Bearded Vulture European Endangered Species Programme (EEP): guidelines for artificial incubating and chick rearing in captivity

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1st version February 2024

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ACKNOWLEDGEMENTS

I would like to thank the former team of the Guadalentín Breeding Centre, Antonio Lucio Carrasco, Alfonso Godino, Fernando Bautista, Manuel del Barco and Elena Macías, for their patience and dedication. Without them it would not have been possible to carry out the incubation study and develop new artificial incubation protocols. Further the new team from Vallcalent Breeding Centre, Raquel Gómez and Albert Porté, together with the author started to test the protocol with other bird species before to implement it by the Bearded Vulture. Also to Dr. Hans Frey technical director of the Richard Faust Zentrum -headquarter of the Bearded Vulture EEP-, and former Bearded Vulture EEP coordinator, and Francisco Rodríguez technical director from the breeding centre Centro de Cría Guadalentín, for their many contributions and advice that have made it possible to substantially improve the document. And, above all, to Chandra David from the California Condor (Los Angeles Zoo), for the hours she has spent revising the text, exchanging her experience on the California Condor captive breeding program with the author, making the document much more comprehensible.

INTRODUCTION

The international Bearded Vulture breeding network (EEP: European Endangered Species Programme) is a collaboration between zoos and similar institutions, breeding centres, and private partners. Between 1978 and 2023, 673 juvenile Bearded vultures were reared successfully as part of the programme. The offspring reared are used for reintroduction/reinforcement projects in Europe – in the Alps, Andalucía, Grands Causses, Corsica and Maestrazgo-Els Ports. Working in collaboration with the Vulture Conservation Foundation (VCF), the ultimate aim of the programme is to establish a European metapopulation of Bearded vultures, creating gene flow between the existing isolated autochthonous populations in Europe (in the Pyrenees, Corsica, and Crete) and with populations in North Africa and Asia.

Between 1978 and 2023, 1161 clutches have been laid, of which a minimum of 632 or a maximum of 668 were double clutches, since in 36 cases the clutch size could not be determined. This represents that 54.4%-57.5% of all clutches have been a double clutch. However, is well known that Bearded vulture chicks display aggressive behaviour while they are in the nest which results in the death of the younger sibling. It was Heinroth (1927), who first described the death of the second Bearded Vulture chick in the wild. But he attributed it to the same behaviour that he has observed in wild kites and buzzards. It was much later, due to the observations carried out in Innsbruck Alpenzoo, the cause of death of the younger chick was determined. The older chick pecks at and holds onto everything that moves and then tries to swallow it every time it is fed. This leads to the death of the younger chick, firstly because it is forced to remain in one corner of the nest where it can't feed and secondly because of the injuries received from the attacks of its older sibling (Thaler & Pechlaner, 1979, 1980). Thanks to these observations, in 1986 Thaler et al. could determine that this behaviour named "cainism" is inborn ("obligatory"), as it is independent of the satiation level of the chick and is more pronounced 5-20 days after hatching. Consequently, for all the above reasons artificial incubation and rearing is used to prevent the cainism behaviour.

As a rule, in double clutches, the full clutch or the 2nd egg is removed from the nest before hatching and the resulting chicks are later returned to their parents or given to foster pairs so that they can be reared naturally and to prevent human imprinting.

But there are also other situations that require the use of artificial incubation and chick rearing. For example, birds with physical disabilities which are not able to incubate the clutch correctly, or for pairs which don't incubate well for various reasons, as well as for birds with behavioural problems that may lead to inappropriate handling of the clutch or chick. This is very typical behaviour observed in birds which have not been naturally reared which can result in damage to eggs or death of the chick. Finally, external disturbances can cause incubation interruptions which make it necessary to remove the clutch and incubate egg(s) artificially.

Occasionally there are more chicks than parents, which forces some pairs to rear two chicks at the same time. Because of the "cainism" behaviour it is not possible to adopt more than one chick in the same nest, being necessary to use different chick rearing technics to ensure a natural rearing.

Cainism behaviour by Bearded Vulture nestlings:

This inborn ("obligatory") behaviour, by chicks less than 1 month old can only be observed when they are awake. In this age their activity in average is around 5%, the rest of the time they sleep. As soon as they are awake, especially because of hunger, the presence of any other chicks stimulates them to react aggressively. Not for nothing, while they sleep it is possible to keep two chicks together in the same nest and be brooded by a single adult (see picture below).

But as soon as they wake up from hunger, they change their attitude completely, attacking everything that moves in front of it (see picture below), and only get stuck when one of them disappears.

GUIDELINES FOR ARTIFICIAL INCUBATION AND CHICK REARING

1. BACKGROUND

In birds of prey it is recognised that the success of hatching depends on the method of incubation. In the peregrine falcon and the bald eagle, using natural incubation -independent of whether it was the parents, adoptive parents or a hen which incubated-, the hatching rate from fertile eggs was 84% and 81% respectively (Cade et al., 1977; Carpenter et al., 1987). On the other hand, if eggs are incubated artificially from the day they are laid, the hatching rate is lower, and most of embryos die during the first phase of incubation or during hatching. Consequently, it is recommended to incubate eggs naturally for at least one third of the incubation period to achieve a high rate of hatching (Carpenter et al., 1987; Cade et al., 1977; Gilbert et al., 1981; Kuehler & Good, 1999).

But in the case of the Bearded Vulture, when the international captive breeding programme began, due to lack of knowledge or lack of foster pairs, a few birds were hand-reared for a longer period than is currently recommended, showing behavioural problems in the reproductive biology once sexual maturity was reached. These alterations made it necessary to remove the clutch and offer it to another breeding pair that was already incubating, or it had to be artificially incubated. Unfortunately, only the eggs adopted by another pair hatched. However, there were not always couples available who could adopt the eggs, and this made it necessary to investigate how to improve artificial incubation. For this purpose, during his stay at the Guadalentín breeding centre (Andalusia), the author together with the former staff carried out a series of comparative studies with a natural pair, a male pair, and a male who was incubating on his own, with the goal to determine which parameters are responsible for a successful embryo development. More than 10.000 observation hours during six breeding seasons were invested, where the number of incubation interruptions per hour and the duration of each interruption, both day and night, were analysed. These observations let to the conclusion that the daily variations in the amount of non-incubation time, the number of interruptions and their duration followed a distinct pattern.

In the case of the single male, once he had accepted the egg immediately after it was laid/offered, at the beginning and comparing with the natural pair, the interruptions were shorter, and the percentage of time not dedicated to incubation was lower (see figure 1). As time went by the male showed increasing signs of exhaustion since he was never relieved from his role in incubating the egg and the average length of interruptions started to gradually increase (0.55-3.91 minutes). Consequently, the average time, which was not dedicated to incubation, also rose (2.87%-15.58%). However, the number of interruptions per hour remained very stable between 1.80 and 3.83 interruptions/hour; average 2.6 interruptions/hour (see figure 2).

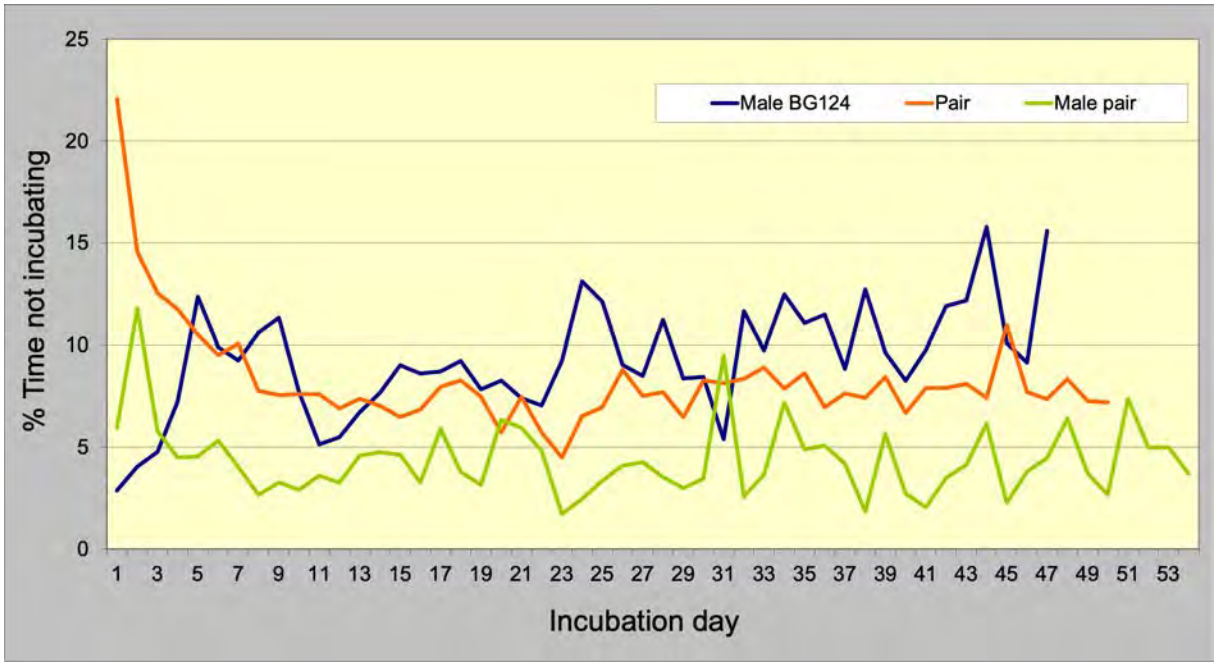


Figure 1: *Daily distribution of the percentage of time not dedicated to incubation from the day the egg was laid until the day before the egg was removed.*

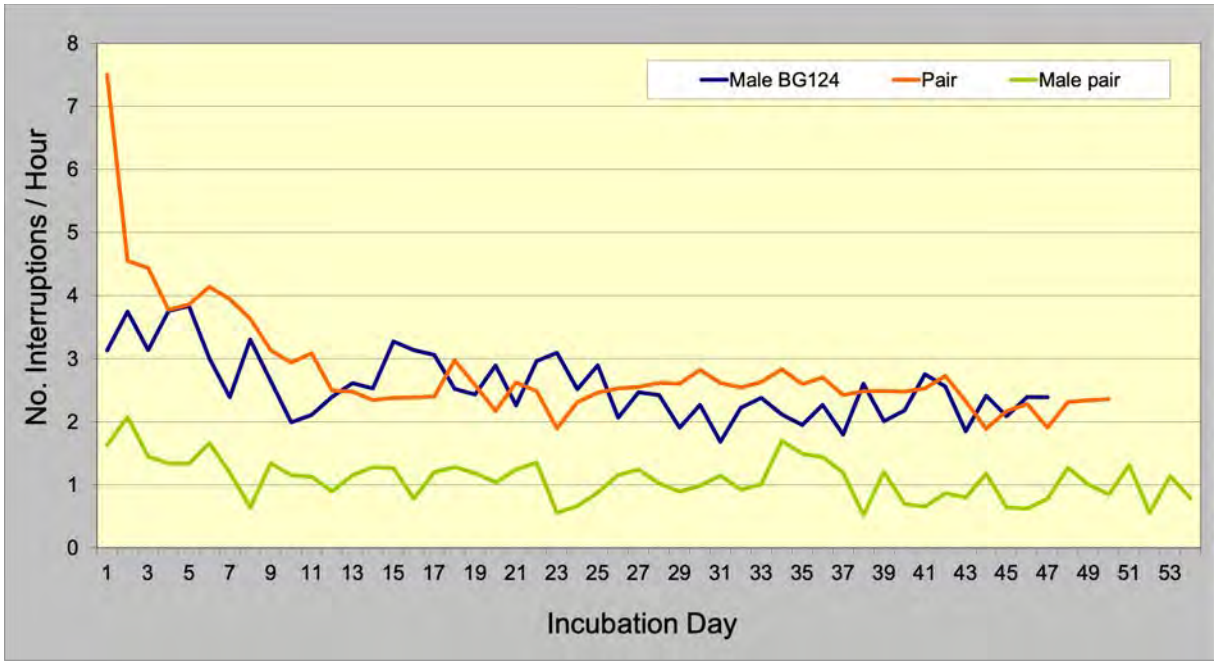


Figure 2: *Daily distribution of the number of interruptions from the day the egg was laid until the day before the egg was removed.*

A similar pattern was observed by the pair of males. As soon they received an egg, the interruptions per hour were very short and the percentage of time not dedicated to incubation was very low (see figure 1 & 2). However, being able to perform exchanges, they showed no signs of fatigue, keeping the length of interruptions very stable throughout the incubation period and consequently also the non-incubation percentage with an average of 4.52% non-incubation/day (see figure 1). Furthermore, they

showed the same pattern as the single male, keeping very stable the number of interruptions per hour during the incubation process, but lower levels from 0.52 to 2.07 interruptions/hour; average 1.1 interruptions/hour half of the single male (see figure 2).

But on the other hand, the natural pair was observed to have significant differences. They displayed more interruptions over the first few days and consequently a higher percentage of time not dedicated to incubation (1st incubation week from 9.51% to 22.06%; average 12.99%). This stabilised from the first week onwards. The reason for this is that the females still haven't entered their incubation stage, making more interruptions per hour in this first week until the second egg has been laid, some of which can be longer than habitually (see figures 1 & 2). But as soon as the female entered the incubation stage, the number of interruptions per hour and their duration remained very stable, having the same number of interruptions per hour like that of the single male. Consequently, also the non-incubation time remained more stable (from the first week onwards 4.49% - 10.97%; average 7.53%), but with higher values than in the male pair (from 1.71% - 11.78%; average 4.52%). Furthermore, the observed differences in incubation parameters between the first week and from the first week onwards in the natural pair, had no influence on the length of incubation for the first, second and third and fourth eggs together (53.9±1.83 n=409; 54.0±1.97 n=191 and 54.4±2.44 n=37 respectively). This is even better reflected if you only take into account the years where most of the partners had video cameras installed in the nests (from 2010), and it was possible to determine the exact laying date (1st egg 53.4±1.46 n=205; 2nd egg 53.0±1.23 n=91 and, 3rd & 4th egg 53.3±2.5 n=16).

Furthermore, in all three cases, more interruptions per hour were detected on hot days than on cold days -particularly on windy days-, changing the adopted posture by the incubating according to the weather. On cold days the body and head are kept much closer to the nest whereas on hot days the head is raised, and the breast may not even completely cover the egg, especially at midday.

Thanks to this study, significant differences in incubation patterns could be detected. The higher number of interruptions per hour and their higher length observed at the beginning by the natural pair, the gradual increase in the time of interruptions (= increase non-incubation time) observed by the single male as incubation progressed due to fatigue, or the stable low number of interruptions per hour and the stable low non-incubation time by the male pair -and both parameters significantly lower as by the other two cases (natural pair and single male)-, had no influence on breeding success. These differences showed that the oxygenation of the egg -which is given by the number of interruptions-, and the intensity of incubation -which depends inversely on the sum of the time of each interruption-, do not determine the success of incubation. These results forced the formulation of new hypotheses, since they must have been other factors that could influences the development of the embryo.

Knowing that the embryo lacks a heart during the first days of its development, but that there are contractile structures in the walls of the existing blood vessels, it was hypothesised that there must be an external stimulus that regulates the contraction of these structures and promotes the movement of fluids, encouraging the exchange of gases.

One of the hypotheses was that at each interruption the egg was exposed to very low temperatures, provoking a thermal shock and consequently the contraction of the blood vessels. As soon as it was re-incubated by one of the adults, the vessels dilated, thus promoting the movement of blood from

the centre of the egg to the surface and vice versa. Furthermore, the incubation interruptions recorded were so short that they made it impossible for the egg to cool down, again demonstrating that refrigeration had no effect on incubation success.

