UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento Biomedicina Comparata e Alimentazione

Department of Comparative Biomedicine and Food Science

Corso di laurea /First Cycle Degree (B.Sc.)

in Animal Care

Conservation of the Egyptian vulture (*Neophron percnopterus*): captive management, breeding and release

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ACADEMIC YEAR 2021/2022

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Summary

In the last decades, worldwide ecosystems have been facing a quick and intense loss of biodiversity; in fact, human-related activities and urbanization of wild areas have been progressively mining the pillars on which many animal species rely on for their survival. This means that, without effective protective measures/actions, together with the already extinct animal species, in the close future many more will be challenged and threatened implying that the extinction rate of the weaker ones will keep increasing.

Luckily, different strategies to solve or slow down this process and thus help to protect/restore the threatened environment, including animal species, have been developed and implemented. One of these involves captive breeding programs for the species which are threatened in the wild, in order to support the size of the wild remaining population by an influx of specimens bred in the safeness of captivity. This, in addition to other *in situ* strategies can prevent the extinction of key species.

The present work focused on the Egyptian vulture (*Neophron percnopterus*), a small Old-World vulture categorized as threatened of extinction by the International Union for Conservation of Nature's Red List of Threatened Species (IUCN): after collection and analysis of the existing scientific literature, a management and breeding protocol for the conservation of this species has been developed. The main idea is that the present protocol could be useful whenever dealing with this species in the captive environment, to provide proper care and fulfil all the species requirements. The attempt is to try to cover every relevant aspect for a successful captive management, including design of the aviary, dietary requirements, indications for a successful breeding (considering also possible issues that can arise with possible remedies), and required measures for a successful release and introduction in the natural environment.

1. Introduction

1.1 Taxonomy

The Egyptian vulture (*Neophron percnopterus*) is an old-world diurnal raptor belonging to the family of *Accipitridae*, the same of eagles, hawks and kites. It comprises small to large raptors with strongly hooked beaks and variable morphology based on diet. Species of the *Accipitridae* family can feed on a wide range of prey items from insects to medium-sized mammals, with some species consuming carrion or fruit.

Specifically referring to the Egyptian vulture, it was formally described by Carl Linnaeus in 1758. It is the sole species of the genus *Neophron*, the oldest branch in the evolutionary tree of to the other living species of vultures and represent one of the basal taxa of the accipitrid lineages (Wink et al., 1996).

1.2 Subspecies

Regarding subspecies, currently there have been described three of them, even though there's quite widespread gradual variation even among population of the same specie considering the gene flow due to the movement of specimen among geographical areas (Donazár et al., 2002).

N. p. percnopterus is the most abundant subspecies, it is distributed throughout southern Europe, northern and central Africa, the Middle East, Central Asia and the Indian subcontinent. It is barely distinguishable by a darker beak tip. *N. p. ginginianus* (Latham, 1790), mainly located in Nepal and India is slightly smaller and the tip of the beak is paler/yellow. *N. p. majorensis* is the latest discovered species, described in 2002: it's endemic to Canary Islands and considered non-migratory (while the other two migrate to Africa in winter), the appearance resembles a lot the *percnopterus* one, with the only difference in size, 0,5 kg heavier and 20 cm larger wingspan (Mishra et al., 2018).

1.3 Physical description

Fully grown adult individuals weigh around 2 kg, with a body length comprised between 58 and 70 cm and a wing span up to 170 cm; recognisable from distance by their flight silhouette, characterized by well-fingered wings and wedge-shaped tail. For what regards their lifespan, it is reported that in captivity they can live up to 37 years (Vulture conservation foundation, 2022).

In general, Egyptian vultures present a yellow to orange bare face skin, with sharp hooked beak that has the same colour of the face and the coloration of the tip that differs among subspecies. Nape and neck have long and lanceolate whitish feathers that gives the characteristic “unpreened” appearance. Primary and secondary wing feathers are black, whereas the rest of the body appear brownish/rusty-white, however this is observed only in wild individuals and it’s due to the fact that, for camouflage reasons, the birds rub themselves in muddy or iron-rich soil, in fact captive-kept specimens have pure white feathers. The terminal part of legs is featherless with pink coloration and each of the 4 phalanges end in a sharp black claw. Nestlings of Egyptian vulture have a dark grey/black plumage that progressively gets lighter in the following 4 years going through different moults before reaching the adult stage.

Even though this species doesn’t present a remarkable sexual dimorphism, it has been observed that the male has a slightly more orange face than the female and it was believed that the blackish spots present under the eyes of some individual were identifying the females, though nowadays it’s proved not indicative due to the presence of them even on males, though the frequency is actually higher in females (Clark and Schmitt, 1998). Besides morphological appearance, behavioural observation and size comparison (the female is slightly larger than the male, as frequent in raptors) are the most reliable assets to evaluate gender. Other methods that are used, common for all birds’ species, are DNA analysis and endoscopy; these methods give clear and precise data but are more invasive, stressful for the animal and expensive, thus performed only if specifically required.

