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Technical Report · September 2023

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English text revision

Maura Andreoni

Recommended citation

Ceccolini G. & Cenerini A., 2023. Technical handbook - Management of captive Egyptian vultures and release of captive-bred individuals. LIFE Egyptian vulture project, LIFE16 NAT/IT/000659, E.5 action, www.lifegyptianvulture.it. Semproniano (Italy).

LIFE EGYPTIAN VULTURE (LIFE16 NAT/IT/000659)

Project implemented with the contribution of the LIFE financial instrument of the European Union

This paper was drafted within the E.5 action of the LIFE Egyptian vulture project.

Data and information underlying the paper derive from activities carried out by the CERM Endangered Raptors Centre Association in the period 2003-2017 and from those carried out since 2018 by the Italian Institute for Environmental Protection and Research (ISPRA), Association CERM and Biodiversità sas within the LIFE Egyptian vulture project (2017-2023).

Acknowledgements

We warmly thank Alessandro Andreotti (ISPRA) for the comments and suggestions provided on the paper and for his long-standing scientific and concrete commitment to the conservation of the Italian Egyptian vulture population. Special thanks also go to the other colleagues of ISPRA who have contributed over the years to the implementation of the LIFE Egyptian vulture project: Arianna Aradis, Andrea Ferri and Pierpaolo Storino.

We thank the Unione dei Comuni Montani Amiata Grossetana for the support provided over many years to the captive-breeding and the contribution to the implementation of the release operations.

Special thanks to Matteo Visceglia and Mariangela Francione for their unwavering and long-standing commitment to protect the species and their willingness to do whatever it takes to achieve that end.

Special thanks also to Giampiero Tirone (CRAS Lago di Vico) for the support provided over many years to the captive-breeding.

We also thank the “pioneers” who contributed to carrying out the first release operations in Apulia, Calabria and Basilicata, the friends and colleagues of the Italian League for the Protection of Birds (LIPU Birdlife Italia), De Rerum Natura sas, the Stazione Ornitologica Calabrese (StOrCaL), the Studio Naturalistico Wildlife Research and the Centro Studi Naturalistici Nyctalus.

We want to thank Carabinieri Forestali for the monitoring and surveillance of the released vultures and for the inspections carried out by the Anti-Poison Dog Units.

Thanks also go to the VCF Vulture Conservation Foundation that has covered the costs of some GPS devices and GPS fees.

We are very grateful to all the volunteers who have helped monitor and support the release of the Egyptian vultures throughout southern Italy. Their work has been incredibly valuable: **Italy** Rosario Balestrieri, Antonino Barbera, Luigi Barraco, Remo Bartolomei, Filippo Bellini, Domenico Bevacqua, Salvatore Bondì, Niccolò Borgianni, Annalisa Brucoli, Andrea Cairone, Matteo Caldarella, Agostino Cantavenera, Giuseppe Cantavenera, Giovanni Capobianco, Andrea Ciaccio, Nicola Cillo, Lino Cirucci, Gianluca Congi, Antonio Luca Conte, Enzo Cripezzi, Andrea Cusmano, Maristella D'Addario, Davide D'Amico, Giacomo dell'Omo, Antonio Dembech, Mario Di Ciommo, Silvia Di Lella, Nino Di Lucia, Fausto Fabbrizzi, Antonio U. Fedele, Pietro Ferrandes, Enzo Ferrantelli, Carmelo Fiore, Carlo Fracasso, Alfredo Galietti, Vittorio Giacoia, Paolo Gattillo, Giuseppe Giglio, Vitantonio Iacoviello, Mariangela Iacovino, Renzo Ientile, Marisa Laterza, Fabio Liberatori, Gaetano Luce, Giacomo Magistro, Maurizio Marchese, Paolo Martinelli, Giuseppe Martino, Maya Massari, Manuel Marra, Mauro Tripepi, Emiliano Montanaro, Victor Muñoz, Loredana Murabito, Eugenio Muscianese, Michele Natale, Nicola Norante, Antonio Onorato, Giulia Pace, Marco Pecoraro, Stefania Pellegrino, Manuela Policastrese, Paolo Maria Politi, Teresa Pollina, Giuseppe Pulicani, Giuseppe Rannisi, Giuseppe Rocca, Salvatore Salerno, Enzo Sciabica, Angelo Scuderi, Salvatore Seminara, Antonio Sigismondi, Francesca Spensieri, Antonio Spinnato, Salvatore Surdo, Fabio Tuccio, Salvatore Tuccio, Salvatore Urso, Giovanni Zaccaria, Manuel Zafarana. **Other countries** Adrian Aebischer, Jovan Andevski (VCF), Volen Arkumarev (BSPB), Marton Arvay (MME), Claudia Feltrup Azafzaf (AAO), Hichem Azafzaf (AAO), Nicholas Barbara (BM), Gellért Bátky, Edward Bonavia (BM), John Borg (NMNH), Vladimir Dobrev (BSPB), Natalino Fenech, Marton Horvath (MME), Mathyas Prommer, Victoria Saravia (HOS), Mark Sultana (BM), Gál Szabolcs, Miklos Vaczi, Herald Xeka (AOS), Xhemal Xherri (PPNEA).

The CERM Endangered Raptors Centre Association

The NGO CERM Endangered Raptors Centre Association develops practical conservation measures in an attempt to prevent the extinction of the Egyptian vulture in Italy, such as:

- captive breeding of the species;

- release into the wild of captive-bred fledglings/juveniles, thanks to the cooperation with Italian and European institutions and associations;

- management of supplementary feeding sites in sensitive areas.

The CERM Association manages 41 Egyptian vultures of different ages and origins (2023) and cooperates in the implementation of the LIFE Egyptian vulture project.

www.capovaccaio.it, www.facebook.com/associazione.CERM/

Foreword

In the Red List of Italian breeding birds, the Egyptian vulture (*Neophron percnopterus*) is classified as “Critically Endangered”. Italy holds around 10-12 Egyptian vulture breeding pairs, most of them located in the southernmost part of the country (7/9 in Sicily, two in Basilicata and one in Calabria). In 2019 a new pair was discovered in Sardinia, outside the historical breeding area. After the dramatic range contraction and the decrease in abundance registered in the 20th century, the trend of the species seems to be quite stable over the last twenty years.

However, the small population size and the restricted and fragmented breeding range expose the species to the risk of extinction in the near future.

The release of captive-bred individuals can help restore the Italian population and is one of the conservation actions implemented in the framework of the LIFE Egyptian vulture project.

This handbook presents the results of 24 years of experience in managing the largest stock of captive Egyptian vultures in the world, hosted at CERM Endangered Raptors Centre located in Rocchette di Fazio (southern Tuscany, Italy). Furthermore, it describes the methods developed between 2003 and 2023 for the release into the wild of 48 captive-bred individuals, the results achieved and the lessons learnt, which can be useful for the conservation of the species.

CONTENTS

1. Captive breeding of the Egyptian vulture at CERM

CERM Endangered Raptors Centre is located in southern Tuscany (Italy) and hosts the largest stock of captive Egyptian vultures (hereinafter referred to as E. vulture) in the world. The Centre was created within the LIFE *Biarmicus* Project and is managed by the homonymous Association.

The captive breeding programme of the species started in 1993 under WWF management, but efforts have been intensified since 2006, when the stock was moved to CERM.

The programme aims at maintaining a captive-breeding stock with a good genetic diversity and yielding captive-bred E. vultures to be released into the wild in Italy.

Most of the captive stock founders were collected long ago from wildlife rescue centres in Spain. CERM currently hosts in Tuscany 38 E. vultures of different age and origin: 10 wild unrecoverable individuals of Spanish origin, one imprinted individual seized form a ship coming from the eastern Mediterranean, 25 individuals born at CERM and two individuals born at the Zoological Garden of Jerez de la Frontera (Spain). Furthermore, two individuals born at CERM are temporarily under care at the CRAS Lago di Vico rescue centre and one young E. vulture, born at CERM, in 2022 is hosted at the CRAS Matera rescue centre.

In the period 1993-2023, 75 chicks were born at CERM and 73 reached the fledging age. 32 E. vultures were born in the framework of the LIFE Egyptian vulture project (2017-2023).

CERM Endangered Raptors Centre

2. General management

2.1 Facilities

CERM is provided with 18 aviaries, a cold storage for food and a service hut. The aviaries are arranged in two groups and placed side by side; each measures 7 m x 4 m x 3 m (L x W x H) and is equipped with perches, runways and a nest-box. They are separated by closed metallic panels.

The aviaries are monitored by a video surveillance system, an irreplaceable tool for an adequate management of the vultures, specifically aimed at monitoring the behaviour of the pairs. The system includes:

- 14 colour Speed Dome PTZ (pan-tilt-zoom) IP camera with 4x zoom, Auto Focus Lens and IR Led;
- two high performance computers;
- a software of video recording with motion detection;
- a high-speed internet access for the remote control.

Aviaries at CERM

2.2 Regular management

The diet of the captive E. vultures is based mainly on rats, mice and poultry to a lesser extent. The daily portion is about 180-200 g of food per capita. The oldest individuals are provided with a powder supplement (based on methylsulfonylmethane, glucosamine and chondroitin) spread on the food to promote articular function and reduce any pain.

Food remains are removed from the aviaries every day and the water troughs are cleaned and refilled regularly. Aviaries are thoroughly cleaned weekly (by hand and rake) to remove pellets, fallen nest material, etc.

The grass covering the ground is regularly cut by hand. During the mowing carried out by means of a low-noise electric lawnmower, the more nervous specimens are temporarily placed elsewhere (even in pet carriers) so as not to stress them. It is essential to avoid the presence of thorny plants in the aviaries (brambles, blackthorns, thistles, etc.), even if newly grown, because they could injury the feet of the vultures and lead to the onset of serious diseases such as the bumble foot. During the breeding season cleaning activities are reduced to a minimum. The use of the lawnmower is absolutely to be avoided and grass must be cut very quickly by hand while food and water are provided. During the summer it is necessary to thoroughly clean the water troughs with abrasive sponges without detergents, to prevent the growth of invasive algae.

