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Breeding Ecology of a Reintroduced Population of Crested Ibis (*Nipponia nippon*) in Henan Province, China

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JW, YB and HN conceived and designed the study. JW conducted the study. JW, JZ, KW and DC collected data. JW and YL analyzed the data. JW, YB and HN drafted, revised and approved the manuscript.

Key words
Crested ibis, Reintroduction, Breeding ecology, Habitat selection, Henan.

ABSTRACT

The crested ibis (*Nipponia nippon*) is a globally endangered bird. Aimed to save and restore the small population in Henan Province, China, a reintroduction program was conducted in the Dongzhai National Nature Reserve in 2007. Wild birds bred in semi-wilderness were reintroduced. Habitat selection and breeding ecology of crested ibis in Dongzhai National Nature Reserve has not been studied. To clarify the breeding status of the crested ibis and to provide more reference for the reintroduction of this species, the process of pairing, nest site selection, egg laying, incubation and brooding of the crested ibis in the wild was studied in Dongzhai National Nature Reserve, Luoshan County, Henan Province, China. From October 2013 to May 2015, a total of 78 captive-bred individuals have been released into the reserve and monitored through radio transmitters or satellite transmitters. Twelve nests of 10 pairs were recorded over three breeding seasons. Sixteen variables of habitat characteristics were measured. The results showed that: 1) the principal component analysis (PCA) revealed that the first four components accounted for 79.31% of the total variance, including vegetation, interference, nest position and food factors, which best reflected the habitat site selection of the crested ibis. The crested ibis were more likely to choose higher tree nests that were closer to residential areas and in a lower slope position in the breeding season; 2) thirty-three eggs were laid in 12 nests, the average clutch size being 2.75±0.75 (range 2-4); 3) twenty-five eggs were hatched, with the average hatching success 78.48%; 4) twenty-three fledglings flew; the average fledging rate: 75.00%; 5) the average breeding success rate was 70.14%. The results will help guide the recovery and expansion of the Chinese crested ibis populations and for other reintroduction programs.

INTRODUCTION

The crested ibis (*Nipponia nippon*) is a globally endangered bird. The bird is included as class I in the Lists of National Key Protected Wildlife of China and is considered a national treasure. Historically, the crested ibis was widely distributed in Japan, China, Korea and the Russian Far East. However, during the early twentieth century, because of deforestation leading to destruction of its habitat, and illegal hunting, its population declined sharply, and the bird became extinct in Russia, Korea, and Japan (Yamashina, 1967; Archibald *et al*., 1980). The wild population in China was also thought to be extinct in 1960. However, four adults and three nestlings were rediscovered in Yangxian, Shaanxi Province in 1981 (Liu, 1981). Since then, massive conservation efforts have been made to restore this small population both in the wild and in captivity. During the past 35 years, concerted conservation efforts, both *in situ* and *ex situ*, have been undertaken. The protective measures taken include local legislation, effective management of nesting and foraging habitats, periodic surveys and monitoring, and public education (Shi and Cao, 2001; Ding, 2004). The wild population and the captive populations increased to approximately 1500 and 500, respectively (Li *et al*., 2016). Bird Life International upgraded the threatened level of the crested ibis (IUCN Red List of Threatened Species) from Critically Endangered (CR) to Endangered (EN) (Bird Life International, 2016).

However, the species is still in danger because of habitat loss, small population size, limited range, winter starvation, persecution, and the effects of disease, which cannot be radically reversed. In addition, with the number

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of crested ibis increasing, the population density of crested ibis in Yangxian County increased, leading to intensified intraspecific competition and an increase in the mortality rate (Ding and Liu, 2007).

Reintroduction of endangered species in their former ranges has helped restore declining bird populations worldwide (Rudolph *et al*., 1992; Armstrong *et al*., 1999). With the number of crested ibis increasing, reintroduction projects have become part of a conservation strategy to save the species (Yu *et al*., 2009). A reintroduction program was conducted in the Dongzhai National Nature Reserve of Henan Province, China, in 2007, in order to establish a new population within the former range. The ultimate goal is to increase numbers in the wild to a healthy population size. The Dongzhai National Nature Reserve received 17 captive bred crested ibis. Of the 17 individuals, four were from the Beijing zoo, and the others were returned by Japan in 2007. By 2013, the number of captive crested ibis in Dongzhai was over 130, which practically satisfied the needs for reintroduction in the wild. A total of 78 individuals were released three times from 2013 to 2015, and some of them bred successfully.

