EAZA BEST PRACTICE GUIDELINES

Aegypius monachus - Cinereous vulture

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First edition 2023

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Citation: Huyghe M.[(1)], Izquierdo P.[(2)], Bureau E.[(3)], Llopis A.[(4)] (2023) EAZA Best Practice Guidelines for the cinereous vulture, *Aegypius monachus*. European Association of Zoos and Aquaria, Amsterdam, The Netherlands.

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DOI: 10.61024/BPG2023CinereousvultureEN

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Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the "Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria". These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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Introduction

These Best Practice Husbandry Guidelines follow the template suggested by EAZA for all their EEPs. The first section gives a detailed overview of our current knowledge on biology of the cinereous vulture. This part includes field data referring to recent conservation action plans and the role of this EEP herein.

The second section deals with cinereous vulture management in zoos. This section includes the experiences exchanged in the workshop held in Antwerp Zoo 2019, where *in situ and ex situ* experts of different vulture species shared their experience to improve the breeding results for cinereous vulture. The former version of the husbandry guidelines published in 2009, is updated with this new information and experiences collected and gained during the past 10 years.
I am very grateful to all people who contributed to those volumes and shared their expertise. Their names are mentioned by the start of the chapter/section they wrote or reviewed: Thank you once again!

Within each chapter a summary is made of the most important points needed for optimal management of the species. However, it is strongly recommended to read the chapter as a whole to interpret it correctly and make the right decisions.

In Section 3, a list of references is presented, and in the appendices the existing protocols are included.

Finally, a big thank you to all experts which helped me running this programme for more than 20 years and shared their experiences: in particular Evelyn Tewes, Hans Frey, Alex Llopis, Iñigo Fajardo, Pablo Izquierdo, the advisors for the programme, Philippe Helsen and Eric Bureau, and the species committee. Last but not least the bird keepers of Planckendael Zoo and Frédéric Verstappen for their continuous support, enormous enthusiasm and hard work!
Thank you to Marina Salas and Dominik Fischer for adding their experience to these guidelines, to Zjef Pereboom for his help in editing the final version and to Allan Muir for reviewing the text.

Hopefully these guidelines will help all people passionate about cinereous vultures to house them in an optimal way, to give them the best conditions and to breed successfully.

Enjoy! And good luck!

Marleen Huyghe, April 2023

SECTION 1: BIOLOGY AND FIELD DATA

BIOLOGY

1.1 Taxonomy

The cinereous vulture (***Aegypius monachus****) is placed in the order of the **Falconiformes**/ Accipitriformes, and the family of the Accipitridae (Hawks and Eagles). It is a member of the old-world vultures (Subfamilies **Aegypiinae and Gypaetinae**) which have been proposed to be monophyletic or polyphyletic with *Gyophierax* (e.g., palm nut vulture), *Neophron* (e.g. Egyptian vulture) and *Gypaetus* (e.g. bearded vulture), based on morphological data. Lerner and Mindell (2005) found strong support of phylogenetic relationships among old world vultures, using molecular data from two mitochondrial genes. Their study shows that **Gypaetinae** is the earlier diverging group, which is genetically quite diverse, whereas the **Aegypiinae** itself form a monophyletic group. It is the only species in the genus *Aegypius*. There are currently no subspecies identified, although small differences are examined between the European and Asiatic populations, namely genetic differences, and differences in size as birds from Southwest Europe are approximately 10% smaller than birds in Central Asia (Poulakakis et al, 2008; Ferguson-Lees & Christie David, 2001).
Common names are European black vulture: cinereous vulture (English), Buitre negro (Spanish), Vautour moine (French), Mönchsgeier (German), Monniksgier (Dutch).

Note: * Aegypius= Greek for "vulture", and monachus= Latin for "hooded"

1.2 Morphology

Figure 1 Adult cinereous vulture (picture Hansruedi Weyrich)

Note the large beak, which is bluish at the edge towards the head. Short feathers cover the head, before they elongate into the 'monk's collar' around the neck.

The head of a cinereous vulture is massive and covered in small dark brown feathers that can be very thick on the head, giving the impression of a small cap. The feathers are dark brown, often with white spots on the wings. It has a firm bill which is almost entirely blackish brown with yellowish patches around the edges and a blue upper part. The eye is a golden brown. The nostrils are circular. The neck collar, composed of fluffy feathers, is shaped like the collar of a monk (see picture). Weight ranges from 7 to 12.5 kg, the wingspan from 250-295cm and the height from 98-107cm (del Hoyo et al., 1994)

Cinereous vultures are one of the largest old-world Vultures and are not sexually dimorphic. Juveniles tend to have black plumage with pinkish skin on the head, neck, and legs. Adult plumage is achieved at approximately six years of age.

1.3 Physiology

Haematologic and plasma biochemical values have been collected in recovered healthy birds with an estimated age of 1-2 years (Seok et al., 2017). In this study haematologic and plasma biochemical reference intervals for captive healthy cinereous vultures were defined. As such these values can be used to evaluate the physical condition of birds in captivity of the same age before release.

1.4 Longevity

Cinereous vultures can live for 20-30 years in the wild (26 years; del Hoyo et al., 1994) and up to 40 years in captivity (39 years; Newton and Olsen, 1990). According to EEP studbook data the oldest registered bird became 49 years old.

FIELD DATA

1.5 Conservation status/ Zoogeography and ecology

Most of the information in this chapter is taken from Birdlife International, 2022, Species factsheet*: Aegypius monachus* and the Flyway Action Plan for the Conservation of the Cinereous Vulture (Andevski et al., 2017).

1.5.1 Zoogeography

Geographical distribution

The International Union for Conservation of Nature (IUCN) and BirdLife International (2022) report the breeding of cinereous vultures in Spain, Bulgaria, Greece, Turkey, Armenia, Azerbaijan, Georgia, Ukraine, Russia, Uzbekistan, Kazakhstan, Tajikistan, Turkmenistan, Kyrgyzstan, Iran, Afghanistan, northern India, northern Pakistan, Mongolia and mainland China, with a small, reintroduced population in France and Bulgaria. Since 2010 the species has begun reproducing once again in Portugal and the breeding population increases continuously. Cinereous vultures

may occasionally breed in Macedonia and Albania, but breeding has ceased in Slovenia, Italy, Cyprus, Moldavia and Romania. According to Mundy et al. (1992) the cinereous vulture is now extinct in Africa (Birdlife, 2022).

Wintering areas are located in Sudan, Pakistan, north-west India, Nepal, Bhutan, Myanmar, Lao' People's Democratic Republic, North Korea and South Korea.

Population numbers

Most recent population estimates are presented in the Flyway action plan for the cinereous vulture (Andevski et al., 2017): the global population is estimated at 9,657-12,306 breeding pairs, with 2.536-2.838 pairs in Europe. The population has increased in Europe, by 48% in the last decade, in particular in Spain, Portugal and France. In Greece, a small and stable (slowly increasing) colony exists of 21-35 breeding pairs, for the Caucasus population estimates are only available for a few countries and seems to be stable. In Asia the population trend is believed to be decreasing although few population numbers are known.
The main strongholds for the species are Mongolia, holding approximately 50% of the species global population, and Spain with more than 20%.

Only partial information is available on trends in the non-breeding (wintering) range of the species; wintering numbers appear to be declining in Nepal and significantly increasing in India and South Korea, due to the establishment of feeding sites.

Table 1 Population estimates per country (Flyway action plan for the Conservation of the Cinereous vulture, 2017)

Country	Breeding pairs	Data quality	Year(s) of estimate	Breeding Population trend in the last 10	Data quality
Afghanistan	No data	M			
Armenia	50	M	2007-2009	stable	M
Azerbaijan	20-100	M	2000-2016	stable	M
China (People's Republic	1760		1991		
France	31	G	2016	small increase	G
Georgia	50	G	1995-2016	stable	G
Greece	21-35	G	2006-2015	moderate increase	G
Iran (Islamic Republic of)	No data				
Kazakhstan	150-300	M	2012	stable	M
Kyrgyzstan	50-60	M	2007		
Mongolia	5000 -7000	P	2016	small decline	P
Portugal	18	G	2016	large increase	G
Russia (Caucasus)	63-102	M	2004	small decline	M
Russia (Altai-Sayan)	71-96	G	2009	moderate increase	G
Spain	2198 - 2258	G	2015	moderate increase	G
Tajikistan	10-100	P	2016		
Turkey	80-200	M	2013	decline	M/P
Turkmenistan	30-32	M	2013	decline	M
Ukraine	15-19	G	2016	stable	G
Uzbekistan	80-120	M	2005	small decline	P

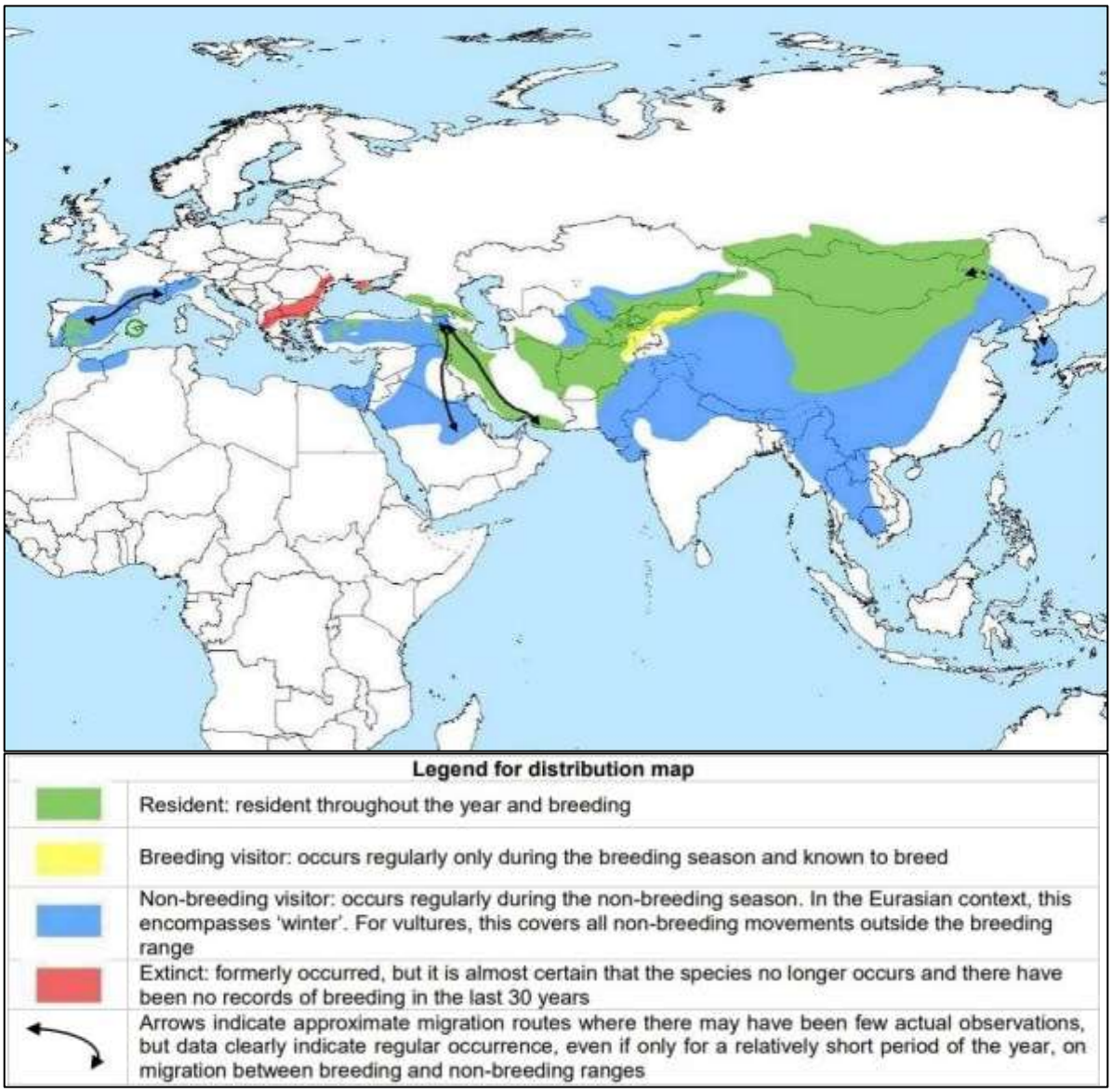


Figure 2 Distribution of cinereous vulture (BirdLife International 2017)

Movements

Research shows that some animals are partly migratory during winter (Bildstein, 2006). Several individuals from the Caucasus (Eastern Europe and Central Asia) migrate southwards from their habitat where they breed during the winter. Some of these birds are nomadic. For example, there are data showing that juveniles and sub-adults hatched in Georgia, migrated to Saudi Arabia and Iran for wintering and then moved back north to Russia. (Gavashelishvili et al., 2012). Many adults and juveniles in Mongolia apparently migrate in autumn to wintering areas in the Republic of Korea (South Korea) (Andevski et al., 2017), while birds from central Asia migrate to the Indian subcontinent, southern China, Russian far East, and the Republic of Korea (Batbayar, 2006).

In Europe, adults tend to be sedentary, with younger birds dispersed over larger areas. In Spain, young birds tend to stay near the various breeding colonies located in the western part and central parts of the Spanish peninsula (Moreno-Opo and Guil, 2007).

Itinerant individual animals between Spain, France, Italy do occur regularly. Few birds are recorded crossing the Gibraltar Strait yearly (Andevski et al., 2017). Birds normally living in the colony of the Dadia-Lefkimi-Soufli Forest National Park in northern Greece are observed to visit a nearby site in Bulgaria on a regular basis. (Vasilakis, Poirazidis and Ellorriaga, 2008).

Threats

Main threats to this species in the wild are poisoning (unintentional), persecution, diclofenac, decreased food availability, habitat alteration and loss, climate change, disturbance, electrocution on energy infrastructure, and collision with energy infrastructure (BirdLife International, 2018; Andevski et al, 2017).

Poisoning is the most severe threat to the cinereous vulture. In general, poisoned baits are not used to intentionally kill vultures but used against predators (foxes, wolves, feral dogs, etc.) of livestock and game animals. Poisoning can also be unintentionally caused by agrochemicals (pesticides), veterinary pharmaceuticals (used in livestock, e.g., diclofenac), and lead ammunition from hunting activities.

Other main threats affecting cinereous vultures are electrocution and collision with electricity infrastructure (wind turbines), where the development of wind farms can be a serious threat in future (Andevski, 2017). In the Balkans, collision mortality through operational windfarms was thought to affect c. 5-11% of the core population (Vasilakis et al*.,* 2016).

The decrease in food availability, more specific the decline of herbivores (wildlife and livestock), resulting in a reduction of animal carcasses in the wild, is considered to have a negative impact on the species, affecting breeding success and distribution (Andevski et al., 2017). Also, disturbance caused by human activities during the breeding season can have a negative effect on breeding success, affecting the number of chicks fledged, even if the disturbance occurs once (González et al., 2006; Zuberogoitia et al., 2008; Margalida et al., 2011; Andevski et al., 2017).

Habitat loss is also thought to be important (Anon, 2004). For instance, in Portugal, there may be suitable foraging areas, but nesting sites are limited (Lourenço et al., 2013).

At present, the persecution or deliberate killing of birds is considered a threat in Central Asia, rather than Europe.

For more information see Birdlife data sheet Aegypius monachus, 2022, Flyway Action Plan for the conservation of the cinereous vulture, Aegypius monachus, 2017 and EAZA Raptor TAG Regional Collection Plan Vultures, October 2022.

1.5.2. Conservation actions

General

A Multi-species Action Plan for African-Eurasian Vultures has been produced (Botha et al., 2017), followed by the Flyway Action Plan for the Conservation of the Cinereous vulture (Andevski et al., 2017). The EU Birds Directive has contributed to the recovery and conservation of the species in Europe, particularly Spain. Co-operation between Spanish government agencies and conservationists under the 'Antidote Programme' also appears to have been effective in mitigating the effects of poisoned baits (Birdlife, 2022).

Supplementary feeding programs have been initiated in Spain, France, Bulgaria, and South Korea to provide a safe, poison-free food source, although there are concerns that the species may be not very prone to feed at conventional feeding stations.

In India, Pakistan, and Nepal, a ban was introduced in 2006 on the production and importation of diclofenac for veterinary use and pharmaceutical firms were encouraged to promote a safe alternative called meloxicam. The use of diclofenac has since declined by 90% across parts of Nepal; however, its complete elimination from the scavenger food chain has yet to be achieved.

In eastern Europe and central Asia, fewer conservation actions are known, although the species occurs within a number of protected areas in the region.
The Balkan Vulture Action Plan's goal is to transfer the expertise gained in the conservation of the species in Western Europe to the Eastern parts of Europe.

Conservation Actions Proposed:

- Survey to determine the species' status and population trends on breeding grounds outside Europe and on wintering grounds.
- Research threats, particularly the decline in abundance of prey species.
- Restore wild rabbit populations in the Iberian Peninsula and the Balearic Islands (Spain) as this may help to increase food availability, particularly during the breeding period.
- Promote cooperation and information exchange among people working on the species, both at a national and international level.
- Remove drugs like diclofenac and other harmful NSAIDs from the environment and control the illicit use of human diclofenac for veterinary purpose.
- Strengthen and enforce legislation regulating trade in pesticides that are used to poison meat baits.
- Increase the rate of prosecution and the severity of judicial sentences for illegal poisoning.
- Protect appropriate breeding habitat.
  (BirdLife International, 2018; Raptor TAG RCP Vultures, 2022).

In addition, the actions listed within the framework the European Species Action plan for the conservation of cinereous vulture and in the Flyway Action Plan (Andevski et al, 2017):

1. Reduce poisoning with poisoning baits /by agrochemicals/ by vet drugs/ by lead.
2. Improve the quality and availability of food resources for cinereous vultures.
3. Reduce the impact of the energy infrastructures on cinereous vulture populations.
4. Improve breeding productivity by adequate protection and management of breeding habitat/ by decreasing human disturbance to breeding the species.
5. Reduce mortality of cinereous vultures though direct persecution.
6. Promote linkage between cinereous vulture populations to restore the species into its former range.
7. Coordinate conservation actions for the cinereous vulture across the global range thought he implementation of the EU species action and the Flyway Action Plan.

Role of cinereous vulture EEP

The cinereous vulture EEP is linked with reintroduction and restocking of the species. The Long-Term Management Plan (LTMP) (Sanchez et al., 2019) has identified as one of its roles to function as a source for reintroduction projects, mainly to re-establish breeding colonies at strategic geographical regions as described in the Multi Species Action Plan (Vulture MSAP, 2017). One action in the Flyway Action Plan for the conservation of the cinereous vulture defines to support and involve the existing cinereous vulture EEP programme in restocking/reintroduction projects (Andevski et al., 2017).

To address this role, a release strategy for cinereous vulture EEP was developed, which determines on basis of the breeding success and the actual number of fledglings, the number of chicks available for release each year (see Appendix 2).

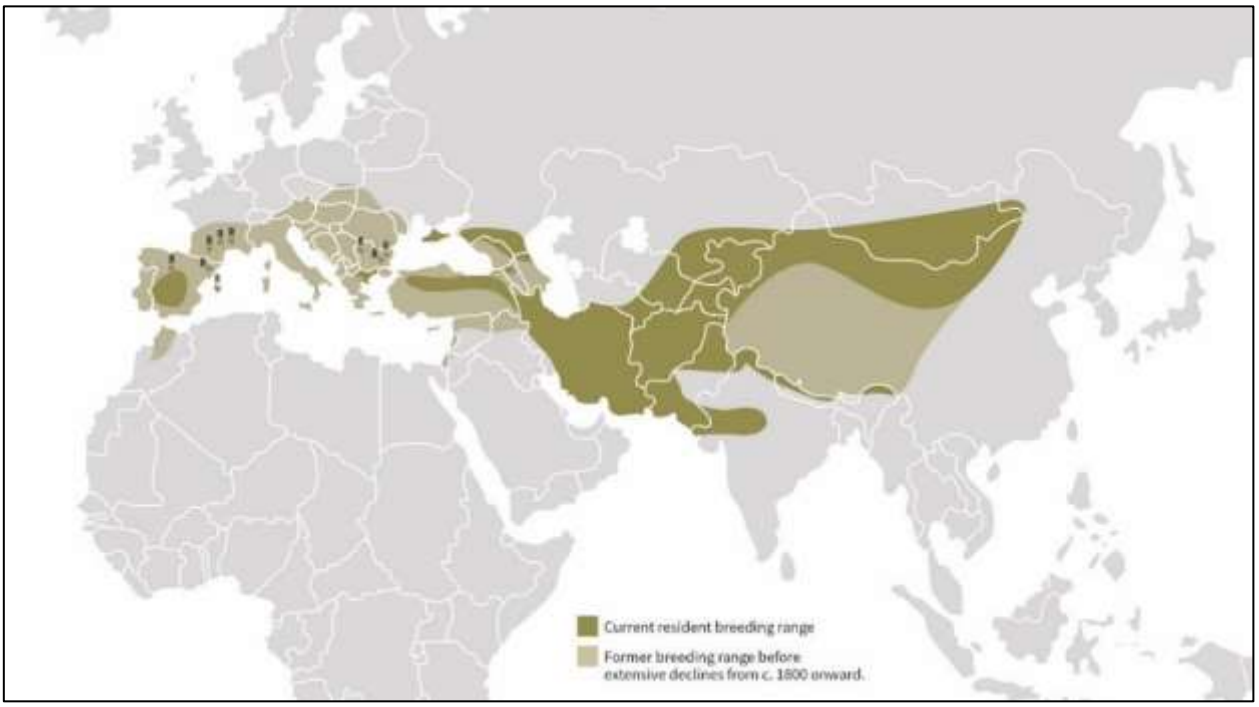


Figure 3 Cinereous vultures originating from the EEP were reintroduced in Mallorca, Spain (Boumort), France (Causse, Baronnies, Verdon) and Bulgaria (Sliven, Kotel). These sites are indicated on the map with "R".

Conservation status

The cinereous vulture is included in the 'Near Threatened' IUCN Red List Category, evaluated by BirdLife International (BirdLife International (2021). It is further listed in the EU Birds Directive Annex I, CMS Appendix II, Raptors MOU Category 1 and listed in Appendix A of the CITES/EEC Community Regulation (Appendix II CITES). A multi species Action Plan has been produced (Botha et al., 2017) and The Flyway Action Plan for the Conservation of the Cinereous Vulture (CVFAP) was additionally finalised in 2017 (Andevski et al., 2017).

1.5.3. Ecology

Natural habitat

A hilly landscape is the most common habitat type used by cinereous vultures in the Mediterranean distribution area (Carrete et al., 2005; Cramp et al., 1980). Cinereous vultures are a tree breeding species, in some cases they breed on the ground when the area lacks suitable trees. In Spain, cinereous vultures occur in scrubland vegetation with few large trees and predominantly nest in *Quercus* tree species. Other possible habitats in Spain are areas with reduced vegetation, converted into eucalyptus and pine plantations. In Greece, cinereous vulture habitat consists of open forest with *Pinus* trees as the main breeding sites. Poirazidis et al. (2004) define the optimal habitat for cinereous vultures in the Dadia Reserve in Greece as areas with mature trees surrounded by openings or with low height vegetation on steep slopes. Open pine forests around big granite mountains have also been identified as cinereous vulture habitat in Spain and Greece. On Mallorca the vultures breed on solitary pine trees growing on sea cliffs scarcely covered with other vegetation. Other habitat types may exist, for example the colonies of ground nesting cinereous vulture colonies in the Mongolian hills. In general, the choice of tree depends on the tree abundance in the area and on the ability of the tree crown to support a cinereous vulture nest. The tree type seems to be less important than height or maturity, and cinereous vultures seem to appreciate a good view from the nest.

1.6 Diet and feeding behaviour

Between different habitats, but also within the same colony, there can be very large differences in type of feeding. (Moreno-Opo et al., 2010). Cinereous vultures mainly feed on carrion from medium sized to large mammals (like sheep, goat, rabbits, and wild ungulates) but also reptiles and insects are recorded as food (Tewes, 1996; Corbacho et al., 2007; Yamac and Günyel, 2010; Andevski et al., 2017).

Animal farming appears to be of considerable importance for the food availability. Changes in the availability of prey over the last 30 years have led to a decrease in the number of rabbits in its diet and an increase in the consumption of domestic ungulates (Corbacho et al., 2007; Costillo et al., 2007; Moreno-Opo et al., 2010). In Mongolia, at least, the species is reliant on livestock numbers for successful nesting (Batbayar et al., 2006), furthermore Tewes (1996) describes food as the most restraining factor for breeding success.

For feeding, cinereous vultures make use of their specialized beak, that is extremely suitable for ripping skin and fat layer of carcasses (Alvarez, 1976). This is probably why cinereous vultures are generally the first species to claim a cadaver. They eat by pulling the meat off the carcass with their beak. To reach areas not readily accessible, they insert their whole head and neck into openings. For meat stuck to the bones the birds slide the sharp edges of their beak along the bones, opening and closing it like scissors. Cinereous vultures feed mainly on muscles and small peripheral scraps of meat and tendon (Moreno-Opo et al., 2010). Fur or feathers are regurgitated in pellets, and bones are digested. If carcasses are scarce, cinereous vultures may hunt live prey e.g. rodents, lagomorphs, reptiles such as lizards and tortoises. Live prey is rarely taken (Batbayar et al., 2006).

1.7 Reproduction

Cinereous vultures are believed to be monogamous. Recent genetic analyses however illustrates individuals do re-pair which is believed to be triggered by breeding failure, loss of partners or a side effect of more dynamic population growth (Helsen et al. in prep.).
Cinereous vultures tend to congregate in very loose colonies and are strongly bound to their traditional nesting grounds. Nests have a distance of 175 to 520 meters between them, although distances from 30 m to 2 km are mentioned too. The nests are huge constructions (145-190 cm wide, 95-300 cm deep) made in the top of a tree. Not all pairs breed every year. According to del Hoyo et al. (1994) only 60% of the couples breed successful every season. Breeding parameters of the species are not well known for the entire breeding range. In Spain, the latest national coordinated census (in 2006) revealed an overall productivity (number of chicks reared/total observed pairs) of 0.60 and a breeding success (number of chicks reared/pairs starting incubation) of 0.68 (De la Puente et al., 2007).