Fig. 1 Egyptian vulture resting on a stone (Oriental bird club, 2022)

1.4 Habitat and distribution

The Egyptian vulture is mainly distributed in the regions surrounding the Mediterranean Sea with the majority of individuals concentrated in the Iberian Peninsula. According to the subspecies, then it can be found endemically in Canary Islands (*N. p. majorensis*) and in the Nepal region (*N. p. ginginianus*). It usually remains in inland environments and it prefers rocky areas such as inland cliffs and mountain peaks, but it is also found in other dry regions like deserts and savanna and eventually shrubland, grassland and wetlands (inland) (Birdlife international, 2021), often comprehended in its home range (up to 7000km^2 for non-breeding individuals) (McGrady et al. 2019).

1.5 Conservation status

The Egyptian vulture is endangered species, as classified by IUCN since 2007; moreover, it is known to be already extinct in some of the historical regions where it was used to be present such as South Africa, Romania and Ukraine.

Between 1980 and 2001 European and middle east populations decreased by roughly 50% while in India in 2003 there was only 20% of the population present in 1990 (Couthbert et al., 2006); breeding pairs in Italian peninsula dropped from 30, in 1970 to only 9 pair in the 90's (Liberatori & Penteriani, 2001). Looking at the *N. p. majorensis subspecies*, endemic to Canary Islands, between 1987 and 1998 the population decreased by 30% (Palacios, 2000).

It's global population size it is currently estimated to be between 12000 and 36000 mature individuals (Birdlife international, 2021).

As many other species, the major threats are represented by human-related activities and, in some cases, even the progresses of human society; an example of this is the sharp decline in feed availability due to the latest regulations in terms of carcass disposal for farmers. No more carcasses were left in the fields for obvious sanitary and public safety reasons (Regulation CE 1774/2002), but this affected scavengers' diet availability with a consequent decrease in population abundance (Dobrev et al., 2016). Other threats include electrocution on electric cables, landscape changes, habitat loss due to urbanization, direct persecution by hunters and disturbances originated by increased mountain tourism (Donazan et al., 2002), that leads to disturbances of nesting sites and force the animals to search for new areas that might be less suitable in terms of climate and feed availability.

1.6 Relevance of captive breeding

In order to support an endangered wild population, it is possible to adopt different strategies. An *in-situ* conservation, aims to act on the natural environment of the species, such as habitat preservation initiatives, creation of corridors to connect patches of safe areas, protect nesting sites, create new reserves, establish new laws or increase reinforcement of already existing laws.
An *ex-situ* conservation can support the wild population by increasing the number of total wild specimens by providing new animals, obtained through captive breeding, to the natural environment. Breeding the animal in captivity allows to provide them a more stable environment, safe from predators and hunters, better disease control and a constant supply of feed, which are frequent causes of low reproduction rate and mortality in the wild (Robert, 2009).

However, captive breeding has its downsides, first of all the reintroduction success with captive bred individuals, tend to be low (compared to translocating wild-born ones) since they're specimens completely dependent on human to survive, they're not used to searching food nor shelter, and across generation the captive bred individuals' can lose traits that might be fundamental to survive in the wild since in captivity natural selection doesn't occur and trait selection act on characteristics that favour the animal in a captive environment rather that a wild ones (e.g. ability to search and find food is not selected and therefore in the wild these individuals will have reduced fitness and so, reduced reproductive potential): it has been estimated that the reproductive capacity in the wild, of released individuals, can decrease up to 40% for each captive reared generation (Robert, 2009).
Moreover to ensure a retention of genetic diversity and to keep low inbreeding rate despite the moderate number of captive kept individual is fundamental to have strict breeding programmes that plan each single mating and try to maximise generation interval to overcome previously indicated problems; the EAZA (European Association of Zoos and Aquaria) takes care of this through his EEP, European Endangered Species Program, financing captive breeding of the species and ensuring to maintain high conservation value of the bred individuals.

2. Discussion

2.1 Captive environment

Due to the limited literature regarding the enclosure design of Egyptian vulture, especially for what concern housing in zoos, in the present subchapter the information including management protocols from closely related species such as griffon (*Gyps fulvus*) and bearded vulture (*Gypaetus barbatus*) have been integrated.

While designing the enclosure it is important to consider that the reproductive potential and the lifespan of the animal are positively correlated with the sense of safety and the lack of stress perceived by the animal (Frey & Llopis, 2014), therefore better management of the birds leads to better results in terms of breeding results.

In the case the birds are kept in a breeding facility, where public is not allowed to enter and where the animals can have greater privacy, the minimum size of the enclosure for each breeding pair can be set at 7m x 4m x 3m (L x W x H), (Ceccolini & Cenerini, 2018) starting from the dimensions indicated when dealing with bearded vulture (6m x 12m x 4m) (Frey & Llopis, 2014), considering that the bearded vulture is approximately 3 times the size of the Egyptian vulture (2 kg for the Egyptian vulture and 7-8 kg of the bearded vulture) (Animalia, 2022).

When designing the enclosure, it is fundamental to consider that larger enclosure doesn't necessarily mean better results; in fact, when staff enters the aviary for maintenance and cleaning the animals might get scared and start flying anxiously trying to escape the "threat" and, since a larger enclosure means more space to gain speed, the bird can get seriously injured by hitting the mash or the structure (Frey & Llopis, 2014).

