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Research article

Comparison of reproductive success between parent-reared and hand-reared northern bald ibis *Geronticus eremita* in captivity during Proyecto Eremita

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Abstract

Modern zoos actively collaborate in the conservation of many endangered species by captive breeding for reintroduction. This paper presents the reproductive success of a captive colony of northern bald ibis *Geronticus eremita* (NBI) at ZooBotánico de Jerez between 1993 and 2013 (21 years). Between 2004 and 2011 was "Proyecto Eremita", a study of the best releasing techniques for hand-reared NBI in Cadiz in order to establish a free-range, self-sustained population in the wild. During this period, first-clutch eggs were artificially incubated and chicks were hand reared until fledgling stage, allowing the pair to produce a second or replacement clutch that was parent reared. This paper compares the colony's reproductive success between the years where one single clutch was reared by the pairs (n=13) and the years of Proyecto Eremita (n=8), with two clutches, the first hand reared and the second parent reared. The reproductive success rate was measured in 300 nests. A total of 268 fledglings reached 2 months old, the age considered here as the reproductive success. Two reproductive variables were significantly higher during the Proyecto Eremita years: mean number of fledglings per nest (1.8 versus 0.3) and overall number of fledglings recorded per year (26.5 versus 4.3). The reproductive success of hand-reared clutches was similar to parent-reared clutches. There was a significant and negative effect of colony size on the percentage of birds paired and on reproductive success. Parent-reared clutches during the Proyecto Eremita showed a higher reproductive success compared to parent-reared clutches outside this period. The data show that a remarkably high percentage of mating pairs outside of the Proyecto Eremita period failed at reproduction, probably due to density-dependent effects. The combination of hand-rearing and parent-rearing methods used in this study was a very effective tool to significantly increase the number of fledglings produced for the reintroduction programme.

Introduction

The introduction of captive-bred animals into nature is a well-known tool used in the conservation of many endangered species. Individuals bred in captivity are later used in (1) reintroduction programmes when the specimens are released to their former distribution areas, (2) introductions when releases occur at non-native areas or the so-called (3) re-stocking or translocation when the movement occurs between areas within their distribution range (IUCN 1996). The number of examples is remarkably high and includes both plants and animals (Maschinski and Haskins 2012; Ewen et al. 2012).

Modern zoos play an important role in the conservation of ex-situ populations of many endangered species. Conservation is achieved by the reproduction of captive- or hand-reared animals in healthy, genetically pure, self-sustained populations. The aim of these programmes is often the reintroduction of captive animals in the wild (Saint Jalme 2002; Gilbert and Soorae 2017; Gilbert et al. 2017).

Well-known examples of successful captive-breeding and (re)introduction programmes include, for instance, Przewalski's horse *Equus caballus przewalski* in Mongolia (Kaczensky et al. 2011), California condor *Gymnogyps californianus* (Walters et al. 2008), Iberian lynx *Lynx pardinus* in Spain (Simón et al. 2012), bearded vultures *Gypaetus barbatus* in the Alps (Schaub et al. 2009) and in Spain (Bustamante 1998), Arabian oryx *Oryx leucoryx* in the Arabian peninsula (Spalton et al. 1999), dorcas gazelle *Gazella dorcas neglecta* in Senegal (Abáigar 2010) or Australian trout cod *Maccullochella macquariensis*

Figure 1. A general view of the northern bald ibis colony in the aviary of ZooBotánico de Jerez. Nests were mounted inside the caves at the back of the aviary.

Figure 2. A close-up view of the NBI nests of the ZooBotánico de Jerez aviary.

(Lyon et al. 2012), among many others. It is important to remark that the release of specimens of captive animals should not be considered a long-term conservation strategy. On the contrary, it should be conducted short term to ensure the survival of species when in-situ conservation methods are not possible or results are ineffective (Snyder et al. 1996).