Food shortage is the most important limiting factor for successful reproduction (Tewes, 1996). Non optimal nutritional conditions can result in lower breeding densities, an increase in the occurrence of pairs that do not lay eggs, later and smaller broods, deserted or neglected nests with chicks, decreased growth of the nestlings and less replacement eggs after loss of the first egg. Generally, older and more experienced pairs have higher reproductive success, because they are more familiar with their terrain and find food easier (Hiraldo, 1984). Breeding success decreases with human disturbance, e.g., when roads are present near the nest or when accessibility to the immediate environment of the nest increases (Morán-López et al., 2006). Another anthropogenic factor is lower sheep livestock density, making it more difficult for vultures to obtain enough food. Breeding failure also increased with natural factors like altitude and ruggedness of the nesting area. Although rugged terrain gives nests more protection against disturbance, and altitude provides slope lifts for soaring, there were nevertheless limitations that were possibly mediated by the climate. Frequent rainfall and high temperatures also caused a decrease in breeding success, probably by limiting foraging behaviour due to rain or because of shade providing behaviour of the parents.

Age at maturity
Cinereous vultures are sexually mature when they are five to six years old (Tewes, 1996). Around this age, they change from black to brown plumage. In the wild, breeding attempts by four-year old individuals have been observed. In captivity, young pairs may show breeding behaviour in the form of copulations and nest building attempts, but without breeding success (Gottschlich, 2008).

Breeding season
Depending on weather conditions, the cinereous vulture breeding season usually starts in January and egg laying occurs from February until April (Hiraldo, 1984; del Hoyo et al., 1994; Suetens, 1992). In Spain the breeding season peaks in late February to mid-March (Mundy et al, 1992). Males and females share incubation and rearing duties.

Clutch size
Typically, females lay a single egg, although occasionally two eggs can be laid. Nonetheless only one chick usually survives (Hiraldo, 1984). The egg is creamy white, sprinkled with red brown spots

and on average 9.17cm long and 6.87cm wide (Tewes, 1996). In the wild, an egg weighs about three percent of an adult's body weight (~250g).

Incubation

In nature the average incubation period is 50 to 57 days (Bernis, 1966; Hiraldo, 1984; Mundy et al. 1992; del Hoyo 1994), with an average of 55 days in nature and 53 days in captivity. Both parents incubate the egg continuously and change over regularly. Birds have been observed to incubate uninterrupted between 6 hours and 5 days (Hiraldo, 1984). The frequency of change-overs increases towards the end of the incubation period (Minneman and Busse, 1984). Because of this continuous presence of one of the two parents the hatching success in the wild was estimated to be 95% (Mundy et al., 1992). If a first brood is lost, a second breeding attempt can occur after approximately 2-3 weeks, although the likelihood decreases later in the breeding season.

Rearing

The hatched chicks are covered with grey-white to grey-brown down. When they are about 30 days old, the first flight feathers begin to grow. At about 60 days of age, they are fully covered in feathers. Both parents are very devoted, active, and very protective. They alternately take care of the young. The parents feed the young by regurgitating ingested food which is then given to the young in small pieces. As the young get older, they peck at the offered food themselves. At around 104 to 120 days of age, the young fledge. However, they remain with the parents for another 2-3 months until about 180 - 215 days and at an age of 4 to 5 years they are ready to breed (Gavashelishvili et al., 2012).

1.8 Other behaviours

In terms of social structure, cinereous vultures are considered semi-colonial, while Griffon Vultures (*Gyps fulvus*) live in dense colonies and Bearded Vultures (*Gypaetus barbatus*) are quite territorial. Cinereous vultures are partial migrants in Mongolia, and the Caucasus range. Juveniles and subadults overwinter on the Korean peninsula and as far as north-eastern Saudi Arabia and southwestern Iran. In southwest Europe adult cinereous vultures are mostly sedentary, while the juvenile birds disperse over larger areas. Movements of individuals to and from Spain, France, Portugal, and Italy have been recorded in recent years. Also, birds from the Dadia-Lefkimi-Soufli Forest National Park colony in north-eastern Greece regularly visit the nearby vulture feeding sites in southern Bulgaria and disperse in the wider range of Rhodope Mountain (Vasilakis et al., 2008; Vasilakis et al., 2016; Vasilakis et al., 2017). Some birds released by hacking in Bulgaria move into Turkey (Ivanov et al., 2022; S. Marin., pers. comm., 2022).

Due to a strong fidelity to former breeding sites the cinereous vulture is able to accept habitat changes well (Tewes, 1996). However, colonies only seem to move a little further away in case of strong disturbances like forestry activities and do not join other colonies or build new ones in other available suitable habitats. As a result, the natural expansion of a population seems to be a difficult and slow process. This is important to know for conservation measures of the species.

Greeting ceremony

Greeting behaviour is observed between mates at carcasses or at the nest. When a cinereous vulture approaches its mate on the nest, a greeting ceremony takes place (Cramps and Simmons, 1980). The bird on the nest starts by raising its collar feathers and the feathers on its back. It makes slow left to right movements, thereby raising the legs abnormally high with spread out toes. The wings touch the nest floor, the tail feathers raised, the neck stretched and the head twisting from left to right. When the partner lands on the nest it assumes the same position. Both birds twist their heads and stretch, making themselves as tall as possible. Next, the tails are lowered, and the wings are being hanged down while the collar and back feathers remain raised. Then both birds touch the other partner's beak, head, and the down in the neck with their beak (also called allopreening). This ritual is probably a combination of ritualized aggressive behaviours (raising feathers and tail) and subjection (lowering the wings, allopreening) (Bernis, 1966; Suetens and van Groenendael, 1966).

Figure 4 Greeting behaviour (picture Hansruedi Weyrich)

Courtship display

The courtship display takes place high up in the sky, making it difficult to observe and describe this behaviour. The male and female fly around each other and give each other symbolical prey. They can make a free fall with entwined feet. This courtship display looks a lot like fighting in the air so both behaviours can be mixed up. Courtship behaviours can be observed on and around the nest too. The vultures will follow one another, inspect potential nest sites and present nesting material. (Fischer, 1974; Minnemann and Busse, 1984). See also section 2, page 18, point 5: Rituals.

SECTION 2: MANAGEMENT IN ZOOS

chapter reviewed by A. Llopis, ex situ vulture expert for the cinereous vulture EEP programme

When housing and managing cinereous vultures in captivity, it is very important to take the following considerations into account:

1. *Cinereous vultures are very sensitive for disturbance → they have to feel safe and secure in their housing*
2. *Cinereous vultures are very picky in choosing their partner and show specific behavioural patterns -> see enclosed pair bonding protocol (pg. 48)*
3. *Close and continuous observation from breeding pairs is required: pair bond behaviour, nest building and raising of chick*

The following chapters of these husbandry guidelines will take these particular qualities as much as possible into account. This should also be done when you plan to house cinerous vultures in your collection.

In December 2019, a workshop was held at Antwerp Zoo, Belgium, to improve and increase the breeding success of cinereous vulture. A very interesting presentation was shared by Iñigo Fajardo and his colleagues from the Junta de Andalucía* in Spain.
**Junta de Andalucia is the administration of the Spanish autonomous community of Andalusia, amongst others responsible for the governing of protected areas, where cinereous vultures occur.*

The presentation included:
"The 7 golden rules observed in wild breeding pairs, to be used in captivity"
Iñigo Fajardo and its team have been performing 20 years of field observations on cinereous vultures in five colonies in Andalucía. From this intense monitoring they selected the following relevant behavioural features to keep in mind:

- High individual variation in behaviour to the point of management
- High social behaviour, in particular in breeding season
- They love privacy from time to time,
- Their life is ruled by rituals, especially during the breeding season
- Strong feelings and long memory: very sensitive and easily traumatised

These rules were translated into the **following 7 golden rules for captivity**
Pictures are from Dani Buron & Inigo Fajardo, Junta de Andalucia, Spain

1. Free mate choice: they invest a huge time in the first years of life in partner choice.

2. Breeding aviaries/pairs must have direct visual contact like in the wild. They like greeting neighbours!! Visual contact seems to have a clear stimulating effect on breeding; additionally, in captivity we observe that zoos where several pairs are housed, have better breeding success.

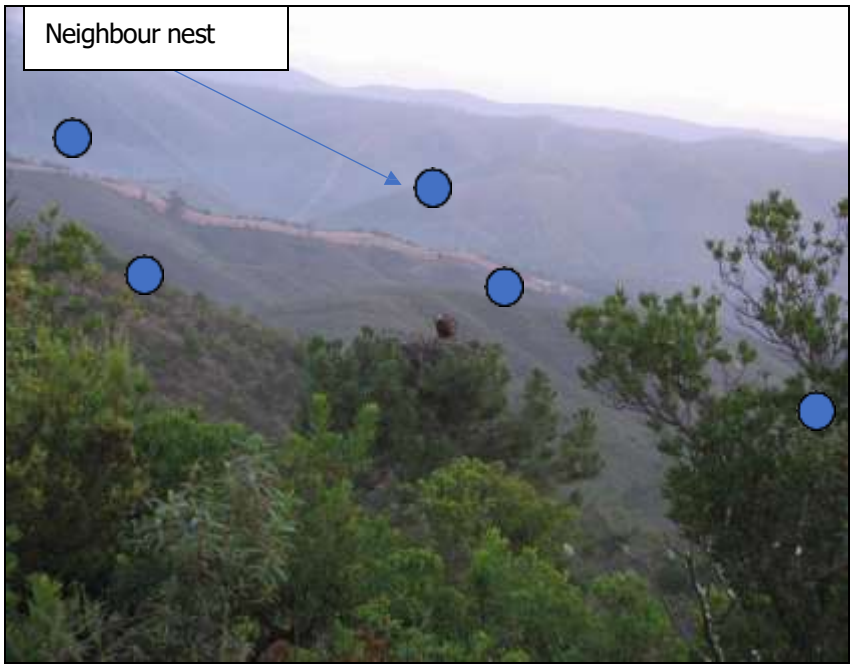


Figure 5 Neighbouring nests in the wild are at visual distance

3. Privacy: They spend many hours on their own (most of their adult lives). Very often members of one pair don't even share foraging areas. Therefore, aviaries should have sections or compartments for them to hide away from each other at will. As soon as the conspecific disappears out of sight, interactions between them and possible aggression is reduced.

4. The nest should be located as high as possible for safety and confidence (in wild colonies, higher nests are home to more relaxed individuals).

Figure 6 Nest located up high in a tree top

5. Rituals and sticks: Perhaps the most ritualistic vulture in Andalucía, especially in the breeding season

Figure 7 Some rituals can be observed in captivity, others – such as nuptial flights - are supressed.

Figure 8 Sticks is the "magic" word: In the wild, most if not all mating occurs after the male has brought in sticks to the nest which are ritually offered to the female (when not food dependent). Aviaries should have a good supply of sticks of different sizes dotted around the ground

6. Sensitive to disturbance: they are easily traumatized. Special care is needed for rehabilitated birds and also founders birds are more sensitive for disturbance.

7. Escort species or socialisation: Griffons and other escort species can be used at least at early stages, as it reduces stress and provides social interactions.

Figure 9 Vultures of all ages visit large carrions even if they do not eat at all.

Figure 10 Intra- and interspecific interactions, particularly by young birds, are important for their development and learning.

Figure 11 Large carrions encourage pair and social bonds. In captivity varied types of food at regular intervals could stimulate these behaviours.

2.1 Enclosure/Housing

The enclosure should be a high and large aviary (min 5 meters high).
We focus on three types of aviaries, as the species is kept both on exhibit and more recently also in (specialised) breeding centres off exhibit:

1. Single aviary on exhibit
2. Mixed aviaries on exhibit and/or socialization aviaries
3. Single aviaries in off-exhibit breeding centres

Cinereous vultures are very sensitive to disturbance and, therefore, larger housing dimensions are required when the species is kept on exhibit in order to reduce additional stress (see also dimensions in 2.1.5). For the same reason, we strongly advise against walk through aviaries.

Visitor position should always be below the birds
Birds perched higher seem to feel safer: this might be because the angle of vision from a high perch causes people on the ground to appear much smaller to the bird than they really are. These people seem to be further away, which implies that the birds feel safer and less stressed (Llopis and Frey, 2014). So, it is important to provide adequate "escape distance" through high perches and a high nest.


Figure 12 Cinereous vultures feel safe on high perches and on a high nest which is far away from the entrance door.

1. Single aviary on exhibit: The enclosure should be in a quiet area of the zoo, where not much unexpected works or traffic is passing. In the breeding season, it should be possible to close the area from the public, in case minimum disturbance cannot be assured. Through educational panels the reasons for closing off the area can be explained. Potentially with the aid of cameras and suitably positioned display screens (ensuring minimal disturbance to the birds) the public, children in particular, can be educated and engaged.

2. Mixed species aviaries: breeding pairs should not be kept in mixed aviaries, as long as the breeding success stays low in the breeding programme and intense monitoring is required during the breeding season. Mixed aviaries can hold older non reproductive birds, surplus birds or juvenile birds (socialisation aviaries). Mixing with other European vultures, with

exception of bearded vultures can be done, as cinereous vultures are not aggressive towards other species (A. Vaidl, pers. Comm.). Aviaries housing several species, or where breeding is not the principal aim, need to be spacious enough.

Figure 13 Mixed species aviary in Rhenen Zoo

3. Breeding centres are located off exhibit in a quiet area of the zoo with minimal disturbance. Breeding aviaries can be built next to each other with a minimum distance of 2m in between. A rectangular shape is recommended as this allows a long distance from the nest in the back to the keepers in the front. The birds will feel safer when they can perch far away from keepers/personnel in the front. Visual contact with other pairs will stimulate the pairs during breeding season (EEP breeding data show that zoos keeping more than one pair have higher breeding success) and the birds will feel comfortable they can watch each other and the environment. In nature, breeding pairs have neighbouring nests and visual contact seems to have a clear stimulating effect on breeding.

Figure 14 Off-show breeding aviaries in Planckendael Zoo

2.1.1 Boundary

Cinereous vultures, should always be kept fully winged in aviaries.

As cinereous vultures are naturally nervous, aviaries should only be closed on one side, the nest side in the back. The birds will be calmer when they have an open view and can see what happens around them: this also allows them to observe keepers and visitors.

Framework and mesh

The **framework** of the aviary should be galvanised or stainless steel. Avoid using paint because this can be toxic. Powder coated with a non-toxic black component has a higher visibility for the birds: recent studies showed that vultures see high contrast objects better e.g., applying black paint on one rotor blade of white wind turbines reduced significantly annual fatality rate (May R. et al., 2020).

Figure 15 Avoid a structure with inside poles as support: Such inside poles can cause deadly collisions.

Welded mesh, chain link wire (only for smaller aviaries) or stainless-steel mesh are the most suitable materials for aviaries. For large aviaries, where birds can speed up, it is recommended to use flexible steel, thickness: min. 2 mm. Chain link should not be used as it is a harder material and birds colliding into it can die.

Recommended mesh size:

- Max. 40x40 mm in zoos with birds on exhibit to exclude martens and ravens from entering the aviary. Smaller mesh size is recommended to avoid entering of small mammals, stealing food (see next paragraph).
- Aviaries off exhibit should have 25x25 mm to prevent smaller predators like rats and small carnivores from entering the aviary.

Figure 16 Mesh of 25x25 mm in an off-exhibit breeding aviary and flexible stainless steel with mesh ≤40x40mm in a socialisation aviary on exhibit

National laws regarding Avian Influenza can require additional measures, so ensure you have enquired and considered this.

NO USE of:

1. netting because cinereous vultures tend to chew on the net, which will lead to escapes and aviaries with roofs in net can collapse with heavy snowfall.
2. glass (panels) to avoid deadly collisions.

The mesh should be attached to the inside of the poles, preventing collisions with the structure, and getting stuck between poles and wire.
The wire should be dug a minimum of 80-100 cm into the ground (to exclude foxes digging their way into the aviary), or the whole bottom of the aviary should be covered with a concrete footing into the ground covered with grass.

One side of the aviary should be closed by a wall or panel to give the birds protection from that side. This is the side where the nest should be placed and is opposite the keepers' entrance door. Newly constructed aviaries should be placed north-south with the closed wall to the North.

Vultures are more secure if they are able to perch high above the keepers, but high enclosures should also be long enough for birds to manoeuvre. To avoid birds flying into the long side in large aviaries, these sides can be made more visible by attaching vertical lats (wood 4cm wide and 2cm thick), every 20-25 cm as visual obstacles, or by using camouflage nets.

Figure 17 The inside area of the aviary is kept open in the socialisation aviary in Rhenen zoo and the fence is made more visible by using camouflage net, fixed at the outside of the aviary.

Figure 18 In Parc des Marmottes, France, the fence is made more visible with wooden lats (Bearded Vulture Housing Guidelines, VCF).

Aviary access should be large enough to allow easy access for wheelbarrows and high enough for keepers. The entrance must be positioned on the far side of the nesting platform to minimise disturbance. The keeper should enter the aviary from the visitors' side, the door should open towards the animals.
A safety lock or cabin is strongly recommended to avoid escape.

Figure 19 First picture: the entrance door is at the same time the keepers' door and is located opposite the nest site. It is also large and high enough to allow entrance with small machinery. Second picture: safety cabin (picture A. Llopis, Valcallent, VCF Spain)

2.1.2 Substrate

Enclosures usually have soil or grass as substrate,

- no sand or gravel as these contain sharp particles.
- no use of weed control mats (woven) because they are made of plastic and the birds might nibble on it and swallow pieces of it.
- provide a good drainage to have a dry substrate in order to avoid muddy grounds. If the substrate isn't drained well, drainage should be improved by adding a layer of gravel underneath.
  Constant humidity and muddy floors can promote/cause aspergillosis and/or bumble foot. It is also a safety hazard for keepers: if they need to catch the birds or by removing clutches/chicks or for adoptions, there is the danger they can slip and injure the bird or break the clutch, etc…

Figure 20: soil or grass is a good substrate

- plants and vegetation should be kept out of the middle of the aviary and should require as minimum maintenance as possible: the birds need space to land and perch, collisions with vegetation should be avoided, open areas are required, and this also minimizes maintenance. Vegetation can be planted outside the aviary to provide shade.
- concrete without any covering or substrate is not suitable.

2.1.3. Furnishings and maintenance

Perches

Perches are essential to discourage birds from sitting on the ground. It is important they can perch on branches with different sizes in order to avoid foot problems. The following is recommended:

- Most of the perches should be at a higher level. The maximum height should be the same as the nest, never higher so that the nesting bird is not inferior to the non-breeding partner (dominance position). Recommended height is minimum 3m high.
  Perches should be assembled in such a way that the birds can "look down" to the public and to the keeper.
- Ladders, stumps, and trunks should be available for all birds to allow easy access to high perches. Special attention should be given to vultures that are unable to fly to give them access to the higher nest and higher perches. Tree trunks or a pile of stones on the floor might be comfortable for such birds too.

Figure 21 Ladders provide easy access to high perches or nesting places

- The middle area of smaller aviaries should contain no perches as they can hinder the birds in flying from one perch to another or can cause collisions. Avoid installing perches across the aviary. In large aviaries such perches can be installed as a resting point.
- A large and comfortable perch should be in close proximity to the nest (useful for parents during changeovers, and for the young during fledging); this perch should be lower than the nest' s edge.
- Perch diameters (minimum 10-12cm; average 12-14cm) must be variable to avoid bumble foot. Perches with and without bark are recommended. Perches should always be round in shape as cinereous vultures are tree breeders.

- Perches to be provided around the whole aviary so the birds can approach each other slowly without having to fly. There should be either no gap or a bigger gap (5cm) between 2 branches to avoid toes or claws getting stuck.
- Distance of the perch to the enclosure mesh should be a minimum of 60 cm to avoid tail feathers to be damaged. Distance between the support arms should be 3-3,5m to ensure enough stability of the perches.
- After each breeding season, in September, perches should be checked for stability and degree of intactness (i.e. absence of rot) and should be replaced in October, if required.

Figure 22 Perches are round shaped and have an average diameter of 12-14cm; they are mounted around the whole aviary.

Access to the nest and to high perches

For the birds through special perches or steps

Cinereous vultures are large soaring birds of prey and access to higher places, such as the high perches or the nest (just by flapping wings) is not easy in smaller environments such as aviaries. Spiral steps can make access a lot easier as the birds can jump from one step to another by opening the wings slightly. This also allows them to bring nest material and food up to the platform easily. Steps should be made of the same material as perches, i.e. round wood. Length is 2m in total, diameter is 12-14cm and height between the steps is 50cm (see drawing below). For handicapped birds the height between the steps should be smaller and adapted to the birds' condition.

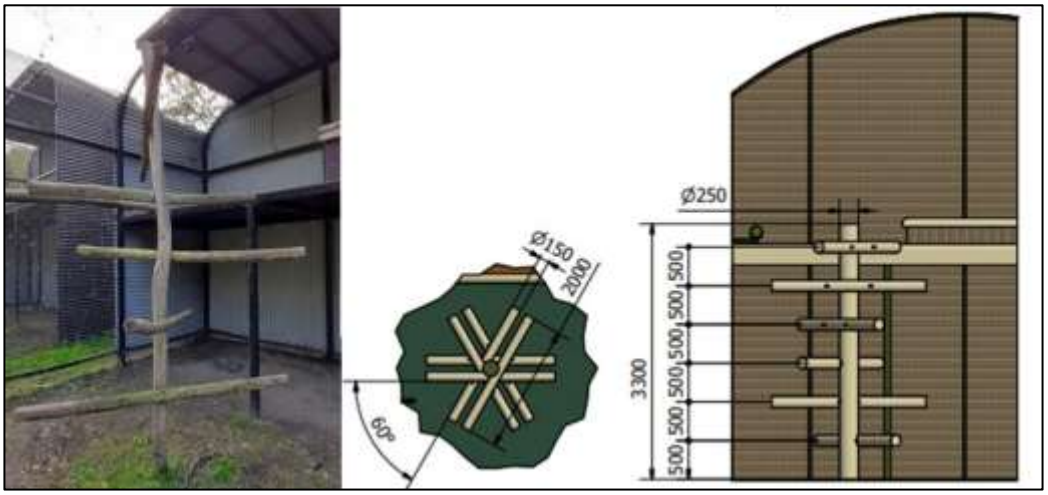


Figure 23 Spiral steps allow for easy access to the nest platform

Figure 24 The ladder is fixed permanently under the nesting platform. When building a new aviary, it is important to foresee a good place to fix a ladder. This picture from the Bearded vulture husbandry guidelines shows a ladder fixed permanently in the aviary to the side, without having to move it.

For the keepers

Access to the nest for monitoring reasons should be done by a ladder or ladder structure that is fixed permanently in the aviary, e.g., to one of the sides, outside the flyway of the birds and with no risk for collision or accident. Experience has taught that the breeding pairs are scared and feel very threatened when keepers enter the aviary from the outside with a ladder. In the worst case this could lead to injuries of the frightened birds flying into the mesh or to damage to the egg and/or loss of the chick. Fixing the ladder to one of the sides, in permanent view will lead to less stress when using it.

Shelter

Individual shelter: The birds should have the choice whether to sit out in the rain, or perch under cover. Enough shelter should be available for each individual bird; this should be the case in particular in mixed aviaries. A minimum of two shelters should be provided, big enough to allow all birds to sit dry. For one bird, the recommended dimension is: minimum 1m to all sides.

Figure 25 Boumort release aviary, Spain (Designed by Ing. Oriol Armet).

Nest: A shelter should cover the nest to the back, min 1.5m from all sides.
The shelter should be a sandwich panel or wood and should well insulated to avoid overheating.

Figure 26 Sheltered high nest with easy access through a big perch (Arnhem zoo). The shelter is installed on the outside of the aviary, which can be a good solution when it is difficult to install inside. A camera installed above the nest allows remote nest monitoring

Pool

Cinereous vultures love to take a bath in fresh water. Therefore, every cinereous vulture aviary must be equipped with a pool for bathing and cooling down: diameter 2m, not deeper than 35cm with a soft access ramp to allow easy access and exit. The birds will also regularly use it for drinking.

Figure 27 Pool (Pictures from Bearded Vulture Housing Guidelines, VCF).

Pools should be located in the first half of the aviary, close to the keepers' entrance to minimize disturbance by entering the aviary too close to the nest. Perches above the pool should be avoided to keep the water clean from dropping faeces.

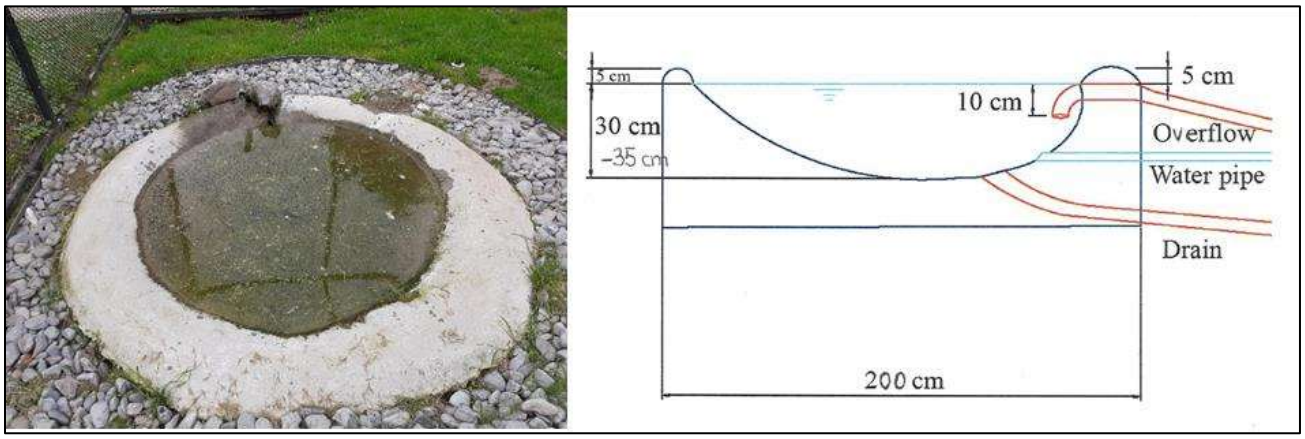


Figure 28 Drawing taken from Bearded Vulture Housing Guidelines, VCF and adapted for cinereous vultures.

