Methodological Guide for Comprehensive Monitoring of Incubation in Captive Breeding

Device selection · Installation · Data processing · Interpretation of incubation parameters

Methodological Guide for Comprehensive Monitoring of Incubation in Captive Breeding

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The use of modern biological tools, data sharing and user-friendly data analysis to optimize the incubation of eggs of endangered bird species in human care

Prague, 2026

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Preface

37.5 and 50 – these are the standard temperature and humidity settings in an incubator, which were used when I first joined the Safari Park in Dvůr Králové and are still used in most institutions today, with very little change. At first glance, this isn't a bad approach; something always hatches. However, as more information becomes available and new studies emerge, it is becoming clear that some bird species are unable to reach these temperature levels; that temperature fluctuations during incubation have a positive effect on embryo development; that even eggs of the same species can have different shells and associated weight loss; which should determine the humidity setting in the incubator, and that the frequency and method of turning affect the position of the embryo within the egg and, in the final stage, the hatching process. These studies would not have been possible without the input data obtained from wild birds, as well as from birds kept in human care. The more data that can be collected, the more accurate the results – and consequently the guidelines – will be. You now have a guide in your hands that will help you identify any incubation problems you may be facing.

Mgr. Michal Podhrázský, Ph.D.
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1. Why do we need a guide to monitoring incubation?

Successful egg incubation is one of the key aspects of keeping birds in human care. Although many zoos and conservation breeding programmes achieve high standards of animal care, the breeding of many species in captivity remains a challenging discipline. To address this, it is essential to monitor the actual conditions to which the embryo within the egg is exposed during incubation. The parameters that fundamentally influence the success of egg hatching and the condition of the hatched chick are, above all, egg temperature, the frequency of egg turning, and nest humidity. Nevertheless, these are rarely monitored systematically during natural incubation in ex-situ breeding programmes. However, incubation problems (whether caused by inappropriate parental behaviour, an unsuitable environment or species-specific requirements) are often difficult to detect without the use of modern technology.

Advances in miniature dataloggers and 3D printing in recent years have opened up new possibilities for non-invasive monitoring of incubation directly in the nest. Artificial eggs containing dataloggers, temperature and humidity sensors placed in the nest, and continuous video recording of the parents' behaviour can now be combined into a comprehensive monitoring system that provides a complete and continuous view of the incubation process without the need to frequently disturb the birds with human presence. However, to fully utilise this potential, it is necessary to select the appropriate equipment, ensure it is installed correctly, and be able to process the data and interpret the final results accurately. It is precisely for this purpose that this methodological guide has been produced. It will serve as your guide to monitoring bird incubation in breeding facilities and, through specific examples, will help you to correctly interpret the data obtained.

This guide focuses exclusively on monitoring natural incubation, that is, the recording and interpretation of the conditions under which a parent incubates eggs in the nest. It does not, therefore, cover artificial incubation, the operation, setting up or maintenance of incubators, or the care of hatched chicks. However, the knowledge obtained can be applied to artificial incubation, for example, as inspiration when seeking the optimal incubator settings.

1.1. Scientific and methodological background

This guide is based on the authors' long-standing experience with research of bird incubation in the wild, which was subsequently followed by the TAČR project (SS07020438), conducted between 2024 and 2026 in collaboration with Czech zoos, primarily Dvůr Králové Safari Park and Brno Zoo. During this period, dataloggers monitoring incubation were deployed for 56 bird species across a wide range of taxonomic groups. A total of 104 installations were recorded, including data from various types of dataloggers. The methodological procedures described in this manual are therefore not merely theoretical recommendations, but practical insights gained from repeated field installations. The manual discusses the risks of errors, improvised solutions and proven procedures that maximise the chances of the birds accepting the devices. This guide is indirectly linked to the Incubase platform (www.incubase.fzp.czu.cz/), which was also developed as part of the aforementioned project. There, you will find data on the artificial incubation of a wide range of species kept in Czech and European zoos.

1.2. Who is this guide intended for

This guide is intended for breeders and curators at zoos and wildlife conservation programmes, as well as for private breeders. It provides practical guidance on selecting and installing a monitoring system, and on the basic interpretation of the data collected to assist with breeding decisions. The manual is written with the understanding that these users generally lack a background in analysis and work under constraints of limited time and resources. Nevertheless, it was not possible to avoid describing some of the more complex computational methods and tools. We therefore assume that the handbook will also be useful for researchers who collaborate with breeding institutions and provide them with data analysis.

2. Incubation of bird eggs: what is it and how is it measured?

2.1. Parameters monitored during incubation

Most bird species ensure embryonic development through contact incubation, in which the parents maintain the conditions necessary for development through direct contact with the eggs. An exception is the megapodes (Megapodiidae), which utilise the heat of the surrounding environment for incubation, generated by the decomposition of organic matter or geothermal activity (Jones 1988). The duration of incubation varies considerably between bird species. It ranges from 10 days for the smallest songbirds to more than 80 days for albatrosses or megapodes (Deeming 2002). The successful development of a bird's embryo depends on the precise regulation of several key environmental parameters throughout the incubation period. These parameters include incubation temperature, humidity, egg turning and the facilitation of gas exchange between the embryo and the surrounding environment (Deeming 2002). Deviations from the optimal values of any of these parameters can lead to a slowdown in embryonic development, morphological abnormalities, reduced hatchability or weak condition of the chick after hatching (Webb 1987; Deeming 2002).

2.1.1. Incubation temperature

The incubation temperature should be maintained throughout the embryo's development at a level above the so-called physiological zero, which is the temperature below which embryonic development ceases. The physiological zero ranges approximately from 23 to 27 °C, depending on the species. At the same time, the incubation temperature should be below the level that can cause irreversible damage to the embryo, typically above 40.5 °C (Webb 1987). The average incubation temperature has been shown to vary between species and most commonly ranges from 35–38.5 °C, measured at the surface of the egg (Webb 1987; Deeming 2002). It was long assumed that the temperature of the egg remains relatively stable during natural incubation. However, studies using continuous recording with dataloggers show that the temperature of the egg fluctuates significantly during incubation and is influenced by a whole range of factors (Figure 1).

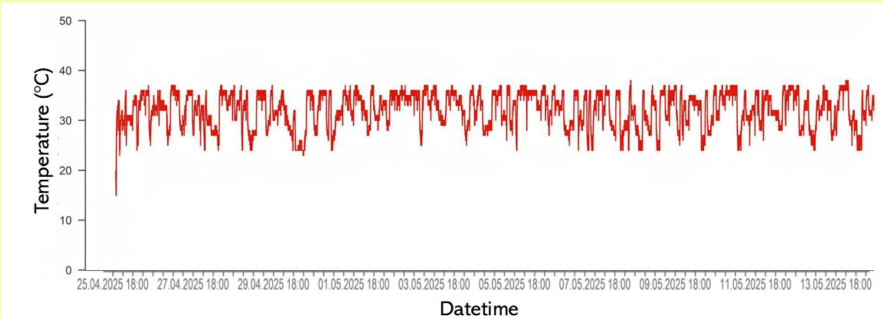


Figure 1: Incubation temperature of the red-crowned crane (*Grus japonensis*).

A key factor is the incubation rhythm, i.e. the frequency and duration of breaks in incubation (in more detail in section 2.1.4.), during which the parent leaves the nest, and the temperature of the eggs equilibrates with the outside temperature (Conway and Martin 2000; Boulton and Cassey 2012). The duration and frequency of these breaks have been shown to vary depending on the incubation strategy, i.e. whether one parent incubates (uniparental incubation) or both (biparental incubation) (Moreau et al. 2018). The outdoor ambient temperature also influences the duration of incubation breaks. At lower temperatures, breaks are shorter to minimise heat loss from the egg (Boulton and Cassey 2012). In extreme heat, when simply warming the egg is of no help, the parent may instead actively shade the nest. The average incubation temperature may also increase in the late season (due to higher ambient temperatures) and is generally higher for eggs in the centre of the clutch (Martin et al., 2007).

Another significant factor influencing incubation is the risk of predation, which can alter the incubation rhythm, for example by leading to shorter or less frequent breaks in incubation (Cresswell et al., 2003).

The measurement of incubation temperature depends on where within the egg it is taken. Heat is transferred from the parent through the shell at the top of the egg, creating a temperature gradient between the warmer area in contact with the parent and the cooler lower part of the egg (Figure 2). The position of the embryo and the magnitude of this gradient change during incubation. In the early stages of development, the embryo is located on the surface of the yolk. As the yolk can move due to its attachment by the egg ligaments, and as the embryo has the lowest density, it is always closer to the upper part of the shell (Figure 2) and is therefore exposed to a higher incubation temperature than the lower half of the egg. This means that the temperature measured at the centre of the egg cannot be considered identical to the temperature to which the embryo is exposed (at least in the first part of incubation).

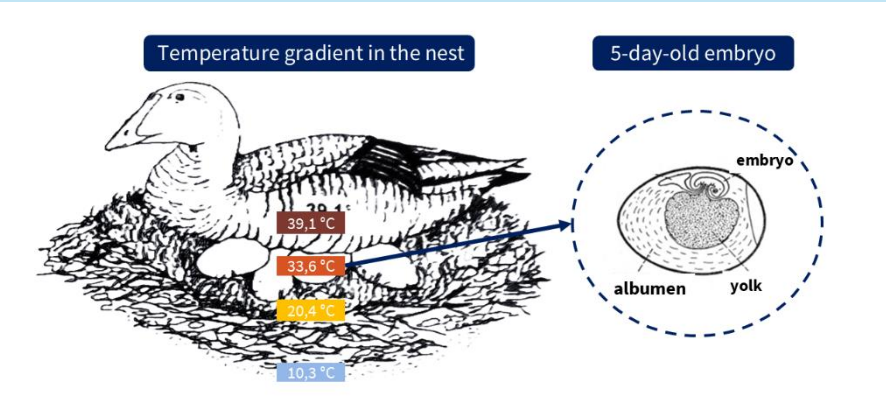


Figure 2: The temperature gradient that arises during incubation and embryo positioning in the egg in the early stages of incubation. Adapted from Deeming 2002.

2.1.2. Incubation humidity and gas exchange

A key parameter of incubation (particularly in artificial incubation) is the loss of egg weight caused by water evaporation through the shell. For successful hatching to occur, the egg must lose a certain proportion of its initial weight by the time of hatching. For most species, this loss ranges from approximately 10–20 % (Ar and Rahn 1980, Incubase 2026, Figure 3). However, the gas permeability of the shell is species-specific and correlates closely with the duration of the incubation period and the weight of the egg (Rahn and Ar 1974; Ar and Rahn 1980).

Gas exchange between the egg and its surroundings during natural incubation occurs mainly during incubation breaks, when the parent temporarily leaves the nest. During artificial incubation, this is usually provided by the incubator's ventilation system. From a practical point of view, therefore, gas exchange does not require active intervention; however, nests with a very compact structure or rearing in unventilated areas may pose a risk (Tullett 1990).

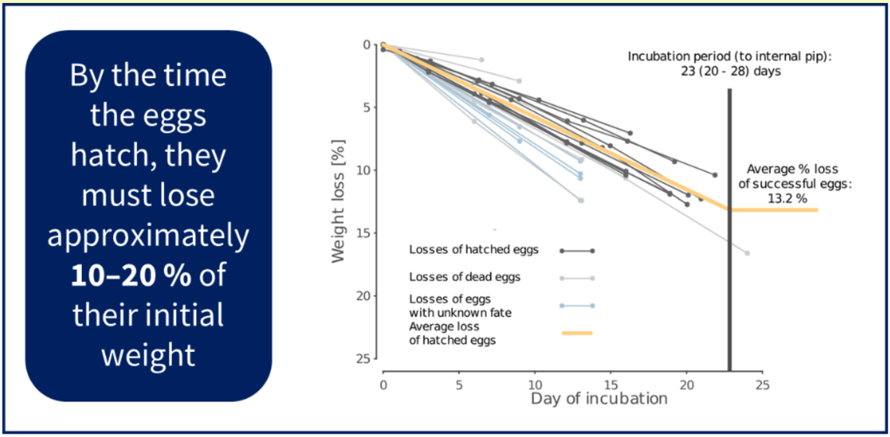


Figure 3: Weight loss of eggs during incubation, using the Chilean tinamou (*Nothoprocta perdicaria*) as an example. For further information, please visit www.incubase.fzp.czu.cz/.

Direct, continuous measurement of humidity during natural incubation remains methodologically challenging, as humidity fluctuates significantly over time and in different parts of the nest, and the available data remain limited. The incubation humidity profile during natural incubation has been studied considerably less than that of temperature. Factors such as the nest's distance from water or the type of nest lining play a role, as some materials may retain water better than others (Ar and Rahn 1980).

2.1.3. Egg turning

Regular egg turning by the parent is essential for normal embryonic development. Turning prevents the embryonic membranes from adhering to the shell, promotes the growth of blood capillaries and the absorption of nutrients from the albumen, ensures the correct orientation of the embryo, and ensures that eggs in larger clutches are heated evenly (New 1957; D. C. Deeming 1989; Boulton and Cassey 2012). Until recently, the frequency and angle of turning were difficult to measure during natural incubation. However, this is gradually changing with the development of miniature dataloggers fitted with movement sensors. Studies to date demonstrate high interspecies variability in the frequency of egg turning (Pešková et al. 2025). Furthermore, egg turning itself is significantly influenced by various types of parental behaviour at the nest (Chapter 8.3.5.), which is influenced by external factors. Egg turning usually occurs more frequently during the day than at night, when the parent tries to move as little as possible to reduce the risk of predation (Pešková et al. 2024). Outdoor temperature may also play a role. If, at any point during the day, the temperature is significantly lower than the optimum incubation temperature, the parents may try to avoid egg turning at that time (Zhang et al. 2017).

2.1.4. Incubation rhythms

The term ‘incubation rhythm’ refers to the pattern of incubation bouts and breaks, or, in the case of biparental species, the frequency with which partners exchange. The specific pattern of the incubation rhythm is usually a compromise between the need to keep the eggs in optimal conditions and the parents' own needs. After all, the parents also need to feed and groom themselves during incubation, just as they need to sleep. This conflict between the needs of the embryo in the egg and those of the parents is more intense in uniparental species, where only one parent incubates the eggs. In biparental species, where both parents incubate the eggs, the form of the incubation rhythm is also determined by conflict between the parents about who will sit on the eggs, when and for how long (Sládeček et al. 2019).

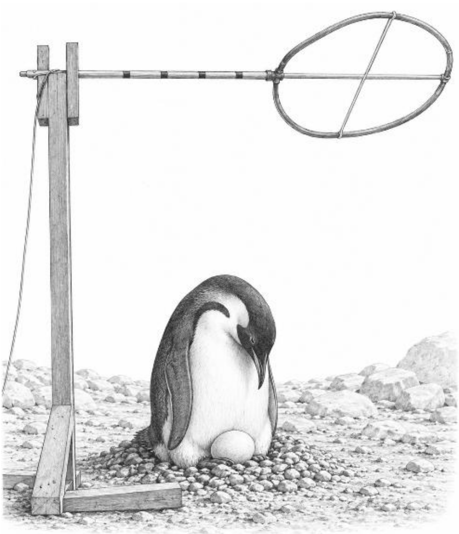
In the wild, we therefore often observe a higher level of incubation presence in biparental species, where parents can provide incubation care consistently throughout the 24-hour cycle (Bulla et al. 2016). However, within a species, nests where both parents are better able to coordinate and share incubation duties receive more incubation care. If one partner (usually the male) significantly reduces their portion of incubation time, the other partner compensates only partially, thereby putting pressure on their less attentive partner. In such cases, however, the eggs receive less care, which may, for example, prolong the incubation period (Sládeček et al. 2019).

Conversely, in uniparental species, we often observe a distinct diurnal rhythm in parental presence during incubation. Breaks in incubation occur mainly during the daylight hours, when it is generally warmer and the parent’s absence from the nest has less dramatic consequences for the eggs (Reneerkens et al. 2011, Trejbalová et al. 2023).

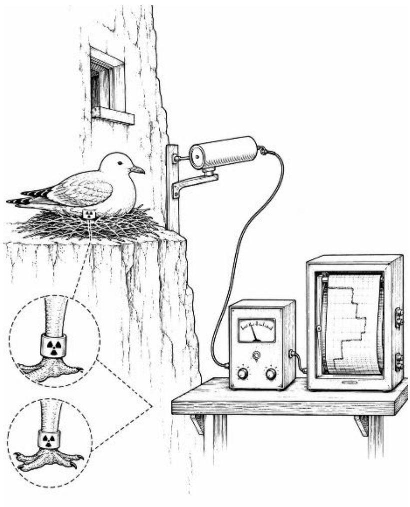
The risk of predation is also a significant factor shaping incubation patterns. Birds generally try to minimise movement around the nest or, if possible, schedule it for times of day when the risk of predation is lower. This is more important for species that are unable to actively defend themselves against predators and rely on their cryptic behaviour (Bulla et al. 2016). Even during the incubation period, parents must attend to other activities, such as preening or sleeping. Although these are activities that can be carried out whilst on the nest, they may reduce the parents' vigilance; consequently, this behaviour also follows a certain rhythm (Brynychová et al. 2020). The resulting incubation rhythm is not an entirely fixed characteristic of the species but rather a flexible strategy that largely adapts to current conditions. Considerable intraspecific variability has been described in many species (Bulla et al. 2016). Parents are able to alter their behaviour, for example, in response to changes in food availability or the loss of a partner. In extreme cases, there may be a shift from normal biparental incubation to incubation by a single parent, even in species where both partners would normally take turns (Bulla et al. 2017).

2.2. From observation to dataloggers

Even in the very early days of biologging, many researchers devoted considerable effort, ingenuity and resources to learning something new about parental care in birds. It is worth mentioning some of the classic (and, within the field, legendary) studies as examples of exceptionally innovative use of available resources, whose contributions were decades ahead of their time.



For example, the first direct measurement of temperature inside an egg using a datalogger was carried out in the 1950s on the emperor penguin (*Aptenodytes forsteri*) (Eklund and Charlton 1959). Recording the temperatures required installing a large device in the immediate vicinity of the incubating bird. This experiment was possible thanks to the extraordinary tolerance and calmness of the emperor penguin, which was able to cooperate with the researchers on this type of research.



The first automatic recording of parental exchanges at the nest was carried out using radioactively labelled rings on the black-legged kittiwake (*Rissa tridactyla*) (Coulson and Wooller 1984). Each bird in the pair was fitted with a ring containing a different concentration of radioactive cobalt. Furthermore, these concentrations were added together when both parents were present at the nest simultaneously. Radioactivity was measured using a Geiger counter and recorded with a pencil on a slowly rotating paper cylinder.