The northern bald ibis *Geronticus eremita* (NBI hereafter) is a critically endangered species (Bird Life International 2000). In Morocco, the population size was estimated at 318 birds in 1994 and was classified as Critically Endangered (Collar et al. 1994). The population has steadily increased over the last years and new colonies have been recently recorded (Aourir et al. 2007). The maintenance of a captive population, according to ex-situ reproductive programs (EEP) held by European Association of Zoos and Aquaria (EAZA), has allowed the reintroduction of captive-reared birds into the wild at different sites throughout their former breeding area during the last decades (Bowden et al. 2010; Collar and Butchart 2014).

ZooBotánico de Jerez has formed part of the NBI EEP held by EAZA since 1991, when a total of seven birds were received from Innsbruck and Berna Zoos. Since then, the captive population has steadily increased as more birds have been received from other zoos. Between 2004 and 2011, was launched "Proyecto Eremita", a study of the best releasing techniques for hand-reared NBI in Cádiz in order to establish a free-range, self-sustained population in the wild. During this period, more than 350 captive-reared NBI were released at Comarca de la Janda, Cadiz, southern Spain. Most of these birds were bred in the colony at the zoo. A few came from other zoos within the EEP to increase the number of released birds.

The NBI population in the wild has been steadily increasing due to the annual release of birds since 2004 and the reproduction of NBI in the wild. The first successful reproduction was observed in 2008, near the location of the release. Since then, reproduction of free-ranging birds has been recorded each year. The Proyecto Eremita ended in 2011. Based on the results, the Agreement on the Conservation of African–Eurasian Migratory Waterbirds (AEWA) approved the development of a reintroduction project at an international meeting held at Jazan (Saudi Arabia) in 2012. Soon after, a second phase of Proyecto Eremita was started, named "The reintroduction project of NBI in Andalusia", which was officially approved by Spanish authorities in 2013. Thus, the reintroduction project has continued since 2012 to date (Aguilera et al. 2012).

Most of the NBI used in the reintroduction project were parent-reared in captivity and came from different EAZA zoos taking part in the EEP. These birds were released into the wild soon after a quarantine period spent at the zoo. In April 2020, a total of 105 NBI were alive and free-living in the wild; the number of breeding pairs was 22 and the pairs were distributed in three colonies (José Manuel López, personal communication, 24 April 2020). For more details about Proyecto Eremita, see Quevedo et al. (2004), Quevedo and Sánchez (2009) and Bowden et al. (2010).

During the Proyecto Eremita years, it was attempted to produce as many fledglings as possible. To achieve this, NBI at the colony were allowed to establish natural pairs and reproduce freely each year. However, the first clutch was removed from the nest and artificially incubated. Chicks that hatched were hand reared until they reached the fledgling stage. Meanwhile, the parents were allowed to produce a second clutch, the so-called replacement clutch. The replacement clutch and the chicks produced were hence reared by their parents. By doing this, two clutches instead of one clutch were obtained per year. This paper analyses the effectiveness of hand-rearing versus parent-rearing methodology by comparing the reproductive success of the colony during the Proyecto Eremita years (n=8) and the years outside of the Proyecto Eremita period (n=13). The paper also analyses the effect of colony size on the colony's reproductive success rate.

Materials and methods

The species

The NBI is a medium-sized, colonial, insectivorous bird that reproduces once a year between March and June. Clutch size ranges between two and four eggs in years classified as good, and between zero and one egg in bad or dry years. The

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Figure 3. Eggs of northern bald ibis in the incubator. Each egg was numbered for identification using a soft pencil. The air sac was also drawn to control embryo development.

Figure 4. Northern bald ibis chicks sunbathing in an outdoor enclosure, in the nursery of ZooBotánico de Jerez. An NBI-dummy was also used to reduce the imprinting of fledglings to people.

incubation period takes 24–25 days and both sexes are involved in the incubation of the eggs. Chicks are nidicolous and fledglings abandon the nest 40–50 days after hatching (del Hoyo et al. 1992).