Management:

The pool must be emptied and filled with a mechanism regulated from outside the aviary, and without a keeper required to enter the aviary. Preferably, fill it with permanently running water to keep it clean from algae and to avoid that it gets frozen in winter and prevent oily substances floating on the surface. A good option can be to pump and filter water.

Nest

It is recommended that the nest should resemble a "tree-type nest" on a wooden platform or on a platform in a tree.

Figure 29 Tree-type nests: first picture shows a tree nest in Riga zoo (picture, A. di Marzio), second picture shows a nest on a high platform in Planckendael zoo

- The nest should be 1.5m wide and 1.5m deep. It can be on a platform or in a tree or on a bigger nest platform across the width of the aviary. The latter is easily accessible for close monitoring. In the second picture, the nest is made higher to achieve a feeling of security for the breeding bird. The height of the edge is 46-50 cm and finished off with a rounded perch of 10cm.

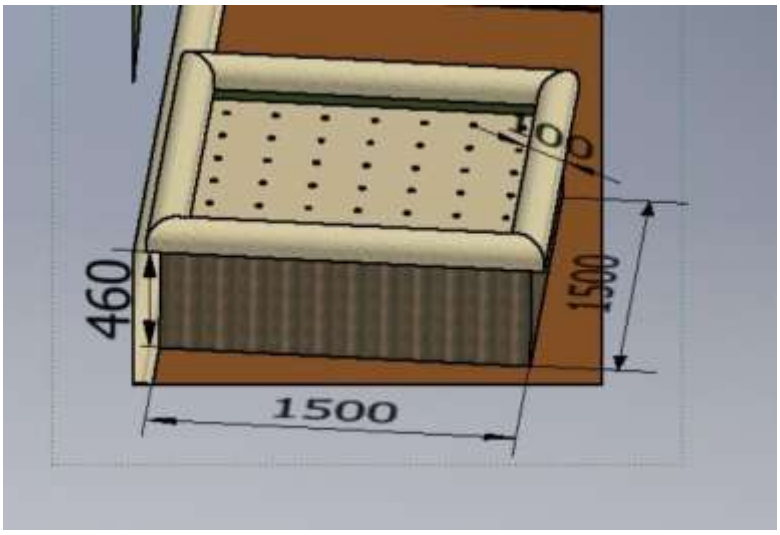


Figure 30: detail of a nest (measurements are in mm)

- A nesting platform should be as wide as the aviary (min. 7m), 2m deep, and should be a minimum 2m from the roof. Cinereous vultures are <u>not</u> cave breeders and need a higher roof to have a better view and feel more secure. It also allows the keeper to stand upright.


Figure 31 The sides of the platform are partially open to allow ventilation and cooling in summer.

- In mixed aviaries: the platform in a tree should be minimum 1.5m x 1.5m, covered, and open on the sides.

  - be placed as far away from the keeper's door and the visitors, with a <u>minimum distance of 14m,</u> but it is highly recommended to provide <u>more</u> distance in aviaries on exhibit. The inside of the nest and the incubating birds should not be visible to the public.
  - be covered by a roof, minimum 1.5m from all sides to provide shelter from the rain and cold, to protect the egg and nestling. The U shape of the platform with protecting wall in the back (see design page 35) protect the birds from wind, rain and heavy sun. The roof should be a

fixed construction. Avoid metal as this can become very hot, the use of a sandwich panel which is isolated is a good option.

- be placed min. 3m high, recommended are 3.5m high; there should be at least 1.5- 2m below the roof to give the adults room for copulation and the nestling room to exercise. Nest height is a relevant factor for successful breeding, with an average nest height of 2.5 m (Tewes et al., 1996).
- Access to the nest: see 2.1.3. "Furnishings and maintenance: access to the nest and high perches".

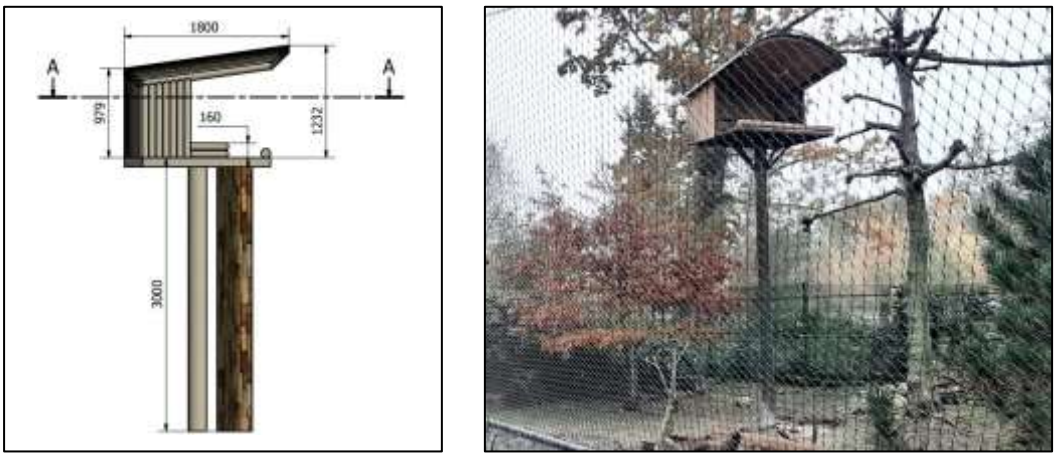


Figure 32 Shelter and nest in dating aviary of Planckendael zoo.

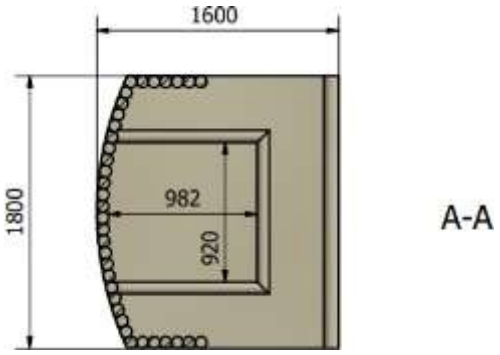


Figure 33 Top view of a shelter/nest platform (courtesy of ir I.I.Fehervari).

Feeding place

Feeding places should be installed near the keeper's door and as far as possible from the nesting platform. This can be made in concrete or tiles. Food can be given through a feeding door of 80cm width and 40cm height, without entering the aviary and disturbing the breeding pairs. It is recommended to give food always at the same time, for example in the morning so that the birds get used to it and feel comfortable.

Figure 34 Examples of feeding doors in Planckendael Zoo

Camera system

Cameras in the aviary should be provided to continuously and closely monitor and observe the birds, without disturbing them. In breeding centres each aviary should have a minimum of two cameras to monitor e.g. pair bond, egg laying, second egg laying, hatching, adoption and fostering, feeding of the chick and fledging. At least one camera should have view of the nest and a second one a full view of the whole aviary (e.g. a dome camera). Preferably a third camera should be positioned in another angle on the nest to assure always visual contact to the egg/chick.

2.1.4. Environment

Wherever possible, aviaries should face away from cold prevailing winds and sheltered areas should be provided in these enclosures. Orientation should be preferably north/south, facing the sun, while the nest and platform should be sheltered from rain, sun, or snow. There is no indoor enclosure required.

No visual barriers between adjacent aviaries need to be provided, but everything should be done to give the birds, in particular breeding pairs, maximum tranquillity.

Aviaries should be situated in areas with minimum disturbance, which is particularly important for breeding pairs. If located in the visitor area, breeding pairs should have a boundary fence to discourage visitors from getting too close to the aviary, which should be at minimum 16 meters from the nest. If necessary, the aviary should be closed for the public during the breeding season. (see also 2.1)

Maintenance work in the vicinity of the aviary must be avoided during the whole breeding season (December - August).

2.1.5 Dimensions

Although square enclosures give room for flying as opposed to long narrow ones, depth is very important for cinereous vultures due to their nervous nature. High enclosures should have sufficient length for birds to be able to get onto high perches and fly to the ground safely.

For these reasons, **rectangular aviaries** are recommended:

- for single aviaries housing one breeding pair on exhibit, the absolute minimum dimensions are: 16m deep x 7m wide x 5m high.

- for breeding aviaries off exhibit the minimum dimensions should be: 14m deep x 7m wide x 5m high.
- mixed aviaries should be a lot larger, and fences should be provided with visual barriers and high perches which serve as obstacles to reduce flying speed and prevent collisions. Some examples of mixed aviary dimensions, used as socialisation aviaries:
  - Planckendael Zoo: 800m², height = 12-24m for 5.5 cinereous vultures
  - Rhenen Zoo: 1500m², height = 7.25- 12.25m for 6.2 cinereous vultures

In general, one can be reasonably sure that a cage is appropriate when the birds do not show panic reactions when a keeper enters the cage, like regurgitating food, flying back and forth, or jumping up and down, flapping the wings, although nervous behaviours are quite common for captive vultures.

Summary: Housing (it is important to read the detailed explanation in the text to fully understand the table)

*Table 2 Summary table. *maximum 40x40cm, recommended to be smaller 25x25cm (see also pg. 22).*

	Topic	Sub-area	Aviary type			Additional recommendation
Housing	dimensions & shape		**Single aviary**	**Mixed (socialisation) aviary**	**Breeding aviary off exhibit**	
		min. dimensions in m (LxWxH)	16x7x5	Larger	14x7x5	
		shape	rectangular		rectangular	in very big aviaries the nest could be placed in the middle, but effective coverage will be a problem
		1 side closed	x	x	x	only closed around the platform in very large aviaries
	mesh	mesh type (mm)	≤40x40*	≤40x40*	25x25	
		thickness (mm)	2	2	2	
	framework	galvanised or stainless steel	x	x	x	
		mesh/stainless steel, NO NETTING	x	x	x	
		80-100cm into ground	x	x	x	
		no inside poles	x	recommended	x	
	perches	Min. 3m high	x	x	x	
		12-14 cm diameter	x	x	x	
		round shaped	x	x	x	
		around the aviary	x	x	x	
		spiral steps (nest access)	x	x	x	
	substrate	grass, ground	x	x	x	
		well drained	x	x	x	

		vegetation at the sides	x	x	x
	shelter	min. 1per bird	x	min. 2 (bigger)	x
		1m all sides	x	x	x
	pool	2m diameter, 35cm deep	x	x	x
		easy access; fill from outside	x	x	x
	nest	min. 3 m high	x	x	x
		size: 1,5m all sides	x	x	x
		nest on platform	x	x	x
		covered (1-1,5m outside the nest)	x	x	x
	feeding panel/shutter	1 at entrance door	x	recommended	x
	ladder	fixed inside	x	recommended	x
	camera system	min. 2(3) cameras/aviary: nest and full aviary	x	x	x

** maximum 40x40cm, recommended to be smaller 25x25cm (see also p. 24)*

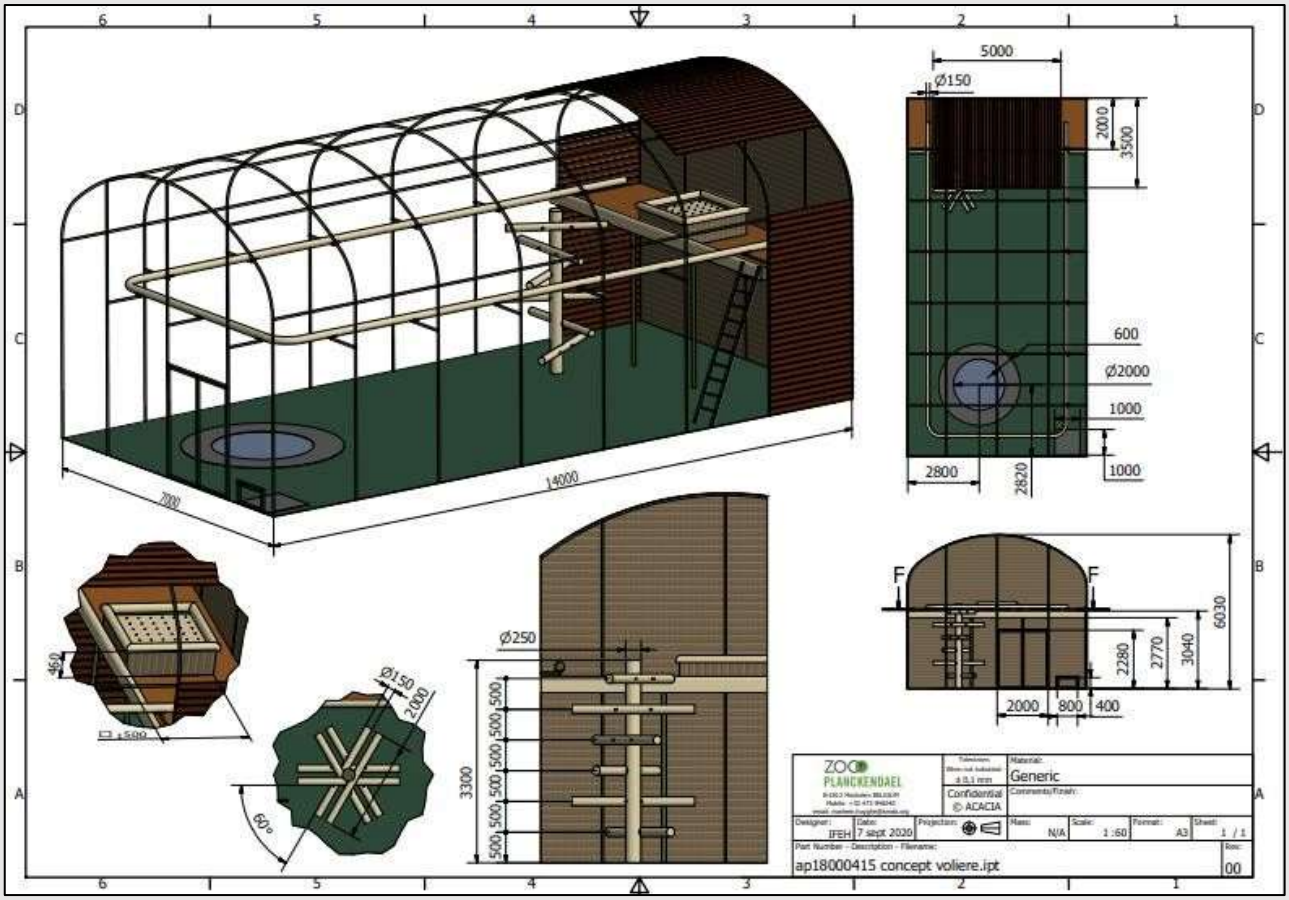


Figure 35 Concept plan of a breeding aviary in breeding centre Planckendael (© ir. I. Fehervari)

2.2 Feeding

chapter reviewed by Eric Bureau, veterinary advisor for the cinereous vulture EEP programme

2.2.1 Basic diets and supplements

General

A diverse diet is recommended, which can be more stimulating to raptors and reduces the chance of mineral and or trace element deficiencies. No poultry (and chicks) should be given due to avian specific infectious diseases (e.g., adenovirus, rotavirus, *Escherichia coli, Mycobacterium avium,* falcon herpes virus, influenza viruses and *Salmonella*). For the same reason (Avian influenza) ducklings must not be given. Furthermore, an intensive one-day old chicken diet might be a risk factor for arteriosclerosis in birds of prey (Legler et al., 2017) (for more details see also 2.7 Veterinary). Animals killed with bullets or lead shots should not be fed because of the danger of lead poisoning (saturnism).

As explained by Forbes (2002), the lifestyle in captivity is very different from the wild. Since captive vultures are less active it is important to adapt the amount of food to the energy required. Excessive feeding can lead to health problems, and therefore fasting days can help manage the risk of overfeeding and of weight overload. 80% of the zoos undertake at least one fasting day per week. Four to 6 meals per week, depending on the fed quantities, seems to be a good basis.

Forbes (2002) suggests that any food given to captive birds of prey should closely resemble food in the wild. Diets different from the food consumed in the wild could potentially have a negative effect on breeding performance and success or could result in nutrient or trace element deficiencies. Especially during the breeding season, a change in diet (or a change in the diet of food species) can have consequences for the essential fatty acid content of the egg yolk, which can result in a negative effect on breeding.

Breeding female raptors have a significantly higher protein and mineral content but lower fat content than non-breeding females. Forbes (2002) proposes that foods rich in proteins and minerals (especially calcium) are important before egg laying, and foods to cover energy requirements during breeding is essential for successful breeding in raptors in general. If boneless meat pieces are given, e.g. beef or horse, and comprise more than 10% of the diet, calcium deficiencies can arise unless supplements (calcium and vitamin D3) are added.
The correct ratio Ca/P (Calcium/Phosphorus) required for a good absorption of Ca is from 1.5 to 2. Red meat contains a lot of P and few Ca (Ca/P = 0.05,) so it's necessary to add nutritional supplements to the diet of vultures when fed with red meat, also if it is with bone. Because even if the bird eats a little bit of periosteum, the quantities are probably insufficient. Beyond vitamin B the meat is poor in other vitamins so a diet with lots of meat must be supplemented by nutritional supplements, even if the meat is fresh.
Some species (e.g. falcons) that are fed an increasing protein level before breeding appear to gain higher fertility. Increased levels of vitamin E before the breeding season might be beneficial to male fertility and to the embryo's viability.

It is very important that the food is always as fresh as possible, even from frozen stock. Frozen food should not be frozen more than 3 months and defrosting should be done under

refrigeration. Cinereous vultures might not eat straight away after the food has been given, and especially very shy and nervous pairs might not eat when keepers or visitors are watching them. Therefore, it can take hours for them to start feeding. If vultures have been given a large feed, it may take longer than 24 hours to put over their crop. Forbes (2002) recommends to feed vultures a couple of days per week (once a day, if possible, in the morning). However, when the birds are rearing young, food cannot be withheld and should be offered daily.

Diet in EEP zoos

The questionnaire studies (Gomis, 2009; Bureau, 2019) show there is a high variety between zoos regarding the food they provide, the feeding regime and the supplements added to the food. In general, the diet of cinereous vultures across zoos includes:

a) carcasses or meat pieces of horse, cattle, calf, goat, sheep and occasionally pig, wild boar, moose, deer and deceased zoo antelope and
b) whole rabbits, rats, mice, guinea pigs, one-day old chicks, chickens, pigeons and quails.

The variety in food items is highly variable between zoos. Some feed up to eight different kinds of food, whereas few others feed the same diet every day, 50% of the zoos include at least five different components in their diets and only 14% of the zoos include only two or less components in their diets.

Some zoos feed animals including the intestines, others without; some feed whole carcasses, others only give pieces of meat on or without the bone. On average a diet is composed of 60% meat with or without bone, 30% of whole prey and 10% of gutted prey. Red meat contains little vitamins (except for vitamins B). A lot of other nutrients are found in viscera, so a diet with little gutted prey must be complemented with nutritional supplements. Some zoos change the diet during the breeding season, feeding more and especially more diverse foods and adding nutritional supplements. Others keep the same diet all year. Mineral and/or vitamin supplements like Carmix©, Carnizoo©, Oceferol© and Forvit© DK meat supplement© being used by most zoos (Gomis, 2009; Bureau, 2019).

The main question of concern here is which diet results in an optimal condition of the vultures and provides them with enough energy and all the nutrients needed during the breeding season. Whether and how diet affects egg quality, and as a consequence, breeding success, remains to be studied. But for now, Gomis' study provides some general suggestions based on the five zoos obtaining the best long-term breeding results.

Even if the alimentation is only a factor influencing reproduction, the entirety of zoos with good long term breeding results are:

- Offering a high diversity of preferably fresh whole preys;
- Providing smaller amount of food regularly (e.g., at least 4 times a week) rather than large amounts once a week;
- A higher food availability and variety during the breeding season (especially whole preys & carcasses); and feeding every day during the rearing period;
- Supplementation of vitamins A, D3, E, B1, and minerals Ca, Mn, Zn, Cu all year round;
- And for some Extra vitamin E supplementation during the breeding season.

These results were confirmed by a questionnaire on food and supplements sent around to member zoos (Bureau, 2019) and the unpublished results of a questionnaire in 2022. These two questionnaires produced the following additional results:

- 57% of the zoos change the diet according to the season and more whole preys were given and with increased frequency.
- Still some zoos do not feed each day during rearing season.
- Only few zoos adapt the diet BEFORE the breeding season starts by giving more diverse food and adding supplements.
- Diversity of food has not really changed: 32% of the zoos offer 3 or less than 3 different kinds of food, while diversity of diet is important to avoid deficiency and malnutrition.
- The quantity of distributed food on average and by day is variable, from 300g to 1.5kg. It of course depends on the quantity of bones in the diet and of the period in the year, but it seems important to measure the quantity of food to prevent overfeeding. A daily diet between 5% and 10% of the birds weight (350 to 1000g) depending on the type of food is recommended.
- 33% of zoos give only frozen food, and half of them also give fresh food; only 24% provide only fresh food. Common deficiencies like shortage of vit E, Vit B1, vit B12, is associated with frozen food. We recommend that frozen storage should not exceed 3 months and if frozen food is used, then the diet must be completed with nutritional supplements

2.2.2 Recommended diet and special dietary requirements

As discussed above, recommending a general diet is difficult. The following paragraphs outline a diet plan that takes into account several theories of raptor diets in general as well as the experience of zoo staff and vets working with cinereous vultures. Any dietary changes should always be discussed with the local veterinarian or the veterinary advisor.

1. General Diet (June-October)

A general diet should include whole prey like rabbits or rats as well as carcass/meat on the bone from cattle, goat, deceased zoo animals like antelope, horse and sheep. When feeding whole prey, no additional supplements are needed because this food contains a good balance of calcium, vitamins, and protein (if the prey was not frozen!). Whenever carcass or meat is fed, a supplement for carnivorous birds (e.g., Carmix, Carnizoo, DK meat etc.),needs to be added to increase the calcium and vitamin level (advice of the producer should be followed). Two fasting days per week are recommended.

Table 3 A diet plan for 1 bird could be

Monday, Tuesday:	Carcass cattle (300-400 gr/bird (without bones) + supplements)
Wednesday	Fasting
Thursday	Rats (=± 250 gr): two big rats per bird
Friday	Carcass goat (300-400 gr/bird (without bones) + supplements)
Saturday	One small rabbit (± 600gr per bird)
Sunday	Fasting

2. Diet before the breeding season (November-January)

It is recommended to increase the amount of proteins and minerals (especially calcium) before the breeding season because the females store these nutrients for reproductive expenditure *before* they are breeding. An increase in protein level might lead to higher fertility (Forbes, 2002); therefore, it is suggested to raise Vitamin E levels one month before the assumed egg-laying, e.g. at Parc des Oiseaux, Villars les Dombes, France, a 5-day course of treatment 200mg/kg per day orally (alpha tocopherol acetate) is done in February.

3. Diet during the breeding season (February-Aug)

Energy-rich food should be given during the breeding season because birds in breeding condition will need more energy. Again, this might not be as important as in the wild but needs to be considered and adapted accordingly in captivity. If a cinereous vulture pair lost their first egg or it was infertile, the calcium and protein levels can be increased to support the female in laying a second egg.

Daily feeding is required when chicks are being reared, and it is recommended to increase the amount of food as a function of the chick's growth. Tewes (1996) advised to offer a great variety of food during the nesting period because it is more nutritious for the nestling, and it stimulates the parents to feed their young. To make sure that nestlings receive an adequate amount of calcium, one should provide whole small preys like rabbit and rat regularly. We do not recommend feeding large pieces of meat with calcium powder added, but only whole preys to make sure the nestling bones grow well.
To monitor the food intake of a bird, we recommend weighing it every time it is handled and at least once a year. For weighing see veterinary chapter 2.7.

2.2.3 Method of feeding

Several problems may arise when a captive diet comprises carcasses and whole prey. Unlike lions and other large cats which eat up everything including the bones, cinereous vultures may not eat straight away and leave quite some waste. If the food is lying around in the aviaries for too long, it may start to rot and attract rats, weasels, stoats as well as flies and other arthropods and decomposing organisms. Depending on the weather, this is sooner or later combined with a nasty smell. Therefore, it is important to determine the correct feeding frequency. The food given should be eaten by the vultures within 1-2 days. Remains should be removed promptly for sanitation.
In hot weather, food should be distributed in the morning and portions should be designed to result in total consumption in less than 5 hours.

During the breeding season entering the cage to clean up the waste should be kept to a minimum in order to keep disturbances low. New enclosures should be designed to allow feeding without entering the aviary and feeding should be done at a regular time daily to maintain routine and minimize disturbance.

2.2.4 Water

Cinereous vultures should always have access to clean water for drinking. Baths should be available year-round and refreshed on a regular basis in warm weather.

Summary: Feeding

1. **The diet should be diverse, preferably fresh whole prey and contain at least 3 different food types per week→ see proposed diet 2.2.2.**
2. **Supplements of vitamins A, D3, E, B1… and minerals Ca, Mn, Zn, Cu… should be added all year round when providing carcasses. Before the breeding season vitamin E supplements should be added.**
3. **During the rearing period, the breeding pair should be fed every day with whole prey like rabbit, rats, guinea pigs,….**
4. **Frozen food should not be stored for more than 3 months and if used, the diet must be completed with nutritional supplements (vitamins).**

2.3. Social Structure

2.3.1 Basic social structure

Monogamous and pair bonded couples

Adult cinereous vultures are monogamous birds and should preferably be kept in pairs. A cinereous vulture pair can be kept with other cinereous vulture pairs in a very large aviary (Pithart, 2001). They will, however, keep some distance and develop a dominance hierarchy which is exercised when the birds want a resting place or during feeding. Aggression towards birds in other pairs is usually not a problem, although it has been reported that pairs will defend their nesting area. Aggression between individuals can take place during communal feeding and dominance fights. Moreover, individuals that are not paired up and juveniles can intentionally attack copulating pairs, causing the copulation to end prematurely. Yet it seems that the eggs of the breeding pairs are usually fertilised despite such interruptions. Nevertheless, since most cinereous vulture pairs are not kept in large aviaries, and disturbance of breeding pairs in general seems to have a negative effect on breeding success (Tewes, 1996a), we recommend keeping different cinereous vulture pairs in separated cages.