The successful breeding of crested ibis in the wild is the key to establishing the wild population, but the changing environment can easily influence the breeding behavior of the crested ibis (Li *et al*., 2002; Ding, 2004; Huang *et al*., 2004). The habitat characteristics and breeding behavior vary among the crested ibis populations in different areas (Zhang *et al*., 2008). Research has also been conducted on reproductive biology and breeding success of the crested ibis in Yangxian County, Shaanxi Province (Yu *et al*., 2006, 2015). However, there are environmental variations such as vegetation and altitude differences between the Dongzhai National Nature Reserve and Yangxian County of Shaanxi. So far, differences in habitat site selection and breeding ecology of crested ibis in these two regions have not been reported. Therefore, studying the habitat site quality and breeding ecology of the crested ibis in Dongzhai will provide more reference for the reintroduction of crested ibis.

From 2014 to 2016, the released birds were monitored using radio-telemetry and colored bands to determine the reproductive potential of captive-reared crested ibis in this habitat.

Through this study, we aimed to record the behavior and reproductive success of the reintroduced crested ibis, and compare reproductive parameters between the released population and the wild population in Yangxian County as reported by Yu *et al*. (2006), and determine the main factors of the habitat site quality in Dongzhai. Furthermore, the results will have implications for both Chinese crested ibis recovery and other reintroduction programs.

MATERIALS AND METHODS

Study area

We conducted a three-year study (from 2014 to 2016) of the breeding ecology of released crested ibis at the Dongzhai National Nature Reserve covering an area of 468 km^2. The area is located in Luoshan County (31°28'–32°09' N, 114°18'–114°30' E) on the northern slope of the Dabie Mountains in Henan province, China (Fig. 1). This area falls in the temperate zone, where the climate is often moderate and humid, with four distinct seasons. The annual average temperature is 15.1 °C, and the frost-free season lasts 227 days in a year. The annual precipitation is up to 1208.7 mm (Xu *et al*., 2006). The main peak in the study area is 827.7 m above sea level. The forest patches are primarily mixed coniferous-broadleaved forests where the dominant tree species include Chinese red pine (*Pinus massoniana*), loblolly pine (*Pinus taeda*), Chinese ash (*Pterocarya stenoptera*) and princess trees (*Paulownia fortunei*).

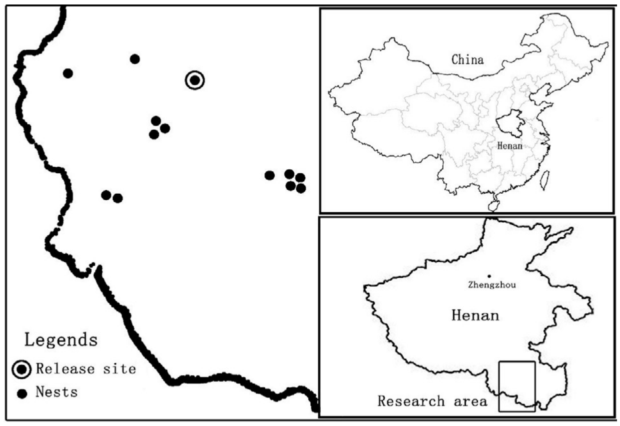

Fig. 1. Map of research area and study nests at surveyed sites.

Release and monitoring

Captive breeding provided an adequate number of crested ibis for release. A total of 78 captive-bred individuals have been released: October 2013 (17 females, 17 males), August 2014 (13 females, 13 males) and May 2015 (9 females, 9 males). All individuals came from the Dongzhai National Nature Reserve. All individuals were held together for more than 6 months before being released (pre-release) in a large aviary (a circular nylon enclosure with a top height of 32 m, tapering to 19.5 m height along the periphery, covering an area of 2800 m^2) containing roost trees and semi-artificial foraging habitat for training in foraging and flying.