As in every structure built for animals, even for the enclosure the chosen material needs to be safe for both animal and keepers, resistant and durable to reduce cost and frequency of maintenance. Wood can be considered a good material since it is softer than metal and might prevent collision-related injury severity, although it requires greater maintenance and cleaning is more challenging. If metal is chosen, internal edges needs to be round-like and, ideally, mesh should be positioned leaving the structure outside to minimize risk of injury for the animals. Moreover, inside the enclosure there must not be frame of structure's support which birds can hit when flying around. (Frey & Llopis, 2014). Round rod dowels are a great solution, since they allow great surroundings visibility form inside the aviary while preventing majority of injuries usually related with the mesh.

Whatever solution is adopted its important that the space between the rods or the size of the mesh is preventing predators and ideally pest from entering the enclosure (habben & Perry-jones, 2016), and to be appropriate even to prevent the hosted birds from getting stuck.

Glass clear windows needs to be avoided since the animal cannot see them and might collide while flying; in the case there's a glass window, it is necessary to apply stickers or other material on it in order to make it evident to the bird.

It's important that the animal has access to sun and rain but to also have a protected part, therefore the roof of the aviary should have a covered portion, below which also nest box is located, and a part made of mesh through which sunshine and rain can pass.

For what regards the sides, at least one needs to be made of wall/obscurant material, undisturbed, to reduce possible stress factors; while dealing with adjoining aviaries, separation walls prevent the birds to see their neighbour avoiding wire gripping behaviours (to reach other birds) and reducing the stressfulness of the environment (habben & Perry-jones, 2016).

When dealing with animals kept in the zoos, it's necessary to take in consideration that the presence of visitors, voices and other noises, represent a great source of stress for the animals.

In order to cope with this external stressor is necessary to increase the size of the aviary, especially the height, doing so, the distance that the animal can stay at a greater distance from visitors, increasing his sense of safety that is require for breeding (Frey & Llopis, 2014).

As reference, the size of the enclosure in a centre opened to public for bearded vulture such as at Barcelona zoo, measures 20m x 10m x 5m (W x L x H) (Frey & Llopis, 2014), considerably larger compared to one closed to public.

Off show accommodation should provide to the animal the same degree of welfare than the on show one: in an off-show context, functionality can be prioritized and some costs to improve the aesthetic aspect of the enclosure can be saved.

Nest box should measure 2m x 1m x 1m (W x L x H) and can be made of wood, one of the long sides is opened facing the inside of the enclosure. Inside the nest box, a small nest area (roughly 70cm x 70cm) is limited by a wood frame.

A breeding facility can greatly benefit from a video-surveillance system, for the aviary in general and specifically for each nest box, in order to keep under control, the situation and spot problematic behaviours between member of the pair or towards the eggs or the chicks/fledglings.

Video surveillance allows to greatly reduce stress on animals by minimizing human disturbances that can be limited to routine cleaning, feeding and general maintenance procedures (Ceccolini & Cenerini, 2018).

Regardless the type of centre, the aviary needs to cope with all the needs of the animal: sufficient space to completely stretch the wings, and ideally, large enough to allow flying, a view to the outside on at least one side of the enclosure, several perching spots of different sizes and position and clean and fresh water (Frey & Llopis, 2014). A particular species-specific need for the Egyptian vulture is the availability of a muddy pond in which the bird can, especially prior the breeding season, bath and stain the white feathers, gaining the characteristic brownish discoloration (Ceccolini & Cenerini, 2018). Moreover, the enclosure has to ensure the safety of the held animals, protection from predators, visitors and pest, at the same time prevent the bird to escape while providing enough opportunities of expressing natural behaviours (Frey & Llopis, 2014).

Maintenance has to minimized and the staff need to be quiet when performing procedures, precise and swift, but at the same time careful. Once a day is necessary to enter the aviary, remove food remains and change water bowls, remove eventual thorny plants grown on the ground (they can injure the feet of the vultures) and also grass should be cut when needed. Electric lawnmower can be used when dealing with calm birds; if it is used in aviaries with easy-to-scare specimens, the animal must be placed somewhere else during the procedure (e.g. Ttansport crate); furthermore, during the reproductive season electric noisy devices must be avoided because every unnecessary disturbance might compromise breeding success (Ceccolini & Cenerini, 2018).

Veterinary checks need to be regularly performed; to avoid disturbance during the reproductive phase, it is recommended to plan one check just prior the breeding season. At that time, it is important to check beak length, considering that, especially in captivity, it might overgrow and affect feeding behaviour. The beak is cut with very sharp bolt cutters, first one side then the other in order to maintain a sharp point. Claws suffer the same problem; therefore, it might be required to trim them to avoid foot problems (Ceccolini & Cenerini, 2018).

2.2 Diet in the wild and in captivity

For what regards its dietary habits in the wild, the Egyptian vulture is an opportunistic scavenger: it means that it usually doesn't actively hunt live prey for feeding, but it searches for already-dead individuals, died from natural causes or leftovers carcasses from other predators.

However, it has been reported that a pair living in Mount Stakar (Bulgaria) actively hunt live tortoises and seems to count on them for a 13% of their diet (Milchev et al. 2012).

Several studies regarding its dietary habits have been conducted by different researchers' groups, collecting and analyzing feeding remains in nests (e.g. bones, meat, feathers, tortoise shell, etc.), in different regions of the species' distribution area, with different results.