With this hypothesis in mind, the author and his new team at the Vallcalent wildlife recovery centre began to experiment with eggs of different species *(Neophron pernopterus majorensis*, *Tyto alba, Lannius minor, Tetrax tetrax, Falco subbuteo and Circus spp*.), which had to be removed from the nest as soon have been laid because of different reasons to incubate them artificially and expose them for 3-5 minutes -depending on the size of the egg-, four times a day at an outside temperature. Seeing that most of the recovered eggs hatched successfully, the same technique was applied to Bearded Vulture eggs, obtaining the same results as in naturally incubated eggs.

It should also be mentioned that three types of incubators of different technology (different heating system, turning system, etc.) were used during the study (INCA 200, ContaqX3 from Brinsea and Falcon C-30-S from Masalles), showing no difference in the results, and corroborating again that there is an external factor to the incubator operation that depended on the incubation success in eggs incubated from day of lay.

*Applying **Thermal shock** on five Bearded Vulture eggs in the Bearded Vulture captive breeding Unit at the Recovery Centre Vallcalent of the Generalitat de Catalunya, Spain.*

2. CHICK NEST CONTROL

On principle we try to avoid as much as possible to disturb breeding birds by entering in the aviary. Furthermore, for new breeding pairs, we suggest leaving the clutch in the nest giving the breeding pair the opportunity to cooperate during this unique experience, which helps to strengthen their relationship. For the above reasons, incubation and the hatching process should take place naturally in the nest, only removing the clutch or the hatchling in the case of a problem. In addition, in very sensitive and first-time breeding birds, any disturbance in the nest can lead to behavioural problems (e.g. inappropriate handling of the clutch when keepers approach to the aviary) that can persist throughout their lives.

Summary:

- ☞ **try to reproduce as naturally as possible (natural incubation and hatching) and only intervene in the case of a problem.**

Nest hatching by first breeders helps to strengthen the relationship between the pair, to promote the natural behavioural development of individuals and to avoid the development of behavioural alterations that can persist for life.

A pair breeding for the first time, will need to gain experience, in order to succeed in rearing a chick successfully. This can take several seasons. Generally, all birds get excited when they hear the chick chirping because it is cold, uncomfortable or hungry (their sclera swells and their heart rate increases) and they also start to salivate. This flow of saliva can be also observed in birds breeding for the first time, showing that it is an innate reflex conditioned by the chirping of the chick but it does not happen when the chick can be seen and is not chirping.

At the beginning these birds will attempt to give the chick food (large bones) or pieces of a size which are not adequate for the age of the chick. Additionally, Bearded Vulture chicks are fed in a different

way that in other raptors. Adult birds present the food in their beak in front of the chick, and it is the chick that takes the food, unlike other raptors where the food is introduced by the parents into the chick's beak. Furthermore, most of the hatchlings present an oedema (accumulation of liquid) at the neck that usually disappears within 24 hours, making it even more difficult for the chicks to take food from the adults' beaks. This requires a lot of experience on the part of the parents on how to present the food, both in the size of the food and the position of the head, so that the chick in one of its attempts to raise its head can pick up the food from the parents' beak. This difficulty makes it quite understandable that in most cases, first time breeding pairs fail in the first years of chick rearing.

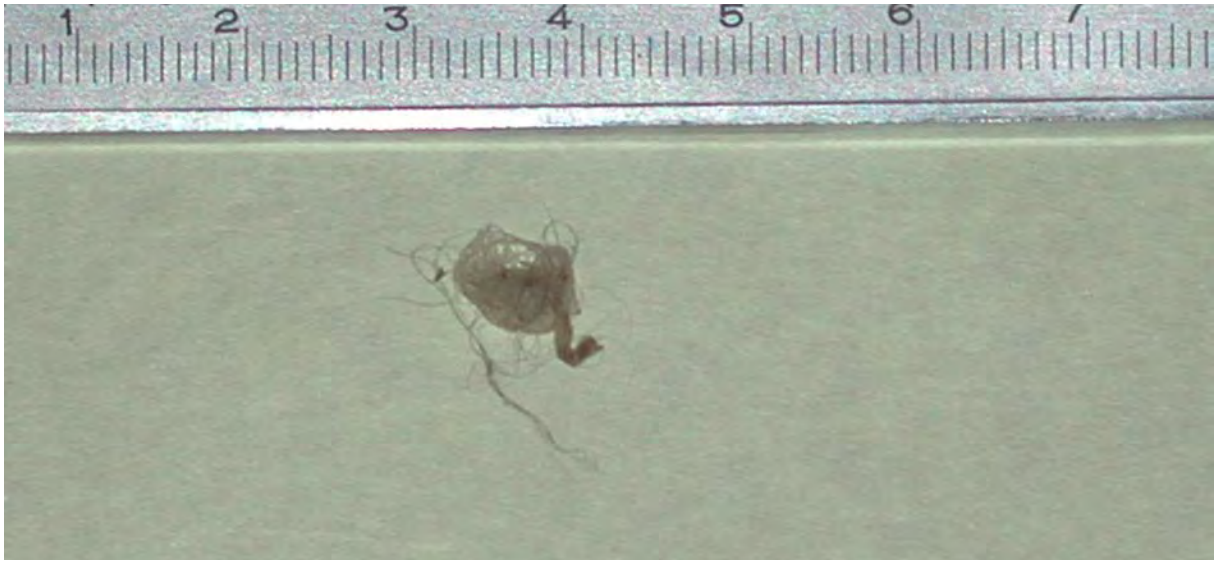
This is where keepers can intervene and speed up the learning process. Supplementary feedings by the keeper keep the chick strong enough so that it continues to chirp for food and the parents continue to try to feed it. Supplementary feedings are prolonged until the adults learn how to feed the chick correctly. This is recognised as soon as the chick gains 10% weight per day without supplementary feedings.

Bearded Vulture chicks are fed in a different way that in other raptors. Adult birds present the food in their beak in front of the chick, and it is the chick that takes the food. This is only possible if the chick is strong enough to raise its head and to have clean eyes to be able to see the food presented by the adult.

It is well known that the second[1] main cause of death in chicks is starvation due to loss of vision caused by rabbit fur stuck in the chick's eyes. As mentioned above Bearded vulture chicks are not fed in the same way as other raptor chicks, where adults will put the food in the chick's bill. Instead, Bearded vulture chicks must take the food from its parent's bill. This means that if the chick is too weak (due to malnutrition) or has vision problems (because it has rabbit fur stuck in its eyes; see picture below), it

[1] The main cause of death in Bearded vulture chicks is yolk sac infections. The second main cause of death is starvation caused by loss of vision due to rabbit fur in the chick's eyes. In inexperienced breeding pairs, another main cause of death is the parents 'inability to feed the chick properly.

will not be able to take the food from its parent's bill and will eventually starve. This is even more likely to happen when chicks are being reared by single adults, because the adult prepares the food right beside the chick. For this reason, it is advisable to skin rabbits before giving them to the birds during the first three weeks of the chick's life. Afterwards, chicks are able to remove stuck rabbit fur in their eyes rubbing his head on the wing.



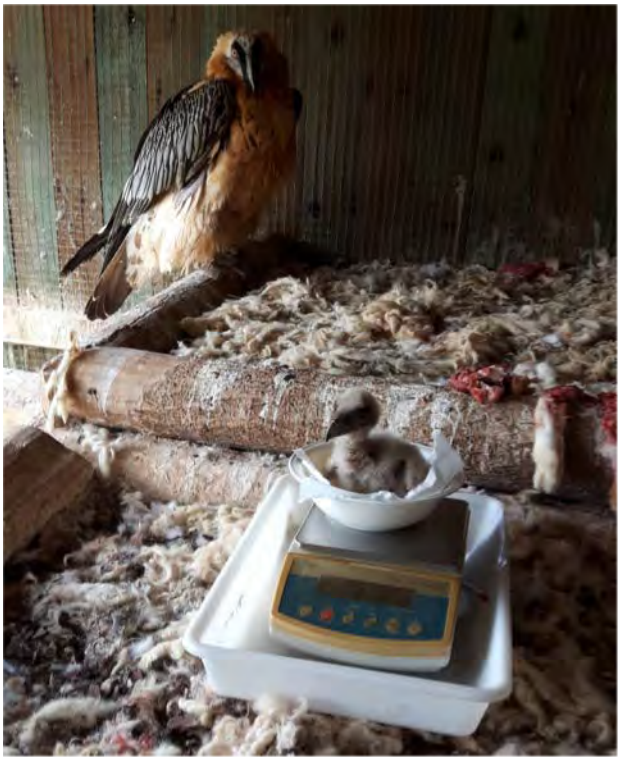
Rabbit fur removed from the eye of a 15 days old Bearded Vulture chick. Rabbit fur with tear fluids can form a lens-like structure above the eye, which can completely obstruct the chick's vision and cause its death, as it is unable to pick up the food from its parent's beak. Therefore, up to the age of 3 weeks, the condition of the eyes must be monitored through video-cameras or directly in the nest.

However, any intervention in the nest must be carried out correctly and with great caution. The adults, in their impulse to protect the chick, can fatally injure it (trample on the chick) or even accidentally kill it as a displaced aggression (redirected behaviour), by picking up the chick with their beak to protect it. For this reason, a protocol was drafted to maintain the number of chick controls in the nest as low as possible. This protocol provides general guidelines on how to proceed but should be adapted to the needs of the chick and the context of each breeding pair.

1. **Chick nest control by natural hatching, particularly with inexperienced breeding pairs:**

   - If the chick **hatched in the morning, nest control should be done 1 HOUR before getting dark** for assuring that the chick is well and strong enough for survive during the night. Check the navel, and if it is not yet fully closed and dry or still shows oedema, **disinfect it with Betadine**. Furthermore, the **chick must also be weighed** to get a reference weight. It can be hand feed 2-3g if it's asking for food or appears very weak (forced feed). But as a rule, if the chick has hatched on its own, the yolk sac provides a minimum reserve of 24 hours. During the chick control process the 2nd egg can be removed for artificial hatching. A **2nd check** should be done the **following day** late morning (11:00-12:00 o'clock). This is the time to offer a **supplementary feeding** (2-3g).

- If the chick **hatched midday-afternoon**, **1st chick check** should be done the **following day** late morning (11:00-12:00 o'clock), giving the adults enough time to try to feed the chick and gain experience. Check the navel, and if it is not yet fully closed and dry or still shows oedema, **disinfect it with Betadine**. This is the time to offer a **supplementary feeding** (2-3g) and where the 2nd egg can be removed for artificial hatching. A **2nd chick check** should be done **1 HOUR before getting dark** to see if the chick has been fed and has maintained or gained weight. If not, the chick must be feed again 3-5g. Always weight the chick before and after feeding to get reference weights and the real amount of food consumed.
- On the following days, two daily checks are carried out with supplementary feeding. The **1st chick control** around 11:00-12:00 o'clock and the **2nd control** should be done **1 HOUR before getting dark.**
- Normally between the 3rd or 4th day of life chicks recover their hatching weight and it is from this moment on that they start to gain weight. Up until this point the daily weight gain is from almost 10% a day. If this is the case, that's the moment when supplementary feeding can be stopped.



Chick controls are always done in the nest. By each control, the weight and the eyes are controlled. The whole process in done in the nest.

- **If at this age the chick is not yet gaining weight**, the **number of additional feedings** per day must be **increased to 4 times per day**. Also, it would be necessary to increase the quantity of offered food by each feeding (The total daily amount of food offered should be increased on the 3rd day to around 25% of his morning weight). Normally adult birds learn in 2-4 days to correctly feed their chicks, but there are exceptions that can take more than a week.
- **At each nest control the chick must be weighed before and after supplementary feeding**. The whole operation is done inside the nest. This implies to enter and smoothly push the adult bird from the nestling. This can be done smoothly with a stick or a shield. In very peaceful birds, this can be done softly with only a primary feather.
- During the **first 3 weeks check the eyes that they are free of hair** (especially rabbit hair). Video cameras installed at the nest site make it possible to check on the chick without performing nest checks in person, which can disturb the adult birds. If this is not the case, the control must be carried out at the nest. In the 1st week every 2nd days. In the 2nd week every 2-3 days and in the 3rd week two controls are enough. **This must be done also done with chicks reared by experienced pairs**.

2. Human hatching assistance for parent reared eggs/chicks:

- If **24 hours after the chick has started hatching** it has not been able to hatch on its own, assisted hatching should be carried out (see point 3.4. Human assistance in hatching). If possible, the whole process should be carried out in the nest. After removing the chick from the egg, a **weight check** and **disinfection of the navel with Betadine** should be carried out.
- As general rule chicks that have required human help in hatching are more weakened, being necessary to start feeding earlier. Further, they require **human support by feeding** and in some cases must even be **force-fed**.
- Chicks that required **assisted hatch in the morning**, **1 HOUR before getting dark** a **supplementary feeding** (2-3g) must be done.
- Chicks that required **assisted hatch in the afternoon**, on the **following day early morning** (2 hours after sunrise) a **supplementary feeding** (2-3g) must be done.
- Thereafter, the number of feedings per day will depend on the vitality of the chick, with a **minimum of 2 feedings per day** and **a maximum of 4 per day**.
- As soon as the chick starts to gain weight, the number of feedings will decrease.
- **At each nest check the chick must be weighed before and after supplementary feeding**. The whole operation is done inside the nest. This implies to enter and smoothly push the adult bird from the nestling. This can be done smoothly with a stick or a shield. In very peaceful birds, this can be done softly with only a primary feather.
- During the **first 3 weeks check the eyes that they are free of hair** (especially rabbit hair). Video cameras installed at the nest site make it possible to check on the chick without performing nest checks in person, which can disturb the adult birds. If this is not the case, the control must be carried out at the nest. In the 1st week every 2nd days. In the 2nd week every 2-3 days and in the 3rd week two controls are enough. **This must be done also by chicks reared by experienced pairs**.

3. ARTIFICIAL INCUBATION

Although the ground rules of the Bearded Vulture EEP is to intervene as little as possible to allow reproduction to be as natural as possible, there are situations that require human intervention and therefore artificial incubation:

- doble clutches, because of “cainism”, the second egg must be removed before hatching and requires artificial incubation.

- in pairs where hatching in the nest has failed in previous years.
- in birds showing incubation behavioural disturbances due to inadequate management during their life or not properly reared during its nestling time.
- Handicapped birds which are not able to handle the egg properly or cannot properly build a nest-bowl, being able to roll the eggs freely throughout the nest with a higher breakage rate.
- when birds stop to breed because of disturbances by different nature (public nuisances, works/repairs in the vicinity of the cage, presence of territorial dominant neighbouring raptors, etc.).

Consequently, egg extraction it will take place at different times of incubation depending on the cause of the need to be extracted:

- in double clutches, the 2nd egg will be removed:
  - principally, by the 1st nest check of the hatched chick from the 1st egg.
  - exceptionally, there are breeding pairs as soon as the chick starts to hatch, they don't take care of the 2nd egg. By these pairs it is recommended to remove the 2nd egg before the 1st egg starts to hatch: 1 week before hatching.
- in pairs where hatching in the nest has failed in previous years, the single or double clutch must be removed 7-10 days before hatching.
- in all other cases the clutch is removed from the nest as soon as the problem appears (stopped to incubate) or immediately after laying in couples that do not incubate the eggs well for different reasons (physically handicapped birds or those with behavioural alterations).

Very important: never leave the nest empty after removing the clutch. Dummy eggs must be offered in order not to interrupt the reproductive cycle of the breeding pair so they can become potential foster pairs.