Egyptian vultures in an aviary

Veterinary check-up

Beyond the routine veterinary check-up, it is important to frequently monitor the beak length. Sometimes, indeed, the beak tends to grow too much, threatening the feeding capacity of the birds. Therefore, whenever the beak seems too long with the tip turning downwards, it is necessary to cut the exceeding part. The operation is carried out by two operators. The first operator holds the vulture belly up, puts his arm around the bird and holds it close to him to keep the wings from spreading. He also immobilises the vulture's feet to prevent it from hurting itself with its claws. The second operator immobilises the vulture's head from behind and keeps its beak closed after gently pushing the tongue inside.

Beak cutting

Subsequently, the same operator begins to cut the tip of the beak using a pair of small clippers (very sharp for avoiding chipping it); the operation is performed by first cutting one side of the exceeding part and then the other one, maintaining the natural pointed shape of the beak and avoiding cutting it too much. It is advisable to control the beak before the beginning of the breeding season to avoid intervening in the laying and incubating phases. The condition of the feet and the length of the claws are also checked at the same time. Should the claws appear too long it is necessary to shorten them. To avoid excessive growth of the beak it is advisable to make available in the aviary large bones with tendons and pieces of flesh, which the birds will tend to tear off with their beaks that inevitably wear out.

3. Breeding

3.1 Preparation of the breeding season

Every CERM aviary has a nest-box measuring approximately 2 m x 1 m x 1 m (L x W x H). Inside each nest-box a corner is bordered by two wooden poles (diameter of about 10 cm) to form a 70 cm x 70 cm wide nest-area. In early February, the nest-corners are fitted out with a layer of dry straw free from molds and in perfect conservation conditions and/or a layer of twigs, sprinkled with an abundant layer of uncarded sheep wool, clean and cut into small pieces not exceeding the length of 4 cm. It is important to cut the wool short to prevent it from getting caught in the vultures' feet and thus being removed from the nest. Sheep wool from old mattresses and pillows is perfectly suited. The thickness of the layers of straw and wool must not exceed the edge of the nest-corner. At the end of the breeding season the material present in the nest is removed and the nest-box thoroughly cleaned. Starting from late March, small pieces of sheep wool are scattered in the aviaries (outside the nest-box) so that the vultures can use them for preparing the nest. At the beginning of the breeding season, it is advisable to provide the potential pairs with a tub filled with muddy water (water mixed with red mud), so that they can take mud baths and colour their feathers.

Nest-corner prepared at CERM

Adult incubating eggs

Small branches and other hard material must be removed from aviaries because they could be brought into the nest and cause egg breakage.

3.2 Pair formation and management

Breeding pair

It is particularly difficult to obtain breeding pairs, due to several critical issues that may arise. Among them the following ones:

- partners do not get along, that is, they ignore or are hostile towards each other;

- partners show signs of courtship behaviour but the male becomes aggressive in an early or advanced phase of the breeding season. At CERM, cases of this kind involved some pairs: the aggressiveness of the males started arising about 10-15 days after the beginning of regular copulations (the most visible display occurs when the male chases the female away from the nest, but warning signs can also be detected);

- the pair is quiet and seems to get along but does not copulate even if the male tries to mate. The female seems to be indifferent and shows faint signs of hostility or escapes even if not attacked. In all these cases it is useless to leave the partners together in the next years.

3.3 Pair behaviour

Tight-knit pair

During the breeding season the behaviour and posture of the vultures provide unequivocal signs of either compatibility or hostility between partners.

Close-knit partners often stand next to each other, do reciprocal grooming (allopreening), stand together close to the nest or work on its preparation (together or separated). Food is shared without any aggressive attitude.

Usually, the male of a tight-knit pair stands upright with the back quite erect, the neck feathers puffed out and the head feathers spread out like a crown. Sometimes he remains next to the female in a submissive attitude, contracts his neck, lowers his head and brings it close to the female, with the back held horizontal and the head feathers down and turned backwards. The vulture makes slow movements while standing close to the female.

The female of a tight-knit pair usually stands with her back down, almost horizontal, neck contracted, neck feathers quite puffed out and head feathers spread out like a crown. She approaches the male in this posture when available for copulation. She can wait for the male in a crouched position, sometimes with the tail slightly raised.

Mating

Behaviour of critical pairs

Aggressive male. He has a very upright posture with extended neck and head and erect head feathers; he walks with extended feet and a quick and purposeful gait (goose-step). If the female approaches the nest, he pursues her by mimicking short pecks or making short attacks. Another typical behaviour is pinching or pulling on the female toe. All these signs lead the female to stop visiting the nest within a couple of days. The aggressive male, on the contrary, works continuously on preparing the nest and remains crouched in the nest for long periods.

Aggressive posturing

Female with aggressive male. She rarely accesses the nest and approaches it with care only when the male is not nearby; she quickly moves away as soon as the male approaches and never stands close to the male, not even at night.

Fighting partners

Male ready to mate with female not available for copulation. The male performs allopreening but the female remains indifferent, with upright posture and extended neck; if the male tries to copulate, the female runs quickly away and moves away whenever the male approaches her. Nevertheless, they can sleep together during the night. Sometimes the male, stressed by the continuous refusals, crouches and mimics a fake copulation or, in other cases, takes a stone and tries to hatch it for a few seconds.

Over the years various techniques have been used to reduce the aggression of the males towards the females during the breeding period but all without success:

- a hooded crow was placed in the aviary of the pair, hoping that the male would turn his aggressiveness towards the crow (the method seems to work with the Bearded vulture);
- two nest-boxes were placed in the same aviary, hoping that the male would defend only one considering it his territory and leaving the other to the female;
- small windows were opened between an aviary and the next one, close to the front perch, so that the males could see each other and thus burn off their aggressiveness.

In the 2018 breeding season a new technique was tested on four pairs. As soon as the males showed signs of aggressiveness, they were moved to a small cage placed in the pair aviary where they could not see the nests. Five days later they were set free. The measure showed to be temporarily successful but not resolutive (three males stopped being aggressive and the pairs went back to copulating but only for a few days).

The only method that seems to be effective foresees the displacement of the male to another aviary for a few days. This allows the female “to take ownership” of the nest so that when the male is back in the aviary, she has a dominant behaviour. Some pairs have returned to mate after this trial separation.

In general, each individual is unique but it was understood that:

- males who have never bred, showing continuous signs of aggressiveness, can mate with the "right" female after having experienced several females before (never losing hope!). A male named Primo, for instance, mated for the first time at the age of 24 and bred successfully, even raising a chick;
- a male usually incubates the eggs laid by his female even if she has been removed from the aviary and often successfully rears the chicks;
- a female without a male does not incubate the eggs but eats them within one day after the disappearance of the male.

4. Egg laying and incubation

4.1 Egg management

About 25 days after the beginning of the copulations a first egg is laid and is usually followed by a second one within 5-6 days (in 2016 at CERM, however, an old female laid a fertile egg 15 days after the first one). Eggs laid by problematic pairs are artificially incubated and are replaced with only one dummy egg per nest. The dummy egg must be placed after it has been heated to around 35°C to avoid it being considered edible food, as the E. vultures like eggs. The eggs can be left to a trustworthy pair for the incubation but for some years CERM has preferred to incubate them artificially, replacing them in the nest with dummy eggs. Anyway, whenever possible the eggs are left to be incubated by the parents for at least five days before being transferred to the incubator because this could have positive effects on hatching. In such a case, however, a pair is left incubating only one egg because sometimes a sort of competition arises between the partners. This can lead to a risky behaviour, such as attempting to incubate one egg each, with the risk of an incorrect incubation or even the abandonment of one egg for a long time.

Newly laid eggs are reddish in colour

Also, pairs showing a normal behaviour during courtship and/or mating may have problems during incubation and/or hatching.

Variability in egg size, shape and pigmentation

Partners fighting during egg incubation

Female attacking the male with a chick in the nest

These are usually due to an anomalous behaviour of the females that may:

- violently attack the male during the incubation of the eggs or the brood of the chicks. Such behaviour, observed at CERM in some pairs, could be caused by an excessive desire of the male to incubate the eggs and brood the chicks; by not allowing the female to relieve him, he triggers her aggression;
- break and eat the eggs just laid;
- eat live newborn chicks.

The females showing the aforementioned anomalies maintained them in the following years so it was necessary to act consequently, collecting the eggs just laid or after few days and leaving the chick to the care of the father only (removing the mother from the aviary).

In two cases the male ate the egg just laid by the female. In one case the pair had already previously exhibited an anomalous behaviour with the male attacking the female during laying. In both cases the male had never worked on preparing the nest. These males had never reproduced before.

Regarding the females laying for the first time in captivity, it has been proved to be essential to constantly monitor the behaviour of the pair through the video control system. This enables to directly observe:

- the moment of laying in order to rapidly collect the first egg and replace it with a dummy egg;
- the moment of hatching, locating a keeper near the aviary so that he can rapidly intervene if the female tries to eat the live chick. To stop her, a loud noise can be produced, and then the female must be removed from the aviary leaving the chick alone in the care of the father.

Dummy eggs

Dummy eggs are prepared using sterile E. vulture eggshells filled with clay and polystyrene that, in the right proportion, allow to obtain an egg with the ideal weight of 80/100 g. For preparing a dummy egg it is necessary to carry out the following procedure:

- the egg is emptied by making a hole in its largest side and letting the content flow out. The egg is then washed and left to dry for at least one day;

- a small quantity of clay is mixed with water until a rather fluid mixture is obtained, which is used to fill the egg slightly more than halfway. Subsequently, small pieces of polystyrene, about 1/2 cm long, are inserted into the egg until the content reaches more or less 1/2 cm from the hole. Finally, the shell is completely filled with the clay mixture, letting it flow out from the hole.

When the egg has dried, excess clay is removed, the shell smoothed with sandpaper and painted with ordinary felt-tip pens.