Data obtained from Bulgarian populations of Egyptian vultures, indicated that they mainly feed on mammals (up to 67%), typically ungulates and especially young individuals, cattle, wild carrions and human wastes (found signs of cooking or other human related processing). They feed even on birds' carcasses (15%), from both wild and farmed ones, and from scaled reptiles (14%). Insects remains have been found too but they're usually ingested with the meat from the decomposing carcasses, and there are no proofs that the animal actively search for them (Milchev et al. 2012).

In general, there's a positive correlation between cattle remains in the nest and the occupancy rate of the territory (across years), while nests with higher portion of remains from wild animals tend to have a lower occupancy (Dobrev et al. 2015) therefore the animals tend to nest and live in regions where they can find cattle carcasses. In some other studies, in some regions of Bulgaria, tortoises remains represented up to 32% of the total analyzed remains and this might indicate a preference for that species though there are many factors to be considered, such as the possible higher density of tortoises in those area and the presence of already dead ones due to the fact that golden eagles catch and drop them on rocky surfaces and then leave them; moreover, tortoises has a very conspicuous amount of remains (shell and other bones) compared to the amount of meat, differently from mammals for example. Due to these factors, such a result might overestimate tortoise importance in the vulture diet (Dobrev et al. 2015).

Different results have been obtained in the Iberian Peninsula, where there seems to be a balanced distribution of feed remains: almost 50-50 between birds and mammals. This study reported even a quite high frequency of visiting rubbish dumps, where vultures feed on meat thrown away by humans; coprophagy has also been reported (Hidalgo et al. 2005).

In Sicily (Italy) feed remains in nest were mainly coming from wild animals, small mammals such as rabbits and wild birds while, in previous studies (before modification on cattle farming), cattle were actually counting half of the feed remaining in nests (Di Vittorio et al. 2017).

To briefly summarize, the Egyptian vulture has a pretty various diet, it can feed on mammalian carcasses and excrements, human food refuses, eggs, invertebrates and other small vertebrates (Dobrev et al. 2015); the proportions greatly vary according to the geographical region, species richness and density in the environment, farming system and proximity to human settlements; this is perfectly in accordance with the definition of opportunistic scavenger.

Something else that must be considered is that these investigations on feed remaining to evaluate the dietary habits, might be biased by the fact that not all the feed is consumed in the nest and not all the feed leaves remaining (such as soft tissues).

For what regards the diet in captivity, as common for other raptors species, the most used food items are mice, rats, and also poultry, the latter however in a lower amount. The daily feed intake of the Egyptian vulture in captivity was reported to be comprised in the range 180-200 g (Ceccolini & Cenerini, 2018). Besides the fact that these animals are scavengers in nature, the quality of the feed and its conservation needs to be appropriate; when dealing with frozen material is always necessary to thaw it completely, making it reach room temperature, before delivering it to the animals. Powdered supplements of vitamins and minerals are also available on the market that can help integrate the captive diet.

In the case the vultures are kept in zoos there might be the possibility to feed whole carcasses of dead animals from other exhibits. This is a very controversial topic; in any case the carcass needs to not be contaminated and there might be country-specific regulations that prohibit this practice. Majority of north America zoos actually feed vultures often with whole carcasses, as this practice demonstrated to enrich the welfare of the animal and also supplement the diet with nutrients not found in commercial mice and rats (Gaengler, 2015). As a general recommendation for feed administration, when dealing with animal destined to the reintroduction into the wild environment, and especially during the breeding season when human interaction might be deleterious for the reproduction success, feed should be delivered in the aviaries through tubes in order to reduce staff disturbances to the minimum.

2.3 Reproduction in the wild and in captivity

Egyptian vulture is a monogamous specie, it breeds once per year and the breeding season slightly changes across its geographical area of distribution, but indicatively occurs in spring (northern hemisphere) between march and may (Rosenblatt, 2007).

Sexual maturity for female is reached at a mean of 6.88 and males at 7.25 years old, although effectively female can reach sexual maturity as early as 3 or as late as 14, while males tend to be older but with a smaller variance, between 5 and 11 years old (Sanz-Aguilar et al., 2017).

Usually such a high variability in first recruitment is detected in females belonging to small populations, they can advance the age of reproduction to increase the time persistence and maintain the population at the carrying capacity for longer (Ferrer, 2004), although this is improbably the case

since the population is declining and already below the carrying capacity of the environment; therefore, the real reason remains unknown.

Male age of reproduction is suggested to be determined by a trade-off between survival and early recruitment, moreover, an important role is also played by demographic mechanisms within a single population and the animal needs to gain experience in foraging and territorial defense, therefore it results that higher breeding success is obtained with older ages (Sanz-Aguilar et al., 2017).

Vultures arrives at the breeding territory at the end of the winter, migrating back from the wintering territory located in the sub-Saharan region of Africa to which they return at the end of the breeding season, August/September (vulture conservation foundation, 2019).

Nesting territories are characterized by the presence of high cliffs, with ledges or cavities suitable for nesting (Donazan et al., 1994), the majority of nest tend to be faced towards east and south, mainly on caves or sheltered ledges (García-Ripollés & López-López, 2006); as mentioned above the birds arrive around February-March and start the courtship process in order to find a mate (although, pairs might be maintained across years); as in other vultures species, courtship behaviors don't include reciprocal feeding (Donazan et al., 1994), and it consists of swooping display and talon grapping (Rosenblatt, 2007).