Continuous video recording as a tool for studying incubation rhythms was first used for the white-rumped sandpiper (*Calidris fuscicollis*) in a study published in 1985 (Cantar and Montgomerie 1985). In this study, the nest was photographed at a rate of one frame per minute, which provided a detailed picture of behaviour at the nest. However, it was also necessary to manually review and analyse over 175,000 photographs, which clearly illustrates the analytical limitations of the methods available at the time.

The progressive miniaturization of electronics during the 1990s and the first decade of this century enabled the development of temperature dataloggers small enough to be placed directly in the nest or inside an artificial egg of relatively small bird species. Subsequent developments in accelerometers also made it possible to record egg movements, and the combination of a temperature sensor and an accelerometer within an artificial egg gradually became the standard method. Over time, the accelerometer was also supplemented by a magnetometer. Computer programmes have been developed for processing camera recordings, which greatly facilitate their detailed analysis (e.g. the BORIS software, Friard and Gamba 2016).

In recent years, methods of automated video analysis based on machine learning and artificial intelligence have been developing rapidly, enabling the detection and classification of animal behaviour without the need for manual assessment. However, their wider use in monitoring incubation is currently limited by, in particular, a lack of sufficiently annotated data for training the models, limited transferability between different bird species and varying camera angles, and the relatively high level of specialist knowledge required for their development and validation.

Over the past decade, our team has been intensively involved in monitoring incubation using dataloggers in wild birds and has extensive experience in this area across a range of bird species and climatic conditions. In our studies, we systematically combine several components of comprehensive monitoring. We primarily use artificial eggs containing dataloggers, temperature and humidity sensors in the nest, RFID sensors to monitor parental exchanges in the nest, and continuous video recording (see Chapter 4). Within the framework of this guide, this system has been adapted for use in ex-situ breeding conditions across a wide range of bird species.

3. Why study incubation in captivity?

Conditions in ex-situ breeding programmes differ from those in the wild in a number of respects; nevertheless, the fundamental biological principles of incubation remain the same, and for most species, their behaviour during incubation in captivity and in the wild is very similar. Systematic monitoring can therefore provide not only new insights about little-known species, but also concrete evidence to improve both natural and artificial incubation. The key benefits of monitoring bird incubation under human care can be summarised into three main categories.

- **Gaining knowledge about species whose incubation behaviour in the wild is unknown.** Species kept in zoos and conservation programmes are often rare, endangered or difficult to study in their natural habitats. For many of these species, therefore, not even basic information on incubation patterns, knowledge of incubation temperature or the need to turn the eggs, is available. Ex-situ breeding programmes allow these parameters to be monitored continuously and under conditions in which the installation of equipment and regular data checks are significantly easier than in the field. The data obtained can thus expand our knowledge of the species' biology and provide a reference basis for further research and breeding decisions.
- **Identifying problems with natural incubation in breeding.** Identifying problems with natural incubation in breeding. If a particular pair is unable to successfully hatch their eggs, monitoring the incubation process is a powerful tool for diagnosing the causes of this failure. It can help us determine whether the parents are warming and turning the eggs sufficiently, how often and for how long they leave the nest, and whether their behaviour is affected by the environment of the enclosure. The causes of problems may include, for example,

disturbances from visitors or breeders' inspections, the presence of other birds in the enclosure, unsuitable nest placement, the enclosure's microclimate, or a lack of coordination between the partners when taking shifts. These difficulties may not be apparent during routine checks, as the parent may remain on the nest, yet their care may still fail to create conditions suitable for embryonic development.

- **Using successful natural incubation as a model for artificial incubation.** If the parents of a given species repeatedly incubate successfully, whilst we are unable to hatch the species in an incubator, the values measured during natural incubation can be used as a biological reference for the incubator settings. The records may show the actual temperature of the egg, the extent of its natural fluctuations, and the frequency of turning. These values cannot always be directly applied to artificial incubation, but they can help refine the existing protocol and determine whether it is appropriate to adjust the temperature, humidity, cooling or egg-turning regime. Monitoring natural incubation thus creates a practical link between natural and artificial incubation.

4. Obtaining incubation data

For comprehensive monitoring of incubation, we use several types of dataloggers, which differ in design, the sensors used and the recording frequency (although it can be usually adjusted). Each device has its own specific application and provides information on different incubation parameters. The main types of devices used are: **1)** artificial eggs with an embedded datalogger recording temperature and egg turning; **2)** temperature and humidity dataloggers recording microclimatic conditions inside the nest and, for comparison, outside the nest; **3)** a camera recording the behaviour of birds on the nest, including interactions with intruders; **4)** an RFID datalogger recording the presence

Figure 4: Components of the comprehensive monitoring system – a camera at the nest recording behaviour, an artificial egg with a datalogger (type: AXY5) recording temperature and turning during incubation, a datalogger measuring temperature and humidity in the nest (type: MSR), an RFID tag recording the presence of a bird (tagged with a chip in its ring) at the nest.

of a parent bird tagged with a chip at the nest (Figure 4).

This chapter provides a brief overview of the various types of dataloggers, followed by a description of typical situations in which the use of a particular device is most appropriate. The aim is to facilitate the choice of a suitable measurement methodology based on the species being monitored, the type of nest and the research objectives.

4.1. Monitoring the internal temperature and egg turning

To measure the internal temperature of an egg and track its movement, it is generally best to place the datalogger directly inside the dummy egg. The main advantage of using artificial egg substitutes over other methods of incubation monitoring is direct contact with the nesting bird and the standardised positioning of the sensors in relation to it. The datalogger inside the egg, therefore, records incubation parameters directly from the position of the incubated embryo.

Artificial eggs with a built-in datalogger must be designed so that their shape, dimensions, weight, colour and surface mimic the natural eggs of the species as closely as possible. Once placed in the nest, the artificial egg is usually accepted by the parents and treated as if it was a real one.

To measure the temperature during incubation, a datalogger fitted with a temperature sensor is placed inside the artificial egg. Depending on the purpose of the monitoring, various types of models can be selected, allowing the temperature to be measured at the centre of the egg or at several points near its surface, thereby capturing temperature differences in different parts of the egg. The temperature sensor records the temperature at regular intervals, enabling an assessment of the temperature regime and fluctuations in the conditions to which the embryo is exposed during incubation.

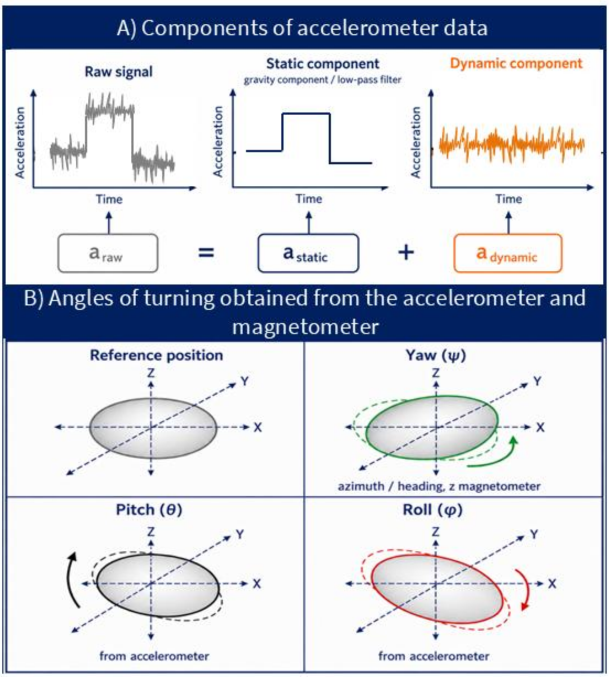


Figure 5: A diagram of the components obtained from data recorded by A) an accelerometer and B) an accelerometer combined with a magnetometer. The recorded movement of the egg consists of changes in angle along three axes – pitch, roll and yaw.

If the datalogger is equipped with a three-axis accelerometer and a magnetometer, changes in the egg's orientation in space can be determined. The accelerometer records acceleration along three axes and makes it possible to distinguish between two basic components of movement: the static component corresponding to the effect of gravity and the dynamic component caused by the egg's own movement (Figure 5A). From the static component, changes in the egg's position relative to the gravitational field can be determined, in particular, the *pitch* and *roll* angles. The magnetometer, on the other hand, measures the orientation relative to the Earth's magnetic field and provides additional information on the direction of the egg's movement in the horizontal direction, i.e. the *yaw* angle (Figure 5B). The combination of an accelerometer and a magnetometer thus enables reconstruction of the egg's spatial position and quantification of its turning during incubation. The data obtained allows the evaluation of the frequency, timing and extent of the egg's turning by the parents during incubation. In general, measuring incubation conditions using artificial eggs fitted with dataloggers can be particularly useful for verifying that incubation is proceeding correctly in parents who are unable to hatch their eggs, or for optimising breeding practices for the artificial incubation of a given species. In such cases, a similarly equipped egg model can also be placed in an incubator to compare the care provided there with that provided by the parent.

4.1.1. Artificial egg

To install the datalogger, you need to obtain an egg into which it can be inserted. The egg replicas must be designed to mimic real eggs of the specific species as closely as possible in terms of dimensions, weight and, ideally, thermal properties. Historically, blown real eggs strengthened shells, or their wooden or plastic substitutes, filled with materials that imitated the real egg properties to varying degrees (for example, modelling clay, wax, water or various types of gels), were used for this purpose

However, we recommend 3D-printed model as the most practical option. 3D printed artificial eggs are described in this guide, and we have had very good results with them. Their main advantages over other methods include the ease with which models can be adapted for individual species, the ease of sharing them, and their low production cost. We have already created suitable models for most bird species, and these are freely available (www.zenodo.org/records/18955995, Chajma et al. in prep). As confirmed by validation tests, the weight of these egg substitutes and their thermal dynamics are relevant to incubation monitoring and fall within biologically meaningful ranges for the actual eggs of the species studied (Chajma et al. in prep).

The following subchapters describe the practical use of the various egg models and the process of their creation. The creation of these models is simple, and all the print data are available. Eggs measuring at least 25.7 × 19.3 mm are suitable for use with dataloggers, which corresponds to the eggs of the Laughing Dove (*Spilopelia senegalensis*). However, for species with smaller eggs, egg models without a cavity for a datalogger are also available for general use in breeding. In the following subchapters, you will find descriptions of the individual types of egg models, comprehensive information on their production, and suitable dataloggers that can be used with egg models to record incubation conditions.

4.1.1.1. Types of artificial eggs

For the majority of bird species, we have developed four types of egg models, differing in design and intended use (Figure 6). The *solid model* (printed in a single piece and filled with filament, with the option to adjust the filling density), and the *shell model*, which only includes an eggshell and must be filled, for example with ultrasonic gel, to achieve the appropriate thermal properties. Both types are primarily intended for standard breeding situations where the only goal is the physical presence of a substitute egg in the nest, for example to regulate egg-laying or to maintain incubation until the chicks hatch in the incubator (Figure 6).

Two further types are essential for the use of dataloggers. The *single-cavity research model* is filled with gel and contains a single central internal cavity for placing the datalogger. This model has a removable screw-on cap and an access cavity for a single miniature datalogger. It records the internal temperature at the centre of the egg, as well as the frequency and angle of turning. The *three-cavity research model* is also filled with gel and contains three side cavities for placing the datalogger, situated approximately in the middle of the egg. The model allows three miniature dataloggers to be inserted simultaneously just beneath the surface of the shell, to measure the surface temperature of the egg and the temperature gradients within it. Like the previous model, this one also allows the frequency and angle of turning to be recorded. The availability

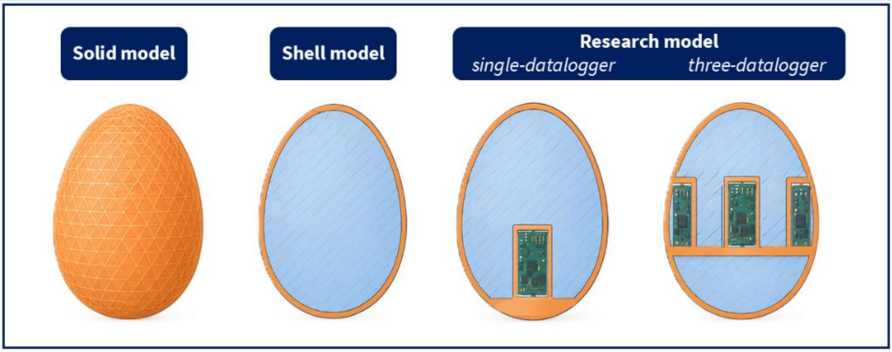


Figure 6: The four basic types of developer artificial egg.

of individual model types is adapted to the size of the specific egg. The full model is available for all of turning to be recorded. The availability of individual model types is adapted to the size of the specific egg. The full model is available for all sizes; the shell model is for eggs ≥ 15 mm in length; the single-chamber research model is for eggs ≥ 25.7 mm in length and ≥ 19.3 mm in width; and the three-chamber research model is for eggs ≥ 45.1 mm in length and ≥ 33.0 mm in width. The three-chamber model is best suited for temperature measurement, as, particularly during the first half of incubation, the embryo in a bird's egg is always located in the upper part of the egg, closer to the shell surface. The measured temperatures, therefore, most accurately reflect those to which the embryo is exposed during incubation.

4.1.1.2. Manufacture of the artificial egg

3D printing

The egg models and all the documentation are available for download from the repository associated with the publication by Chajma et al. (in prep.) at www.zenodo.org/records/18955995. Here you will find models for printing in *.stl format and detailed information on modelling and printing eggs. Commonly available FFM printers are sufficient for printing the artificial eggs (Figure 7). We recommend using non-toxic and thermally stable PETG or PLA filament. A light-coloured (white) filament is suitable for subsequent colouring of the egg. The layer height should not exceed 0.2 mm to ensure the shell is waterproof. To further ensure the model's strength and water resistance, we recommend printing with at least three perimeter walls using a standard 0.4 mm nozzle and staggered internal seams with random placement.

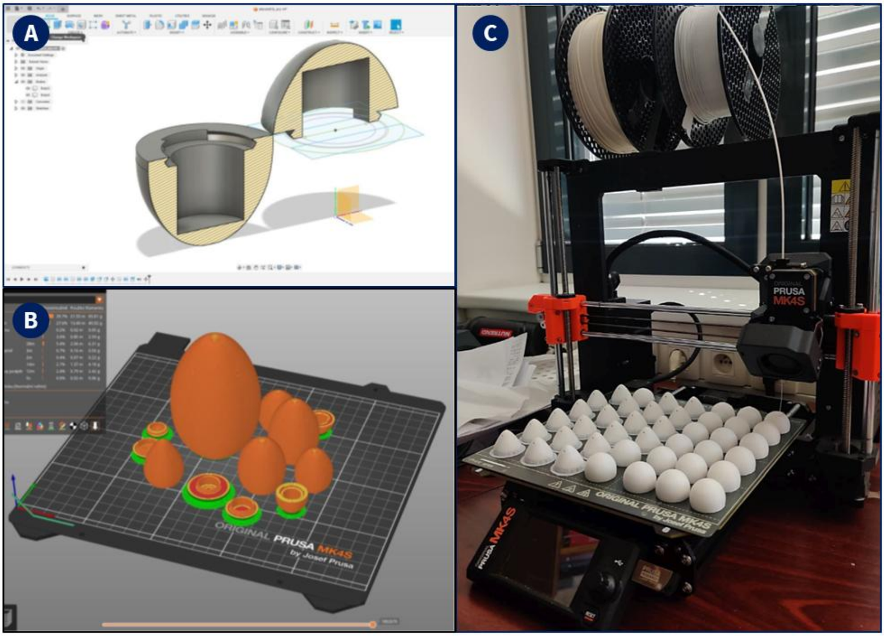


Figure 7: 3D printing of artificial eggs – A) modelling eggs, B) preparing eggs for printing, C) printed eggs.

Gel filling

For filling models with an internal cavity, it is necessary to add a print pause at 95% of the egg's final height. That is, before the top of the shell is sealed, when the filling hole is large enough, but the egg can already hold a sufficient amount of filling material. The exact percentage at which printing should be paused may vary slightly depending on the shape of the specific egg and the filling tool.

We recommend fill egg with commercially available ultrasound gel diluted with water to approximately 55% by volume. This gel has suitable density, viscosity and thermal properties, and also a low purchase price. If necessary, the gel may be replaced with another inert, non-toxic liquid of comparable density and thermal properties; however, in such cases, it is essential to check the final weight of the egg and its resistance to filling leakage before placing it in the nest.

Colouring and visual adjustment

Before you start painting, you must thoroughly inspect the printed egg and repair any leaks in the filling. In most cases, superglue is sufficient to fix these, or you can melt a small piece of filament onto the affected area at a low temperature using a 3D pen or a soldering iron. Surface imperfections can be removed with fine-grit sandpaper. Then cover the egg with a base coat of paint to ensure a smooth surface for further colouring.

For safety reasons, use only non-toxic, water-soluble acrylic paints. Figure 8 A) shows a universal colour palette which, when combined, will allow you to reproduce the colour range of most birds' eggs. For the base layer, choose a suitable colour according to the specific species. Dilute the paint with water in a ratio of approximately 1:3 and apply it evenly using a paintbrush or an airbrush (which allows for the application of a thin, even layer of paint). You must then allow the paint to dry completely and repeat the process two or three times as required to achieve even coverage and a smooth surface on the egg, ensuring that the filament layers are not visible, particularly at the pointed end of the egg.

If the eggs are spotted (e.g. waders, birds of prey), once the final layer of paint has dried, you can proceed to paint the spotted pattern. To do this, apply paint diluted in a ratio of 1:5 using a comb or brush to create fine droplets that mimic the natural markings on the shell.

If the eggs are spotted (e.g. waders, birds of prey), once the final layer of paint has dried, you can proceed to paint the spotted pattern. To do this, apply paint diluted in a ratio of 1:5 using a comb or brush to create fine droplets that mimic the natural markings on the shell. If the egg needs to have a faded or dirty appearance (e.g., pelicans), use very diluted brown or black paint (1:10), applied by hand, leave it to dry partially, then carefully wash it off. This creates a subtle residual colouring similar to the natural dirty/wear on the shell. Once the colouring is complete, leave the egg to dry thoroughly. Before installation, ensure the paint is not sticky and the filling is not leaking.

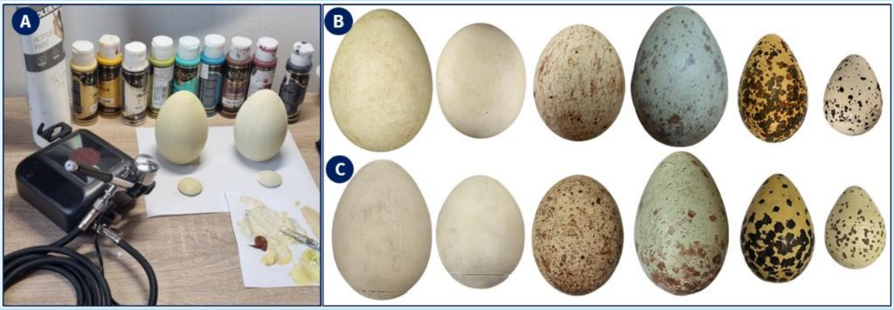


Figure 8: A) accessories for painting eggs, including a range of basic colours, B) real eggs used as templates for painting, C) painted artificial eggs.