Eggshell

Dummy egg

Part of the CERM equipment

4.2 Artificial incubation

CERM equipment for artificial incubation

- 4 incubators
- 3 brooders
- 1 flat candling lamp (homemade)
- 1 Buddy Digital Egg Monitor
- 1 precision balance
- 1 digital calliper for measuring the egg size
- 3 high precision digital thermometers
- 2 glass thermometers for incubators
- 3 digital hygrometer/thermometer
- 1 Egyptian vulture puppet (homemade, professional since 2018)
- 3 batteries 12 volts, 70 A/h, anti black-out
- 3 uninterruptible Power Supply, anti black-out
- 8 dummy eggs weighing about 100 gr

Preparation for artificial incubation

Whenever possible, the eggs are left to the parental incubation for at least five days and then moved to the incubator. If necessary, they can be moved to the incubator as soon as they are laid, good results are achieved anyway. Before placing the eggs in the incubator, it is necessary:

- to sterilize the machine and check its working conditions for several days. A solution of alkyl-benzyl-dimethyl-ammonium chloride (10 mg/l of distilled water) is used for sterilization. The same solution is used to refill the water tank, which maintains the humidity;
- to carry out a gentle cleaning of the eggs, removing any residues of excrement using a cutter and paper soaked in the above-mentioned disinfectant solution;
- to indicate on each egg, with a soft pencil, the number of the pair and the laying sequence (e.g. 7/2 = pair n. 7, second laid egg).

Incubation parameters

- Incubation period: 41 - 43 days.
- Temperature: 37,4°C - 37.5°C (37°C after the chick has hatched).

- Humidity: about 45 - 50% (about 65% after the chick has broken the shell).
- Rotation in daylight: manually 90° and 180° every 3 hours in the interval 08:00 - 23:00 (6 times).
- Rotation at night: automatically 90° every 3 hours in the interval 02:00 - 05:00 (2 times).
- Cooling outside the incubator for 10 minutes 3 times during daylight.

The eggs are marked with a red and a green dot at the opposite end for a better monitoring of the rotation and avoiding errors. Manual rotation is always counter-clockwise.

From the 25th day of incubation onwards, it is crucial to maintain the eggs in the incubator with the small end always downwards so that the chicks can position themselves correctly for hatching, influenced by gravity (with the legs in the small end).

Ideal eggs weight loss during incubation

The incubation period lasts about 42 days (range: 41 - 43). During the artificial incubation it is crucial to monitor the egg weight loss. The reference is an ideal weight loss of 15% ± 1% in 40 days which can be ensured by adjusting the relative humidity. An Excel spreadsheet, featuring both a table for data entry and a graph for visual representation, has been created to track weight loss trend and adjust the relative humidity of the incubator accordingly. The relative humidity must be increased if the weight decreases more than expected or reduced if the weight loss is too low. However, it is important to keep in mind that humidity adjustments have limited effects on the weight loss trend after 2/3 of the incubation period. Temperature must never be changed.

The following parameters (table 1) are inserted in the spreadsheet:

1) data of the laying parents;

2) laying date;

3) egg weight at the time of collection;

4) days elapsed between laying and collection;

5) the two diameters of the egg, measured with a calliper.

Table 1 - Necessary data to monitor the eggs weight loss

EGG WEIGTH & DENSITY LOSS TECHNIQUES							
Egg imput data			Egg calculates data				
Egg code	11/1-2023		Pip date	22/05/2023	Weight loss		
Year	2023		Hatching date	25/05/2023	14%	15%	16%
Pair	11		Laying weight (gr)	96,47	96,47	96,47	96,47
Male	Melchiorre		40 days weight (gr)	82,00	82,96	82,00	81,04
Female	Patricia		Daily weight loss (gr)	0,36	0,34	0,36	0,39
Laying date	13/04/2023		Volume (cm^3)	87,82	Density loss		
Collection date	19/04/2023		Laying density (gr/cm^3)	1,0985	1,0985	1,0985	1,0985
Collection hour	8.30		40 days density (gr/cm^3)	0,9338	0,9447	0,9338	0,9228
Days in the nest	6						
Collection weight (gr)	94,30						
Lenght cm	7,036						
Width cm	4,947						

By G. Ceccolini

Through these parameters the following data are automatically calculated:

1) weight assumed at onset of laying;

2) 15% ideal weight loss and weight loss in the acceptable range of 14%-16%;

3) a table and a graph reporting, in the 40 days concerned, the daily weights calculated at 14%-15%-16%;

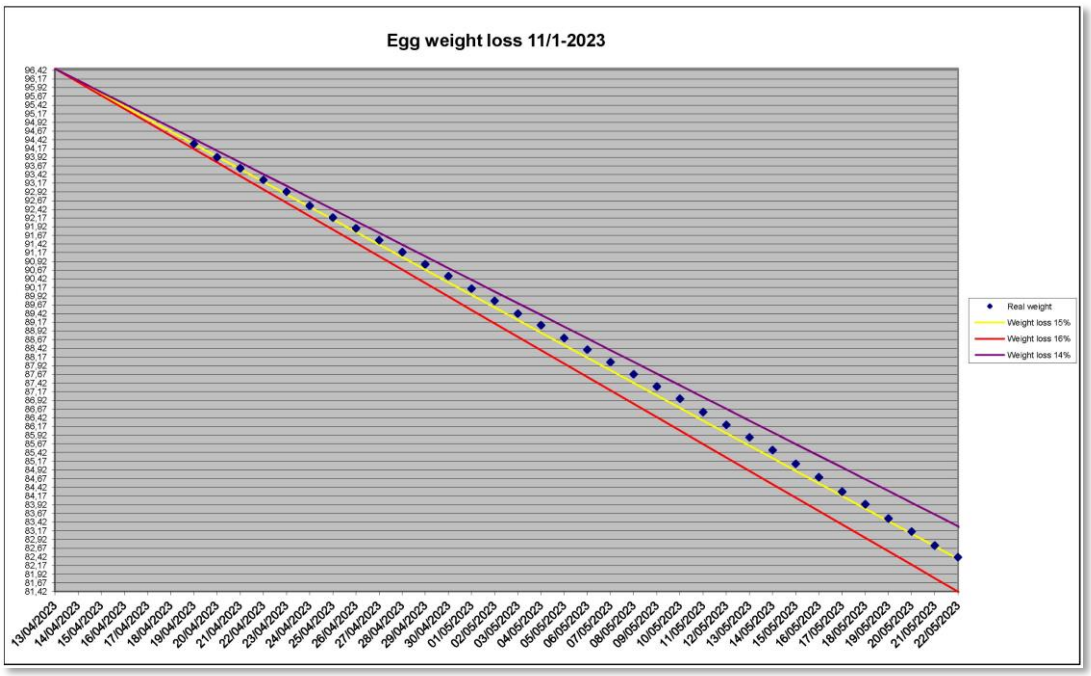
4) the egg density at onset of laying and after 40 days (for statistical purposes).

The eggs are weighed every day at the same time in coincidence with one of the periodic coolings. The collected data are inserted in the spreadsheet (table 2) in order to obtain a graph allowing a quick monitoring of the weight loss trend (graph 1) and to adjust the relative humidity, if needed.