The nest is formed by old rags, hair or fur (Rosenblatt, 2007) in order to soften it and make it cozier for the chicks. Especially during the pre-laying period, the female is rarely left alone by the male, copulations starts roughly 25 days before laying the first egg, mating seems to never occur in the nest (Donazan et al., 1994) and copulations occurring during foraging trips are difficult to record since they can move as far as 20 km away from the nest in search of food (Oppel et al., 2016). So, in general copulations observed in the nesting territory reveal that the Egyptian vulture as a high copulation rate with a mean of 2 times a day, that increases approaching laying date, with each successful copulation lasting a mean of 13.7 seconds. Male paternity-guarding strategy is indeed based on mating frequently and not leaving alone the female, even though copulation during foraging trips (out of nest territory) has not been confirmed it might be probable since the animal spend their time together even then and copulations have been recorded during foraging in observation areas. Mating has been recorded also during the nesting and fledgling phases; this indicates that besides being fundamental to fertilize the eggs and acting as a way of ensuring parenthood by the male, copulation is probably a way of maintaining pair bond (Donazan et al., 1994).

Egyptian vulture lays 1 or 2 eggs per season (Oppel et al., 2016) and usually the time between the first and the second egg (inter egg interval) is quite long, ranging between 3 and 8 days (Donazan et al., 1994). The mother tends to incubate the eggs alone for the first days before sharing duties with the male, parental cares are indeed shared, feed is brought to the nestling from both parents that either

carry it with feet or swallow and then regurgitate it, territory defense duty is also shared by the pair (Rosenblatt, 2007).

Hatching of chicks takes around 42 days, between 39 and 45 (Balaban & Yamac, 2018; Rosenblatt, 2007), and due to this considerable window of time usually a chick is more developed and coordinated then the other (Rosenblatt, 2007). Nesting period last between 70 and 90 days, after that chicks are fledged (Balaban & Yamac, 2018) and can be seen flying around in the nesting territory along with parents (Rosenblatt, 2007).

When studying reproduction of a species several data are collected in order to have a picture of how well a species is doing in nature and if it's coping with environmental threats:

1) The first parameter examined is the mean breeding success, meaning the number of chicks born from a pair that engage breeding. Across the geographical range of the Egyptian vulture many studies have been conducted with the following results: in 2018 Turkey was the country where the highest breeding success was detected (1.15), followed by Italy (0.99), Macedonia (0.93), Spain (0.6-1.1) and Bulgaria (0.73) (Balaban & Yamac, 2018; Liberatori & Penteriani 2001).

2) A second parameter is the mean productivity, so the number of chicks successfully fledged per monitored pair; in this are considered even couples that haven't even start to breed so rate is going to be equal or lower than the breeding success; higher productivity has been recorded in Italy (0.99), Spain (0.8-1.1) then Turkey (0.85), Macedonia (0.84) and Bulgaria (0.63) (Liberatori & Penteriani 2001; Balaban & Yamac, 2018).

3) A third parameter is mean fledging rate, (considering only successful pair, so only the couples that fledged at least one chick) which was reported to have the highest value in Turkey and Bulgaria (1.3), followed by Italy (1.27), Spain (1.17-1.22) and Macedonia (1.19) (Balaban & Yamac, 2018; Liberatori & Penteriani 2001). Fledged young leave the family at the time of migration (august/September) going towards wintering territory in Africa (Rosenblatt, 2007).

Besides the encouraging breeding parameters, the global population of Egyptian vultures still lowering; this cannot be addressed to a specific environmental factor, and although suitable nesting sites and feed availability have been pointed out to be important in the selection of nesting territory, they doesn't affect enough the breeding outcome to justify a such sharp decline of the wild population; stochastic events causing death of individuals such as illegal poisoning, direct human persecution and collision with wind turbines are constantly contributing in reducing the number of breeding animals (Oppel et al., 2016). Therefore, there's not an actual, fundamental problem affecting significantly the reproduction in the wild, some variables might influence it lowering it a bit but the main cause of decline is not coming from reproduction problems.

Reproduction in captivity present many different challenges and obstacles but, dealing with a controlled and protected environment has its own benefit. Effectiveness of captive breeding rely first of all on the suitableness of the aviary in which the animal is supposed to reproduce, appropriate nutrition is also fundamental; those parts have been extensively covered in paragraphs 2.1 and 2.2.

Once established the right environment and feeding, the pair formation is probably the most challenging part; the two individuals might, just by personal preferences, not like each other, the male can get aggressive towards the female while approaching the breeding season, or the pair can be quiet and stable but not engage copulation (Ceccolini & Cenerini, 2018). In the wild the birds would choose by themselves the mate that they want to breed with but this is not possible in captivity so, when the two specimens are chosen to form a pair the introduction to each other has to be gradual and slow. First contact needs to be only sensorial so there's no risk of aggression, for example the two cages holding the animal can be put close to allow them to see each other. Once possible hostile behaviours are not detected, the animals can be set free in the same aviary and strictly monitored for a while.