3.1. Artificial incubation protocol

It's recommended to **start up and check the incubator before the pair have laid** (according to laying records of the pair), having previously cleaned and disinfected. It is also very important to take special attention on the ambient temperature (20°-24° C) and humidity (ideal 40%, but <60%) in the room where the incubators are placed.

Before placing the removed egg in the incubator, it is recommended that a number of checks are carried out:

- weigh the egg
- check that the eggshell is intact. By candling it, any cracks, holes, or extreme porosity due to lack of calcium can be seen very well. **Depending on the size of the imperfections**, they **should be sealed** to prevent possible infection or inadequate egg weight loss.
- to evaluate the **level of dirt** (primarily faeces) adhering to the eggshell. **Never clean it with water**, as it can promote microbial invasion. Just clean it with soft paper towels. **Never scrape off dirt with a scouring pad** that could damage the cuticle of the egg. The **egg cuticle**, the outermost part of the egg, with a thickness from µm, covers the pores preserving the inside of the egg from microbial contamination.

Summary:

☞ **before placing the egg in the incubator check its weight, if the eggshell is intact and the level of dirt.**

☞ **Never scrape off dirt with a scouring pad that could damage the cuticle of the egg.**

Depending on when the egg is removed from the nest, we distinguish two scenarios:

1. artificial incubation since the egg has been laid (lay day = 0 day)
2. artificial incubation just during the last incubation days (egg has been removed during the last 7-10 days before hatching when air cell displacement has already started).

For both cases the parameters used for artificial incubation of bearded vultures (see table below) are very similar to those used with other birds of prey, **mimicking natural incubation as much as possible**:

Temperature: 37.3°C – 37.5°C
It is recommended to use incubators that automatically renew the air inside the incubator during the turning process. In this way, they promote a temperature fluctuation during the egg turning, simulating what happens in the nest during the turnover by adults.

Humidity: 25%-35% humidity (adapting to the evolution of the daily weight loss of the egg).
It is recommended **not to use the automated humidity system**, as it does not mimic the natural incubation process. In the nature Bearded Vultures are breeding in dry areas -like in several habitats in Spain-, or in very humid areas -like in several areas in the Alps-, but in none of them is the humidity constant throughout the incubation process. It also varies each time the adults carry out an incubation exchange. That's why we advise **to use a water bowl**, filling it with distilled water in such a way that the humidity inside the incubator does not exceed 35%, and refilling it with water every 24 hours. In this way, during the evaporation of the water, the humidity inside the incubator decreases slowly, but should not fall below 25% (for more details see chapter 3.2. Egg control; Weight). It is only advisable

to increase the humidity above the recommended values, if the egg tends to lose more than necessary due to a defect in the shell (poorly formed, cracks, etc.).

Egg turning: it should be turned at least 5-10 times a day, with adequate hygiene precautions if this is carried out by hand and not always do them in the same direction. If you have an automatic rotate egg system, you can set it for once every 2 hours.
It is **recommended** to use incubators where **different parameters can be set for the day and for the night**. This makes it possible to mimic the natural incubation process, decreasing the night turning intervals (at least every 3 hours).

In case the egg has been **removed soon after had been laid**, we add an **additional management protocol (Thermal shock)**. Furthermore, the automatically turning of the egg, should be 4 times a day removed for 5 minutes from the incubator and exposed to outside temperature (it doesn't matter if the outside temperature is under 0° C). Additionally, the egg should be turned manually 180° with each thermal shock assuring a full turning during the whole incubation. This thermal shock helps the movement of the fluids, improving gas exchange, and helps the healthy development of the embryo.

***Thermal shock**. Additionally, to the automatically turn of the egg, the egg should be exposed 4 times per day for 5 minutes to outside temperature. Further with each thermal shock the egg is turned manually 180°.*

It is very important do not confuse thermal shock with egg cooling:

- **By egg cooling**, the egg is exposed to a temperature below the incubator for a certain period of time in order to reduce the temperature inside the egg. In reaction to this **drop in temperature inside the egg**, the embryo tries to maintain its temperature by using the energy resources at its disposal (the yolk). 1/3 of the yolk is required for embryo development, another 1/3 is used as energy and the last 1/3 is used for hatching. Consequently, any energy expenditure above normal would have a negative effect on hatching, as energy resources within the egg are limited.

☞ **By thermal shock**, the egg is exposed for a short time to low outdoor temperatures, not being able to reduce the temperature inside the egg, but it does cause a **contraction of the blood vessels of only those on the surface of the egg**. This recognises that only the blunt end of the egg is cold, the rest stays just as warm. As soon as it is no longer exposed to the outside temperature, the blunt end immediately returns to the temperature of the egg.

Independently if the egg has been removed from the nest later or sooner, in both cases, **once the air cell starts to enlarge (between 7-10 days before hatching) the egg is no longer turned, but the thermal shock is still maintained without manual 180° egg rotation.** Once the displacement of the air cell has started, it is checked daily by drawing the displacement with a pencil through an egg candler. The displacement varies, being in the first days a couple of millimetres per day and at the end reaching more than a cm. The egg must always be held with the displacement facing upwards.

The air cell displacement starts 7-10 days before hatching and it is checked daily by drawing the displacement with a pencil through an egg candler. The displacement itself can vary between a couple of millimetres in the first days and more than a cm in the last days.

In this phase (at hatching time), the proper position of the embryo is with the head-under-right-wing, slouched neck and upside down, with the embryo's vertebral spine parallel to the longitudinal axis of the egg and the head directed towards the air cell in the large end of the egg (see figure 3).

The embryo breaks the egg membrane approximately 3 days before hatching and starts to breathe with its lungs (**internal pip**). From this moment onwards, the embryo can be heard

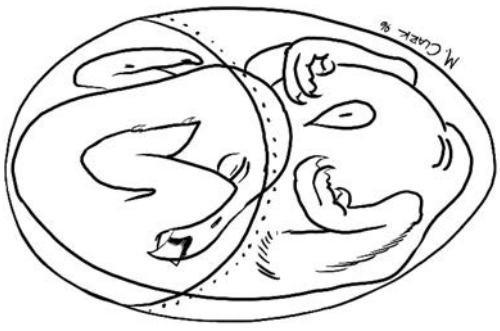
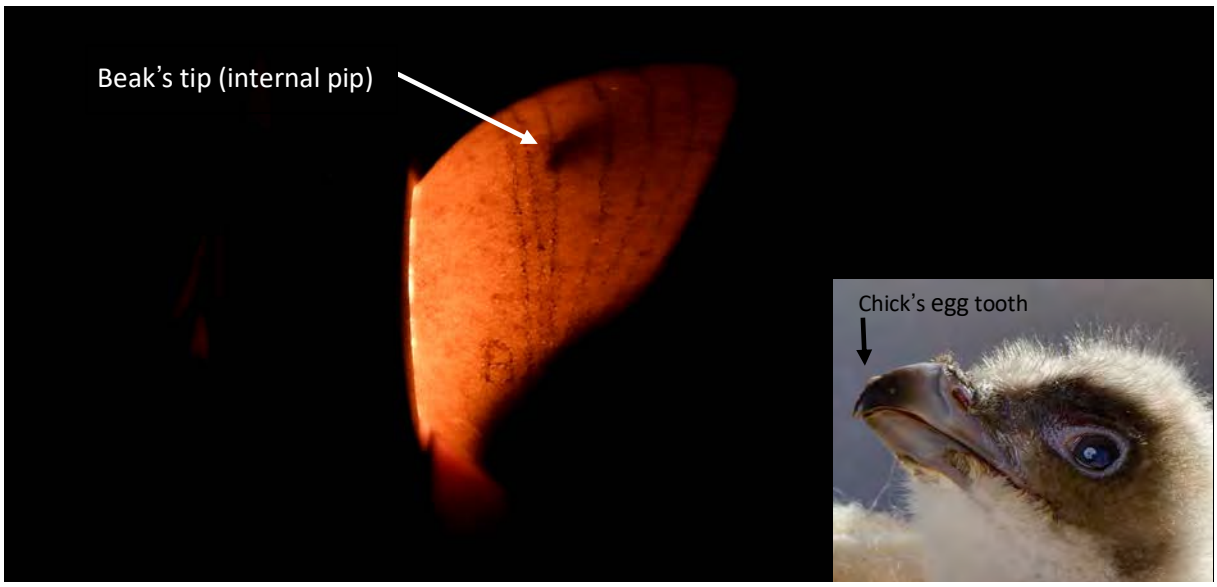
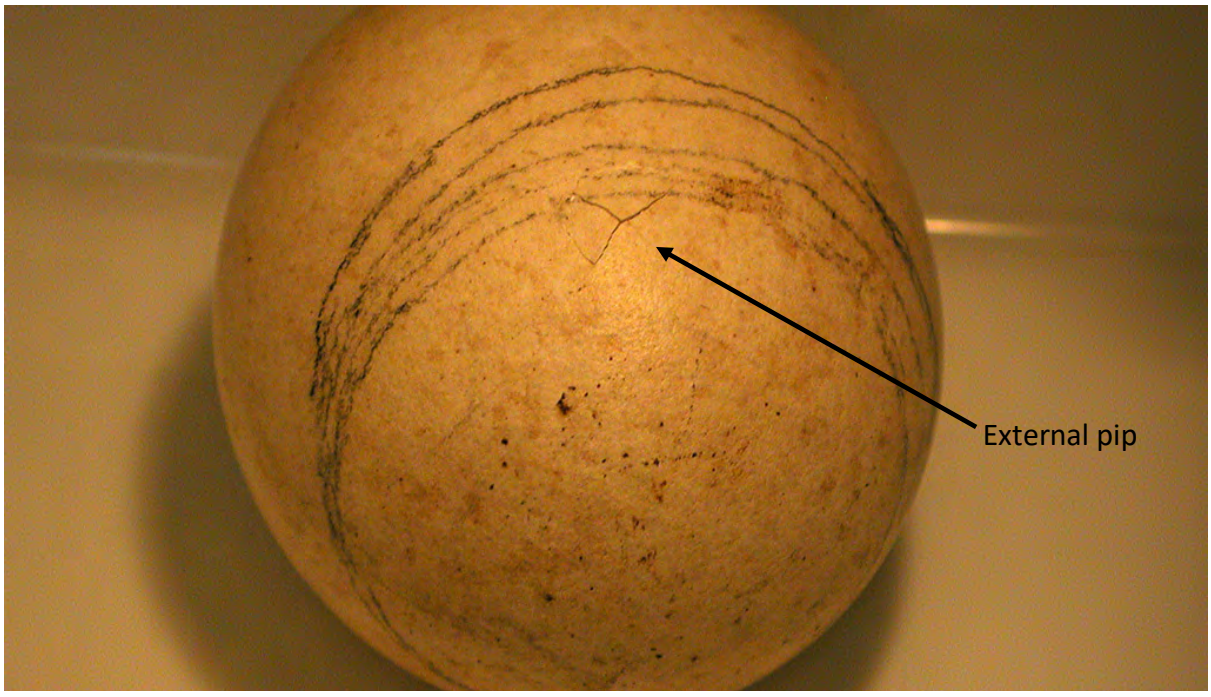


Figure 3: *Proper hatching position of the embryo with the head-under-right-wing; M. Clark©.*

vocalizing. By candling the egg, it is possible to see the beak of the chick in upper part of the air cell and at the bottom of the air cell, the shoulder pushing the egg membrane towards the inside of the air chamber (see picture below). **Thermal shock is maintained without 180° manual egg rotation**.



One to three days before hatching the chick pecked the shell with its egg tooth **(external pip)**. At this point humidity should be raised to 80% and the **thermal shock stops**.



If the embryo is positioned correctly, it opens the egg in an anti-clockwise direction, starting from where is has done the external pip. Hatching may go on for several hours and is occasionally interrupted by rest periods. To facilitate hatching and to prevent the egg from rolling around in the tray during the hatching process, the **egg should be fixed with paper or cotton wool**.

Incubation parameters:

Age	Temperature	Humidity	Thermal shock	Turning	Illumination
From the Beginning until air cell starts to displace	37.3°C – 37.5°C	25-35%	4 times a day during 5' exposed to outside temperature and turned manually 180º	10 times a day	darkness
When air cell starts to displace since internal pip			4 times a day during 5' exposed to outside temperature	-	
From the external pip to hatching		80%			light
During the first 5-10 hours of chick's life		Ambient level			

Summary: we try to mimic natural incubation as much as possible

- ☞ **Thermal shock should be always done until the chick pips the eggshell.**
- ☞ **Temperature fluctuation happens every time the adults carry out an incubation exchange. This is why it is recommended to use incubators that automatically renew the air inside the incubator during the turning process, promoting temperature fluctuation.**
- ☞ **In the nature, humidity varies constantly depending on the climate. For this reason, we recommend the use a bowl, which, by slowly evaporating the water it contains, helps a gradual daily change in humidity. The fluctuation should be between 25% and 35%.**
- ☞ **Only increase the humidity above the recommended values, if the egg tends to lose more than necessary due to a defect in the shell (poorly formed, cracks, etc.).**
- ☞ **By natural incubation, breeding pairs turn their eggs at each interruption (short incubation rest, changing incubation position or exchange), the interruptions being irregular in time. Additionally, the number of interruptions during the night are much lower than during the day. This is why it is recommended to use incubators where different parameters can be set for the day and for the night and turning frequency is based in different and random algorithms.**
- ☞ **Do not confuse thermal shock with egg cooling.**

3.2. Egg control

Throughout the whole artificial incubation process, the egg must be monitored to control the good development of the embryo, especially if several eggs are kept in the same incubator, there is a danger that one egg could abort and contaminate the rest of the eggs. That is why it is so important to determine as soon as possible if an egg is infertile/aborted, to be removed immediately from the incubator. For this purpose, different methods are used, each of them depending on the nature of the egg (grade of pigmentation) and incubation time, being egg weight loss one of the parameters monitored during the whole incubation process.

Weight loss

On average, the weight loss of a Bearded Vulture egg during the entire length of incubation period (52-54 days) is **on 14% (n= 43 monitored hatched eggs; 34 artificial incubation and 9 natural incubation; Centre de Fauna Vallcalent 31 eggs and Centro de Cría Guadalentín 12 eggs)**. However, some

successful hatched eggs showing a lower weight loss during the incubation process, reaching even a loss of less than 10% (range 9.09%-17.06%), although humidity has been adapted to its weight lost evolution.

As examples, the 9 artificially incubated eggs that hatched of female BG 115 showed an average weight loss of 13.68% (range 11.72% - 15.40%). Of these 9 eggs, 4 were artificially incubated from the day of laying (= day 0), 3 were removed from the nest with three days of natural incubation, 1 with five and the last with 6 days of natural incubation. Another example is the 11 artificially incubated eggs of female BG103 with an average weight loss of 14.17% (range 12.08% - 15.27%), of which 6 were artificially incubated from day 0. On the other hand, the 8 controlled eggs from the day of laying of the female BG041, showed an average weight loss of 15.04% (range 13.10% - 16.75%). Most interestingly, the two eggs artificially incubated from the day of laying showed a weight loss of over 16%, well above the other two females (13.68% and 14.17% respectively). In contrast, the 6 naturally incubated more than the first half showed an average weight loss of 14.51%, similar weight loss to the artificially incubated eggs of the first two females. And as a last and most extreme case, the two artificially incubated eggs from day 0 and 1 of the female BG658 have shown the highest and slightest loss of the 43 eggs monitored (17.16% and 9.09% respectively). In all artificially incubated eggs, an attempt was made to adapt the humidity according to the weight loss (target of 16%), within the humidity range established for Bearded Vulture eggs (25%-35%).

Finally, it must be considered that during the hatching process, the **weight loss once the chick has already made the external pip is much higher than during the previous days**, losing more than 1-1.5g per day. This high weight loss per day has a significant influence on the total loss, especially in cases where it takes a few days between the external pip and hatching.