Table 2 - Excel spreadsheet to monitor the eggs weight loss

EGG WEIGTH & DENSITY LOSS TECHNIQUES					PAIR: 11		EGG CODE: 11/1-2023						
Days	Date	Incubatrice	Temp. °C	Humidity %	Hour	Real weight	Weight loss 15%	Weight loss 14%	Weight loss 16%	Real density	Density loss 15%	Density loss 14%	Density loss 16%
0	13/04/2023						96,47	96,47	96,47	0,00	1,10	1,10	1,10
1	14/04/2023						96,11	96,13	96,08	0,00	1,09	1,09	1,09
2	15/04/2023						95,75	95,80	95,70	0,00	1,09	1,09	1,09
3	16/04/2023						95,39	95,46	95,31	0,00	1,09	1,09	1,09
4	17/04/2023						95,02	95,12	94,93	0,00	1,08	1,08	1,08
5	18/04/2023						94,66	94,78	94,54	0,00	1,08	1,08	1,08
6	19/04/2023	3	37,4	45	10,00	94,30	94,30	94,44	94,16	1,07	1,07	1,08	1,07
7	20/04/2023	1	37,4	45	14,20	93,92	93,94	94,11	93,77	1,07	1,07	1,07	1,07
8	21/04/2023	1	37,4	45	14,20	93,60	93,58	93,77	93,38	1,07	1,07	1,07	1,06
9	22/04/2023	1	37,4	45	12,00	93,27	93,21	93,43	93,00	1,06	1,06	1,06	1,06
10	23/04/2023	1	37,4	40	12,00	92,93	92,85	93,09	92,61	1,06	1,06	1,06	1,05
11	24/04/2023	1	37,4	36	12,30	92,52	92,49	92,76	92,23	1,05	1,05	1,06	1,05
12	25/04/2023	1	37,4	45	12,10	92,18	92,13	92,42	91,84	1,05	1,05	1,05	1,05
13	26/04/2023	1	37,4	48	12,00	91,87	91,77	92,08	91,45	1,05	1,04	1,05	1,04
14	27/04/2023	1	37,4	45	12,00	91,53	91,41	91,74	91,07	1,04	1,04	1,04	1,04
15	28/04/2023	1	37,4	42	12,00	91,19	91,04	91,41	90,68	1,04	1,04	1,04	1,03
16	29/04/2023	1	37,4	42	12,45	90,84	90,68	91,07	90,30	1,03	1,03	1,04	1,03
17	30/04/2023	1	37,4	40	12,00	90,50	90,32	90,73	89,91	1,03	1,03	1,03	1,02
18	01/05/2023	1	37,4	40	12,00	90,14	89,96	90,39	89,52	1,03	1,02	1,03	1,02
19	02/05/2023	1	37,4	40	12,00	89,79	89,60	90,06	89,14	1,02	1,02	1,03	1,02
20	03/05/2023	1	37,4	40	12,00	89,42	89,24	89,72	88,75	1,02	1,02	1,02	1,01
21	04/05/2023	1	37,4	40	12,00	89,08	88,87	89,38	88,37	1,01	1,01	1,02	1,01
22	05/05/2023	1	37,4	40	12,20	88,72	88,51	89,04	87,98	1,01	1,01	1,01	1,00
23	06/05/2023	1	37,4	40	12,20	88,38	88,15	88,70	87,60	1,01	1,00	1,01	1,00
24	07/05/2023	1	37,4	40	12,10	88,02	87,79	88,37	87,21	1,00	1,00	1,01	0,99
25	08/05/2023	1	37,4	40	12,00	87,67	87,43	88,03	86,82	1,00	1,00	1,00	0,99
26	09/05/2023	1	37,4	40	12,00	87,32	87,06	87,69	86,44	0,99	0,99	1,00	0,98
27	10/05/2023	1	37,4	37	12,00	86,97	86,70	87,35	86,05	0,99	0,99	0,99	0,98
28	11/05/2023	1	37,4	36	12,00	86,59	86,34	87,02	85,67	0,99	0,98	0,99	0,98
29	12/05/2023	1	37,4	36	12,20	86,22	85,98	86,68	85,28	0,98	0,98	0,99	0,97
30	13/05/2023	1	37,4	36	12,00	85,86	85,62	86,34	84,89	0,98	0,97	0,98	0,97
31	14/05/2023	1	37,4	36	12,00	85,49	85,26	86,00	84,51	0,97	0,97	0,98	0,96
32	15/05/2023	1	37,4	36	12,00	85,10	84,89	85,67	84,12	0,97	0,97	0,98	0,96
33	16/05/2023	1	37,4	36	12,20	84,71	84,53	85,33	83,74	0,96	0,96	0,97	0,95
34	17/05/2023	1	37,4	38	13,20	84,29	84,17	84,99	83,35	0,96	0,96	0,97	0,95
35	18/05/2023	1	37,4	35	12,00	83,93	83,81	84,65	82,96	0,96	0,95	0,96	0,94
36	19/05/2023	1	37,4	35	12,00	83,52	83,45	84,32	82,58	0,95	0,95	0,96	0,94
37	20/05/2023	1	37,4	35	12,00	83,15	83,09	83,98	82,19	0,95	0,95	0,96	0,94
38	21/05/2023	1	37,4	35	12,00	82,74	82,72	83,64	81,81	0,94	0,94	0,95	0,93
39	22/05/2023	1	37,4	45	12,00	82,41	82,36	83,30	81,42	0,94	0,94	0,95	0,93
40	23/05/2023	1	37,2	65	12,00	82,08	82,00	82,96	81,04	0,93	0,93	0,94	0,92
41	24/05/2023	1	37,2	65	12,00	81,75	81,64	82,63	80,65	0,93	0,93	0,94	0,92
42	25/05/2023						81,28	82,29	80,26	0,00	0,93	0,94	0,91

Graph 1 - Weight loss trend of one egg



Monitoring of eggs during incubation

Home-made egg candler

With the exception of the moment of cooling and weighing, the eggs are handled as little as possible but, in any case, with care and wearing latex gloves. From the 15th day, the eggs can be candled every 5-6 days in order to check the embryo growth and the growth and tilt of the air cell. Even if no air cell is visible, it does not mean that the egg is infertile because sometimes the shell is very dark and, in such a case, candling can give unclear results. Only an anomalous weight loss trend (less than expected) and a bad smell emanating from the egg give certainty of infertility. When there are no certainties, it is therefore advisable to transfer the egg to another incubator so that it does not infect other eggs in case of spontaneous break.

After 30 days of incubation, it is possible to check if the egg is viable by gently placing it on a flat and smooth surface. The egg is observed closely and lightly tapped sideways to make it unstable. The egg will swing and finally stop but if the embryo is viable, it will continue moving autonomously with jerky motions for some seconds, due to the embryo movements. In any case, if the egg does not move on its own, it could be a false negative because the embryo might not be so athletic!

The Buddy Digital Egg Monitor can help check the egg fertility and assess the chick well-being before hatching without any risk of damaging the embryo by the traditional candling. The device provides real-time monitoring of the chick's heart rate using electronic sensors that amplify 20,000 times the chick's heart beat in the egg.

Digital Egg Monitor

5. Hatching

5.1 Hatching process

During the hatching process it is important not to be anxious and impatient to see the chick coming out of the egg. Care should be provided to chicks, if necessary, for example when they take too long to hatch or when they are very big (unable to rotate inside the shell) or when the eggs are abnormally shaped (e.g. oblong). Chicks definitely need to be assisted if they are malpositioned in the egg. From the moment the chick breaks the internal membrane until it hatches, it usually takes a little more than two days.

Egg during hatching

Day 1: the chick enters the air cell, breathes and chirps (internal pip). In the incubator, the temperature is decreased to 37°C, humidity remains unchanged (45-50%), egg rotation stops and the egg is placed on a piece of a soft terry towel.

Day 2: the chick breaks the shell. The time between the internal pip and the first hole in the eggshell (a very small crack, sometimes difficult to be detected) is variable but usually less than a day. Temperature is still at 37°C and humidity is increased to 65%.

Day 3: the chick hatches. After the first eggshell break, chicks of E. vultures usually take 24 to 36 hours to hatch (although in 2018 one chick successfully hatched on its own after 44 hours). Temperature is at 37°C and humidity at 65%. After one hour the residues of the shell must be removed and, if necessary, the dry cord cut off. The operation must be carried out with care avoiding stretching the cord if it is still attached to the shell. The umbilicus is then gently swabbed and disinfected. The terry towel must be replaced with a clean one and covered with a paper towel.

Natural hatching

5.2 Artificial hatching

At CERM there is often the need to intervene and help the chicks hatch out of the egg. The reasons why natural hatching often does not occur are not precisely known, but it seems to depend on the incubation system and/or on the parameters of the very last hatching phase, certainly not on the quality of the eggs and the chicks. Indeed, the weight of the chicks at the end of the incubation period is always within the ideal range. Furthermore, when the eggs about to hatch are moved from the incubator to a nest in CERM aviaries and incubated by a pair or by a single male 100% of chicks hatch naturally.

This occurs both when the eggs are transferred to a nest as soon as the chicks have already entered the air cell and when they have pipped the first hole in the eggshell a few hours before. This indicates that the last day/day and a half of incubation is crucial for natural hatching and that the artificial incubation method during the last stage of the process needs to be modified.

Unfortunately, artificial incubation and hatching become indispensable when trusted pairs or males are not available. The expertise of the breeders significantly minimizes the risks of incubation failure and the risks for the chicks immediately after hatching are also reduced (in the nest they can be killed by one of the parents, usually by the female).

Artificial hatching

At CERM between 2017-2023, 43 eggs were artificially incubated up to the hatching phase. Nine of them were moved from the incubator to a nest after the internal pip or after the eggshell had already been cracked. Eight chicks hatched naturally, whereas one had to be helped out from the remaining part of the eggshell. All of them survived hatching in good health.

34 eggs spent the entire incubation process inside the incubator. Only five chicks hatched naturally, whereas 29 hatched artificially. 28 of them survived hatching in good health, one died from the consequences of delayed intervention.

When to intervene and how to help chicks hatch

There are not fixed deadlines beyond which the breeder can be certain that the intervention is indispensable.

In this delicate phase, the chick must be regularly monitored and its beak/head watched as much as possible (without opening the incubator) to understand if it needs help and, if does, determine the appropriate moment in which to provide it.

Attention must be paid to following points:

- a large hole in the eggshell and/or the beak always positioned in the same place. These signs indicate the lack of rotation of the chick inside the egg;
- vitality of the chick. When the chick becomes non-responsive to stimuli such as cold and disturb and the sounds emitted change or weaken (they are normally rapid and strong “chock” repeated a few times) it is urgent to intervene;
- the shape of the beak. As soon as the mandible becomes slightly asymmetrical in relation to the maxilla it is urgent to intervene.

It should be kept in mind that a too early intervention could jeopardize the possibility of a natural and safe hatching and expose the chick to the risk of serious problems if the yolk absorption is not yet completed. On the other hand, a late intervention can lead the chick to suffer from neurological symptoms due to compression of head and neck, which could even lead the chick to death.

Chicks that experienced mild hypoxic stress during hatching may have a deformed head, oedema in the head and neck area, and show weakness. They usually recover completely within a few hours, although sometimes their head keeps tremble over the next few days and they need to be fed very carefully.

When a chick requires human intervention to hatch from the egg great care must be taken. The process can take from twenty minutes to a few hours, depending on several factors. The main and most urgent purpose is to free the chick’s head and neck from the shell.

The intervention begins around the hole pipped by the chick. Using scissors, tweezers and fingers, small pieces of the shell are gently and slowly removed. The inner shell membrane must subsequently be removed, after having verified the presence of the capillary plexus and its condition. Cotton swabs soaked in warm water are used to separate the inner shell membrane from the chick.

If the chick is ready to come out, the removal of the membrane can cause only very small blood leaks, which can be stopped by pressing on the tip with a cotton swab soaked in cold water. If the external blood circulation is still active, the need of the intervention should be re-evaluated (probably the yolk has not been completely absorbed yet).

From time to time during the process, the egg should be placed back in the incubator to allow the chick to warm up. When head and neck are no longer surrounded by shell the chick can be left in the incubator inside the remaining eggshell, keeping the upper part of its body slightly lifted. The chick will complete the hatching on its own or can possibly be helped later.