The aviary

ZooBotánico de Jerez (size 6.5 ha) is located in the west part of Jerez de la Frontera city, Cadiz, in southern Spain (36.689009, −6.150112). Birds are at an aviary-type enclosure (16x11x5 m) with a vertical wall in one end of the aviary and artificial caves that are used by birds for nesting. A circular 3 m^2 pond, some bushes (e.g. *Pittosporum tobira, Rhamnus alaternus, Plumbago auriculata, Ipomoea* sp. and *Cotoneaster* sp.) and horizontal poles, used as perches, are also available (Figure 1). Fresh alfalfa and small branches were provided ad libitum during the reproductive season to facilitate nesting. During these years, the number of birds kept in the aviary varied from seven NBI in 1991 (see introduction) to a maximum of 50 birds in 2013.

Figure 5. A foster parent characterised with a black t-shirt and an ibis-like helmet observing northern bald ibis fledglings sunbathing at ZooBotánico de Jerez during one of the Proyecto Eremita years.

Captive breeding

Every year, the NBI colony was observed during the reproductive season between March and June at least three times a week. Identification of the birds was possible as all the birds in the colony were colour-ringed with a unique alpha-numeric code. Standard 10x40 binoculars were used to watch the birds. The task of observing the whole colony took between 30–45 min by one observer between 1000 and 1400.

Two birds were considered a pair when they exhibited typical reproductive behaviours, like nest building, defence of the nest against conspecifics, incubation or feeding of hatchlings. Copulations were not considered a good indicator of pairing since many extra-pair copulations have been recorded in the colony (unpublished data). Every year, the location of each nest and the identity of paired birds were noted in a scaled map of the colony.

Variables used to define the reproductive behaviour and breeding success of the colony were as follows: (1) colony size, measured as the number of birds available at the colony on 1 March each year; (2) identity of both members of each pair; (3) identity of unpaired birds, and (4) reproductive success per clutch. In this study, reproductive success was measured as the number of fledglings per pair reaching 2 months old. Because nests were located in caves at >3 m height, neither clutch size nor hatchling success could be recorded (Figure 2).

Parent- versus hand-rearing reproduction

During the years of Proyecto Eremita, the first clutch was removed and artificially incubated in the zoo laboratory. All eggs were

cleaned with soap and warm water and then disinfected with ultraviolet light, before being placed into a M240-C Massalles incubator at 37.4ºC and 60% humidity. Eggs were checked three times a week for embryo development with an ovoscope. After 1 week of incubation, eggs with no embryo development were considered infertile and hence discarded.

Chicks were hand reared using artificial nests consisting of a plastic bucket with the bottom full of alfalfa. During chick development, they were first provided with water in a syringe and then food up to five times a day under temperature-controlled conditions. During the first two weeks, chicks were kept in artificial nests at an ambient temperature of 33–35ºC. To keep this temperature constant, a heating lamp was mounted above the artificial nests. A combination of chopped chicken, beef heart and mice, supplemented with multi-vitamins (Calfostonic® INVESA, Laboratorio Industrial Veterinaria S.A) was provided as food.

Fledglings of 10–14 days old were kept under supervision at outdoor conditions (a small roofed aviary, isolated from the public view) for sunbaths (see Figures 3 and 4). Feathered fledglings of 1 month old were carried by car to an outdoor aviary settled at Zahara de los Atunes, 60 km south of Jerez, for acclimatisation (Figure 5). Fledglings at this aviary were kept under daily supervision by personnel of Proyecto Eremita following a procedure similar to that used at the zoo aviary. Birds were released after reaching between 10 and 24 months old. For more details, see Bowden et al. 2010; Cuadrado 2003; 2013; Cuadrado et al. 2007; 2017; Quevedo and Sánchez 2009.

