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Breeding Biology of Isolated Captive Dalmatian Pelicans (*Pelecanus crispus*) at the Shanghai Zoo, China

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Abstract

The breeding biology of a captive, isolated population of Dalmatian Pelicans (*Pelecanus crispus*) at the Shanghai Zoo, China, was studied from 2007 to 2019. The breeding age of the Dalmatian Pelicans was estimated at three to four years old, and they started breeding in late October or early November. The clutch size varied between one and two eggs per nest, and the eggs were 83.2 ± 4.8 mm in length, 55.6 ± 2.9 mm in breadth and 136.1 ± 21.5 g in weight. The fertility fluctuated slightly, with an average rate of 38.7% ± 9.7%, but remained consistently low. The survival rate of chicks fluctuated substantially from 0% to 100%, with an average survival rate of 68.6% ± 27.6%. The averaged values of observed heterozygosity, expected heterozygosity and polymorphism information content were 0.468, 0.465 and 0.446, respectively. It indicated that the population had a low heterozygosity and genetic diversity. There was a significant change in the breeding index compared to that recorded 40 years ago, which indicates that inbreeding depression has occurred in this small, isolated, captive population of Dalmatian Pelicans. These results can be used to improve management of Dalmatian Pelicans in captivity.

Keywords

Egg Size, Expected Heterozygosity, Fertility, Inbreeding Depression, Isolated Population, Survival Rate

1. Introduction

The range of the Dalmatian Pelican (*Pelecanus crispus*) extends from southeast Europe through central Asia to eastern China, where the species occurs in three separate populations (Shi et al., 2008). The eastern Asian subpopulation, which breeds in Mongolia and migrates to southeast China for the winter, is in serious

danger of extinction due to habitat degradation and human impacts (Batbayar et al., 2007) and is considered functionally extinct (BirdLife International, 2018). This pelican species is listed as an endangered species and is nationally protected as a Category I key species in China.

More than 12 Dalmatian Pelicans were introduced to the Shanghai Zoo from Inner Mongolia, China, in 1959 and reared on Swan Lake at the zoo (Xu et al., 2018). The first records of breeding among these birds occurred in the 1970s (He et al., 1984), and artificial incubation was carried out to increase reproduction beginning in the 1980s (He et al., 1984; Shi, 1989; Xu et al., 2014). Young birds were raised by hand and released into the original population to stabilize it. The present population size exceeds 40 individuals (not including the Dalmatian Pelicans transferred to other zoos) and became the largest population of Dalmatian Pelicans undergoing captive breeding in China. However, there are many problems associated with breeding among the members of this small, isolated population at Swan Lake that have persisted since the beginning of this century, such as low hatchability, high chick mortality (Xu et al., 2014), and failed introductions of hand-reared young birds into the original population (Ma et al., 2011). Thus, there is an urgent need to learn as much as possible about the breeding biology of this population for its effective management (Xu et al., 2018).

Previous studies of this captive Dalmatian Pelican population have mainly focused on its breeding parameters (such as clutch size, egg dimension, egg weight and so on) (He et al., 1984), artificial incubation conditions (Shi, 1989; Xu et al., 2019), natural incubation behavior (Zhou et al., 2011) and incubation conditions (Xu et al., 2018), and diseases (Ma et al., 2011; Xu et al., 2014). The purpose of this study is to assess reproduction in this small, isolated Dalmatian Pelican population after almost 70 years of captivity and to provide basic breeding information for the management of captive Dalmatian Pelican populations.

2. Methods

The captive Dalmatian Pelicans in Shanghai Zoo have been reared in the 3.4 ha Swan Lake area of the zoo. The pelicans (more than 30), gather on one of the islands, which cover an area of 40 m^2 and is separated from the mainland by water, to build nests during the breeding season. Zookeepers feed the pelicans once a day, but the birds are also able to hunt and eat the live fish that occur in the lake. The pelicans have access to all areas of the lake and are free flying. During the breeding season, zookeepers add nesting materials to the island, including grasses and tree branches, but the pelicans are also able to gather other natural materials to their nests. More detailed information on the lake and animals is provided by He et al. (1984) and Xu et al. (2018).