5.3 Malpositioned chicks (egg *dystocia*)

At CERM four cases of chicks malpositioned in the egg occurred (head towards the small end of the egg and legs towards the large end, i.e. upside down from the normal position). Three of them were assisted and successfully hatched as they had been able to pip at the small end giving the opportunity to intervene. The way to realize that a chick has an incorrect position in the egg is to

candle the egg about 39 days after the onset of the incubation. If the inner membrane separating the air cell from the chick appears irregular (warped or broken by the legs) a malposition of the chick is likely. The chick pips the shell in the small end of the egg and earlier than expected (both internal and external pip). It is often necessary to carefully check the shell because the chick only opens a small hole that is not easy to detect (most often it consists of small cracks of the shell which can be more easily detected by touching, see 5.1 Hatching). The reasons for malposition can be different. In our case the cause was mainly related to the automatic rotation of the eggs that led them to have the small end up, causing the embryo to position itself incorrectly misled by the anomalous tilt of the egg (process starting about 15 days before hatching). Since 2016, the eggs have been manually rotated in daylight every three hours, taking care to maintain them with the small end always downwards: since then, no more cases of malpositioned chicks have occurred.

Assistance to malpositioned chicks

To prevent the death of the upside-down chick, it is first necessary to free the neck and then the head by removing tiny pieces of shell around the hole in the egg. This procedure must be carried out very carefully for no longer than five consecutive minutes using scissors, tweezers and cotton buds soaked in cold or lukewarm water, avoiding breaking the underlying membranes. The egg is then placed back into the incubator for at least half an hour to allow the underlying membranes to dry. The incubator is set at 37°C temperature and 65% humidity. At a later stage, other tiny pieces of membrane are removed but only if they have dried in the meantime and the vessels have receded, otherwise the chick could bleed. This process must continue until the shell fragments are completely removed. Once neck and head have been freed (the process takes about two days) the remaining part of the shell is left untouched so that it surrounds the rest of the chick body. In fact, it is crucial for the chick to have the time to completely reabsorb the yolk sack. During the intervention, any incorrect movements or the opening of a too large hole in the shell can cause the death of the chick, attributable to damage to the umbilical area (disembowelling) or exposure to the risk of septicaemia due to infection of the incompletely reabsorbed yolk sac.

Intervention on a malpositioned chick

Once the yolk sack has been reabsorbed and the umbilicus closed, the chick, if necessary gently stimulated, will autonomously start the last phase of hatching by pushing with the legs and the nape. As in the case of a natural hatching, one hour after the chick has come out from the egg, any residues must be removed and the dry cord, if necessary, has to be cut (see 5.1 Hatching). The whole process, between shell crack and hatching, takes about 3 days. For information on how to assist refer to following website:

backyardchickens.com/articles/step-by-step-guide-to-assisted-hatching.64660/

In 2023 two chicks failed to break the shell probably due to a malposition though they were not completely upside down. They started chirping one and two days earlier than expected and it was

necessary to intervene. As soon as freed from the shell one of the chicks had a heavily deformed head and oedema in the head and neck area however it completely recovered after two days. The artificial hatching took place 41 days after laying. The other chick did not suffer any consequences thanks to a prompt intervention since the egg was opened 40 days after laying. In both cases the opening procedure was performed quite rapidly, leaving the chicks inside the remaining part of the shell for several hours after taking care to remove the shell that was squeezing head and neck.

6. Incubator hatched chicks

6.1 Management of incubator hatched chicks

Ten hours after hatching, the chicks are transferred to a brooder, where the temperature is settled at 37°C to be decreased by about 1°C per day in the following days. Thereafter temperature can be lowered more quickly. It is important to carefully monitor the reaction of the chicks to these temperature adjustments.

A two-day-old chick just placed into the brooder

Humidity is kept above 60% and slowly decreased over the next few days until it reaches 50%. To avoid hip dysplasia (splayed legs) the chicks are placed in small concave containers lined with small piece of rags creating a sort of nest; a paper towel is laid on the rags and changed very often. In the following days the paper towel is replaced with a small terry towel to guarantee a faster absorption of the faeces, essential for keeping the chick clean. It is important that the legs are always kept close to each other and not open out (the chicks should not be placed on smooth, flat surfaces). The chicks are covered with a thin white towel imitating the parents' body; in this way they get used to the gentle pressure of the towel and willingly accept the possible incubation by adults. It is important to keep in mind that:

Chick in a brooder

- if a chick cheeps incessantly it is not fine;
- a curled up and not active chick may be cold;
- a chick stretching neck and legs is hot. Warning: too much heat seriously damages the chicks.

Eighteen-day-old chicks in a brooder

Several actions must be undertaken to prevent incubator-hatched chicks from being imprinted on humans. First of all, the contact with the breeders must be avoided, especially when the chicks are between five and 15 days old. When a pair or a single male are available for the adoption, the chicks are moved to the nest by five days of age. Otherwise, each operation close to the chicks must be carried out with the operator adequately disguised (covered with a vest, mask and dark glasses) in silence and wearing on the arm which supplies food and water a glove puppet in the shape of the neck and head of an adult E. vulture.

Good hygiene practices are required to prevent or reduce the risk of infections (e.g. food must be stored hygienically, chicks must be handled wearing gloves, water must be supplied fresh).

Chicks while feeding

Chicks are fed for the first time about six hours after hatching, as soon as they start to peck around keeping the head upright. They are fed with fresh meat pieces about every 2-3 hour or when they appear hungry. However, it is important not to overfeed them especially during the first days of life, when the digestive system has yet to get used to the new feeding regimen. They should never be fed with food straight from the refrigerator (they would die!); cold food must always be brought to room temperature (e.g. by keeping it in the palm of the hand, protected by a latex glove). During the first days of life the chicks are fed with small pieces of mice or rats, excluding bones, fur, stomach, bowel and liver. When the chicks can master their neck (trembling is gone) it is possible to give them some water, with caution and preferably before feeding. The “false” beak of the glove puppet, retaining some water drops, is very suitable for the purpose: chicks peck at it, and are able to swallow some water. Syringes must be avoided to prevent the risk of choking the chicks. Just a few days after hatching the chicks drink a lot. At the age of 7-10 days, they are fed four times per day and they can take small pieces of food placed in front of them.

Small pieces of young mice tail and muscle can be supplied with small and not sharp pieces of bone since chicks are now able to regurgitate pellets. Supplements with vitamins and bone meal are also provided. Water is given drop by drop by using a small piece of a waterlogged sponge inserted into the beak of the glove puppet. At the age of two weeks, the chicks are fed three times per day, at the age of three weeks they can completely eat and drink on their own.

A chick while drinking

Self-feeding chick

6.2 Transfer of chicks to the aviaries and their adoption

Whenever possible, at the age of 1-5 days chicks hatched in the incubator are transferred to the nest of a pair or a single male (which can be both biological or foster parent), where a dummy egg had previously been placed. Half shell of an E. vulture egg is placed in the nest together with the chick; later the dummy egg and the half shell are removed.

In the presence of trusted adults, the eggs can be placed in the nest during hatching, when the chick is chirping inside the egg.

If the chick has to be hand-reared for a long time because there are no available foster parents, an adult can be temporarily placed close to the brooder, otherwise the chick can be placed in a small cage inside an aviary of adults, if its age and the external temperature allow it. In this case small pieces of food are placed daily near the cage in order to attract the adults and make them easily visible to the chick. It is advisable to have an adult male in the aviary because males are usually curious and willing to approach chicks.

Chick just moved to a nest

Chicks separated by a net

Whenever all available pairs and/or males are rearing chicks, a newborn chick born in incubator may be inserted in a nest removing an older chick (age >15-20 days) in order to avoid its imprinting to humans. The older chick may be placed in a small cage in an aviary of adults. Multiple displacements of chicks may be arranged.

When reliable adults are already rearing, one or more nestlings at the age of at least two weeks can be added to the nest. In these cases, especially when ages are very different, it might be useful to temporarily install a low separation net, which can be removed if the chicks will get used to a peaceful cohabitation.

The net enables to avoid risky contacts between the chicks and to favour a quiet socialization. The net, if rapidly installed, usually does not cause problems to the adults, which can easily circumvent it to reach the chicks placed at the two sides. Of course, these operations must be carried out only under regular video control.

Fledglings are ready to be released at the age of about 80 days; if it is expected to release them in the following years it is necessary to transfer them to aviaries which are completely closed in order to reduce contacts with the breeders as much as possible. It is particularly important that they do not associate food availability with humans; for this reason, food has to be supplied by means of a tube. Otherwise, they might approach humans, with a high risk of being killed.

A two-month-old chick with a parent

7. Comments

7.1 Fitness of the captive-bred individuals

The results of the releases performed in Italy reveal that most of the captive-bred individuals adapt easily to the wild especially if they are released in their first calendar year.

Captive breeding and population reinforcement. Sara's story

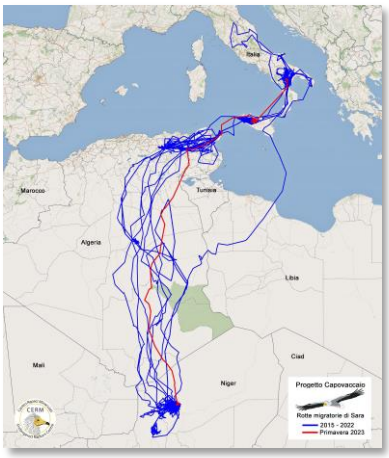
Usually, the life of satellite transmitters is limited to 4-5 years, so in most cases they do not enable to track the movements of the released E. vultures until they start breeding, at the age of 6-7 years. This was not the case with Sara's transmitter, which was still working in 2023, eight years after the release. Sara was born at CERM in 2015 and released three months later in Apulia by the CERM Association with the support of the Italian League for the Protection of Birds (LIPU, Italian partner of Birdlife International).

The long-lasting functioning of Sara's transmitter, together with the monitoring carried out by Italian ornithologists as part of the LIFE E. vulture project, enabled to ascertain that in 2022 and 2023 she successfully bred in Italy. For the first time in Europe there was evidence that a captive-bred E. vulture bred in the wild.

During seven years, Sara's movements have been tracked by means of the Microwave Telemetry satellite transmitter fitted on her back. The transmitter enabled to capture the milestones of her life. In 2015, during her first post-natal (autumn) migration, Sara crossed the Mediterranean Sea from Sicily to Libya with a 550 km non-stop flight over the sea and reached the sub-Saharan-wintering grounds on the 21th of September. Overall, she covered 3.980 km in 28 days. Sara spent in Africa the first four years of her life (mostly in Niger and in other countries of the Sahel Belt) and moved every summer from the age of two to North Algeria (Atlas Mountains).