Prior to release, all released individuals were uniquely marked with digital colored bands for field identification. Digital colored bands were worn on their left tibias. Sixteen individuals were fitted with lightweight radio transmitters (model TRX-1000S; battery life 24 months; 3-element directional antenna, Wildlife Materials Inc., USA) weighing 19 g, approximately 1.3% of the body weight of a crested ibis, or satellite transmitters weighing 30 g, accounting for approximately 1.8% of the body weight (Platform Transmitter Terminal, sun function). The radio transmitters and the satellite transmitters were installed using the "Backpack" method. The "goal-directed method" was used to conduct wireless tracking (Mech and Barber, 2002). That is, the radio tracker determined the individuals' activity orientation, quickly approached this direction, and finally found the target. The marked individuals were geographically pinpointed using Global Positioning System (GPS) after they flew away. Then, the GPS sent the activity locations of the banded individuals to the users once an hour.

Bird visits and surveys

We observed and recorded the reproductive behavior of crested ibis with the aid of a spotting scope (GEOMA 65A 20-60 x 60mm), binoculars (SICONG 10×42mm) and cameras during three breeding seasons from February to June in the years 2014 to 2016. We identified the potential breeding pairs by observing their bands, and tracking the target nest sites. Breeding processes were observed daily from a commanding height. Egg laying, clutch size, incubation, hatching rate, fledging rate, breeding success rate and fledging dates were recorded. Chicks, 20 to 25 days old, were banded along with bands around nesting trees. After the end of their breeding, a sampling quadrat of 10 m×10 m (100 m^2) surrounding the nesting tree was subsequently marked to measure the habitat characteristics. A tape measure, box and needle, GPS, altimeter, and diastimeter were used to measure 16 variables of habitat characteristics.

In accordance with the position of nesting trees on the mountain slope, we assigned slope bottom, middle and top values as 1, 2 and 3, respectively, and gave flat ground a value of 0. Similarly, we assigned the top, middle and bottom of nesting trees to 1, 2 and 3. For nesting tree, we assigned loblolly pines, princess trees, Chinese ash and Chinese red pines as 1, 2, 3 and 4, respectively.

Statistical analysis

SPSS for windows (version 20.0) was used for data analysis. To determine how the effects of different factors influencing the crested ibis habitat selection, principal component analysis (PCA) was employed. We used the *T*-test to examine differences of crested ibis habitat characteristics and breeding process between wild and reintroduced populations. We report means ± SD unless otherwise mentioned. Statistical tests were considered significant at $P < 0.05$.

Table I.- The general characteristics of crested ibis nests.

Habitat factors	Min	Max	Mean	SD	VC
ALT (m)	34.00	193.00	104.70	46.62	0.45
SPO	0.00	2.00	1.00	0.94	0.94
SGR	0.00	36.00	14.10	13.70	0.97
DPF (m)	2.00	2600.00	373.90	807.35	2.16
DST (km)	1.00	3700.00	1.78	1.29	0.72
DMV (m)	18.00	1500.00	646.80	589.83	0.91
DRA (m)	30.00	510.00	254.00	147.04	0.58
NTS	1.00	4.00	3.30	1.06	0.32
DBH (cm)	19.00	56.00	39.30	12.54	0.32
NTH (m)	10.40	18.30	13.92	2.01	0.14
AAH (cm)	7.20	18.00	11.12	3.25	0.29
ADE	1.60	4.30	2.62	0.83	0.32
NHE (m)	7.50	13.50	10.36	1.75	0.17
NLO	1.00	3.00	2.20	0.63	0.29
NDI (m)	0.00	2.80	1.05	0.96	0.91
NCO	27.00	90.00	63.10	22.65	0.36

ALT, altitude of the nest size; SGR, slope gradient where the nest tree was located; SPO, slope position where the nest tree was located; NTS, nest tree species; DBH, diameter of the breast height of the nest tree; NDI, nest distance straight line distance from the nest to the trunk of the nest tree; NTH, nest tree height from the bottom of the nest tree to the top of nest tree; NLO, nest location, the nest is located in the vertical position of the nest tree crown and the position of the nest tree crown is divided into top, middle and bottom parts; NHE, nest height from the bottom of the nest tree to the nest; NCO, nest coverage of the canopy of the nest; AAH, average arbor height in a sampling quadrant of 10m x 10 m (100m^2); ADE, arbor density in a sampling quadrant of 10m x 10 m (100m^2); DPF, distance to paddy field straight-line distance from the nest to the nearest paddy field; DMV, distance to motor vehicle path straight-line distance from the nest to the nearest motor vehicle path; DST, distance to stream straight-line distance from the nest to the nearest stream; DRA, distance to residential area straight-line distance from the nest to the nearest residential area.