The color of the gular pouch can be used as an indicator of breeding. The pouch turns from yellow to bright orange-red when the pelicans begin to breed. The total number of successful breeding pelicans was 18 individuals (nine pairs), of which six pelicans (three breeding pairs) were chosen to study breeding behavior

through all-occurrence sampling. Because pelicans are diurnal animals (Ma et al., 2011), observation started at 6:00 hr until 18:00 hr. Breeding behavior was record for only one pair of pelicans each day. Eight one-year-old pelicans, which were some of the artificially incubated and hatched chicks, were used to determine age at first breeding and these birds were released back into the original population in 2008.

Every year, the zookeepers visit the island to collect the eggs for artificial incubation three to four times during the nesting and reproduction period. There were about 8 - 9 breeding pairs every year. During this visit, nest parameters, including the inner diameter, cup depth and distance between nests, are measured using a steel measuring tape. These parameters were measured for 58 nests in total from 2009 to 2011. The clutch size was estimated by counting the eggs in the nests. The length, breadth and weight of 67 eggs were measured to the nearest 0.1 mm using calipers, and the eggs were weighed to the nearest 0.1 g in 2010, 2016 and 2017. The incubation period of Dalmatian Pelicans is approximately 32 days (He et al., 1984). The eggs were opened to determine fertility if they did not hatch after 32 days, and chick survival was defined as a chick reaching 3 months of age. Data on pelican fertility and chick survival rate were continuously collected for 13 years from 2007 to 2019.

In order to understand the genetic status of the captive isolated population, 16 Dalmatian Pelican progenies blood samples were collected from 2010 to 2012. Based on the PCR amplification of 12 microsatellite loci, the genetic diversity was analysed using Popgene Version 1.32 and NTSYS 2.10e (Machado et al., 2009; Hickman et al., 2008; Zhou et al., 2018). The Polymorphism information contents (PIC) were calculated according to Botstein's (Botstein et al., 1980) formula.

All statistical tests were performed using SPSS Statistics 19. Each individual equally contributed to the data; therefore, we calculated averages (mean ± SD) for the nonparametric statistical analysis, and the results were considered significant at $P < 0.05$.

3. Results

3.1. Breeding Date and Age

The Dalmatian Pelicans at the Shanghai Zoo began breeding in late October or early November. If their nests were destroyed, the pelicans would re-nest until March of the following year. The number of incubating birds every year was approximately 20 individuals. Eight hand-reared young Dalmatian Pelicans were released into the lake in 2008. The gular pouch of these pelicans became orange-red in 2010, after which they successfully paired and laid eggs with other pelicans, suggesting that the breeding age of Dalmatian Pelicans is three to four years old.

3.2. Courtship Pattern

The Dalmatian Pelican is a colony-nesting species, and many breeding pairs

share a common nesting area where each pair builds its own nest. The breeding pair relationship is established through courtship, which includes three stages: 1) courtship display stage-during the early breeding period, males attract females through the bright coloration of their gular pouch and the sound made by their large beaks clacking together; 2) determining pair relationship stage-males collect nesting material (usually large tree branches) for females, and a breeding pair is successfully formed if a female accepts it; 3) body touch and copulation stage-males tend to bite the female's neck or wing and jump onto her back for copulation. At the Shanghai Zoo, the Dalmatian Pelicans were observed to pair bond with captive Great White Pelicans (*P. onocrotalus*) and laid eggs that produced hybrid offspring.

3.3. Nest Material and Size

The Dalmatian Pelicans build their nests within a common area on the island. The nest materials include grass, tree branches, reeds, small willow branches and tree leaves. From 2009 to 2011, 58 nests were selected for measurement (Table 1), but there were no significant differences among years, and the nest data were combined to calculate the average nest size. The nests were 37.8 ± 3.0 cm in diameter with a cup depth of 9.4 ± 1.8 cm. Furthermore, the nests were very close together, with the distance between them being 79.1 ± 13.4 cm.