In the spring of 2019 Sara returned to Italy for the first time and has returned every year since then, spending over there part of the spring and summer.

During the 2019 autumn migration she made a stopover on Pantelleria Island together with Tobia, another captive-bred E. vulture released in 2015 by the CERM Association in a different place, located in Calabria. In the following autumn migration Sara was observed on Pantelleria again, this time together with a wild-hatched juvenile, thus acting as a guide for an unexperienced bird during its first migration.



Routes of Sara 2015-2023

In 2022 Sara started her fourth spring migration one month and half earlier than in previous years. She left Niger on February 26th and reached Italy in March. In Sicily she was sighted and photographed paired with a "wild" partner, the two vultures showing an

unmistakable reproductive behaviour. Then Sara and her partner moved to mainland Italy (Basilicata region) and nested in a hollow already used in the past by another pair of E. vulture.

They raised two chicks. On September 18th Sara started the autumn migration and on October 10th she reached her usual wintering ground in Niger.

In 2023 Sara bred again in the same nest, raising one chick.

Fledglings in the nest of Sara (2022)

Sara's nesting proves that restocking programmes with captive-bred birds can be effective for the reinforcement of threatened E. vulture populations. Excellent news for the species and a strong incentive to continue these actions in Italy.

7.2 Shortcomings of the captive breeding at CERM

The number of captive birds hosted at CERM is too high in relation to the available space. Some individuals should be transferred to other centres to reduce overcrowding. This would make it possible:

- to build one or more large aviaries where adults could be grouped in winter, so that they can mate freely. Natural pair formation can be an important factor in successful breeding;
- to distance the breeding aviaries by at least 70-100 m. This measure could prevent the breeding pairs from hearing the sounds emitted by nearby pairs while copulating. It might reduce the aggressiveness of the adults and consequently favour a successful breeding;
- to equip aviaries with additional structures (e.g. internal divisions enabling the partners to stay temporary separated, flaps enabling the breeder to access the nest area from the back).

Furthermore, the translocation of some individuals to other centres would significantly reduce the risk of infectious diseases.

CERM is managed by a small staff. It is crucial to enlarge the number of specialized operators by training new qualified personnel in Italy or elsewhere in Europe, based on the long-standing experience of the CERM staff.

7.3 Concluding remarks

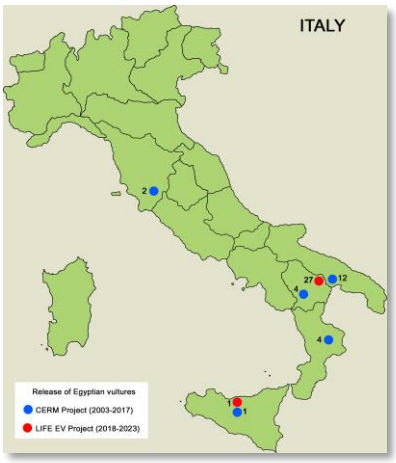
The productivity of captive E. vultures is very low worldwide, therefore the number of young available for restocking programmes is quite limited. This suggests that the collection of wild fledglings from populations with a good conservation status could contribute to enhance the reinforcement programmes.

To prevent potential negative effects on wild population, strict criteria should be defined for selecting the nests where chicks/nestlings could be taken from (i.e. death of one of the pair's partners, nests with high risk of breeding failure, nests with two chicks, etc.). To this end, an accurate monitoring of the breeding pairs should be conducted, possibly supported by video surveillance systems.

A tight international cooperation among different actors (captive-breeding centres, ornithologists and biologists involved in the conservation of the wild populations, public authorities) should be established to ensure the availability of a good number of captive-bred individuals and/or wild fledglings to be released into the wild every year.

8. Release of captive-bred Egyptian vultures, general overview

The restocking with captive-bred individuals is one of the main conservation actions of the LIFE Egyptian vulture project. The releases were carried out during 2018-2023 by De Rerum Natura sas under the coordination of ISPRA and with the cooperation of the CERM Association and Biodiversità sas. The activity is in continuity with the restocking programme which was launched by the CERM Association in 2003.



Over the whole period 2003-2023 51 captive-bred E. vultures were released in Italy.

During 2003-2017, the CERM Association released into the wild 23 captive-bred individuals. Sixteen of them were born at CERM Endangered Raptors Centre, five had been donated by some European zoological gardens and two were fledglings born from confiscated wild eggs (robbed in Turkey). These vultures were released experiencing three different methods: hacking, delayed release and fostering. Most of these vultures were released in Apulia, Calabria and Toscana using the hacking method (18), four individuals were released in Basilicata through the delayed release method and one more in Sicily using the fostering method (2003). Hacking was used for the first time in 2004 whereas the first delayed release took place in 2017.

Between 2018 and 2023, in the framework of the LIFE Egyptian vulture project, another 28 juveniles born at CERM Endangered Raptors Centre were released into the wild in Basilicata and Sicily regions using both the hacking and the delayed release methods.

9. Release operations 2003-2017 *(CERM Association, Egyptian vulture project)*

In the period 2003-2017, the CERM Association managed the release of 23 captive-bred E. vultures by using the fostering (1), hacking (18) and delayed release methods (4).

Fledgling in the wild nest (Fostering)

The first individual was released in Sicily in 2003 through the fostering method. The fledgling was placed at the age of 61 days in the nest of a wild pair rearing two wild siblings of similar age. The fate of the bird is unknown since the GPS stopped sending signals a few days after release.

The fostering method involves a rigorous observation of several nests to identify the most suitable one, both in terms of age of the chicks (the “natural” chick must have a similar age to that of the “intruder”) and for the possibility of post-intervention in case of fights between the fledglings. The supply of food in the surroundings is indispensable to support the pair and prevent fledglings from competing for food.

Following this experience, this method was no longer used for various reasons. First of all, it reveals the location of a nesting site, making it more exposed to disturbance and nest robbery, two serious threats that can cause the wild pair fail breeding. Furthermore, the placement of a new fledgling can trigger fights between nestlings leading to possible injuries of both individuals. Lastly, it should not be forgotten that it is extremely difficult to find nests with the required characteristics when the release is carried out to restock a very small population, such as the case of Italy.

In the period 2004-2015, 18 E. vultures were released by the hacking method in three different regions: Tuscany (2), Apulia (12) and Calabria (4). All of them were historical nesting sites for the species.

Hack box in Tuscany

Release in Tuscany

Single release operations took place in Tuscany and Calabria and in both cases the fledglings were hosted in a hack box for the adaptation phase. In Tuscany the release took place in 2005 in the south of the region (SAC/SPA Monte Labbro e Alta Valle dell'Albegna, Rocconi Natural Reserve, province of Grosseto).

In Calabria the vultures were released in 2015 in a valley located in the central-eastern part of the region (SPA IT9320302 Marchesato e Fiume Neto, province of Crotone).

Release in Calabria

In Apulia releases were carried out during 2004-2015 in the Gravina of Laterza, a canyon located in the westernmost part of the region (SAC/SPA Area delle Gravine, province of Taranto). The area is part of the Murge, a large karstic plateau extending between central Apulia and north-eastern Basilicata, also including the Murgia of Matera, located just to the west, where further releases took place after 2017.

In the Gravina of Laterza canyon the fledglings spent the adaptation period in a small cave in the rocky faces, closed with a net until they were ready to fly, usually after 4-5 days. Food and water were provided through tubes descending from above.

Gravina of Laterza

Preparation of the hack site

Fledglings in the hack site

Aviary and feeding platform in the Appennino Lucano N. Park

In 2017 four E. vultures in their second calendar year were released through the delayed release method in Basilicata (SPA Appenino lucano, Appennino Lucano National Park). They spent 15-23 days in an aviary installed in a pasture, located at a distance of 5 km from a nesting site occupied by a wild pair. The delayed release method by using an adaptation aviary has not been used in the following years, especially due to the difficulty of finding a suitable place, remote and quiet, where the juveniles could spend some weeks/months. During the release operations, the surveillance of the E. vultures was carried out through video-surveillance systems and by direct observation from vantage points.

Immatures in the adaptation aviary

All the 23 vultures released in 2003-2017 were equipped with VHF tags and 17 of them with GPS tags (Argos or GSM) fitted with backpack harness.

The releases were carried out by the CERM Association with the support of various organizations, public bodies and many volunteers, which collaborated in different tasks such as preparation of the release site, surveillance, care of the vultures during the adaptation period, post-release management (provision of supplementary feeding, monitoring, rescue of birds in need, etc.).

10. Release operations 2018-2022 *(LIFE Egyptian vulture project)*

Hack box in the feeding site of Matera

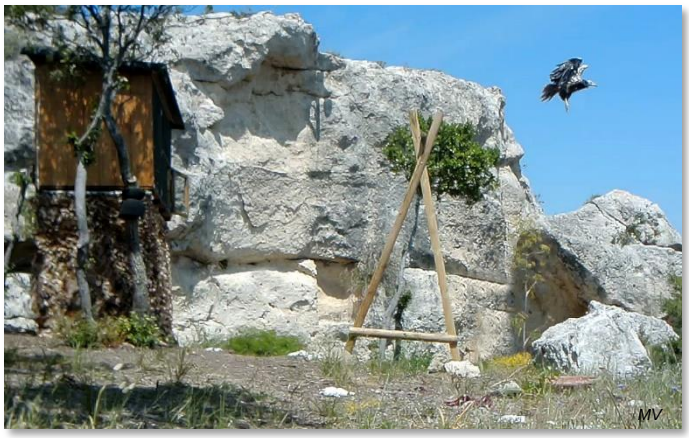
Fledgling in the feeding site of Matera

Within the framework of the LIFE Egyptian vulture project seven releases were carried out in the period 2018-2022 in south-eastern Basilicata (SAC/SPA Gravine di Matera, Murgia Materana Regional Park). The area is characterised by a complex of karstic canyons, which engraves the plateau of the Murgia Materana, close to the border with Apulia. Here nests one of the few E. vulture pairs still occurring in Italy.