Lifespan and maintenance of artificial eggs

Experience to date has shown that the artificial 3D printed eggs have a very good durability. When used with a datalogger, unlike other types of dataloggers installed in or around nests, they are practically unaffected by nesting birds. If the gel filling leaks out over time, the eggs can be easily repaired. Treat the leak with superglue, once it has dried, gently smooth the surface with sandpaper and reapply the paint to the repaired area following the procedure described above. Once removed from the nest (after datalogger removal) the egg can be washed with water without any problems and left to air dry.

Artificial egg – practical notes

- Egg substitutes are generally well accepted by nesting birds in the wild and in zoos
- Acceptance may be problematic for some species (e.g. cranes, sensitive species) – a faithful visual imitation of an egg significantly increases the chances of acceptance
- The egg substitute should match the colour and pattern of the species' natural egg as closely as possible (however, it is not necessary to imitate the detailed pattern exactly, the aim is to approximate the overall visual impression)
- Use photographs of real eggs of the target species as a reference, ideally taken directly from the specific breeding facility or from the specific female to which the substrate will be applied
- If eggs of the target species are highly variable and photographs from the breeding facility are not available, prepare several substrates reflecting the individual colour forms
- Photographs of eggs can be found and shared via the Incubase platform (www.incubase.fzp.czu.cz/) enter the species name in the Incupedia section
- If the size of the egg allows, mark both parts of the artificial egg with a permanent marker, indicating the species or pair for which the egg is intended

4.1.2. Choosing a datalogger for an artificial egg

Choosing a suitable datalogger for an egg depends on its technical specifications and size. The main limiting factor is the dimensions of the datalogger (which are primarily determined by the size of the battery), as it must fit into the internal cavity of the egg (12.4 x 7.0 x 24.5 mm). At the same time, the datalogger must not move freely within the cavity, as such movement would skew the record of the egg turning. The artificial egg may not be completely watertight, so it is recommended that the used datalogger be encapsulated or sealed to protect it from any ingress of water. However, the datalogger is not normally exposed to the egg's gel filling.

In terms of sensor equipment, a temperature sensor is the absolute minimum requirement, and a combination of an accelerometer and a magnetometer is essential for monitoring egg turning (see above). The sampling rate should be adjustable, as the optimal data collection interval may vary depending on the specific research question. Given that installing the datalogger in the nest usually requires temporarily disturbing the nesting bird and interrupting incubation, it is recommended to choose a datalogger with a longer battery life and sufficient memory capacity to handle at least 4 days of continuous recording. This will minimise the frequency of necessary interventions in the nest required to replace the device.

Another advantage is the option for delayed data collection, i.e., the ability to configure the datalogger in advance and select a later start time. An even greater advantage is the option to start data collection only at the time of installation, without needing to connect the device to a computer. This feature is important, for example, with species where we do not wish to interrupt incubation and want to install the dataloggers precisely at the moment of a natural incubation break; however, the timing of this break may vary during incubation and must be accurately identified.

The range of such devices is therefore currently very limited. A large proportion of the data presented in this methodological guide was obtained using the DAL2 and DAL3 dataloggers developed by the authors' team, which, in addition to temperature, recorded humidity and light intensity. However, these dataloggers are not commercially available. For this reason, this methodological guide focuses primarily on the AXY5 XS datalogger, which is currently the only commercially available device suitable for use in the artificial egg described here. Its technical specifications, configuration and practical application are described in detail in the following chapter.

4.1.2.1 AXY5 – datalogger for measuring the temperature and egg-turning

The AXY5 XS is a commercially available miniature datalogger manufactured by the Italian company TechnoSmArt Europe (www.technosmart.eu/axy-5-xs/), designed for monitoring animal behaviour. The device's compact dimensions (21 × 11 × 7 mm, weight 1.8 g; Figure 9) currently make it the only commercially available datalogger that can be directly integrated into the artificial egg model described in section 4.1.1, the design of which has been adapted specifically for this device. The AXY5 XS is a commercially available miniature datalogger manufactured by the Italian company TechnoSmArt Europe (www.technosmart.eu/axy-5-xs/), designed for monitoring animal behaviour. The device's compact dimensions (21 × 11 × 7 mm, weight 1.8 g; Figure 9) currently make it the only commercially available datalogger that can be directly integrated into the artificial egg model described in section 4.1.1, the design of which has been adapted specifically for this device. The device is equipped with three sensors: a temperature sensor, an accelerometer and a magnetometer, which enable the recording of temperature and the egg turning during incubation. With a temperature sampling rate of 1 Hz (once per second), an accelerometer sampling rate of 10 Hz, a magnetometer sampling rate of 2 Hz, and a fully charged battery, the datalogger will operate for approximately 18 days, which covers at least half the incubation period for most species, and for some species, even the entire incubation period. A practical advantage of the AXY5 is the ability to start data collection just before installation in the nest using a magnet. Configuration modes can be exported (*.sch file) and shared with other breeders using the same datalogger model.

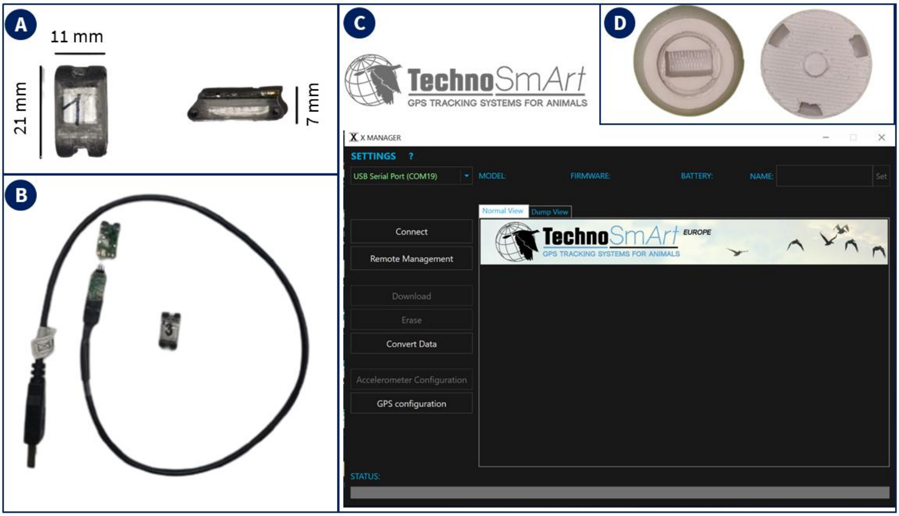


Figure 9: A) the AXY5 XS datalogger and its dimensions, B) the datalogger's power cable, C) the X Manager software environment, D) the internal layout of the cavities for inserting dataloggers into the artificial egg.

Starting and configuration

A special four-pin cable and the *X Manager* software are used to communicate with the datalogger. Always use the latest version of the software available on the manufacturer's official website (www.technosmart.eu/downloads/). Connect the cable to the PC and launch the *X Manager* programme. In the top-left corner, select the *USB Serial Port* input. Swipe the magnet along the side of the datalogger until the indicator light starts to flash faster and then slower. Whilst it is flashing, connect the datalogger using the cable and select the *Connect* option. Delete any previous data (if stored) using *Erase*. In the *Accelerometer configuration* tab, select the settings for each sensor. For the accelerometer, we recommend setting the sampling rate to 10 Hz, the measurement range to 2 g and the resolution to 8 bits. For the magnetometer, we recommend a sampling rate of 2 Hz. For the temperature sensor, a sampling rate of 1 Hz is sufficient. Confirm the settings by clicking the *Send configuration* button. Then disconnect the datalogger using *Disconnect*. The status of the datalogger is indicated by an indicator light when a magnet is brought close to it. Moving the magnet along the side of the datalogger triggers a flashing sequence, the pattern of which indicates the current status of the device (see Table 1).

Table 1: AXY5 datalogger status indication.

Flashing pattern when a magnet is swiped along its side	Datalogger status
It flashes faster, then slower (for a total of 20 seconds)	Switched on; can be connected to a PC whilst it is flashing
One a short flash	No change in status
One long flash and several short flashes	Switched off

Launching and installation

Configuration can be completed in advance, but recording starts by swiping a magnet across its side. The indicator light will begin to flash more rapidly, then slowly and regularly, for approximately 20 seconds. If it is not connected to a computer, the datalogger is active from this point and records data. Place the switched-on datalogger into the egg so that the contacts are facing outwards from the egg, and screw the lid on.

For eggs with cavities for three dataloggers, insert the datalogger with the battery (silver side) into the centre of the egg and the sensor plate (green side) facing outwards so the temperature at the egg surface is recorded. If possible, point the contacts outwards from the egg. If you use a different position, write it in the installation form.

Switching off and data download

Once you have removed the egg from the nest, take the datalogger out of the egg. Use reasonable force, but as the entire device is encased in epoxy, it should be able to withstand the pressure. Switch off the datalogger by swiping a magnet along its side (Table 1). Store the datalogger in this way until the data is downloaded.

To download data, connect the cable to your PC, launch *X Manager* and set he input to *USB Serial Port*. Switch on the datalogger using the magnet and, whilst it is flashing, connect it to the computer via the cable and select *Connect*. In the programme, select *Download*. The data will be saved as a set of compressed files with the extensions *.ard and *.memDump. To convert the data, select *Convert data*, locate the saved *.ard file and, in the *Conversion preferences* tab, set the output table format. The data will then be saved to a *.csv file. The procedure for processing and analysing the data after download is described in section 7.1.

Charging

The datalogger is charged using the same cable used to connect it to a PC. If the red indicator light is on, charging is in progress. If the green indicator light is on, the datalogger is fully charged. The charge level, expressed as a percentage, is displayed in *X Manager*. Check the charge level at least one week before deployment to allow time for recharging if necessary. To ensure the long-term reliability of the datalogger, it is advisable to check the device at least once every six months when it is not in use for extended periods and to recharge the battery to at least 50% of its capacity.

AXY5 - limitations and practical notes

- Configuring and running the datalogger are two separate steps (data recording will only start after magnet activation)
- Choose the orientation of the datalogger in the cavity for three-cavity substrates so that the temperature sensor points to the surface of the egg
- Datalogger should not move freely in the cavity, if necessary, fix the datalogger firmly with paper; for three-cavity eggs, always place the paper from the inner side (battery) to minimize the temperature distortion
- Make sure, that both parts of the egg are tightly screwed
- For species with longer incubation times, replace the datalogger during monitoring with another piece
- A lower recording frequency can be selected to extend the data collection period
- Specific recommendations and limitations for each species group are given in Chapter 4.6.

4.2. Monitoring of temperature and humidity in the nest

Temperature and humidity dataloggers in the nest are among the most commonly used tools in incubation studies. They can be used for a wide range of species, including those with eggs too small to use an artificial egg with a datalogger. In practice, it is usually a pair of temperature or temperature-humidity sensors or two separate dataloggers. The first sensor (or datalogger) is placed in the middle of the nest between the eggs and records the conditions to which the eggs are immediately exposed; the second sensor (or datalogger) is placed within 1 m of the nest and serves as a reference record of the ambient temperature (and humidity) in the given environment.

Compared to the datalogger inserted into the egg, this solution offers greater design freedom, because only the sensor itself needs to be placed into the nest. The body of the datalogger (including the battery) may be bulkier and out of the bird's reach, for example, buried in the substrate near the nest or mounted outside the nest box. By comparing the temperature records from both sensors, it is possible to determine the incubation rhythm, i.e., the alternation of incubation sessions and incubation breaks, as reflected in the temperature balance between the two sensors. This can detect a prolonged incubation interruption, which would indicate the parent's inability to hatch the egg successfully.

However, the measured temperature is strongly dependent on the sensor's exact location relative to the clutch and the parent's body. For this reason, the data from these devices provide reliable information mainly about the course of incubation rhythms, but not about the absolute temperature at which eggs are actually exposed. This is better captured by a datalogger located in the egg, which is in direct contact with the body of the incubating bird (Chapter 4.1.). Another complication is that the sensor in the nest can change position; thus, during incubation, it may be buried deeper in the lining or removed out of the nest, which can distort or completely invalidate the data for the relevant period. However, this datalogger is suitable for supplementing the information obtained from an artificial egg.

4.2.1. MSR – datalogger for temperature and humidity measurement in the nest

MSR is a datalogger designed to measure temperature (or humidity, depending on the model) in the nest and its immediate surroundings. It is commercially available from the Swiss company MSR Electronics GmbH (www.msr.ch). We recommend the MSR145 waterproof IP67 in a silicone case with two temperature and humidity sensors on cables of at least 20 and 40 cm in length. Two external probes (on cables of different lengths) allow simultaneous measurement of conditions inside and outside the nest. The datalogger can be set in advance, with a delayed start at a preset time, or turned on with the side button.

Starting and configuration

A special cable and *MSR.exe* software are used to communicate with the datalogger. Unscrew the protective cap on the datalogger connector, connect the cable and connect it to the PC. Start program *MSR.exe* and select *Setup* (blue S icon in the left panel). The configuration window opens; the port should be recognized automatically by the computer. In the configuration window, go to the *Format memory* tab and select *Format* to delete the previous data. Formatting takes approximately 80 seconds and must not be interrupted. Then go back to the *Basic settings* tab and set the temperature record parameters (*t1* and *t2*) to 1s and humidity (RH) to *t1* (this setting will collect temperature and humidity data at the same time, see the recommended settings in Figure 10). In the *Start at*, set the expected start time for data collection corresponding to the planned installation. Alternatively, you can choose the *Start and Stop by push button* option, which can be slightly less reliable. Deselect the *"blue LED flashes with t1"* option to turn off the flashing of the LED light that is unsuitable in the nest or in its immediate vicinity. Confirm with the *Write basic settings* button and close the window with the *Exit* button. Disconnect the cable and screw the protective cap back onto the connector.

The recommended setting is designed to capture both short-term temperature fluctuations during incubation breaks and long-term trends throughout the incubation period.

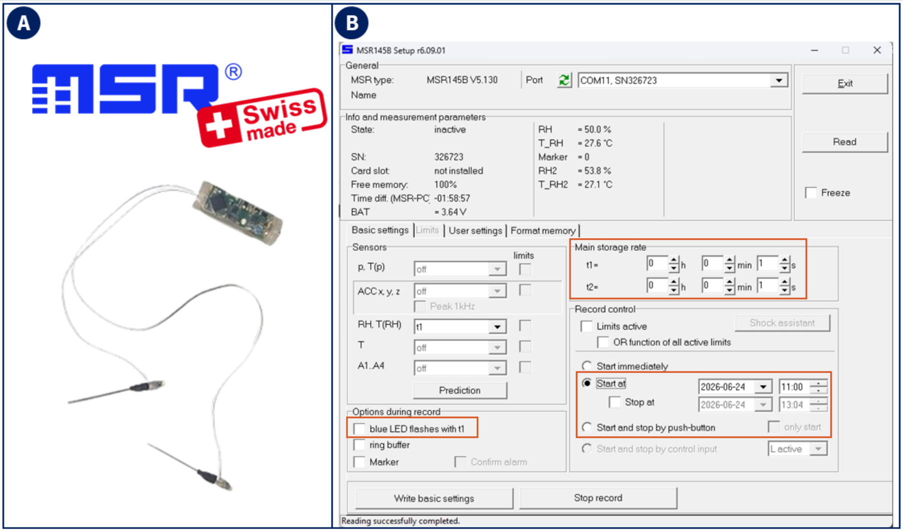


Figure 10: A) MSR datalogger, B) MSR environment. The main settings boxes are framed in red.

Launching and installation

Insert the sensor on the longer cable (after downloading, the data form this sensor are labelled T_RH2, RH2) vertically into the lining in the middle of the eggs. Place the sensor on a shorter cable (data labelled T_RH, RH) outside the nest in the surrounding area. This sensor serves as a reference for environmental temperature and humidity. Keep the body of the datalogger out of the bird's reach so it is not damaged or pulled out. Although the datalogger is waterproof, it is advisable to protect its body from moisture and dust with plastic packaging.

Switching off and data download

Connect the cable and connect the datalogger to the PC. Start *MSR.exe* and select *Reader* (blue R icon in the left panel). For *„Record is active! Terminate record?"* select *Yes*. Choose *Select all* and confirm with *Ok*. The data is downloaded, and the software displays the path to the saved file with the extension *.msr. After downloading, a window with a graph of the recorded temperature and humidity data opens automatically for quick visual inspection. Here you can immediately check whether the datalogger has been working properly, whether the sensor has been correctly placed in the nest and whether incubation continued. Data can be exported from this window to *.csv format via *File→Export time window as a text*. Warning, before exporting data, mark all variables in the graph and check that they are displayed. Data processing and analysis, including visualisation of temperature and humidity, is described in Chapter 7.

Charging

MSR is charged via the same cable that connects it to the computer. You can check the charge status in *MSR.exe*. Verify the charge status when configured before the scheduled installation.

MSR - limitations and practical notes

- The biggest practical limitations are the sensors themselves and the need to install them in the nest
  - for destructive species (e.g. parrots), there is a risk of pulling out and damaging the cables
- Specific recommendations and limitations for each species group are given in Chapter 4.6.
- Be very careful when handling the MSR datalogger cable
  - The connector contains fragile pins that can be easily damaged, so never force the cable (damaged cable disable communication with the datalogger and its charging)

4.3. Recording behaviour on the nest

Cameras and photo traps are widely used tools both in nature and in ex-situ breeding. Continuous monitoring of incubating pair or individual can be essential for successful breeding. The analysis of the recording in the relevant software allows to compose an etogram of incubation behaviour, i.e. a systematic record of the frequency and length of individual types of behaviour (breaks, parental exchange, modifications of nest, preening, food gathering, interaction with other individuals, etc.), even without the need to combine with other dataloggers.

The camera also serves as an additional tool for dataloggers inserted into eggs and sensors placed in the nest. This will add context to the data collected by other dataloggers. It allows you to link the measured values of temperature and egg movements with the specific behaviour of the parent on the nest. Thanks to the camera recording, for example, the reason for frequent interruptions of incubation can be identified. Similarly, it is possible to observe the interaction of the incubating bird with other individuals of the same or other species in the enclosure and the influence of this behaviour on incubation conditions. This method can also be used to verify the compatibility of a particular pair in egg care.