Statistical analyses

Data were checked for the assumptions of normality and homoscedasticity prior to the use of statistical tests. In case of data not satisfying normality assumptions, non-parametric statistics were performed. Data represent mean ±SD, range: minimum and maximum values, and n: sample sizes. Mann-Whitney U tests, Wilcoxon Z tests and Kruskal-Wallis X^2-tests were used to carry out the comparisons among samples as well as rs, Spearman rank order correlation. Analyses were performed using IBM SPSS Statistics 20 software. Significance level 0.05 was selected.

Table 1. Reproductive data of the northern bald ibis *Geronticus eremita* colony of ZooBotánico de Jerez between 1993 and 2013. During the Proyecto Eremita (PE) years, first clutch was removed, eggs were artificially incubated and chicks were hand reared. Birds were allowed to reproduce again (in the replacement clutch) and chicks were parent reared. In both cases, reproductive success was measured as the number of chicks reaching 2 months old. Values represent mean ±SD. [1] RPT-Reproduction type [2] N – normal years, only parent-reared clutches, [3] PE – Proyecto Eremita – two clutches per pair, first clutch hand reared and secont clutch parent reared. *Number of birds reaching 2 months old per nest.

Year	RPT[1]	Colony size	No. of non-paired birds	No. of paired birds	No. of pairs	0*	1	2	3	4	5	Young fledged per pair	Total young fledged	No. of young fledged (hand reared)	No. of young fledged (replacement clutch)
1993	N[2]	28	4	24	12	9	2	0	1	0	0	0.42±0.9	5	-	-
1994	N	32	8	24	12	6	2	1	1	2	0	1.25±1.6	15	-	-
1995	N	32	4	28	14	11	2	1	0	0	0	0.29±0.6	4	-	-
1996	N	49	15	34	17	16	1	0	0	0	0	0.06±0.2	1	-	-
1997	N	49	15	34	17	17	0	0	0	0	0	0	0	-	-
1998	N	46	16	30	15	15	0	0	0	0	0	0	0	-	-
1999	N	46	18	28	14	14	0	0	0	0	0	0	0	-	-
2000	N	38	4	34	17	13	3	1	0	0	0	0.29±0.5	5	-	-
2001	N	43	13	30	15	10	4	1	0	0	0	0.40±0.6	6	-	-
2002	N	46	14	32	16	12	4	0	0	0	0	0.25±0.4	4	-	-
2003	N	50	26	24	12	12	0	0	0	0	0	0	0	-	-
2004	PE[3]	50	30	20	11	0	3	3	5	0	0	2.18±0.8	24	22	2
2005	PE	32	4	28	15	5	6	2	1	1	0	1.13±1.1	17	16	1
2006	PE	46	28	18	10	2	1	4	2	1	0	1.9±1.2	19	18	1
2007	PE	40	20	20	15	3	6	3	3	0	0	1.4±1.0	21	14	7
2008	PE	39	13	26	18	0	6	6	6	0	0	2.0±0.8	36	28	8
2009	PE	27	3	24	15	0	6	3	4	1	1	2.2±1.2	33	28	5
2010	PE	39	15	24	18	3	2	9	4	0	0	1.78±1.0	32	21	11
2011	PE	25	7	18	15	0	4	7	4	0	0	2.0±0.7	30	17	13
2012	N	36	14	22	11	7	1	3	0	0	0	0.64±0.9	7	-	-
2013	N	30	8	22	11	5	3	3	0	0	0	0.82±0.8	9	-	-
Total					300	160	56	47	31	5	1	0.89±1.1	268	164	48