These results shows that the **embryo has the capacity** to adapt within a normal range to external climatic factors, being able **to regulate by itself weight loss**.

However, abnormalities do occur, as happened in 2014 when it rained throughout most of Europe during the incubation period, causing most of the eggs to abort/putrefied (12 from 20 laid eggs), as high humidity promotes the immigration of germs and contaminates the egg.

All eggs before being placed in the incubator for the first time must be weighed on a precision decigram scale. Subsequently, a **weight check is recommended every 3 days**, so that the incubator humidity can be adjusted within the range of 25%-35% according to the egg weight loss. It is only advisable to increase the humidity above the recommended values, if the egg tends to lose more than necessary due to a defect in the shell (poorly formed, cracks, etc.).

Summary:

- ☞ **The average weight loss of the egg during its 52-54 days of incubation is on 14%.**
- ☞ **Check the weight of the egg before introducing the egg for the first time in the incubator, and every 3 days thereafter.**

- ☞ **The embryo has the capacity to regulate by itself weight loss if the external climatic factors are within a normal range. Therefore, adjust incubator humidity within the range of 25%-35% according to the egg weight loss.**

- ☞ **Only increase the humidity above the recommended values if the egg tends to lose more than necessary due to a defect in the shell.**

Egg candling

To rule out infertile, aborted or rotten eggs as soon as possible, is recommended daily egg candling to check the development of the embryo. Fertility can be determined from the sixth to seventh day. Due to the thickness of the Bearded Vulture eggshell, candling can only be done with candlers adapted for ostrich eggs. At the point of hatching, the egg thickness measures 0.67 mm in infertile eggs (N = 22) and 0.63 mm in hatched ones (N = 62) (Frey et. al. 1995). Furthermore, depending on the pigmentation of the eggshell, different parameters can be monitored by candling. For more pigmented eggs there are other devices to monitor its development (see photos below of Laseroscope and Buddy).

While **candling unpigmented eggs,** pay special attention to:

- Determine is the egg is fertile. For eggs removed at the beginning from the nest, confirm if the egg is fertile by the appearance of the germinal disc and vessels from the seventh day of incubation. Further check/evaluate vessel growth during the following 7 days. (See point 3.3 Embryo development).

- the growth of blood vessels, their thickness and that they are maintained throughout the incubation period.

- the growth of the chick and its movements.

For **all eggs** take special attention to:

- check the membrane between the air cell and the rest of the egg to see if it remains intact during the whole incubation period.

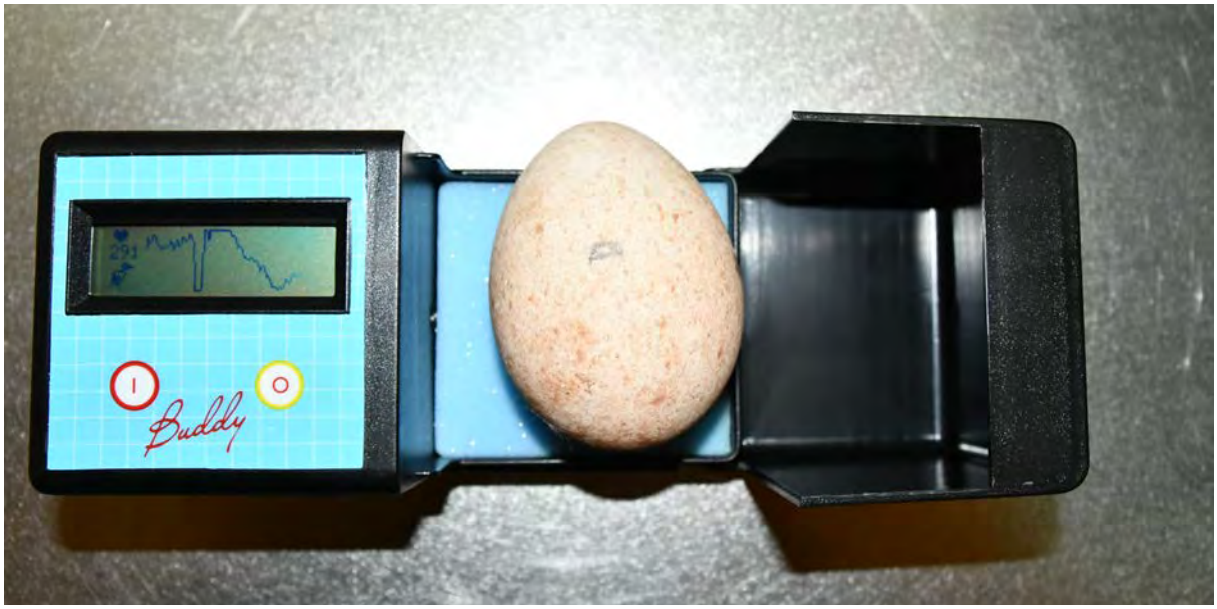
- The progression of the air-cell.

- if the chick has pipped the air cell **(internal pip)**.


Egg candling: *depending on eggshell pigmentation, different parameters can be monitored. By unpigmented eggs (left picture) the vessels growth and the embryo evolution can be monitored from the beginning of incubation. By pigmented eggs (right picture), only the air displacement and internal pick can be monitored.*

Buddy MK2

One of the ways to determine if the embryo is alive in pigmented eggs is to use the Buddy, where movements and heart rate can be registered. Such a device has been designed primarily for falcon, hawks and eagle-sized eggs. Therefore, depending on the size and form of the Bearded Vulture egg, it is often difficult to detect heartbeat and movement, particularly the heartbeat if the embryo is moving a lot. With experience **from 25 days of incubation onwards**, the vitality of the embryo can be monitored by using the Buddy.



Heart rate and movement after thermal shock of a 28-day old embryo using the Buddy.

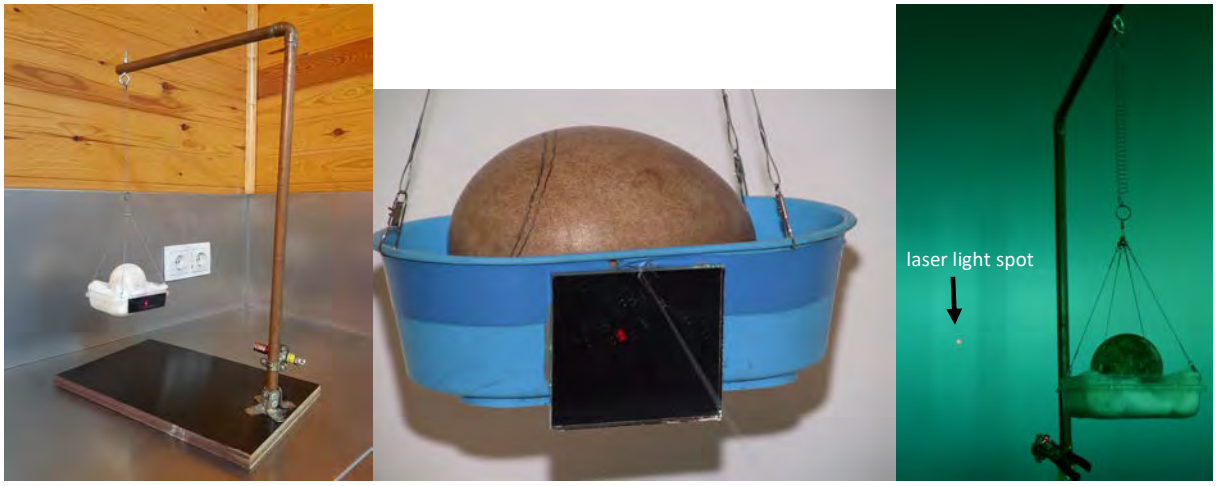
Heart rate can vary greatly depending on the activity of the embryo (if the embryo is sleeping or awake). In moments of high activity (after thermal shock, when it chirps in the air chamber, during internal/external pick or hatching) heart rate can exceed 300 beats per minute. But if these high

pulsations are sustained in longer periods (even exceeding 400 beats per minute) and accompanied by a chirp of displeasure when the chick is already in the air-cell, or vice versa, if the embryo's heart rate and movements progressively decrease, and the chick is no more chirping, human intervention is recommended (see point 3.4. Human assistance in hatching).

Laseroscope

A second way to determine if the embryo is alive in pigmented eggs is to use the laseroscope. The laseroscope determines if the chick is alive by detecting and **making the movement of the embryo more noticeable, by projecting its movement with a laser beam** against a wall at a certain distance from the device (Pichler, 2000). The bigger the distance between the laseroscope and the wall, the better the embryo's movements are visible, the ideal distance being about 3m. The whole process should be carried out in a dimly lit room, so that the projection of the laser beam can be better detected.

The device consists of a basket, into which the egg is inserted. The basket itself is attached by a string and a spring to a solid structure. The spring must be of such elasticity that when the egg is inserted into the basket it must elongate by at least twice its length. This solid structure has a foot at a certain distance from where the basket hangs. On this foot is a laser pointer which is projected onto a mirror attached to the front of the basket. Once the egg is inserted, the basket tends to move slightly along the spring like a pendulum, and each time the embryo moves, its movements are transferred to the basket and therefore to the mirror where the laser beam is projected. To minimize the pendulum effect, a fishing line can be tied into the basket with the foot of the device. The movements of the embryo are recognisable by the sudden change of the pendulum movement projected to the wall. These changes may be of amplitude, a sudden change of direction, or small jumps. In an infertile or aborted egg, these changes do not appear and the pendulum movement also disappears at the end, leaving a fixed luminous point on the wall**. With experience and knowledge of your own device** (laseroscopes are not on the market and each one knows its sensitivity depending on the spring used) it is possible to **recognise the vitality of the embryo**. In general, **from 35 days of incubation onwards**, the movements are very well visible with the laseroscope. However, **with experience, slight movements can already be detected within 25 days of incubation**.



Laserovoscope. *The left picture shows the design of the device, which contains a basket attached to the structure with strings and a spring. At the front of the basket there is a mirror that reflects the laser beam coming from a laser pointer attached to the front of the basket. The middle picture shows the laser beam reflected on the mirror. The right picture shows the projection on the laser beam on a wall, which comes from the laser pointer attached to the structure and reflected in the mirror attached to the basket in a dimly lit room (Pichler, 2000).*

Summary:

- **The laseroscope enlarges embryo's movement by projecting its movement with a laser beam against a wall.**
- **From 35 days of incubation onwards, movements are well visible with the laseroscope (25 days of incubation onwards with experience).**
- **With experience it is possible to recognize the vitality of the embryo.**

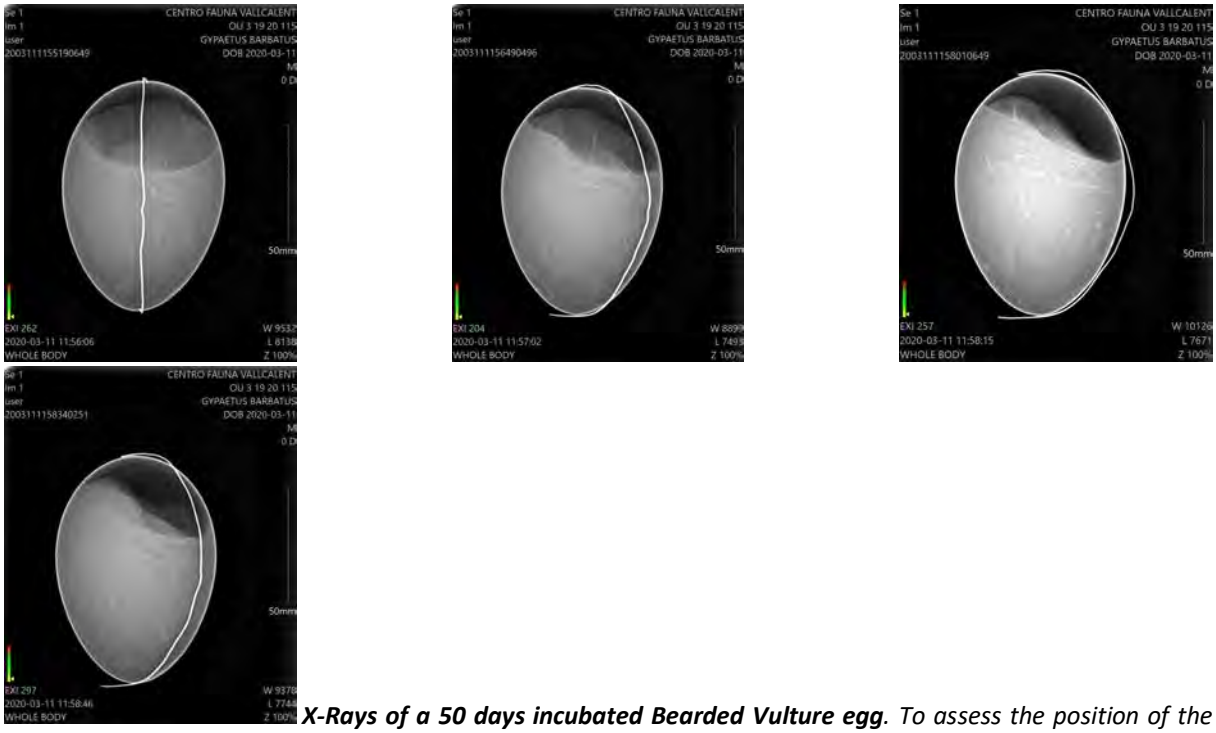
X-Ray

In cases where the chick does not break the air cell or fails to hatch, an X-ray is recommended to do to determine the location of the head and to rule out malpositioning. To correctly assess the position of chick's head, it is necessary to take 4 radiographs from different planes, turning the egg in its long axis each time by 45°. To facilitate the analysis of the radiographs and to know the positioning of the egg, it is recommended to fix a wire along the long axis of the egg with insulating tape at both ends of the egg. The wire should be fixed over the spot where the maximum progression of the air cell is located. This protocol was drafted and is used in Los Angeles Zoo for the California Condor (per. comm. Chandra David, 2017):

1. The first radiograph has the wire straight up and down on the vertical and all rotation must be done clockwise.
2. The second view is taken with the wire rotated 45°.
3. The third view is done by turning the egg again by an additional 45°.
4. The last view the egg is rotated additionally 45°.

The X-Ray settings for Bearded Vulture eggs are as follows:

- 46kV
- 100 mA
- 63mS



X-Rays of a 50 days incubated Bearded Vulture egg*. To assess the position of the chick's head, four X-rays are required, rotating the egg clockwise by 45° each time. As a reference point for correct rotation, a wire should be fixed over the spot where the maximum displacement of the air cell is located. In these X-rays we can see the correct position of the Bearded Vulture chick during the air cell displacement process. (California Condor protocol, per. comm. Chandra David, Los Angeles Zoo 2017).*

3.3. Embryo evolution

By artificial incubation it is very important to recognize as soon as possible any infertile or aborted egg to discard and avoid contamination of fertile eggs. However, this is only possible with unpigmented eggs where embryo development can be monitored from the start of incubation. On the other hand, in pigmented eggs it is possible to determine the state of the eggs from day 25 of incubation, by recording embryonic movements and heart rate using the Buddy and the Laserovoscope. A candler (ovoscope) can be also use by pigmented eggs to follow the air cell displacement and the internal pip, when the chick introduces its head in the air cell and starts to chirp.

In relation to embryo development, **after six days of incubation**, in unpigmented eggs, it is possible for the first time to determine whether the egg is fertile or not. At this stage it can be observed that **the shadow of the yolk has become more denser and has doubled in size**.