When choosing a camera, it is important to consider several parameters. The main thing is the possibility of continuous recording and the related sufficient capacity of data storage and power supply. The camera should be able to be powered by a separate power supply (battery or powerbank) and should not require a Wi-Fi connection. To interpret data from other dataloggers, it is not necessary to take a camera recording throughout the incubation period, three days of recording is enough. Incubation takes place continuously even at night, so the camera should have an infrared vision sufficient for reliable behaviour resolution even at low levels of light, without disturbing the incubating bird. For the same reason, sufficient resolution (minimum 1280 × 720 px) and frame rate (minimum 10–15 fps) are required for reliable behaviour recognition and identification of specific individuals. The camera should capture nest along with its immediate surroundings.

Figure 11: A) the camera in the enclosure, B) the powerbank hidden in the substrate, C) the camera placed in a fake stone.

It is important that the camera attachment can be adjusted to specific options in the enclosure. For outdoor aviaries, at least basic resistance to moisture and dust is appropriate, or the ability to place sensitive parts (e.g., the power supply) in a protective cover (Figure 11). Currently, relatively small cameras are available to fulfil the above-mentioned conditions. Choosing a particular camera model is always a compromise among recording quality, longevity (battery and storage), and enclosure conditions. We recommend masking the camera before installation by painting. The masking should match the environment in which the camera will be used. In general, brown tones with irregular spots have proven effective at masking, helping to blur the device's outline and reduce its conspicuousness.

Commonly available home security cameras are best suited for recording behaviour at the nest. We have good experiences with the Wyze and Neos

Smart Cam models, which are affordable. In terms of specifications, the E1 and E1 Pro models from Reolink, for example, are also suitable.

4.3.2. Camera Wyze

Of the available devices, we recommend the Wyze camera (www.wyze.com). The recording period of these cameras depends on the capacity of the connected power bank and the microSD card used to store the recordings. When using a 30,000 mAh power bank, you can expect approximately 3–4 days of continuous recording. The camera does not require any specialised software. Data is downloaded directly from the microSD card using a standard card reader.

Figure 12: A) a complete set for recording nest behaviour: a powerbank, power cable, microSD card, camera, and stand; B) installation of the camera near the nest.

Starting and time synchronisation

Insert the microSD card into the slot on the bottom of the camera and gently press until it clicks into place. Connect the camera to a power source using a cable (microUSB or USB-C, depending on the camera model). At this point, write down the start time to the nearest second. The camera will start automatically once the cable is connected. About 10 seconds after connecting the cable, you'll hear a click, and the camera will start continuously recording.

To ensure synchronization with data from dataloggers, you must synchronize the time, as these types of cameras often lack their own accurate clock (RTC module) to maintain the correct time over the long term. Immediately after starting the recording, point the camera at your phone or PC screen displaying the exact time, including seconds. Let the camera record the displayed time for 5–10 seconds while slightly adjusting the angle of the screen. If the camera is recording correctly, the indicator light on the camera (usually on the front, depending on the model) will flash red. After checking the recording, cover the indicator light with black tape so that the red light does not disturb the incubating bird.

Installation

The recommended Wyze camera model is equipped with a magnet and a joint at the bottom for adjusting the viewing angle. Choose a location for the camera where the sun shines directly into the camera for the shortest possible time each day (usually from the south side of the nest). There are several mounting options. For nests without a large nest lining, such as those of ground-nesting birds (ducks, waders), we have found it effective to use a plastic stand that can be placed in the substrate (Figure 12). If this is not possible, the camera can be attached with adhesive tape to a solid object (a branch, the wall of a cage, a support structure, or the wall of a nest box) without using a stand. The camera can also be hidden in a stone mound or an artificial rock, depending on the conditions in the enclosure (Figure 11C).

Thanks to the cable, the powerbank can be placed farther away from the nest and covered with substrate or buried. Always protect the power bank from moisture and dust by enclosing it in a waterproof plastic bag (Figure 11B). Secure the opening where the cable enters the bag with tape. Adjust the camera's angle so that the nest is centred in the frame and the recording provides an overview of what is happening around the nest. If you need to visit the nest while the camera is recording, again, at the beginning and end of your time in the enclosure, display the exact time of your visit on the camera (e.g., on your phone) and write this time down on the installation form (Chapter 5.1.).

Deinstallation and data download

Turn off the camera by unplugging the cable from the camera or the powerbank. Remove the microSD card by gently pushing it inward into the slot until it pops out. Insert the card into a microSD card reader and connect it to your PC. Copy the entire "record" folder from the card to your computer's hard drive. Due to the large amount of data, it's important to make sure that all recordings have been downloaded. Three days of recording can take up to 18 GB of space on this type of camera. After successfully copying the data, format the memory card. Insert the card back into the camera and recharge the power bank for future use. The procedure for analysing camera recordings and creating ethograms is described in Chapter 7.3.

Camera - limitations and practical notes

- Recording duration depends directly on the power bank's capacity (Before installation, always check the charge status of the power bank and consider whether its capacity will be sufficient for the planned monitoring period)

- If a longer recording is required, after three days
    - replace the powerbank and SD card (no need to completely disassemble the camera)
    - after turning the camera back on, record the exact time on the camera again
- Accurate time synchronization of the camera is crucial for properly linking the camera footage with other collected data; otherwise, it is not possible to reliably correlate a parent's behaviour with corresponding temperature fluctuations, etc.
- In direct sunlight, the phone's display may be unreadable when showing the time due to reflection. Choose an appropriate angle for the phone, or shade the display with your hand if necessary
- The camera's placement must balance conflicting requirements
    - a detailed view of the nest
    - minimal disturbance to the monitored animal
- For sensitive species install the camera at a greater distance and carefully camouflage it
- Specific recommendations for individual species groups are provided in Chapter 4.6.
- Continuous recording generates a large amount of data
    - Before installation, verify that the camera's internal memory or SD card has sufficient capacity, as well as the storage space for archiving recordings
- When using a different type of camera, verify before the first installation
    - the camera's field of view and resolution and battery life
    - the approximate size of recorded files for a specific period

4.4. Why is continuous monitoring necessary?

We know from nature that incubation conditions fluctuate significantly depending on many external factors (Chapter 2). Natural short-term fluctuations in egg temperature are a common part of incubation and occur very frequently in some species (Webb 1987; DuRant et al. 2013). At the same time, it appears that natural fluctuations in incubation temperature may not only be tolerated but may also represent an important factor influencing the phenotype of hatchlings. Incubation conditions shape growth, metabolism, thermoregulatory abilities, immune response, and post-hatch behaviour, and may therefore be critical for the survival of chicks (DuRant et al. 2013). The same is likely true for other incubation parameters.

It is therefore appropriate to monitor incubation conditions continuously over a period of several days, as short-term measurements may not accurately reflect the actual values of incubation parameters. To evaluate fluctuations in incubation temperature, parental exchanges, egg-turning frequency, or reactions to disturbances, it is necessary to obtain at least a two-day continuous record that captures the normal daily and nighttime variability of incubation conditions.

Long-term monitoring (throughout the entire incubation period) is particularly important in cases where, at first sight, the parents appear to be incubating perfectly, but the eggs repeatedly fail to hatch successfully. The cause may be, for example, occasional longer interruptions in incubation after a few days, after which the parents resume sitting on the clutch, or changes in their behaviour during the incubation period that can only be detected by observing the entire incubation or a substantial portion of it.

4.5. Further monitoring options

RFID

In addition to the dataloggers described in the previous chapters, other types of devices are also used in the study of incubation monitor parental behaviour at the nest. This includes the use of RFID readers, which record the presence of a specific individual (parent) at the nest. Using this device requires marking incubating parents with an RFID chip beforehand; when used in the field, the chip is typically inserted into a ring or flag and attached to the bird’s leg. In breeding programs, however, many species are already chipped for record-keeping, which could be utilised for this type of monitoring. The reader itself consists of a coil and a recorder. The coil is placed around the nest and, at an adjustable frequency (once per second), records the presence of a bird with a chip within inner perimeter. If another individual with a chip enters the coil, it is also recorded, allowing tracking of any chipped individual's presence at the nest.

Unfortunately, there are currently no commercially available devices for this type of monitoring. Therefore, studies published to date have relied exclusively on devices developed directly by research teams. An example of such a device is ZAYDA 2.1 (developed as part of the TAČR project TJ02000199), which combines continuous recording of temperature and humidity in and out of the nest with recording of the presence of a tagged individual at the nest (Figure 13). This combination makes it possible to verify whether a specific parent was actually present at the nest at a given moment and whether he was incubating an egg. The disadvantage of this approach is the need to first capture the parent and fit it with an RFID chip (if not already chipped), which represents a further intervention.

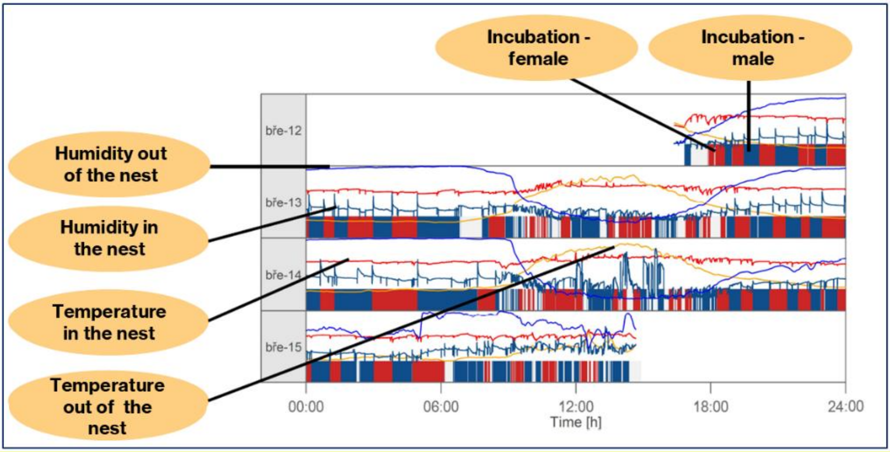


Figure 13: A graph showing the microclimate inside the nest over three days of incubation. The temperature measured inside the nest is shown in red, and the temperature outside the nest is orange. Humidity inside the nest is in light blue, and humidity outside the nest is dark blue. The graph also shows the presence of the male (blue boxes) and female (red boxes) at the nest, as tracked by RFID chips. White gaps indicate that the nest was not incubated by either parent. The data were collected using a ZAYDA 2.1 datalogger.

Voice recorders

Voice recorders or automatic acoustic dataloggers are used to record vocalizations at the nest, which can help identify individual birds and detect chick hatching, disturbances, or predation events.

Telemetry

VHF and UHF radio transmitters and GPS transmitters that transmit data via the GSM network or satellites are most commonly used to track individuals in the wild. They allow researchers to monitor where and when an individual breeds and what foraging trips he takes during incubation. Some transmitters are also equipped with an accelerometer that records activity levels, typically through overall dynamic body acceleration (ODBA), which can also be used to determine whether an individual is incubating the nest or rearing chicks. The disadvantage is the cost and the need to capture the bird and attach the transmitter. Generally, this solution is not widely used in captivity. Similarly, the aforementioned AXY5 datalogger can be attached to a bird chick to continuously monitor its body temperature and physical activity.

4.6. Selection criteria and characteristics of specific groups

No monitoring system is universally suitable for all bird species or in all situations. Based on experience to date, the monitored species can be divided into several groups according to the restrictions and recommendations that apply to them (Figure 14). However, there may, of course, be exceptions for specific species, individuals, or pairs.

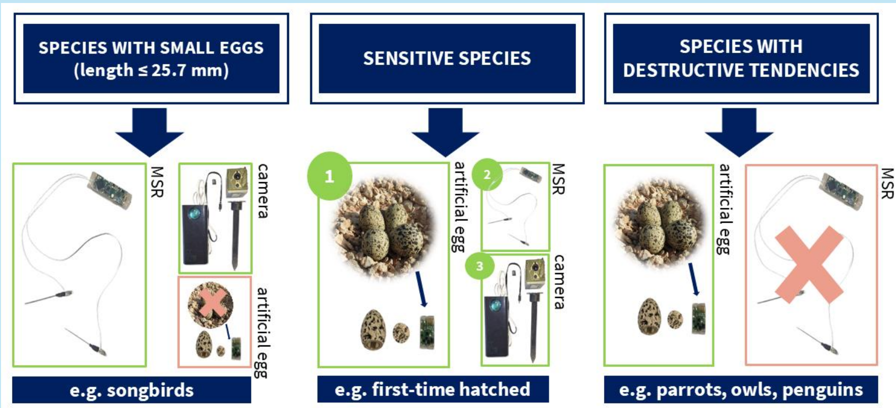


Figure 14: Choosing the right dataloggers for specific groups of birds. Suitable dataloggers are shown in green, and unsuitable dataloggers for a given group are shown in red.

One of the most significant limitations of using dataloggers for a particular species is the size of its eggs. This determines whether an egg substitute with an integrated datalogger can be used and, if so, which type of artificial egg (with one or three dataloggers) can be chosen (Figure 15 and

Section 4.1.1.). For very small species (typically songbirds), the current size of available dataloggers is incompatible with insertion into a proper-sized imitation of their eggs. In these cases, we recommend using a combination of nest sensors and a camera to monitor the incubation process.

Special attention should be paid to species that are sensitive to disturbance (e.g., the first breeding of specific species). For species prone to abandoning their nests when disturbed, it is advisable to minimise the number of visits and to conduct any interventions (if possible) during natural incubation breaks. In such cases, an artificial egg with a datalogger is a suitable solution, as it can be installed quickly and does not require further visits to the nest for the duration of the battery life. If the parents accept the egg, the system can be expanded during subsequent installations to include sensors placed inside the nest or a camera. When installing a camera, however, start with a more distant installation, even if this means reducing the ability to observe certain types of behaviour at the nest. This approach minimises the risk of the birds abandoning the nest while also allowing you to identify equipment that might cause stress and lead to nest abandonment. Similarly, if monitoring is conducted on a species for which there is no prior experience, and there is reason to believe the species will be sensitive to new elements, we recommend proceeding step by step. Start by testing the acceptance of an artificial egg with a datalogger.

For some species (such as cranes), the egg substitute must mimic a real egg very precisely, which is crucial for the egg to be accepted. We therefore recommend paying close attention to the colouring of the egg substitute for problematic species and using photographs of real eggs shared via Incubase (subsection 4.1.1.2).

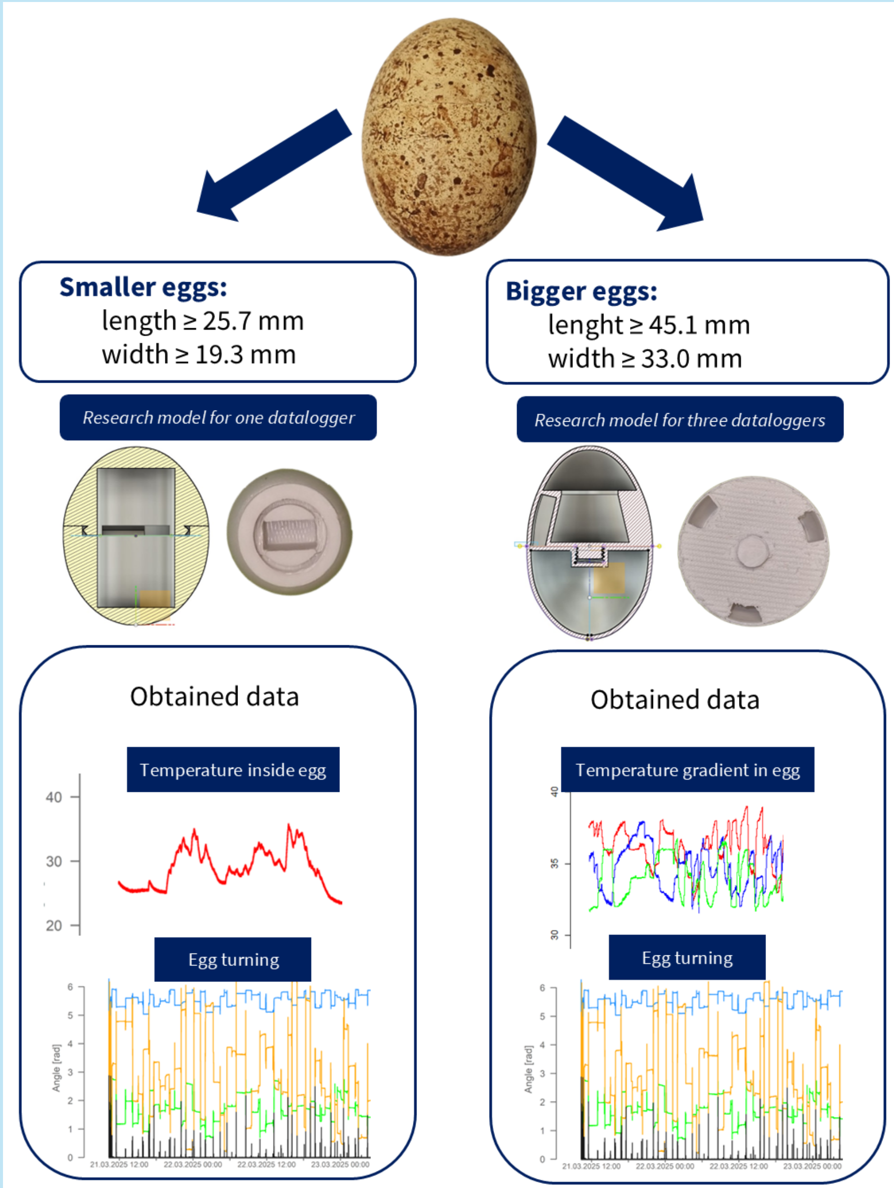


Figure 15: Decision-making regarding the use of a specific type of egg model and the types of data that can be obtained from both types of egg models.

Another criteria for selecting a datalogger is the risk of damage of the monitoring equipment by the birds themselves (Figure 14), as some species tend to destroy dataloggers (typically parrots, owls, or penguins). This usually occurs during nest adjustment, when birds actively manipulate objects in the nest; if they find cables from temperature sensors, they often destroy them. For these species, it is recommended to use only the egg logger and to place other sensors or cables outside the birds' immediate reach. Therefore, avoid using temperature and humidity sensors inside the nest. A camera can be used, but during installation, it is important to carefully hide the cables and not install it too close to the birds. Immediate proximity could lead to attempts to remove or damage the device. We have had good experience with species that nest in nest boxes by attaching the camera to the ceiling of the box and leading the power cable out of the box (as verified, for example, with the African penguin) or by installing cameras in stone mounds or artificial rocks (Figure 11).

5. Operating the monitoring system

This chapter describes best practices for installing dataloggers, maintaining installation records, and provides practical recommendations for the placement of individual device types. A simplified checklist of steps for installing dataloggers (AXY5, MSR, and cameras) is provided in Appendix 9.1.

