Breeding program for the Dalmatian pelican – *Pelecanus crispus*

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Preface

This report was written for my Master's thesis at the Animal Breeding & Genomics (ABG) group at Wageningen University & Research (WUR). During my studies of biology, I delved into the fields of ecology, genetics and bioinformatics. I am interested in the use of genetics for the management and conservation of wildlife populations. Therefore, I am grateful to have had the opportunity to work on this project about the breeding of Dalmatian pelicans in captivity.

The idea for the project came from Jeroen Kappelhof (PhD student at WUR and research officer at Rotterdam Zoo) who was one of my supervisors. Through his work at Rotterdam Zoo, he learned about the problems with the breeding and genetic management of the Dalmatian pelicans. Together with dr. Jack Windig (senior researcher at ABG (WUR)), who is an expert on breeding circles, he proposed this study to find out if a breeding circle could be a suitable management strategy for the species. Together they guided me throughout my thesis and provided me with useful discussions, knowledge and feedback.

Furthermore, this research would not have been possible without the help of Maarten Vis (Curator of Birds at Rotterdam Zoo). Alongside sharing his experience and allowing me to collect samples, it is largely through his efforts that we were able to reach a large number of survey respondents. And of course, the extensive contributions of the survey respondents have also been crucial for providing insights into the current management and breeding of Dalmatian pelicans in zoos.

With this study, I hope to have contributed to a better understanding of the management and breeding behaviour of Dalmatian pelicans. I also hope to have provided a basis for improving the ex situ management of the species which can lead to more consistent breeding and better preservation of genetic diversity.

Abstract

English

The Dalmatian pelican (*Pelecanus crispus*) is kept in multiple European zoos. However, breeding of the species is inconsistent and it is difficult to keep a studbook because Dalmatian pelicans live in groups. First, a survey was sent to zoos that keep Dalmatian pelicans, to assess the current management and breeding. Secondly, simulations were performed to test whether a breeding circle could be a suitable management strategy. And lastly, it was tested if high-quality DNA could be isolated from plucked breast feathers. Dalmatian pelicans need 10 or more breeding pairs in a group to breed successfully. A breeding circle consisting of the 15 largest EAZA populations reduced the inbreeding rate from 3.36% to 0.96% when 5 females were exchanged every 6 years. Increasing the exchange frequency and number of exchanged females resulted in lower inbreeding rates. High-quality DNA was successfully isolated from feathers, providing possibilities for future genetic research and management.

Nederlands

De kroeskoppelikaan (*Pelecanus crispus*) wordt gehouden in meerdere Europese dierentuinen. Het fokken van de soort is echter erg inconsistent en het is lastig om een stamboek bij te houden, omdat kroeskoppelikanen in groepen leven. Als eerste was een enquête verzonden naar dierentuinen die kroeskoppelikanen houden. Ten tweede waren simulaties uitgevoerd om te kijken of een fokcirkel een geschikte beheerstrategie zou kunnen zijn. Tot slot was getest of DNA van hoge kwaliteit kon worden geïsoleerd van geplukte borstveren. Kroeskoppelikanen hebben 10 of meer broedparen nodig in een groep om succesvol te broeden. Een fokcirkel bestaande uit de 15 grootste EAZA-populaties verminderde de inteeldsnelheid van 3.36% naar 0.96% wanneer er 5 vrouwtjes iedere 6 jaar werden uitgewisseld. Het verhogen van de uitwisselingsfrequentie en het aantal uitgewisselde vrouwtjes resulteerde in lagere inteeldsnelheden. DNA van hoge kwaliteit was met succes geïsoleerd uit veren, wat mogelijkheden biedt voor toekomstig genetisch onderzoek en beheer.

Introduction

Almost a third of the species assessed by the International Union for Conservation of Nature (IUCN) (2020) are currently threatened with extinction. We are currently experiencing the sixth major global extinction event in the earth's history (Amato & DeSalle, 2012; Ehrlich et al., 2010). Loss of biodiversity not only threatens the functioning of ecosystems but also has large implications for humanity. Preserving biodiversity is important because it provides many bioresources that are useful for humans like food, materials, and medicine (Díaz et al., 2006; Frankham et al., 2010), and ecosystem services such as prevention of zoonotic disease outbreaks, pollination, and entertainment (Frankham et al., 2010; Keesing & Ostfeld, 2021; Pongsiri et al., 2009). Species conservation is more important than ever.

Even though the main goal of species conservation is to preserve the in situ population, ex situ management can support in situ conservation (Farhadinia et al., 2020). Ex situ populations can serve as backup populations from which animals can be released to supplement or restore wild populations (Frankham et al., 2010). The European Association of Zoos and Aquaria (EAZA) currently manages more than 400 EAZA Ex situ Programmes (EEPs) for different species (EAZA, 2022b).

Captive populations are usually small with limited genetic diversity (Willoughby et al., 2015). These populations are often founded by a limited number of animals and natural gene flow with other populations is absent. Because of that, captive populations have a high risk of inbreeding depression, which are the negative effects of inbreeding. The offspring of related individuals are more likely to obtain alleles that are identical by descent. Rare recessive deleterious alleles are, therefore, more likely to be expressed, resulting in a potential loss of fertility and higher vulnerability to diseases (Jiménez-Mena et al., 2016; Xu et al., 2022). Lower fitness caused by inbreeding depression also makes animals less suitable for reintroductions (Frankham et al., 2010; Jiménez-Mena et al., 2016). Breeding programs should aim to minimise the rate of inbreeding to sustain a healthy population (Lacy, 1995).

Breeding programs can either focus on the level of individuals or populations. However, pedigree information that is necessary for individual-based breeding programs is often not available (Wang, 2004). One of the reasons for this is that many animals live in groups with multiple males and females (Frankham et al., 2010; Wang, 2004). Paternities are hard to track in these species, making conventional genetic management strategies useless (Frankham et al., 2010; Jiménez-Mena et al., 2016). When pedigrees are absent, it might be better to manage inbreeding on a population level.

Inbreeding happens more often in smaller populations because there is a higher probability that two mating individuals are related. Yet, the debate is still ongoing on whether one large population or multiple small populations are better for species conservation: the so-called SLOSS debate (Ovaskainen, 2002; Tjørve, 2010). Both strategies have strong and weak points. A large population contains more individuals, resulting in less inbreeding. However, multiple smaller populations retain more genetic diversity between populations and have a higher reproductive fitness, and thus, produce more offspring (Margan et al., 1998). Keeping the species in multiple smaller populations also protects the total population from risks like disease outbreaks and institutional financial insecurity (Frankham, 2008; Smith et al., 2023). To get the best of both strategies, captive populations are often treated as one large population that consists of smaller subpopulations between which animals are exchanged (Frankham et al., 2010; Margan et al., 1998).

Breeding circles do not rely on pedigree data and can be used to manage inbreeding on a population level (Windig & Kaal, 2008). With this strategy, the populations are ordered in a rotational pattern through which animals of the same sex are exchanged. Each population provides a certain number of individuals for the neighbouring population (Mucha & Komen, 2016; Windig & Kaal, 2008). Donor and recipient populations always stay the same, so every zoo only needs to make agreements with its two neighbours. Another benefit is that a breeding circle can be ordered in such a way that transport distances between zoos and, thereby, costs can be minimised (Kappelhof & Windig, 2021; Mucha & Komen, 2016). A breeding circle is also better for long-term conservation compared to maximal avoidance of inbreeding, which is a more complex rotational breeding system (Windig & Kaal, 2008). All in all, a breeding circle is thus a relatively easy, but also effective strategy for the ex situ management of animals that live in groups.

The Dalmatian pelican (*Pelecanus crispus*) is a species for which a breeding circle could be useful. This large species of waterbird is kept in multiple zoos across Europe (Birdlife International, 2018; EAZA, 2022a). The species' range in the wild spans from South-East Europe to the East of China (Birdlife International, 2018;

Kurstjens et al., 2021). The Dalmatian pelican is classified as near-threatened by the IUCN (Birdlife International, 2018) and at least many European populations in the wild are at risk of extinction (Doxa et al., 2010). In zoos, Dalmatian pelicans are popular exhibit animals (Brouwer et al., 1994; EAZA, 2022a). In August 2022, the Dalmatian EEP contained 515 animals in 57 institutions (*ZIMS Species Holdings*, 2022). Due to the declining wild population (Birdlife International, 2018), breeding Dalmatian pelicans in captivity is important for the conservation of the species. Yet, few captive pelican populations breed consistently and there seems to be a minimum colony size at which the birds start breeding (Brouwer et al., 1994). Moreover, since Dalmatian pelicans breed in colonies and cannot be mated individually, it is hard to keep a studbook for the species (Brouwer et al., 1994; Kurstjens et al., 2021). Using a breeding circle would, therefore, be more suitable than MK.

Even though a breeding circle can be set up without pedigree information, it might still be useful to assess the genetic status of captive populations. Simulation tools can be used to initially design a breeding circle based on inbreeding predictions. However, the results of the simulations might not align with the real-world situation (Windig et al., 2019). Monitoring the level of inbreeding will be important to check if the applied breeding strategy works as expected and allows for a quick response when necessary. Inbreeding can be measured by observing the amount of heterozygosity in the animals' genome (less heterozygosity means more inbreeding) (Frankham et al., 2010; Kardos et al., 2015, 2016). Dalmatian pelican DNA can be collected from blood and tissue samples (Kennedy et al., 2013; Shuangye et al., 2021), however, feather samples are considered less invasive and stressful for the animals (Bello et al., 2001; Dai et al., 2015) and can be obtained easily during routine handling of the animals (Jeroen Kappelhof, personal communication). Generally, it is possible to gain high-quality DNA from feathers (Bello et al., 2001), however, it has not been tested yet for Dalmatian pelicans. Testing this could be useful for future research on the genetics of the species.

To design an effective and functional breeding strategy for Dalmatian pelicans, I will first investigate the current knowledge on the breeding success of Dalmatian pelicans in zoos. Secondly, I am going to model different breeding circles to find the optimal strategy. This part of my research will be similar to the research by Kappelhof & Windig (2021). And lastly, I want to find out if it is possible to gather DNA from plucked Dalmatian pelican feathers for genetic research. The research questions are as follows:

1. What are the differences in breeding success for the Dalmatian pelican between different European zoos?
    - How successful are the different zoos at breeding Dalmatian pelicans?
    - What do zookeepers think are the most important factors affecting the breeding success of Dalmatian pelicans?
    - How can the breeding success of Dalmatian pelicans in captivity be improved?
2. What is the effect of using a breeding circle for captive Dalmatian pelican populations on maintaining genetic diversity?
    - What are the benefits of using a breeding circle in comparison to one big population and no exchange between zoos?
    - What is the effect of the number of animals exchanged between zoos?
    - What is the effect of the frequency of exchanging animals between zoos?
    - What is the effect of differing the minimum population size of the subpopulations in the breeding circle?
    - What would a breeding circle look like in practice and how viable would it be?
3. Is it possible to gain sufficient DNA from Dalmatian pelican feathers to conduct research on their genetics?

Materials & Methods

Management & breeding success in zoos

A survey was made to investigate the current management and important factors for the breeding success of Dalmatian pelicans in zoos. The survey was designed in consultation with Maarten Vis and Jeroen Kappelhof (Rotterdam Zoo). First, some questions were asked about the contact details of the respondent. Questions about the consent of using the answers, referencing the respondent and their institution and follow-up questions were added to the end of the survey. Content-related questions were included based on different categories: group composition, enclosure, flight, diet, breeding success, health & breeding circle.

Questions about group composition were asked to get an idea about the size and sex ratio of the zoo population. These data could also be gathered from ZIMS Species Holdings (2022), however, the group composition might have changed at the time the respondent filled in the survey. By including these questions, the survey response could be linked to the situation at the time of answering.

To get an overview of how Dalmatian pelicans are kept in different zoos, questions about the characteristics of the enclosure were added. This included questions about the enclosure type, size, water area and indoor area. A question was also included to find out if specific elements were added to the enclosure to promote breeding.

Many pelican species are deflighted to keep them in open enclosures (Haase et al., 2021). Deflighting birds can have a negative effect on breeding success (Bračko & King, 2013; Reese et al., 2020), however, that does not necessarily have to also be the case for pelicans (Klös, 1968). Questions were added to find out which deflighting techniques are used for Dalmatian pelicans and what the effect of deflighting is on the breeding success of the species.

Maarten Vis indicated that even though Dalmatian pelicans are a freshwater species, they are sometimes fed saltwater fish in some zoos. It was also interesting to know if supplements were added to the diet. Therefore, questions were added to get an idea of the diet composition of Dalmatian pelicans in the different zoos.

Furthermore, questions were included about the breeding success within the zoos. This was done to get an insight into which zoos have been successful at breeding Dalmatian pelicans and why. Questions about health were also included to find out if there were indications of inbreeding depression.

Finally, a short explanation about breeding circles was given, followed by a question about the opinion of the respondent about the suitability of applying this management strategy to the captive Dalmatian pelican population. It was also included to get an idea about the current challenges of exchanging Dalmatian pelicans.

The survey was sent to EAZA-affiliated zoos that keep Dalmatian pelicans according to data from ZIMS Species Holdings (2022). Survey answers were gathered from November 2022 up to February 2023. The zoos that responded to the survey are indicated in Table 1. Two respondents wanted to remain anonymous. These are not indicated in Table 1. The survey was made using Google Forms. The raw script of the survey can be found in Appendix I.

Effects of a breeding circle

Simulations

Simulations were done to test the effects of a breeding circle. The simulations were based on the current EAZA populations (Table 1) to keep them as close to the real situation as possible. The EEP contained 515 Dalmatian pelicans which were distributed over 57 institutions (*ZIMS Species Holdings*, 2022). Dalmatian pelicans with unknown sex were omitted from the simulation.

The zoo population of Réserve Zoologique de la Haute-Touche (Obterre) had a very skewed sex ratio. However, it also had a large population and was therefore still interesting for incorporation into a breeding program. To make the population more functional, an even sex ratio with 10 animals of each sex was used in the simulations. This can also be done in practice by exchanging Dalmatian pelicans with zoo populations that are too small to participate in the breeding program.