3.4. Clutch Size and Egg Weight and Dimension

The captive Dalmatian Pelicans at the Shanghai Zoo usually laid 1 - 2 eggs. In 2010, 2016 and 2017, 18, 22, 27 eggs were measured in terms of their length, breadth and weight. There were no significant differences among the three years (Table 2), and the dimensions of the eggs from the three years were combined to obtain a mean value of 83.2 ± 4.8 mm for length and 55.6 ± 2.9 mm for breadth. The weights of the eggs from the three years were also combined to obtain a

Table 1. Nest size of the Dalmatian pelicans at the Shanghai Zoo from 2009 to 2011.

Items	2009 (n = 18)	2010 (n = 28)	2011 (n = 12)	Mean value	*P*
Inner diameter/cm	38.8 ± 2.4	37.4 ± 2.2	37.4 ± 1.8	37.8 ± 3.0	0.079
Depth of nest/cm	10.0 ± 1.9	9.1 ± 1.9	9.0 ± 1.3	9.4 ± 1.8	0.198
Distance between nests/cm	81.5 ± 8.2	78.9 ± 16.9	75.9 ± 10.4	79.1 ± 13.4	0.546

Table 2. Dalmatian Pelican egg weight and dimensions at the Shanghai Zoo in 2010, 2016 and 2017.

Items	2010 (n = 18)	2016 (n = 22)	2017 (n = 27)	Mean value	*P*
Weight/g	146.4 ± 19.1	132.6 ± 16.8	132.1 ± 24.6	136.1 ± 21.5	0.056
Length/mm	84.2 ± 3.8	82.5 ± 4.8	83.0 ± 5.4	83.2 ± 4.8	0.533
Breadth/mm	56.9 ± 2.8	54.8 ± 2.2	55.4 ± 3.1	55.6 ± 2.9	0.068

mean value of 136.1 ± 21.5 g.

3.5. Fertility and Survival Rates

Data on the fertility and chick survival rates of Dalmatian Pelicans in 2007-2019 are shown in Figure 1. The fertility rate of the Dalmatian Pelicans dropped from 54.7% (2007) to 23.5% (2019) over the 13 years and remained consistently low. The average fertility rate was 38.7% ± 9.7%. The chick survival rate substantially fluctuated from 0% (2010) to 100% (2012 and 2015), and the average value was 68.6% ± 27.6% (Figure 1). The low survival rate from 2009 to 2011 was caused by disease. The survival rate began to rise after 2012, mainly due to the identification of an effective medicine for the treatment of the disease which caused by bacteria Escherichia coli (Xu et al., 2014).

3.6. Genetic Diversity Analysis

Data on the genetic diversity analysis of 12 microsatellite loci are shown in Table 3. The number of alleles ranged from 2 to 6, with an allele mean of 3.833 ± 1.267. Under Hardy-Weinberg equilibrium, the expected heterozygosity varied from 0.272 to 0.719, with a mean of 0.465 ± 0.148, whereas the observed heterozygosity varied from 0.305 to 0.883, with a mean of 0.468 ± 0.185. The polymorphism information content varied from 0.059 to 0.692, with a mean of 0.446 ± 0.204.

Based on the genetic distance within the Dalmatian Pelican population, the systematic tree was constructed by UPGMA (Figure 2). The results of clustering analysis showed that the 16 pelicans were mainly descended from two pairs of parent birds and the genetic similar coefficient of individuals No. 5 and No. 6 was 0.92.