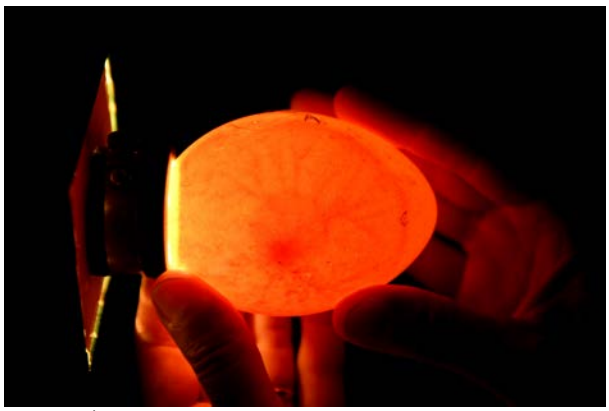
Egg candling by unpigmented egg. *On the left picture a four days incubated egg. On the right a six days incubated egg, where we can see that the shadow of the yolk has become denser and has doubled its size, showing that the egg is fertile (lay day = day 0).*

At seven days of incubation it is the first time that the **germinal disk and first vessels can be observed**. Subsequently it is possible to follow the **growth of the vessels system on average until the 13th incubation**, a time when the curvature of the egg made it impossible to monitor its growth (see below).

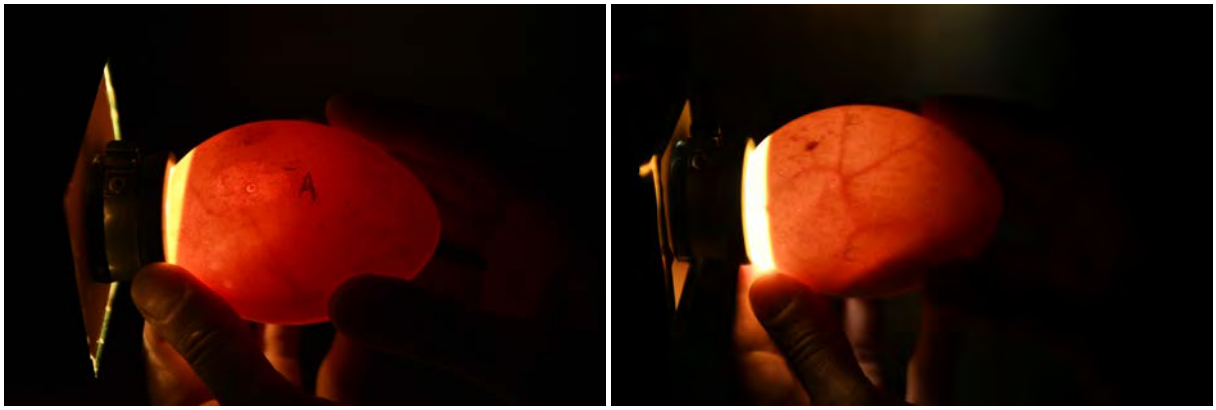
Incubation day	6th		7th		8th		9th		10th		11th		12th		13th	
Vessels growth	wide	large	wide	large	wide	large	wide	large	wide	large	wide	large	wide	large	wide	large
Size in cm	**Not visible**		1,74	2,18	2,93	3,37	3,69	4,38	4,45	5,65	5,25	6,69	5,90	7,39	6,10	6,30

Left: vessels system growth from the 8th to the 13th incubation day.
Right: vessels system growth from the 7th to the 12th incubation day.

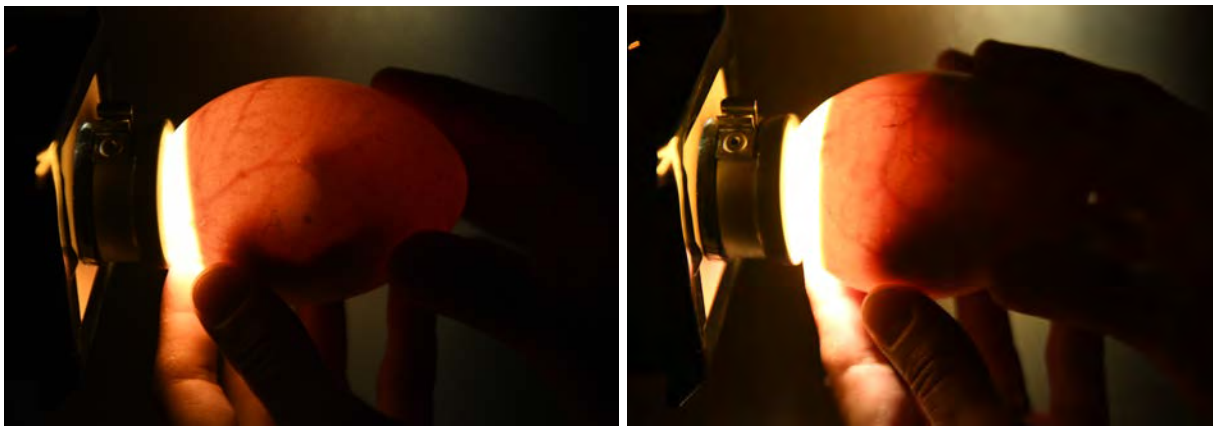
Afterwards, because of the growth of blood vessels in the secondary plane makes everything more diffuse, in some cases, it can be difficult to visualise the blood vessels until around the 21st day of incubation when some major vessels appear and later, the growth of the embryo can be visualised.



Left: 14th incubation day, where vessels and embryo growth are good visible by unpigmented eggs.
Right: 19th incubation day. Because of the vessel's growth in the secondary plane, everything becomes more diffuse, and only the embryo can be visualised.

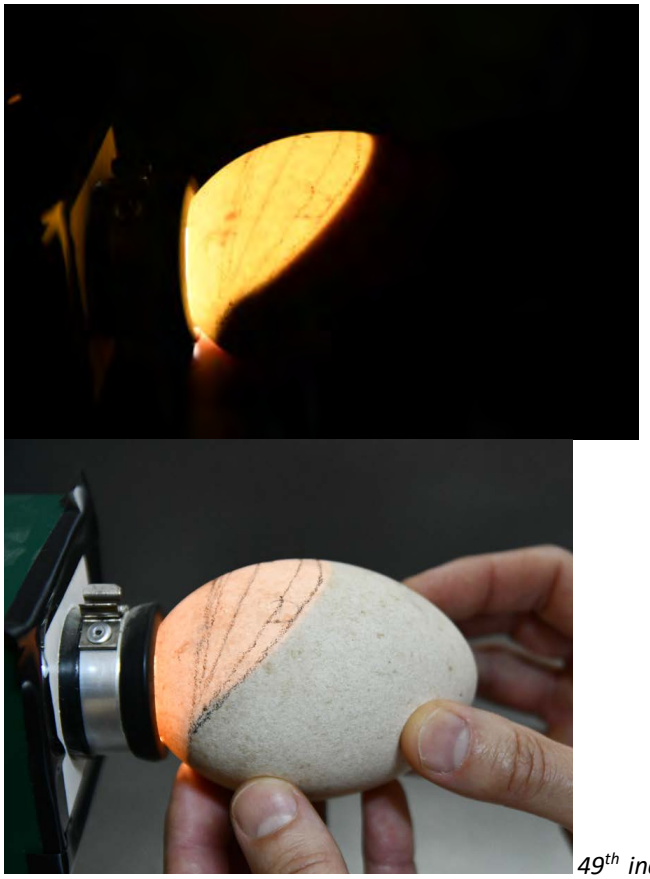


Left: 21st incubation day, major vessels are visible.
Right: 28th incubation day. The embryo with its annexes and blood vessels can be well identified.



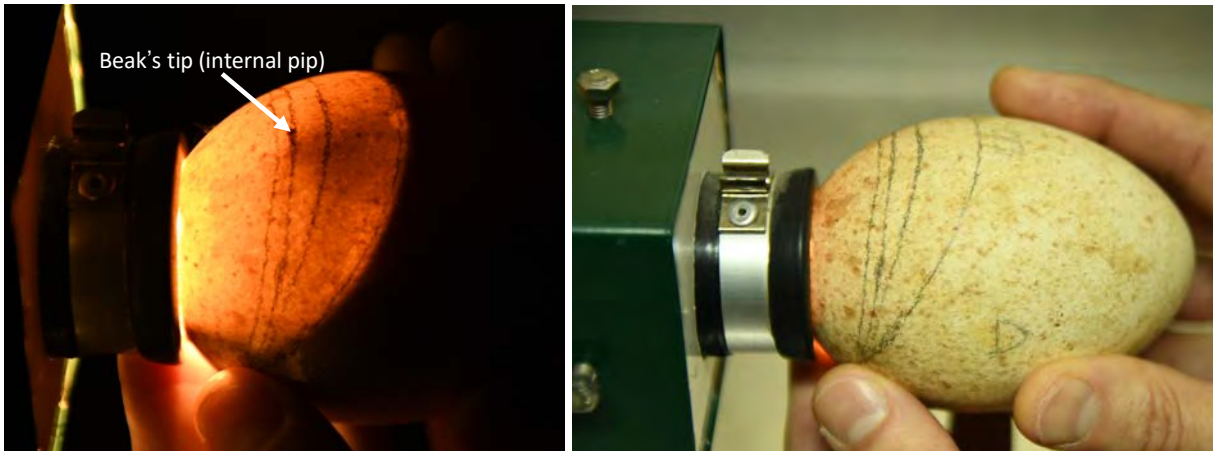
Left: 35th incubation day and Right: 42nd incubation day. It can be seen how the embryo increases in size.

Between the 43rd and 46th incubation day, air cell starts to displace and can take between 6-9 days, when the chick pecks the air cell (internal pick) and starts to chirp (mostly between the 48th and 51st incubation day). The total displacement of the air cell can vary from just below about 20mm to about 35mm.



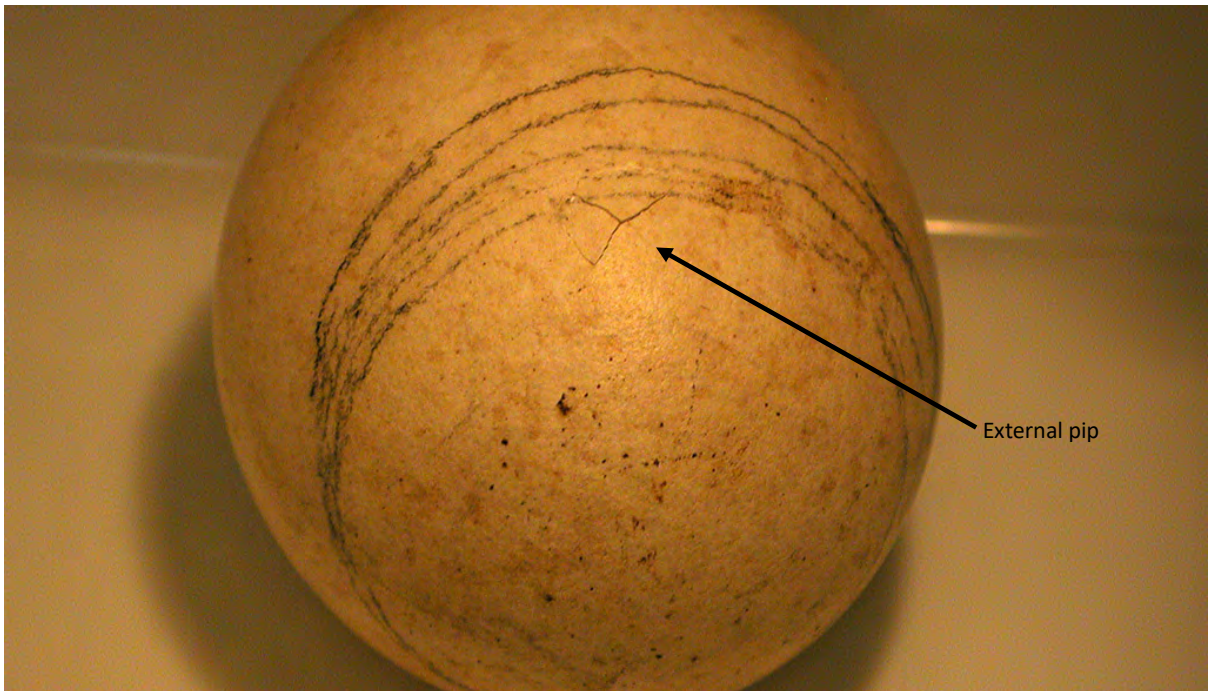
49th incubation day, the fifth day of air cell displacement. The air cell displacement started on the 45th incubation day. In six days, the air cell displaced in total 34.6mm.

In average, between the 48th and 51st incubation day, the chick pecks the air cell, introducing the beak in it (**internal pip**). These can be perfectly observed by candling, independent if the egg is pigmented or not.

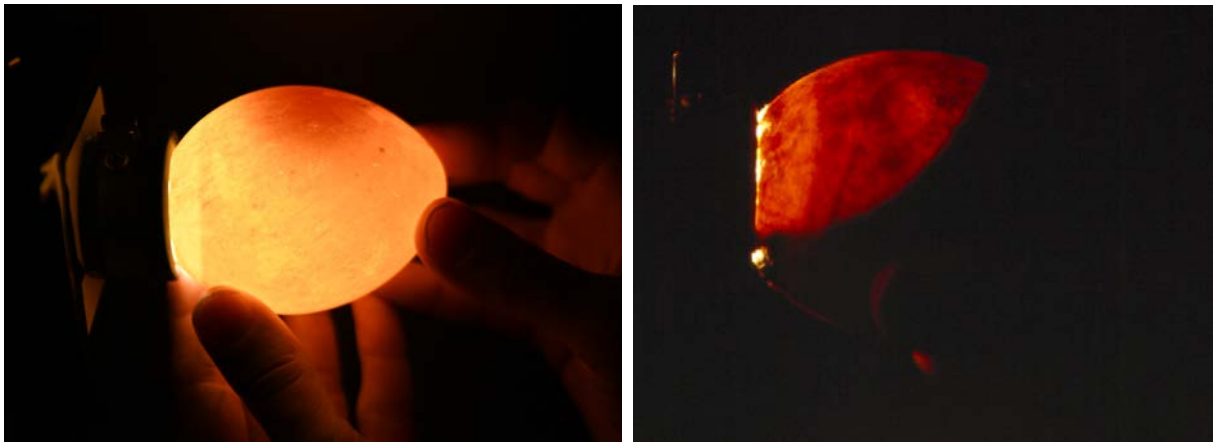


Internal pick on the 51st incubation day. The air cell displacement started on the 48th incubation day. In three days, the air cell displaced in total 23mm. The chick hatched on the 54th incubation day.

And finally, one to three days before hatching the chick pecks the shell with its egg tooth (**external pip**).



On the other hand, by infertile eggs, the shadow of the yolk doesn't increase and always remain floating when the egg is rotated during candling. In rotten eggs, the dividing line between the air cell and the rest of the egg always remains the same when the egg is rotated during candling, because of the rotten liquid in the egg. And finally in aborted eggs, we can observe how the blood vessels are disappearing, and if the egg was already in the last stage of incubation, we can no longer register any movement or heartbeats. Later the membrane between the air cell and the rest of the egg breaks down, and it is no longer clean and continuous.



Left picture: infertile egg. The shadow of the yolk doesn't increase and always remain floating when the egg is rotated during candling.

Right picture: rotten egg. The dividing line between the air cell and the rest of the egg always remains the same when the egg is rotated during candling, because of the rotten liquid in the egg.