Table 1: EAZA Dalmatian pelican population. This data was retrieved from ZIMS in August 2022. The zoos that filled in the survey are also indicated.

Zoo	Country	Survey**	Number of animals			N
			Males	Females	Unknown sex	Males + Females
Rotterdam	Netherlands	✓	22	21		43
Aywaille	Belgium	✓	13	18		31
Villars-les-Dombes	France	✓	14	14		28
Obterre	France	✓	19 (10)*	1 (10)		20 (20)
Schmiding	Austria	✓	8	7	2	15
Val-de-Reuil	France	✓	7	6	3	13
Sainte-Croix	France		6	8	2	14
Budapest	Hungary		7	4	4	11
Berlin Zoo	Germany		5	9		14
Mechelen	Belgium	✓	7	7		14
Bern	Switzerland	✓	5	8		13
Poznań	Poland		7	6		13
Prague	Czech Republic	✓	5	7		12
Moscow	Russia		4	3	4	7
Jurong	Singapore		1		9	1
Dvůr Králové	Czech Republic	✓	6	4		10
Vienna	Austria	✓	5	5		10
Augsburg	Germany	✓	3	6		9
Clères	France				9	
Fort-Mardyck	France	✓	6	2	1	8
Leeuwarden	Netherlands	✓	2	7		9
Leipzig	Germany	✓	3	6		9
Mulhouse	France		5	4		9
Münster	Germany	✓	6	3		9
Nürnberg	Germany	✓	4	5		9
Trégomeur	France	✓	3	6		9
Amersfoort	Netherlands	✓	4	4		8
Berlin Tierpark	Germany	✓	4	4		8
Overloon	Netherlands	✓	4	3	1	7
Zagreb	Croatia		4	4		8
Le Guerno	France	✓	4	3		7

Pont-Scorff	France		3	2	2	5
Salzburg	Austria		4	3		7
Agrate	Italy		3	3		6
Basel	Switzerland		3	3		6
Chomutov	Czech Republic		1	5		6
Paignton	UK	✓	2	4		6
Torino	Italy		3	3		6
Le Pal	France	✓	1	4		5
Maubeuge	France		5			5
Herberstein	Austria	✓	2	2		4
Karlsruhe	Germany		1	3		4
Le Vigen	France	✓	2	2		4
Lisieux	France		4			4
Opole	Poland	✓	3	1		4
Heidelberg	Germany	✓	3			3
Jurques	France		2	1		3
Kaliningrad	Russia			3		3
Košice	Slovakia		2	1		3
La Teste-de-Buch	France	✓	1		2	1
Ayzac-Ost	France	✓	1	2		3
Walsrode	Germany		1	2		3
Barcelona	Spain		2			2
Tallinn	Estonia		1	1		2
Izmir	Türkiye				1	
Ballaugh	Ireland		1			1
Gelsenkirchen	Germany		1			1
Total			245 (236)	230 (239)	40	475 (475)
Mean			4 (4)	4 (4)	1	8 (8)

* The numbers used in the simulations are given in brackets

** Two additional respondents who wanted to remain anonymous filled in the survey

Pointer

The simulations were done using the software Pointer (previously known as GenManSim) (Windig & Hulsegge, 2021). This program was originally developed to simulate genetic management for Dutch golden retriever dogs (Windig & Oldenbroek, 2015). However, Pointer is not limited to dogs and can be used to simulate the breeding of other species of captive animals. The software can also be used for the simulation of breeding circles (Kappelhof & Windig, 2021; Windig et al., 2019; Windig & Hulsegge, 2021).

A detailed description of the software can be found in Windig & Hulsegge (2021). Pointer uses stochastic simulations of individual animals. It generates a dataset based on the input with characteristics for each animal (sex, age, subpopulation, relatedness & alleles). Only breeding animals and animals that could breed in the future are simulated. Culled animals are replaced by new-born animals so the population will stay constant. However, populations can still become smaller when the birth rate is too low. The program simulates a cycle with mating, births, migration between subpopulations, culling and replacing culled animals. After that, the cycle repeats with the new population. This goes on for as many years as given in the input. The population and subpopulations are defined by giving the numbers of males and females in the input. Relatedness can also be added, however, that was not used in this study. Population composition can be easily obtained from studbook data. Each litter has a random sire and dam assigned from the population. A female can only have one litter per year, while a male can mate with different females based on the given input parameters.

Migration between subpopulations can be added to the input. Pointer allows for multiple schemes, so different migration patterns can be simulated per simulation cycle. The scheme is visualised as a table where the number of animals migrating between individual subpopulations can be defined. This can either be done as probabilities or numbers of animals. For this study, the migrating animals were defined by fixed numbers. Longer exchange intervals were created by adding empty migration schemes for the simulated breeding cycles without exchange.

Inbreeding in small populations can be hard to predict. Because there are only a few individuals, the life history of each animal can have a significant impact on the development of the population. Chance effects like genetic drift allow replicate populations to significantly diversify from the original population (Frankham et al., 2010). Therefore, stochastic simulation gives a more useful output than a deterministic model if the simulation is repeated multiple times. By having multiple repeats, a range of possible outputs will be generated. It will not be possible to predict the exact outcome, but Pointer will give a good indication of the effectiveness of a specific breeding program setup.

The most important output for this study is the rate of inbreeding (ΔF). Pointer estimates ΔF using the following equation:

$$\Delta F = (1 - F_n)^{L/n}$$

With n being the number of years, F_n is the average inbreeding in the last year and L the average generation interval. L is also given by Pointer. This equation was also used to calculate the average inbreeding rates for the individual zoos as Pointer does not output these values.

Pointer has the option to select a proportion of champion sires that sire a specific proportion of the next generation. This is useful for situations where a few males disproportionally contribute more to the gene pool than other males, like for instance a champion dog whose genes are highly valued (Leroy, 2011) or animals that live in polygynous systems like Hamadryas baboons (*Papio Hamadryas*) (Kappelhof & Windig, 2021). However, because pelicans only mate with one female per breeding season, the number of champions was set to 0.

Pointer also has multiple options to change the breeding policy. Dalmatian pelicans form couples each mating season (Kurstjens et al., 2021), thus the male mates with only one female per year so the maximum number of females serviced/male/year was set to 1. As females were not restrained from breeding, their maximum number of nests (litters in Pointer) was set very high (1000). The other parameters for the breeding policy were set to their default values.

All simulations were run over 99 years with 40 repeats.

Age structure

Dalmatian pelicans have overlapping generations. The population was divided into age classes of 3 years because Dalmatian pelicans can breed until an old age. At Rotterdam Zoo, a dam was recorded having two offspring at 42 years of age (ZIMS database). However, this is more an exception than the norm, as most births come from animals up to 30 years old. The age structure of the parents that was used in the simulation (Table 2) was based on studbook information from multiple EAZA zoos (Amersfoort, Augsburg, Basel, Berlin Tierpark, Berlin Zoo, Bern, Budapest, Chomutov, Villars-les-Dombes, Dvůr Králové, Fort Mardyck, Le Guerno, Leeuwarden, Mechelen, Mulhouse, Nürnberg, Overloon, Poznań, Prague, Rotterdam, Salzburg & Vienna). For pelicans of which the parents were known, the age of the parents was calculated at the time the animal hatched. Few animals were born from parents older than 30 years (2 from sires, 2 from dams). These outliers were removed from the data. Pointer uses rounded percentages that add up to 100%. Rounding up the offspring percentages for the dam yielded a total of 99%. To make it workable for Pointer, 1% was added to age class 22-24.

Table 2: The age structure used for the simulations. The table shows the percentage of offspring the different age classes contribute to the next generation.

Age class (year)	% Offspring sires	% Offspring dams
1-3	3	3
4-6	22	8
7-9	8	11
10-12	16	18
13-15	16	19
16-18	14	21
19-21	9	12
22-24	9	3
25-27	2	3
28-30	1	2

Clutch size distribution

The clutch size distribution was determined using the studbook data described under "Age structure". It was found that, per dam, 85% of the successful nests raised one offspring and 15% of the successful nests raised two offspring. The mean age of the dams was 24.25y and each dam had produced on average 1.75 clutches. Even though the literature puts the age of sexual maturity around 3 years (Brouwer et al., 1994; Klös, 1968; Xu et al., 2022), the age of sexual maturity was assumed to be 1 year based on the studbook data. However, the simulations still correspond to the literature because only a small fraction of the pelicans in the age class 1-3 years breeds (Table 2).

It was calculated that female Dalmatian pelicans have on average 0.226 clutches every 3 years. Subsequently, the probabilities of having certain amounts of offspring over 3 years can be calculated. The probability distributions can be found in Table 3. A dam has a 21% chance of having offspring within 3 years (Table 3). The number of clutches can then be calculated for each simulation by taking 21% of the least represented sex in the population (e.g.: $N_m = 59,\ N_f = 63\ \rightarrow\ \#clutches/3y\ =\ 59 * 0.21 = 12$).

Table 3: The offspring distribution over 3 years. The table shows the probability that a dam gets a certain number of offspring within 3 years. The table shows the distributions of the probability of having a certain number of offspring and of having a certain number of offspring if the dam has offspring within the 3 years. In the second column ("% Probability"), 1% was added to the probability of having 3 offspring over 3 years to get the rounded values to add up to a total of 100%.

#Offspring / 3 years	% Probability	% Probability (if ≥1 offspring)
0	79	
1	16	79
2	4	19
3	1	2

Breeding circles

Studbook data (*ZIMS Species Holdings*, 2022) and data by Brouwer et al. (1994) suggested that a population of at least 10 pelicans is necessary to get them to start breeding. Brouwer et al. (1994), however, also suggested that a population of 20 pelicans might be the minimum. Both values are probable. Maarten Vis indicated that N=10 might be enough, but N=20 could be preferable (personal communication). Therefore, simulations were run with only populations larger than or equal to both minimum values. A smaller group of Dalmatian pelicans might also breed if they share their habitat with another breeding species of pelican. However, it is not preferable to keep multiple pelican species together as they can hybridise (Brouwer et al., 1994; Klös, 1968). In the simulations, it is assumed that the pelicans live in monospecific groups.

Two sets of simulations were done where zoo populations smaller than the minimum population size (N_{min}) were left out. The zoos were ordered in the breeding circle based on their location. The two breeding circles based on the current populations are shown in Figure 1. These will be called the '4 Zoos' and '15 Zoos' scenarios, with N_{min}=20 and N_{min}=10 respectively.

Different combinations of the number of animals exchanged and exchange intervals were tested for the two simulation sets. Only females were exchanged because if both sexes were exchanged, animals from the same donor zoo could mate with each other in the recipient zoo. There was no particular reason to exchange females instead of males. The results were expected to be similar because Dalmatian pelicans only have one breeding partner per breeding season and both sexes are equally represented in the population. For both the scenarios '4 Zoos' and '15 Zoos' either 1 or 5 female Dalmatian pelicans from the first age class were exchanged to their neighbouring zoo per exchange event. Exchanging 10 female pelicans was also tested for the '4 Zoos' scenario. This was not done for the '15 Zoos' scenario because with N_{min}=10 it was more likely that there were not enough females available for exchange.

To test the effects of different exchange intervals on inbreeding, simulations were run where pelicans were exchanged every 3, 6, 12 or 21 years. These values were chosen based on the longevity of the species and logistical aspects (a larger exchange interval means less effort). Soulé (1980) suggested that inbreeding for conservation should be kept under 1% per generation. A value of 1% inbreeding per generation was used as the baseline to determine if the simulated amount of inbreeding was acceptable.

The genetic diversity at the end of the simulations was calculated by: $1 - f$. Here, f is the kinship value after 99 years. Kinship is used to calculate the genetic diversity instead of inbreeding because inbreeding can be very large within subpopulations while the genetic diversity between subpopulations is still high. Kinship shows the relatedness between all animals in the population and is, therefore, a better estimator for the total genetic diversity.

A third breeding circle was designed after the '4 Zoos' and '15 Zoos' scenarios had been simulated. The goal was to develop a scenario that was effective while also realistic and contained the best properties of

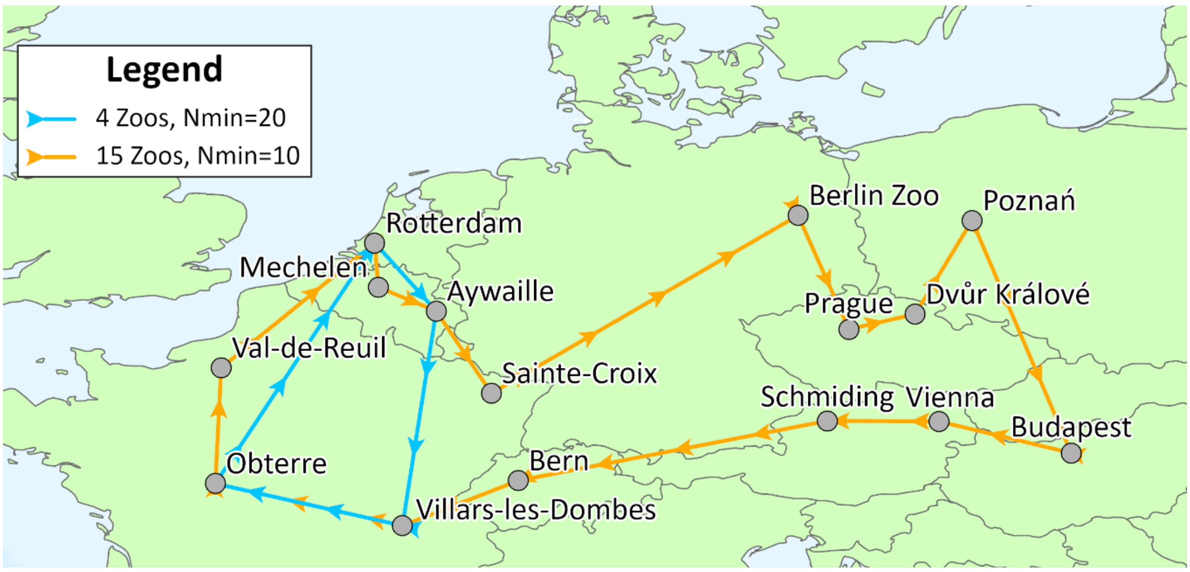

Figure 1: The '4 Zoos' and '15 Zoos' scenarios. The '4 Zoos' scenario is shown in blue. This breeding circle includes the zoo populations with N_{min}=20. The 15 'Zoos' scenario is shown in orange. This breeding circle includes the zoo populations with N_{min}=10.

the other two scenarios. The transport distances were kept short in this breeding circle, while only zoos that were already relatively large were included. These were the four zoo populations from the '4 Zoos' scenario (Aywaille, Obterre, Rotterdam & Villars-les-Dombes) and the four zoo populations with N_{min}=10 closest to these zoos (Bern, Mechelen, Sainte-Croix & Val-de-Reuil) (Figure 2). As stated before, the population of Obterre would be changed to 10 males and 10 females (Table 1). This will be called the '8 Zoos' scenario. This breeding circle was simulated with the exchange of 1, 5 or 10 female Dalmatian pelicans every 3, 6, 12 or 21 years.