RESULTS

Nest site selection

Seventy-eight captive-bred individuals (39 females, 39 males) were released; twenty birds (25.64%) paired whereas 58 birds (74.36%) remained unpaired. Twelve nests of ten paired birds were recorded over the three year period from 2014 to 2016 (1 in 2014, 7 in 2015 and 4 in 2016). Among these, the proportion of successful nests, which gave rise to the fledging of at least one chick, was 83.33% (10 nests); unsuccessful nests, that produced no chicks being 16.67% (2 nests). Successful and partially successful nests were 60% (6 nests) and 40% (4 nests), respectively. All the nests were on trees (seven on Chinese red pines, three on Chinese ash, one on loblolly pine and one on princess tree). The characteristics of successful nests are shown in Table I.

Table II.- The eigenvectors of the crested ibis nest-site selection.

Component	Eigenvalue	Contribution	Cumulative contribution
1	4.54	28.39	28.39
2	3.63	22.68	51.07
3	2.33	14.56	65.63
4	2.19	13.68	79.31
5	1.37	8.57	87.89
6	0.91	5.69	93.58
7	0.48	2.97	96.55
8	0.36	2.27	98.82
9	0.19	1.19	100.00

Numbers 1 to 9 refer to the first up to the ninth principal component.

The results of the principal component analysis (PCA) revealed that components one through four accounted for 79.31% of the total variance (Table II). The first principal axis showed that the nest tree height (NTH), nest height (NHE), diameter of the nest tree (DBH), distance to stream (DST) and average arbor height (AAH) were the higher loads, which mainly represented the vegetation factor and food factor. Distance to residential area (DRA), nest distance (NDI) and distance to motor vehicle path (DMV) contributed more to the second principal axis and were regarded as interference factors and nest position factors. The large loads of third principal axis were nest location (NLO) and distance to paddy field (DPF), which are known as nest position factors and food factors. In the fourth principal axis, the loads of slope position (SPO) and slope gradient (SGR) were high and are considered as topographical factors (Table III). Furthermore, the largest loads among the first four principal components were NTH, DRA, NLO and SPO, in decreasing order (Table III).

Breeding behavior

The crested ibis laid eggs from February 16th to May 15th (Table IV). A single clutch of 2-4 eggs by each pair was laid in two-day intervals. Thirty-three eggs were laid from 2014 to 2016 (two each in five nests, three each in five nests, and four each in two nests). The average clutch size was 2.75±0.75 (n=12) (Table IV). There was a slight fluctuation in clutch size during the three year period. The proportion of fertilized and unfertilized eggs was 81.82% to 18.18%, respectively.

Table III.- Rotated component matrix of eigenvectors in crested ibis nest-sites.

Variable	Component			
	1	2	3	4
NTH	0.96	0.04	-0.21	0.05
NHE	0.94	-0.19	0.15	0.08
DBH	0.71	0.34	0.45	-0.22
DST	-0.70	0.01	-0.30	0.43
AAH	0.66	-0.17	-0.40	-0.23
ALT	0.58	0.49	0.49	0.16
DRA	-0.15	0.94	-0.05	0.18
NDI	0.07	0.81	0.37	0.03
DMV	0.04	0.78	-0.09	-0.11
NLO	0.17	-0.03	-0.92	0.09
DPF	0.33	0.06	0.86	0.22
SPO	-0.11	0.29	-0.16	0.87
SGR	0.07	0.15	0.49	0.77
NTS	-0.05	-0.31	-0.01	0.70
NCO	-0.03	-0.11	-0.19	-0.69
ADE	-0.01	0.28	-0.00	0.09

For abbreviations, see Table I.

As an asynchronous hatching bird, the incubation period of the crested ibis was 28 to 33 days. In this period, males and females take turns incubating the eggs. With an increase in hatching days, the frequency of taking turns incubating also increased from 2 to 6 times a day. Furthermore, our investigation found that two of ten pairs (010/018, 022/017) laid two nests. The eggs in their first nest were all unfertilized and abandoned. After that, they made another nest, laid eggs and bred successfully. The average hatching rate of all eggs was 78.48% (Table IV).