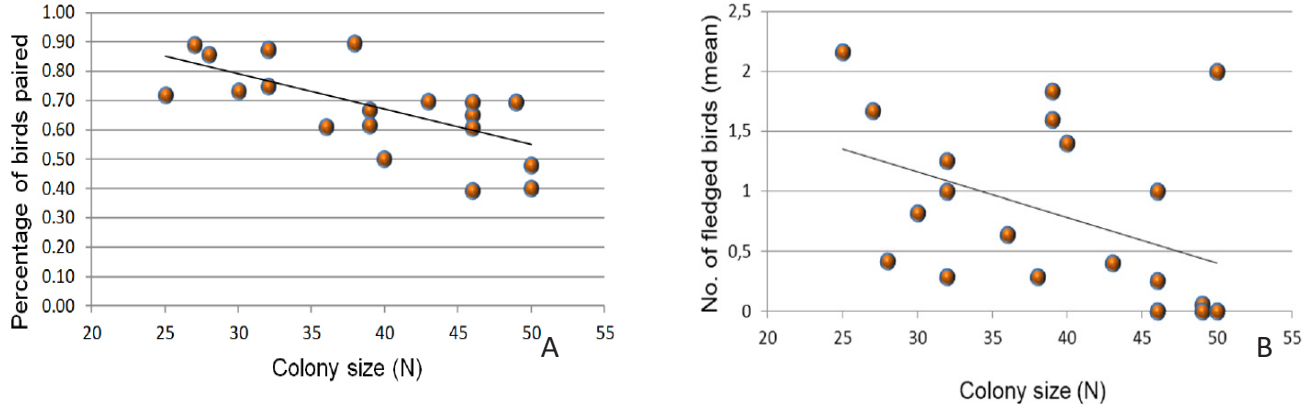

Figure 6. Effect of colony size on the percentage of birds that were paired each year (A) and mean reproductive success (B). Reproductive success was measured as the number of chicks reaching 2 months old. In this figure, data of each year were pooled for calculations. The regression lines were highly significant (P<0.01 in both cases, n=21 years).

Results

Colony size

Colony size varied greatly between years (Table 1). The number of birds in the aviary was minimum in 2011 (n=25) and maximum in 2003 and 2004 (n=50). On average, colony size was 39.0±8.2 birds (n=21 years). Similarly, the number of pairs recorded also varied between 9 pairs (in 2006 and 2011) and 17 (in 1996, 1997 and 2000). On average, the number of pairs was 12.9±2.5 pairs (Table 1). Furthermore, the comparison between the number of paired and non-paired birds varied greatly among years during the 21-year period (X^2_{20}=80.2, P<0.001).

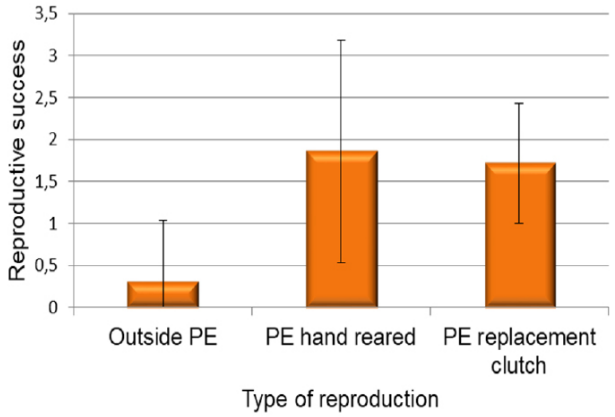

Figure 7. Reproductive success of NBI clutches reared under different breeding conditions: parent-reared clutches outside of the Proyecto Eremita period, hand-reared clutches and parent-reared clutches during the Proyecto Eremita period. Reproductive success was measured as the number of chicks reaching 2 months old per nest. Bars represent mean values +2 SD. See results for statistical significance of differences among groups.