Figure 2: A visual representation of the '8 Zoos' scenario. This breeding circle consists of the four zoo populations with N_{min}=20 and the four closest zoo populations with N_{min}=10. Smaller populations can be increased by adding Dalmatian pelicans from zoos outside of the breeding

After this scenario was tested, the populations of the last four zoos were increased so they contained at least 10 males and 10 females (Table 4). Realistically, this could be done by bringing in Dalmatian pelicans from zoos outside of the breeding circle. Similarly, an extra set of simulations was done where the populations were increased to at least 15 male and 15 female Dalmatian pelicans (Table 4). Of course, there are many ways to design a breeding circle and it could look very different if one was created in practice.

Another breeding circle was simulated to test the effects of maximising the amount of included Dalmatian pelicans from the EAZA population. However, only Dalmatian pelicans from within the EU and Schengen area were included because cross-border transport of animals can be difficult due to regulations (Jeroen Kappelhof, personal communication). All Dalmatian pelicans of known sex from EAZA zoos within the EU and Schengen area were divided over populations of at least ten males and ten females (Table 5). Practical aspects like the current zoo facilities and transport distances were left out. Because of this, it did not matter which zoos were included, therefore the subpopulations were not assigned to a specific institution. The breeding circle was simulated with the exchange of 1, 5 or 10 female Dalmatian pelicans every 3, 6, 12 or 21 years. This will be called the 'Maximised' scenario.

Controls

Two sets of control simulations were run to find the extreme values for inbreeding. To get the lowest amount of inbreeding, three simulations were run to test the effects of keeping animals in a single large

Table 4: The populations used in the '8 Zoos' scenario. this breeding circle includes the four largest zoos and the four closest zoos with at least 10 animals. For two sets of simulations, the populations were increased so they would have at least 10 or 15 animals per sex.

Zoo	Original size			N_{min}=20			N_{min}=30		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
Rotterdam	22	21	43	22	21	43	22	21	43
Aywaille	13	18	31	13	18	31	15	18	33
Villars-les-Dombes	14	14	28	14	14	28	15	15	30
Obterre	10	10	20	10	10	20	15	15	30
Mechelen	7	7	14	10	10	20	15	15	30
Val-de-Reuil	7	6	13	10	10	20	15	15	30
Sainte-Croix	6	8	14	10	10	20	15	15	30
Bern	5	8	13	10	10	20	15	15	30
Total	84	92	176	99	103	202	127	129	256

population. First, this was done for the complete EAZA population (N_{male}=236; N_{female}=239) (Table 1). Secondly, this simulation was run while combining the populations of the '4 Zoos' scenario, and lastly, while combining the populations of the '15 Zoos' scenario. There was no exchange in these simulations because each simulation only had one population.

Another two simulations were done for the '4 Zoos' and '15 Zoos' scenarios but without exchange. These simulations give the maximum amount of inbreeding as there is no gene flow between populations. The results of the breeding circle simulations should end up somewhere in between the inbreeding rates of the controls.

In total, 73 different simulations were run (3x 'One Population', 2x 'No Exchange', 8x '15 Zoos', 12x '4 Zoos', 36x '8 Zoos' & 12x 'Maximised').

Table 5: Subpopulations of the 'Maximised' scenario. For this scenario, the Dalmatian pelicans within the EU and Schengen area are redistributed into populations consisiting of at least 10 males and 10 females.

Subpopulation	Males	Females	Total
1	12	11	23
2	12	11	23
3	11	11	22
4	11	11	22
5	11	11	22
6	11	11	22
7	11	10	21
8	11	10	21
9	11	10	21
10	11	10	21
11	11	10	21
12	11	10	21
13	11	10	21
14	11	10	21
15	11	10	21
16	11	10	21
17	11	10	21
18	11	10	21
19	11	10	21
20	11	10	21
21	11	10	21
Total	233	216	449

Possibility DNA Isolation

In Rotterdam Zoo, ziplock bags are used to store feather samples from vultures (Maarten Vis, personal communication). The feathers are then stored dry and cool. This method is very practical, however, using a fixative might be better to preserve the DNA in the samples. Therefore, both methods were tested to find out which is better to preserve DNA.

Breast feathers were plucked from five Dalmatian pelicans at Rotterdam Zoo during a routine wing clipping event. Per pelican, a handful of feathers were taken of which half was stored in ziplock bags. The other half was cut at approximately 2cm from the tip of the calamus and stored in 2mL tubes with 1mL DESS solution (composed of dimethyl sulphoxide, disodium EDTA and saturated NaCl). All samples were stored for two weeks at 4°C.

DNA isolation was done using the *Qiagen DNeasy Blood & Tissue Kit*. For each sample, three feathers were used. The feathers were cut just before the barbs, so only the calamus was used. This region of the feather contains most of the DNA (Olsen et al., 2012). The calami were then cut in the longitudinal direction. The cut samples were placed into 1.5mL Eppendorf tubes. 180µL Buffer ATL and 20µL proteinase K were added to the Eppendorf tubes. The samples were incubated at 56°C at 400rpm for 40 min. After incubation, 200µL *Buffer AL* was added and mixed by vortexing. 200µL ethanol was added and the sample was vortexed again. The mixture was pipetted into a *DNeasy Mini spin column* that was placed in a 2mL collection tube. The sample was centrifuged at 8.000rpm for 1 min. After centrifuging, the spin column was placed into a new 2mL collection tube. 500µL *Buffer AW1* was added and the sample was centrifuged again at 8.000rpm for 1 min. The spin column was placed into a new 2mL collection tube. This time 500 µL *Buffer AW2* was added and the sample was centrifuged at 13.300rpm for 3 min. The spin column was transferred to a clean 1.5mL Eppendorf tube. The DNA was eluted two times by adding 100µL *Buffer AE* to the centre of the spin column, incubating for 1 min at room temperature and centrifuging at 8.000rpm for 1 min. However, it might be better to do the last step only once with 50µL *Buffer AE* because the sample was very diluted.

The quality of the isolated DNA was tested by gel electrophoresis. The electrophoresis sorts the DNA fragments by their lengths. Longer fragments indicate less degradation of the DNA. 10 drops of 2µL loading dye were pipetted onto a paper. To every drop, 1µL of one of the samples was added. Then the samples were loaded into the gel. 2µL of *GeneRuler Express DNA Ladder* was loaded next to the samples to indicate different fragment lengths. The gel was run with 110V for 30min.

Results

Management & breeding success in zoos

Survey response

Survey answers were gathered from November 2022 up and until January 2023. From the 57 contacted zoos, 32 filled in the survey. Most respondents filled in the survey just after the survey was first shared and after a reminder that was sent after 12 days (Figure 3). A second reminder was sent after 47 days to the six zoos with the largest groups that had not yet filled in the survey. Three of them responded.

Four zoos also replied to the invitation but did not fill in the survey. Two of them did not have Dalmatian pelicans anymore. One zoo only had one pelican that was a hybrid between a Dalmatian pelican and a pink-backed pelican (*Pelecanus rufescens*). The fourth zoo only had Dalmatian pelicans since 2022 and had therefore not enough experience to answer the survey.

Nearly all respondents fully filled in the survey. Only follow-up questions were often not answered because it was not relevant. Questions from *Section 8* of the survey were also often left unanswered (Appendix I). This was because *Section 8* was a follow-up part if the respondent indicated that their zoo had breeding pelicans at that moment. Furthermore, the respondents that did fill in this

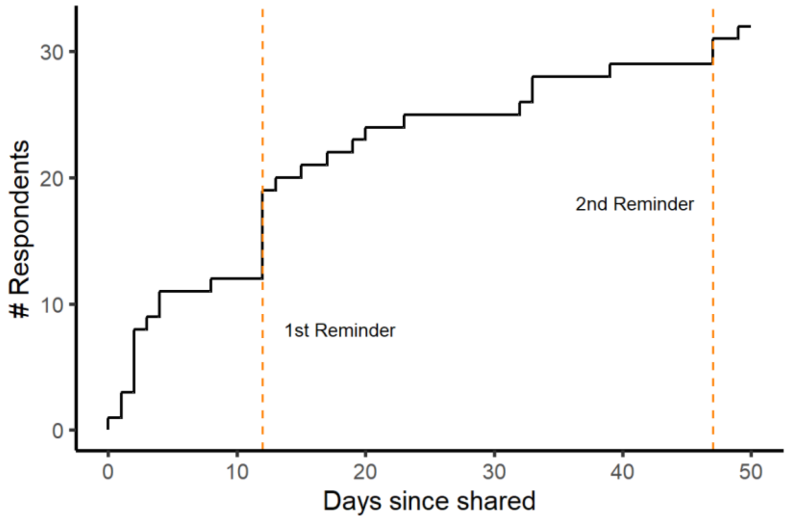

Figure 3: Survey response over time. After 12 days, the first reminder was sent to all zoos that had not yet responded to the survey. After 47 days, a final reminder was sent to the six zoos with the largest groups that had not responded. The reminders are shown by the dotted lines.

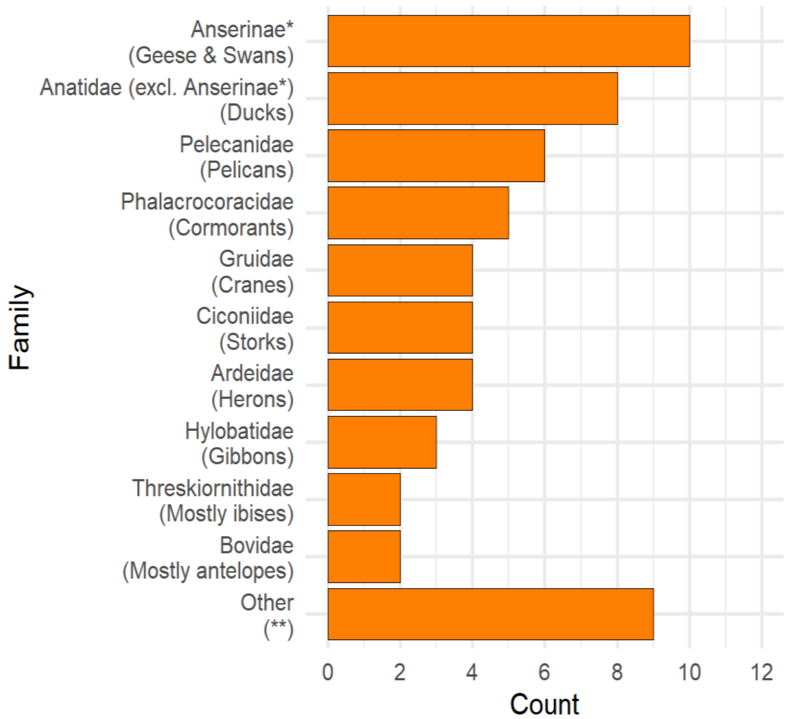

Figure 4: Animal families that Dalmatian pelicans often share their enclosure with. Many of the mentioned birds, like ducks, geese, herons and cormorants, are free-ranging.

*** Subfamily of *Anatidae* which contains geese and swans.**

**** The category "Other" contains nine families that were counted once (*Accipitridae*, *Anhimidae*, *Atelidae*, *Cebidae*, *Laridae*, *Numididae*, *Scopidae*, *Tapiridae* & *Phoenicopteridae*).**

part were often not able to answer the questions. *Section 9* also did not yield many results. Only three respondents experienced unexplainable health issues within their Dalmatian pelican population. Still, the survey can be considered very successful based on the large number of respondents and fully answered questions. A list of the respondents and their affiliations can be found in Appendix II.

Enclosure

Most zoos keep their Dalmatian pelicans in an outside pond (Table 6). They are also sometimes kept in a moat that serves as a barrier around another enclosure. The specifics of Dalmatian pelican enclosures at different European zoos can be found in Table 6.

Dalmatian pelicans are often kept together with other animals. Next to multiple animal families, 36 different species have been indicated by name. Sometimes, the answers were not clear enough to determine the exact species (for example: waterfowl or African hoofstock). Only animals from which the family could be determined were included in the analysis. Because we are looking at the family level, different species within the same family that are held in the same zoo were counted as one. For example, if a zoo keeps their Dalmatian pelicans together with marabou storks and yellow-billed storks they are counted together as one because they are both part of *Ciconiidae*. Waterfowl was mentioned two times, but these were not counted because it was unclear if they were ducks, geese or swans.

Most often, Dalmatian pelicans share their enclosure with ducks and geese (*Anatidae*), but they are also commonly kept together with other species of pelican (Figure 4). Five different pelican species were mentioned, but the great white pelican (*Pelecanus onocrotalus*) was mentioned most often (n=5). As Dalmatian pelicans are often kept outside in an open enclosure, many of the habitat-sharing species are free-ranging. For example, captive cormorants used to be kept with Dalmatian pelicans at Rotterdam Zoo in the past. Nowadays, the Dalmatian pelicans share their exhibit with free-ranging cormorants and herons that are attracted by the fish that is fed to the pelicans (Maarten Vis, personal communication). Free-ranging animals are also included in Figure 4.

Table 6: Dalmatian pelican enclosure characteristics in different zoos. Ruben Holland mentioned that the Dalmatian pelicans at Zoo Leipzig (Germany) are kept inside during avian influenza outbreaks. This might also be the case for other zoos, but this was not specifically asked in the survey. The amount of water is given for the outside enclosures.