The rearing period varied from 39 to 46 days, and both the parents together fed the chicks. The amount of food needed and the feeding time of birds increased with the growth of nestlings. However, there was no significant difference in the frequency of feeding between parents,

Table IV.- Reproductive status of reintroduced crested ibis during 2014-2016 in Dongzhai, Henan Province.

Year	Bird banding number of breeder	Parental age	Clutch size	Egg laying date	Fledgling date	Hatching number	Fledging number	Breeding success rate (%)
2014	010/017 (♂/♀)	3/5	2	-	-	2	1	50
2015	022/017 (♂/♀)	4/6	4	2.16	-	0	0	0
	022/017 (♂/♀)	4/6	2	5.15	7.23	2	1	50
	010/018 (♂/♀)	4/4	3	2.26	5.4	3	3	100
	035/005 (♂/♀)	5/5	2	3.9	5.21	2	2	100
	019/050 (♂/♀)	6/3	2	3.11	5.25	2	2	100
	040/042 (♂/♀)	3/3	3	3.10	5.28	3	3	100
	008/053 (♂/♀)	4/4	3	-	-	3	3	100
2016	035/005 (♂/♀)	6/6	4	2.25	5.9	3	3	75
	040/042 (♂/♀)	4/4	3	4.2	6.15	2	2	67
	010/018 (♂/♀)	5/5	2	-	-	0	0	0
	010/018 (♂/♀)	5/5	3	5.2	7.12	3	3	100

- means the date is unclear.

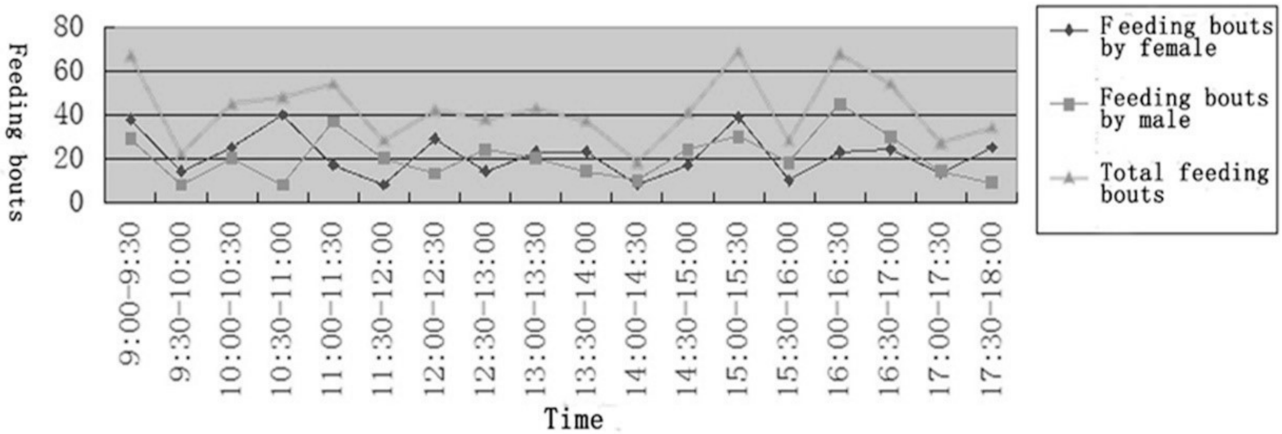

Fig. 2. Relationship between feeding time and feeding frequency of brood chicks during brooding stage.