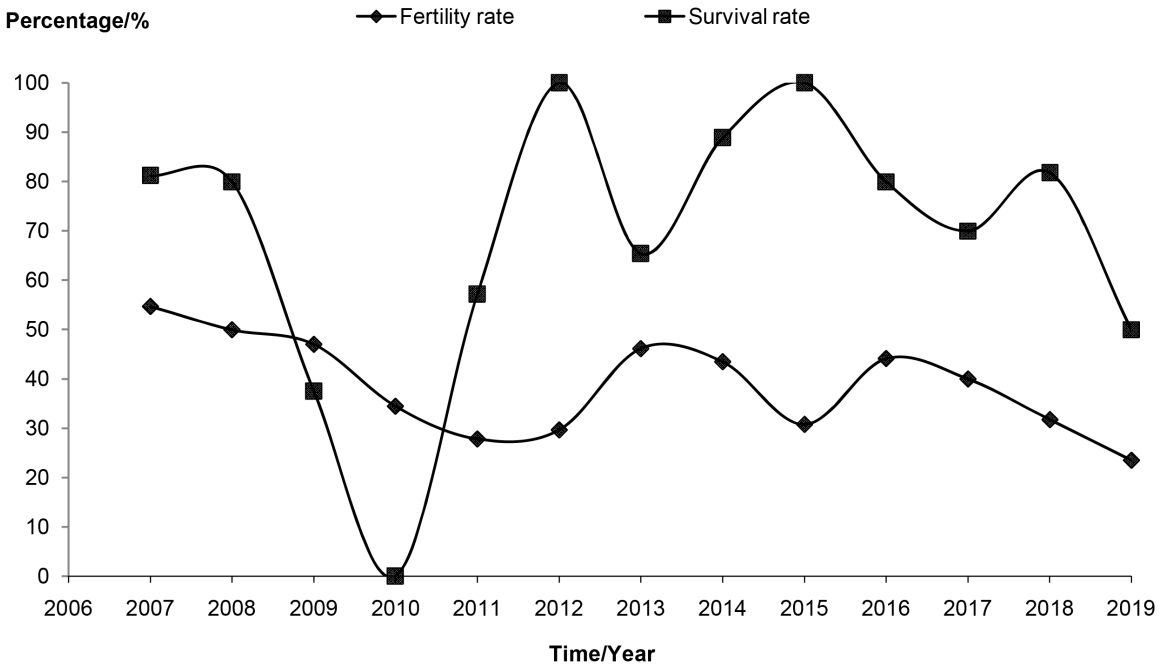

Figure 1. Fertility and chick survival rates of Dalmatian Pelicans at the Shanghai Zoo from 2007 to 2019.

Table 3. Microsatellite statistics of the Dalmatian pelican.

Locus	NA	NE	HO	HE	PIC
PeErH09-11	2	1.753	0.570	0.719	0.337
PeErH09-13	5	3.282	0.305	0.331	0.683
PeErH10-01	3	1.910	0.523	0.540	0.421
PeErH10-03	5	3.048	0.328	0.334	0.606
PeErH10-04	4	2.909	0.344	0.418	0.611
PeErH10-05	2	1.133	0.883	0.716	0.059
PeOnH10-08	6	3.282	0.305	0.272	0.692
PeOnH10-09	3	1.471	0.680	0.533	0.265
PeOnH10-12	4	3.282	0.305	0.415	0.662
PeOnH10-13	4	1.707	0.586	0.422	0.352
PeOnH10-14	3	2.246	0.445	0.542	0.410
PeOnH10-15	5	2.909	0.343	0.332	0.252
Mean	3.833	2.411	0.468	0.465	0.446
SD	1.267	0.792	0.185	0.148	0.204

Abbreviations: NA, number of alleles; NE, number of effective alleles; HO, observed heterozygosity; HE, expected heterozygosity; PIC, polymorphism information content.