Zoo	Enclosure	Outdoor (m^2)	Indoor (m^2)	Time indoors	Island	Water
Amersfoort	Aviary	3000			✓	17%
Augsburg	Outside Pond	2200	220	Nov-Mar		80%
Aywaille	Outside Pond	2500			✓	25%
Ayzac-Ost	Outside Pond	200				50%
Berlin Tierpark	Outside Pond	7000	30	Nov-Mar		50%
Bern	City River	6325			✓ (floating)	50%
Dvůr Králové	Outside Pond	7500	48	Nov-Apr		89%
Fort-Mardyck	Outside Pond	950				40%
Heidelberg	Outside Pond	10000				50%
Herberstein	Outside Pond	1500			✓ (floating)	33%
Le Guerno	Outside Pond	7000			✓	80%
Le Pal	Outside Pond	10000	60	Dec-Feb, during cold weather	✓	85%
La Teste-de-Buch	Outside Pond	2000			✓	80%

Le Vigen	Outside Pond	1700				2%
Leeuwarden	Aviary	1085	25		✓	29%
Leipzig	Outside Moat	2800	25	During avian influenza, during cold weather	✓	89%
Mechelen	Outside Pond	200			✓	45%
Münster	Outside Pond	4000	60	During cold weather	✓	25%
Nürnberg	Outside Pond	9873		During cold weather	✓	75%
Obterre	Outside Pond	30000			✓	85%
Opole	Swimming Pool	1650	107.5	Nov-Mar, during cold weather		67%
Overloon	Outside Pond	400				50%
Paignton	Outside Pond				✓	70%
Prague	Outside Pond	1000	30	Jan, during cold weather	✓	50%
Rotterdam	Outside Pond	1500			✓	91%
Schmiding	Outside Pond	8000	35		✓ (solid & floating)	70%
Trégomeur	Outside Pond	2500	20		✓ (floating)	50%
Val-de-Reuil	Outside Pond	20000				
Vienna	Outside Pond	2360			✓	28%
Villars-les-Dombes	Outside Pond	10000			✓	80%
-	Outside Pond	5700			✓	
-	Bird park structure, two outside ponds	8201.13			✓ (floating)	29%

Flight

All zoos, except for Dierenpark Amersfoort, where the Dalmatian pelicans are kept in a walkthrough aviary, have at least some deflighted birds. Figure 5 shows the deflighting techniques that are most commonly found in zoos. In some zoos, deflighting techniques differ between the birds. Often, older individuals are pinioned because regulations were different in the past.

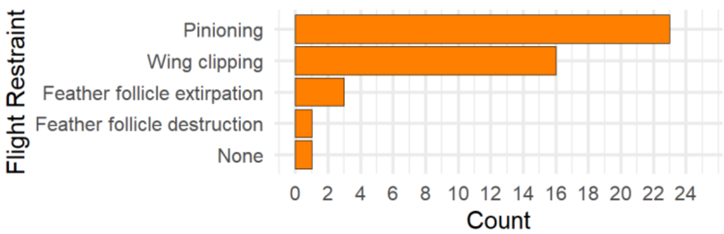

Figure 5: Deflighting techniques for Dalmatian pelicans found in zoos. In some zoos, deflighting techniques differ between the birds.

Diet

Dalmatian pelicans are solely fed freshwater fish in 27 of the zoos. The other five zoos give a mix of saltwater and freshwater fish. At Zoo Heidelberg, the diet consists of around 80% freshwater fish and 20% saltwater fish that are leftovers from other animals. At Vienna Zoo, saltwater fish is added to the diet when the quality of freshwater fish is poor. However, that is seldom necessary. At Tierpark Bern, the Dalmatian pelicans were given saltwater fish to test if this would induce breeding. Interestingly, the opposite is done at Zoo Planckendael (Mechelen). There they only feed freshwater season seven months of the year during the breeding season. The other months the Dalmatian pelicans are given saltwater fish for economic reasons. At Paignton Zoo, trout was added to the diet for breeding when they were fed herring. Currently, they receive roach and the extra trout is no longer given. At Monde Sauvage Safari Parc (Aywaille), it was also observed that the Dalmatian pelicans started breeding when their diet was changed from saltwater to freshwater fish. Multiple zoos also indicated that they increase the quantity and/or quality of the fish during the breeding season.

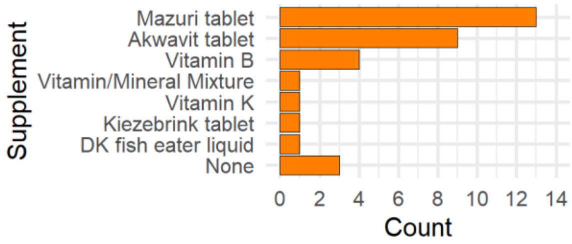

Figure 6: Supplements added to the Dalmatian pelican diet in European zoos.

The fish is often supplemented with vitamins. Mazuri fish eater tablets and Akwavit fish eating tablets are most commonly given to the Dalmatian pelicans. Three respondents indicated giving one Mazuri fish eater tablet per bird per day. At another zoo, they receive half a tablet per bird per day. And at Zooparc de Trégomeur the Dalmatian pelicans receive one Akwavit fish eating tablet per bird per day. As it was not specifically asked, the other respondents did not indicate the quantity of supplements that were given.

Important factors for breeding

Currently, only 9 out of the 32 zoos that filled in the survey are still breeding with their Dalmatian pelicans. However, 20 zoos have been successful at breeding Dalmatian pelicans in the past.

The answers to the question “What factors do you think are important for breeding Dalmatian pelicans?” were grouped into 11 categories based on keywords (Table 7). The category “Other” included the factors: good indoor lighting (UV-light), clean water, couple training, animals outside all year, genetically valuable birds, stalling breeding until spring and avian influenza regulations. All factors within this category were only mentioned once. The counts of the answers are given in Figure 7. Group effect was most commonly given as an important factor for breeding Dalmatian pelicans (n=18). Further analysis shows that zoos that had breeding success on average also have larger groups (Wilcoxin test, $p=2.625*10^{-4}$) (Figure 8). Having an island in the enclosure for breeding was mentioned four times. This was included in the category: “Breeding area”.

Furthermore, 25 respondents think that Dalmatian pelicans need a minimum population size before they start breeding. Of whom 22 indicated a minimum population size. A N_{min} of 10 birds was mentioned the most (n=8). Some respondents indicated N_{min} for males and females separately, but they all gave an equal sex ratio (n=10).

Table 7: Categories into which the answers to the survey question "What factors do you think are important for breeding Dalmatian pelicans?" were grouped. The answers were grouped based on the keywords given in the table.

Category	Keywords
Care	Care
Breeding area	Breeding area, island, nesting area
Enclosure size	Enclosure size, pool size, space
Feeding	Freshwater fish, feeding
Flight	Flight, pinioned
Group effect	Group effect, group size, social stimulation, large colony
Nesting material	Nesting material
Safety	Predation, predator avoidance, safety, feeling of security
Social structure	Age, sex ratio, partner choice, demography, stable colony
Tranquillity	Tranquillity, quiet, peace, no disturbance, no stress
Other	…

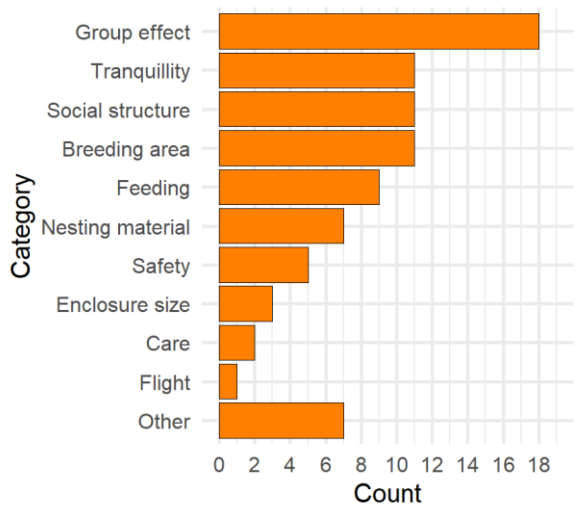

Figure 7: The number of times an answer within a specific category was given to the question: "What factors do you think are important for breeding Dalmatian pelicans?". The category "Other" included the factors: good indoor lighting (UV-light), clean water, couple training, animals outside all year, genetically valuable birds, stalling breeding until spring and avian influenza regulations.

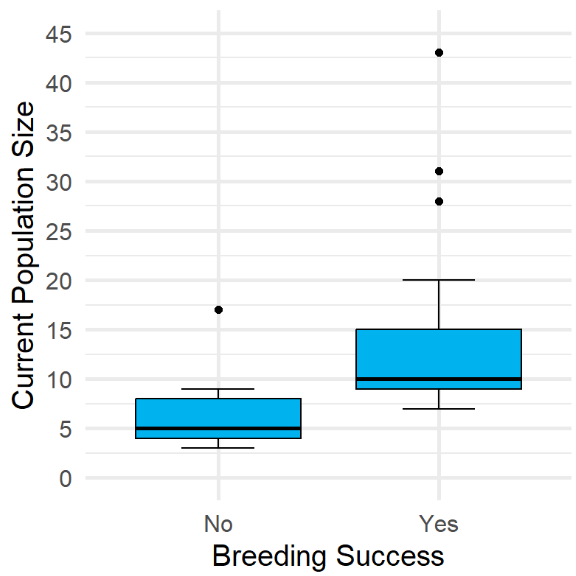

Figure 8: A comparison of the current population sizes of zoos that did and did not have breeding success. Zoos that had breeding success have larger groups (Wilcoxin test, $p=2.625*10^{-4}$).

Effects of a breeding circle

Effects on inbreeding

The average generation interval for all simulations was 13.7y (13.4y – 14.4y). Managing the different zoo populations as a single large population resulted in the lowest rate of inbreeding over 99 years (0.490% for N_{min}=20) while managing the populations separately without exchange resulted in the highest rate of inbreeding (1.91%, N_{min}=20) (Figure 10). When the different populations were managed as a breeding circle, the rate of inbreeding was somewhere in between the 'No Exchange' and 'One Population' strategies (0.730% for N_{min}=20, exchange interval = 6 years, and the number of females exchanged = 5). Exchanging animals with a breeding circle can thus help to reduce inbreeding.

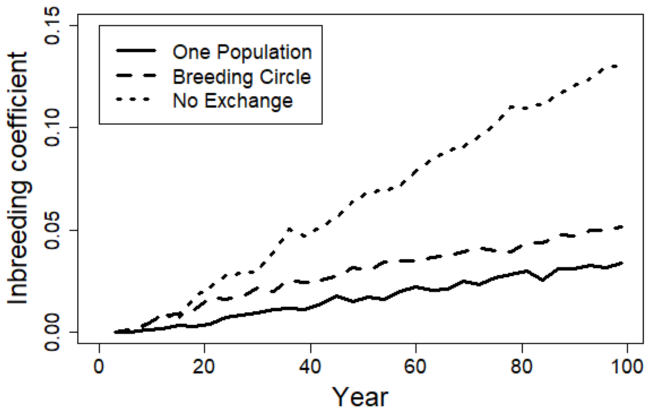

Figure 9: A comparison of three breeding strategies: no exchange, one population & breeding circle. The Y-axis shows the average inbreeding of 40 repeats. All simulations were done based on four zoos (Rotterdam, Aywaille, Villars-les-Dombes & Obterre). 'One Population' managed these four zoos as one population, resulting in a minimal amount of inbreeding. 'No Exchange' managed the four zoo populations without any exchange, resulting in a maximal amount of inbreeding. 'Breeding Circle' allowed the exchange of 5 females of 1 to 3 years old every 6 years.

Without exchange, the rate of inbreeding over 99 years was 3.36% for N_{min}=10 and 1.91% for N_{min}=20 (Table 8). As expected, the inbreeding coefficient for N_{min}=10 was higher. Even though there are more animals in the total population, without exchange, the inclusion of smaller breeding populations only increases inbreeding. The rate of inbreeding after 99 years was 0.131% if the current EAZA population was simulated as one large population.

Table 8: The rate of inbreeding over 99 years for the simulations with one population, a breeding circle and no exchange. The breeding circle simulated the exchange of 5 females of 1 to 3 years old every 6 years. The results are given for simulations run where different zoo populations were included based on their current size.

	Total EAZA population	15 Zoos, N_{min} = 10	4 Zoos, N_{min} = 20
One Population	0.131% (0.118% - 0.144%)	0.224% (0.210% - 0.238%)	0.490% (0.452% - 0.529%)
Breeding Circle		0.960% (0.913%-1.01%)	0.730% (0.678%-0.782%)
No Exchange		3.36% (3.28% - 3.44%)	1.91% (1.85% - 1.97%)

Figure 11A & B show the results of the breeding circle simulations based on the current distribution of Dalmatian pelicans over EAZA institutions according to the ZIMS database. If zoos were included with N_{min}=10, the rate of inbreeding exceeded the threshold of 1% per generation for most scenarios. Only exchanging 5 birds every 3 years yielded consistently low inbreeding (0.524% (0.493% - 0.555%)). For a breeding circle which includes only zoos with N_{min}=20, it was possible to keep the rate of inbreeding below the threshold value for five scenarios. Even though inbreeding was always lower when 10 animals were exchanged compared to 5, the results are often similar.

Furthermore, for N_{min}=10 the rate of inbreeding varied between 1.32% in the largest subpopulation (Rotterdam, N=43) and 5.91% in the smallest subpopulation (Vienna, N=10) when there was no exchange (Table 9). With a breeding circle where 5 female pelicans were exchanged every 6 years, the rate of inbreeding only varied between 0.694% (Rotterdam, N=43) and 1.54% (Dvůr Králové, N=10). Smaller subpopulations benefit the most from exchanging animals. Their rate of inbreeding decreases more when animals are exchanged using a breeding circle compared to larger subpopulations (Figure 12). The same effect was found in other studies on breeding circles (Kappelhof & Windig, 2021; Windig et al., 2019). For about half of the zoos (Rotterdam, Mechelen, Aywaille, Sainte-Croix, Villars-les-Dombes, Obterre & Val-de-Reuil), the rate of inbreeding dropped below the 1% per generation threshold when the breeding circle was tested.