Analysis of EAZAs EEP breeding data demonstrates that breeding success is better in zoos keeping more than one pair. This is also observed in breeding centres where several pairs are housed next to each other. It is encouraged that zoos should try to keep more than one breeding pair, especially as keeping more pairs increases the knowledge on the species.

Females in a breeding pair can occasionally show aggression towards their partner on the nest, chasing him away and pecking him immediately when approaching the nest.

Observations show that the female's behaviour can be very unpredictable; sometimes pairs can sit peacefully in the nest together or build on the nest for hours before suddenly chasing the male away. Other days they can be very harmonious and affectionate towards each other with no aggression. Sometimes this was observed following a failed copulation attempt of the male (Gottschlich, 2008).

Observing the behaviour of cinereous vultures in captivity can provide important information about the degree of pair bonding of a pair and provide clues about whether a pair will be successful as a breeding couple. Many details are still unknown, but we recommend engaging in daily observations of pairs at the start of the breeding season to get an impression of their social behaviours. In section 2.4.1, an overview of commonly observed behaviours (pair formation ethogram) can be found that can be used as a reference. Ongoing studies will hopefully result in more detailed information about the relation between social behaviours and breeding success in the near future. If you do not observe these or similar behaviours it is strongly advised to contact and discuss this with the studbook keeper.

Socialisation aviaries

Within natural populations social interactions are common and believed to be important (see also section 2 – 7 rules, p. 16). It has been questioned how limitations in these kinds of interactions affect social learning and eventually impact breeding success (workshop Antwerp, 2019).
Young birds being removed once they are fully fledged (minimum 4 months old) and subadult cinereous vultures should be housed in large "socialisation" aviaries where they can establish a natural pair bond. The process of pair bonding can take several years and is still not yet well known and needs further research in the future.

The potential role of these larger aviaries holding a group of birds should be investigated in future. Young individuals might learn specific skills from older individuals and as such these socialisation aviaries could hold different "type of birds" together (experienced + unexperienced – juveniles). Juveniles might benefit from observing experienced couples during the breeding season, either within or in close proximity of the socialization aviary. This role could be played by old or overrepresented experienced pairs within these socialization aviaries as they can be kept in in proximity of an aviary housing several juveniles. As such they can be used for educational purposes of both the juveniles and the public.

Figure 36 Dating/socialisation aviary (picture: Jonas Verhulst/Zoo Planckendael)

Methodologies used to evaluate/ discriminate good from bad pairs (cfr pairing/ re-pairing),
The breeding performance of a couple is evaluated in a longer time span, and this for the last 5 years. However, in the recent workshop for cinereous vulture, held in Antwerp in 2019, it is agreed to recommend:

1. that pairs that are not successful in a two-year time window should be re-paired. This should be assessed in a case-wise manner as other factors may play a role: the aviary, its direct surroundings, veterinary aspects, and local husbandry practices should be evaluated, to check whether husbandry forms part of the problem.
2. Re-pairing should ideally be done preferably in either a dating aviary or in a more social environment in which different aviaries are in close contact, enabling more efficient switching of birds meanwhile creating a more natural and standardized way to screen interactions and evaluate mate choice. This could e.g., be implemented within specialized breeding centres or in socialisation aviaries.
3. However, at present this is not yet possible to achieve as only very few socialisation aviaries and some breeding centres are existing. Therefore, in a first phase, aspects listed within the "married at first sight protocol" are implemented and tested and evaluated yearly. On basis of several analyses a checklist has been created to either form new pairs or to re-pair couples that prove to be unsuccessful. Once re-paired and proven successful these new pairs can go back to an individual zoo.

Tool: "Married at first sight" → Factors to take into account when re- pairing birds:

1. Minimize age between partners: preferably < 5 years.
2. Previous breeding experience of the partners (=estimated via number of previous breeding attempts) plays a significant role in breeding success.
   In particular statistics on breeding success show that the more experienced the male, the more chances for success for breeding. Therefore, rotation of good, experienced parent reared captive males should be considered whenever possible.
3. Birds involved in a transfer should be genetically re-sexed if not PCR sexed in the last five years.
4. Age class of the female should be taken into account: reproduction tends to decrease when females reach an age of 32 years. Experienced non reproductive older birds can be paired up and used as foster parents.
5. Relatedness between individuals (kinships).
6. Founders (yes or no)?
7. Two hand reared birds should preferably not be paired up: one should be parent reared and preferably have experience in breeding successfully before *.
8. Previous breeding experience: the birds should have never been paired up before.
9. Origin: if known, birds with similar origin (Iberian >< Eastern), should be paired up.
10. Timing to pair up two birds: introduction in October – at the latest just before the breeding season starts.

** At the Antwerp workshop 2019, it was suggested that pairings between two hand-reared birds should not be restricted as based on experiences in other species.*

2.3.2 Changing group structure

New mates should be introduced well outside the breeding season (see Appendix 5) and monitored for several months to establish whether the birds can become a potentially successful breeding pair. The ethogram provided (p.47) can be used to evaluate a pair's behaviour.
Introducing a bird with an already existing pair has to be observed closely as aggression might occur towards the new 'intruder". This has been reported by introducing a female in an existing male-male pair.
In the large aviaries used for pairing up (subadult) cinereous vultures, aggressive interactions may occur when feeding and once a pair has formed. It is recommended to transfer the newly formed pair to a separate aviary to evaluate whether the observed pair bond establishes further.

New imports (e.g., confiscations or rehabilitated birds) should follow the same procedure as young or unsuccessful EEP birds. Depending on their age they will be introduced in a socialization aviary or a more dating focussed setting.

2.3.3 Sharing enclosure with other species – mixed species exhibits

EAZA EEP data show no differences in the ability of egg laying or raising young successfully between cinereous vulture pairs housed in mixed species aviaries versus birds housed as solitary pairs. In 2019 a survey indicated that 64% of the aviaries hold single pairs and 36% are mixed aviaries.

Other species, however, do seem to cause disturbance, which can negatively influence the breeding success. There are several examples of mixed-species aviaries where breeding efforts of cinereous vultures were interrupted by other species, or where cinereous vultures show intolerance towards birds in their aviary or in neighbouring aviaries.
Therefore, to optimise the breeding success of cinereous vultures it is recommended to house no other species in the aviary at all. This also allows to monitor the pair closely.

In a non-breeding situation, however, several cinereous vultures, with or without other bird species may reside together without problems, and even be the dominant species. The list below is given for species with which cinereous vultures are/were mixed without big problems.

Cinereous vulture mixed with:

- *Gyps fulvus and Neophron percnopterus, Falco naumanni, Geronticus eremita, Grus virgo, Oxyura leucopcephala, Spatula clypeata, Marmaronetta angustirostris, Aythya baeri, Urocissa erythroryncha, Coracias garrulus, Catreus wallichii, Burhinus capensis, Recurvirostra avosetta, Tringa totanus, Vanellus vanellus, Haematopus, Columba livia, Streptopelia turtur*
- *Gyps fulvus, Geronticus eremita*
- *(1.1) Terathopius ecaudatus*
- *(1.1) Milvus milvus*
- *Gyps fulvus, 0,1 Milvus milvus*
- *(1,1) Pernis apivorus and (1,1) Strix uralensis*
- *Ovis aries*
- *(4.4) Gyps fulvus and (1.1) Sagittarius serpentarius*

- *Gyps fulvus, Gyps rueppellii*
- *(1.1) Neophron percnopterus*
- *(0,1) Gyps fulvus, (1,1) Asio flammeus, (4,4) Pyrrhocorax pyrrhocorax(1,1) Neophron percnopterus*
- *Milvus milvus, Sarcoramphus papa*

Summary: Social Structure

1. **Breeding cinereous vultures should be housed in pairs and in separate aviaries. Close observation and monitoring the couple's pair bond behaviour is essential.**
2. **Chicks and subadult birds should be housed in socialisation / dating aviaries.**
3. **Evaluation of the breeding behaviour is communicated to the studbook keeper yearly, which will allow the latter to prepare recommendations to re-pair unsuccessful pairs.**

2.4. Breeding

Authors: Pablo Izquierdo (GREFA, Spain) & Marleen Huyghe (Zoo Planckendael), reviewed by Frédéric Verstappen & Olivier Vercauteren (Zoo Planckendael)

2.4.1 Mating

Courtship and pair bonding

At the beginning of the breeding season the vultures start to approach potential partners. Often the first contact is induced by flying next to another bird onto the same branch or perch, keeping a distance of 2-3m. Then the greeting behaviour follows, where one bird starts to turn the head to the side, sometimes with raised feathers. If the other bird responds by greeting back, they walk closer towards each other, and the greeting behaviour continues. In the pair bond ethogram below the different behaviours, which we should observe in a good pairing, are described in detail

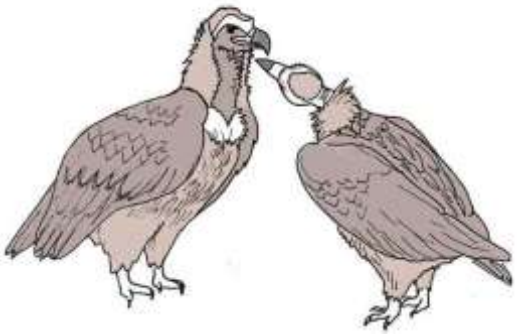
In large aviaries which can keep several birds, social behaviours can be readily observed, and usually dominance relationships are established between the birds. Such "dating or socialisation aviaries" can be used to pair up cinereous vultures that had problems pairing up with a partner in another zoo participating in the EEP programme or to pair up young vultures as they mature.

Pair formation ethogram (June 2020) → checking on "pair bond" behaviours
Drawings by Maartje Leenders

APPROACHING

APP +	**Approaching**	Walking or flying next to the partner, mostly followed by greeting and ruffling. Often resulting in standing in very close proximity or flying to the nest together

GREETING



GR +	**Greeting**	Rotating the head with raised feathers, sometimes touching the head of the other vulture with the beak, the other vulture doing the same movement.
AGR +	**Attempted greetings**	Same as greeting but the other vulture is not doing the same movement

Figure 37 Greeting behaviour

ALLOPREENING

AP +	**Allopreening**	Picking of neck and head feathers of another vulture (note which individual)
AAP +	**Attempted allopreening**	Allopreening while the other vulture rejects or is aggressive

Figure 38 Allopreening behaviour

BUSY WITH BRANCHES

PB	**Picking up branches**	With beak
NB	**Nest Building**	Arranging branches or fine material in and around the nest
NM	**Busy with nest material**	Walking on ground or platform and exchanging or/and collecting branches or twigs. Sometimes partners help each other and carry large branches

Figure 39 Busy with branches

COPULATION

CO +	**Successful copulation**	The male jumps on the back of the female. She spreads her wings slightly and lifts her tail and the male pushes down his tail next to the female's tail and they mate. They screech. **IMPORTANT**: observe closely that the position of the birds is correct, the tail of the female is pushed aside and upwards and the tail of the male downwards so that cloacas are touching.

ACO +	**Attempt to copulate**	The male tries to copulate, but female responds aggressively

Figure 40 Copulation. Pictures: Jens Schuurmans, Dani Buron, Katja Wolfram & Zoo Planckendael

Nest building "STICKS" is the magic word!

Some pairs start earlier than others the breeding season: in Spain some pairs start already in October to build the nest on a platform or a prepared nest area, while in other institutes, they start later, in December - January.
In early pairs, sticks should be offered from October on, in other pairs from December on. Offer small portions –2 to 3 times a week- in order to trigger the birds to start nestbuilding which is important for pair bonding and synchronization of the pair. Nest building is mostly linked with weather conditions: offer nesting material on sunny days or days without rain.

Figure 41 Different types (thickness) of branches can be used (30 cm in length); to give in small portions (pictures-Zoo Planckendael)

Longer branches of pine tree can be given, too. If good nest builders, the birds themselves will break these into smaller pieces, using claws to grab these and pulling with the beak

Figure 42 Longer branches (Picture: P. Izquierdo, GREFA, Spain)

How long branches should be provided depends on the behaviour of the pair and their nest building capacities. Good nest builders with a shy and nervous conduct, can be disturbed by giving nesting material and this could delay egg laying. Here it is recommended to provide enough branches and stop providing branches a month before egg laying date. With pairs which are no good nest builders you can continue to provide small twigs and soft material till one week before egg laying (see also below). And the week before egg laying only soft material should be given.

Nest quality: it is very important for breeding success!

Partners in breeding pairs can differ considerably in the amount of effort they put into nest building behaviour and/or collecting nesting material.

A well-built nest is very important to avoid breakage of the egg and to ensure your pair will be successful. It is essential to check the quality of the nest: if you observe not enough nest building when providing enough branches, you can help the birds by offering a fully built nest.

Figure 43 Typical nest in the breeding pairs in Planckendael zoo. (Pictures: Olivier Vercauteren, Planckendael zoo, Belgium)

Figure 44 Nestbuilding in pair in GREFA breeding centre (P.Izquierdo, Grefa, Spain)

If no or not enough nestbuilding is observed (if you have a camera on the nest this can be followed) it is a good stimulation to offer a fully/ready built nest:

Offering a well-built nest will trigger the birds for picking up branches and do nest building. Nest building will help pair bonding and synchronization of a pair.

How to do?

1. Start from a firm and stable wooden frame in which the nest will be built; this will avoid that the egg can roll out of the nest during incubation.
2. To have a solid base you make a basic layer of chopped pine bark or sand; we use grass sods (dug out) as base, which we turn upside down.
3. Then start with thicker branches for the edges of the nest and add smaller twigs for the inner side.
4. The central inner part is covered with moss and grass. This to avoid breakage of the egg

The last weeks before egg laying, only soft material for the centre of the nest should be given, like *Miscanthus*, moss or grass (but no wool)

Remark: It is possible that the pair or one partner destroys your nest. Nevertheless, an artificial nest is a very good stimulation.

Figure 45 In the above pictures it is shown how you can prepare the nest. Here the centre of the nest is filled with soft material like moss and grass. Pine branches were added.

Copulations

Cinereous vultures start copulating approximately 20 to 30 days before egg laying. They may occasionally copulate earlier, but they are not yet in breeding condition then.

Copulations generally take place in or in close vicinity of the nest and last between 30 seconds up to 3 minutes. Initially, copulations occur once or twice a day, but the frequency increases to three to four times a day when the egg laying date draws nearer (Minneman and Busse, 1984).

Keepers' attitude

Disturbance can negatively affect breeding success. Aviary size, distance from the visitors to the nest and distance from the keepers' entrance should be maximised (see housing chapter). We recommend leaving cinereous vultures alone as much as possible, in particular during the breeding season and to enter cages only when strictly necessary.

Food can be offered via a feeding door or can be pushed through the bars or wire of the aviary to avoid entering. Cage cleaning should be minimised, and jobs like felling trees, mowing, chain sawing and other noisy tasks that are not done on a regular basis should be avoided during the breeding season.

2.4.2 Egg laying and incubation

One of the main reasons for the low reproductive output of the breeding programme is that more than half of the eggs are not fertilized, and eggs frequently break during incubation. Possible

reasons have been proposed such as nervousness or aggressive behaviour by breeding birds, disturbance, poor pair bond or a poor quality of the nest (Tewes & Sanchez, 1989; Tewes, 1996[a]; Pereboom et al., 2005).

2.4.2.1 Egg Laying

Egg laying occurs from February to April (EAZA studbook data).

Figure 46 Egg patterns can differ from totally white to very speckled/patched (pictures Olivier Vercauteren, Planckendael Zoo)

Egg breaking

Egg breaking is a common problem in the captive population (Pereboom et al., 2005). About 30% of the breeding pairs broke an egg at least once. Often this happens when one bird tries to displace the other from the nest to take over incubation, thereby breaking the egg in the struggle. The birds can also unintentionally break an egg when startled as a result of disturbances.

Figure 47 Freshly laid egg (Picture: P. Izquierdo, GREFA, Spain)

Nest quality may be another cause of egg breaking. When the vultures are not very active in building the nest, make sure the nest is strong and horizontal with a soft centre to prevent the egg from breaking. Providing extra nesting material may stimulate the birds in improving their nest. Also,

including turned grass sods and/or a thin layer of sand at the bottom of the platform may protect the egg in case of a poorly built nest. Adding a preformed nest is also an option when birds are not skilled builders. When the nest is not suitable or when the parents have broken their egg in the past, it is advisable to replace a subsequent egg by a dummy and place it in an incubator (see below).

Dimensions for a dummy egg: should be 10 cm long and 7 cm broad, with an approximate weight of 240 grams, as this is the mean weight of a fresh laid egg.

Replacement clutch
When an egg breaks, disappears or is lost, it might be replaced by a replacement clutch. This will depend if this happens in an early stage of incubation (around 10-13 days) and on the pair itself (not all pairs will do a replacement clutch).

Summary: Breeding

1. **Use the pair formation ethogram to check on "pair bond" behaviours.**
2. **Check on nest quality; if not good, offer more nesting material and/ or offer a well-built nest**
3. **No good nest at egg laying → remove the egg for artificial incubation and offer a dummy egg**
4. **Artificial incubation: need for trained staff, good equipment and correct environment → see also next paragraph (2.4.2.2)**

2.4.2.2 Artificial Incubation

Parental or natural incubation always gives a better hatching rate (Tewes, 1996). However, artificial incubation is an option that should be considered when the breeding pair repeatedly failed to successfully incubate the egg or broke the egg during incubation. The recommendation and decision whether or not to remove an egg for artificial incubation **should be made on a case-by-case basis.** Ideally, a hatchling should be returned to its parents as soon as possible to give them the chance to raise a chick and gain experience (see section adoption, p. 70). It is therefore recommended to give the pair a dummy egg to stimulate them to continue incubation, or else they will never accept the hatchling.

In general, we use two different techniques: artificial incubation from the day the egg has been laid (day 0) and artificial incubation after 10 days of parental incubation. The second technique with natural incubation of the egg during the first 10 days gives a better hatching rate (Tewes, 1996).

Artificial incubation is a good option to increase low breeding success. By pulling the first egg soon (day 0 or day 1) after laying, the pair might lay a replacement clutch (not all pairs lay a replacement egg). The lay date of the first egg must be early enough (at present we recommend at the latest 16th of March) to have enough chances for a second egg laying (see also paragraph on egg pulling).

Incubator set up and monitoring
An incubator must be cleaned, disinfected, and, when possible, checked by a specialist prior to use. Ambient temperature and humidity in the room where the incubators are placed should stay fairly constant day and night: temperature between 20-24ºC and humidity 50-60%. Preferably, incubators ought to be run and checked for at least two weeks before the first eggs are expected (according to breeding records in the facilities) to certify its proper function, and to ensure constant temperature.

The **recommended incubation temperature is 37.0ºC** with an allowed maximum deviation of +/-0.2ºC. Starting relative **humidity should be around 50%** (Gomis, 2009)

Figure 48 Vulture egg in a Masalles incubator (Picture: P. Izquierdo, GREFA, Spain)

Note: Advise for reliable incubators: Masalles incubators – Falcon model: check with the manufacturer to adapt the rollers for vulture eggs; website: *www.masalles.com*

Turning: The egg should be turned at least every 2-3 hours. Most incubators have an automatic turning system: this should be on every 1-2 hours. However, most incubators are unable to turn the eggs enough, so they should be turned 2-3 times extra per day by hand 180º degrees - every time in the opposite direction!

Weighing ~ managing weight loss: During incubation, it is essential to weigh the egg on a regular basis (e.g., every 2–4 days) in order to review its weight loss through evaporation. The weight needs to decrease by 15% +/-3% ideally, and the humidity in the incubator must be adjusted accordingly. If humidity is too high, the egg cannot lose weight, if it is too low, it will lose weight too quickly. Since the individual make-up of the eggshell partly determines the rate of evaporation, this needs to be taken into account when adjusting humidity. For adequately monitoring weight loss, data should be plotted into a graph (see p. 56-58 for examples).

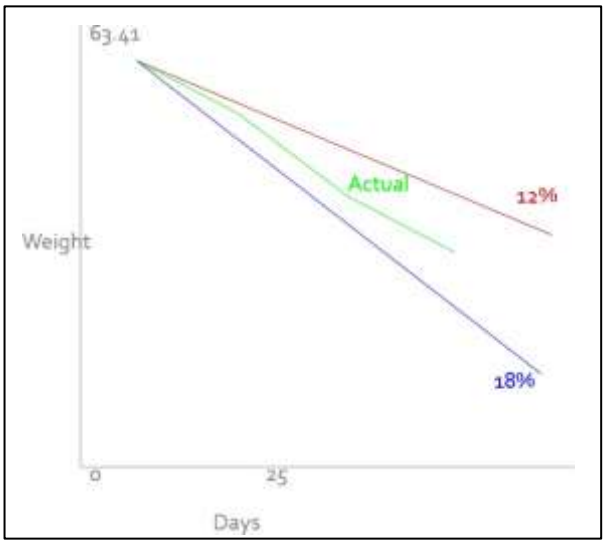


Figure 49 a 15% weight decrease should be aimed for

Determining weight loss trend in absence of a fresh weight

An egg can be taken away after 10 days of parental incubation (to avoid breaking or disappearance of the egg), as several days of natural incubation will increase the survival rate of a fertilized egg (germ). In this case the following procedure should be followed:

a. Weigh the egg and candle it to determine the fertility stage (see also point 3. Determine fertility stage of the egg)
b. As there is no initial weight, a weight loss trend can be calculated to manage the weight loss of the egg, by using the following formula:

$$Start\ wt = \frac{Today\ wt}{1 - (\frac{(Days\ incubated * 0{,}15)}{Inc})}$$

Start wt = weight of the egg when laid
Today wt = today's weight
Days incubated = days between start and today's weight
Inc = total incubation period

c. Go to paragraph above on: Weighing ~ managing weight loss during incubation

On the next pages, two examples are included, one of a 17% weight loss (used at GREFA breeding centre in Spain) and one of 12% weight loss (used at Planckendael breeding centre, Belgium); both with hatching success. Future experiences will make it possible to refine advise.

The second example is a weight loss sheet which can be obtained from the studbook keeper.

WEIGHT LOSS SHEET

Egg code: BN24.1	**Weight after pulling:** 235,18g	**Fresh weight:** 239,73g

Days of natural incubation: 0	**Incubation start date:** 15/04/21	**Laying date:** 15/4/21

Observations:

A high weight loss tendendy is observed on the second measurement (day 5), so humidity is increased to 75%.

Weight measurements							
Day	**Weight**	**Day**	**Weight**	**Day**	**Weight**	**Day**	**Weight**
1	239,73	16		31		46	202,83
2		17		32	213,78	47	
3		18	224,34	33		48	
4		19		34		49	
5	235,18	20	223,01	35	211,34	50	
6		21		36		51	
7		22		37		52	
8	232,42	23	220,92	38	208,9	53	
9		24		39		54	
10		25	219,31	40		55	
11	230,01	26		41	206,67	56	
12		27	217,88	42		57	
13	228,4	28		43		58	
14		29		44	204,44	59	
15	226,77	30	215,72	45		60	

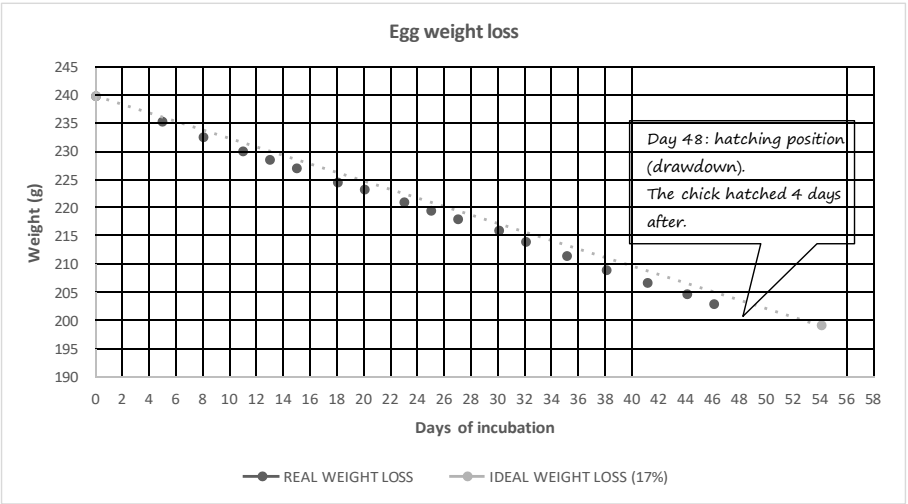


Figure 50 Example 1 of a weight loss sheet

Artificial incubation of cinereous vulture egg

Species & nr egg: **cinereous vulture** (pair male Id x female ID) - nr.