5.1. Maintaining the installation records

Keeping installation records is one of the most frequently underestimated steps when using dataloggers. Accurate documentation makes it possible, years later, to reliably assign the collected data to a specific nest and species. Detailed records allow the correct interpretation of anomalies caused by disturbances during the breeder's visits to the nest. These factors are absolutely essential when reusing data, for example, in comparative studies. By adhering to the principles and guidelines described below, you can prevent yourself from missing out on the opportunity to participate in larger-scale research projects with your data in the future or from having to repeat data collection. Without a traceable context, hard-earned and unique data can easily become practically worthless.

For each installation, we recommend recording at least the following information (see also the form template in Appendix 9.1.):

- Unique installation identification *(ID installation*)
- Bird species
- Identification of an individual or a breeding pair (*ID parents/nest*)
- Number of eggs in a clutch
- Incubation stage/date the first egg was laid/start of incubation

- Type of datalogger used
- Datalogger identification number *(ID AXY, ID MSR, ID SD)*
- (Date and exact time the dataloggers were activated)
- Date and exact time the enclosure was visited for installation
- Aviary location or number
- Any additional notes regarding monitoring: an egg logger replaced a real egg that was taken to the incubator/an egg logger was added to the existing clutch, etc.

During data collection and when removing the dataloggers, we recommend recording the following:

- Date and exact time range of presence in the aviary during data collection
- Date and time when monitoring ended
- Name and location of the directory with the downloaded data

If possible, we recommend writing down additional information:

- Fate of the nest (reason for failure)
- Number of hatched eggs (or their fertility)

Notes regarding specific devices

See Chapter 9.1 for an example of installation form structure. The form must include the numbers (IDs) of all deployed devices. For AXY5 dataloggers, you must first write a number of the datalogger directly in the software and also write this number on the side of the datalogger where the battery is located, so that the device can be securely identified. MSR dataloggers, have their own six-digit serial number located in the upper right corner of the device (e.g., SN 326723), which is unique and can be used for identification. This number is also included in the file after the data is saved. For cameras, we recommend numbering the microSD cards used, as they contain the actual recordings and can be used across different cameras.

If additional visits to the nest are necessary during the monitoring period (for inspection, equipment replacement etc.) it is essential to always record the exact start and end times of each visit. During the visit, the birds are disturbed and behave unnaturally; therefore, in subsequent analysis, these segments must either be removed from the data or, at a minimum, taken into account in the interpretation. It is equally important to record the time of the final removal of the dataloggers. For example, if it is not possible to turn off the datalogger on-site or if we forget to do so, we must retroactively exclude the data recorded after the datalogger is removed from the nest.

5.2. Reducing disturbance during installation

A key principle for reducing stress in animals is to prepare all equipment as much as possible in advance. All dataloggers should be launched, configured, and tested for proper functioning before entering the enclosure. Serial numbers should be written down, and the installation methods for sensors and cameras should be planned in advance. We recommend bringing materials that enable multiple mounting options for the camera and nest sensors (wire, tape), since the specific conditions at the nest cannot always be fully predicted in advance, and being unprepared prolong the time the bird is disturbed. In terms of installation order, it is best to install the camera first. It's a good idea to connect and start the camera before entering the enclosure, as this allows you to synchronize the time in a calm environment (point the camera at the time display and write time in form), thereby shortening the actual time spent near the nest. In this case, be careful not to disconnect any cables while moving the camera, as this will interrupt and ruin the recording. In any case, the entire installation should be as brief and as non-disruptive as possible.

5.3. Installation of an artificial egg

The installation method varies depending on whether the species has a variable or fixed number of eggs per clutch. For species with a variable number of eggs, the artificial egg with the datalogger can simply be added to the clutch without removing any of the species' own eggs. For species with a fixed number of eggs, such as the four-egg clutches typical of many waders, it is recommended to remove one of the species' own eggs, store it in an incubator (at least) for the duration of the monitoring period, and replace it with a dummy egg. If dummy eggs are routinely used to replace the species' own eggs, we recommend equipping at least one of these eggs with a datalogger. The best time to replace an egg with a artificial egg is during an incubation break or during a natural shift of partners at the nest, when neither parent is present. If such a moment cannot be predicted, it is necessary to disturb the incubating bird during the replacement.

5.4. Installation of temperature and humidity sensors

The method of installing the sensor in the nest (MSR) depends on the type of substrate in which the nest is located. For soft and adequately deep substrates, the pin provided with the sensor itself is sufficient for fixing. For a more secure attachment, V-shaped wires can be used to attach the cable directly to the nest lining. If installing the datalogger in a nest box with minimal lining, tape can be used to attach it. It is recommended to embed the sensor cable as deeply as possible into the nest lining so that the bird is not tempted to actively pull on it. A visible, freely hanging cable increases the risk that the device will be pulled out and damaged. An additional precaution is to paint the cable a colour that matches the nest lining, thereby making it less conspicuous.

5.5. Camera installation

When installing the camera, it is necessary to pay attention to the field angle so that the view covers the entire nest area and its surroundings, while also ensuring a stable and secure power supply for the entire planned recording period. Particular attention must be paid to precise time synchronisation. Accuracy of time synchronisation depends on the length and distance from which the time is displayed to the camera (Section 4.3.2), as well as on the angle of inclination of the display device relative to the camera lens. As with nest sensors, no part of the power cable should remain visible. Secure the cable with wire or adhesive tape and paint it a neutral colour matching the surrounding substrate, thereby minimising the risk that a bird will notice it and begin to manipulate with it.

5.6. Device recording duration and control

During monitoring, it is recommended to check that the devices are actually recording data. For cameras, it is important to verify the orientation angle and the recording status by checking the indicator light (Chapter 4.3.). Unfortunately, for other dataloggers, verification is only possible by connecting them to a computer. The operating time of each device and its recommended settings are listed in Table 2.

Table 2: Recording duration for each type of recommended datalogger

Device	Recording duration	Setting
AXY5 (artificial egg)	18 days	acm 10 Hz, mag 2 Hz; temperature 1 Hz
MSR (nest/ambient)	24 days/4 months	1 Hz/0,1 Hz
Camera Wyze	3-4 days	powerbank 30 000 mAh, microSD card 32 GB

5.7. Deinstallation of dataloggers

The timing of the datalogger deinstallation should be based primarily on minimising disturbance to birds. Although the durability of the devices varies, it is recommended to consider removing all devices at once after the entire clutch has hatched, thereby minimising the number of nest disturbances.

At the end of the season, it is important to ensure that the equipment is stored properly. Dataloggers should be protected from extreme cold and heat. We recommend checking a datalogger that has not been used for approximately three months and, if no further use is currently planned, charging it to 50% of its battery capacity. Long-term storage with a low battery can damage the battery or shorten its lifespan.

6. Data management

Correct organisation, archiving, and management of data are an integral part of data collection. Since different types of dataloggers generate large amounts of data in various formats, it is essential to establish a uniform system for storage, file naming, and writing of supplementary information from the very beginning. A standardised procedure facilitates subsequent analysis, data sharing, and long-term archiving.

6.1. Export and data transfer

After monitoring ends, it is necessary to download data from all devices used. Most of the dataloggers used in incubation monitoring allow data export via software provided by the manufacturer in text form (files *.csv, *.txt, etc.). The exact procedure for downloading data varies by device type and is described in the sections of Chapter 4 dedicated to individual dataloggers.

After export, it is always recommended to verify that the data has been successfully saved, that the files have the expected size and that they contain the entire measurement interval. Only then is it recommended to erase (format) the datalogger memory.

6.2. Data organisation and file naming

As time passes and the number of datasets grows, keeping track of downloaded files naturally becomes complicated. It is therefore strongly recommended to adhere to basic data management and storage principles from the very beginning. For long-term data storage, we recommend creating an organised directory structure, for instance, by year, species, and individual installations. An installation record in form should be created for each installation (i.e., each specific monitoring run at a particular nest); this record is crucial for linking data from all the devices used (see Chapter 9.1). In this form, the installation should have assigned a unique identifier (installation ID) that unambiguously identifies the monitoring activity and ideally includes information about the year, species, and installation rank (e.g., 2026_Burhinus_grallarius_1, or the abbreviated version 26_burgra_1). It is also advisable to use this same identifier in the filenames of all files associated with the installation, ensuring that individual datasets can be easily linked and located.

6.3. Common mistakes in data management

Overwriting original data

Original files exported from dataloggers should never be modified. We recommend backing them up immediately after downloading. Any editing, filtering, or analysis should be done only on working copies of the data. Keeping the original files allows you to verify the correctness of the modifications made or to revert to the original data at any time.

Time zones

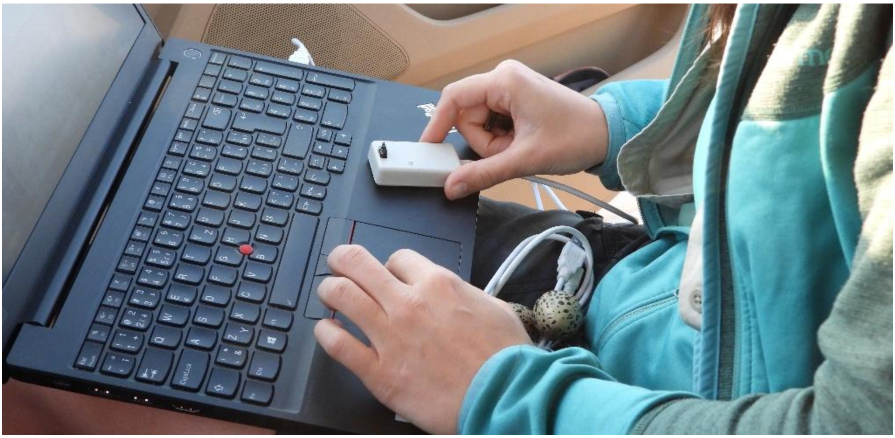
One of the most common sources of error is the incorrect interpretation of time data. Some dataloggers store time in Coordinated Universal Time (UTC; e.g., AXY5), while others take the time zone setting from the computer on which they were configured (e.g., MSR). Combining multiple devices can easily lead to a time shift between individual data files. Therefore, it is advisable to record the exact time at which a particular datalogger begins recording data for each installation. Writing the correct time is also important when using a single datalogger. Many aspects of incubation behaviour show circadian rhythmicity; therefore, accurate determination of the beginning and end of the night is essential for the correct interpretation of results.

Work with video files

Video recordings represent the largest part of the data in terms of volume. A recording of three days of incubation can be 20 GB in size. For the cameras recommended in this methodology, a large number of separate video files are created during recording (for the Wyze camera, one file every 60 seconds). When copying them, a file may exceptionally be omitted, which subsequently disrupts the time continuity of the video and may prevent proper analysis. For this reason, after completing monitoring, we recommend that all video files be first packaged into a single archive (e.g. ZIP) and only then copied or transferred between storage locations. After transfer, it is advisable to verify the completeness of the archive file and the number of individual videos.

Regular backup

All data should be stored on at least two independent storage locations. A combination of a local disk and network or cloud storage is ideal. Backups should be performed continuously during monitoring, not at the end of the project.



7. Data processing and visualisation

Data collection itself is only the first step in monitoring incubation. The data acquire their real value only through their correct processing, visualisation, and interpretation, which is usually the most demanding part. Given that time series analysis often requires at least basic knowledge of working with data and statistical tools, we recommend that in the case of larger projects, you establish cooperation with an expert with experience in the field of data analysis. At the same time, however, there are tools that allow users without advanced programming or statistical knowledge to perform basic processing and visualisation. In this chapter, we present procedures and tools for the initial processing of data obtained using the methods described in this manual.

7.1. Application accelR8

For basic visualisation and pre-processing of data from the accelerometer, magnetometer, and temperature sensor (i.e., data from AXY5 or DAL2/DAL3 dataloggers inserted into the artificial egg), the online application accelR8 (Chajma & Sládeček 2023) is available at https://bergmaps.fzp.czu.cz/accelr8/. The application can load, calibrate, summarise, and visualise recorded data from an accelerometer, magnetometer (Figure 16), temperature, humidity and light sensor and recognise general hidden states (e.g. different types of movement or activity) in them using a hidden Markov model (Hidden Markov Models, Leos-Barajas et al. 2017). This application will provide you with general insights into the data obtained on temperature and egg turning. It is also possible to use a separate script to calculate some specific metrics, which can be found, for example, in the publication Pešková et al. 2024.

7.1.1. Data preparation and loading

The application can automatically import data in two supported formats: the native AXY5 datalogger export and the original DAL2/3 export. The maximum size of the analysed file is limited to 2 GB. For AXY5 dataloggers, the application uses a tab-separated *.csv export. The AXY5 format is recognised automatically when the header starts with the columns *Tag ID, Timestamp, X, Y, Z.* These five columns are mandatory. *Tag ID* contains the datalogger or record identifier, *Timestamp* contains the date and time, including milliseconds, in the format *YYYY.MM.DD hh:mm:ss.sss*, and *X, Y, Z* contain acceleration values for the three accelerometer axes in *g*. Additional columns are imported automatically if present: *Temp. (°C)* for temperature, *MagX, MagY, MagZ* (the three magnetometer axes), and *Batt. V. (V)* for battery voltage, and *Metadata* for operational datalogger information. Empty values from sensors sampled at a lower frequency are expected and do not need to be filled in before import.

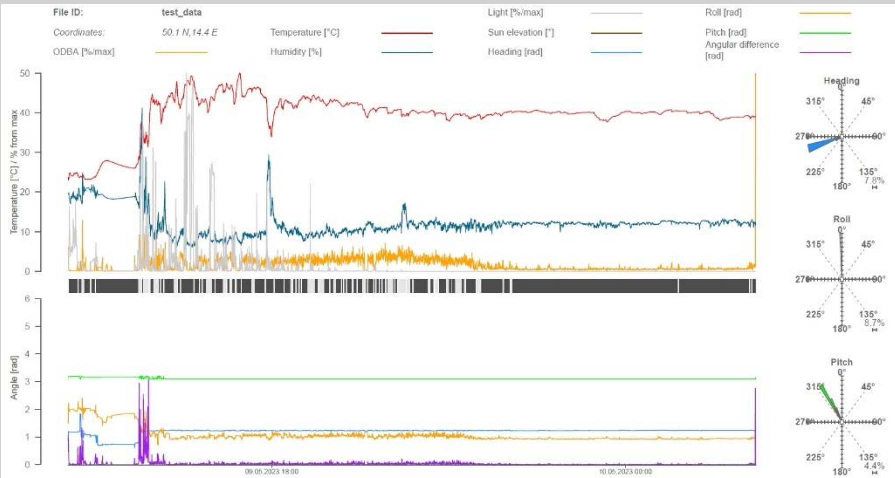


Figure 16: The accelR8 application interface with visualized data. For demonstration purposes, data from the DAL3 datalogger were used; unlike the AXY5, this datalogger also contains information on light intensity and humidity. The upper graph shows temperature in red. The lower and right graphs show movement along three axes—pitch, roll, and heading (or yaw)—see Section 4.1.

7.1.2. Processing procedure

After uploading the data file, enter the geographical coordinates of the collection location in the WGS84 standard. The application assumes that the time in the datalogger is recorded in the local time zone and, based on the supplied coordinates, automatically converts the measurement time to UTC and calculates the height of the sun above the horizon, which can help in interpreting the data in relation to the time of day. For data from the accelerometer and magnetometer, automatic calibration can then be performed, which minimizes the systematic error of the sensors.

The next step is to prepare and the data. Here you select the variables to be summarized and specify the size of the summary window in seconds (for example, average in 1 second). This step is mainly used to convert the data to a uniform sampling rate and to speed up further graphing. Basic descriptive statistics are available (mean, median, minimum, maximum, standard deviation) and, for the magnetometer, angular functions (angular distance and angular difference). To reduce noise, the *dif1* (first-order difference) or *log1* (natural logarithm) transformation can be applied to the selected variables, or both at the same time.

Then you can proceed to predict hidden states. Here, select the number of hidden states that the model should distinguish, select predictors and transformations, and run the model. Before running, it is recommended to under sample (*rarefy*) the data for a faster preview, and to remove (trim) the initial and final sections of the record that were affected by manipulation during installation or removal of the datalogger.

The result is a two-line actogram (Figure 16), which displays the measured values (movements in three axis, temperature etc.) in the upper part and the predicted states (which are not relevant in this context) in the lower part, supplemented by pie charts showing the distribution of angles in the three axes of the magnetometer. The chart can be saved as a *.png file, the prediction output and the model summary can be downloaded for further processing or archiving.

7.2. Application incub8

For automatic processing of data from temperature and humidity sensors in the nest (e.g. MSR), the online application incub8 (Chajma & Sládeček 2020, developed with the support of TAČR, grant no. TJ02000199) is available. The application is available at: www.bergfzp.shinyapps.io/incub8/. To detect incubation and its breaks, the application uses Hidden Markov Models (Leos-Barajas et al. 2017). The model can be trained in two ways. For a simpler variant, the *em* method is used, in which the model learns to distinguish individual states on its own, without the need to have any confirming data on the actual behaviour of the bird. The second option is the *loo* method, which can be used when you have verified data on the bird's actual behaviour, typically obtained from a camera recording, for at least part of the recording. This reference data then allows a more accurate estimate of the model parameters and, at the same time, verifies its reliability by comparing the prediction with reality, and subsequently allows for predicting behaviour on a larger dataset.

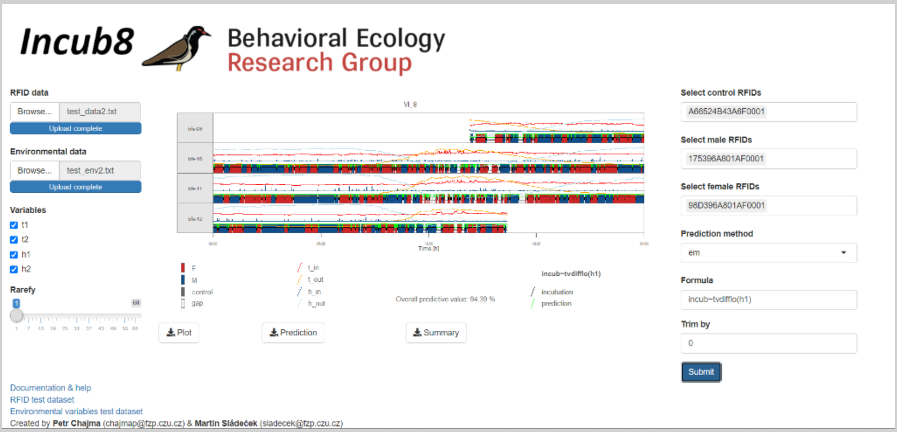


Figure 17: The Incub8 application interface displaying data from a temperature-humidity datalogger and RFID. For a description of the graph, see Chapter 4.5, Figure 13.