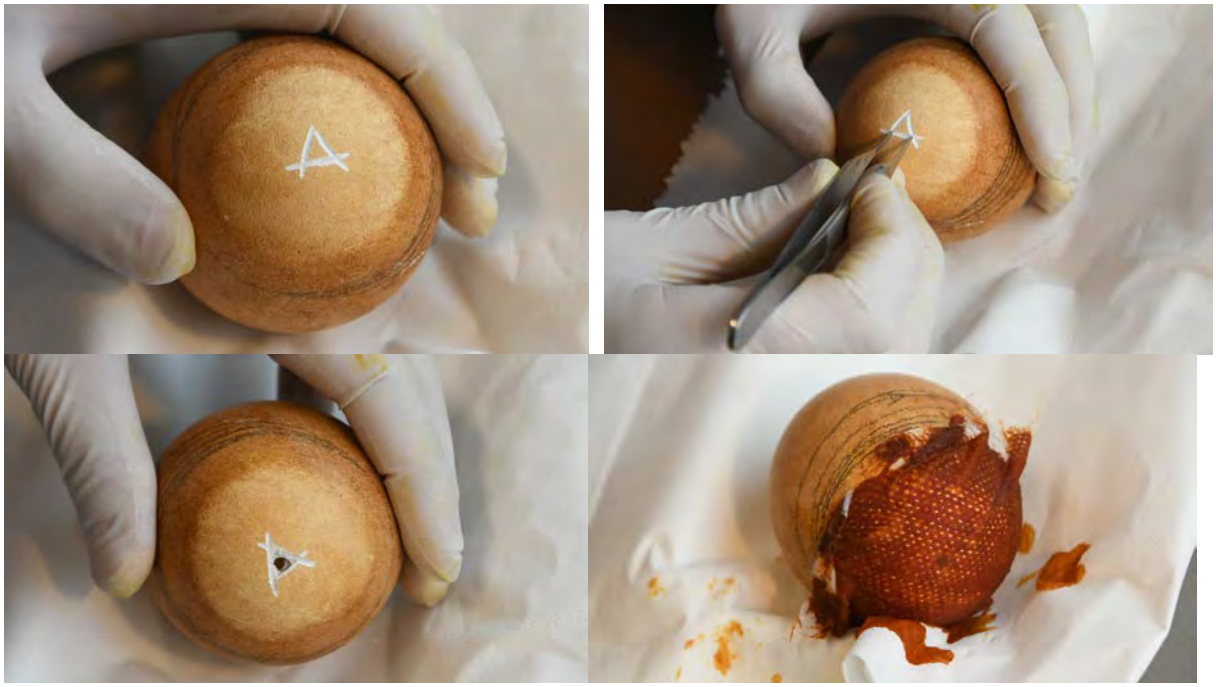
3.4. Human assistance in hatching

There are many reasons why a fertile egg fails to hatch. However, in some cases, especially when the embryo is close to hatching, it is possible to intervene and help the chick to survive. In this stage hatching problems occur especially when:

1. the chick is very large and cannot move its head, preventing it from being able to make the internal pip or external pip,
2. if the chick is too weak and is not able to hatch,
3. if the membrane has stuck to the neoptile when drying, impeding the chick's ability to rotate and continue hatching or
4. bad positioning

In the first case, when the chick is too big and cannot pip internally or externally, it can be heard chirping of displeasure loudly and frequently at first. At this stage, with the Buddy we can detect a constant high heart rate, even exceeding 400 beats per minute (not to be confused with sporadic high heart rates that occur when the embryo is active, e.g. after heat shock, or if the embryo is awake and very active), and intense movements, showing its discomfort. As the oxygen is consumed, the embryo becomes weaker, its heart rate and movements decrease progressively until they stop to chirp, and the heart rate is around 100 beats and even below. This is the latest time at which human intervention is required. The same goes for a weak chick that does not have enough energy to perform its own hatching. In the Buddy we observe very low heart beats.

In both cases we perform a 1-1.5cm square hole in the eggshell in middle of the air cell. In case the chick has not made the intern pip, but is already chirping and radiographs confirm good positioning, the hole shall be made in the air cell near the membrane, giving us the possibility to access the membrane and make an opening without damaging possible blood vessels. In general, as soon the hole is done, chicks immediately start to chirp more strongly. Afterwards the eggshell-hole should be covered with gauze soaked with Betadine and return it to the hatcher keeping like this for next 24-48h, depending on chicks' reaction, how long it was already chirping in the egg and the hatching date. If we are under 54th incubation day, there is enough time to wait. If it is approaching 60 days of incubation, several checks should be made every 2-4 hours, each time removing a little more shell and checking whether the egg membrane is already dry. Afterwards, the eggshell-hole should be covered again with a gauze soaked in betadine.



Chick BG 1045 needed hatch assistance since was chirping continuously of discomfort. *On the 20th of March at midday internal pick. 21st of March from the morning on continuously chirping of discomfort with periods of heart rate >400 beats/min. After disinfecting the eggshell with Betadine, with a Dremel the shell was drilled without damaging the membrane, removing the piece of shell with tweezers. Afterwards the whole was covered with gauze soaked with Betadine. Immediately the chick stopped to chirp of discomfort and the heart rate stabilized between 133-240 beats/min. 22nd of March the chick did the external pick and a day after hatched without human help.*

In the third case, when chicks are stuck during hatch by becoming stuck to the membrane, they chirp of discomfort and show an elevated heart rate. Furthermore, as they cannot turn its head in the egg, the chick can only open a big hole and/or they stop the hatching progress (see pictures below). As they do not have breathing problems, the chick doesn't become weaker and don't stop to chirp of discomfort.

Two examples of chicks having problems by hatching. ***Left picture:*** *the chick makes a large opening on the external pick as it cannot turn its head.* ***Right picture:*** *the chick makes a large opening on the external pick and stop hatching after opening a 1/3 of the eggshell. In both cases the chick chirped of discomfort continuously.*

In these cases, with a tweezer's pieces of shell from the flat pol (air cell) should be removed. In this area they are no blood vessels, and a hole can be opened and locate the head position. Then slowly continue to open the egg and control that the blood vessels of the membrane are already dry, ensuring not provoking any haemorrhage. In case they still contain blood, the opening is covered with gauze soaked with Betadine and checked again after 3-4 hours. It is very important not to remove the chick early, as the yolk sac may still not be completely reabsorbed. Remember that in average 24-48 hours may pass between external pip and hatching.

***Chick BG 1036 needed hatch assistance since the membrane stuck on its beak**. 3rd of March at 15:00h internal pick. 4th of March at 21:00h external pick. 5th morning the chick was chirping of displeasure. The open shell was removed, and the hole was covered with gauze soaked with Betadine. 6th of March, during the day the membrane stuck to the chick was removed gradually covering afterwards with gauze soaked with Betadine. 7th morning at 7:30 hatched by itself (Centre de Fauna Vallcalent, Lleida, Spain).*

Chicks that do not react quickly after opening and show weakness, it is recommended that antibiotics be administered immediately, even if they are not yet extracted from the egg (see chapter 4.).

Summary:

- **Human help is needed if the hatchling doesn't stop to chirp of discomfort with a heart rate >400 beats/min.**
- **The latest time at which human intervention is required, is when its heart rate and movements decrease progressively until they stop to chirp, and the heart rate is around 100 beats and even below.**

☞ **Never extract the chick from the egg immediately after having opened a hole in the eggshell. Time must be allowed so that the blood vessels can dry out and the chick can absorb the yolk sac.**

4. 1ST WEEK HAND-REARING

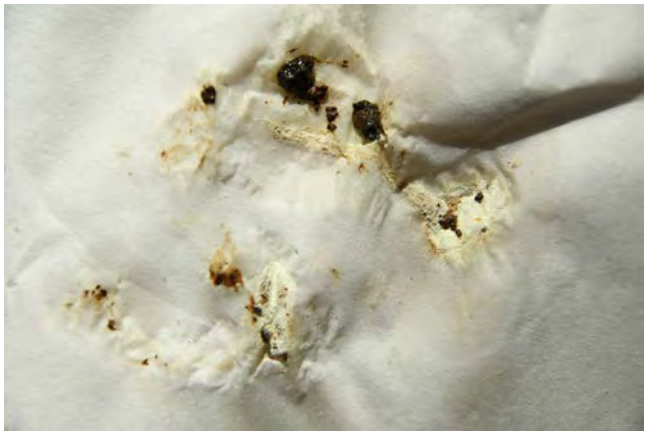
Once the chick is out from the egg, the navel and the umbilical cord must be disinfected with "Betadine" (iodine). The disinfection should be repeated very 2-3 hours until the navel is completely closed and the umbilical cord dry. During the first 5-10 hours the chick is left to dry in the incubator at 37.3ºC - 37.5ºC and with natural humidity levels. Then, its first down (neoptil) is rubbed with a smooth toothbrush. At the same time, the temperature is reduced (by tenths of a degree) depending on the behaviour of the chick.

If the temperature is too low, the chick starts cheeping, tries to move into a corner and can have diarrhoea (the faeces are white mixed with some darker parts, which are sandy in consistency). As soon as the temperature is corrected, the chick is given black tea (1-2 ml in drops) and is not fed for a short time. On the other hand, if the temperature is too high, the chick starts cheeping, lies face down and pants. The chick becomes very nervous and may vomit. Once the temperature is corrected, the

chick is given camomile tea and is not fed for a short time. Both vomiting and diarrhoea cause problems with the chick's thermal regulation and it is sometimes necessary to raise the temperature by a couple of tenths of a degree and in some cases to raise it above the temperature of artificial incubation. Furthermore, if the loss of liquid is excessive, it is necessary to subcutaneously inject serum.[2] When the temperature is optimal, the chick doesn't chirp (or at least it does so only occasionally, for example before excreting), it "dreams" and makes movements as though it was cleaning its plumage. Further the chick evacuates by completely separating faeces (solid and brownish) from urine (white liquid). It is very important to cover the chick with a cloth so that it gets used to being covered later by its adoptive parents. The cloth should be light so that the chick doesn't overheat.

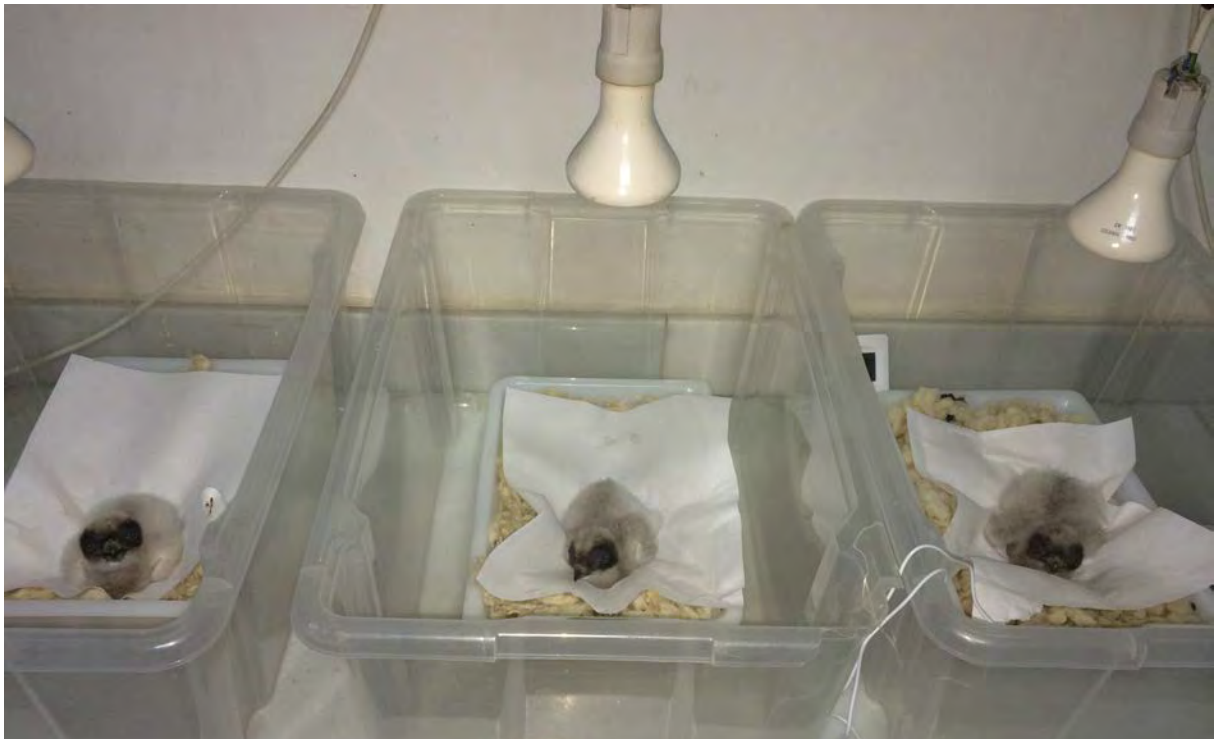
Left picture: *perfect evacuation where the solid part (faeces) and the liquid part (urine) are perfectly differentiated, showing that the chick is at its ideal temperature.* ***Right picture:*** *diarrhoea, where faeces and urine are completely mixed. The temperature is not adapted to chicks needs.*

Nevertheless, during the first days, until the digestive system is fully functioning, the solid part of the first evacuations is still excreted separately (see picture on the right). It takes several days until the solid part (faeces) is excreted jointly.



Brooders or a ceramic lamp keeping the chick in a plastic box can be used as a heat source for brooding the chicks. Both techniques have advantages and disadvantages to be considered. Nevertheless, in both regularly monitoring of the chick is needed.

[2] The cocktail against dehydration for a chick: 0.2ml of Catosal (Vitamin B12), 0.05ml of vitamins A, D3 and E (the first time the cocktail is administered these quantities should be quadrupled); 0.03ml of Methionine; 1ml of 5% glucose which should be diluted to 3ml with Lactated Ringer Braun solution. This should be injected every 2-3 hours in the nape of the neck.



Chicks reared with ceramic lamp and plastic box technique. *This technique allows the chicks to regulate itself its temperature needs approaching or moving away from the heat source. But this requires that chicks are large and strong enough to move on its own. By young chicks the lamp must be raised or lowered according to the temperature needs of each chick (Centro de Cría Guadalentín).*

Brooders provide a stable temperature, independent of possible temperature fluctuations in the brooding room. But they do not offer the possibility that the chick can regulate itself, moving closer to or further away from the heat source according to its needs. The rearing technique with a ceramic lamp and plastic box, is a perfect method for a few days' old chick when their heat requirements are constantly changing depending on food intake. In general, after feeding, because of the large amount of food eaten, they require a lower temperature due to the digestion process. But as the night/early morning approaches, when the digestive tract is empty, a higher temperature is required, and the chick by itself can approach to the heat source. But this requires the chick to be large and strong enough to move on its own. By young chicks the lamp must be raised or lowered according to the temperature needs of each chick.

Chick mortality usually occurs during the first few days after hatching, the principal causes being inadequate temperature regulation when the chick's behaviour is not understood or correctly interpreted, septicaemia (the chick dies during, or a couple of hours after, hatching), and infection of the yolk sac (the chick dies approximately one week after hatching). Weak hatchlings, or those that seem to want to vomit (and those who need to be extracted from the egg) may have a yolk sac

infection. In these cases, we treat them with antibiotic: Enrofloxacine 2.5%: 0.1 ml/2x/day for 3 days, maximum 4 days. Please take care; Bearded vultures are very sensitive to any drugs. **Enrofloxacine is the recommended antibiotic for Bearded vultures (all ages).**

1st meal is given when the chick asks for food (in general 10-24 hours after hatching), but not before it has already excreted its first faeces (meconium, greenish colour). Strong hatchlings are given food held with tweezers in front of the beak. These birds can take the food themselves. Nevertheless, some of them they still have a natural oedema at the back of the neck, being not able to hold their heads up for long. This disappears by the second or third day and from then onwards they can hold their heads up. For this reason, when these chicks are first fed, they must be helped to support their heads (see picture below). In the first few days, strong chicks are fed every 3 hours (only during the day). It is important to observe the crop, which should be empty before they are fed again. The pieces of food should measure 3-5mm. Later they are only fed 3-4 times a day. Often the chick starts shivering from cold and tiredness after eating and it is advisable to raise the temperature of the incubator by 0.5-1ºC for 15-30 minutes. Further use always **pre-heated trays**, it helps to maintain the warmth of the chick during feeding.