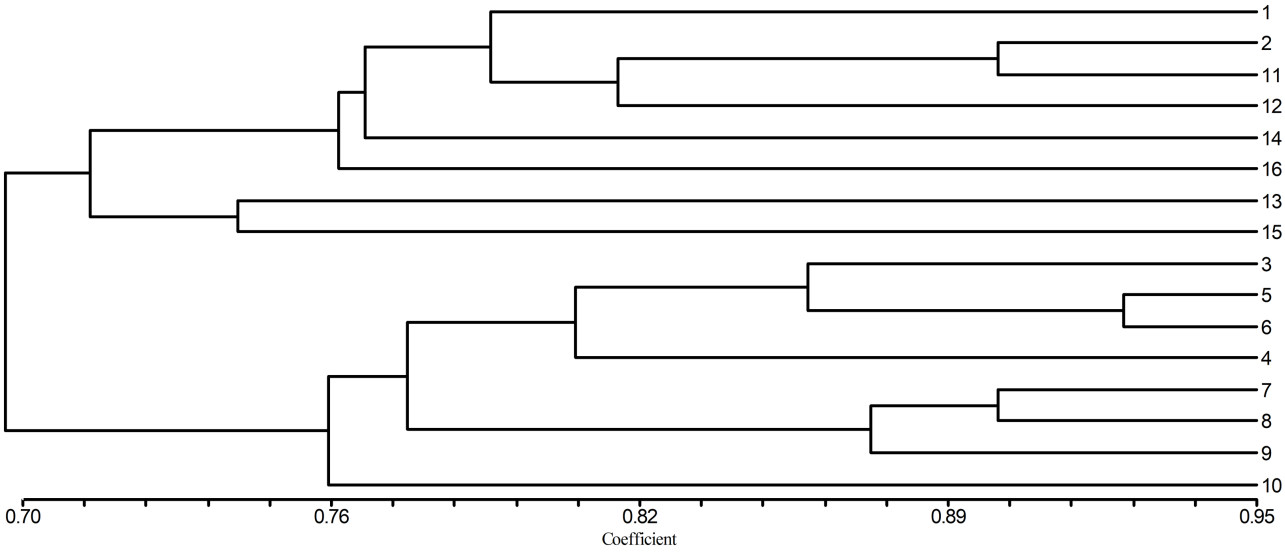

Figure 2. The dendrogram of the 16 individuals of pelican.

4. Discussion

In comparison to the values (He et al.,1984; Shi, 1989) recorded 40 years ago, significant decreases were observed in terms of clutch size and egg dimensions and weight. The clutch size decreased from 2 - 3 (He et al., 1984) to 1 - 2 (present), and the eggs decreased in both length and width. The egg weight fell from 159 g

to 136 g over the past 40 years. These parameters are also lower than those in the wild Dalmatian Pelican population. The fertility rate of the captive Dalmatian Pelican population dropped from 66.7% (Shi, 1989) to 23.5% (2019), while chick mortality has increased and has substantially fluctuated. The chicks and young birds are easily infected by bacteria and parasites, leading to death (Ma et al., 2011; Xu et al., 2014). The genetic variation within the small, captive Dalmatian Pelican population has also become low after nearly 70 years of isolated development (Zhou et al., 2018), indicating that inbreeding depression has occurred because the decline in egg mass, size, fertility and the chick survival may be a consequence of the reduced genetic variation. It is therefore urgently necessary to introduce new individuals to the population to improve its genetic structure and facilitate its sustainable development.

The Dalmatian Pelicans at the Shanghai Zoo were found to be able to form breeding pairs with the captive Great White Pelicans and produce hybrid offspring. He et al. (1984) also observed the same results, but the eggs were infertile. Zhou et al. (2018) reported that the DNA of the Dalmatian Pelicans contained some genetic fragments from Great White Pelicans. This coincided with observations that the hybrid offspring had some external features from both Dalmatian Pelicans and Great White Pelicans. To prevent hybridization between the two species, the following recommendations should be followed: 1) separate the Dalmatian Pelicans from the Great White Pelicans and rear them separately; 2) forbid the release of hybrid pelicans into the original Dalmatian Pelican population. Dalmatian Pelicans sometimes breed alongside Great White Pelicans in the wild (Crivelli et al., 1998), but the mechanism causing the reproductive isolation of these two species is unclear. Understanding what causes their reproductive isolation might be beneficial for the management of Dalmatian Pelicans in captivity.

Furthermore, in the wild, Dalmatian Pelicans begin breeding in spring (Crivelli et al., 1998; Batbayar et al., 2007). The lengthening of the days in spring generally induces breeding in many types of birds, as their breeding cycles are initiated by changes in day length. However, at the Shanghai Zoo, the Dalmatian Pelicans were found to reproduce in winter when the daytime becomes shorter. Ambient temperature may play an important role in the breeding cycle of Dalmatian Pelicans, similar to the photoperiod. However, the role of ambient temperature in the breeding cycle of Dalmatian Pelicans will require further study and investigation in the future.

5. Conclusion

Inbreeding depression has occurred in this small, isolated, captive population of Dalmatian Pelican. It is urgently necessary to introduce new individuals to the population to improve its genetic structure and facilitate its sustainable development.

Dalmatian Pelicans at the Shanghai Zoo were found to be able to form breed-

ing pairs with the captive Great White Pelicans and produce hybrid offspring. It is necessary to separate the Dalmatian Pelicans from the Great White Pelicans and rear them separately to prevent hybridization between the two species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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