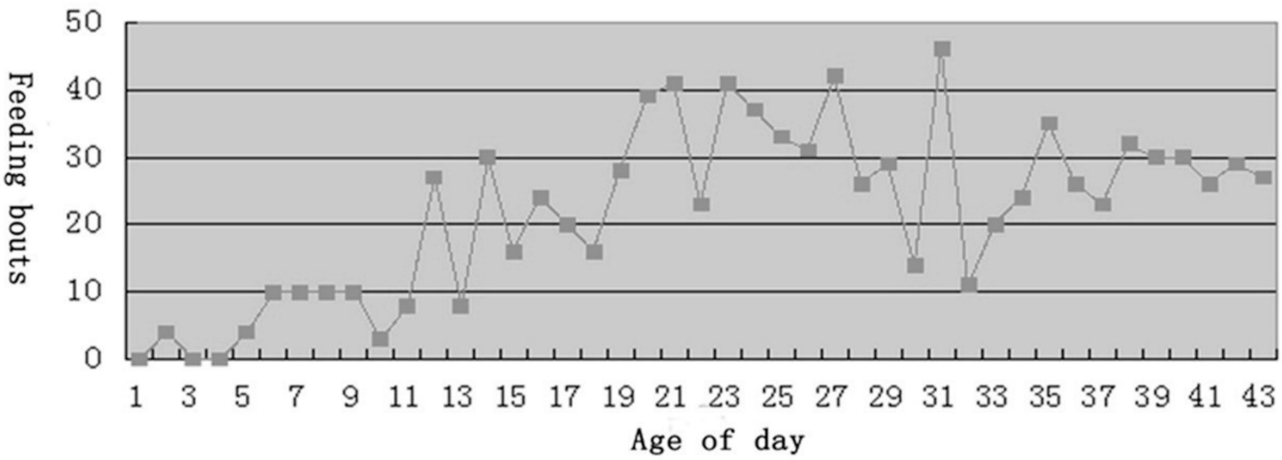

Fig. 3. Relationship between the age of chicks and the number of feedings.

and they were complementary. The peak of feeding periods occurred mainly between 9:00 to 10:00 hours and 15:00 to 16:30 hours (Fig. 2). Nestlings cannot obtain basic food on their own; the feeding frequency by the parents started to increase in the first six days of hatching. The time of feeding peaked on day 32, then began to decline, and stabilized until the chicks left the nest (Fig. 3). A total of 23 young birds fledged: one in 2014, 14 in 2015 and eight in 2016. The average fledging rate was 75.00%, and the average breeding success rate was 70.14% (Table IV).

DISCUSSION

Wei *et al*. (2004) and Wang *et al*. (2016) considered that sheltered places and the distribution of food resources in the habitat site selection were important parts of the bird survival strategy and that the birds needed to balance these two components to increase fitness. In the present study, the first four principal components, which are collectively known as the predator factors, include the vegetation factor, which mainly concern the concealment of the crested ibis, the interference factor, which mainly concerns not being discovered or preyed upon by predators, the nest position factor, which is mainly related to the steadiness of the nest or concealment of the crested ibis, and the topographical factor, which mainly concerns the concealment and escape of the crested ibis. The distance to a stream and paddy fields were related to food and were defined as a food factor. However, the load of distance to stream was negative in value in the first principal component, which indicated that the distance between the nest and stream was large (Table III). The paddy fields was the main factor of food, which was located in the third principal component. Thus, the survival requirements were obviously higher than the choice of food resources. In summary, for the breeding habitat site selection of the crested ibis, the primary choice was the predator factor followed by the food factor.

In this study, the largest loads among the first four principal components were nest tree height, distance to residential area, nest location and slope position. That is, the four variables listed had a high correlation. If the nesting tree was tall, the crested ibis could not only improve the height of the nest location but also reduce in and out barriers of the woodland area. In addition, the advantages of tall nesting trees mainly included strong resistance in order not to be easily affected by adverse factors (weather, natural enemies) and could ensure the safety of nests, eggs and nestlings, which was in accordance with the existing research (Li *et al*., 2001; Zhang *et al*., 2008; Menaa *et al*., 2016). Nests were closest to residential areas in this study. We speculated the reasons may be as follows: (i) The crested ibises were bred in captivity before being released and thus were acclimated to humans. (ii) The people in the reserve had a higher awareness of animal protection compared to people in other regions and therefore reduced the number of predators of the crested ibis. (iii) The paddy fields were generally distributed near residential areas. Low slopes means that more land can be used as farmland and the distribution of streams is increased *i.e.,* the area may be more suitable for foraging. The slope position also influenced the width of nests through the growth of vegetation. These may be trade-offs of the crested ibis in terms of food factors and predator factors.

Climate change affected the date of egg laying (Wu *et al*., 2012). Due to the temperature increase around the world, approximately 60% of studies reported that the breeding period started earlier than ever before (Dunn, 2004). The wild crested ibis breeding period was generally from mid-March to early April (Yu *et al*., 2006). In this study, the breeding period started earlier than that in Yangxian i.e. from February 16. The average temperature at Dongzhai (15.1°C) was higher than that at Yangxian (12.5°C) (Yu *et al*., 2015). Hence the higher temperature in the spring may have led to the early start of breeding compared to that at Yangxian.