The proposed '8 Zoos' scenario yielded similar results (Figure 11C, D & E). The rate of inbreeding was similar in the simulations with N_{min}=20 compared to the breeding circle '4 Zoos' scenario which also had N_{min}=20. The simulations with N_{min}=30 yielded lower inbreeding rates compared to all the other tested scenarios.

Table 9: The rate of inbreeding within individual zoos with and without a breeding circle. The breeding circle is based on the '15 Zoos' scenario. The breeding circle simulated the exchange of 5 females every 6 years. The zoos are ordered based on their position in the breeding circle. The highest and lowest values are indicated with ↑ and ↓ respectively.

Zoo	No Exchange	Breeding Circle
Rotterdam	1.32% ↓	0.694% ↓
Mechelen	4.08%	0.930%
Aywaille	1.92%	0.790%
Sainte-Croix	3.82%	0.991%
Berlin Zoo	4.66%	1.14%
Prague	4.68%	1.26%
Dvůr Králové	5.77%	1.54% ↑
Poznań	4.40%	1.08%
Budapest	5.43%	1.49%
Vienna	5.91% ↑	1.01%
Schmiding	3.86%	1.26%
Bern	4.78%	1.15%
Villars-les-Dombes	2.15%	0.869%
Obterre	2.69%	0.952%
Val-de-Reuil	4.23%	0.876%
Total population	3.36%	0.960%

Effects on genetic diversity

The differences in genetic diversity were not as big between the simulations as for inbreeding. The most genetic diversity was retained when the total EAZA population was managed as a single population (Table 10). A breeding circle where 5 females were exchanged every 6 years retained more diversity than corresponding 'One Population' scenarios. However, the difference was very small. The same can be said for the simulations without exchange between subpopulations.

The genetic diversity after 99 years did not vary much for different parameter combinations within the breeding circle scenarios. However, differences could be observed between scenarios. The '4 Zoos' scenario retained the least amount of diversity over 99 years while the 'Maximised' scenario retained the most diversity over 99 years (Table 11). Furthermore, the retention of genetic diversity seems to be predominantly correlated with the total number of animals and the number of subpopulations in the breeding circle.

Table 10: The genetic diversity after 99 years for the simulations with one population, a breeding circle and no exchange. The breeding circle simulated the exchange of 5 females of 1 to 3 years old every 6 years. The results are given for simulations run where different zoo populations were included based on their current size.

	Total EAZA population	15 Zoos, N_{min} = 10	4 Zoos, N_{min} = 20
One Population	99.039% (99.025% - 99.052%)	98.24% (98.21% - 98.27%)	96.27% (96.17% - 96.36%)
Breeding Circle		98.42% (98.40% - 98.45%)	96.40% (96.34% - 96.47%)
No Exchange		98.67% (98.64% -98.69%)	96.64% (96.56% - 96.72%)

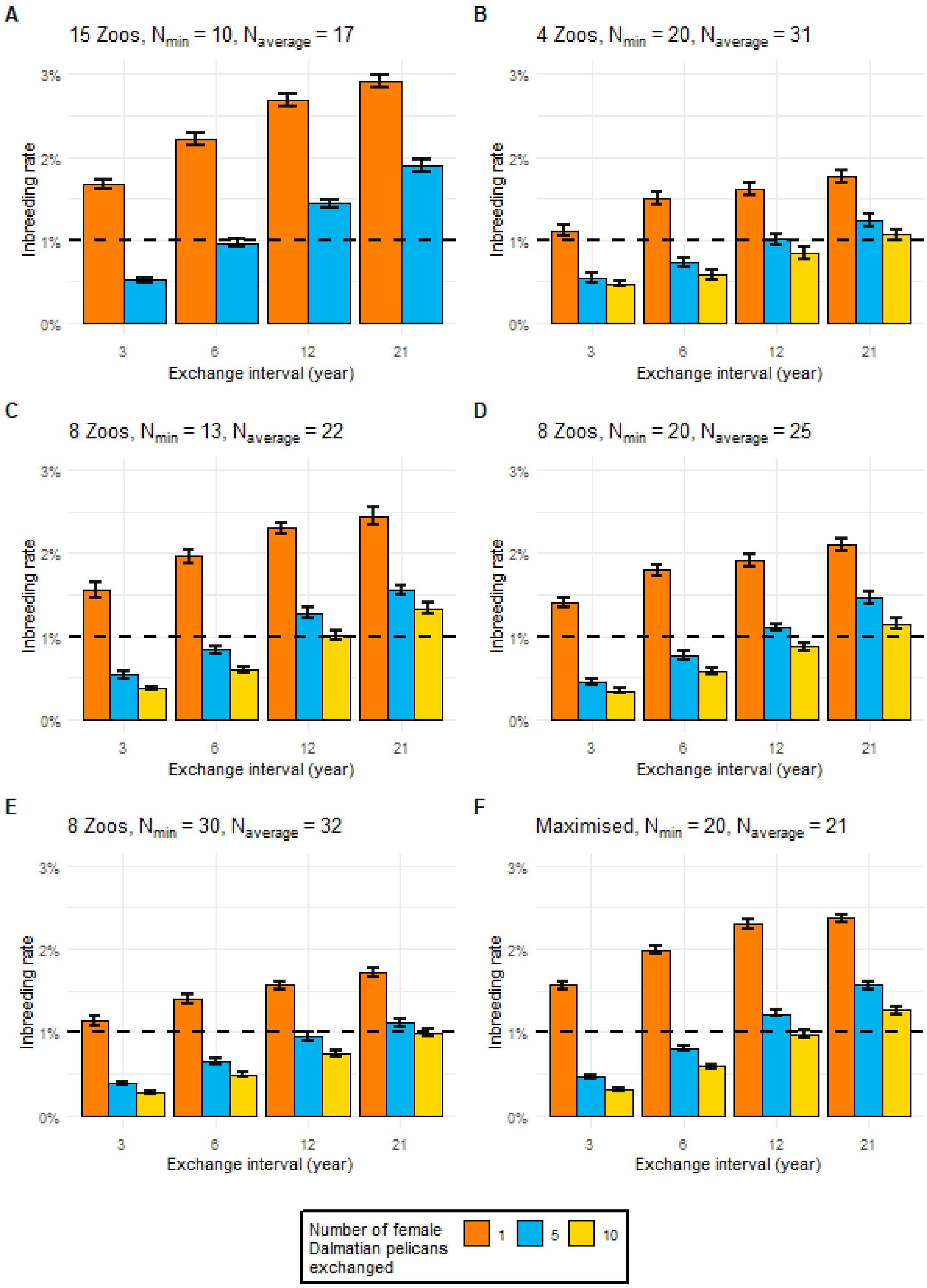

Figure 10: Inbreeding rate after 99 years for different breeding circle (BC) scenarios. The inbreeding rate is the average of 40 repeats. The error bars show the minimum and maximum values. The dashed line shows the threshold value of 1% inbreeding per generation. The number of female Dalmatian pelicans that was exchanged is shown by the colour of the bar. A and B show the results of simulations of breeding circles based on the current distribution of Dalmatian pelicans over the subpopulations, limited to subpopulations with N_{min}=10 and N_{min}=20 respectively. C, D and E show the results of simulations based on a suggested breeding circle that could be scenario in the future. This breeding circle consisted of the four zoos with N_{min}=20 and the four closest zoos with N_{min}=10. C shows the inbreeding if the population sizes would remain the same. For the other two scenario, the populations were enlarged so they contained at least 20 (D) or 30 (E) Dalmatian pelicans. F shows the results of a maximised breeding circle where all the Dalmatian pelicans from EAZA institutions within the EU and Schengen area were redistributed into subpopulations consisting of at least 10 males and 10 females.

Table 11: Properties of the six different breeding circle scenarios that were simulated. For each scenario, the average retained diversity was calculated over the different parameter combinations. The lowest and highest retained diversity are given in between brackets.

Scenario	N_{min}	#Animals	#Subpopulations	$\mu_{animals/zoo}$ (σ)	Average retained diversity	Inbreeding rate
15 Zoos	10	261	15	17 (9)	98.50% (98.40% - 98.61%)	Figure 11A
4 Zoos	20	122	4	31 (8)	96.45% (96.30% - 96.64%)	Figure 11B
8 Zoos	13	176	8	22 (10)	97.65% (97.51% - 97.82%)	Figure 11C
8 Zoos	20	202	8	25 (8)	97.93% (97.83% - 98.02%)	Figure 11D
8 Zoos	30	256	8	32 (4)	98.32% (98.26% - 98.40%)	Figure 11E
Maximised	20	449	21	21 (1)	99.12% (99.06% - 99.19%)	Figure 11F

The results of the 'Maximised' breeding circle scenario are given in Figure 11F. For only four of the twelve parameter combinations of this breeding circle the rate of inbreeding would stay well below the threshold value.

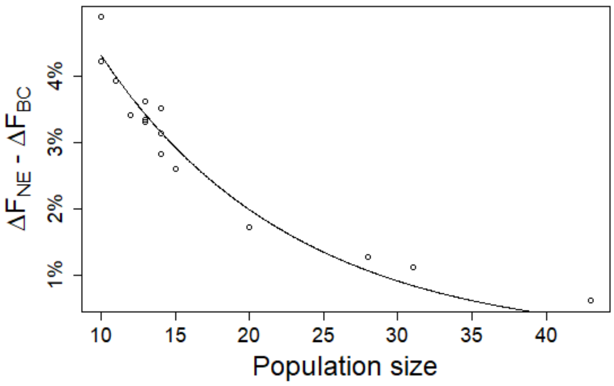

Figure 11: The difference in the rate of inbreeding between a breeding circle (BC) strategy and no exchange (NE) by population size for individual zoos. Smaller zoos seem to benefit more from exchanging pelicans using a breeding circle. The simulated breeding circle exchanged 5 female pelicans every 6 years.

The effectiveness of a breeding circle is influenced by multiple factors. First, if more animals are exchanged per exchange event, the inbreeding will decrease. However, this decrease does not seem to be linear because an increase from 1 to 5 animals exchanged decreases the inbreeding rate much more than an increase from 5 to 10 animals exchanged (Figure 11). Secondly, increasing the frequency of exchange also decreases the rate of inbreeding. And thirdly, the average size of the subpopulations also seems to influence the rate of inbreeding. Breeding circles with a larger average subpopulation seem to have lower inbreeding rates overall compared to those with a smaller average subpopulation (Figure 11 & Table 11). This is especially clear when looking at the three '8 Zoos' scenarios. Between those, only the total amount of simulated animals and the average subpopulation size differ. The overall inbreeding rates are also much lower for the '8 Zoos' scenario with a similar total population size compared to the '15 Zoos' scenario (N=261 vs N=256 respectively) (Figure 11A & E). Whereas, the '8 Zoos' scenario with a similar average subpopulation size as the 'Maximised' scenario ($\overline{N}_{sub}$=22 vs $\overline{N}_{sub}$=21 respectively) shows similar overall inbreeding rates even though the total population sizes differ greatly (N=176 vs N=449 respectively).

Possibility DNA Isolation

DNA was successfully isolated from all the feather samples. Both storage methods yielded DNA. The results of the gel electrophoresis can be found in Figure 13. All samples show bands of very long reads (>5kb). Lanes 1, 3, 4, 5 & 10 also show hints of isolated RNA of 1kb and 500b. There is no indication that DESS solution is a better storage method than ziplock bags to preserve DNA from feathers.

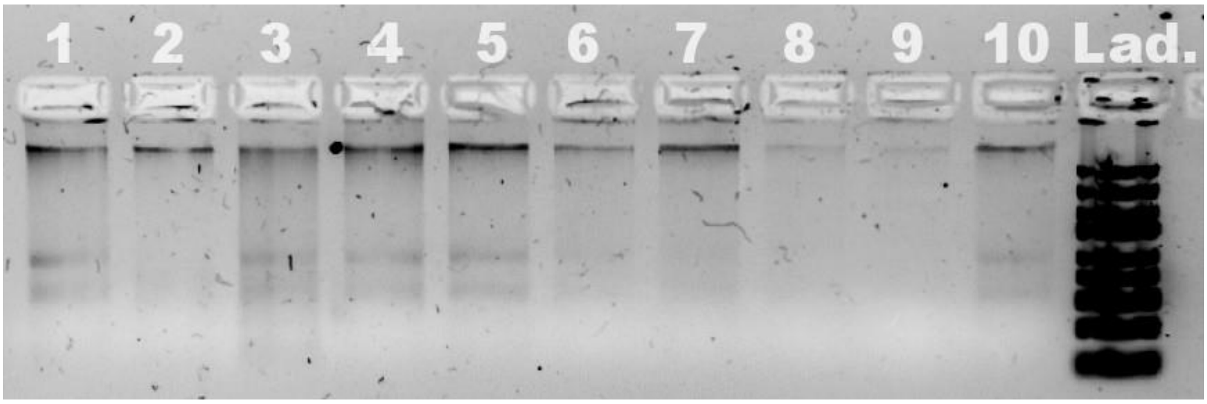

Figure 12: Results of electrophoresis of Dalmatian pelican DNA extracted from feathers. Lanes 1 to 5 show the results of five individuals where feathers were stored in a ziplock bag. Lanes 6 to 10 show the results of the same individuals respectively where the feathers were stored in 2mL tubes with DESS solution. The right most lane contains the GeneRuler Express DNA Ladder.

Discussion

This research provides insights into the current management and breeding success of Dalmatian pelicans in zoos, limiting inbreeding using a breeding circle and the possibility of using plucked feathers for genetic research. Breeding success varies a lot, both between and historically within institutions. Group effect was mentioned most often as an important factor for breeding Dalmatian pelicans. After that, a good social structure, tranquillity and a suitable breeding area were most important. Yet, every factor is likely connected to the other factors. A suitable breeding area, like an island, can also result in less disturbance and a higher feeling of safety. A large group also needs a good social structure for breeding (equal sex ratio, presence of experienced individuals, etc.). Therefore, multiple factors should be taken into account when breeding Dalmatian pelicans. To improve the success and consistency of breeding, more large subpopulations must be created. This could be done by merging smaller groups. More active management of the EEP could help to coordinate these changes. Furthermore, genetic testing could aid to identify genetically valuable individuals for the breeding program.