To positively evaluate the new formed pair, there are some specific behaviours that should be monitored: allopreening (grooming each other), is a powerful sign of compatibility, while feeding the animal shouldn't compete and share the meal quietly; male always makes slow movements while close to the female and tend to keep an upright posture, sometimes it might also show a "submission" sign by bending horizontally close to the mate. Female normal posture is horizontal, when approaching the male for copulation the tail can be a bit lifted (Ceccolini & Cenerini, 2018).

Aggression in the male is manifested by extended neck, fast gait and chasing or even biting toes of the female; female might not be available for copulation in that case she won't crouch and will escape when the male tries to mate, this can lead the male to frustration and can happen that he gets a stone in the nest and try to hatch it.

Aggressiveness is frequent in males that has never met before; in general, is possible to cope with this aggressive manifestation (not 100% effective) by placing the male in a cage inside the aviary (without visual access to the nest) as soon as aggressive behaviour occur and leave it there for around five days, after being set free the bird should be back to quiet (Ceccolini & Cenerini, 2018).

Nests are prepared prior to the breeding season: different soft-like material can be used, for the bottom layer straw is perfect and it needs to be clean and without any mould. On top of that it is added uncarded wool (cut in small pieces <4cm so claws don't get tangled); it's important that any stick or rocky/hard material is present in the nest because it could lead to egg-breaking. From the beginning of the breeding season (around march) more uncarded wool can be spread around the aviary so the birds can bring that to the nest and they will eventually improve it by themselves adding their

own plumages, this besides encouraging natural behaviour is also an enrichment and at the same time can be a good indicator for compatibility if both parents are engaged in it.

Copulation start around 25 days prior to the laying of the first egg and, usually, after 5-6 days another egg is laid, with the inter egg interval possibly increasing up to 15 days.

It is fundamental to monitor the couple, especially in this phase because after the laying of the eggs many more problems can arise (video surveillance come really in handy in this case). The fact that a pair seems doing well before laying doesn't imply that there won't be problems later on; for instance, the female can get aggressive towards the male and the two might fight to compete for egg incubation and fight in the nest frequently leads to egg breaking. Male might also eat just laid eggs; in general, these events are predictable by abnormal behaviour in phases prior the laying (such as not cooperating in nest enrichment) but, as mentioned above, they might just be unpredictable.

Important thing of this species is that the male can actually incubate the eggs even if the female is removed while the other way around she just eats the eggs after male removal, and this is important to keep in mind while thinking about parental fostering, a single male can be used while a single female cannot. The best practice is so to artificially incubate the egg to avoid any kind of behavioural problem to threaten the future newborns; although, in order to not create problem on the parent side, it is important to substitute the real eggs as soon as they are laid (also here video surveillance is quite necessary for perfect timing) with "fake" clay eggs (exactly similar to the real ones and preheated at 35 °C to not be perceived as food and try to be ate by the female) (Ceccolini & Cenerini, 2018).

2.4 Egg hatching and chick nursing in captivity

Total egg incubation period lasts 42-43 days the right temperature is between 37.3 and 37.5 Celsius degrees and humidity between 45 and 50%.

First thing when dealing with egg incubation is to check the equipment, everything needs to function properly, temperature and humidity needs to be correctly maintained constant, eventual rotating system for the eggs should work smoothly and an emergency power source should be available as safety to guarantee continuity even in case of power interruptions.

All the materials and machines have to be sterilized with a solution of alkyl-benzyl-dimethyl-ammonium chloride (20 mg/l of distilled water). The same solution will also be used to gently clean the eggs after the collection and is also the one used in the incubator to create the right humidity. Right after the collection of the eggs from the nests, they're carefully examined and eventual excrement residues are scrapped away with a cutter before disinfecting them with the solution as said

above. After marking each egg with an identifying code, the eggs have to be measured (both diameters) and weighted, they will be weighted every day until the hatching to monitor the weight loss. Eggs are handled as little as possible and always wearing latex gloves.

Eggs should be positioned with the small end facing downwards, turning is suggested to be done manually during the day (8am-11pm), 90° counter-clockwise rotations every 3 hours (6 times) and automatically during the night (2am-5am), 90° every 3 hours (2 times). Marking the egg with different colour spots at poles can help in avoiding mistakes in the direction of the rotation. Eggs are also removed from the incubator 3 times during the daylight, 10 minutes each to cool down resembling wild conditions in which parents might briefly leave the nest; in this occasion the eggs are weighted.

Ideal weight loss across the whole incubation period is 14 % in 40 days, adjustments on the humidity percentage can be done to regulate the weight loss, higher humidity will slow it down while lower humidity will speed it up; changes in humidity shouldn't be done after 2/3 of incubation phase.

Starting from 15th day of incubation is possible to candle the egg to monitor the growth of the embryo and the tilt of the air cell; from day 30 is also possible to check viability of the embryo by gently un-stabilizing the egg on a flat surface and it should keep moving by itself due to embryo movements. Abnormal trends of weight loss and bad smell coming from an egg might indicate that the embryo is not viable anymore, therefore it is recommended to isolate the egg in question to avoid problems to other eggs in case of rupture (Ceccolini & Cenerini, 2018).

Hatching process usually take three days: the chick breaks the internal membrane and starts chirping; as soon as this happens, rotation has to be interrupted, the egg placed on a towel and temperature should be lowered at 37 °C while humidity is kept steady.

On the second day usually, the chick breaks the egg shell: at that point is time to increase humidity up to 65%.