7.2.1. Data format

The application works with a single data file in *.txt format with comma-separated values and a header containing precisely named variables (uppercase and lowercase letters are distinguished). The file must contain a column with the date and time of the measurement, datetime, which must be written in the standardised format shown in the sample file (YYYY-MM-DD hh:mm:ss). At the same time, at least one of the following variables must be present: temperature in the nest *t1*, temperature outside the nest *t2*, humidity in the nest *h1* and humidity outside the nest *h2*. The maximum file size is 50 MB. We recommend following the sample file below exactly to avoid an error during import.

```
"datetime","t1","t2","h1","h2"
"2019-03-09 14:15:21",29.0578,37.8964,38.504,22.7692
"2019-03-09 14:15:22",29.0845,37.9098,38.2294,22.7524
"2019-03-09 14:15:23",29.0845,37.9231,38.0432,22.7844
```

7.2.2. Data upload and analysis

After uploading the data to the application, you choose which variables should be displayed and then used in the prediction model. For faster preview rendering and testing of several model variants, it is advisable to use the *rarefy* function, which by default uses only every 30th record. However, for the final analysis, it is necessary to return this setting to 1 so that the model works with the entire data set and achieves full accuracy.

Since the original acquired data is usually loaded with noise, the application offers several recommended transformations that improve its informational value. For temperature data, we recommend the *negdiff* transformation, which performs particularly well at detecting long incubation intervals. For humidity data, the *difflo* and *tvdifflo* transformations are more suitable, as they capture even short intervals more sensitively, with *tvdifflo* being better suited to noisier data. You will usually achieve the best results by combining the transformed temperature (*negdiff*) and the transformed humidity (*difflo* or *tvdifflo*) in a single model.

After setting the variables and transformations, select the prediction method. The options are: *none* for simply displaying data without prediction, *loo* or *em*. The application then plots the incubation actogram, i.e. a graphic record of the temperature and humidity, along with marked incubation and incubation break sections (Figure 17). For a detailed description of the actogram, see Figure 13. If you have reference data available, the application also automatically calculates the sensitivity and accuracy of the model and displays a comparison table of actual and predicted states.

If necessary, the initial and final sections of the record can be excluded from the analysis using the *trim by* function, which is especially suitable if parts which were affected by the presence of a person during the installation or uninstallation of dataloggers. Than you can download the plotted graph in *.png format and the state prediction itself in the form of a *.txt file (0 for a break, 1 for incubation). The application also allows processing data from RFID reading devices described in Chapter 4.5.

7.3. Video analysis in the BORIS program

For systematic processing of camera recordings of nest behaviour, we recommend the freely available software BORIS (Behavioural Observation Research Interactive Software; Friard and Gamba 2016), available at www.boris.unito.it. The program allows you to create your own ethogram of the observed behaviour and then enter each behaviour directly during video playback using a simple interface with keyboard shortcuts, significantly speeding up a process that would otherwise require more complex manual recording.

7.3.1. Ethogram and event types

A key step before starting any analysis is to create an ethogram (an example can be found in chapter 9.2), i.e. a list of types of behaviour that can appear in the video recording. Here, you assign them the keyboard shortcuts that you will use when analysing the videos. BORIS distinguishes two basic types of behaviour:

State events are longer-lasting behaviours that are recorded by pressing the same keyboard shortcut to start and end them. Typical categories of state behaviours in incubation studies include the presence of a parent on the nest, incubation itself (direct body contact with the eggs), shading the nest, or sleep (recognisable, for example, by closed eyes or a head tucked into feathers).

Point events, on the other hand, are short-term or repetitive behaviours without a clearly defined end, recorded as a single moment. Typical examples are arranging nest material, turning an egg, or preening feathers. Some behaviours may be classified as point events even though they actually last for a certain period of time, if it is difficult to clearly determine the moment of their end.

For point actions that can be repeated in a short time (for example, frequent feather preening), it is advisable to set a fixed time interval within which the action is recorded as a single occurrence. In practice, an interval of 30 seconds has proven to be effective. This approach prevents the results from being distorted by an excessive number of very short, almost immediately consecutive records.

Once an ethogram is created, it can be used to analyze all videos and does not need to be recreated. Therefore, it should include all possible behaviours across species. An example of a universal ethogram can be found in Chapter 9.2.

7.3.2. Preparing the recording

Before determining the behaviour, it is necessary to assign identification data to each video recording, such as the installation ID, nest ID, andto enter the exact date and time of the start of recording. This step is crucial for later synchronisation of the observed behaviour with data from other dataloggers (AXY5, MSR). Use the *Add all media from directory* option to upload all recordings from the given folder (Figure 18). For the recommended camera type, this is 60 files corresponding to 1 hour of recording.

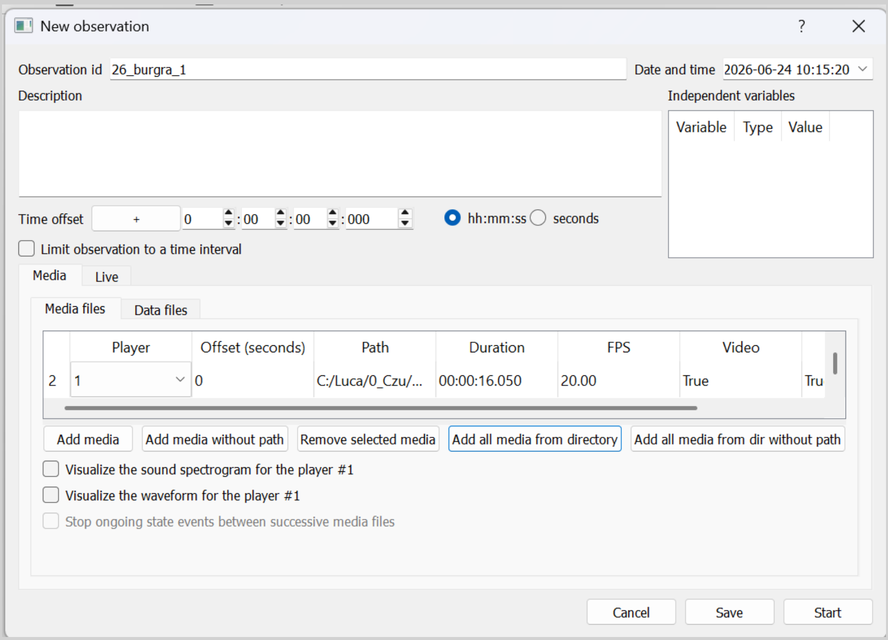


Figure 18: BORIS Program Interface: When creating a new project, it is important to set the start time correctly (upper-right corner). When uploading videos from Wyze cameras, it is important to select "Add all media from directory", this will allow you to upload all the videos in one folder together.

7.3.3. Determining behaviour and data export

After uploading the videos, the individual recordings will play in sequence in the BORIS program, which allows you to speed up, slow down, stop, or rewind the recordings. During playback, record individual categories of behaviour using the assigned keyboard shortcuts. The result can be exported as a *.csv table of individual events with their exact times (including durations for state behaviour), which can be further processed and linked to data from other dataloggers. BORIS also allows you to view basic summary statistics directly in the program interface (Figure 19). You can visualise the frequency and total duration of individual behaviour categories and view them on the timeline.

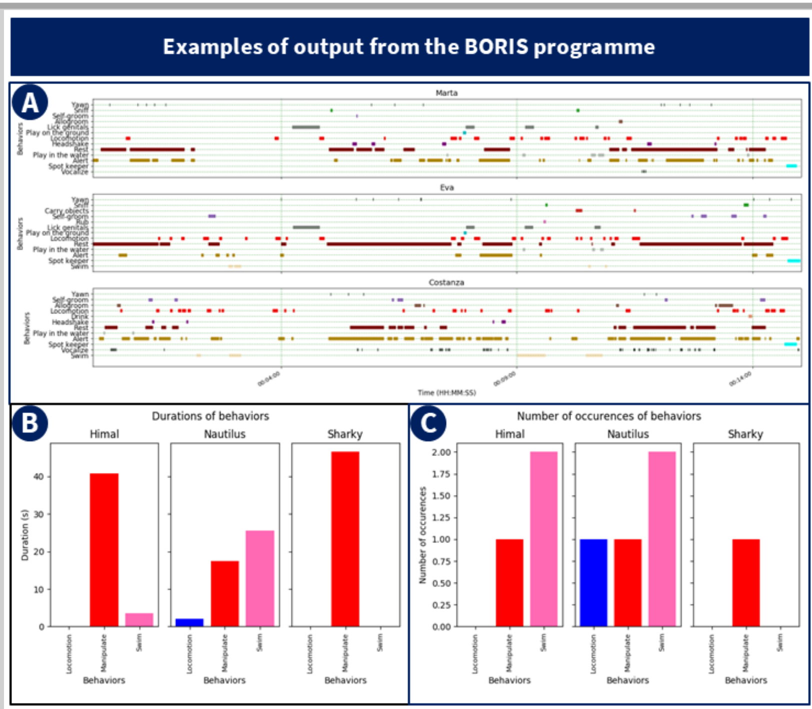


Figure 19: The BORIS program allows the creation of an actogram based on specified behaviour (A); it also allows the creation of summary graphs showing the duration of selected state behaviour (B) and frequency graphs of selected point behaviour (C). Source: www.boris.unito.it/user_guide/plot/.

8. Interpretation of results

This chapter focuses on the practical interpretation of data obtained using the devices described in the previous sections of this guide. Based on real data sets, examples of their processing and evaluation are presented here, both as summary analyses across different bird orders and as specific examples that illustrate the characteristics of selected species. Although the conversion of measured values into specific recommendations for breeding practice is not unambiguous due to species variability, different breeding conditions and a limited amount of reference data, the chapter tries to offer an interpretative framework that can serve as support for decisions on the optimisation of incubation conditions. The aim is not to establish universal recommendations, but to demonstrate the possibilities for using the data obtained to evaluate the incubation process and to identify the causes of potential incubation failures.

8.1. Temperature and humidity during incubation

This chapter focuses on interpreting temperature and humidity data obtained from the dataloggers described above. Examples of various bird species show the possibilities for evaluating the temperature recorded by an egg with a datalogger, as well as by dataloggers in the nest and out of the nest. The possibilities for monitoring humidity in the nest and the basic principles for interpreting the data obtained are briefly presented. The chapter also includes an overview of measured incubation temperatures for selected taxonomic groups and a comparison of these temperatures with values commonly used in artificial incubation. The specific examples given illustrate the fact that the natural course of incubation is significantly more variable than might appear from the settings commonly used in incubators (see also chapter 2.1.).

8.1.1. Microclimate in the nest

For a general idea, it is important to visualise the temperature in the nest and compare it with the temperature outside the nest (Figure 20). This data can be obtained, for example, from the MSR datalogger described above. The internal temperature usually varies within a few degrees, but there are often larger fluctuations towards the values of the external temperature at a given moment. These fluctuations show the incubation breaks, which usually last several minutes. In general, it is visible that the incubation temperature is maintained by the parents above the colder and below the warmer external temperatures. Maintaining the incubation temperature within a certain range is essential for proper embryonic development. By using temperature probes placed inside and outside the nest, we can also determine when the nest hatched or was predated (although this may be a relatively rare phenomenon in captive breeding).

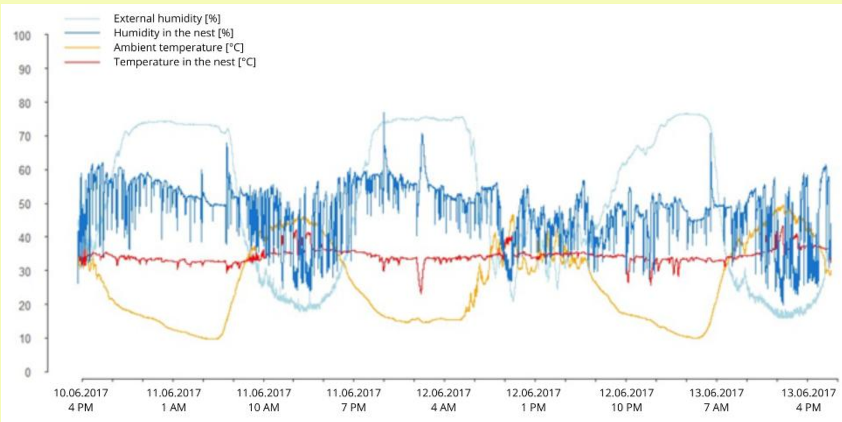


Figure 20: Temperature and humidity in the nest - the temperature measured in the nest is red, the temperature outside the nest is orange. The humidity in the nest is dark blue and the humidity outside the nest is light blue.

It is also evident from the graph (Figure 20) that the humidity in the nest is not stable, but fluctuates within a certain range compared to the outside humidity. However, the humidity in the nest never reaches the extreme (high or low) values measured at the same time outside the nest.

Incubation temperature can also vary depending on the stage of incubation. Precocial species in the wild need newly hatched chicks to leave the nest at the same time, so they must hatch synchronously. This can also be important in breeding, as management with chicks of the same age is easier. However, when laying one egg per day, a full clutch is completed after several days, and eggs laid earlier may begin to develop in the meantime if outside temperatures are high. Or the earlier laid eggs

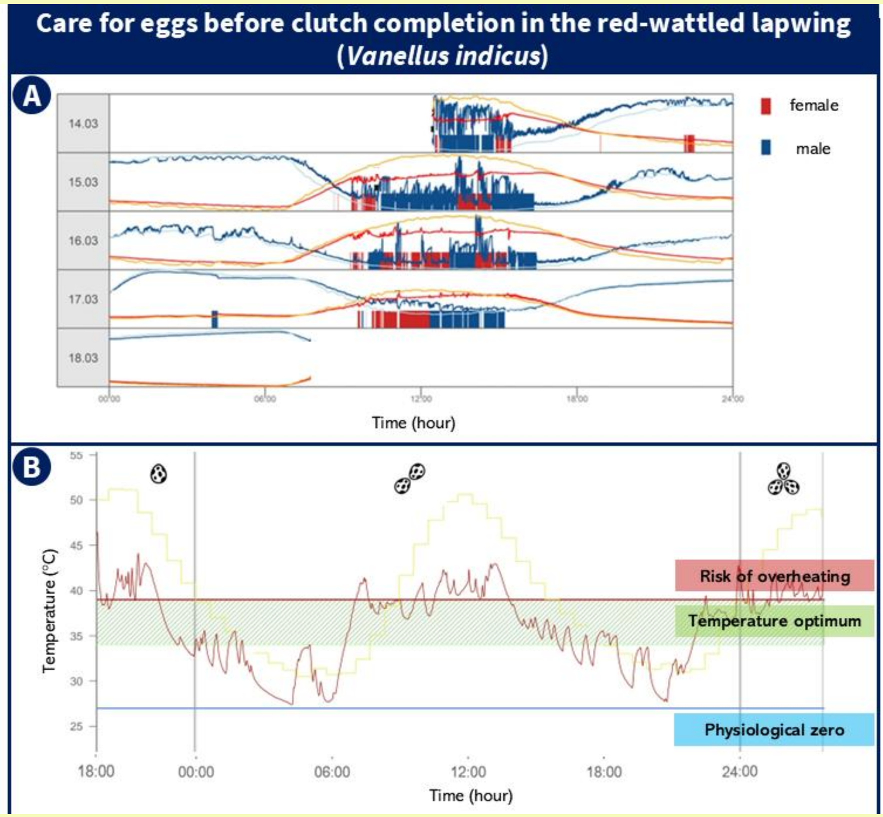


Figure 21: Caring for a clutch before its completion in red-wattled lapwing (*Vanellus indicus*). A) Data from the MSR temperature and humidity datalogger in and outside the nest, linked to the RFID recording of the presence of parents on the nest. B) Data from the egg with the AXY5 datalogger togehter with the outdoor temperature. Red curve – internal temperature in the nest/egg logger, yellow curve – outdoor temperature, dark blue curve (only A) – humidity in the nest, light blue curve (only A) – outdoor humidity.

can be exposed to extremely low temperatures that can damage the embryo. One of the mechanisms of synchronisation is incubation care during the laying process, when the parent tries to maintain the temperature around physiological zero so that the embryo stops developing, or at least slows its development, and the whole clutch hatches at once. In this situation, the parent does not incubate during the whole day, but only at extreme (high) outdoor temperatures (Figure 21).

Some bird species may be vulnerable to temperature fluctuations during incubation. On the contrary, other species have a relatively wide tolerance to fluctuations in incubation temperature. The collected data allowed us to look at the average temperature measured by dataloggers in the eggs of different species and their range. The values of the measured temperatures for individual orders compared to the temperature ranges used for their artificial incubation can be found in Figure 22.

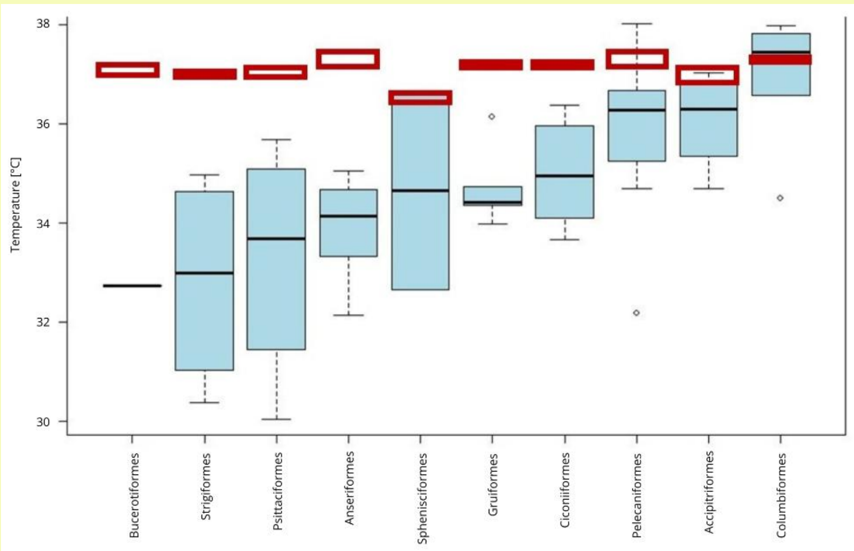


Figure 22: The blue boxplots show the range of temperatures measured by the datalogger in the egg during natural incubation. The box represents the interquartile range (25th–75th percentile), the horizontal line inside the box indicates the median. The whiskers show the range of values up to 1.5 times the interquartile range, and individual points represent outliers. The temperature commonly used in an incubator for the given order is red.