Left picture: *1st excreted faeces named meconium, composed of amniotic fluid, mucus, bile, and cells that have been shed from the skin and intestinal tract.*
Right picture: *1st meal after hatching. By this strong chick it could still not hold its heads up for long, having to be assisted.*

***2 days old hatchling** already holding its head without human help.*

Only weak chicks, after being treated with antibiotics, **need to be force-fed**: the head is held in the hand and 3-5mm sized pieces of food are introduced into the mouth. 1st meal can be already given 5-10 hours after hatching or extraction from the egg. In order not to tire them additionally with digestion, it is recommended to feed them with a maximum of 1-2g and every 2 hours, with a higher number of feedings per day than the rest of the healthy chicks. First feedings can be done with soft tissues (muscle, heart, liver) to lighten digestion. Generally, these chicks recover after 2-3 days and start requesting food and feeding like stronger chicks. Weak chicks are very sensitive to temperatures changes, being very important to use pre-heated trays by feeding the chick.

The chicks are fed recently killed young rats/mice (up to a week old) **with their bones crushed and their intestines removed**. The rats/mice should be **always skinned**, since a 5-day old chick has great difficulties regurgitating a pellet after being fed whole mouse/rat. It is recommended for the firsts feedings to offer pinkies (still with pink skin and <5g). As the chicks grow, they are given older rats/mice (fuzzies: still have not opened their eyes yet but have developed a coat of fur). A 5-6 day old strong chick can already be fed with weaned mice. The young rat/mouse must be cut into 3-5mm pieces with scissors and the pieces are mixed well with the keepers' saliva. At all ages it is very important to include bones in their diet to prevent rickets.

The weight of a Bearded Vulture hatchling is about 150.9g on average (n= 270 chicks). As in other birds of prey, weight is lost on the second day. In Bearded Vultures this is 4.44% of the body weight and birth weight is not regained until the third or fourth day. Average daily growth during their first 2 weeks of life is 10.4±2.02% of their weight. The daily needs of soft tissue increase rapidly from 10% to 25-30% of the hatchling´s weight.

Table 1: Average morning weight in grams and food quantity needs in % of weight by nestlings

DAY	1	2	3	4	5	6	7	8	9	10	14	21	28	40	60	120
Weight	150	145	147	157	171	186	204	223	244	268	421	930	1400	2500	5000	5500
%	1	11	19	20	25	25	25	+30	+30	+30	+30	+30	+30	25	10	10

Summary:

- **Immediately after hatching disinfect the navel and the umbilical cord with "Betadine" (iodine) and keep it in the hatcher at same temperature and with natural humidity levels.**
- **As soon the first down (neoptil) is dry, rub it with a smooth toothbrush and reduce the temperature 0.5ºC temperature.**
- **Bearded Vulture chicks are very sensitive to temperature. Any temperature failure can produce the loss of the chick. Take especial attention on chick's behaviour and the form of its evacuations to adjust to the right temperature.**
- **1st feeding not before 10-24h after hatching, but not before it has already excreted its first faeces (meconium).**
- **Weak chicks need to be force-fed.**
- **Chicks are fed with recently killed and skinned young rats/mice, with their bones crushed and their intestines removed. Food pieces are always mixed with keepers' saliva.**
- **Don't feed only with meat. Chicks will show rickets.**

5. ADOPTION

It is well known that hand-reared Bearded Vultures -especially the males-, are not able to reproduce with their conspecifics when they reach their sexual maturity, being also useless for releases. Additionally, from 14 days old starts the species imprinting process, being necessary to return the chick to its parents/foster pair before this age to ensure imprinting to its conspecifics. Nevertheless, from one week of age, the chick starts to become aware of who is feeding it. That's why it is recommended to adopt the chick on an age around seven days old and/or with a weight of 200g. Later adoptions, the chicks may react aggressively against the adults, and the adults, if inexperienced in rearing, may respond aggressively with fatal consequences. **The adoption should be only done if the chick can hold its head upright and easily pick up food with its beak from the adult's beak**.

5.1. 1ST option

5.1.1. Single adoption

Once the chick is ready for adoption, this should **only be done in pairs that are incubating**, never in pairs that have stopped incubating. By interrupting the reproductive cycle, they lose the stimulus to breed and as a rule react aggressively or ignore it, they may even kill the chick. Every year huge efforts are invested to ensure that all chicks are raised naturally, being necessary to transfer chicks through Europe between EEP partners where foster/experienced pairs are available.

During the adoption process, before the chick is placed in the nest, it is advisable to remove the clutch/dummy eggs. Only some couples do not accept this abrupt change, and in these pairs, it is recommended to adopt the chick with only one egg in the nest (always remove the second one). In experienced pairs, the adults usually adopt the chick immediately, approaching from behind the chick, lying down on it very carefully and ignoring any aggressive reaction from the chick.

In inexperienced pairs, however, the adoption process can take up to several hours, with the adult cautiously trying to lie down on the chick, getting up every time the chick reacts aggressively. In most cases the adoption is successful when the exhausted chick stops pecking at the adult and once brooded by the adult, feels its warmth.

Adoption six days old chick by an experienced breeding bird. *The experienced bird step by step approaches behind the chick, until it is lying down on top of it, ignoring any aggressive reaction from the chick. During the adoption food without fur must be always offered on the edge of the nest. In this way the adult can approach the chick that is chirping with food in its beak and perform the adoption more calmly.*

However, chicks are often not fed sufficiently during the first few days, and it is necessary to feed them by hand at least once a day, preferably one hour before dusk. When the chick is adopted by an inexperienced pair, it is often necessary to prolong the supplementary feeds until the adults learn to feed the chick correctly (follow nest control protocol; see point 2.). There are cases in which adoption has not been completed even after 2 or 3 weeks (the chicks are generally not fed properly). In these cases, it becomes necessary to transfer the chick to another pair. All adult birds, at the moment of adoption, get excited when they hear the chick cheeping because it is cold, uncomfortable or hungry (their sclera swells and their heart rate increases) and they also start to salivate. This flow of saliva has also been observed in other birds breeding for the first time and is an innate reflex conditioned to the cheeping of the chick, given that it does not happen when the chick can be seen but is not chirping.

At the beginning, inexperienced birds will attempt to give the chick food (large bones) or pieces of a size which are not adequate for the age of the chick. That's why daily monitoring of the chick's weight is crucial to determine whether the adoption process is progressing correctly.

Nevertheless, there are several points to consider increasing the chances of a successful adoption:

- The adoption should be done at the **warmest time of the day**, late morning or midday. This is important to prevent the chick from suffering from hypothermia in case the adoptive parents take too long to warm the chick (particularly by inexperienced foster pairs).
- **Never do the adoption in raining days**, as the parents would adopt with wet plumage and could cause hypothermia in the chick.
- The chick should always be **feed to maximum before the adoption**. This makes a sated chick feel like sleeping and react less aggressively, and with a full crop, it is more difficult for it to lift its head to peck aggressively against the adults.
- Offer several pieces of **food on the edge of the nest**. Especially in inexperienced pairs, the presence of food and the chick's chirping gives them the opportunity to discharge their excitement with food, and they can approach the chick with food in its beak and perform the adoption more calmly.
- The whole **adoption process must be monitored** from distance, but close enough to be able to act quickly in case the adults react aggressively and start pecking repeatedly the chick or picking it up with their beaks. Ideally, one person should monitor via surveillance cameras and a second person should be hidden near the cage in continuous telephone contact with the person in front of the cameras.

Summary:

- ☞ **Adoption should be only done by pairs that are incubating.**
- ☞ **Adoption should be done at the warmest time of the day.**
- ☞ **The chick should be feed at maximum before the adoption.**
- ☞ **Weights checks in the nest must be done to determine whether the adoption process is developing correctly.**
- ☞ **Never do the adoption on rainy days.**

5.1.2. Multiple adoptions using two or more pairs

Occasionally there are more chicks than parents, which forces some pairs to rear two chicks at the same time. Due to the "cainism" behaviour in Bearded Vulture nestlings, adopting more than one chick in the same nest is not possible. It is crucial that the two chicks have no physical contact and are kept apart with visual contact only. This separation makes it impossible for a single adult to brood both hatchlings at the same time, making it necessary that during the period when they still need to be warmed, they must be reared by different pairs. As soon as one of the two chicks is one month old and has started to thermoregulate, which have previously been reared on their own by another pair since they were a week old, a double adoption can take place. It is advisable to do double adoptions before the flight feather cannons appear on the older chick (approx. 35 days old), as they are sometimes rejected by the adults because of the difference in looks. At first, if the nights are very cold, and the adults do not brood the older chick in the supplementary nest, it is necessary to remove it before dark so that it spends the night indoors, without contact with humans. Early in the morning the chick is put back into the supplementary nest. From 45 days of age, the chicks are already able to withstand freezing nights without being warmed by adults.

In conclusion, **chicks in a double adoption**, such as the exchange of chicks between pairs**, should preferably resemble each other as closely as possible**, as large differences between them (size, presence of down or feathers, etc.) may cause the adults to dislike and reject the offered chick.

Double adoption can be done in the same nest divided with a piece of wood into two parts (if the nest is large enough for a division) or a lower supplementary nest is installed next to the main nest (15cm lower). This separation (wooden partition or height difference) makes it possible for the chicks to see each other but prevents them from attacking each other. As the chicks get older, the separation should be elevated to ensure the chicks mustn't be able to get over the partition because they will fight with each other. **As a rule, the smaller chick is placed in the main nest and the larger chick in the supplementary nest**. However, not all pairs are capable of rearing 2 chicks at once since they don't recognise which of the chicks is chirping to be fed. In these cases, it sometimes works very well to separate the adults and each of them takes care of one chick. As soon the chicks leave the nest and start to explore the aviary, the barrier can be removed. Juvenile Bearded Vultures are removed from their parents' cage a couple of months before the next reproductive cycle begins (usually in August). In this way chicks are guaranteed a natural development and problems between chicks and parents are avoided when the next reproductive cycle begins (they will often viciously attack each other), which can inhibit the reproductive cycle.

Double adoption. *Left picture: on the right hand of the picture a 15-day-old chick is placed in the main nest. On the left of the picture the 24-day-old chick is transferred to the supplementary nest. Middle picture: as the chicks get older, the barrier should be elevated to ensure no physical contact because they will fight with each other. Right picture: both chicks have already fledged being possible to remove the barrier (pictures from CC Guadalentín).*

Thanks to double adoptions, it becomes possible for two pairs that were each raising a chick of similar age to ultimately raise three chicks by transferring one of these two chicks to the other pair (double adoption). This allows the pair from which the chick was removed to adopt a third chick. And so on, so that a centre with a certain number of breeding pairs can breed twice as many chicks. Furthermore, in some cases it is possible to adopt more than two chicks to the same pair, as long as the chicks are kept separate from each other. It is even possible to separate the pair and have each adult alone raise two chicks by double-adopting as described above. In all cases it is very important to closely monitor the development of each chick, as sometimes adults under so much pressure can fail to feed the chicks properly. In multiple adoptions, as soon as **nestlings are over 45 days old**, it is recommended to **offer additonally chopped food** to ensure an adequate intake of daily food.

Summary:

- **A double adoption can be done as soon one of the chicks has started to thermoregulate (around one month old). This chick must be reared previously as a single chick under another pair.**
- **Chicks for double adoptions should preferably resemble each other as closely as possible, as large differences between them (size, presence of down or feathers, etc.) may cause the adults to dislike and reject the offered chick.**
- **The older chick (4 weeks old) is introduced in the supplementary nest and the younger introduced in the main nest.**
- **Until 45 days old, take special attention on night temperatures. If the adults are not brooding the older chick and the nights are freezing, the nestling should spend the night indoors without human contact.**
- **Always ensure that the separation between the two nests prevents physical contact between the nestlings.**

- ☞ **The separation can be removed as soon the chicks fledged.**
- ☞ **As soon as chicks are over 45 days old, it is recommended to offer chopped food to ensure an adequate daily food intake.**
- ☞ **Very important to closely monitor the development of each chick.**

5.2. Other options

As has been mentioned above, each year huge efforts are invested to ensure that all chicks from one week of age are naturally reared, being necessary to transfer chicks between EEP partners where foster/experienced pairs are available, for the purpose of single or multiple adoptions. Remember that hand-reared Bearded Vultures are not able to reproduce with their conspecifics when they reach their sexual maturity, being also useless for releases.

With the restrictions of the Covid19 pandemic that we suffered in 2020 and 21, being the borders closed for any transfer, also movement of people between municipalities, regions and countries were forbidden, it was impossible to move chicks for adoption. In the context of this exceptional situation, the involvement of inexperienced pairs/birds in chick rearing was necessary. Double/triple adoptions were also necessary to carry out only using one pair, becoming necessary to draft **special emergency plans**. This led to the need to develop special protocols:

- In the case where the couple lacked experience in rearing and/or stopped incubating, making adoption unfeasible, the **Nest-Box protocol** was implemented.
- In the case where the couple had no experience in rearing two chicks, the **double/triple adoption protocol** was implemented.

5.2.1. Nest-Box protocol

This protocol was drafted for pairs not able to adopt a chick for different reasons (has stopped incubating prior to chick adoption, no rearing experience, react aggressively against the chick, etc.). The chick is introduced in a box installed at the nest platform to maintain visual contact with their conspecifics throughout the entire rearing period. This requires a longer period of artificial rearing without human visual contact.

A box with a nest inside is built in the nest platform with the goal that the chick will always have visual contact with its parents, promoting imprinting on their species. To avoid that adults having physical contact with the chick and injuring it, the box itself has to be covered with a welded wire mesh with a hole size not bigger than 2.5cm. Further, the mesh needs to be installed in both sites, as in the past two chicks have been killed by a non-breeding adult bird because the holes were too big and there was not a double mesh. **The back should be closed to prevent seeing what is on the other side and trough**

a small door the chick can be fed without human visual contact. Inside the box, a nest has to be installed and filled with wool for keeping the chick warm.

How to proceed is as follows:

1. During the first 7-10 days (depending on outside temperature) the chick has to be kept indoors and has to be fed without human contact.

2. Afterwards the chick has to be put in the box during the day and fed from behind a visual barrier. At the beginning, during the hottest hours, and as the chick grows, the hours outside get longer. After 3 weeks, if outside temperatures are not under 5ºC, the chick can stay 24h outside. It's recommended to cover the chick with wool to keep it warm enough, particularly during the night. In case the temperature drops, a **heating lamp or a heating pad** should be installed to keep the chick warm. It's very important to ensure the chick cannot nibble on the electrical wire and be electrocuted.

3. The number of feedings has to be reduced to a few as possible. This implies that the feedings must be bigger in quantity (at 3 weeks of age receiving 2-3 feedings per day). This requieres calculating the needed food each day on a theoretical basis. During the period where the chick spends the night indoors, weight control has to be done to follow its growth. As soon the chick spends 24 hours outside, weight controls has to be avoided as much as possible to reduce human contact. At this stage, the estimated food requirements is calculated on the basis of the Bearded Vulture EEP growth tables (see table pg. 37).