Applying a breeding circle can greatly reduce the inbreeding rate in ex-situ populations, especially in smaller subpopulations. Exchanging more animals per exchange event decreases the overall inbreeding rate. The inbreeding rate can also be reduced by increasing the frequency of exchange. However, exchanging more animals and/or increasing the frequency of exchange also makes the management more intensive and costly. A too-frequent exchange might also disturb the social structure of the subpopulations. Larger subpopulations result in less inbreeding. The stability of these populations might also be better. A breeding circle with multiple subpopulations of ≥10 breeding pairs where 5-6 young individuals of the same sex are exchanged approximately every 6 years might be best suitable for the breeding of Dalmatian pelicans in captivity. The interval between exchanges can be bigger depending on the exact sizes of the subpopulations.

Group size

Dalmatian pelicans are social animals that breed in large colonies in the wild (Catsadorakis et al., 2015; Kurstjens et al., 2021). They nest together in units most often consisting of 6 to 10 nests (Crivelli et al., 2008). Laying dates are earlier for larger colonies (Doxa et al., 2012). However, the laying date varies a lot, especially for smaller colonies. Most zoo populations are significantly smaller than the colonies found in the wild. Some colonial birds need social stimulation to reproduce, called the 'Fraser Darling effect' (Darling, 1938). This might explain why the breeding success of Dalmatian pelicans is higher for larger populations. Even though this theory has since been disputed (Orians, 1961), the effect can still be observed. Different zoos have used mirrors and statues to initiate breeding within different flamingo species (Rees, 2011). However, these techniques were not considered very effective even though they did manage to increase nest-building behaviour. A study on the effects of different stimulation techniques like adding mirrors and pelican statues to the enclosure and playing recordings of pelican sounds could be useful to improve the breeding of Dalmatian pelicans in small populations.

Furthermore, housing the pelicans in smaller groups than those found in the wild could also affect the well-being of the animals. Captive great white pelicans that live in smaller groups were found to have higher stress levels than those that were kept in larger groups (Haase et al., 2021). Even though it has not been tested yet, this could also be the case for Dalmatian pelicans. Larger colonies could provide a better feeling of security and therefore make the birds more resistant to disturbances. The higher stress levels associated with smaller group sizes, next to the Fraser Darling effect, could be part of the reason for the low breeding success in small Dalmatian pelican populations.

Even though the survey results suggest that a N_{min} of 10 individuals can be enough to breed Dalmatian pelicans, it might still be better to keep them in larger groups. Firstly, the inbreeding rate is higher in smaller subpopulations. Dalmatian pelicans can be exchanged less often when the subpopulations are larger. Secondly, larger groups are less vulnerable to events that could cause breeding to stop (Brouwer et al., 1994). Having reliable breeding populations in the breeding circle is important to ensure that there are always enough young individuals that can be exchanged.

The survey results are based on the experience of keepers and curators, however, more quantitative research could be done to support their opinions. It was found that zoos that had breeding success historically currently have larger groups of Dalmatian pelicans. This could support the idea that group size is important for breeding. However, these results only hold true under the assumption that the groups have always been similar in size. In a recent study by Mooney et al. (2023), the effects of flock size on the probability of reproduction in different flamingo (*Phoenicopteriformes*) species were modelled using data from the ZIMS database. Similar research could be performed to get more accurate data on the optimal group size for breeding success.

Flight

According to Bračko & King (2013), birds are most often kept in open enclosures because of the lack of barriers between visitors and the animals, the ability to mix with other species and the high costs associated with building an aviary. To prevent escape, Dalmatian pelicans in captivity are often restrained from flying, like many aquatic birds in zoos (Bračko & King, 2013; Reese et al., 2020).

Legislation on flight restraint can differ vastly between countries (Reese et al., 2020). Therefore, also a range of different deflighting techniques can be observed between and within zoos. Differences within zoos are often due to a change in management or exchange of pelicans between zoos that use different deflighting techniques. Hence, pinioned pelicans are often older individuals.

Birds that are often deflighted generally use other forms of locomotion, like swimming and walking, and mainly use flight to escape from predators. When zoos, remove the threat of predators, these birds do not need to fly and can resort to other natural behaviours (Haase et al., 2021; Reese et al., 2020). Deflighted great white pelicans also do not seem to experience significantly more stress than flight-capable individuals (Haase et al., 2021). But interestingly, wing-clipped individuals tend to have higher stress levels than those that are irreversibly deflighted. This could be caused by the frequent handling of the animals for clipping which could be stressful for the birds (Haase et al., 2021). Deflighted animals can also, in a way, be considered more free because they do not need to be housed inside and can get access to a larger area to roam and forage (Hesterman et al., 2001). Still, flying is a natural behaviour for most birds which they will perform if allowed to do so (Reese et al., 2020).

For many species of bird, symmetry is very important for partner selection and wings are often used in courtship behaviours. Some birds also use their wings to maintain balance during copulation (Reese et al., 2020). Wing clipping could therefore have a negative effect on the ability of a bird to reproduce (Bračko & King, 2013; King, 2008). However, this might not be a problem in pelicans, because they make less use of their wings during partner selection (Klös, 1968). Most respondents also did not indicate that flight restraint influences the breeding success of Dalmatian pelicans (Figure 7). Two of the respondents that indicated that deflighted birds could experience problems with copulation based their information on other bird species (like flamingos), and not directly on pelicans. Frédéric Verstappen indicated that Dalmatian pelicans copulate mainly on their nest and have no problems with finding balance (survey results). Yet, Aude

Haelewyn-Desmoulins suggested that the inability to fly could have a negative effect on the body mass and overall health of the birds (survey results). Thomas Lipp also indicated that deflighting might have a small negative effect on the fertilisation rate of the eggs (survey results). But the exact effects of deflighting on the welfare and breeding success of Dalmatian pelicans are hard to determine, as almost all zoos keep flight-restrained birds and a good comparison with flight-worthy individuals cannot be made.

Overall, the inability to fly does not seem to negatively influence the breeding behaviour of Dalmatian pelicans. Deflighting could also mean that the Dalmatian pelicans can be kept in a larger outside enclosure and, therefore, in a larger group. A large group of deflighted Dalmatian pelicans is expected to be better for breeding than a smaller group of flight-worthy individuals that is kept in an aviary.

Diet

In the wild, Dalmatian pelicans forage predominantly in freshwater areas and therefore mainly eat freshwater fish. However, some zoos also feed them saltwater fish. This could be because saltwater fish is cheaper (Maarten Vis, personal communication). Yet, some zoos might just include saltwater fish in the diet that are leftovers from other animals (Eric Diener, survey results).

Saltwater and freshwater fish differ in nutrient content (Khalili Tilami & Sampels, 2018; Pokras, 1988; Steffens, 1997). Multiple zoos indicated a positive effect of feeding freshwater fish on breeding. The difference between a saltwater and freshwater fish diet could, thus, be a significant factor in the breeding success of piscivorous birds like the Dalmatian pelican.

Freshwater fish are better at synthesising docosahexaenoic (DHA). DHA is linked to improved growth, fertility and offspring performance (Lee et al., 2019). The lower DHA content in their diet could explain the lack of breeding in Dalmatian pelicans that are solely fed on saltwater fish. On the other hand, increased DHA concentrations have a negative effect on oxidative stability (Lee et al., 2019). The higher oxidative stress in birds that feed on freshwater fish seems to be balanced by higher carotenoid concentrations which function as anti-oxidants (Surai et al., 2001). Carotenoids are especially effective within the embryo (Surai et al., 2001), which indicates their importance for reproduction and offspring development.

Vitamin B1 (thiamine) and E degrade quickly in dead fish. Therefore, it is recommended to supplement the diet of piscivorous animals with these vitamins (Bernard & Allen, 1997; Crissey et al., 1998; Pokras, 1988). The survey results indicate that *Mazuri fish eater tablets* and *Aquavit Fish Eating* are often given to captive Dalmatian pelicans. However, these supplements do not contain DHA. Possibly because they might have mainly been developed for marine animals. Giving DHA supplements to Dalmatian pelicans that are fed saltwater fish might solve the breeding issue. Still, more research is needed on the topic. The fact that the birds stop breeding could also indicate other negative health effects caused by a saltwater fish diet. For now, it would be best to feed Dalmatian pelicans freshwater fish because that is their natural food source and it promotes breeding.

Furthermore, there is no indication that the availability of freshwater fish is a characteristic specific to Dalmatian pelican breeding sites. Dalmatian pelicans in South-East Asia have been observed to migrate between coastal areas in China and their more inland breeding site in Mongolia (Shi et al., 2008). But the Dalmatian pelicans mainly migrate between wetlands near the coast and intertidal areas. There is no indication that freshwater fish is only available at their breeding site, and therefore a cue for breeding. Based on European populations, winter-related climate conditions might be a better predictor of breeding (Doxa et al., 2012). The migration to coastal areas in winter could also be explained by better climatic conditions (Bounas et al., 2022).

Nesting area

Catsadorakis & Crivelli (2001) listed twelve properties that a Dalmatian pelican nesting area should have (Table 12). Their conclusion was based on the research they did on wild Dalmatian pelicans at Lake Mikri Prespa in Greece. Yet, their conclusions are similar to the results from the survey.

Dalmatian pelicans should be safe from predators. In captivity, eggs and chicks are preyed upon by animals like rodents, snakes and foxes (Bračko & King, 2013). Aviaries can keep birds safe from local predators. However, most Dalmatian pelicans are kept in open enclosures (Table 6). An indoor area can provide safety against predators during the breeding season, but a predator-free outside nesting area is preferable (Matthias Papies, survey results). At Zoo Leipzig, eggs were also never fertile when the Dalmatian pelicans bred indoors (Ruben Holland, survey results). However, the crowdedness of a small indoor area could stimulate breeding behaviour (Maarten Vis, personal communication). Interestingly at Zoo Schmiding, the Dalmatian pelicans are free to choose to use the indoor area, but they never do so (Andreas Artmann, survey results). An island can also provide a breeding area that is inaccessible to terrestrial predators. However, the Dalmatian pelicans will still be vulnerable to predatory birds, including cormorants (Bračko & King, 2013; Brouwer et al., 1994).

The island should have a gentle sloping edge so it is easily accessible by the Dalmatian pelicans (Brouwer et al., 1994; Catsadorakis & Crivelli, 2001). Young pelicans that get into the water could drown if they are unable to get back onto the island. Older animals can also injure themselves when they have trouble getting out of the water. Especially deflighted birds are at risk, as pelicans will try to lift themselves out of the water by beating their wings (Brouwer et al., 1994).

Catsadorakis & Crivelli (2001) found that breeding success was low on free-floating islands. However, they also indicate that the success might be higher if the rafts have more of the other listed characteristics (Table 12). At Tierpark Bern, Dalmatian pelicans have successfully nested on a floating island (Meret Huwiler, survey results). As the enclosure is located on the river Aare and can experiences fluctuating water levels. The floating island might prevent nests from being washed away. Yet, a floating island has to be constructed carefully as the anchor cables can break when the water level becomes too high (Catsadorakis, 2017). Still, for zoos floating platforms could be more suitable nesting areas than in the wild, as weather conditions and fluctuating water levels pose less of a risk.

Fixed wooden platforms are also suitable for nesting, as long as they are easily accessible (Catsadorakis, 2017). However, artificial islands from stone might be more durable (Cheshmedzhiev et al., 2022).

Table 12: Properties that a Dalmatian pelican nest site should have according to Catsadorakis & Crivelli (2001). The right column indicates if the properties were also mentioned in the survey.

	Catsadorakis & Crivelli (2001)	Indicated in the survey
1.	Be inaccessible to mammalian predators.	✓
2.	Be free of disturbance by humans.	✓
3.	Be as large as possible and certainly over $80m^2$.	✓ (A large nesting area was mentioned by multiple respondents as an important factor.)
4.	Perhaps provide some protection from the prevailing winds.	
5.	Be as close as possible to other nest sites.	
6.	Be in contact with a large open water area.	✓
7.	Provide unobstructed view of more than 180°.	
8.	Have a combination of live vegetation and open unvegetated spaces.	
9.	Contain readily usable nesting material.	✓
10.	Be resistant enough to last for more than one year.	
11.	Not be free floating.	
12.	Be high enough not to be affected by wave action, but have gentle sloping edges to facilitate access by the birds.	

Mixed species

Several zoos keep their Dalmatian pelicans in a mixed species enclosure. They are most often kept with other *Pelecanidae* and *Anatidae* (Figure 4). Keeping multiple species together can have both positive and negative effects, depending on the species. At Paignton, Dalmatian pelican eggs were stolen by lar gibbons that they share their island with (Peter Smallbones, survey results), while at Le PAL Zoo, the Dalmatian pelicans started breeding when they accidentally mixed with great white pelicans (Nicolas Géli, survey results). Even though Dalmatian pelicans often nest together with great white pelicans in the wild (Catsadorakis et al., 2015; Doxa et al., 2012) and keeping them together with other pelican species can increase social stimulation, it is not recommendable to do so. Different pelican species can hybridise (Brouwer et al., 1994; Klös, 1968), which results in animals that are useless to the breeding program. Cormorants can stimulate breeding in pelicans but have also been observed to eat chicks (Brouwer et al., 1994). They also tend to dominate pelicans when they have to compete for food and nesting space. Still, most zoos indicated that they did not observe specific effects influencing the breeding success of their Dalmatian pelicans that were caused by keeping the birds in a mixed-species enclosure.

Breeding Circle

A well-developed breeding program is important to sustainably keep animals in captivity. Limiting inbreeding reduces genetic drift and consequently the chance of rare deleterious alleles reaching high frequencies in the population. This reduces the risk of genetic diseases getting expressed and thus sustains the welfare of the animals (Jiménez-Mena et al., 2016). Furthermore, a good breeding program conserves genetic diversity, which is important if reintroductions from captivity might become necessary for in situ conservation in the future. Less inbred animals have a higher chance of survival after release into the wild and are better able to adapt to changing conditions (Frankham et al., 2010; Jiménez-Mena et al., 2016). Conserving diversity for reintroduction can be very relevant for the Dalmatian pelican as a study already has been done on the possibility of reintroducing the species in the Netherlands (Kurstjens et al., 2021).