The temperature during natural incubation differs significantly from that under incubator conditions. For some groups, the measured values may not be accurate, as they may have been influenced by a small sample, an atypical pair, or other factors. However, it is probable that bird eggs have a greater tolerance for a wider range of incubation temperatures than was assumed and used in practice in incubators, where a constant temperature with minimal fluctuations is set. The temperature used in artificial incubation is generally higher (Figure 22). The averages and ranges of measured temperatures for individual orders are provided in the Appendix (Chapter 9.3).

8.1.2. Incubation rhythm

The incubation rhythm of a given individual or pair can be determined accurately from the temperature recorded by the datalogger in the egg. The actogram of incubation in Dalmatian pelicans (Pelecanus crispus) from Safari Park Dvůr Králové shows a continuous incubation period, carried out exclusively by the female (Figure 23). Occasionally, a very slight drop in temperature is noticeable, possibly due to a short interruption in incubation. However, the temperature is very constant and the female almost never interrupts contact with the eggs. On the last day shown in the graph, a drop in temperature is visible. This can happen for several reasons: the uncut data (the datalogger was therefore removed from the nest, which is why the temperature dropped) or the datalogger was rejected by the parent (the artificial egg is no longer heated together with the clutch).

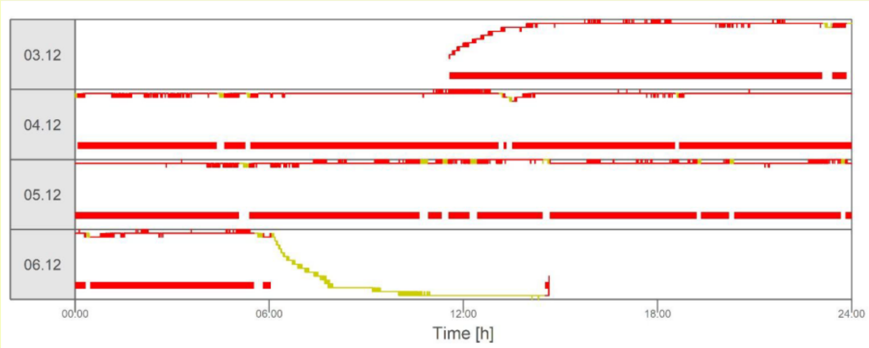


Figure 23: Dalmatian Pelican (*Pelecanus crispus*) incubation. The thin yellow-red line shows the temperature measured by the datalogger in the egg. The lower thick red line shows the model-estimated incubation session and its interruption, the incubation break. The date is shown on the left side of the graph.

A different strategy is evident in the red-crowned crane (*Grus japonensis*) (Zoo Olomouc, Figure 24). The temperature fluctuations are more pronounced than in the previous case, and there are frequent alternations of incubation sessions and breaks. Here, both parents incubate the nest. This frequent exchange at the nest results in temperature fluctuations, as the alternation cannot be carried out without a drop in temperature.

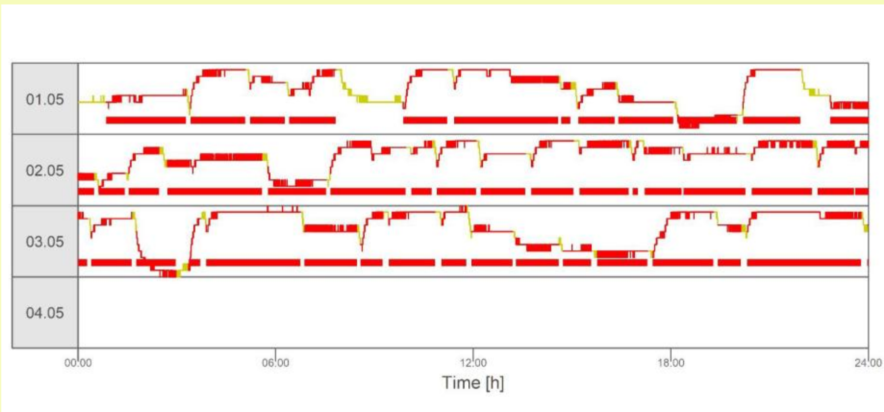


Figure 24: Incubation of a red-crowned crane (*Grus japonensis*). The thin yellow-red line is the temperature measured by the datalogger in the egg. The lower thick red line is the model-estimated incubation session. The date is on the left side of the graph.

8.2. The frequency and angle of egg turning

Egg turning can be monitored using an egg with a datalogger that contains a three-axis accelerometer and a magnetometer (see chapter 4.1.). This system allows recording of the frequency of egg movements and the size of the turning angle, including small changes, over time. The obtained data showed variation in the intensity of egg turning among species. The differences are manifested both in the turning frequency, i.e. the number of movements per hour, and in the total angular change per hour. Some species turn eggs very often and by a large angle during incubation (Figure 25 – C,D; rainbow lorikeet (*Trichoglossus moluccanus*) frequency 135.84 rotations/hour, angular change 427°/hour). This can be caused by intentional manipulation, as well as by small movements from normal contact between the parent and the clutch (chapter 8.3.5). In other species both frequencies and angles are low (Figure 25 – A, B; Dalmatian Pelican (*Pelecanus crispus*) frequency 9.91 turns/hour, angular change 36°/hour).

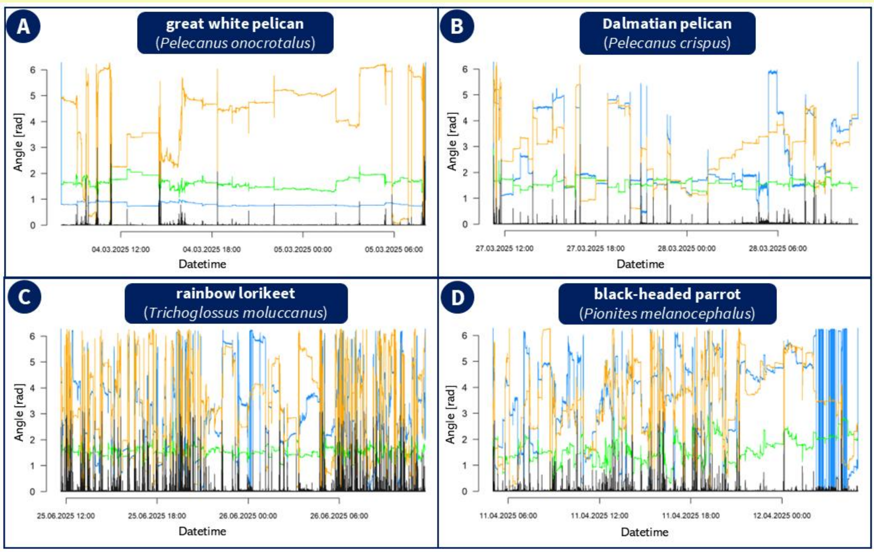


Figure 25: Examples of egg turning in all three axes. Blue – roll, green – pitch, blue – heading (yaw), black – the amount of total angle change.

This variability indicates that we cannot establish a single universal turning setting valid for all species. When comparing data for all orders, the highest turning frequency is evident in the orders Psittaciformes, Bucerotiformes and Columbiformes (Figure 26). For most of the other orders monitored, the turning frequency was low. When interpreting the values, it is necessary to take into account the ecology of the given species and the type of nest. A low turning frequency alone should not automatically be considered a problem if it corresponds to the natural behaviour of the given taxon.

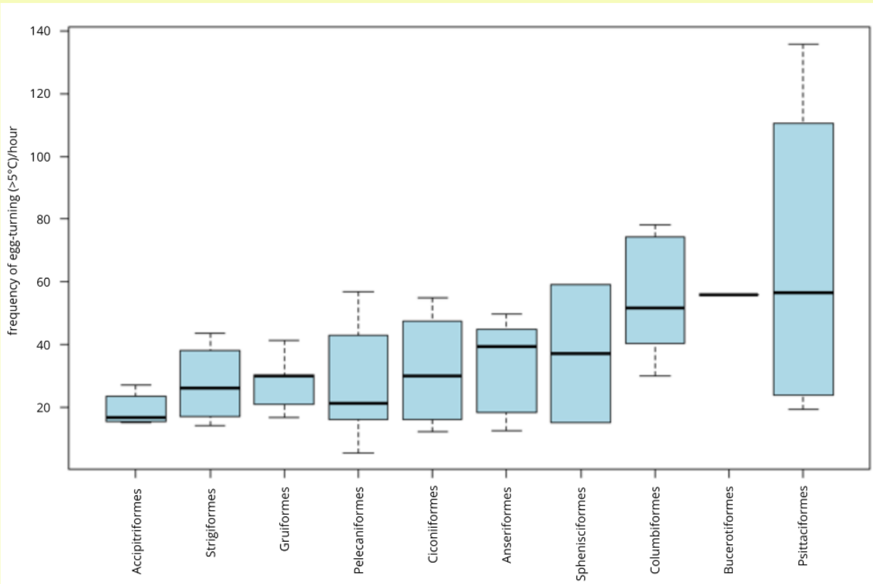


Figure 26: Egg turning frequency across studied bird orders. The blue box represents the interquartile range (25th–75th percentile), the horizontal line inside the box indicates the median. The whiskers represent the range of values up to 1.5 times the interquartile range.

A recently published study demonstrated differences in egg turning between precocial and altricial species (Pešková et al. 2025). It is evident that the frequency of turning varies between groups with the different developmental maturity of the hatchlings. The highest frequencies were found in altricial species, while the lowest were found in precocial ones

(Figure 27). This result suggests that the frequency of turning may be related to different embryonic requirements and to different yolk-to-albumen ratios in the egg. This is because eggs of altricial species contain significantly more albumen than yolk compared to eggs of precocial species, and egg turning is necessary for the absorption of nutrients from the albumen (Chapter 2.1.3.).

Practical interpretation of data should always be species-specific. In species with naturally high turning rates, a sudden drop may signal a change in parental behaviour, disturbance, or other problems. In species with naturally low turning rates, low values may not be a problem.

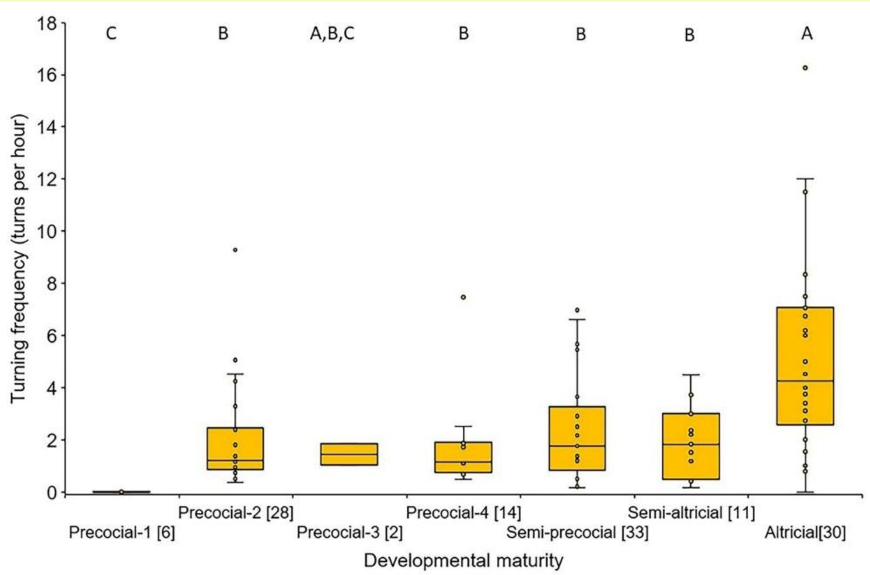


Figure 27: Egg turning frequency in groups with different developmental maturity of hatchlings. The box represents the interquartile range (25th–75th percentile), the horizontal line inside the box indicates the median. The whiskers show the range of values up to 1.5 times the interquartile range, and individual points represent outliers. Grey points show individual measured values. Different letters above the boxplots indicate statistically significant differences between groups ($p < 0.05$) (Pešková et al. 2025).

8.3. Behaviour during incubation

Continuous camera recording allows the determination of specific parent behaviour on the nest. While a datalogger in the egg or a nest sensor will record fluctuations in the temperature and humidity of the egg or its movement, only the camera recording will answer the question of what caused it. The presence of a specific parent on the nest and specific parent behaviours, such as sleeping, preening, or interaction with other individuals, are variables that cannot be obtained from datalogger data but are key to understanding what happens during incubation.

Systematic analysis of camera recordings using a predefined ethogram (see chapters 7.2 and 9.2) is an indispensable part of comprehensive incubation monitoring. The result is not only a description of typical incubation behaviour for a given species or pair, but above all, a tool for revealing the real cause of problems identified by other dataloggers. Analysis of video recordings will help reveal problems such as too long or too frequent incubation breaks, insufficient synchronisation between partners, excessive disturbance by other individuals, or the reason for parents' inability to warm the eggs effectively. The following subsections present examples of observed behaviour and illustrate their interspecific variability using data obtained within the project. For the following studies, video recordings from 21 nests of 20 bird species kept in zoos were analysed.

8.3.1. Sleep during incubation

Analysis in the BORIS program (chapter 7.3.) revealed significant interspecific variability in the time that incubating birds spend sleeping on the nest, which is one of the basic self-maintenance activities. The average hourly sleep time on the nest (Figure 28) among the species studied ranged from zero (cattle egret, *Bubulcus ibis*) to approximately 38 minutes (African penguin, *Spheniscus demersus* and red-crowned crane, *Grus* *japonensis*). Very low values were also recorded for the bush stone-curlew (*Burhinus grallarius*), the Abdim's stork (*Ciconia abdimii*), the marabou stork (*Leptoptilos crumeniferus*) and the great horned owl (*Bubo virginianus*).

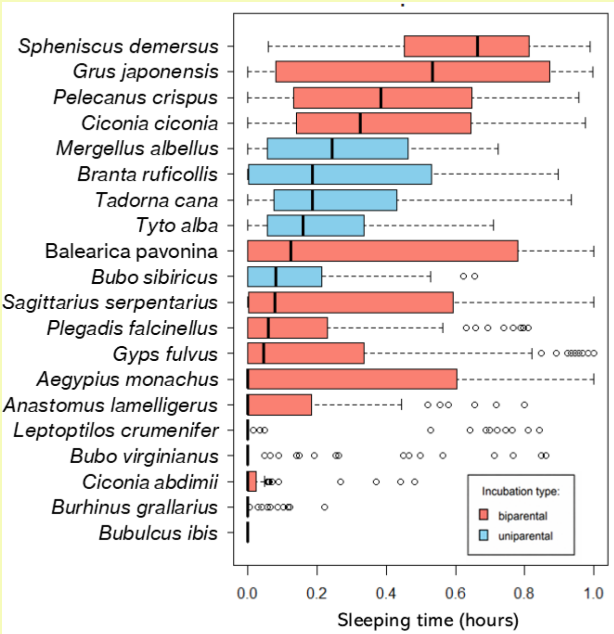


Figure 28: Sleep duration during incubation for individual species. The box represents the interquartile range (25th – 75th percentile), the horizontal line inside the box indicates the median. The whiskers represent the range of values up to 1.5 times the interquartile range, and individual points represent outlier observations (Němcová 2026).

Although at first glance, the explanation for these differences is suggested by the type of incubation, the analysis showed that uniparentally and biparentally incubating species do not statistically differ in average sleep time. However, biparental species show significantly higher variability. The great horned owl, with its unusually short sleep, is an exception among uniparentally incubating species.

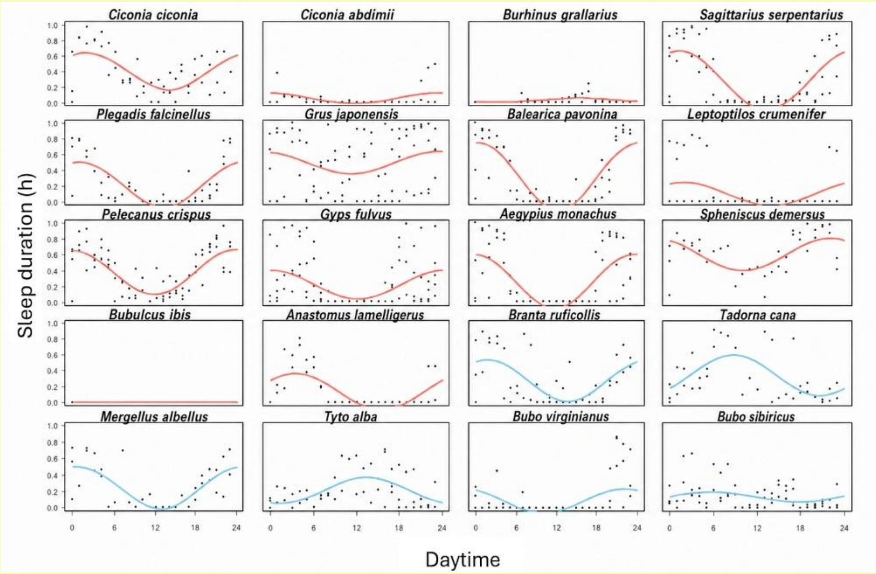


Figure 29: Distribution of sleep time during the day in individual bird species. The graphs were supplemented with model curves estimated by a linear mixed model, which show the course of the circadian rhythm of sleep. Species are marked according to the type of incubation: red curve – biparental, blue curve – uniparental (Němcová 2026).

Sleep in most species showed a circadian rhythm (Figure 29). In three quarters of the species studied, such as the glossy ibis (*Plegadis falcinellus*), the secretarybird (*Sagittarius serpentarius*) or the red-breasted goose (*Branta ruficollis*), sleep was concentrated in the night hours. In contrast, a smaller proportion of species, such as the South African shelduck (*Tadorna cana*) and the barn owl (*Tyto alba*), slept more during the day. Approximately 15% of the species studied, including the bush stone-curlew, Abdim's stork and the cattle egret, showed an overall very low proportion of sleep without a pronounced circadian rhythm.

The length of individual continuous sleep episodes also varied significantly between species. In most species, shorter episodes predominated, with longer episodes occurring less frequently, especially at night. Species such as the marabou stork, griffon vulture (Gyps fulvus), or African openbill (Anastomus lamelligerus) showed a wide range of sleep episode lengths, whereas in the bush stone-curlew, glossy ibis, or red-breasted goose, mainly short sleep episodes were recorded. Their duration increased during the main sleep phase.

An important finding is the effect of disturbance on the amount of sleep. It has been shown that with increasing frequency of visits by other individuals in the vicinity of the nest, whether intraspecific or interspecific, the total sleep time decreased (Němcová 2026). This result suggests that incubating birds respond to potential threats by being alert at the expense of rest. This may be a crucial finding when interpreting data from captive breeding with higher animal densities or with greater movement of breeders. In particular, in uniparentally incubating species, it is appropriate to pay special attention to disturbance during incubation, since these parents have almost no opportunity to rest outside the time spent sitting on the nest.