Rojo (#1401) x Chica (# 1307) -

Weigth (g) after pulling:	Fresh weigth:
251,35	251,35

number of incubation days mean: 50-57

51

Incubation start date

15-3-2022

Laying date

15-3-2022

Picture of the egg

Weigth loss

Day	Percentages 10	12	15		Weigth	RH used	RH	Date
1	250,86	250,76	250,61	T=37C	250,7	30%	OK	16-3-2022
2	250,36	250,17	249,87		250,06		OK	17-3-2022
3	249,87	249,58	249,13		249,47		OK	18-3-2022
4	249,38	248,98	248,39		248,9		OK	19-3-2022
5	248,89	248,39	247,65		248,3		OK	20-3-2022
6	248,39	247,80	246,91		247,7		OK	21-3-2022
7	247,90	247,21	246,18		247,15		OK	22-3-2022
8	247,41	246,62	245,44		246,59		OK	23-3-2022
9	246,91	246,03	244,70		245,99		OK	24-3-2022
10	246,42	245,44	243,96		245,35		OK	25-3-2022
11	245,93	244,84	243,22		244,8		OK	26-3-2022
12	245,44	244,25	242,48		244,23		OK	27-3-2022
13	244,94	243,66	241,74		243,71		OK	28-3-2022
14	244,45	243,07	241,00		243,21		OK	29-3-2022
15	243,96	242,48	240,26		242,57		OK	30-3-2022
16	243,46	241,89	239,52		241,85		OK	31-3-2022
17	242,97	241,30	238,78		241,46		OK	1-4-2022
18	242,48	240,70	238,04		240,84		OK	2-4-2022
19	241,99	240,11	237,30		240,14		OK	3-4-2022
20	241,49	239,52	236,56		239,78		OK	4-4-2022
21	241,00	238,93	235,83		239,23		OK	5-4-2022
22	240,51	238,34	235,09		238,57		OK	6-4-2022
23	240,01	237,75	234,35		238,06		OK	7-4-2022
24	239,52	237,16	233,61		237,53		OK	8-4-2022
25	239,03	236,56	232,87		236,95		OK	9-4-2022
26	238,54	235,97	232,13		236,35		OK	10-4-2022
27	238,04	235,38	231,39		235,87		OK	11-4-2022
28	237,55	234,79	230,65		235,23		OK	12-4-2022
29	237,06	234,20	229,91		234,8		OK	13-4-2022
30	236,56	233,61	229,17		234,27		OK	14-4-2022
31	236,07	233,02	228,43		233,7		OK	15-4-2022
32	235,58	232,42	227,69					16-4-2022
33	235,09	231,83	226,95					17-4-2022
34	234,59	231,24	226,22					18-4-2022
35	234,10	230,65	225,48		231,48		OK	19-4-2022
36	233,61	230,06	224,74		230,95		OK	20-4-2022

37	233,11	229,47	224,00	230,42		OK	21-4-2022
38	232,62	228,88	223,26	229,82		OK	22-4-2022
39	232,13	228,28	222,52	229,11		OK	23-4-2022
40	231,64	227,69	221,78	228,47		OK	24-4-2022
41	231,14	227,10	221,04	227,69		OK	25-4-2022
42	230,65	226,51	220,30	227,5		OK	26-4-2022
43	230,16	225,92	219,56	226,27		OK	27-4-2022
44	229,66	225,33	218,82				28-4-2022
45	229,17	224,74	218,08	225,75		OK	29-4-2022
46	228,68	224,15	217,34	224,91		OK	30-4-2022
47	228,19	223,55	216,60				1-5-2022
48	227,69	222,96	215,87	223,63	kipkast 65%	OK	2-5-2022
49	227,20	222,37	215,13	222,92	external pip	OK	3-5-2022
50	226,71	221,78	214,39				4-5-2022
51	226,22	221,19	213,65				5-5-2022
52	225,72	220,60	212,91	malposition, peeled out			6-5-2022
53	225,23	220,01	212,17	birth weigth 176,8g			7-5-2022
54	224,74	219,41	211,43				8-5-2022
55	224,24	218,82	210,69				9-5-2022
56	223,75	218,23	209,95				10-5-2022
57	223,26	217,64	209,21				11-5-2022
58	222,77	217,05	208,47				12-5-2022
59	222,27	216,46	207,73				13-5-2022
60	221,78	215,87	206,99				14-5-2022

Legend RH (RH = Relative Humidity)	
OK	Weigth loss is OK. Relative humidity can be maintained
-	Too little weigth loss but still OK. Reduce relative humidity preferably a little
↓	Weigth loss is far too little. Relative humidity must be reduced
+	Too much weigth loss but still OK. Increase relative humidity preferably a little bit.
↑	Weigth loss is too high! Relative humidity must be increased

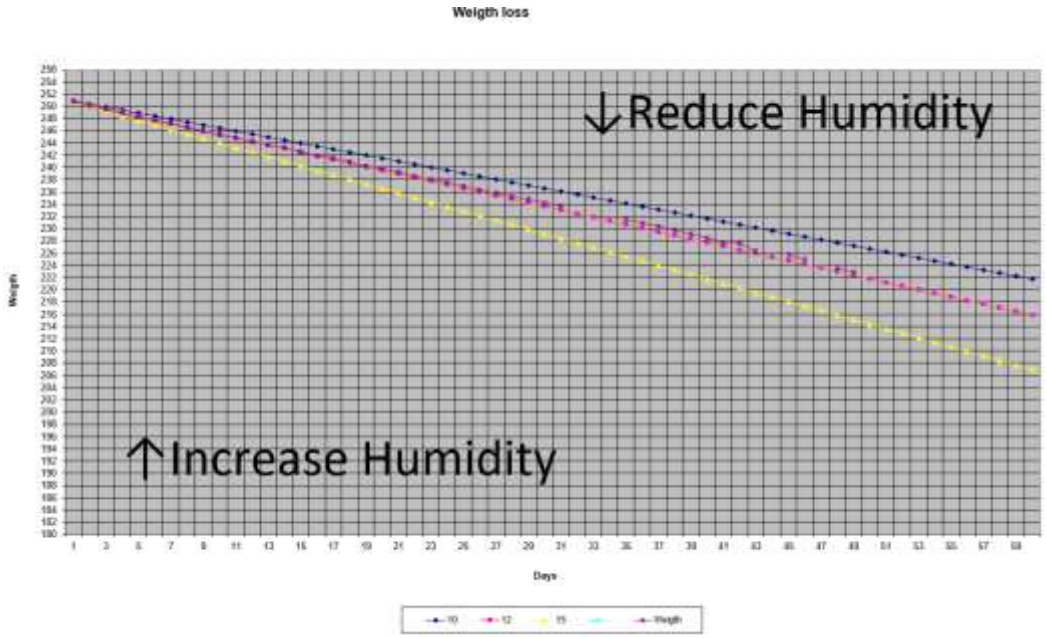


Figure 51 Example 2 of a weight loss sheet

Candling to determine the fertility rate of the egg

To establish whether the egg is fertilised, it can be candled 6-7 days after laying to determine whether small veins can be detected. Vulture eggs can be extremely difficult to candle, especially after 2-3 weeks. As water balance changes within the developing egg, shell membranes become opaque. Opaque membranes do not occur in an un-developing egg, but a rotting egg may also appear dark. During early incubation, use a high quality candler or tactical flashlights (>5000 Lumen).

Fertilisation can be best seen 7 to 10 days after laying: it is essential to candle several consecutive days to see the development in the egg.

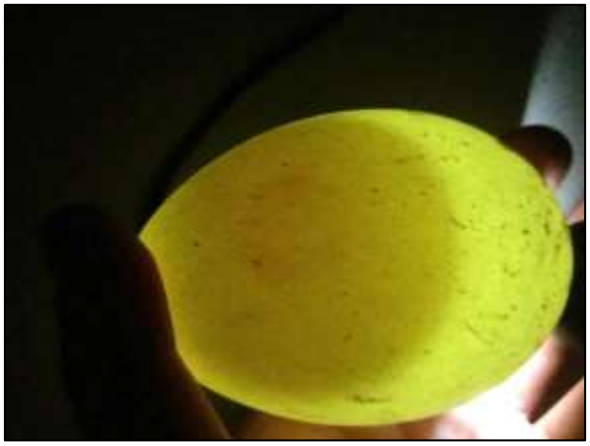


Figure 52 Fertilised egg after 7 days incubating.

1. How does a fertile vulture egg look?
    a. small veins can be detected when the eggshell is not too thick.
    b. chorioanibiotic (chorion) membrane can be seen (herein the veins will develop) → hold your egg horizontal and you will observe the membrane.

Figure 53 a) small veins, and b) chorioanibiotic membrane

2. From day 35 on, fertility can be checked with an egg buddy which will show heart rate of the embryo (not always easy to use!)

Figure 54 Egg buddy

3. From day 35-40 on: movements in the egg can be examined by placing it on a flat surface for about 30 seconds.

Figure 55 When the air cell starts to move and there is an internal pip, stop turning the egg.

About two days before hatching, increase humidity to 70 - 80%. If needed, move the egg to a hatcher with this humidity.

Egg pulling

It is possible to induce a second clutch for the species as long as the egg is pulled early (preferably the day of laying) and the pair has laid the first egg early in the season. The 1st egg can be removed when it is laid, **before or on normal** laying date of the pair. If later there is a risk the female will not re-lay, and you lose a potential foster pair.

From studbook data we notice that after mid-March less than 30 % of pairs re-lay so we do advise not to pull the egg after this date.

There's still a higher risk of embryo mortality when the eggs have not been naturally incubated and it is crucial to have the proper incubation facilities and trained staff to be successful with artificial incubation in this case.

It is NOT recommended to pull the egg from newly established pairs, as it could disturb their pair formation because of a negative experience. Leave them at least for two successful breeding seasons rearing a chick, without pulling the egg.

2.4.3. Contraception

Contraception should be avoided as much as possible and should only be used for reproductive management when specifically advised by the studbook keeper.

2.4.4. Hatching

Hatching usually occurs after 51-57 days

1. Surface for hatching should not be even, or slippery to avoid sliding of chick's legs.
2. Stop turning the egg when the embryo is positioning for internal pip; candling can show that the embryo is ready for the internal pip. This is usually 2-3 days before hatching.
3. Once internal pip has occurred, the embryo will remain more or less inactive for, at most 24 hours, before breaching the shell.
4. If the chick does not manage to breach the shell after 24 hours, assistance is needed. See below.
5. After first breaching of the shell, hatching should normally take from 24 to 48 hours. For assistance see below.
6. Check whether the yolk sac is resorbed. Disinfect the navel and umbilical cord with betadine (iodine). If yolk sac is not well resorbed, see under hatching assistance.
7. Keep the chick in the hatching machine for a few hours until dry. Humidity should be 50%.
8. Brush the chick's feathers with a smooth brush.
9. Weight the chick.
10. Move it out to a brooder with temperature of 36ºC or put it in a brooder or incubator on 36ºC.
11. No feeding for the very first 24 h (enough nutrition is available from the yolk; avoid overfeeding). Warm water is given to the chick with a pipette if it wants to drink.

Afterwards, the temperature is reduced (by tenths of a degree) depending on the behaviour of the chick.

Hatching assistance

Drawdown (or displacement of the embryo before the internal pip) in the species usually takes around 24 hours. 24 hours after drawdown has ended, internal pip should be observed by candling. If the embryo is unable to breach the shell 24 hours after the internal pip, a small hole can be drilled in the eggshell in the air sac area with sterile material (a needle or wooden awl). The hole should be covered with a gauze pad soaked in a disinfectant, e.g. betadine.

Figure 56 A small hole in the egg shell in the air sac area

Hatching should normally take from 24 to 48 hours after the eggshell was first breached by the chick. If hatching exceeds more than 72 hours, no action needs to be taken as long as the hatchling calls loudly. If, however, calling diminishes, the chick is in trouble and may need assistance.

The hatchling can be safely helped out of the egg by carefully breaking away small pieces from the shell without contacting the eggshell membranes. BE CAUTIOUS AND VERY PATIENT!!! Be aware that if this is done too early, the eggshell membrane may start bleeding. Cover the hole in the egg with a gauze soaked in disinfectant to avoid dehydration. If you notice the yolk sack has not been resorbed, one should wait until it has, mean while trying to keep up sterility, and maintain the advised temperature and humidity until the yolk sack is fully resorbed.

Figure 57 Egg shell removal must be done carefully. No blood vessels should be damaged during the assistance. (Picture: P. Izquierdo, GREFA, Spain)

Figure 58 Egg opened and protected with a gauze soaked in disinfectant. (Picture A. Llopis, VCF Spain)

Malpositions

A chick, which is pipping at the wrong side of the egg or below the air cell, or which head is over the wing, will need assistance for hatching.

When you start removing egg pieces, you can progress from the pip site, open a hole and try to locate the head position. Slowly continue opening the egg (from pip to hatch may take 48 hours or longer), without provoking any haemorrhage. Check on the yolk sack: when this has not been resorbed one should wait until it has. In the meantime:

- Replace the cap
- Maintain humidity
- Monitor membranes for drying
- Return and check in 2 hours

<u>X ray to gain more information on malposition (if possible)</u>

- When assessing hatching assistance, it is useful to obtain an X-ray image to determine if the embryo is in actual need of assistance and malpositioned.
- Situate the egg on a radiolucent padded surface, placing the egg in its balance position and fastening it to the chassis by means of radiolucent pads to prevent it from turning, so that the head is on the top and centre of the egg. In cinereous vulture eggs, the values used were 43-46 Kv and 8 mA. These values may vary depending on the X-ray equipment used.
- Up-side down and left-wing-over-head malpositions are easily detected by this method.

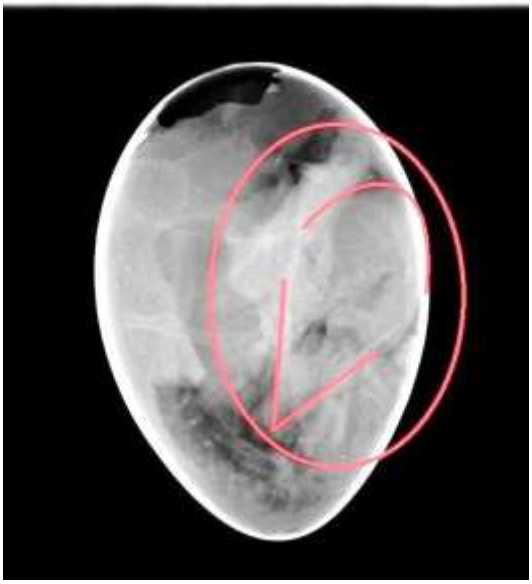


Figure 59 X-ray image of a malpositioned embryo (in red circle: position of the head). Up-side-down chicks can be easily detected. (Picture: P. Izquierdo, GREFA, Spain)

No full resorption of the yolk sac

If after hatching assistance, the yolk sack unexpectedly appears not to have been resorbed after all, it may need to be retracted manually to prevent infection of the umbilical cord. Hygienic precautions are essential. The yolk sac needs to be tied and cut with sterile material, or sterile gauze should be applied with physiological NaCl solution. Disinfection of the umbilicus is carried out using a 2% tincture of iodine.

Genetic sampling (see Appendix 6 for a detailed protocol)

- The remains inside the eggshell and the eggshell itself should be collected and sent to CRC lab in Antwerp Zoo for sexing and DNA samples.
- From the chick a buccal swab should be taken for DNA sexing.
- When the chick dies, a Post-mortem protocol should be followed.

Summary: Artificial Incubation

1. **Artificial incubation: need for trained staff, good equipment and correct environment.**
2. **In case of egg pulling for 2nd egg laying: not recommended in newly established pair and only when 1rst egg is laid before or on normal laying date and before 16th of March (more research on 2nd egg laying in future should be included in the guidelines).**
3. **Fertility can be determined 7 (-10) days after laying through candling.**
4. **Hatching occurs after 50-57 days; when assistance is required, be careful and patient.**
5. **Take a buccal swab for DNA sexing → see Appendix 6 for protocol.**

2.4.5. Development and care of young

Hatchlings weigh on average 110 grams at birth (about 3% of the body of the female) (Mundy et al, 1992). The feeding is done by both parents. Initially chicks will eat regurgitated pieces of meat, skin and bones torn into pieces by the parents. In the last few weeks before fledging parents provide meat on the nest (Hiraldo, 1984; Minneman and Busse, 1984).
Recently two zoos experienced aggression or a dispute between the partners (in two experienced pairs) after the chick hatched. It is observed that one or both partners continuously wanted to take care of the young, causing in one zoo a fight in take overs, in which the young was killed. In the second case the female did not tolerate the male on the nest. The bird did not want to leave the chick and the nest, so she had nothing to feed and started feeding nesting material. The situation was monitored closely, and the chick was fed properly and food was provided to the nest, so that the female could eat something without leaving the chick. After two days the situation normalised, and the male was tolerated again on the nest. So, it is VERY important to monitor the pair very closely.

Cinereous vulture chicks spend about 14 weeks on the nest before taking their first flight (Wilbur and Jackson, 1983; del Hoyo, 1994). In the four weeks before fledging nestlings start to exercise (wing beating, jumping, short flights on the platform) (Tewes, 1996b). When a nestling is selected for the reintroduction programme it will be removed at an approximate age of 12 weeks when it can feed independently.

At an age of 4 months, chicks can be separated from the parents for releasing or when they can have company of other chicks (social interactions are important for young birds). They should be removed at the latest at an age of 5 to 5.5 months because it has been observed that later removal may delay next egg laying and as such influence (prevent) 2nd egg laying. Chicks should be removed earlier when it is observed they disturb the parents. They should be housed with conspecifics or other vultures to keep them company.

2.4.6. Hand-rearing and foster rearing

General considerations

Hand rearing of cinereous vulture chicks should be avoided as much as possible because it results in imprinting on humans. Adult imprinted cinereous vultures will not keep normal distance from humans and therefore cannot be used in reintroduction programs. They also are in general more difficult to pair up and are less successful in breeding and rearing chicks than foster or parent reared birds. Hand-reared birds seem to be less successful in incubating and in rearing chicks to adulthood than (Foster) parent-reared birds (Pereboom et al., 2005)

Imprinted cinereous vultures have occasionally been used as a foster parent or a breeding partner but this was only successful due to a lot of keeper assistance.

If, however, there are no possibilities to avoid hand rearing, imprinting should be reduced to the minimum. We recommend using a hand puppet resembling a cinereous vulture when feeding, while the keeper stays out of sight. Social contact to conspecifics is vital for a normal development and can be realised by housing the chick in a nest box in front of conspecifics (Appendix 4 EEP nest

box protocol). Socialising should be continued: hand reared and nest box reared chicks should be housed with other young chicks of the same age, as soon as possible, from the age of 3 months on, in a crèche till they are old enough to move to a dating/socialisation aviary. This methodology has been tested for the first time in 2021 and should be evaluated on its efficacy in the coming years when these chicks attain adulthood.

Figure 60 Hand rearing a cinereous vulture chick (Picture Olivier Vercauteren, Planckendael Zoo)

The use of foster parents is obviously the most preferred alternative. Foster parents should be of the same species as cross fostering, e.g. by griffon vultures, will cause wrong imprinting.

When a chick will be fostered by foster parents the nestling should first be raised by hand for the first 7-10 days for optimal results: Proper feeding behaviour by the foster parents may initially take a while, and hand rearing will make the chick strong enough to cope with this.

2.4.6.1 Hand rearing

During the first 7 days of life

After hatching, a chick is placed in a small box or bowl on tissue paper, and it must be weighed regularly to monitor its growth. The nestling can be kept in an incubator maintained at about 36ºC initially or under a warming lamp. Temperature can be lowered 1ºC every 24 hours (If the temperature is too low, the chick starts cheeping and will try to move into a corner; if the temperature is too high, the chick starts cheeping, lies face down and pants).

The first feeding can occur after 24 hours, when the chick is gaping actively. Pre-digested food is not required, but the chick should be fed with fresh born mice/rat, cut into small pieces. When the mice or rat are not fresh born, then the skin and the digestive system should first be removed. The pieces of food should measure 3-5mm.

Food is offered with a pair of tweezers. The daily amount of food can be increased to approximately 20% of its body weight and the daily increase of weight should be around 10% (this is a mean increase!).

Figure 61 When hand rearing for 7-10 days, no hand puppet is needed as the period is short

The feeding frequency should be 4 times a day. Never feed a nestling when its crop is still full or when he is not hungry. If the crop is full, reduce to 3 feedings. If the chick does not gain enough weight, increase to a 5th feeding.

If the nestling is soiled, stains have to be removed with lukewarm water.

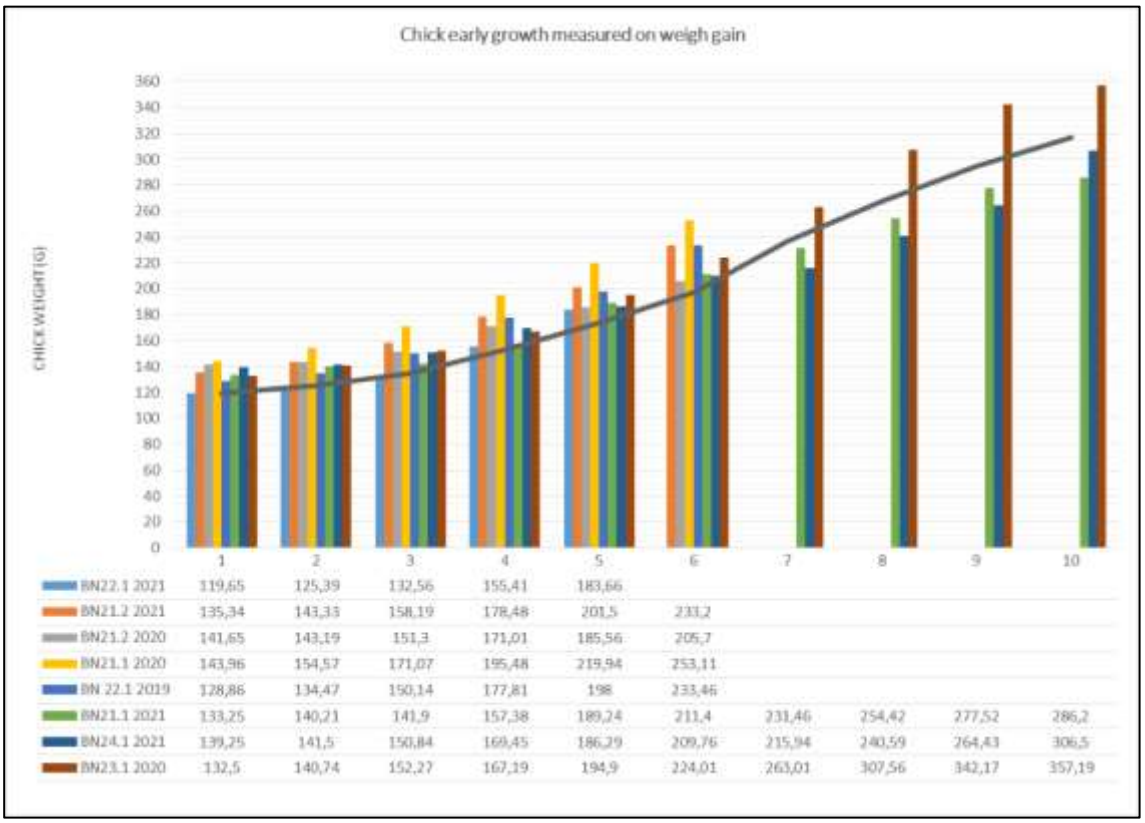


Figure 62 Evolution of the weight in chicks, hand reared for 6-10 days (data P. Izquierdo, GREFA, Spain)

2.4.6.2 Foster rearing

Foster rearing after 7 days: Chick's adoption

1. FIRST OPTION

As soon the chick has been one week hand-reared, the chick should be transferred for **adoption** to a zoo or breeding centre which keeps a breeding pair with experience in rearing a chick. Cross species fostering should be avoided as this leads to wrong imprinting. The studbook keeper will advise and assist with finding suitable foster parents.

For administrative requirements foster parents should be selected **long before** a chick hatches. The nestling needs to be placed with its new parents within two weeks after hatching, preferably at 7 days. When a nestling is older it starts showing dark parts in its plumage, making some parents not accept it.

Pairs experienced in rearing nestlings are best suited as foster parents. Although foster parents having no nestlings are preferred, occasionally pairs can rear their own and a foster nestling at the same time. At Villars les Dombes (Parc des Oiseaux), France a very young nestling of a few days was added to a nestling only a few days older, and at GREFA breeding centre, Madrid, Spain, a chick was introduced to a foster pair already rearing a chick which was 10 days older (35 and 45 days old): both birds developed properly, and no fighting occurred during their time together. Only at the first contact there was little aggression and therefore it is recommended to respect the age of 45 days to minimize the risk. At Planckendael, a chick of 18 days old was moved to a nest with a chick of 25 days old and was adopted successfully.

Figure 63 Two nestlings of the same age

Figure 64 Two nestlings with an age difference of 10 days (Picture P. Izquierdo, GREFA, Spain)

The chick can be placed in the nest of the foster parents after the first feeding in the morning. Behaviour of the birds and chick development should be closely monitored without disturbing the animals. If necessary, chicks can be provided with extra food on the nest (see Appendix 3: adoption guidance and data collection).

Natural rearing -reared by its conspecifics- parents or foster pairs- is **always** the first option to choose.

2. SECOND OPTION

If natural rearing cannot be done because the adoption failed or the transfer of the chick cannot proceed, it is necessary to rear the chick with visual contact to its conspecifics to avoid human imprinting. For that a "**Nest-box rearing protocol" was developed**: see Appendix 3 for details.

- **With 7-10 days old** (depending on the outside temperature) the chick has to be put in the box during the day and fed from behind, without human contact. At the beginning it should stay only outside during the hottest hours of the day and as the chick grows, the hours outside get longer.

Figure 65 Nest box (Picture: Parco Viva Natura, Italy)

Figure 66 Nest box (Pictures: St Polten, VCF Austria)

- **With 4 weeks**, if outside temperature is not under 0°C, the chick can stay 24h outside. With a safely installed heating lamp or mat the chick can stay outside for the whole day and taken inside for the colder nights and to control its weight in the first weeks. The chick can be covered with straw to keep it enough warm. If temperatures are lower than 0°C, the chick should stay the night indoor without human contact.

Figure 67 Nest box (Pictures: St Polten, VCF Austria)

- **If the box is on a covered nest platform with cold wind circulation,** it is recommended to close 2 of the 4 sides: the back side and the side way from the parents in order to avoid infections of the respiratory tract of the chicks.

- **The number of feedings has to be reduced to a minimum**. This implies that the feedings must be bigger in quantity. With 3 weeks, 2-3 feedings per day should be enough. This requires to calculate each day needed food on a theoretical basis. As soon as the chick is 24h outside, weight controls should be avoided as much as possible to reduce human contact (2x/month). That's why it will be very important to calculate the needed food following a mean daily growth of 9,7% in the first 30 days. During the period where the chick will spend the night indoor, it will be possible to control its growth.

- **Feeding should always be done** with a puppet to avoid visual human contact.

Figure 68 Puppet rearing bearded vulture. (Pictures: Parco Natura Viva 2020, Italy, VCF)

- The nestling starts to feed alone from the bowl **with an age of 3 weeks**. From this age on, chopped food should be offered in a plate, reducing progressively the following days the feeding with the help of a puppet. This will encourage the chick to feed on its own.