4. From the 3rd-4th week, chicks should to be feed from a plate giving them the ability to learn to eat on their own. Generally, once they have reached 35-40 days old, they are able to eat chopped food without help.

5. As soon the chick is around 75 days old, an attempt can be made to remove the nest box, giving the chick and the adult birds a chance to interact with each other. This should only be done under strict control of the behaviour of the adults. If the adults react aggressively, the chick should continue to grow in the nest box, and a further attempt should be made after 15 days. At the age of 4 months at the latest, the nest box can be removed. At this age, nestlings are dominant and are already strong enough to defend themselves against possible attacks by adults.

Zoos' experiences which implemented this protocol:

- *Parco Natura Viva (Italy)*

Parco Natura Viva zoo implemented successfully the Nest-Box rearing protocol.

In 2019 the pair produced its first chick, which was reared by the female alone. The male was removed from the aviary just before the adoption, because fights between the pair were observed as soon the chick pipped through the air-cell and started to chirp, being necessary to remove the hatching egg.

In 2020 after one month of incubation, fights between appeared, damaging the 2nd fertile egg. Consequently on the 26th of February -two days after the fights-, the 1st egg was removed for artificial incubation. The chick hatched on the 12th of March and until the 25th was reared indoors without human visual contact.

On the 25th of March, when the chick was already 13 days old, at midday it was introduced in the Box for 3 hours. The following days from 10:00 to 16:00 o'clock and from the 29th, when the chick was already 17 days old, it spent all daylight hours in the Nest-Box and was only fed there (three times per day).

As soon the chick was 24 days old, it spent, for the first time, 24h outside. On the 26th of May, when the chick was 75 days old, the Nest-Box was removed and the chick was introduced directly in the nest. One hour later the chick started to ask for food and it took one hour more before the female started to feed it.

It should be noted that since the chick was introduced in the box the chick was always asking for food and the female was attentive and ate near the box.

Nest-Box rearing: *Picture right top: Staff from Parco Natura Viva installing the Nest-Box on the nest platform. Picture left: as soon the chick was introduced in the Nest-Box, the female was always attentive to the chick and ate in front of it, trying several times to feed the chick through the mesh. Picture middle: Until the chick was not able to take food by itself, it was fed three times a day with a puppet and with no visual contact with human. Picture right: with 75 days old, the Nest-Box was removed and the female took care of the chick (pictures from Parco Natura Viva).*

5.2.2. Double/Triple adoption protocol

This protocol addresses the adoption of a second/third chick in two/three nests, respectively, using only one pair. These additional chicks have not been reared previously by another pair. Generally, with double clutches, the 1st chick hatches in the nest, and is reared by its parents, while the 2nd is hatched/kept in the incubator and adopted by a foster pair with 7-10 days old. In exceptional situations where moving chicks for adoption is not possible, it becomes necessary to rear the second chick by its parents. The additional chick has not been reared previously by another pair. To avoid human imprinting, once the second chick is over 7 days old, the chicks are exchanged daily until the first chick reaches 3-4 weeks of age, marking the time for a definitive double adoption. This requires a longer period of artificial rearing without human contact.

For this technique, a supplementary nest has to be built near the main nest or use one big nest and divide it into two (e.g. with a wooden trunk).

How to proceed is as follows:

1. During the first 7-10 days (depending on outside temperature) the 2nd chick has to be kept indoors and has to be fed without human contact.

2. As soon the 2nd chick is already 7-10 days old, chicks are daily exchanged, keeping always one in the nest and the other indoor alternatively. **The daily exchange takes place** as soon as the chick in the nest has been fed by the adults. At the same time, the chick that is indoors in the morning, before transferring to the nest, is not artificially fed, even if it chirps for food. This reduces the number of artificial feedings.

3. As soon the 1st chick is 3-4 weeks old (depending on outside temperature), the 1st chick is moved to the supplementary nest and the 2nd chick introduced in the main nest.

4. This is the crucial moment where the adults must be very well monitored, to ensure that they feed both chicks sufficiently. In several cases as soon chicks are around 40-45 days old, chopped food can be offered in a plate. At that age they are able to start to eat alone and this ensures that the amount of daily ingested food is appropriate to the age of the chicks in case the adults do not feed them enough.

5. It's very important to install a wooden plank between both nests as soon nestlings start to move (between 40-50 days old) to avoid fighting between them. The height of the wood is adapted to the chicks' ability to move. Remember "cainism" lasts for the entire duration of the stay in the nest.

Zoos' experiences which implemented this protocol:

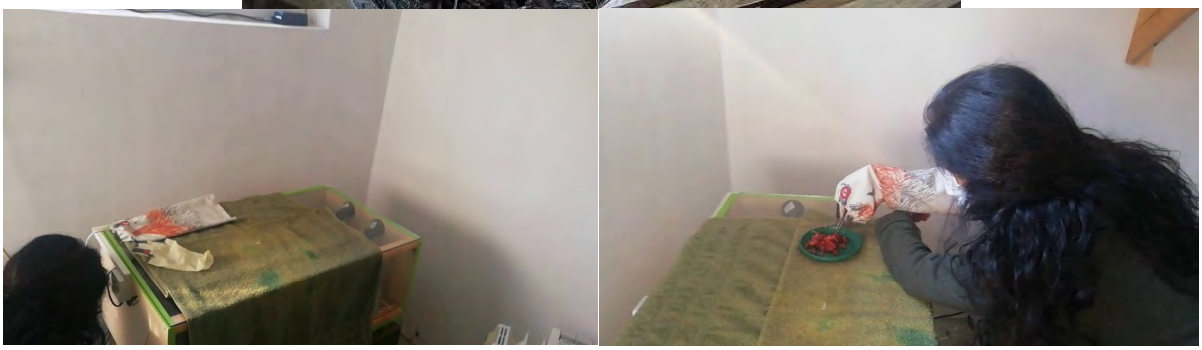
- *Green Balkans Recovery Centre (Bulgaria)*

Green Balkans Recovery Centre implemented successfully the double adoption protocol.

In 2016 the breeding pair from Green Balkans produced for the first time a double clutch and both eggs were fertile. The pair reared the 1st chick successfully. The second was sent to RFZ for adoption. Since then they have reared a chick every year, having enough experience in rearing a single chick. 2nd chicks were always sent to another centre for adoption.

In 2020 the pair laid a double clutch. On the 9th of March the 1st egg had to be removed because of hatching problems (chick BG1067). On the 16th, when the chick was already 7 days old, it was adopted by its parents and the 2nd egg was removed for artificial hatching. The egg had already an unusual big hole, showing again hatching problems, being needing human intervention (chick BG1078).

On the 25th of March, when the 2nd chick was already 9 days old, in the early afternoon it was transferred to its parents' nest and the older chick removed and transferred indoors. From this moment on, every day the chick in the nest was removed after being fed by its parents and replaced by the chick kept indoors, which received its first feeding of the day by its parents. The chick kept indoors was placed in a brooder and fed with a simple puppet without visual contact with humans.



<u>Picture above:</u> between the 25th of March and the 3rd of April daily chick exchange. <u>Pictures left and right:</u> the indoor chick fed with a simple puppet in a brooder without human contact (pictures from Green Balkans).

On the 3rd of April, when the 1st chick was 25 days old and the 2nd 18 days, both chicks spent the whole day outside. The older chick was introduced in the supplementary nest and the younger in the main nest. The younger chick was removed during the night only and no longer needed to be fed by the staff. On the 18th of April, when the 2nd chick was 33 days old and was a weight of 2kg, it spent the first night outside. At that time the parents were no longer keeping the chicks warm, but they were still feeding both chicks.

On the 11th of May, the older chick jumped over the wooden plank which separated the two nests. The next day the older chick was moved to the right platform where a third nest was urgently built.

6. FOOD QUALITY AND QUANTITY DURING THE CHICK-REARING PERIOD

6.1. Food quality during the chick-rearing period

Chicks are fed by the parents on the day they hatch or, at the latest, on the following day. For the first few days, the food consists of small pieces of meat roughly the size of a lentil, free of skin, hair or hard tissue, mixed with saliva. This was observed by Thaler & Pechlaner (1979, 1980) in the pair at the Alpenzoo Innsbruck. In some cases, some of the meals during the first few days of the chick's life are made up almost entirely of saliva. This use of saliva has also been observed in the White-tailed eagle, Eagle owl, Bald eagle, and Lappet-faced vulture (Fentzloff, 1983; Wiemeyer, 1981; Mendelssohn & Marder, 1989). These authors also refer to the fact that saliva provides the chick with fluid to slake thirst, enzymes that help to digest food, and calcium to promote bone formation. A study carried out on Eagle owls showed that the amount of calcium in the saliva increased during the period of chick rearing (Fentzloff, 1983). This explains why chicks that are fed artificially (hand-reared) on the same diet as if they are fed by their parents (meat only) often get rickets (Fentzloff, 1983; Thaler & Pechlaner, 1979, 1980; Wiemeyer, 1981).

Generally speaking, the diet for pairs that are rearing a chick should be the same as described above (in 'Recommendations Regarding Food Quality'). However, the chick's diet is composed principally of soft tissue: 100% soft tissue for the first four weeks, and 70% soft tissue thereafter. Therefore, sufficient quantities of meat should be available to the birds every day. Furthermore, food should be given fresh (not frozen and thawed).

Rats, guinea pigs, and rabbits weighing up to three kilos with the digestive tract removed are the best food to provide during the chick-rearing period. The parent birds prepare the food for the hatchling themselves. Normally, they remove the fur from the food outside the nest, before transferring it to the nest.

It is well known that most losses of Bearded vulture chicks happen during the first few days of a chick's life. The second[3] main cause of death in chicks is starvation due to loss of vision caused by rabbit fur stuck in the chick's eyes. To understand this problem, it is important to know that Bearded vulture chicks are not fed in the same way as other raptor chicks, where adults will put the food in the chick's bill. Instead, Bearded vulture chicks must take the food from its parent's bill. This means that if the chick is too weak (due to malnutrition) or has vision problems (because it has rabbit fur stuck in its eyes), it will not be able to take the food from its parent's bill and will eventually starve. This is even more likely to happen when chicks are being reared by single adults, because the adult prepares the

[3] The main cause of death in Bearded vulture chicks is yolk sac infections. The second main cause of death is starvation caused by loss of vision due to rabbit fur in the chick's eyes. In inexperienced breeding pairs, another main cause of death is the parents 'inability to feed the chick properly.

food right beside the chick. For this reason, it is advisable to skin rabbits before giving them to the birds during the first three weeks of the chick's life.

During this period, it is necessary to pay special attention to the chick's eyes. Nest checks should be undertaken every 2-4 days for the first two weeks of the chick's life, to ensure that the chick's eyes are clean and do not have fur stuck in them. Video cameras installed at the nest site make it possible to check on the chick without performing nest checks in person, which can disturb the adult birds.

During the first four weeks of its life, the chick is fed on 100% soft tissue. To prevent chick deaths caused by fur sticking in their eyes, feed on skinned rabbits during the first three weeks of the chick's life.

Summary:

- **Ensure that sufficient soft tissue is available every day. During the first four weeks of a chick's life, it is fed on 100% soft tissue. Thereafter, it is fed on 70% soft tissue.**
- **Rats, guinea pigs, and rabbits weighing up to three kilos, with the digestive tract removed, are the best foods to provide birds with during the chick-rearing period.**
- **Avoid offering rabbits with fur during the first three weeks of the chick's life. Fur can stick in the nestling's eyes and cause its death.**
- **Avoid feeding principally on frozen food during the chick-rearing period.**

6.2. Food quantity during the chick-rearing period

The average weight of a Bearded vulture chick on hatching is 155.4g (n= 164 chicks). As in other birds of prey, weight is lost on the second day. In Bearded vultures this weight loss amounts to 4.47% of total body weight and birth weight is not regained until the third or fourth day. On average, chicks put on around 10% of their own body weight every day during the first two months of their life. The daily soft tissue requirement increases rapidly from 10% to 25% of the chick's weight.

A one week-old chick weighing 200g will therefore consume 25% of their own body weight – roughly 50g – every day. This is equivalent to one weaned rat weighing 70-80g. Consumption increases rapidly

and daily, so that by one week later, the two week-old chick's requirements have doubled. By the time the chick is three weeks old, it may be triple the weight of the two week-old chick. At three weeks, the chick weighs around 1 kg and consumes 250-300g of food per day. That is equivalent to an adult (reproductive age) rat, which weighs around 500g with skin and entrails. At 35 days old, the chick reaches its maximum food consumption of around 500g per day -around double that of an adult bird. This can be achieved by feeding at least one fresh rabbit per day. This level of food consumption is maintained until they leave the nest.

It is very important to gradually increase the quantity of food provided as the chick grows, bearing in mind that during its last months in the nest a chick can eat half a kilo of food per day. The only way of knowing exactly how much food to provide is by observing how much food is left every day or by increasing the quantity if all the food has been eaten. The quantity of food required by a pair rearing a chick that is more than a month old is therefore 1000-1200g per day (equivalent to around 1500-1800 gross weight).

Summary:

- **Chicks up to 5 days old consume 25% of their body weight daily.**
- **Aged 35 days, chicks reach their maximum food consumption of 500g/day, which is maintained until they leave the nest.**
- **The quantity of food consumed by a pair with a chick that is more than one month old is 1000-1200g per day.**

BIBLIOGRAPHY

CADE, T.J.; WEAVER, J.D.; PLATT, J.B. & BURNHAM, W.A (1977): The propagation of large falcons in captivity. *Raptor Research* **11** (1/2): 28-48.

CARPENTER, J.W.; GABEL, R.R. & WIEMEYER, S.N. (1987): Captive Breeding. En: Pendleton, B.A.G.; Millsap, B.A.; Kline, K.W. & Bird, D.M. (Eds.): Raptor Management Techniques Manual. National Wildlife Federation, Washington, p. 349-370.

FREY, H., KNOTZINGER, O., LLOPIS, A. (1995): The breeding network - an anlysis of the period 1978 to 1995. FCBV, Annual Report 1995, 13-38.

GILBERT, S.; TOMASSONI, P. & KRAMER, P.A. (1981): History of the captive management and breeding of Bald eagles *Haliaeetus leucocephalus* at the National Zoological Park. *Int. Zoo Yb.* **21**: 101-109.

HEINROTH, O.M. (1927): Die Vögel Mitteleuropas. Hugo Bermüller, Berlin-Lichterfelde.

KUEHLER, C. & GOOD, J. (1999): Artificial incubation of birds eggs at the Zoological Society of San Diego. *Int. Zoo Yb.* **29**: 118-136.

PICHLER, A. (2000): Entwicklung eines nichtinvasiven Verfahrens zur Vitalitäts- und Aktivitätsdiagnostik an Vogelembryonen. Inaugural-Dissertation, Veterinärmedizinische Universität Wien.

THALER, E. & PECHLANER, H. (1979): Volierenzucht und Handaufzucht beim Bartgeier (*Gypaetus barbatus aureus*): Beobachtungen aus dem Alpenzoo Innsbruck. *Gefiederte Welt* **2**: 21-25.

THALER, E. & PECHLANER, H. (1980): Cainism in the Lammergeier or Bearded Vulture at the Alpenzoo Innsbruck. *Int. Zoo Yb.* **20**: 270-280.

THALER, E., MASCHLER, S., STEINKELLNER, V. (1986): Studien zur Postembryonalentwickling dreier Altweltgeier: Bartgeier, *Gypaetus barbatus aureus* (Hablizl 1788), Schmutzgeier, *Neophron percnopterus* (Linné 1758) und Gänsegeier, *Gyps fulvus* (Hablizl 1783). *Ann. Naturhist. Mus. Wien* **88/89**: 361-376.

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