Zhang *et al*. (2000) determined that when bird breeding failed, and if the parents had time and energy to reinvest, it was possible to repeat the process. In this study, one of the pairs (022/ 017) laid four and two eggs in two nests in 2015. In 2016, there was another pair that laid two and then three eggs in two separate clutches. The eggs in the first nest were unfertilized, however all eggs in the second clutch hatched successfully. The breeding may have been affected by the early egg laying date and the abundance of food in Dongzhai.

Trivers (1972) argued that most females of monogamous birds provided more parental care than males in rearing chicks, while a study on black skimmer, *Rynchops niger*, showed that males provided more parental investment than females (Burger, 1981). This study revealed that the brooding time by female and male birds was relatively balanced, with no significant difference, and the result was in agreement with previous studies (Shi and Cao, 2001).

Existing research showed that altitude was an important factor in the crested ibis nest site selection (Li *et al*., 2001, 2002, 2006). This study demonstrated that the average altitude (m above mean sea level) of nest sites in Dongzhai (104.70 ± 46.62 m, n=10, 34-193 m) was significantly lower than that in Yangxian (806.1 ± 179.7 m, n=271, 450-1200 m) (P=0.000) (Yu *et al*., 2006) (Table V). The altitude of Yangxian County is in the range of

Table V.- Summary of breeding parameters for wild crested ibis in Yangxian County in 1981–2004 studied by Yu *et al*. (2006) and for released birds in Dongzhai County in 2014–2016 (present study).

	Crested ibis at Yangxian	Crested ibis at Dongzhai	Difference	Significance
Altitude of nest sites (m)	806.10 ± 179.70 (n =271, 450-1,200)	104.70±46.62 (n=10, 34-193)	t =-47.85, P =0.00	*
Height of nest trees (m)	22.60 ± 3.80 (n=204)	13.92±2.01 (n=10)	t =-13.69, P =0.00	*
Height of nests (m)	15.00 ± 4.90[a] (n=21)	10.36±0.55 (n=10)	t =-8.40, P =0.00	*
DBH of nest trees (cm)	43.00 ± 21.50 (n=204)	39.30±12.54 (n=10)	t =6.48, P =0.00	*
Clutch size	2.84 ± 0.77 (n=271)	2.75±0.75 (n=12)	t =-0.41, P =0.69	ns
Hatching rate	80.20% (n =271 clutches and 770 eggs)	78.48% (n =12 clutches and 33 eggs)	t =-0.16, P =0.88	ns
Fledglings/successful nest	2.24 ± 0.80 (n=201)	2.3±0.82 (n=10)	t =0.23, P =0.82	ns

[a] Data from Shi and Cao (2001); * significant ($P < 0.05$); ns, not significant.

450 to 1200 m, and the most suitable altitude was at 650 to 800 m in the Garden Township where the region had abundant paddy fields (Liu et al., 2003; Yan et al., 2015). In this study, the altitudinal range of the study area was 34 to 840 m. The crested ibis selected low hilly areas close to human activities and rich in paddy fields, where they could easily obtain food. However, they did not select high altitude areas with few paddy fields. This suggests that an abundance of food was the key factor in crested ibis nest site selection, instead of altitude; this is in agreement with related research studies (Li et al., 2001; Zhai et al., 2001). This may be related to the environmental differences in the two regions leading to differing preferences of altitude.

The quality of nest sites had a direct impact on the breeding success rate of birds and the change in population number (Robertson, 1995). In Yangxian and Xixiang Counties of Shaanxi Province, although nest site characteristics of crested ibis changed, they had no significant effect on the clutch size, hatching rate and fledging rate. Ma *et al*. (2000) indicated that the nest site quality of crested ibis had not changed significantly in the past 20 years. In this study, compared with the wild population of Yangxian, although the released individuals at Dongzhai had significant differences in altitude, the height of the nest tree and the height of the nest had no significant effect on the clutch size, hatching rate and fledglings per successful nest (Table V). Hence, it was inferred that there was no significant change in the nest quality in the two regions.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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