As soon as the chick is **2.5-3 months old,** it's possible- if parents don't react aggressive - to remove it from the nest box and introduce it with the parents. If this cannot be done, the bird should be moved to a socialisation or dating aviary in consultation with the EEP coordinator. This will allow the chick to continue to grow up with conspecifics.

Figure 69 Socialisation of a nest box reared chick with parent reared chicks.

Fixed foot ring for chicks

Chicks on the nest can be ringed from an age between 33-37 days. A closed band with diameter of 28mm is recommended. The chick should be weighed at the same time, to have an indication on its body condition.

Figure 70 Fixed foot rings

Figure 71 Weighing the chick

Summary: Hand Rearing & Foster Rearing

1. **Hand-rearing should be avoided because of imprinting on humans.**
2. **Foster rearing should be done ONLY with experienced cinereous breeding pairs (and after 7 days of hand-rearing).**
3. **Contact the studbook keeper to assist in finding suitable foster parents**
4. **Nest box rearing is recommended if no natural or foster rearing is possible**

2.4.7 Population management

The breeding programme for the cinereous vulture is an EAZA Ex-situ Programme (EEP), monitored under the EAZA umbrella. A Long-Term Management Plan (LTMP) was held for the species in 2018 (Sanchez et al., 2019). In this plan the following roles, target and to do's were defined:

The roles for this EEP:

1. Function as a demographically stable, genetically healthy, and behaviourally competent insurance population that can function as source for reintroduction.
2. Function as source for reintroduction projects, mainly to re-establish breeding colonies at strategic geographical regions as described in the species' action plan (Andevski et al., 2017).
3. Generate funding for *in situ* and *ex situ* conservation of the European black vulture.
4. Generate awareness of the species to the public and relevant stakeholders such as farmers and schools about European black vulture conservation, ideally in collaboration with *in situ* partners.
5. Be used in research that aims to counter threats to the species in the wild, such as by testing tools and methodologies.

Target

The cinereous vulture EEP can potentially easily fulfil its role as genetic reserve. However, with the current population parameters, the population size is projected to start to crash. Especially in the coming 15 years, it will be very challenging to maintain the population size at the target of at least 170 birds.

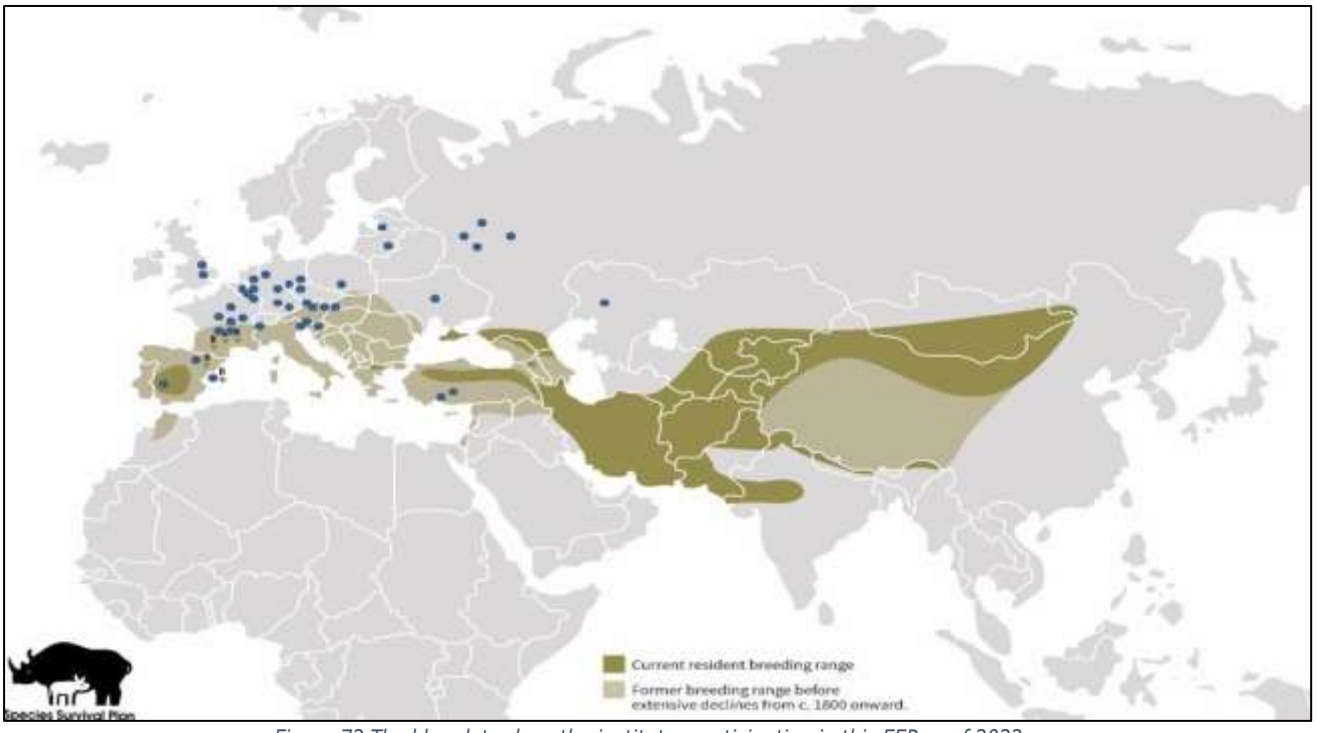


Figure 72 The blue dots show the institutes participating in this EEP as of 2022.

The reproduction the last 5 years has been more successful, with the achieved hatch rate being twice (1.9) as high as the expected one. If this positive trend can be continued it can be expected that the population will remain more or less equal over time, and even grow from 2033 on. (E. Fienig, pers.comm.).

Recently a release strategy was developed to have a more solid, flexible and clear method in place that starts from the primary role of this population (back-up) and looks at opportunities for releases (second role of the EEP) taking into account current space limitations and long-term population trends. (see Appendix 2).

2.5. Environmental Enrichment

Edited by Marina Salas, Animal Welfare Scientist for the Antwerp Zoo Centre for Research and Conservation

We can enhance the quality of life of zoo-housed cinereous vultures by providing environmental enrichment, which is a combination of techniques aimed to provide environmental stimuli necessary for optimal psychological and physiological welfare. Therefore, environmental enrichment programmes must be based on detailed knowledge of the natural history of the species and consider the species' behavioural biology. The practice of enrichment should be integrated as a basic principle of zoo animal husbandry and should always consider an individual animal's needs and changing requirements over time.

There are several types of environmental enrichment that can be categorised as feeding, tactile, structural, auditory, olfactory, visual, social, human-animal, and/or cognitive.

Good environmental enrichment programmes can have the following positive consequences:

- Increase the diversity and performance of species-appropriate behaviours
- Increased space use in the enclosure
- Reduction of the time dedicated to display abnormal behaviours or prevent their development
- Reduction of the time dedicated to display undesired behaviours such as net biting
- Increased general activity, foraging, and exploratory behaviours
- Decrease in the frequency and intensity of aggressive interactions
- Decrease in the prevalence of some diseases
- Decrease in the (chronic and acute) stress responses
- Increased reproductive success

Environmental enrichment programmes also aim to increase the animal's sense of control and ability to choose, since the perception of unpredictable situations, being unable to choose and not having a sense of control over the environment, can have a negative impact on welfare.
Occasionally, environmental enrichment can have negative effects on animal welfare. For example, in animals housed in groups, environmental enrichment can cause an increase in aggressive behaviours by stimulating competition for resources that did not exist before. However, this problem usually disappears by increasing the number of items, devices, or feeding points used in the enrichment, so that the animals do not have to compete for the rewarding resources.

It is essential to measure the effectiveness of the environmental enrichment in order to ensure that the resources are being used effectively and that the enrichment being used does indeed procure animal welfare benefits. This effectiveness can be evaluated by comparing the behavioural outcomes against a pre-enrichment behavioural baseline. The habituation of animals to the enrichment and the consequent loss of its positive effect in the short or long term should also be frequently measured. The so-called 'SPIDER' framework can be a useful tool to implement and evaluate environmental enrichment and it includes six stages: **Set goals**, **Plan** an approach to enrichment, **Implement** the enrichment, **Document** outcomes, **Evaluate** those outcomes in comparison to the set goals, and **Re-adjust** implementation if necessary.

Environmental enrichment can improve the vultures' mental and physical health by allowing them to engage in rewarding behaviours that are related with positive experiences. Such behaviours may involve food, space, temperature, social partners, activities such as dustbathing, among others.

Carcasses

Supplying carcasses (whole or partial) can be a form of enrichment because carcasses can stimulate the vultures to perform natural feeding behaviours and increase the time spent foraging, tearing apart muscles, tendons, and sticking heads inside the carcass to remove meat. Vultures will need to use their whole body to tear the meat and scrape carcasses to the bone. Adding carcasses, tendons, and/or preys with skin will increase the opportunity for the vultures to use their beak and display podomanipulation.

It is important to consider that, in order to keep a balanced diet, the carcasses given for enrichment must be calculated into the animals' dietary needs.

Perches

It is advisable that cinereous vultures are stimulated to fly as long a distance as possible. Therefore, perches should be placed appropriately within the enclosure to achieve this purpose. Interior posts are not recommended because they hamper the flying.

Perches can also make the enclosure more interesting for the birds, so it is recommended to offer a variety of perching sizes (diameter and/or length) and location. The perches can be wrapped with mats of different textures (sisal rope, jute rope, artificial grass, carpet, etc). The chewing of the mats needs to be monitored and the items removed if they become a hazard for the birds.
Re-perching periodically and changing the perches' location can stimulate activity and increase enclosure exploration and use. However, it must be noted that cinereous vultures tend to be nervous animals that do not like changes. So, replacing or removing perches or regularly placing new items in the enclosure is not advisable during the breeding season.

Water and sunbathing opportunities

The birds can benefit from the provision of natural bathing opportunities (such as perches exposed to the elements, pools, or sprinklers) for water bathing as well as perches receiving direct sun for sunbathing. To avoid competition for resources, all these resources should be available to all the vultures at the same time.

Covered shelter
There should be plenty of areas for the vultures to escape and/or hide from conspecifics (or other species in mixed exhibits), as well as visual barriers from other animals and zoo visitors. To provide optimal welfare to all birds within an enclosure, these shelters and visual barriers should be accessible to all the vultures at the same time.

Nesting material
The provision of cardboard boxes stuffed with nesting material or piles on the floor with different items can stimulate nest building, dragging material with the beak and gifting 'presents' to other vultures and potential partners.

Examples of material that can be provided: wool, pine sticks, sticks, twigs, non-toxic plants branches, bark, fringes and flowers, leaves, grass, colourful shed feathers (e.g., from macaws or flamingos), pinecones, etc.

Tree branches
Cinereous vultures have been observed to nibble on and torn tree branches to pieces, so these items can be offered to increase beak and podomanipulation. The birds will also have to use their feet to stabilize the item while tearing apart.

Mirrors
Shatter-proof mirrors can be used to stimulate curiosity and sensory senses (visual, auditory and/or tactile).

Puzzle feeders
Creating a challenge to obtain the food can increase foraging time and also stimulate natural feeding behaviours. However, the animals should be capable of solving the problem or meeting the challenge presented to them, otherwise frustration may arise.

<u>Examples of puzzle feeders:</u>

- Ball with lots of holes in it and pieces of meat stuffed inside. The animals will have to tear them out of the ball.
- Tubes with pieces of meat inside, attached to a wooden frame. The vultures will have to stick their beak or head inside the tubes to take the meat out.
- Forage box with meat inside, filled also with hay or twigs so they have to tear and search for their food. Leave the box open for easier access (especially at the beginning while learning how to interact with the item) or make holes so they must stick their heads inside the box.

Figure 73 Forage boxes (Pictures by Jessica Smith)

Since cinereous vultures appear to be of neophobic character, we must take into consideration that it may take several days before the vultures approach a (new) enrichment item. We suggest to keep offering enrichment opportunities to them frequently, allowing them enough time to interact with the items, and wait longer than with other species before regarding those items as useless for the enrichment of cinereous vultures. However, any enrichment item should be removed if, after monitoring the vultures' behaviour, it appears to be a hazard for the animals or encourages the development of undesired behaviours (such as abnormal behaviours or an increase in aggressive interactions, even after multiple items have already been provided in order to avoid competition for resources).

2.6. Handling

2.6.1 Identification and Sexing

Reviewed by Philippe Helsen, scientific advisor for the cinereous vulture EEP programme

Identification

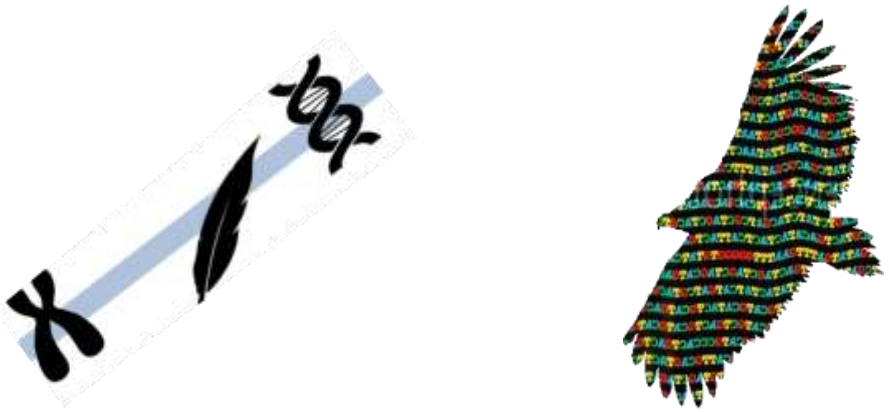
Identification of individuals is usually done by using coloured plastic DARVIC rings with numbers. They are inexpensive, easily recognisable and do not impede the birds. The disadvantage of plastic rings is that some vultures lose their ring after nibbling on it. Metal rings are an alternative, but they are often small and have a similar appearance making them less easy to read. Closed metal leg bands are small but have the advantage to apply easily for CITES documents. We strongly advise the use of two types of rings, to allow identification in case one is lost.

Besides rings we recommend that all vultures in the EEP programme should have a microchip because this prevents mistaken identification. Often these identities are the only ones accepted by CITES or other authorities, depending on local regulations.

Individuals can be recognisable by their appearance, like differences in plumage (especially the white spots on the wings), the coverage of feathers on the head, the colouring of feathers on the head, although these features are not permanent and change when the animal moult. More permanent particularities like a hanging wing, or bumblefoot can also make birds easily recognisable.

Although long-distance identification is usually not of any particular significance for captive animals, bleached feathers in the vultures' wings can be used but this identification will disappear when the bird moults. We do not recommend using wing tags as this technique is invasive and also temporary as the hole though the wing enlarges and the tag can be lost.

Sexing- collection of biological samples for the EAZA Biobank



Sexing

Determination of the sex is essential to create successful breeding pairs in the EEP breeding programme and to assure equal sex-ratios in the dating/ socialization aviary. To practically organise release, it is very important and helpful to know the sex of hatchlings as soon as possible.

By far the least invasive and less stressful method, is to **sex** birds using DNA sexing typing which can be performed on either blood, freshly plucked feathers or buccal swabs samples. Blood sampling should be performed by a vet and ideally blood is stored in standard EDTA tubes. Feathers can be stored in paper envelopes in an area of low humidity and temperature (e.g. RT*) until further analysis. Buccal swabs are an easy methodology for sampling DNA from hatchlings. Chicks which are a bit older can be feather sampled on the nest.

**RT= Room Temperature*

Figure 74 Feather sampling to allow DNA sexing typing.

Gender identification should be performed shortly after sample collection to facilitate population management and more specifically to create transfer recommendations. At present there is a collaboration with Antwerp Zoo's Centre for Research and Conservation of which is carrying out

the sexing of all EEP birds for free. These samples will be stored for later population management purposes. For a detailed protocol see Appendix 6.

Collection of biological samples (e.g., for the EAZA Biobank)
For each individual it is recommended to collect a blood sample (high DNA yield) for running genetic research and for long-term storage in the EAZA Biobank. Blood sampling should be performed during veterinary screening or when transferring birds between aviaries or institutes. For a detailed protocol see Appendix 6.

However, this might not always be possible (e.g., stressed birds, availability of a vet or another person competent to collect samples). In such cases 3-10 breast feathers, a buccal swab and/or dried eggshells can be collected in this order of preference. Ideally both feather and blood sample types are collected to have a back-up if one of the samples fails.

Figure 75 Blood sampling and storing

All samples should be labelled individually mentioning:

1. the unique sample ID
2. sample type
3. date of sample collection

And, if applicable

4. the preservative being used (e.g., EDTA).

EDTA blood samples can be stored in the fridge (4°C) for 1 week prior to sending out for sexing, research, or banking by normal post. If longer storage is needed, samples can be frozen and are transferred in a frozen state. Samples from one individual should be packed in one container (e.g., Zip lock bag) including absorbent material. Ideally the receiving institute is notified samples will be shipped in order to assure the package arrives at the correct place and time.

When sending samples, please label all tubes but also add the unique ids of all samples on paper including when the samples are taken.

Weighing and veterinary check up
When the bird is in hand, it should be weighed in order to have a correct idea of its condition. Weighing can be done with a weight scale or by putting the bird in a bag and weighing it. A cinereous vulture weighs from 7-10 kg depending on its age and its sex. For more details see chapter on veterinary care.

2.6.2 General Handling

Cinereous vultures are very sensitive to handling or to disturbance in general. When upset, they might hurt themselves, break their egg or abandon their nest. For that reason, it is often emphasized that animals should only be caught when strictly necessary and that all handling (like for changing cages, transport, and veterinary checks) should be postponed until completion of the breeding cycle. Vultures will vomit when they are upset (Parry-Jones, 2000), therefore she advises not to feed the vultures on the day of catching them.
In the socialisation aviary of Rhenen zoo, training /conditioning is used to manage capture of birds in this large aviary: Birds are trained to come on a platform where food is provided and to enter a smaller aviary in which handling is less stressful and more controlled.

Therefore, it is very important **to prepare all actions** which should be done on beforehand and to minimize the time required for handlings to an absolute minimum. In the following paragraphs the different actions are mentioned to consider and to prepare before catching the bird.

Equipment needed:

- rings
- blood tubes and envelopes for blood and feather samples to be taken
- weighing scale

For chicks/hatchlings the following extra equipment is needed: depending whether the chick hatches following artificial incubation, a swab can be taken, and eggshell remains should be collected.

- buccal swab for hatchlings
- envelopes for eggshell remains after hatching

For parent reared chicks on the nest: depending on the age

- buccal swab
- feather collection

2.6.3 Catching / Restraining

Catching can best be done with a net.

Figure 76 Catching and restraining a vulture with a net

After catching a cinereous vulture, it is important to restrain the head first because vultures can inflict very nasty bites. However, one must make sure that the bird does not choke or gets strangled. When the head is restrained with one hand, the feet should be held with the other hand. Then tuck the wings into the bird's body and hold the bird under your arm against your body.

Figure 77 The recommended way of handling a cinereous vulture.

When doing a veterinary examination, the head can be covered with a hood so that the view of the bird is blocked, and he will get calmer when handled for e.g., blood sampling.

Figure 78 a vulture with a hood

2.6.4 Transportation

vultures should be transported in a pet-box respecting the IATA rules. The floor of the pet-box is very smooth and slippery; therefore, a non-slippery carpet or wood chips (no straw, as this could cause aspergillosis) should be placed inside, to make it more comfortable for the bird. Never place the crate with bird in direct sunlight.

Figure 79 Large pet boxes used for dogs are easy transport crates.

Dimensions should be: height = 75cm, width = 54 cm and length = 100cm.
IATA Live Animal Regulations allow the use of a rigid plastic container described above or a wooden crate with meshed ventilation openings (Container requirement 20; IATA 2023).

Figure 80 Transport crate

For transport of chicks for adoption the following crate can be used:

Figure 81 Brinsea Brooder TLC-40 – with transformer for use in the car.

2.6.5. Safety

When catching a cinereous vulture, it is important to restrain the head first because vultures can inflict very nasty bites. Once the head is restrained with one hand, the feet should be held with the other hand. Then tuck the wings into the bird's body and hold the bird under your arm against your body. There are no extra special conditions for safety as this is not an aggressive species.

However, in walk-through aviaries it was experienced that the species likes to interact with visitors e.g., pulling laces of shoes. So, it is not recommended to house them in a walk-through aviary.

Before transport, several documents need to be applied for. Please note that different countries require different forms. Cinereous vultures are CITES (II-A) listed: for a transport in Europe, a CITES certificate needs to be requested, for transport to countries outside Europe, a CITES permit is necessary.

2.7 Veterinary: Considerations for health and welfare

chapter reviewed by Dr. Eric Bureau, veterinary advisor for the cinereous vulture EEP programme and Dr. Dominik Fischer, veterinary advisor for the EAZA Raptor TAG.

2.7.1 Health & Welfare

General recommendations

Cinereous vultures are rarely sick. At least one thorough check-up of each bird should happen yearly. This includes:

1. monitoring the body weight
2. perform a physical examination particularly of
    a. Body condition and weight
    b. Check of skin and feathers also for ectoparasites (esp. in young birds)
    c. Pulmonary and cardiac auscultation
    d. Feet and talons (esp. check for pododermatitis)
    e. Ophthalmic, otic and oral examination
    f. Cloacal condition
    g. Uropygial gland examination
    h. Trans coelomic palpation
3. Screen for parasites and give specific antiparasitic treatment if needed; recommended to do this 2 times/ year, in particular for young birds
4. Coproculture (Salmonella…) tests
5. Carry out a blood tests for chronic diseases (protein electrophoresis)

Monitoring the body weight

Weighing can be done with a weight scale or by putting the bird in a bag and weighing it. A cinereous vulture weighs from 7-10 kg depending on its age and sex. If possible, continuous weight monitoring can be done by installing stationary weight stations in the inside enclosure e.g., in perches.

It is very important to monitor the weight as vultures, which are too fat tend to develop pododermatitis osteoarthritis, atherosclerosis, infertility…
An overly low weight can evoke weakness and immune suppression resulting in a chronic illness (aspergillosis, renal failure, avian tuberculosis…)

Bumble foot (pododermatitis)
Bumble foot is a vascular necrosis and infection of ventral feet tissue, and it is one of the most common physical problems in captive raptors. It may be caused by pressure necrosis (e.g., when a bird leans on a single foot while recovering from the injury of the other foot), improper husbandry conditions such as dimensions or surface material of the perch, inappropriate conformation of the ground (e.g. too rough surface), obesity or puncture wounds. Vitamin deficiencies (e.g., vitamins A, B, and E) and nutritional imbalances may act as predisposing factor like insufficient hygiene inside the enclosure. Microbiological culture and radiographs should be performed to estimate the severity and prognosis of this painful disease.

Affected birds may need a thorough veterinary treatment including analgesia and antibiotic therapy, and in advanced stages surgery (Parry-Jones, 2000).

As it is linked with inadequate husbandry and diet it is recommended:

- To offer perches and branches of different diameters and material (e.g. appropriate padding material) to prevent bumble foot problems.
- Avoid overweight and nutritional deficiency, in particular vitamin A, B and E deficiencies.
- It is important to check at least once a year and preferably more the condition of the feet, as early cases of pododermatitis are much easier to treat than in advanced stages.

Cataract
Cataract, being defined as opacification of the lens inside the eye, is quite common when birds become very old. The sight reduces and they can become blind. However, it can also occur in younger birds due to multiple reasons and therefore a yearly check of the eyes must be done.
Lenticular opacification is easy detectable during basic ophthalmologic examination.
The degree to which the bird is affected by the decreased vision, health and age will often dictate whether surgery for cataract removal is needed. Unilateral cataracts are of little clinical importance. The deep narcosis associated with this procedure can be risky and therefore in case of very old birds a treatment should be balanced against the potential benefits of such surgery.

Birds suffering from cataract should not be changed from enclosure as they know their housing environment well.

Trauma
Trauma is the number one cause of death. Therefore, correct husbandry conditions are essential: to avoid collisions, the aviary should not contain inner poles and boundaries can be made visible by using wooden lats. For correct husbandry, we refer to section 2. Regularly the consequences of a trauma are not visible with the naked eye and require a specific veterinary examination including an ophthalmoscopy and radiography.

Flight restraint, wing clipping and pinioning
Pairs with flight-restrained males were found to be less successful in breeding than pairs with full-winged males. We assume that such males have problems during copulation (keeping balance when mounting a female). Pinioning or wing clipping is primarily used with vultures that are kept in roofless aviaries, but this **is not recommended for animal welfare and reproductive reasons.**

Ectoparasites

There are various ectoparasites that affect cinereous vultures, including ticks, Mallophaga chewing lice, "flat" flies, maggots and mites. At Planckendael only lice are found on cinereous vultures. Treatment is done with a spray for pets with Fipronil as active agent. As it contains alcohol, it damages the feathers when administered directly on the feathers. It is recommended to spray the product on a tissue and apply it as such on the back at the base of the neck (to avoid preening the product).

Internal parasites
Internal parasites mainly affect young birds, in general adult birds in good health are less often affected. Clinical signs may include problems to swallow (dysphagia), anorexia, weight loss, diarrhoea and death, the latter especially in nestlings, young, immune suppressed and stressed individuals (associated with a high parasite burden leading to intestinal obstruction and anaemia). Tracheal and air sac worms may cause a marked inflammatory response, dyspnoea, voice change, head shaking, and extension of the neck with open beak (gasping).

It is recommended to perform a parasitic coproscopy on samples from three consecutive days to avoid false negative (in particular for coccidea and *Serratospiculum* sp.). Deworming should be done when needed and anti-parasitic drugs and dosages must be selected carefully and specific to the detected kind of parasite. CAUTION: anthelmintics containing benzimidazoles (especially fenbendazole) are toxic to vultures; to treat nematodes it is recommended to use avermectines such as ivermectin or moxidectin).

Caseous lesions in the mouth, under the tongue or around the choana, and in the upper digestive tract of vultures, may be caused by a flagellated protozoan parasite (*Trichomonas gallinae*). Especially young birds may become infected by eating infected pigeons or chickens, resulting in impaired swallowing and breathing. Metronidazole-like drugs such as carnidazole and ronidazole are used to treat the infection. In severe cases additional antibiotics and antifungals, analgesia and sometimes even surgery is indicated.