Reproductive success

In this study, the reproductive success was measured as the number of fledglings per nest reaching 2 months old. Reproductive success was recorded in a total of 300 nests (183 during the 13 years outside the Proyecto Eremita period and 117 within the 8-year Proyecto Eremita period). Overall mean reproductive success was 0.88±1.11 (range=0–5, n=300). However, it was around six times higher during the Proyecto Eremita years (1.81±1.0, n=117) than during the years outside of the Proyecto Eremita period (0.3±0.7, n=183). The difference was highly significant (U=2696.5; Z=−11.947; P<0.001, Table 1). Similarly, the total number of chicks (n=268) was significantly higher (U=0.00; Z=−3.781; P<0.001, Table 1) during the Proyecto Eremita years (26.5±7.1, n=8 years and 212 fledglings) compared to the years outside of the Proyecto Eremita period (4.3±4.4, n=13 years, and 56 fledglings).

The difference between the Proyecto Eremita years (n=8 years) and the other years (n=13) was mainly due to a higher number of chicks produced per clutch during the Proyecto Eremita period, as colony size (U=42; Z=−0.728; P>0.05), percentage of paired birds (U=40; Z=−0.870; P>0.05) and the number of pairs (U=45; Z=−0.515; P>0.05) did not vary between periods.

Effect of colony size on NBI reproductive success.

Overall, colony size significantly reduced the reproductive success of NBI at the colony. Not only were colony size and the percentage of paired birds negatively correlated (r_s=−0.707; P<0.01, n=21), but also colony size and reproductive success (r_s=−0.482; n=21; P<0.05, Figure 6).

Comparison between parent- and hand-reared clutches

When the data of the 21-year period were pooled, the reproductive success of parent-reared clutches was remarkably lower (0.49±0.8 chicks, range=0–4, n=211) than hand-reared ones (1.84±1.1 chicks, range=0–5, n=89) which proved differences to be highly

significant (U=3424.5; Z=−9.501; P<0.001). As a result, the hand-rearing method was more effective than the parent-rearing one.

Moreover, if the analyses are restricted to the Proyecto Eremita years (n=8 years), the reproductive success of hand-reared clutches was very similar to that of replacement clutches: respectively, 1.86±1.3 chicks, range=0–4, n=28 and 1.71±0.7, range=1–3, n=28, which showed no significant differences (Z=−0.460; P>0.05).

However, the reproductive success of parent-reared clutches during the years outside of the Proyecto Eremita period was significantly smaller than that of parent-reared or replacement clutches during the Proyecto Eremita period (respectively, 0.3±0.7 chicks, range=0–4, n=183 and 1.71±0.7, n=28, U=420; Z=−8.876; P<0.001, Figure 7). In other words, the colony's reproductive success was remarkably lower during the years outside of the Proyecto Eremita period because most pairs failed to reproduce.

Discussion

Every year the NBI colony was allowed to establish natural pairs and freely reproduce in the aviary, producing only one clutch per year. During the Proyecto Eremita years, however, the first clutch was removed, eggs were artificially incubated, and chicks were hand-reared until fledgling stage. By collecting the first clutch, birds were allowed to produce a replacement clutch that was parent-reared by the pair. This methodology greatly increased the number of chicks available for reintroduction during the Proyecto Eremita period. Moreover, the methodology produced not only two clutches instead of only one, but also the reproductive success of replacement clutches was significantly higher than that of parent-reared clutches during the years outside of the Proyecto Eremita period.

The hand-reared method used during the Proyecto Eremita period produced more chicks per nest on average than the parent-reared clutches when the 21-year period was considered (this study). Avadich (2006) observed similar results in Oriental white ibis *Threskiornis melanocephalus.* However, during the Proyecto Eremita years, the reproductive success of hand-reared and parent-reared clutches was very similar, suggesting that the artificial method used in hand-reared clutches was as good as parent-reared or replacement clutches. Furthermore, the small reproductive success obtained in the colony during the years outside of the Proyecto Eremita period suggests that nest failure was remarkably high. The logical question is therefore: why do NBI pairs show a remarkably low reproductive success when birds are allowed to reproduce naturally?