A total of 25 birds were released: 13 with the hacking method in the second half of August, when they were 76-87 days old and the other 12 with the delayed release method, when they were in their second (6 individuals) and third calendar year (6). Most delayed releases took place between late July and early August; only four birds were freed in May. All the vultures were released after 3-5 days of adaptation spent inside a hack box.

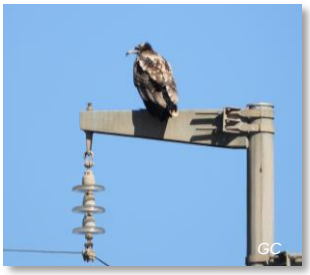
Between 2018 and 2021 the hack box was installed within the fence of a supplementary feeding site located about 1.8 km from the Matera canyon, whereas in 2022 the hack box was placed in a small flat area close to the rock faces. Both areas were monitored by a remote-controlled video-surveillance system powered by solar panels and consisting of two cameras, one of which monitoring the inside of the hack box and the other the outside area. All the released E. vultures were equipped with backpack mounted GPS devices and VHF tags.

To prevent mortality, 324 poles of the medium voltage power lines around the release area were insulated by E-Distribuzione S.p.A., one of the project



Release by hacking in Matera canyon

partners. Furthermore, periodic preventive inspections were carried out by the Anti-poison Dog Units of the Carabinieri forestali. Personnel of the Carabinieri forestali also ensured the surveillance of the release area with the cooperation of project team experts and external subcontracted ornithologists.



Egyptian vulture on a pylon

Insulation of power lines

Inspection by an Anti-poison Dog Unit

11. Release methods and stages

11.1 Pre-release management

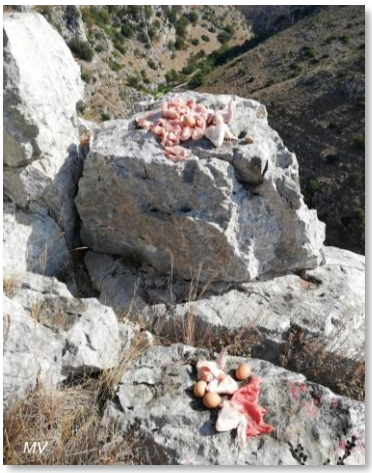
Food supply in a hack box

A few weeks before release, the variety of food supplied to the juveniles in the stock aviaries was increased to get them used to recognize and feed on a larger variety of items (while usually rats, mice and chicks are mainly used to feed juveniles).

The behaviour of the vultures inside the hack box/rocky hollow/aviary was monitored through webcams and direct observations were made from very large distance.

In the hack box/aviary, food and water were supplied through special flaps located in the back side, thus preventing the vultures from having any visual contact with the keepers and linking the availability of food to human presence. In the rock cavity, food and water were supplied by tubes descending from above.

Quantity of food (about 200 g per day per individual) was not increased either during the adaptation period or in its last phase since it has been seen that, once free, many juveniles wait some days before feeding despite having access to easy food. This behaviour enables them to lose weight to get fit and fly well.



Temporary feeding site

A few days before release, different foods were scattered around the adaptation site and in other strategic sites nearby (parts of sheep, pigs and cows, eggs, etc.). This is useful to attract other E. vultures and scavenging raptors, and for juveniles to identify the area as a safe source of food should they move far away soon after release.

The carcasses of animals killed in road accidents were not used due to the risk that they could have been poisoned before being run over or contain residues of lead ammunition.

While the vultures were still in the stock cages they were marked with microchips, metallic and coloured plastic rings, and a few days before being placed in the hack box/aviary, fitted with GPS and VHF transmitters.

11.2 Release and post-release management

The vultures were released by opening a small window of the hack box or aviary. In the case of the fledglings hosted in the rocky cave, the net was removed by pulling down some ropes from below.

Release of an immature Egyptian vulture

Field monitoring

Fledgling in a feeding site

Tub with water used by fledglings

The vultures usually left the facility within hours. Only a few of them decided to remain inside and spend their first night there. In that case the window was closed again before sunset to avoid any risk of predation during the night and reopened the following day. Only a few juveniles returned to the hack site (in the rock face cave used in Apulia).

The first two weeks after the release are very critical for the juveniles as they are inexperienced and must acquire flying skills, learn to find food, identify safe roosts, learn to interact with conspecifics and other species, etc. In few words they have to learn how to survive in the wild.

A team of experts constantly monitored the vultures remotely by means of the GPS tags but field inspections were also carried out. Observations were made from great distances to check their state of health and their progress in flying. Monitoring was also carried out by webcams and camera traps installed in the supplementary feeding stations nearby.

In case of doubts, VHF tags were used to detect the position and reach individuals supposed to be in difficulty, if the morphological conditions enabled it to.

If GPS signals showed that one or more vultures frequently visited the same site, this was inspected to ensure there were no risks for the birds.

After release, abundant food of different types was scattered around the hack site and placed in strategic points hardly accessible by terrestrial carnivores and frequented by the juveniles. When larger carcasses were also left on the ground, their bellies were cut and the heads opened in order to make them more attractive to scavengers. Tubs with water were placed at supplementary feeding sites and close to the release sites. They were constantly used by the E. vultures and other birds for drinking and bathing. Especially in summer, the availability of these tubs can avoid the vultures to perform long flights in search of water, thus reducing the risk of running into possible threats. In preventing mortality, food supply played the same role. Food and water were supplied until all juveniles left the release site.

It usually took at least one week for the vultures to start making long flights around the release site. The fledglings spent an average of 16 days (min 6 - max 27) in the release site, then they started migrating and tried to reach Africa or southern Italy. They left the release site at an average age of 95 days (min 84 - max 106 days).

11.3 Support and monitoring of the released vultures

Juvenile Egyptian vulture

Temporary feeding site in Linosa Island

Temporary feeding site in the Sila N. Park

After the start of the autumn migration, the monitoring of the vultures was mainly carried out by analysing satellite data to track vulture movements and detect possible behavioural anomalies.

To follow and guard the released individuals during their movements and migration, a network of field operators was created involving the project team, many volunteers and the officers of the Carabinieri forestali. The team also included ornithologists in Tunisia and Malta.

Based on sharing the real time GPS positions of the vultures, this network proved to be effective in rescuing birds, providing food support, preventing poaching and gathering relevant information on the species behaviour. The network made it possible to promptly rescue one injured vulture and three individuals in trouble and to find the carcasses of most of the dead released individuals in time to ascertain the cause of death and retrieve their GPS tags.

Temporary supplementary feeding sites were also activated along the migration route, in stopover sites or in areas where the vultures wintered in Italy.

In the last years, the released vultures could also count on five permanent feeding sites. One was located in Apulia (built by the Regione Puglia and managed by LIPU), two in Calabria (managed by ISPRA) and two in western Sicily (operated by ISPRA and the CERM Association). The latter were located in a stopover site which was visited by E. vultures during the autumn migration before crossing the Mediterranean Sea to reach Tunisia. They were also frequented by hundreds of black kites and other scavenging raptors.

A feeding site in Calabria region

Immature in a feeding site in Sicily

Immatures in a feeding site in Sicily

12. Problems occurred in the post-release period

The remote monitoring through satellite tags and the network of field operators made it possible to follow the movements of 43 of the 47 captive-bred E. vultures released in Italy in the period 2004-2022 and to know the fate of most of them.

In the six months after release, 18 individuals reached their wintering grounds in southern Italy or Africa and 22 died or were brought back to captivity. The satellite tags of three individuals stopped sending signals and therefore the fate of those birds remained unknown (table 3).

Table 3 - Outcome in the first six months after release

Calendar year	N.	Alive	Dead	Recaptured	No data
1	27	48.15% (13/27)	48.15% (13/27)		3.7% (1/27)
2	10	40.00% (4/10)	30.00% (3/10)	30.00% (3/10)	
3	6	16.66% (1/6)	33.33% (2/6)	16.66% (1/6)	33.33% (2/6)
1-3	**43**	**41.86% (18/43)**	**41.86% (18/43)**	**9.30% (4/43)**	**6.98% (3/43)**

The causes of death of 18 individuals that died in the first six months after release are reported in table 4.

Table 4 - Causes of death in the first six months after release

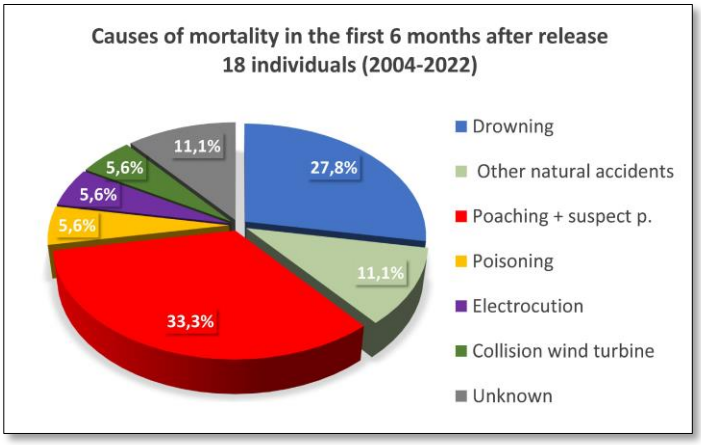
		Natural cause of mortality		Anthropogenic cause of mortality					
Calendar year	Number of individuals	Drowning	Other natural accident	Suspected Poaching	Poaching	Poisoning	Electrocution	Collision with wind turbine	Unknown
1	13	30.77% (4/13)	7.69% (1/13)	23.07% (3/13)	15.38% (2/13)	7.69% (1/13)	7.69% (1/13)		7.69% (1/13)
2	3		33.33% (1/3)					33.33% (1/3)	33.33% (1/3)
3	2	50.00% (1/2)			50.00% (1/2)				
1-3	**18**	**27.78% (5/18)**	**11.11% (2/18)**	**16.67% (3/18)**	**16.67% (3/18)**	**5.56% (1/18)**	**5.56% (1/18)**	**5.56% (1/18)**	**11.11% (2/18)**
		38.89%		**50.00%**					**11.11%**

Poaching

Electrocution

Collision with wind turbine

Graph 2



Four individuals were found weak or in bad state of health a few days/weeks after release. They were captured and rescued. The carcasses of eight E. vultures were found. The death of the other nine individuals was assumed on the basis of tracking data. One young male E. vulture was crippled in Basilicata by an aerogenerator few days after his release and, though rescued and alive, he has been considered as dead in table 3 and table 4 since he cannot longer be released.