Intoxication

Cinereous vulture can be victim to acute lead intoxication, lead can be present e.g. in wild game and therefore wild game should not be included in the diet.
Cinereous vultures are also sensitive to iatrogenic intoxication and poisoning. They are sensitive to pesticides, such as carbamates (e.g. carbofluran or aldicarb) and organophosphates (e.g. parathion or chlorpyrifos), and some rodenticides (e.g. coumarin derivatives). Therefore, rodent and arthropod control inside the institution must be done very carefully, considering undesired side effects.

The following drugs are toxic or possibly toxic for vultures and should therefore be used with special care or preferably avoided: Enrofloxacin (IV), Neomycin, Procain penicillin, acyclovir, fenbendazole, flubendazole, carprofen, diclofenac, flunixin, ibuprofen, ketoprofen, phenylbutazone.

Arteriosclerosis

This is a diffuse or local degenerative condition affecting different parts of the walls of blood vessels (mainly internal and medial tunics of arteries) and the entire circulatory system. This thickening of the arterial wall and the reduction of vessel lumen affects the blood flow and may cause acute myocardial infarcts, syncope, seizures and death due to hypoxy. The intensive feeding of one-day old chicks (including egg yolk) has been hypothesized as risk factor for arteriosclerosis in birds of prey (Legler et al., 2017). It is most often seen in older birds on high-fat diets, but vascular changes may occur in young birds as well. Furthermore, it is also a risk for kidney failure because of the fatty contents of the yolk sac.

Diagnosis is usually made postmortem, but in living vultures, a radiographic examination might identify arteriosclerotic mineralization (increased density and prominence of the great vessels and the caudal lung field) and blood chemical analysis might help to monitor levels of cholesterol and triglycerides.

Arthrosis

This is a degenerative condition of cartilages and bone structures of different joints, usually affecting old individuals. Treatment is depending on the clinical signs and consist of analgesia and supportive care. The enclosure may be modified to a disability-friendly husbandry, simplifying access to perches and elevated spots by providing plants or boards. Analgetic and anti-inflammatory treatment includes the daily application of drugs including meloxicam and in some cases gabapentin. Low level laser therapy and leech therapy have been demonstrated to aid in the supportive treatment of arthrosis with providing analgesic effects.

West Nile virus (WNV)

West Nile virus (WNV) belongs to the Flaviviridae family (arbovirus): currently, 9 lineages are identified of which 4 reported in Europe. The number of WNV cases has increased a lot in the last years in European countries (Fischer et al., 2019). In general, most birds are subclinical infected, but WNV may cause severe and fatal disease and clinical signs are:

- sudden death,
- central nervous signs (e.g., disorientation, head tremor, torticollis, uncoordinated and staggered movements,
- depression, apathy,
- anorexia,
- shedding of greenish urine/urates,
- ocular disorders including blindness
- ruffled feathers, feather disorders and/or feather loss.

Cinereous vultures are sensitive for this virus, there are several confirmed cases. Dead birds should therefore be screened on the virus. It is recommended to apply mosquito control measures with removal of breeding grounds for mosquitos, adding fish to ponds, identification of

mosquito species, control with *Bacillus thuringiensis* and/or mosquito traps (e.g. CO_2-light or pheromone traps).

In endemic areas it is advised to vaccinate the birds. In absence of vaccines for birds, recombinant or killed vaccines for horses have been used successfully and without side effects in large falcons: inactivated vaccine Duvaxyn® WNV (Fort Dodge), the recombinant live RECOMBITEK®-Equine WNV vaccine (MERIAL) and two newly designed DNA vaccines (Fraunhofer Institute, IZI Leipzig). In Italy EQUILIS WEST NILE from MSD Animal Health has been used for this species. EQUIP WNV® vaccine from Zoetis has also been tested in a large scale of diurnal raptors (including griffon vultures and bearded vultures) and owls with success and without secondary effects.
In recent studies, it was recommended to use the killed vaccines twice (with a 3 weeks interval between the injections) for initial immunization with a yearly booster injection. However, the interval of booster vaccinations, the duration of protection and the development of antibody titres following vaccination are currently under investigation. Thus, whenever possible, blood should be collected at each vaccination and submitted for serology testing in order to monitor the situation in your facility and the ex-situ population.

Treatment is limited to symptomatic and supportive care (fluid support, artificial feeding, anti-inflammatory treatment using meloxicam and anti-infective treatment of secondary infections).

Usutu virus (USUV)
This virus is similar to WNV and also belongs to the Flaviviridae family (Arbovirus). It has spread across all of Europe in the last few years. Clinical signs in susceptible birds may be similar to a WNV associated disease, but most infections in cinereous vultures seem to be subclinical. There are no clinical cases reported in cinereous vultures but in Californian condors and bearded vultures. As for West Nile virus increased screening is recommended. Treatment is similar to West Nile virus.

Avian Influenza/ Bird flu
In the past avian flu cases were restricted to late autumn, winter and early spring, but since 2022 there is a strongly increasing number of epidemics occurring all over Europe and all over the year. Especially the Avian influenza virus (AIV) subtypes H5 (such as H5N1, H5N2 or H5N8) and H7, but also other subtypes, may cause highly contagious diseases with dramatic consequences for raptor populations. Affected birds usually die fast, but may show clinical signs such as depression, apathy, ruffled feathers, inappetence, diarrhoea, cyanosis, oedema, haemorrhages, dyspnoea and central nervous signs (such as ataxia, seizures, head tilt).

For prophylaxis, a biosecurity plan is vital to limit the risk of virus introduction into the collection. Specific prophylaxis includes vaccination. Studies on birds of prey on H5 inactivated vaccine against Avian Influenza have shown that the vaccine is working without side effects (Lierz et al. 2007). Since 2006 many cinereous vultures have been vaccinated in France without sides effects too (using Nobilis® Influenza H5N2, MSD). Initial immunization consists of two injections of 0.75ml vaccine per bird with a six week interval. In the following years, one booster vaccination is considered to be sufficient, but there are also recommendations that a bi-annual booster vaccinations should be done before and after the breeding season, in December and July.

However, within most countries in Europe vaccination against avian influenza is still not allowed, but emergency vaccination might be permitted under specific biosecurity and diagnostic conditions to counter crisis circumstances. If possible, it is recommended to vaccinate your birds.

Newcastle Disease (ND)
This disease is caused by the avian paramyxovirus-1 (PMV-1 or Avulavirus 1) and most raptors become infected by direct contact to sick birds or by eating infected prey or food animals (especially poultry and pigeons). Neurologic signs (torticollis, tremors, ataxia), conjunctivitis, respiratory signs, vomiting, anorexia and diarrhoea and sudden death have been reported especially in juvenile raptors. There are no detailed reports about ND in cinereous vultures, but it has been suspected as cause of death in bearded vultures (Lublin et al. 2001). In endemic areas, it may be advisable to vaccinate raptors with inactivated PMV-1 vaccines, but specific vaccines for raptors are not available in most countries and a full protection cannot be achieved by vaccination.

Herpesvirus
Infections with herpesviruses may be harmful for different raptors, in particular for falcons, owls and some eagle species. However, there are specific herpes viruses described in vultures as well. Herpes virus is mainly transmitted by ingestion of infected pigeons, as the herpes virus of pigeons is very similar or identical to the viruses of raptors. Similar viruses may be transmitted by wild birds or via direct contact to infected individuals or their excrements. Affected birds usually become depressed and anorexic; they suffer from inflammation and necrosis of liver and spleen, which may be associated to enlargement of liver and spleen and to the shedding of lime-green urates. Infected birds of prey usually die within few days after displaying the first clinical signs or even prior to clinical disease.

Aspergillosis
Fungi of the genus *Aspergillus* are regarded as the main cause of this disease. Despite overwhelming fungal exposure, usual environmental fungal concentrations of the fungi are not dangerous for healthy birds. Weakness, young age, immunosuppression, injuries, illnesses, or suboptimal and unhygienic husbandry conditions, prolonged antibiotic treatment and corticosteroid therapy may predispose raptors to develop the disease. Aspergillosis is not regarded contagious between birds.
Weight loss, dyspnea and/or sometimes a loss or change of the animals' voice, anorexia, weakness, and lime green urine are some of many various possible signs.
Aspergillosis needs to be treated as fast as possible.

Bacterial Diseases
As obligate scavengers vultures are used to bacterial pathogens and adapt by time even to very potent bacterial toxins such as the toxin of *Clostridium botulinum*. Moreover, zoonotic bacteria including *Salmonella* spp., *Campylobacter* spp., *Chlamydia psittaci* and some *Mycobacterium* species haven been reported in free-ranging and captive vultures, in most cases not associated to clinical disease. However, in predisposed individuals bacterial pathogens may act as debilitating co-factor worsening the individual health situation. Moreover, cases of avian mycobacteriosis have been described in vultures too, associated with granulomatous splenitis and hepatitis and severe clinical disease. To limit the risk of bacterial diseases, hygiene measures (especially of the

feeding and drinking sites) and a careful selection of the source of food animals are recommended.

2.7.2 Post mortem protocol

→ see Appendix 6 for sampling protocols

Necropsy

When a cinereous vulture dies, we advise to perform a full necropsy, preferably using a standard necropsy form. A copy of the necropsy report should be always sent to the studbook keeper. For ZIMS users, the necropsy should be entered in the ZIMS for Medical database, which can be consulted by the veterinary advisors of the programme.

Every embryonic mortality should - if possible - be subject to an ovautopsy and in particular a bacteriology. See also 2.9. Recommended research.

Samples should be collected for the EAZA Biobank, and if possible, sampling for screening for Usutu and West Nile virus (WNV):

Sampling for EAZA Biobank

Additionally, some blood and tissue of the dead birds should be collected for future DNA research (EAZA Biobank) and send to Antwerp Zoo. For the protocol see Appendix 6.

1. For recent deaths, a blood sample should be collected.
2. Collect a 1 cm^3 tissue of an internal organ (preferable liver).
3. Collect external tissue samples (e.g., toe pad or part of the tongue).
4. Place each sample in a plastic tube (such as a 2ml screw-cap tube).
5. Label tube with studbook ID, ring number and any other unique identifier.
6. Freeze at -20°C or -80°C immediately or preserve in ethanol (70-90%).

Sampling for USUTU/WNV screening

Given the clinical significance of these diseases, all bird holders are encouraged to put in place a robust disease surveillance protocol. Different European countries have different surveillance and monitoring regimes in place. Holders should therefore contact the government veterinary authority in their country and organize a sampling scheme.

Summary: Veterinary

1. **At least one check-up of each bird should happen annually: this includes a physical examination (in particular feet condition), a weight control, a parasite treatment (if needed), coprocultural tests and, if possible, blood sampling for at least plasma protein electrophoresis (indicative of an inflammatory response).**
2. **Vaccination against Avian Influenza and West Nile virus is recommended in endemic areas and if legally allowed in the country.**
3. **Post-mortem examinations should always be performed, also on dead embryos. And results should be entered in ZIMS for Medical database. Samples should be collected for the EAZA Biobank and, if possible, for screening on Usutu and West Nile virus.**

2.8 Conservation

In 1986 the Black Vulture Conservation Foundation (BVCF) established a conservation programme for cinereous vultures. Its aim was to create a captive breeding programme with captive vultures present in European zoological gardens and breeding centres and use their nestlings for reintroduction into former habitats where the reasons for previous decline could be eliminated, and to put a halt to more captures of wild adult birds. In 1987, the European Endangered Species Programme (EEP) for cinereous vultures was started by EAZA (European Association of Zoos and Aquaria).

Reintroduction projects were carried out in Mallorca from 1984 - 1990 and in three regions in France, Les Causses (1994-2004), Baronnies (2004-2018) and Verdon (2005-2020) and in Spain, Catalonia, in Boumort - Alinya (from 2007-2017). Reintroductions now started in Bulgaria in 2018 and are ongoing in Sliven, Kotel and in Vratza. For these projects young chicks of 3 months old, born in zoos, are released by hacking together with wild birds from recovery centres in Spain.
Other conservation projects for the species are carried out in in Spain (La Demanda, 2017-(ongoing)) and have recently been started in the Southern Rhodopes in 2022.

Additionally, campaigns against the illegal use of poison have been running since 1996 in Spain (including Mallorca), Portugal, France (including Corsica), Italy (including Sardinia) and Greece and more recently in the Balkans (Birdlife, 2022). To increase the struggle against the illegal use of poison, the Junta de Andalucía has installed a special anti-poison unit in its region. The Vulture Conservation Foundation has established with the help of this unit in the Balkans a wildlife crime academy to train people for investigating illegal poisoning incidents in 7 Balkan countries.

2.9 Recommended Research and sample collection

In view of long-term studies into the basic breeding biology and breeding success of the captive population of cinereous vultures we recommend to regularly perform basic behavioural

observations and collect material for DNA analyses and make these available to the studbook keeper. In the near future special attention should go to study the impact of the diet composition on breeding success and the observed embryonal death.

Behavioural monitoring, in particular quality of pair bonding

To allow proper evaluations of breeding pairs we recommend performing regular observations, which will allow taking management measures to improve breeding success. An ethogram, specifically designed to evaluate pair bonding, which can be easily used by keepers or students, is included in section 2.4.1.

Installation of a camera to observe an egg, a nestling, or the birds' behaviour closely without disturbance is a big advantage in zoo-kept vultures. A camera should be installed, preferably focussed on the nest platform, at least two months before the breeding season so the birds can adapt to its presence. To easily check the birds and their offspring, a monitor and recorder can be used. This is particularly advantageous for difficult pairs that show a high degree of nervousness. During critical periods in the breeding season like hatching or fledging or when an egg was broken, such recorded scenes can provide useful information to take appropriate management decisions. In view of a large-scale ongoing study at Planckendael we recommend sending the results of student projects or keeper observations to the EAZA EEP coordinator.

Where to install camera's:

At Planckendael vulture breeding centre, a Hikvision player is used- type: DS-7732NI-I4: it can play, rewind and record images (4MP, 25X optical zoom)

There are three cameras (type: DS-2DE4A425IW-DE) per aviary: two on the nesting platform, one fixed on the left and one PTZ with zoom function on the right (see picture – orange bullets). There is one PTZ camera in the front with zoom function which covers the whole aviary. The latter one is used when chicks are fledged to observe and monitor them from close by.

Note: it is important to make the wires unreachable for the birds as they will try to chew and pull when they can reach these.

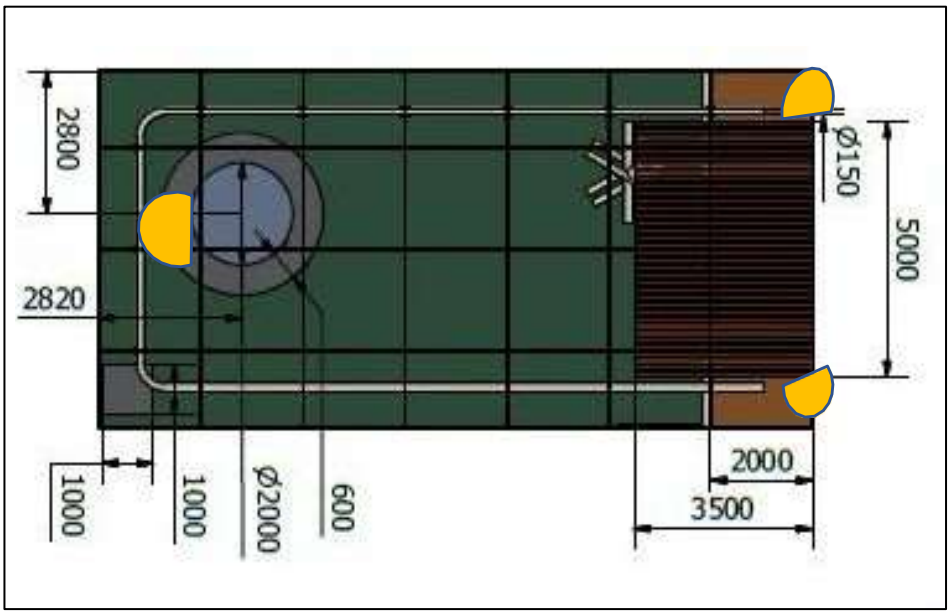


Figure 82 Camera locations (in yellow) in Planckendael aviaries.

Origin of captive birds and genetic compatibility and breeding success

The Centre for Research and Conservation at Antwerp Zoo is investigating the role of genetics in breeding success in cinereous vultures. Using studbook data as well as DNA from captive individuals from the EEP collection, the aims are to define relatedness per pair and evaluate whether genetic incompatibility between paired birds may be the cause for low breeding success in the EEP. To this end, it is important to collect feather and preferably blood samples of each individual in the ex-situ EEP population. For more information see section 2.6.1 Identification and Sexing.

Nutritional analyses

The results of the nutrition questionnaire (Bureau, 2022) provide some interesting suggestions for diet composition. However, continuous research is needed, especially regarding whether and how diet affects egg quality and breeding success. More generally, a more thorough evaluation is needed of the nutritional value of the diets provided and the role of the supplementation of vitamins and minerals. More specifically, we need studies on the impact of vitamin E levels on both sexes prior and during the breeding season, and further investigations into the adverse effect of artificially high dietary vitamin A levels on vitamin E levels.

Embryonal death and fertility

Over the last years it was noticed that quite a substantial percentage of fertilised eggs die during artificial incubation, varying from 16-31% in the last 3 years, but with an increasing number of artificially incubated eggs in these years. Therefore, every embryonic mortality should, if possible, be subject of an ovautopsy and in particular bacteriology.

Further investigation should show whether this might be related to nutrition or to genetic incompatibility or other factors, such as incorrect parameters for artificial incubation.

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SECTION 4: APPENDICES

Appendix 1: Contact info

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Koningin Astridplein 20-26, 2018 Antwerp, Belgium
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Appendix 2: Release strategy for cinereous vulture EEP for 2022-2023

Marleen Huyghe & Philippe Helsen, 24/06/2022 (07/12/2022)

The EAZA EEP for cinereous vulture has always played an important role in release projects of the species in Europe. It started in 1986 with the BVCF, releasing chicks in Mallorca, then in France, Spain and Bulgaria. At present 64 chicks have been released.

More recently (2019) the **EEP goals** have been redefined as part of the species LTMP (Long Term Management Plan) and comprised two major pillars:

1. guarantee a sustainable reserve population representing the species' natural genetic diversity, representing 11% of Western/Iberian genes and 89 % of Eastern/Asian genes.
2. be a source for reintroductions or conservation translocations

This strategy explains how both goals can be achieved on the long term.

In release projects nowadays birds from Spanish recovery centres are used at the start of release projects, as they are available and fixate easier on the site if they are released in a group of at least 6-10 birds. In addition, EEP chicks can be released at a very young age to increase and restore genetic diversity.

The rationale behind the current strategy?

The continuous release of the majority of all chicks born in the EEP population in the past in combination with a low breeding success has resulted in substantial **ageing** of the population .The last years several actions have been undertaken to improve breeding success: In 2019-2020, a thorough exercise was carried out in which birds - that were unsuccessful in breeding for the last 5 years - were **re-paired** on basis of several criteria (see below) and from 2022 on, **2nd egg laying** is promoted on a larger scale.

In 2020 an analysis was performed on breeding performance of existing pairs further increasing our understanding in factors affecting breeding success (e.g., age of the female, age difference within couples and previous breeding experiences). Pairs that proved to be unsuccessful over the past 5 years were reformed bearing in mind these parameters, meanwhile keeping an eye on the genetic goal of this population (e.g., focus on re-pairing founders, minimizing kinship within pairs and where possible combine individuals from the same geographic region). Reforming 19 new pairs from previously unsuccessful birds is believed to positively impact population growth of the ex-situ population.

Furthermore, it was recommended in 2022 to induce 2nd egg laying, whenever possible, which implies that one or both eggs are incubated artificially. More specifically the first egg is removed in 1) pairs which proved to be successful breeders before, and 2) if the 1st egg was laid close to normal laying date of the pair and not later than 16/03, as from this date onward the chance to replacement clutches is $\leq$ 30%. As a positive side effect, the focus on artificial incubation will decrease breakage and/or disappearance of eggs both observed at above normal frequencies within this EEP.

Building on these two interventions breeding success in 2022 and the following years is expected to increase. For 2022, we already notice a 50% success in 2nd egg laying, which is promising. On the mid to long term the foreseen increase in reproductive success will increase population growth rates which eventually can impact demographic health of the population. As the population will grow in the next years, we might have **shortage of available spaces**, in particular for socialisation aviaries, where juveniles are housed to pair up.

This document proposes a dynamic strategy to assess the number of chicks needed to achieve long-term genetic and demographic health of the EEP. This number combined with the yearly achieved reproductive output of the population directly portraits the number of chicks available for release. This process is dynamic, in that each year this strategy will be revised in function of the needs of this EEP.

In 2022, the actual managed EEP population consisted of 142 birds. Using PMx a simulation was run to evaluate how many chicks are required to keep a stable EEP population of this size over the coming 20 years.

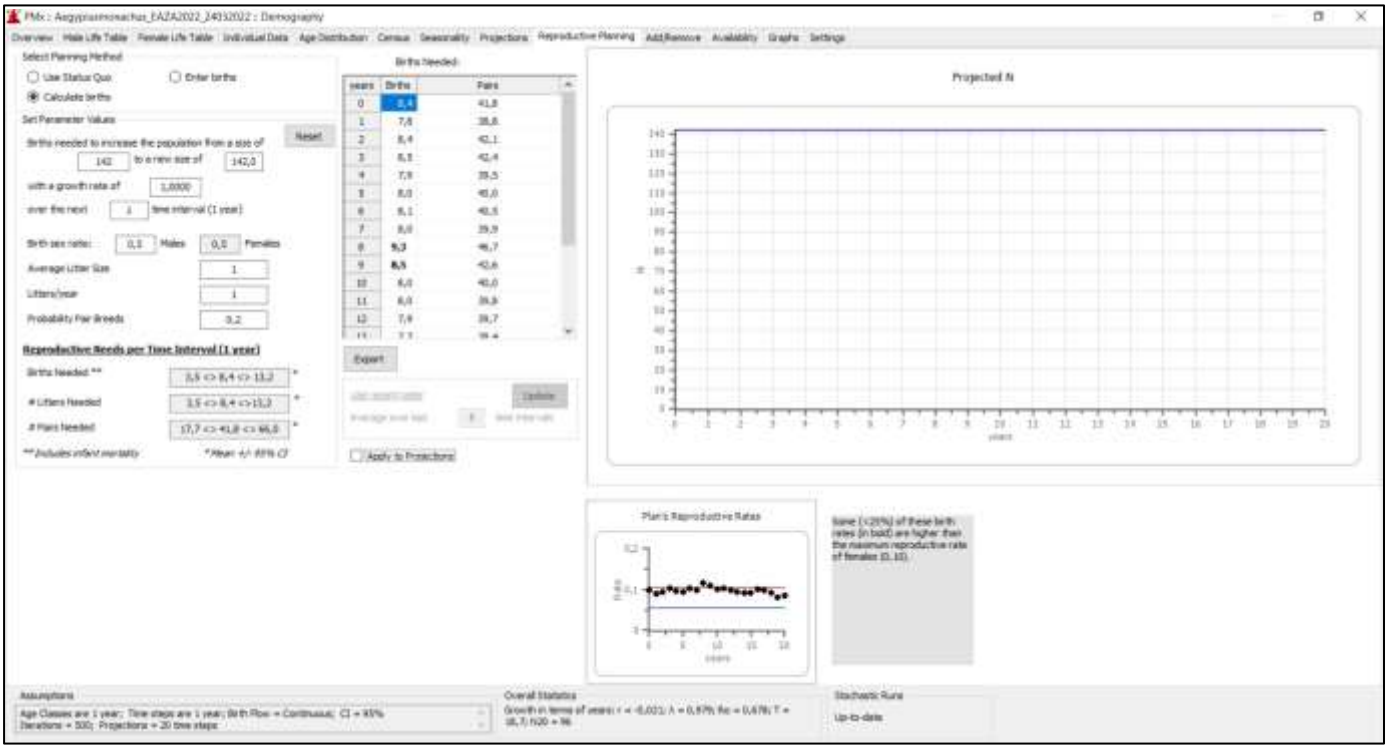


Figure 83 PMX calculation for the cinereous vulture EEP with a λ=1; productivity of pair =0,2; 8,1 hatchlings needed (infant mortality included, mean calculated over 21 years); Productivity of the pair is calculated from the ratio of number of breeding pairs on hatchlings over the last 8 years (2013-2021).