Firstly, the possibility that food resources were scarce during the reproductive season is excluded. In fact, diet was provided ad libitum and birds of all ages, including chicks, exhibited very good body condition throughout the reproductive season (authors' observations). In addition, starvation was never identified as a cause of death on necropsies routinely performed on deaths of all ages. To support this idea, feather gloss and red colour of the head of adult ibises (both signs of health condition) showed normal and healthy appearance (Galván et al. 2017).

Secondly, reproduction might have failed due to egg-development failures during the incubation phase (Kalita et al. 2013; Erns et al. 2016). In fact, common problems during incubation have been recorded, such as the occurrence of infertile eggs, embryonic malposition at the end of the incubation phase, dead-in-shell embryos, and finally, chicks with part of the yolk not completely absorbed in 1–2 days after hatching (own data). However, these problems have been recorded in a very low number of eggs each season (approximately <5 % of eggs, unpublished data). As the reproductive success obtained in hand- and parent-reared clutches during the Proyecto Eremita period was very similar (see Results), it is suggested that the problems encountered during the egg incubation phase are probably the same regardless of the rearing method.

Thirdly, frequent social interaction between members of different pairs have been recorded during the incubation phase, such as aggressions or the theft of nest material (González 2014). Similarly, Bowden et al. (2003) reported aggressive interactions between reproductive pairs and non-reproductive birds resulting in nest destruction and clutch failure in the wild. In fact, 20% of the pairs produced no chicks, 60% produced one egg, 15% produced two eggs and only 5% bred three chicks. Weather conditions, especially rainfall, have a great impact on the reproduction of this species in the wild (Aguilera et al. 2012). In the aviary, agonistic encounters were recorded in situations when new food items were provided as environmental enrichment (Deza Rollán 2011). Moreover, NBI chicks that shared the artificial nests during hand rearing, showed frequent aggressive, and sometimes very aggressive, interactions. In some cases, the most aggressive individuals, often the largest ones, were removed and put in different artificial nest to reduce aggression. These aggressive interactions among siblings have also been recorded in the aviary nests during parent rearing.

Overall, aggressive interactions among individuals are common during the reproduction phase, both in nature and in captive conditions. This study found a negative effect of colony size on reproductive success. Frigerio et al. (2016) found a similar density-dependent effect in another study performed with the species. Thus, social interference is remarkably common in the aviary and presumably an important factor that influences significantly both nest failure and low reproductive success during years outside of the Proyecto Eremita period.

However, two studies emphasise the importance of NBI social interactions, at least in captivity. In an experimental study on captive NBI at the Bronx Zoo (New York), the authors observed a significant increase in reproductive success after playing communication signals of NBI of the same colony used as acoustic enrichment (Clark et al. 2012). Another study conducted at Alpen and Stuttgart zoos highlighted the importance of early social stimulation in the development of NBI chicks in the nests. Chicks reared in isolated conditions showed difficulties interacting with members of the colony after reaching adult stage, probably as a consequence of stress during growth phase (Tintner and Kotrschal 2002). Assuming that social factors might play an important role in reproductive success, the present study enforces the idea that there are remarkable differences among individuals or pairs, in their reproductive success.

In the colony, the pairs with a higher reproductive success were composed of the oldest and most experienced NBI birds (González 2014). Other studies have shown similar age-related effects (Holmes et al. 2003). Their reproductive success was remarkably higher and similar to that obtained using hand-rearing methods during the Proyecto Eremita period. Outside of the Proyecto Eremita years, the mean reproductive success of the colony was extremely low because most of the recorded pairs failed during reproduction. The reasons for nest failure are unclear, but density-dependent effects of colony size on reproductive success found in this study suggest that competition for space and aggressive interferences with conspecifics could play an important role. According to this experience, the combination of hand- and parent-rearing techniques is a highly recommendable tool to increase the number of fledglings produced every year for reintroduction projects.

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Conflict of interest

The authors declare no potential conflict of interest in this article.

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