The third and last day is when the complete hatching occurs; after one hour more or less all the shell residues must be removed and the chick should be gently cleaned, disinfected and the dry cord needs to be cut, the bedding towel should be changed and a white paper towel can be positioned on top of the fledgling to resemble parental care (Ceccolini & Cenerini, 2018).

Mispositioned chicks can be spot by candling the egg, usually the head of the embryo is located towards the round end but in some cases, it can be directed towards the small end; this can happen for several causes, one of them can be incorrect position of the egg in the incubator (with small end upwards). Mispositioned chicks tend to start the hatching process a little bit in advance, once the hatchling breaks the egg shell (frequently just a crack) it'll need assistance in getting out due to the improper posture and higher resistance of the small end. Egg shell and membrane should be

removed gently and slowly trying to free the neck and the head of the chick with tweezers; it's a tedious process and it's important to wait until blood vessels recede from the portion of membrane that you're willing to remove before doing so otherwise the chick might bleed and die; after each intervention the egg has to be put back in the incubator.

Freeing of the neck should take around two days, the rest of the shell is left in place to allow the absorbing of the yolk sack, at that point the fledgling should push the shell away, after that it needs to be cleaned, disinfected and the dry cord needs to be cut, bedding towel is changed and a white paper one is put on top of it, as is done with all the other birds (Ceccolini & Cenerini, 2018).

Around 5 hours after hatching the newborns chicks can be transferred to a brooder. It is important to not let the bird stand on a flat surface otherwise it might develop legs problem, therefore is recommended to create a concave "nest" with soft material and place the chick in there with a towel below, changing it frequently as it gets dirt and wet.

Initial temperature is 37 °C and it'll be lowered by 0.5 °C each day until heating is not needed anymore, humidity is also gradually lowered from 60% to around 50%; while decreasing temperature is necessary to observe the response of the chicks, in fact body posture can suggest that the bird is too cold (curled position and inactive) or too hot (stretched limbs and neck). Even in the brooder the chicks should be covered with a white paper towel to resemble parental presence.

As soon as the fledgling start pecking around and rising his head food is provided, rat and mice small pieces of meat (without bones and fur) needs to be at room temperature (not refrigerated) and can be offered to the bird with tweezers wearing a painted glove or with a fake Egyptian vulture puppet (see "imprinting and how to avoid it" paragraph below). Feed is provided as they demand it but is important to not overfeed them (look at crop size) on the first days.

It is recommended to limit human interaction as much as possible especially between day 5 and 15 since it is a really sensitive period for the young. Best rearing method is to bring back the fledglings to the parents, foster couple or to a single male with half of the egg shell that is going to be removed shortly after along with the clay egg that was already in the nest and that have been incubated until that moment. If no trustable adult is available to foster the chick is at least important that the fledgling sees adult individual by placing them in a cage close to them or vice versa.

Young Egyptian vulture can be released at 80 days of age, but, if it is necessary to wait the following year for the release, the birds should be hosted in a closed cage to limit their interaction with keepers and avoid imprinting to humans (Ceccolini & Cenerini, 2018).

2.5 Imprinting and how to avoid it

Imprinting is rapid learning that occurs during a brief receptive period, typically soon after birth or hatching, and establishes a long-lasting behavioural response to a specific individual or object, as attachment to parent, offspring, or site (www.dictionary.com, 2022).

Usually imprinting occur in the nest, towards parents, but when dealing with hatchlings coming from incubator or in general newborns in the early stages is important that imprinting process doesn't occur towards humans and this becomes fundamental if the bird is going to be released in nature. In fact, if the fledgling understand that feed and cares comes from human he might search for them once adult and free in nature, leading to potential life-threatening situation with poachers (Ceccolini & Cenerini, 2018).

There are several ways in which is possible to reduce human influence onto young Egyptian vulture: as mentioned in the paragraph above during the feeding process is possible to wear a glove on which is drawn an adult individual (fingers black as the beak and yellow/orangish hand with an eye), otherwise is possible to use a more detailed puppet with beak, face and feathers to wear on the hand while feeding the chicks. In any case the caretaker should always be completely covered with a suit that hides his appearance to not habituate the bird to the human morphology (Ceccolini & Cenerini, 2018).

Fig. 2 Egyptian vulture puppet to handfeed chicks avoiding imprinting (@associazione.cerm)

In general, less time the chick spends under full human care the better it is so choosing a foster pair or a single male if the biological parents are not available should always be considered as the best option. If this is not possible is important to not treat the fledgling like a pet, play recording of the adults calls during the feeding (wildlife veterinary trust, 2018) or by placing an adult in a cage by the chick to get it familiar with his own kind.

If fostering couple are limited it can even be considered to swap the chicks in the nest in order to make them spend the most sensible period (between day 5 and 15) with the adults and then switch back to human care after that and put again another younger chick with the adults (Ceccolini & Cenerini, 2018).

Young individuals approaching release should still have less contact as possible with keepers so the aviary should be closed, not allowing them to see outside and feed should be provided through a tube to avoid them to link human and food availability (Ceccolini & Cenerini, 2018).

2.6 Release methods

Talking about releasing birds in nature, specifically raptors, we can adopt different solutions, fostering, hacking and delayed-release (Vulture conservation foundation, 2022).