The calculations show that **the EEP needs on average 8 hatchlings per year for the next 20 years to come**. The number however slightly differs over years. For the current year 8.4, here rounded to 9 hatchlings are needed. When translating this number in number of fledglings needed, a 0.25 infant mortality at 30 days needs to be taken into account, resulting in the **need for 6 fledglings**
*** Mortality rates for hatchlings and fledglings are calculated by the PMx programme. The yearly more detailed figures kept by the studbook keeper are conform these.*

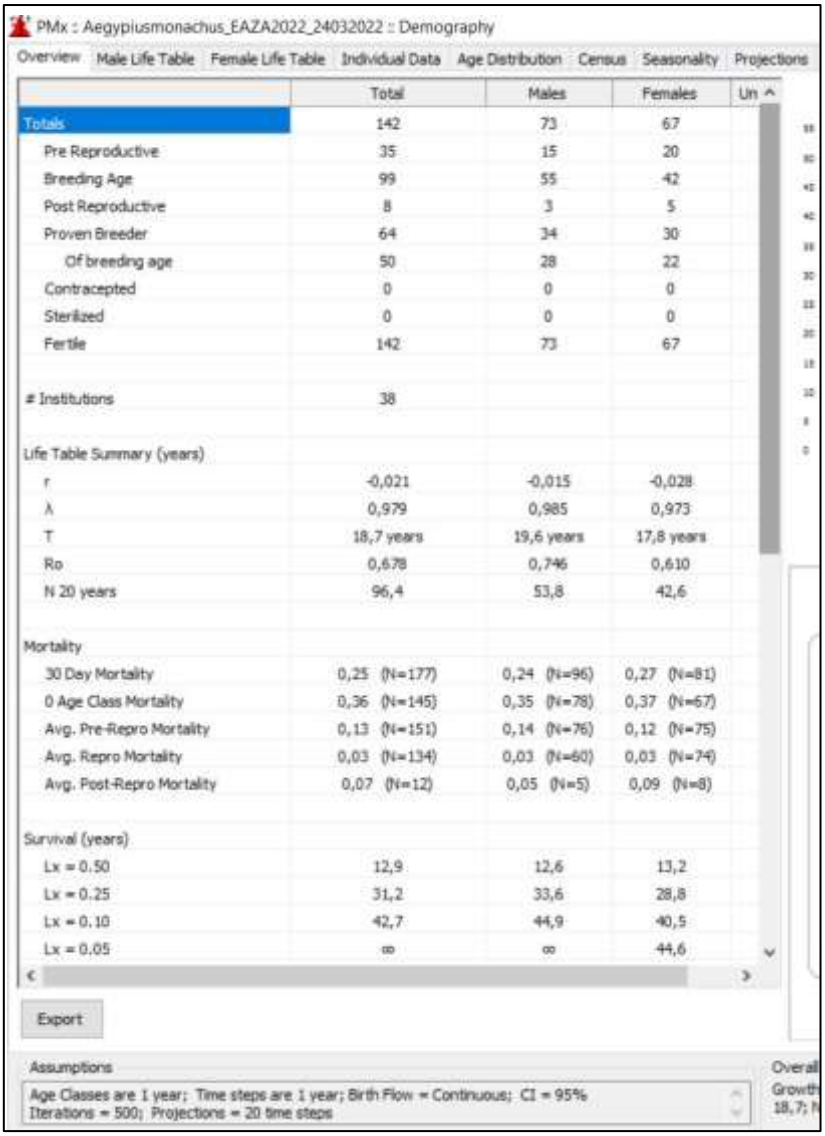


Figure 84 screen shot from PMx Demography

The average number of annual offspring in the EEP is included in the table below. In the last 5 years (2017-2021) the EEP produced on average 7,6 hatchlings or 5,2 fledglings, which is not enough to have a λ=1.

Table 4 average number of annual offspring in the EEP

	2017	2018	2019	2020	2021	average from 2017-2021
Pairs*	45	45	48	41	41	44
Total hatchlings	7	6	12	3	10	7,6
Total young (<30d)	6	4	9	2	7	
Total fledglings (>120d)	6	4	9	1	7	
Total young (>12mo)	6	3	9 (5)	1	7	5,2

**Pairs in this table are pairs which are actively managed, including post reproductive (=non laying) pairs. It only includes pairs from which information was received from the participating zoo.*

What is the actual situation?

1. The juvenile birds in the EEP are housed in "socialisation aviaries" for several years to allow them to pair up. By keeping all young birds ex situ, we (will) need extra space for a longer period: at present the EEP includes two socialization aviaries, and some zoos (#1-2) are candidates to create extra socialisation aviaries. However, we will reach maximum capacity soon when we manage to increase the productivity by the formation of new pairs and second egg laying.
2. The genetic policy is to retain equal genetic contributions from each founder in the programme. Some pairs are reproducing well and are or will soon be overrepresented in the population.
3. The current population is male biased, so we need to invest in reaching a more balanced sex-ration which can be achieved by keeping more females in the population.
4. There is again a small but unpredictable influx from non-releasable recovered birds from French release sites and Spanish recovery centres.

Summarised we use the following ranked criteria to select birds to be released:

1. Mean kinship (the higher the better).
2. Sex should be chosen in function of the needs of the programme. If possible, preferably males should be selected as they **tend to stay closer to the release site.**
3. Zoos which have not yet released, giving all participating zoos the chance to release one day.

Assuming the methodology of re- pairing unsuccessful birds and 2nd egg laying is successful, productivity will increase and the EEP will produce more than an average of 7,6 hatchlings or 5,2 fledglings in the coming years. This result should be shown clearly in the breeding seasons of 2022 and 2023.

On basis of the above information and considerations/assumptions, we propose a **release strategy**, based upon the actual number of hatchlings which will be re-calculated each year.
It starts from the number of hatches needed to keep the ex-situ population demographically stable (mean of 8 hatchlings for the coming 20 years). It also takes into account that minimum 2 chicks should be released at the same time because social contact between young birds is important, and release should be done together.

This results in the following formula:

$$\mathbf{max.\ birds\ available\ for\ release} = (\mathbf{realised\#hatchlings} - \mathbf{required\#hatchlings}) \times \mathbf{0,75}$$

the maximum number of birds available for release depends upon the realised number of hatchlings minus the required number of hatchlings, times hatchling survival rate (0.75).

Combined with the recommendation to release minimum to two chicks, this gives for 2022/23:

Table 5 Number of hatchlings produced in the EEP and chicks available.

	2022			2023		
# hatchlings	≤ 10	11	≥ 12	≤ 9	10	≥ 11
# chicks available for release	0	1	≥ 2	0	1	≥ 2
recommendation	No releases	No releases (less than 2 chicks available)	Release (use formula above)	No releases	No releases (less than 2 chicks available)	Release (use formula above)

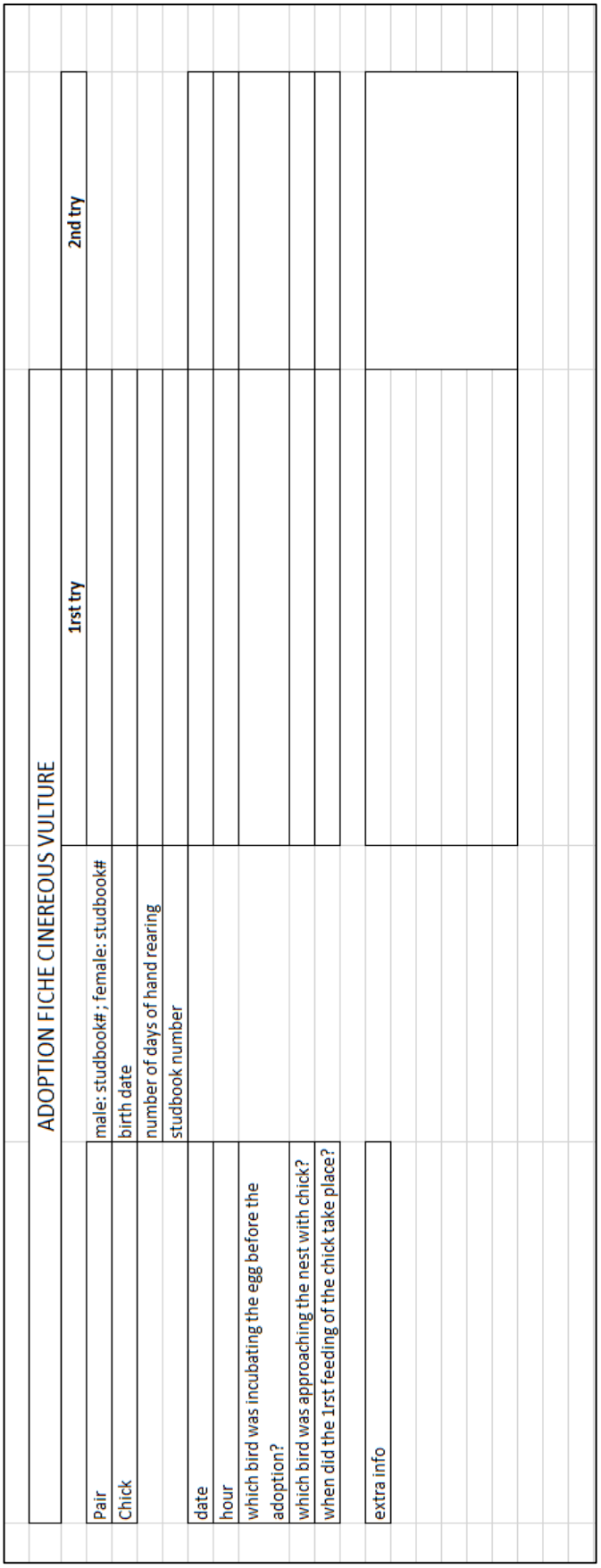
With a realised number of hatchlings of 13 in 2022, a maximum number of 3 chicks can be released.

If, based on the above-mentioned yearly tables, there's an overproduction of hatchlings within the EEP the following criteria will be used to select to most appropriate candidates for release:

1. Individuals with high kinship values will be selected over low MK individuals.
2. Physical and behavioural readiness to be released.

This strategy will allow us to reply to the demand of our release partners for EEP chicks, and at the same time taking into account the actual fragile situation of the programme and the estimated growth/ increase of the breeding success following different measures taken from 2020 on.

Appendix 3: Datasheet for use in case of adoption



ADOPTION FICHE CINEREOUS VULTURE		1rst try	2nd try
Pair	male: studbook# ; female: studbook#		
Chick	birth date		
	number of days of hand rearing		
	studbook number		
date			
hour			
which bird was incubating the egg before the adoption?			
which bird was approaching the nest with chick?			
when did the 1rst feeding of the chick take place?			
extra info			

Appendix 4: EEP cinereous vulture nest box protocol

NEST BOX REARING PROTOCOL for HAND REARING WITHOUT HUMAN VISUAL CONTACT AND IN VIEW OF THE PARENTS- 27/05/2021- first version – DEVELOPPED for KAZAN ZOO

All pictures are from VCF, Spain.

ONLY WHEN NO ADOPTION OR FOSTERING IS POSSIBLE AND IN CONSULTATION WITH THE EEP COORDINATOR

Introduction

Keeping in mind that only natural reared chicks are suitable for release, capable to survive in the wild without human help and able to reproduce when they arrive to their sexual maturity, this protocol will prioritize the quality of the chicks over the quantity, with the aim to obtain chicks with the conditions above described.

Hand raised birds are not able to reproduce with their conspecific when they get their sexual maturity. The pair bond process with a conspecific essential for successful reproduction and natural rearing is impossible for imprinted birds. Therefore, inside the cinereous vulture EEP such a rearing method is **NOT** recommended.

Background

In general fostering or adoption for chicks which cannot be raised by their parents will be sought for as first option. During the breeding season 2020/21, because of the Covid19 restrictions, it was not possible to perform chick transports for adoption. Therefore, another solution had to be sought for.

In Austria on 19th of May 2021, we tried to have a 22-day old chick fostered by a breeding pair, but the chick was not accepted. Based on the experiences in the Bearded vulture EEP (A. Llopis, pers.comm.) in 2020 and on former experiences in imperial eagles (2002 in Guadalentin, A. Llopis, pers.comm.) and lesser grey shrikes (Valcallent, 2007) - which successfully developed and used a "nest box" rearing protocol - a protocol for cinereous vultures was developed.

Below you will find some examples of the nest boxes used for these species:

Figure 85 Nest box for imperial eagles, fixed at the outside of the aviary (nest of eagles is in a tree of the middle of the aviary)- picture A.Llopis, VCF

For lesser grey shrikes the nestbox is constructed in the outside aviary

Figure 86 Nest box for lesser grey shrikes, picture and protocol A. Llopis, VCF.

For bearded vultures the nest box is placed in the nesting cave:

Figure 87 Nest box used for bearded vultures

Figure 88 Pictures by Parco Natura Viva, 2020, Italy & A. Llopis, Vallcalent, Spain (Bearded vulture EEP, VCF)

PROTOCOL & RECOMMENDATIONS

(all pictures, St Polten, Austria, 2021, H. Frey (Bearded Vulture EEP,VCF))

Before implementing the protocol, it will be necessary to construct **a nest box**. This nest box should preferably be installed nearby the nest platform in order to have frequent visual contact between nestling and adult pair/parents. Access from the back to feed the chick will be necessary in order to avoid visual contact with humans.

Construction of the NEST BOX on the nesting platform or in the neighbourhood of the nest (pictures VCF, Austria)

- It is essential to offer the chick/s the opportunity to be raised in visual contact with its parents. This can be done by introducing the chick/s in a box near the nest of the parents. The box should be covered with a welded wire mesh with a hole-size not bigger than 2.5cm. Nest box dimensions are minimum 1m x1.5m on 1.2m (l)x(w)x(h).

Figure 89 Nest box installed nearby the ground nest of the breeding pair

- Very important: adults should not have the possibility to hurt the chick: the mesh has to be installed at both sides. This to safeguard the possible loss of a chick by attacks by the adults through the mesh.

Figure 90 Double mesh

- The **back** should be closed and with a small door from which the chick can be fed without visual contact.

Figure 91 There is a construction to give water though a pipe from the outside, without disturbing the chick

- **Inside the box a nest** with straw of high quality has to be installed (vital to keep the chick warm). The box should be big enough to hold one chick and to allow it to move when it grows and becomes bigger

Figure 92 Nest material should be straw to keep the chick warm

- If possible, **a heating lamp or heating mat** should be installed to keep the chick warm. Make sure the chick cannot nibble on the wire and be electrocuted : the wire should be protected with a metal layer!

Figure 93 The chick should be put on the heating mat protected with a towel

- Depending on the geographical location of the aviary, in particular in colder regions, **2 or 3 from the 4 walls should be closed**. If possible retractable glass panels can be installed which can be removed/opened with sunny weather and placed back/closed with colder outside temperatures.

Figure 94 Two walls are protected, the back and the side; the chick can see the parents' nests and the rest of the aviary; the roof protects the chick from rain. The keeper can approach the nest box from the back, invisible for the bird inside.

Figure 95 Pictures from Bearded vulture nest box rearing protocol, Parco Natura Viva, 2020, Italy

REARING PROTOCOL:

1. The first 7 days – Hand rearing

Hand rearing should be done following the **guidelines** (see 2.4.6) and without visual contact; a puppet can be used to avoid human contact with the keeper.

Figure 96 Hand rearing a cinereous vulture chick with a puppet (Picture Olivier Vercauteren, Planckendael Zoo)

After hatching, a chick is placed in a small box or bowl on tissue paper, and it must be weighed regularly to monitor its growth. The nestling can be kept in an incubator maintained at about 37°C initially or under a warming lamp. Temperature can be lower in the following days. The first feeding can occur after 24 hours, when the chick is gaping actively and feeding frequency should be five to six times a day. Feeding can be offered by using a "vulture-like "puppet to minimize the imprinting and from behind a curtain so that the chick does not see the keeper

The daily amount of food can be increased to approximately 20% of its body weight and the daily increase of weight should be around 10% (this is a mean increase!). Never feed a nestling when its crop is still full or when he is not hungry. Pre-digested food is not required. To avoid a lack of minerals which could negatively affect the development of the bones, the chick is fed recently killed **one day old mice cut into small pieces** or young rats (up to a week old), **always skinned with their bones <u>crushed</u> and with their intestines**. The pieces of food should measure 3-5mm. The rats are cut into pieces with scissors and the pieces are mixed with some drops of water. *

As the chick grows, they are given 2 to 3 weeks old rats **<u>without skin, as the skin is difficult to regurgitate for the chick</u>**. Meat consisting of beef, cattle heart, should not be the main food and if fed, a <u>multi-vitamin/calcium mixture should be added</u>.
If the nestling is soiled, stains have to be removed with lukewarm water.

** Instead of crushing the bones, the bones of young rats can be cut with strong scissors without problems (pers. communication, A.Llopis, VCF).*

Figure 97

2. After 7 days: chick(s) adoption

1. FIRST OPTION

As soon the chick has been one week hand-reared, the chick should be transferred for **adoption** to a zoo or breeding centre which keeps a breeding pair with experience in rearing a chick. This should be done in close consultation with the studbook keeper who knows where such pairs are available. Natural rearing -reared by its conspecifics- parents or foster pairs- is **always** the first option to choose.

2. SECOND OPTION

If natural rearing cannot be done because the adoption failed or the transfer of the chick cannot proceed, it is necessary to rear the chick with visual contact to its conspecifics to avoid human imprinting. For that we will use the **Nest-box rearing protocol**:

- **At 7-10 days old** (depending on the outside temperature) the chick has to be **put in the box during the day** and **fed from behind, without human contact**. **At the beginning it should stay only outside during the hottest hours of the day** and as the chick grows, the hours outside get longer.
- **At 4 weeks**, if outside temperatures are not under 0°C, **the chick can stay 24h outside**. With a safely installed heating lamp or mat the chick can stay outside for the whole day and taken inside for the colder nights and to control its weight in the first weeks. You can always cover the chick with straw to keep it enough warm. If temperatures are lower than 0°C, the chick should stay the night indoors without human contact.
- **If the box is on a covered nest platform with cold wind circulation,** it is recommended to close 2 of the 4 sides: the back side and the side away from the parents in order to avoid infections of the respiratory tract of the chicks.
- **The number of feedings has to be reduced as maximum as possible**. This implies that the feedings must be bigger in quantity. With 3 weeks 2-3 feedings per day should be enough. This requires to calculate each day needed food on a theoretical basis. As soon as the chick is 24h outside, weight controls should be avoided as much as possible to reduce human contact. That's why it will be very important to calculate the needed food following

a mean daily growth of 9.7% in the first 30 days. During the period where the chick will spend the night indoor, it will be possible to control its growth.

- **Feeding should always be done** with a puppet to avoid visual human contact.

Figure 98 Picture: Parco Natura Viva 2020, Italy, VCF

- The nestling starts to feed alone from the bowl **with an age of 3 weeks**. From this age on, chopped food should be offered in a plate, reducing progressively the following days the feeding with tweezers. This will encourage the chick to feed on its own.
- As soon as the chick is **2.5-3 months old,** it's possible- if parents don't react aggressive - to remove it from the nest box and introduce it with the parents, while observing continuously! If this cannot be done, the bird should be moved to a socialisation or dating aviary in consultation with the EEP coordinator. This will allow the chick to continue to grow up with conspecifics.

Appendix 5: Guidelines for introduction of new pairs

17/08/2020

New pairs are introduced in September-October before the breeding season starts. This will allow birds to settle down and to know each other before the breeding season starts beginning of January. It is very important that pair bonding can start to establish before the breeding season. In the breeding season, birds could be more aggressive, and this makes pair bonding difficult.

Below is a check list as a guidance to use:

1. Prepare your aviary: clean and check all perches on stability before introducing both birds: it is important that the birds feel safe in this aviary and no or only minimal disturbance is caused by daily management (feeding, cleaning).
   Cinereous vultures are extremely sensitive for disturbance, in particular during the breeding season, so it is important to avoid works or sudden interventions in the aviary or in the neighbourhood of their aviary.
   a. Provide enough high perches (round shape), so that birds can take distance and choose where to sit. Preferably perches are installed all around the aviary (60cm distance from the fence), giving the birds always the possibility to maintain distance between them, while preserving the same height. This in order to avoid that the subordinate bird has to be on the ground to maintain distance and as such finds itself more vulnerable on the ground.
      Perches should not be higher than the nesting platform to avoid that the breeding bird feels vulnerable on the nest (towards the higher positioned, dominant bird). Diameter of the perches should be average 15cm.
   b. Prepare the nest: provide a nest bottom (see also p.52); preferably the nest should be as high as possible and in the back of the aviary, far from the keepers entrance door, so that the birds feel safe.
   c. If possible, install a camera on the nest. This will allow to monitor the pair when there are copulations, at egg laying and during hatching and rearing process.
2. Check whether a blood sample should be taken. This should be taken upon leave of a bird (protocol for blood sampling included in Appendix 6). Samples will be sent to Philippe Helsen, scientific coordinator for the breeding programme: philippe.helsen@kmda.org; postal address: Centre for Research and Conservation/ Antwerp zoo, Koningin Astridplein 20-26, 2018 Antwerpen.
3. The new bird will only be introduced after the other partner has left and preferably the new bird is introduced some days after its old partner has left.
4. Females tend to be dominant in this species. Therefore, newly arrived males should be introduced first in the aviary without the female present, and 5-7 days later when the aviary is known to the new bird the female can be introduced. If the new partner is a female, she can be introduced without an adaptation period. If it is preferred that she gets to know the aviary first on her own, this can be done, but then the introduction period should be done as described above, male first and then later female.

Close monitoring is required: this species is social and will in general not react aggressively, but they might be pecking to the partner. It is important to provide feeding on minimum 2 different locations in the aviary so they can feed independently from each other.

5. Daily management: it is recommended to feed always at the same time (e.g. 10h in the morning) so the birds know this routine. In the husbandry guidelines a diet plan is included, which can be followed. During pair bonding take special attention that both birds get food, preferably cut the food into various pieces or offer more smaller preys, giving the subordinate specimen always the possibility to get food.
6. Sticks are the magic trick! Offer sticks, in small portions, every day or every other day; sticks at this period will trigger the birds for nestbuilding which is important for pair bonding and synchronization of the pair. Nest building is mostly linked with weather conditions: offer nesting material on sunny days or days without rain.
7. If you have to do some enclosure management (cleaning pond,…), organize this at another time (e.g. in the afternoon) than feeding time. Food should not be associated with a bad experience.
8. Look at your birds! Monitor: daily observations by the keepers should be done to identify the behaviours as described in the "protocol for pair bonding". Cameras can be very helpful for monitoring, because these give access to behaviours without disturbing the birds and – when placed in the bird house- keepers can look at the images several times per day. In case of frequent aggressive behaviour between the 2 birds, the studbook keeper should be contacted for advice.

Appendix 6: Sampling protocols for Blood, Feathers, Tissue, Swab and Egg shell containers

Whenever logistically possible, a blood sample should be taken (high DNA yield). However, this might not always be possible or recommended (e.g. stressed and or young birds, availability of a vet or another person competent to collect samples). In such cases 3-10 breast feathers, a buccal swab and/or dried eggshells can be collected in this order of preference. If possible different sample types are collected, in order to have a back-up if one of them fails.

When sending samples please label all tubes but also add the unique ids of all samples on paper including when the samples are taken.

Blood sampling

1. Take 100-300 µl of blood from the brachial vein using a sterile syringe.
2. Collect the blood in a standard EDTA tube or in 1ml lysis buffer (100mM Tris Hcl pH 8.0,100mM EDTA, 0.5% SDS, 10mM NaCl).
3. Mix thoroughly by gentle inversion of the tube a couple of times.
4. Label each tube with a studbook and/or ring number or unique identifier that correlates with the datasheet.
5. Transfer the tube in a plastic container/bag with absorbent material (enough to absorb sample content).
6. Please forward samples asap to the postal address listed below. For storage >7 days prior to sending, blood EDTA tubes should be stored in a fridge (4°C) or freezer (-20°C).

Feather sampling

1. Collect 3-10 breast feathers by direct plucking making sure the rachis/quill is intact.
2. Do not touch the rachis or quill as human skin cells are easily transferred. Pen feathers (those with a residual blood supply) are also useful.
3. Store the feathers in a plastic bag or a paper envelop. Do not mix feathers from different individuals in the same bag.
4. Mark each bag or envelop with a studbook and/or ring number or unique identifier.
5. Feathers may be stored at room temperature (in the dark) until transported to the lab.

Buccal swab

1. Sampling should **not** be performed shortly after the bird of interest has eaten.
2. Gently rotate the sterile foam-tipped part of the buccal swab 5-10 times against the inner cheeks and across the tongue.
3. Break or fold the swab if needed and place them in a plastic tube /container (often provided by the manufacturers).
4. Label each tube with a studbook and/or ring number or unique identifier.
5. Send the swab asap to the lab facilities. If temporal storage is needed, place the swab in a cold and dark environment (e.g. fridge) until shipment.

Egg shell remains

1. Whenever eggshells remain unutilized during fostering processes parts of the eggshell can be collected for DNA analysis.
2. Separate parts of the egg, containing most internal blood vessels. Whenever needed eggshell remains can be broken in smaller pieces. Avoid outer parts to touch the inner parts at any stage.
3. Leave eggshells to dry in a "sterile" environment.
4. Transfer the eggshells in a plastic bag or close the drying container.
5. Label the bag with a studbook number, ring id or any other unique identifier.
6. Samples can be kept dried in a dark environment at RT prior to shipment.

Post Mortem (including early chick mortality)

1. Whenever a blood sample can still be collected (recent death) follow the guidelines as described above.
2. Collect a 1 cm^3 tissue of an internal organ (preferable liver).
3. Whenever no PM analysis is planned external tissue samples can be collected (e.g. toepad or part of the tongue).
4. Place sample in a plastic tube (such as a 2mL screw-cap tube).
5. Label tube with studbook id, ring number of any other unique identifier.
6. Freeze at -20°C or -80°C immediately or preserve in ethanol (70-90%).

If you need a kit to collect a sample or swab of your cinereous vulture(s), please contact Philippe Helsen to provide you with one.

CITES: General rules according to CITES regulations:

Within the EU: there is no need for CITES export/ import permits, but exemptions may apply, so check national rules applying for blood/ feather samples for scientific research.

Outside the EU: CITES export permits must be applied for at the national CITES office. Remember to apply for permits ahead of shipping samples.

PLEASE SEND ALL MATERIAL ASAP TO:

KMDA-CRC
Philippe Helsen
Molecular Lab
Koningin Astridplein 20-26
2018 ANTWERP
BELGIUM

Such samples will be used for long-term management of the EEP population. For questions/comments please contact:

Philippe.helsen@kmda.org or Frederic.verstappen@kmda.org

Appendix 7: Egg swapping in cinereous vulture to check on fertility through candling

Report made by the bird keepers at Planckendael zoo (March 2023)

Figure 1 Incubating bird (08h56)

Figure 1 Keepers enter the aviary and the bird leaves the nest (08h57)

Figure 2 Keepers climb to the platform with nest with pre-heated dummy (08h 57)

Figure 3 Keepers take the incubated egg away and remove protruding twigs (8h 58)

Figure 5 The pre-heated egg is put in its place (8h 58)

Figure 4 The keepers leave the nest platform and safely pass on the incubated egg (8h 58)

Figure 5 The keepers left the aviary and the bird immediately returned to its nest (9h 01)

Figure 6 The bird goes right back to incubating (9u 02)

- Breeding pair (VBC 6): Zlatan x Imke (ID 241032 x 241040); # 1303 x # 1703
- Egg checked on 21/03/2023 or day 7 of incubation → egg fertilized
- Weight of the egg: 243.77 grams