 × 6.24 cm, Bent 1926; 10.06 × 6.29 cm, Stephenson and Smart 1972; and 10.13 × 6.29 cm, Stephenson and Smart 1972). Wild-laid eggs appear to be larger than captive-laid eggs, but 1 published account was based on eggs from a single captive pair at San Antonio Zoo, San Antonio, Texas (mean = 9.42 × 6.08 cm, Stephenson and Smart 1972). We found that the volume of fertile eggs was greater than those with no embryo, and eggs laid by novice females were smaller than those laid by experienced females. These findings were based on non-randomly collected eggs and need to be confirmed. Egg volume was a more sensitive measure of the size of the egg since weight varies during incubation, and length × width do not take into account the variability in shape.

Nest Outcome

Only 30% of clutches hatched, substantially below the expected rate for the AWBP (60%, Kuyt 1981), but not dissimilar from the Florida sandhill crane (38%, Nesbitt 1988). Low hatchability due to environmental limitations characteristic of Florida may be the reason for a small, but sustained, population of resident sandhill cranes in Florida. The most common type of failure, pre-hatch failure (41% of clutches) was attributed to a variety of factors ranging from flooding to drought to human disturbance and interference by other cranes, and was relatively constant from year to year. Human-related disturbance was often difficult to document, but could be roughly estimated at less than 10% of clutches. Egg predation is difficult to assess (nests with shells or missing eggs); however, no more than 24% of clutches failed in this way. It is surprising that more clutches are not taken by alligators which are common in Florida wetlands. Cattle were rarely a problem unless the nesting marsh dried. We had insufficient data to evaluate causes for failure to fledge, but a few pairs were able to repeatedly fledge chicks.

Incubation-failure clutches (fully incubated) represented a significant proportion of failed clutches and were characterized by: 1) high inter-annual variability, 2) low apparent fertility, and 3) smaller egg volume. We found no evidence that these clutches failed due to timing of nesting, location of nest, disease, or experience of the female. The variations in apparent fertility of clutches, hatchability, and fledging success suggest the influence of annually fluctuating factors such as climate or disease.

Diseases that cause early embryo mortality could not be confidently ruled out as a cause for incubation-failure clutches. Bacterial culture of egg fluids did not result in the isolation of a consistent pathogen of concern; however bacterial overgrowth in autolytic eggs might prevent such a finding. A new and incompletely characterized "wasting syndrome" of young cranes, believed to be caused by infectious bursal disease (IBD) virus, causes emaciation, immunosuppression, and death in young cranes. This virus is not known to be vertically transmitted in the chicken (hen to egg). Titers to IBD virus are highly prevalent in subadult and adult sandhill and whooping cranes in Florida (Spalding et al. 2008, Candelora et al. 2010). Immunosuppression and subsequent predisposition to infection with other diseases occurs in domestic chickens and also appears to occur in cranes. Two release groups of young cranes were particularly impacted by wasting syndrome, and the survivors of the outbreak would have first nested in 2004. Only one of the incubation-failure clutches was parented by a wasting syndrome survivor, making this an unlikely sole cause for incubation-failure. One male and 4 females recovered from wasting syndrome and exhibited no evidence of fertility. There is insufficient information currently to implicate IBD virus as a cause for infertility or early embryo mortality.

Parentage

We found no difference in reproductive performance between isolation- and parent-reared chicks raised in captivity and released in Florida. Both rearing techniques differ considerably from the wild learning experience. Exposure to "captive" parents may enhance the nesting and parental interaction experience of the chick, but lack of predator avoidance behavior by parents protected by confinement may make chicks more susceptible to predation.

The released offspring with high RV scores were not evenly distributed among the captive parent pairs. Several captive pairs produced both high and low scoring offspring, whereas others produced offspring with only low RV scores. Identification of the pairs that consistently produce non-productive offspring should allow managers to increase the success of released birds by eliminating them from release cohorts. Further evaluation of these captive pairs may reveal genetic, physiologic, or behavioral problems that are being passed on to their offspring. The presence of non-productive birds is problematic because they can prevent potentially productive mates from being successful. The finding that some pairs are better at producing productive offspring than other pairs is not unexpected and has been noted in other species. Nesbitt (1992) found that 26% of adult Florida sandhill cranes produce 62% of young.

We found that whooping cranes released into Florida varied significantly in their potential to reproduce. Being able to identify traits conducive to reproductive success, and improving the quality of birds to be released, should lead to greater success for the project. This is especially true when generation time can prolong the time necessary to evaluate the release effort. Survival in the presence of bobcats is likely learned by chicks observing appropriate adult response to a predator. Power lines present a more difficult problem to solve. Although placing markers on the lines can help to deter some strikes, they can only be a partial solution.

The presence of delayed and unproductive birds in the flock equally limits productivity. More intense management of the flock by removal or exclusion of unproductive birds will likely improve productivity. Research directed at identification of individual birds and genetic lines more likely to be productive would be necessary to meet this objective.

Climate associated variability in nesting, fertility, hatchability, and fledging is addressed in Spalding et al. (2009). Other aspects of reproductive health are poorly understood due to limited or lack of data in this study, such as the influence of infectious disease, nest microclimate and incubation behavior on hatchability, and could be studied in the Florida sandhill crane.

The future survival of the Florida whooping crane population depends upon the ability of wild-fledged chicks to reproduce with a higher rate of success than their parents, their ability to overcome current low numbers, especially of males, and the preservation of suitable wetlands without hazardous power lines across them. Although introduced resident whooping cranes in Florida reproduced very poorly when compared to the wild AWBP, it may be more appropriate to compare them with the Florida sandhill crane which is also resident and therefore exposed to similar habitat-related limitations on reproduction. Egg collections and other reproductive health information could lead to more rapid answers to unanswered questions in this study due to the inability to obtain of appropriate samples. Such a comparison may elucidate and clarify some of the reasons for the poor productivity of whooping cranes released in Florida.

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