Three individuals disappeared during the autumn migration between Sicily and Tunisia but, on the basis of the available data, it was suspected that they were shot at sea. Therefore, they have been classified in the category “suspected poaching”.

During the first autumn migration most birds died due to human-related causes, mainly poaching and electrocution. The only natural causes of mortality were natural accidents some days after release and drowning during the crossing of the Mediterranean Sea.

13. Comparison of results Italy/Bulgaria

The comparison of data referred to fledglings released by hacking (26) and juveniles in their second/third calendar year released by delayed release (14) allows to assess which method guarantees better results. As reported in table 3, captive-bred individuals released by hacking had higher post-release survival rates during the first six months after release (50.0%) than those released through delayed release (35.71%). Thus, hacking resulted to be the best method to release captive-bred E. vultures in Italy.

This finding is in contrast to the results obtained in Bulgaria. Here, the release of captive-bred E. vultures started in 2016. Between 2016 and 2021, 13 captive-bred individuals were released through the delayed release method and nine by hacking (table 5). The birds released through delayed release showed to have much higher probabilities of survival in the first six months (69.23%) than those released by hacking (22.22%). (Arkumarev et al., 2022[(1)]).

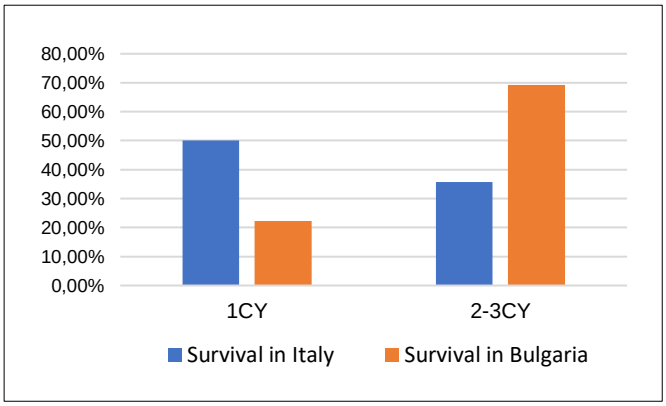
Table 5 - Post-release survival rates during the first six months after release, Italy vs Bulgaria

Calendar year	Survival in Italy*	Survival in Bulgaria
1	50.00% (13/26)	22.22% (2/9)
2/3	35.71% (5/14)	69.23% (9/13)
	45.00% (18/40)	**50.00%** (11/22)

** The percentage was calculated including four individuals, which were recaptured, therefore lost for the reinforcement purpose.*

[(1)] Arkumarev, V., Saravia-Mullin, V., Dobrev, V., Dobrev, D., Klisurov, I., Bounas, A., Ivanova, E., Kret, E., Vaidl, A., Oppel S. & Nikolov, S.C. 2022. Reinforcement Strategy for the Egyptian Vulture (*Neophron percnopterus*) in Bulgaria and Greece. Technical report under action C3 of the LIFE project “Egyptian Vulture New LIFE” (LIFE16 NAT/BG/000874). Bulgaria 76 p.

Graph 3



It is worth mentioning that both methods have been implemented in the two countries in different ways.

Hacking In Italy the first calendar year fledglings were placed in a natural cave on a rockface or in hack boxes installed in a feeding site or on cliffs. The cave and the hack boxes were closed with a net for 4-5 days, then the net was removed leaving the vultures free to fledge (usually they did it in a few hours, rarely in the following day). Furthermore, most released birds were born early in the season (within 10 June), so they could be ready to migrate in late August-early September, when weather condition are more favourable and a certain number of adult E. vultures are still in Italy. The presence of adults at the migration onset is supposed to be relevant, because inexperienced juveniles can find the right migration route following adults. In Bulgaria the juveniles spent some weeks in the hack site; moreover, some birds were released even if born late in the season.

Delayed release In Italy most of the individuals released in their second/third calendar year spent few days in a hack box or in an aviary (4-23). Furthermore, most of them were released in summer because it was not possible to find a suitable place where to keep the birds for a long time. Conversely, in Bulgaria, the second/third calendar year vultures were released in May after spending about 6-8 weeks in an adaptation aviary located inside a feeding station.

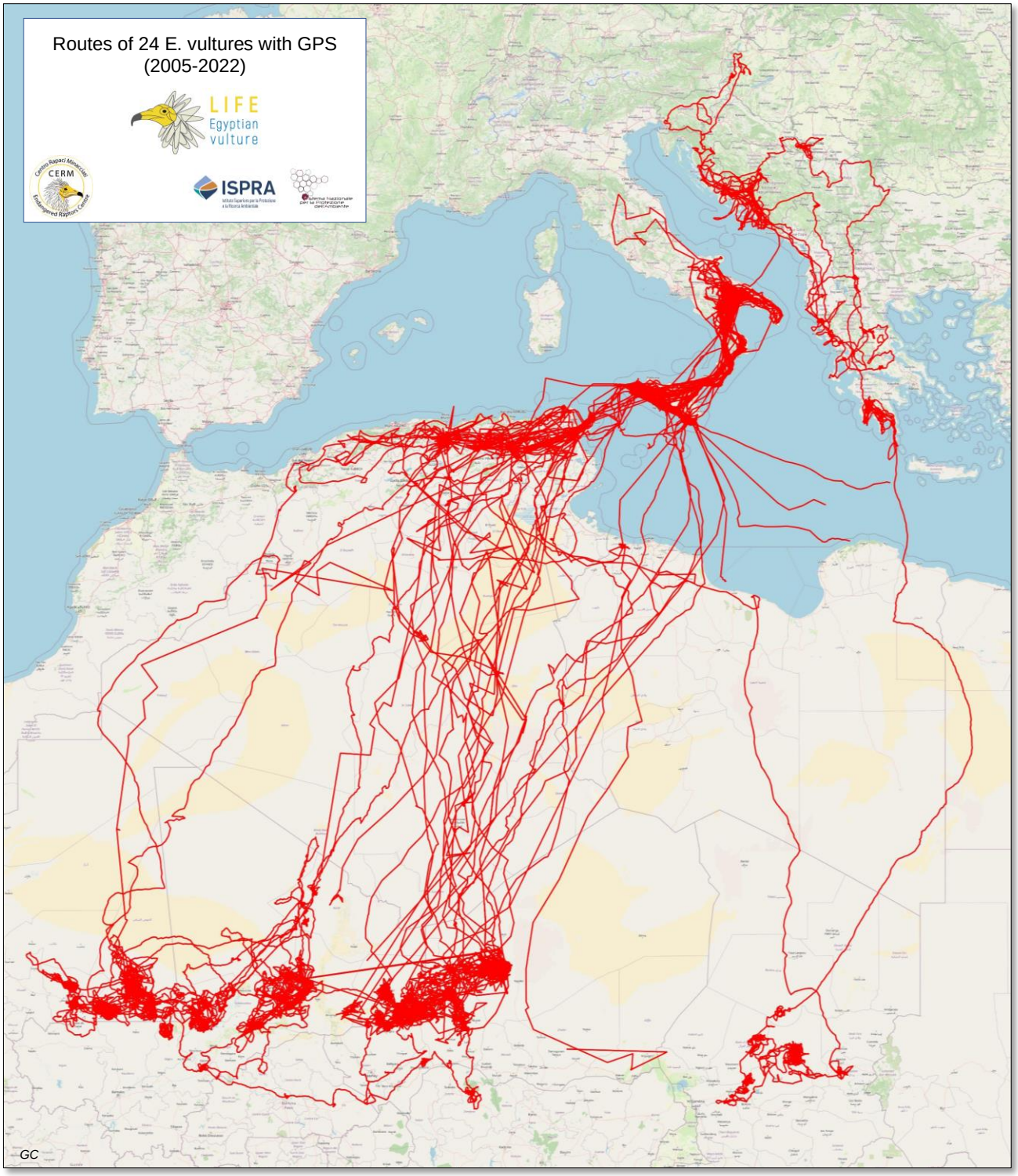
Concluding remarks The differences in the release techniques employed in Italy and Bulgaria may have had some influence on the achieved results. However, other factors may explain the contrasting results obtained in the two countries:

- the origin of the individuals released in Italy and Bulgaria was not the same; differences in the captive-breeding protocols may have had consequences on the fitness of fledglings and juveniles and the potential imprinting on humans;

- the environmental context in which the vultures spent the adaptation period and the following months could have greatly influenced the mortality rate. Among the most relevant factors conditioning the restocking results at the local level are worth to be mentioned the potential threats (electrocution, poisoning, poaching, etc.), the availability of food and the presence of a number of other sub-adult E. vultures that can make easier the acclimatization of the released birds.

The comparison between the restocking programmes carried out in Italy and Bulgaria reveals that it does not exist one method better than another in absolute terms. To maximise results, the release technique must be tailored on local context, keeping in mind different aspects.

Finally, these results suggest that an exchange of captive-bred individuals between the reinforcement programmes in Italy and Bulgaria would lead to increase the survival probabilities of the released birds. E. vultures born at CERM late in the breeding season could be released in Bulgaria through the delayed release, whereas fledglings born earlier in the season in Bulgaria or in other captive-breeding centres could be released in Italy by hacking in the summer of the same year of their birth.


Routes of 24 E. vultures with GPS
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