However, it can be hard to develop a good breeding program for animals that live in groups. It is not always possible to keep a pedigree of each animal. There is also no control in mate choice when multiple mates are available in a group. A breeding circle can be a suitable solution for the genetic management of group-living animals. It combines the benefits of several small populations and a single large population, i.e., the SLOSS debate (Kappelhof & Windig, 2021). The subpopulations retain their specific diversity, while the inbreeding rate is reduced through the exchange of animals between subpopulations (Margan et al., 1998). This research indeed shows that a breeding circle can be used to reduce the inbreeding rate in separated populations of group-living animals. Even though it has been tested for the specific case of Dalmatian pelicans in EAZA institutions, a breeding circle can also be a suitable genetic management strategy for other species, as long as the breeding circle is optimised for the situation in question (Witzenberger & Hochkirch, 2011).

Exchanging Dalmatian pelicans between zoos using a breeding circle can reduce the rate of inbreeding considerably in the species. However, exchanging only one animal per event was never enough. A shorter exchange interval could still be tested, but that might prove to be very intensive in practice. It is better to exchange 5 animals per exchange event than 10 animals because fewer animals have to be moved while the resulting rate of inbreeding is similar. However, this similarity could also be due to not enough young Dalmatian pelicans being available for exchange. In that case, the numbers of actual Dalmatian pelicans being exchanged might have been similar for the two strategies, resulting in similar outputs. This similarity might disappear if larger subpopulations are used that have more animals available for exchange. This could also happen if both sexes were to be exchanged, which effectively doubles the number of available animals. Yet, exchanging both sexes will increase inbreeding as mating between closely related individuals becomes possible. Furthermore, exchanging multiple birds between two populations at the same time could lead to faster adaptation to the new situation. However, it is more important that the recipient population has a decent size (Maarten Vis, personal communication).

The sizes of the subpopulations have a big effect on the overall rate of inbreeding within the breeding circle. Even though smaller subpopulations benefit from larger subpopulations in the circle (Figure 12), the effect also works the other way around. The benefits of larger subpopulations are reduced by the higher inbreeding in their smaller neighbouring populations. The ‘4 Zoos’ scenario (N_{min}=20) yielded lower inbreeding rates than the ‘8 Zoos’ scenario which includes four extra subpopulations that were enlarged to 20 Dalmatian pelicans (Figure 11B, D). The smaller ‘4 Zoos’ scenario probably benefits from the fact that the average size of the subpopulations is higher than that of its larger counterpart ($\overline{N}_{sub}$=31 vs. $\overline{N}_{sub}$=25). Yet, it is still recommendable to include more subpopulations in the breeding circle because a larger proportion of the EAZA population would be included in the breeding program. But it might also be beneficial to increase the minimal population size to reduce the rate of inbreeding in the subpopulations and to reduce the frequency at which Dalmatian pelicans need to be exchanged.

The ‘Maximised’ scenario which was based on a redistribution of captive Dalmatian pelicans within the EU and Schengen area (Table 5) includes a total of 449 animals. However, the rate of inbreeding could still only be kept below the 1% per generation threshold for four tested parameter combinations (Figure 11). Including this many animals yields the highest preservation of genetic diversity (Table 11) because there are more subpopulations that contain their specific diversity. This scenario, however, is not realistic. If the main aim is to reduce inbreeding, combined with the logistical aspects, setting up a smaller breeding circle would be recommendable.

Most of the concerns about the application of a breeding circle are related to the risk of disrupting the social structure of the subpopulations which might reduce the breeding success. Multiple recipients noted that it is unwise to separate successful breeding pairs. However, this should not be a problem if only young individuals that have not mated will be exchanged.

Still, Dalmatian pelicans can already mate when they are one year old and should thus be exchanged every year. That could be logistically challenging. It is also possible to exchange only animals that are one year old at the moment of the exchange. However, there might not be enough pelicans born per year to have a large enough group of immature pelicans to exchange at a certain moment in time. Ideally, one batch of chicks is born every few years. This could be realised using a contraception policy (Kappelhof & Windig, 2021) or by changing the diet.

One advantage of breeding circles is that they work for populations where it is difficult to keep a studbook. Yet, even though Dalmatian pelicans live in groups, it is still possible to keep track of the kinship between individuals. Offspring can be easily linked to their parents because Dalmatian pelicans only have one partner per breeding season and the young are reared on the nest. Individuals can be ringed to identify them. A concern could be that the chicks have to be ringed on the nest because they might become indistinguishable from other chicks when they eventually leave the nest. Ringing the chicks on the nest could disturb the colony and negatively affect the further development of the breeding season. However, the parent-offspring relationship can also be identified by genetic testing and multiple zoos have shown to be capable of keeping records (*ZIMS Species Holdings*, 2022).

There are other reasons to apply a breeding circle in the case of Dalmatian pelicans and other group-living animals. Even when studbook records are available, it could be hard to successfully mate preferred breeding partners. Pair formation might be impossible to force when the animals are kept in a large group. If the couple is kept isolated from the rest of the group, they will most likely not breed due to the lack of social stimulation. Isolating breeding pairs could also be more expensive due to the need for separate housing facilities.

Limitations simulations

As Pointer was designed to simulate the breeding of dogs (Windig & Oldenbroek, 2015), it has not been optimised for the breeding of longer-living species with few animals in each year class, or even no animals in some year classes, like Dalmatian pelicans. This was circumvented by simulating age classes consisting of multiple years. Yet, there are some limitations. Pelicans breed yearly and can change their partner each year (Kurstjens et al., 2021). However, when you simulate in steps of more than one year, each clutch of a female will have the same sire for those years. This could result in higher kinship between offspring compared to the real world. Still, Dalmatian pelicans only get a few successful clutches within three years. Therefore, the effect of having the same mate for three years might be minimal. Three respondents also indicated that their pelicans have the same breeding partner each season, four did not know and one respondent indicated that this was not the case in their zoo. The limited partner choice in captive populations could result in birds pairing up with the same breeding partner more often than would happen in the wild. Therefore, Dalmatian pelicans having only one mate for three years might not be unlikely.

The simulations were performed under the assumption that the individuals in the starting population are unrelated and non-inbred. Yet, this is seldom the case for zoo populations which often originate from a few founders (Frankham et al., 2010; Windig & Kaal, 2008). Furthermore, in situ populations of threatened animals which depend most on these breeding programs, usually already show some levels of inbreeding (Ekblom et al., 2018; Li et al., 2014). There are clear indications that inbreeding is also present in captive Dalmatian pelicans (Li et al., 2014; Xu et al., 2022). Inbreeding would therefore be higher in practice than indicated by the outputs of the simulations. These results should be interpreted carefully. Nonetheless, the starting population for the breeding program can be created of less related animals through genetic research. However, this could be very labour-intensive and costly to do for the whole population. It might also require a lot of reshuffling of the current zoo populations.

Furthermore, parameters for breeding success have been based on the historical situation with inconsistent breeding. If the management conditions were to be improved, more chicks are expected to be born. While this would be good news for the near-threatened species, a contraception or egg removal policy might need to be applied to sustainably keep the species in captivity. The simulations did also not account for a possible reduction in fertility that might be caused by increased inbreeding (Xu et al., 2022). Inbreeding rates in the practical situation will likely differ from the simulation results (Windig et al., 2019). Therefore, active monitoring and adjusting of the breeding program will be necessary to ensure proper genetic management.

DNA isolation

It is possible to collect high-quality DNA from Dalmatian pelican feathers. DNA was isolated from samples that were stored using the two tested methods. However, it was expected that storing in DESS solution is better than in ziplock bags for long-term preservation. The dry winter conditions at the time of sample collecting might have helped preserve the feathers as little moisture was likely trapped in the feathers. Higher humidity could result in more water being collected with the samples which could result in faster degradation of the DNA after collection (Taberlet et al., 1999; Vili et al., 2013). More so, the feather samples were also very fresh (two weeks), which could be the reason for the high yield. However, Aslam et al. (2023) were able to perform high-quality PCR with feather samples that had been stored at room temperature (~25°C) for five years. But the fragment lengths were short (100bp & 500bp). This might be suitable for microsatellite genotyping and accurate base calling (Amarasinghe et al., 2020; Chan et al., 2008; Guichoux et al., 2011; Yannic et al., 2011; Zou et al., 2010) but the older samples might not contain the long fragments that are needed for accurate contigs (Amarasinghe et al., 2020; Sefc et al., 2003). For now, it is safe to say that ziplock bags can safely be used for the short time storage of feather samples. Silica gel can be added to ensure that the samples remain dry (Taberlet et al., 1999).

The successful isolation of DNA from Dalmatian pelican breast feathers provides a practical technique for DNA collection. Keepers and researchers can easily collect feather samples during routine handling of the birds. Collecting feathers is also considered to be less stressful for the birds than blood collection (Bello et al., 2001; Dai et al., 2015).

The ease of collecting and storing allows for more genetic research on the species. This will be useful to fill the current gaps in the studbook and to identify genetically valuable individuals (Koepfli et al., 2019; Presti et al., 2013; Russello & Amato, 2004; Witzenberger & Hochkirch, 2011). Genotyping can be done with microsatellite markers that have been developed for other pelican species (de Ponte Machado et al., 2009; Xu et al., 2022). Inbreeding can be measured (Caballero et al., 2021; Koepfli et al., 2019; Townsend & Jamieson, 2013) and, thereby, the effectiveness of a breeding program for the species can be monitored. Furthermore, zoo populations often originate from a few founders (Frankham et al., 2010; Windig & Kaal, 2008). Genetic research can be used to estimate the size and timing of this bottleneck which could give insight into the historical development of the diversity in the ex situ population (Culver et al., 2008; Frankham et al., 2010; Groombridge et al., 2009; Hartmann et al., 2014). And lastly, it might also be interesting to compare captive and wild individuals. This could be especially useful to find out if the captive population is comparable in genetic diversity to the wild population and could be used for a potential reintroduction of the species.

Conclusion

Group size is the most important factor for breeding Dalmatian pelicans in captivity. To ensure breeding, groups should consist of at least five breeding pairs. However, larger groups are preferable as they are more resistant to changes and therefore could provide more consistent breeding. Furthermore, Dalmatian pelicans should have access to a quiet and safe breeding area like an island. The ability to fly was not considered an important factor for breeding as many deflighted pelicans have successfully bred. There are also indications that feeding saltwater fish can prevent breeding behaviour and the diet can be used to delay the breeding season.

A breeding circle could be a useful strategy to manage the genetic diversity of captive populations of Dalmatian pelicans. More frequent exchange results in a lower inbreeding rate. The same counts for exchanging more animals per exchange event. Breeding circles consisting of larger subpopulations yield less inbreeding. Therefore, only including groups containing at least ten breeding pairs is advisable. More quantitative research must be done to determine the optimal group size.

This study also shows the possibility to isolate high-quality DNA from plucked Dalmatian pelican breast feathers. This allows for non-invasive and practical sample collection. Accessible samples allow for more genetic research which can be used to, among other things, the identification of genetically valuable individuals, the measurement of inbreeding and the monitoring of a potential breeding program.

To improve the breeding of Dalmatian pelicans in captivity, it would be useful to enlarge some more zoo populations to around 10 breeding pairs. This can be done by bringing in birds from smaller zoo populations that will not be part of the breeding population. Genetic testing could help to identify genetically valuable birds for the EEP. Preferably, zoo populations near the four zoos which already have ≥20 Dalmatian pelicans (Rotterdam Zoo, Monde Sauvage Safari parc (Aywaille), Parc des Oiseaux (Villars-les-Dombes) and Monde Réserve Zoologique de la Haute-Touche (Obterre)) are enlarged. The population at Monde Réserve Zoologique de la Haute-Touche should also be restructured to create an even sex ratio. A breeding circle should then be set up between these zoo populations.

To keep the inbreeding rate acceptable, around 5 individuals should be exchanged every 10 years (somewhere between 9 to 12 years seems optimal) from each zoo to its neighbour in the breeding circle. More frequent exchange is also possible, but that is not necessary to keep the rate of inbreeding below the 1% per generation threshold. Additional simulations should be done to test the proposed breeding circle before it is implemented. The true inbreeding should be expected to be higher than the results of the simulations. Therefore, it is better to use a stricter inbreeding threshold (<1% per generation). For the same reason, it is also recommendable to keep monitoring the inbreeding when the breeding circle is in use.

Dalmatian pelicans in zoos that are not part of the breeding circle should not be allowed to reproduce, due to the high risk of inbreeding. These populations could be used to house birds that are not useful for the breeding program. If the breeding circle becomes too successful, excess birds could be moved to populations outside the breeding circle, or a contraception policy might need to be used to only allow offspring in specific years.

All in all, the findings of this study, open up many possibilities to improve the breeding, genetic management and genetic research on Dalmatian pelicans.

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Supplementary material

Supplementary data associated with this study can be found through the data management plan in Appendix III.

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Appendix I

Dalmatian pelican survey

By: Stijn Kouwenberg

- … -> Open answer
- • -> Multiple choice
- ○ -> Tick boxes

Section 1

Survey on Dalmatian pelicans in captivity

For my MSc thesis at Wageningen University & Research, I want to find out which factors determine the breeding success of Dalmatian pelicans (*Pelecanus crispus*) in captivity. To do so, I would like to ask you a few questions about your own experience and the current management of Dalmatian pelicans within your zoo. This survey will take about 30 minutes to fill in.

Section 2

Contact Details

Please fill in the following details so I can identify to which zoo the survey answers are referring.

Full name

…

Associated zoo

…

Your function within the zoo.

…

Email

…

Section 3

Population

The following questions are about the group composition.

How many Dalmatian pelicans do you currently keep in total? (males, females, unknown)

…

Could the zoo potentially house a larger population of Dalmatian pelicans?

- Yes
- No

If you answered "Yes" to the previous question, how many Dalmatian pelicans would you like to add to your group? (males, females)

…

Section 4

Enclosure

Enclosures can greatly vary between zoos. The following questions are about the characteristics of the Dalmatian pelican enclosure in your zoo.