Fostering consist in placing a growing-up nestling (around 60 days old) that is approaching release period in a wild nest, already held by a couple and with wild nestling of similar age inside. Best would be to choose a nest with only one chick but successful results were obtained also with nest containing two chicks, in that case an artificial feeding station were present close to the nest to support the parents in the food provision for the extra chick; both wild nestlings and both parents accepted the new entry straight away and started feeding it (Di Vittorio, 2006).

Hacking method can be considered a soft-release one since it implies an acclimation period during which the bird gets used to the natural environment in which it'll be released and human interactions are almost absent. It is generally performed by creating an artificial nest, a nest box in the wild, closed with a net, in a safe and controlled spot where nestlings, at around 80 days of age (Ceccolini & Cenerini, 2018), ready to be released, are left alone, provided with feed and water, for some days. After the acclimation period (4-7 days) the net is opened (possibly automatically) and the birds should fledge by themselves (Progetto capovaccaio, 2022).

To help the released animals might be useful to provide some kind of post-release actions such as provisions of feeding station close to the area (Mihoub et al., 2014). Since now, hacking demonstrated to be the most effective releasing method for Egyptian vulture (Vulture conservation foundation, 2022).

Delayed release can be helpful especially in cases where the released bird is going to migrate right after, since migration is a really challenging journey and might lead to death of the just introduced juveniles (Life Egyptian vulture, 2018). With the delayed release technique, the bird is

kept under human care until the next spring (in which it should be back at the breeding territory after wintering in Africa), this will allow the animal to be older and possibly fitter to face the journey at the end of the summer; in the meanwhile, is important to keep the animal in a closed aviary (Ceccolini & Cenerini, 2018) to reduce human contact as previously stated. Release is still performed with hacking method but delayed several months.

Prior to release each bird is usually marked with a metal identification ring on the leg and a short-term radio transmitter on the tail to monitor movements in the following days, if founds are available birds are also equipped with a GPS tracker to monitor long term movements (Progetto capovaccaio, 2022).

Fig. 3 Young Egyptian vultures in pre-release cage, Hacking method (@associazione.CERM)

2.7 Existing conservation programs

Egyptian vulture species is protected in Europe by the habitat directive (92/43/EEC) and the bird directive (79/409/EEC); the project "LIFE Egyptian vulture" aims to implement and establish actions to promote the conservation and the protection of the Egyptian vulture specifically in Italy and in the Canary Islands. These actions include the monitoring and protection of existing nesting sites, creation and management of artificial feeding stations, intervention to make power lines as safe as possible to avoid electrocution of individuals, enforcement activities to prevent illegal poisoning and finance ex-situ captive breeding (Life Egyptian vulture, 2018).

CERM (Threatened raptor center) is an NGO association that run the LIFE Egyptian vulture project in Italy (southern Tuscany). Built in 2006, it is today the world largest captive breeding center of Egyptian vultures: the center has 18 video-surveilled aviaries that in 2016 hosted 42 specimens. Besides the captive breeding and release of specimen (hacking method) to restock wild population,

the center also manages artificial feeding stations and carry on monitoring programs to assess wild population condition (Progetto capovaccaio, 2022).

Vulture MsAP (Multi-Species Action Plan) is an international agreement involving several stakeholders such as international conventions, national governments, NGOs, research institutions, universities, private companies and local communities. The aim is to implement conservation actions in order to preserve and recover populations of the 15 species of African-Eurasian vultures and, by 2029, bring back population abundances to safe levels. Actions include local reduction of direct threats for the vultures such as prevent poisoning and electrocution but also ensure safe and quiet environment in the nesting areas (Botha, 2017).

3. Conclusion

Egyptian vulture global population decreased dramatically in the last decades, and IUCN in 2007 listed this small old-world vulture as endangered. Since the drop in the abundance of the species is not directly linked to difficulties or problems in reproduction (as might be for other species), breeding this animal in captivity is not particularly challenging and it is possible to obtain successful results in terms of production, as proven by existing captive breeding facilities. Of course, it is fundamental that all the species requirements are met, including appropriate housing, proper feeding and with a suitable nest,

Nursing of chicks and releasing methods share pretty much the same protocols among vultures; minor differences can be found in incubation duration and environmental parameters. A specific care must always be paid in avoid imprinting and limit human interaction.

Despite successful results might rise hopes and lead to think that captive breeding has the power to counteract population drop, the reality is far more complicated and *ex situ* conservation measures has always to be paired with *in situ* actions. It is indeed merely effective to restore the wild population, while threats and death causes are still present in the natural environment.

The present protocol collects a series of indications for a successful management of captive Egyptian vultures and it is aimed at helping any care taker dealing with this species in the routine work. It is important to stress out that a conservation program requires a holistic approach, where actions need to be taken considering different aspects; even though there's still much more improvements that can be made to implement protocols, the existence of international conservation programs and institutions taking care of the Egyptian vulture already place ourselves in the right path.

4 Acknowledgments

First of all, I would like to thanks my supervisor professor Marco Cullere, who patiently followed me in the final steps of my university career, guiding me in the writing of this thesis.
I want to thanks my parents since they gave me the possibility to study. I want also to thanks my friends and housemates with whom I shared many amazing memories that I'll carry with me and will always make me smile while reminding of them.

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