Can you give a short description of the enclosure? For example: enclosure type (free-flight aviary/outside pond/...), noteworthy features

…

Are the pelicans kept inside or outside?

- Inside
- Outside
- Both

If you answered "Both" to the previous question, what time of the year are the pelicans kept inside?

…

What is the size of the enclosure? (size outside, size inside)

…

What is the water-to-land ratio of the enclosure?

…

Are the Dalmatian pelicans kept in a mixed species enclosure?

- Yes
- No

If you answered "Yes" to the previous question, with what other species do the Dalmatian pelicans share their enclosure?

…

Do you know of specific positive and/or negative effects that other species have on the breeding success of Dalmatian pelicans?

…

Are there things added to the enclosure **specifically** to facilitate breeding?

- o No
- o Yes, solid island
- o Yes, floating island
- o Yes, nesting material
- o Yes, fencing around the nests
- o Other …

Section 5

Flight

Many zoos do not have the option to keep birds in large free-flight aviaries. To prevent birds from escaping, they are often restrained from flying. This is especially the case for waterbirds. There are many ways to restrain birds from flying and different countries have different regulations on this. The following questions are about the techniques that are currently used for Dalmatian pelicans and the possible effects of this on breeding behaviour.

Are the Dalmatian pelicans in your zoo restrained from flying?

- o No
- o Yes, by pinioning
- o Yes, by wing clipping
- o Yes, by brailing
- o Yes, by feather follicle extirpation
- o Yes, by feather follicle destruction
- o Other …

Do you think that restraining Dalmatian pelicans from flying also affects their ability to breed? And why?

…

Section 6

Diet

The following questions are about the diet of the Dalmatian pelicans in your zoo.

What do you feed your Dalmatian pelicans?

- Freshwater fish
- Saltwater fish
- Other …

In the wild, Dalmatian pelicans mainly eat freshwater fish. If you feed them something else, what is the reason for that?

…

Are there special supplements added to the diet? If so, which supplements?

…

Do you change the diet during the breeding season? If so, what do you change and why?

…

Section 7

Breeding Success

The breeding of pelicans in captivity is generally very inconsistent. The following questions are there to get a better idea about the factors that influence the breeding success of captive Dalmatian pelicans.

Has your zoo been successful in breeding Dalmatian pelicans in the past?

…

What factors do you think are important for breeding Dalmatian pelicans?

…

It has been suggested that pelicans need a minimum population size before they start to breed. Do you also think that this is the case?

- Yes
- No
- Other …

If you answered "Yes" to the previous question, how many individuals do you think a population of pelicans needs for them to start breeding?

…

Do you currently have Dalmatian pelicans that breed?

- Yes [Go to section 8 (Breeding Characteristics)]
- No [Go to section 9 (Health)]

Section 8

Breeding Characteristics

Do the pelicans have the same partner each breeding season?

- Yes
- No
- Other …

How many eggs do all of your Dalmatian pelicans combined lay on average per breeding season?

…

How many of those eggs are fertile?

…

How many of the fertile eggs hatch?

…

How are the eggs incubated?

- By the parents
- Using an incubator
- Other …

How are the chicks reared?

- By the parents
- By hand-feeding
- Other …

Do you also remove eggs?

- Yes
- No
- Other …

If you answered "Yes" to the previous question. How many eggs are removed on average per year and why are those eggs removed?

…

Section 9

Health

Did you recently experience health issues within your population of Dalmatian pelicans without a clear cause?

- Yes
- No

If you answered "Yes" to the previous question, what kind of health issues did you experience?

…

Section 10

Exchange between Zoos

Zoos often exchange animals with each other. I want to find out the extent to which this is also the case for Dalmatian pelicans.

Do you often exchange Dalmatian pelicans with other zoos?

- Yes [Go to section 11 (Exchange)]
- No [Go to section 12 (Breeding circle)]

Section 11

Exchange

The next questions are about the details of exchanging Dalmatian pelicans with other zoos.

How often do you exchange Dalmatian pelicans with other zoos? And how many Dalmatian pelicans do you exchange?

…

What are your reasons for exchanging Dalmatian pelicans with other zoos?

- ○ We take in pelicans to grow our population

- We send out pelicans when our colony grows too big due to births
- To reduce inbreeding
- We take in rescued pelicans
- It is recommended by the studbookkeeper
- Other …

Are there problems commonly associated with exchanging Dalmatian pelicans or similar animals between zoos? And if so: what problems?

…

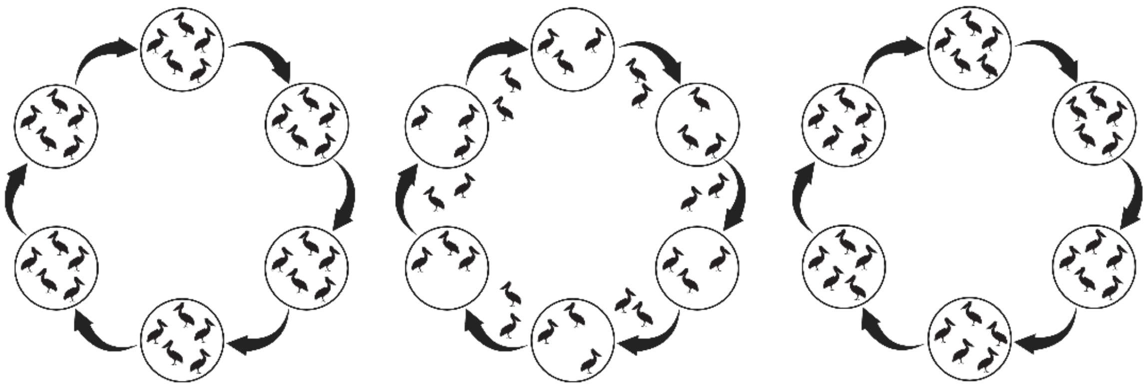
Section 12

Breeding Circle

A breeding circle is a rotational breeding strategy in which a certain amount of animals is moved to the neighbouring population. An advantage of a breeding circle is that a pedigree is not necessary. This makes it a useful strategy for breeding group-living animals. Furthermore, the donor and recipient populations are always the same. This means that a zoo only has to make agreements with the zoo it receives animals from and the zoo it donates animals to. However, these agreements should be made for a long time because animals will be exchanged periodically. The breeding circle can also be set up in such a way that the distances between donor and recipient populations are minimised, reducing transport costs and stress for the animals.

For more information about breeding circles check out Windig & Kaal, 2008 and Kappelhof & Windig, 2021.

Breeding circle in which each population donates two animals to its neighbouring population.



What are important considerations that should be taken into account when setting up a breeding circle?

…

Do you think problems will arise when applying the breeding circle management strategy? If so: what kind of problems?

…

Section 13

Finalisation

I would like to ask you to fill in the final wrap-up questions.

Is it okay if your name and/or organisation will be mentioned in my report?

- Yes
- No

Is it okay if I contact you with additional questions?

- Yes
- No

If you have anything else you would like to say, you can do that here.

…

Section 14

Thank you!

Thank you for filling in this survey! Feel free to contact me at stijn1.kouwenberg@wur.nl if you have any questions or remarks regarding the survey, the topic, or my research.

Appendix II

Table 13: List of all the respondents and their affiliations. The two anonymous respondents are not indicated in the table.

Respondent	Zoo	Location	Country
Alexandre Petry	Parc de Branféré	Le Guerno	France
Andreas Artmann	Zoo Schmiding	Schmiding	Austria
Anthony Dubois	Parc Animalier des Pyrénées	Ayzac-Ost	France
Antonin Vaidl	Prague Zoo	Prague	Czech Republic
Aude Haelewyn-Desmoulins	Parc Zoo du Reynou	Le Vigen	France
Diana Koch	Tiergarten Nürnberg	Nürnberg	Germany
Elodie Trunet	Zoo Bassin d'Arachon	La Teste-de-Buch	France
Eric Bureau	Parc des Oiseaux	Villars-les-Dombes	France
Eric Diener	Zoo Heidelberg	Heidelberg	Germany
Frédéric Verstappen	Zoo Planckendael	Mechelen	Belgium
Laetitia Lassalle	Biotropica	Val-de-Reuil	France
Maarten Vis	Rotterdam Zoo (Diergaarde Blijdorp)	Rotterdam	Netherlands
Marcel Alaze	Allwetterzoo Münster	Münster	Germany
Martha Moritz	Tierwelt Herberstein	Herberstein	Austria
Matthias Papies	Berlin Tierpark	Berlin	Germany
Mélanie Bourdu	Zooparc de Trégomeur	Trégomeur	France
Meret Huwiler	Tierpark Bern	Bern	Switzerland
Michal Podhrázský	Zoo Dvůr Králové	Dvůr Králové	Czech Republic
Nicolas Géli	Le PAL Zoo	Le Pal	France
Nils Dijkgraaf	DierenPark Amersfoort	Amersfoort	Netherlands
Patrick Roux	Réserve Zoologique de la Haute-Touche	Obterre	France
Peter Smallbones	Paignton Zoo	Paignton	UK
Ronald Renson	Monde Sauvage Safari Parc	Aywaille	Belgium
Ruben Holland	Zoo Leipzig	Leipzig	Germany
Sabine Ketelers	Bio-Topia (Parc Zoologique Fort-Mardyck)	Fort-Mardyck	France
Simone Haderthauer	Vienna Zoo	Vienna	Austria
Steven van den Heuvel	ZooParc Overloon	Overloon	Netherlands
Thomas Lipp	Zoo Augsburg	Augsburg	Germany
Wiene van de Bunte	AquaZoo Leeuwarden	Leeuwarden	Netherlands
-	Zoo Opole (Ogród Zoologiczny w Opolu)	Opole	Poland

Appendix III

Data Management Plan

Annex to MSc thesis – Data management plan

Data management plan belonging to the MSc thesis performed at the Animal Breeding and Genomics Group by Stijn C.W. Kouwenberg, completed in March 2023.

Agreements

1. The data used in this thesis project have been described in this document and have been stored in a systematic manner (at least in separate folders for all sections as described below). Data includes all data as mentioned in the results section of your report.
2. The data management plan has been discussed with the MSc thesis supervisor and he/she has agreed on the location for data storage.
3. In case of confidentiality, contact details of the responsible person from the company/institution that has ownership of the data are mentioned in this document.
4. The data can be found through Jack J. Windig, email: jack.windig@wur.nl

Section A - Raw data

File names	Received from	On date
EAZA Population Dalmatian pelican.xls	Retrieved from ZIMS Species Holdings.	August 2022
Survey_Results.xlsx	This file contains the answers to the survey.	January 2023
op_Cur_Nmin20_20221216.txt	Generated using Pointer.	December 2022
bc_Cur_Nmin20_r6_n5_f_20221216.txt	Generated using Pointer.	December 2022
ne_Cur_Nmin20_20221216.txt	Generated using Pointer.	December 2022
bc_Cur_Nmin10_r6_n5_f_20221216.txt	Generated using Pointer.	December 2022
ne_Cur_Nmin10_20221216.txt	Generated using Pointer.	December 2022
Results_Simulations_R_input.txt	The data was generated using Pointer and manually added to this file.	March 2023

Comments: I used Pointer to simulate the data needed for my study. Pointer generates output files like bc_Cur_Nmin20_r6_n5_f_20221216.txt. From these files, inbreeding, generation interval and genetic diversity values were manually added to Results_Simulations_R_input.txt.

Section B – Data analysis

File names	Created in	Remarks
Population_Demographics_v02.xlsx	October 2022	This file contains the data and calculations that were used to determine the offspring per age class and the offspring distribution.
Graph_F_over_t_comparison.R	November 2022	This script was used to generate: Breeding_Strategy_Comparison.png
dF_for_subpopulations.R	November 2022	The output from this script was manually added to: Results_Individual_Zoos.csv
Results_Individual_Zoos.csv	November 2022	This file was used as input for: Effect_BC_by_N.R
Effect_BC_by_N.R	November 2022	This script was used to generate: Effect_BC_by_N_v2.png
Bar_charts_combined_v3.R	November 2022	This script was used to generate: Bar_charts_BC_results_dF_20230321.png

Survey_Factors_for_Breeding.csv	February 2023	This file was used as input for Bar_plots_survey_results.R to generate: Survey_Factors_for_Breeding_final.png
Survey_Flight_Restraints.csv	February 2023	This file was used as input for Bar_plots_survey_results.R to generate: Survey_Flight_Restraints_final.png
Survey_Mixed_Species_02.csv	February 2023	This file was used as input for Bar_plots_survey_results.R to generate: Survey_Mixed_Species_02_final.png
Survey_Supplements.csv	February 2023	This file was used as input for Bar_plots_survey_results.R to generate: Survey_Supplements_final.png
Bar_plots_survey_results.R	December 2022	This script was used to generate: Survey_Factors_for_Breeding_final.png, Survey_Flight_Restraints_final.png, Survey_Mixed_Species_02_final.png & Survey_Supplements_final.png
Boxplot_Group_Effect.R	March 2023	This script was used to generate: Survey_Breeding_by_Group_size_v02.png.
Graph_response_over_t.R	March 2023	This script was used to generate: Survey_Response_over_Time.png

Section C – Final data

File names	Created in	Remarks
Breeding_Strategy_Comparison.png	December 2022	Figure 10 in the report.
inbreeding_rates_subpopulations_bc_Cur_Nmin10_r6_n5_f_20221216.csv	December 2022	Data is used in table 9 in the report.
inbreeding_rates_subpopulations_ne_Cur_Nmin10_20221216.csv	December 2022	Data is used in table 9 in the report.
Effect_BC_by_N_v2.png	December 2022	Figure 12 in the report.
Bar_charts_BC_results_dF_20230321.png	March 2023	Figure 11 in the report.
Survey_Response_over_Time.png	February 2023	Figure 3 in the report.
Survey_Mixed_Species_02_final.png	March 2023	Figure 4 in the report.
Survey_Flight_Restraints_final.png	February 2023	Figure 5 in the report.
Survey_Supplements_final.png	February 2023	Figure 6 in the report.
Survey_Factors_for_Breeding_final.png	February 2023	Figure 7 in the report.
Survey_Breeding_by_Group_size_v02.png	February 2023	Figure 8 in the report.