8.3.2. Preening during incubation

The same sample of video recordings as in the previous chapter was used to monitor time spent preening (autopreening) on the nest during incubation. The data showed that this behaviour is also highly species-specific, and even in this case, uniparentally and biparentally incubating species did not differ statistically (Figure 30). For example, in the bush stone-curlew and the Abdim's stork, preening was almost not observed during incubation. On the contrary, the Dalmatian Pelican engaged this activity very often, with preening taking up approximately 17% of the observed time, which is by far the most compared to the other species studied.

Allopreening (mutual preening of feathers between partners), occurred in only a few species and usually at low levels. In the white stork (*Ciconia ciconia*), it occurred approximately in 5% of the observed time, and in the African penguin and the Abdim's stork it reached approximately 1.67% of the observed time. In the other species, allopreening was rare or absent.

Preening was also subject to a circadian rhythm, but with an increased frequency during the day (compared to sleeping). A strong rhythm was observed in particular in the glossy ibis, the smew (*Mergellus albellus*), the black crowned crane (*Balearica pavonina*) and the secretarybird. In these species, the preening rhythm is in exactly the opposite phase to the sleeping rhythm. On the contrary, in the cinereous vulture (*Aegypius monachus*) and the Siberian eagle owl (*Bubo sibiricus*), the relationship was the opposite. An interesting finding is the different reaction of uniparentally and biparentally incubating species to disturbance in the nest area. While in biparental species the presence of other individuals had no effect on the frequency of preening, in uniparental species a higher number of intruders led to a decrease in this activity. A uniparentally incubating bird that cannot exchange on the nest with a partner, in a time of increased disturbance, prioritises vigilance not only at the expense of sleep, but also at the expense of feather care.

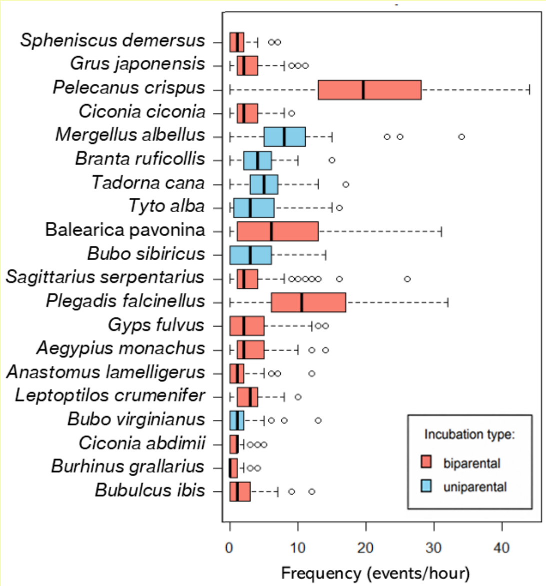


Figure 30: Preening frequency during incubation in individual species. The box represents the interquartile range (25th–75th percentile), the horizontal line inside the box indicates the median. The whiskers represent the range of values up to 1.5 times the interquartile range, and individual points represent outliers

8.3.3. Manipulation with nest material

The analysed video recordings showed that nest building is not an activity occurring only before the start of incubation, but it also continues during the whole incubation (Figure 31). Incubating birds actively maintain, rebuild or upgrade the nest even when the clutch is already incubated. Nest building occurred in both biparentally and uniparentally incubating species. The highest frequency of manipulation with nest material was recorded in the glossy ibis, red-breasted goose, Abdim's stork, secretarybird, red-crowned crane and white stork. In species that build large nests from branches, continuous subsidence or loosening of nest material may occur, so regular nest adjustments help to maintain stability and the appropriate position of the eggs. At the same time, it may involve restoring the nest's insulating properties, which is important for maintaining optimal incubation conditions for the entire clutch.

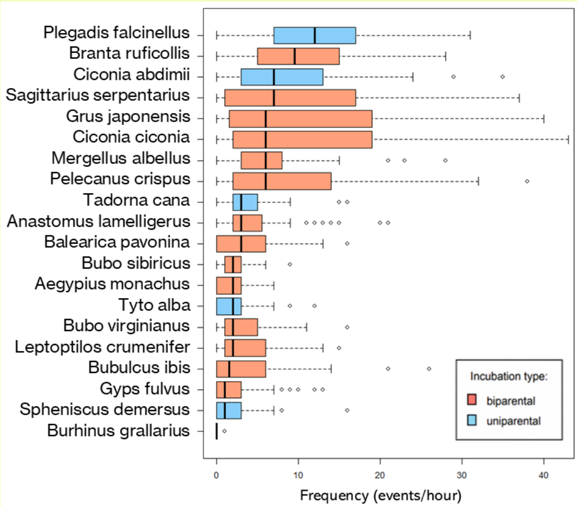


Figure 31: Manipulation with nest material and lining during incubation for each species. Box represents interquartile range (25th–75th percentile), horizontal line inside box indicates median. Whiskers indicate range of values up to 1.5 times interquartile range, and individual points represent outliers.

On the contrary, a very low frequency of nest modifications was recorded in the cattle egret, griffon vulture, African penguin or bush stone-curlew. These are, with the exception of the egret, species nesting (in the given zoo) on the ground, in which there is no risk of collapse or fall of the nest in the absence of its adjustments. In some species, avoiding manipulation with nest material can again be an anti-predation strategy.

To conclude, the nest is a dynamic structure. Its maintenance continues even after eggs are laid and incubation begins, and nest lining significantly contributes to microclimate regulation and clutch stability. Nest modification is part of ongoing parental care.

8.3.4. Interspecific interactions

The video analysis also showed the defensive reactions of incubating birds to intruders. Increased vigilance (see chapter 8.3.1.) increases the energy demands on the parents during incubation. If a direct attack occurs, the incubating bird runs towards the intruder or at least assumes a threatening pose, this means that incubation is interrupted, which usually lasts as long as the intruder is near the nest. Aggressive reactions to intruders during incubation represent a very important behavioural indicator in breeding, as they directly disrupt the continuity of incubation.

The frequency of intraspecific and interspecific interactions involving the incubating individual (including humans, although this phenomenon was not very frequent) was again very variable among species (Figure 32). The Dalmatian pelican showed the highest values, with the number of reactions significantly exceeding those of all other species. This is because it is a colonial species that was also specifically placed in an indoor enclosure with a large number of other individuals during the incubation period. However, if we look at the incubation record (Chapter 8.1.2., Figure 23), we are surprised by its continuity. Although the graph (Figure 32) shows only direct attacks, the attack involved the beak striking an individual in the immediate vicinity and did not led to interruption of incubation. In general, this species is accustomed to disturbance from other individuals and copes with it very well. High frequency was also observed in glossy ibis, secretarybird, marabou stork, smew and cattle egret. On the contrary, almost no defensive reactions were recorded in species such as the barn owl, African shelduck, or red-breasted goose, which may be due to their nesting in boxes that provide them with adequate peace.

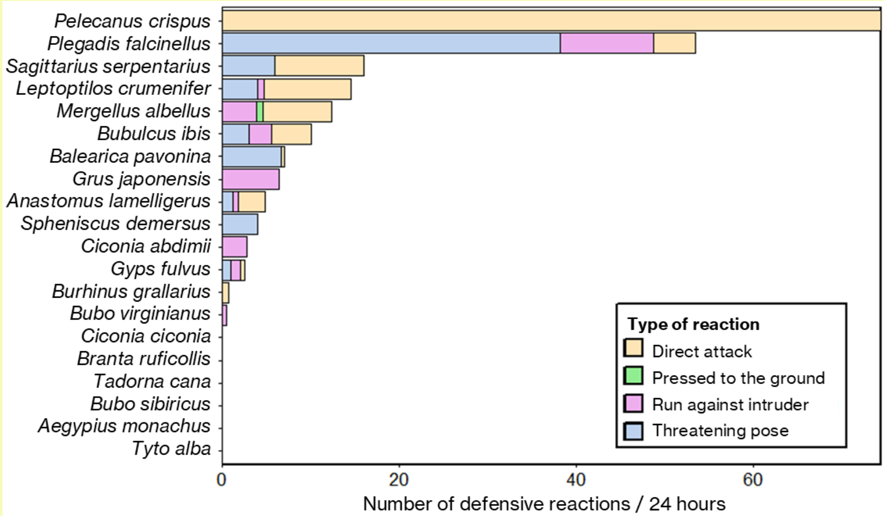


Figure 32: Frequency of defensive responses in incubating birds by species.

In the glossy ibis and secretarybird, is noticeable not only higher frequency, but also a greater variety of reactions. For example, in the glossy ibis, the most common responses were a threatening pose and running away from the intruder, both of which clearly require interrupting incubation.

It can be concluded from the data that, particularly for species kept in group enclosures, the number of interactions may be influenced by how many other individuals are present (and can come near the nest), whether visual barriers are present, and how far apart the nests are. A low number of aggressive reactions does not necessarily indicate a low level of stress, but it may reflect that the species is not aggressive. However, the bird can remain constantly alert during incubation (stop sleeping or preening on the nest), which can lead to exhaustion, especially in uniparental species.

8.3.5. Egg turning: purposeful behaviour or by-product?

The combination of data from the egg logger and camera recordings for 40 nests of 32 bird species allowed us to distinguish whether egg movement (e.g. egg turning) was caused by direct manipulation (intentional movement), other parental behaviour on the nest (unintentional movement), or external environmental influences (wind, movement of a branch with a nest). Movements resulting from normal incubation behaviour accounted for an average of 76.2% of all events, while purposeful egg manipulations by the beak accounted for 18.8% and passive environmental movements for 2.5%. This shows that eggs are not only turned during incubation when the bird actively manipulates them with its beak, but also when sitting on the nest, standing up, stepping over, flew from the nest, or maintaining the nest lining.

A single intentional movement caused an average angular change of 9.2°, while an unintentional movement only 3.7°. Environmentally induced movements had an even smaller effect, approximately 1.2°. Targeted egg manipulations, therefore, had a greater mechanical effect. However, after accounting for both the frequency and magnitude of the movements, the main contribution to the eggs' total angular change still came from unintentional movements, which accounted for 65.1% of the total turning. Intentional movements contributed 33.0%.

In general, there was a large variability among species in the proportion of intentional and unintentional movements in the total egg turning (Figure 33). In species with a larger number of eggs, the proportion of intentional movements was higher, which may be related to the fact that small body movements may not affect all eggs equally in a larger clutch. Therefore, the parent may use its beak more often to turn a particular egg. There was no difference in egg turning whether the parents were incubating in wild or captivity.

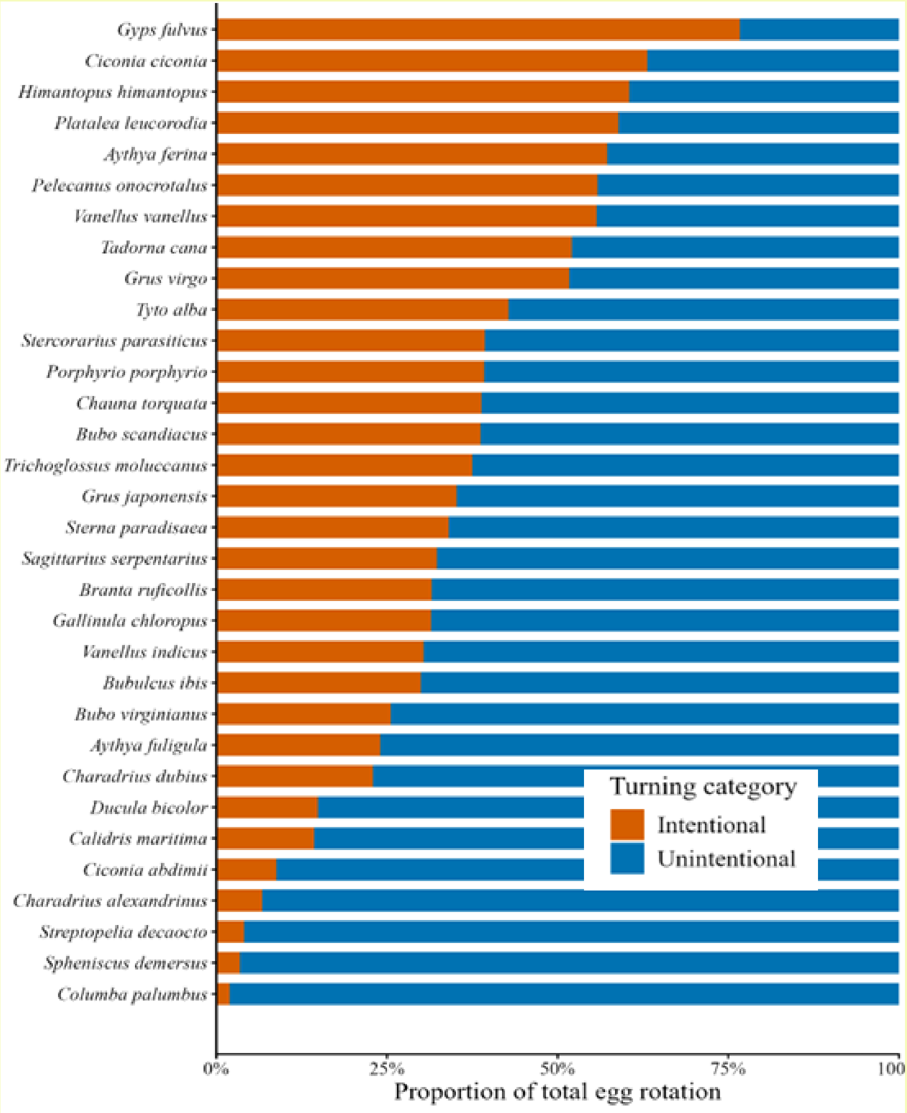


Figure 33: The proportion of each type of behaviour causing egg turning in the total egg turning for a particular species. Red - intentional egg turning; blue - nonintentional behaviour causing egg turning (Matějková et al., in prep).

9. Supplementary material

9.1. Protocol templates

- page 93 – checklist of steps for installing the dataloggers
- page 94 – form for writing installation details

COMPREHENSIVE INCUBATION MONITORING – INSTALLATION CHECKLIST

AXY5 – datalogger in plastic egg

☐ Recharge the battery, format the memory

☐ X manager: accelerometer 10 Hz,2g,8-bit; magnetometer 2Hz; temperature 1×/s

☐ Start data collection by magnet (indicator light flashes faster → slower ~20 s)

☐ Write down the data logger ID and the start time to the form

☐ Place it in the holder (correctly oriented) and close it

⚠ Battery life ~18 days (at 1 Hz)

MSR – temperature and humidity sensor in the nest

☐ Recharge the battery, format the memory

☐ MSR.exe: sampling frequency 1 Hz, delayed start time (*Start at*)

☐ Write down the data logger ID and the start time to the form

☐ Install the sensor on the longer cable to the nest lining between the eggs and the shorter cable ~1 m from the nest

☐ Hide the cables in the nest lining / secure them with wire or tape

☐ Hide the device body in a plastic bag out of the bird's reach

⚠ Beware of destructive species, and sensors moved out of the nest

Camera **Wyze**

☐ Recharge the power bank, format the SD card, insert it into the slot

☐ Write down the SD card ID in the form

☐ Once connected to the power bank, wait until the camera starts recording (you will hear a click) and note this time in the form (accurate to the seconds)

☐ Check functionality – if the indicator light flashes red, cover it with black tape

☐ Point the camera at a display showing the exact time from multiple angles

☐ Mount the camera ~1m from the nest (the frame includes nest + surroundings)

☐ Hide and secure the power cable (using wire or tape)

☐ Place the power bank out of the bird's reach in a plastic bag

⚠ Battery life ~3 days (30,000 mAh power bank)

Date nest visit	Time start	ID installation	Species	ID parents/nest	Num eggs	ID AXY5	AXY5 note	ID MSR	MSR note	ID camera/SD	CAMERA START TIME	Time end	Note (incubation start, fate of the nest)

How to use the form: Fill in a new row on the form for each visit to the nest. At least, an entry should be made when installing or removing data loggers, on each subsequent visit to the nest, or when replacing devices (e.g., the camera's power bank). Mark all visits related to a single installation with the same installation ID, which uniquely links all records for the same nest and the same set of data loggers. For quick identification, you can add a symbol to the installation ID to indicate the action associated with the data loggers, for example, ↓ for installing equipment and ↑ for removing equipment.
Enter the exact start and end times of the nest visit in the form. Enter the identification numbers of all devices used (AXY5, MSR, camera/SD card) in the relevant columns, along with any notes on their condition or method of installation (e.g. the orientation of the data loggers within the egg). It is absolutely essential to also record the time the camera started recording during installation, as this is used for subsequent time synchronisation of the camera recordings with data from the other dataloggers. In the last column, write all important information about the nesting, such as the estimated date of the first egg laid or the final fate of the nest.

9.2. Universal ethogram for the BORIS

Behaviour code	Behaviour type	Description	Key
incubation	State event	the bird is incubating and is in contact with the eggs	i
on the nest	State event	the bird is on the nest, incubating/standing above etc.	o
M on the nest	State event	female on the nest (if distinguishable)	u
F on the nest	State event	male on the nest (if distinguishable)	y
sleeping	State event	bird sleeping on the nest	s
stop video	Point event	determining the end time of videos for subsequent videos	x
vocalization	Point event	vocalisations	v
egg turning	Point event	egg manipulation/turning	t
building	Point event	manipulates nest material	b
preening	Point event	feather preening	p
mating	Point event	copulation of the pair on nest	m
courtship	Point event	court rituals	c
allopreening	Point event	mutual preening with partner	l
partner exchange	Point event	partner exchange	e
walk from nest	Point event	leaving the nest by foot	w
flight from nest	Point event	flying from the nest	f
interspecies visit	State event	another species in the immediate vicinity of the nest	j
intraspecies visit	State event	an individual (same species) in the immediate vicinity of the nest (not a parent)	n
attack	Point event	incubating bird attacking another animal	a
aggressive running	Point event	running out of the nest towards another animal	r
wing display	Point event	threatening posture with outstretched wings	d
pressed to ground	Point event	pressed down to the ground when in danger	g
feeding	Point event	gathering or processing food	k
incubation feeding	Point event	feeding or offering food by a partner	z

9.3. Incubation parameters obtained for individual orders

Table 3: Temperatures recorded in individual orders of birds.

Order (number of species)	Temperature (mean)	Temperature (median)	Maximal temperature	Minimal temperature	25 % quartile	75 % quartile	Standard deviation
Bucerotiformes (1)	32.73	33.00	37.00	24.00	31.92	34.00	1.94
Strigiformes (4)	32.83	33.16	35.71	23.55	32.02	34.07	1.85
Psittaciformes (3)	33.27	33.69	37.58	26.79	31.88	34.74	2.13
Anseriformes (5)	33.90	34.24	37.85	26.88	32.63	35.46	2.06
Sphenisciformes (1)	34.64	36.00	37.58	25.21	33.50	36.38	2.85
Gruiformes (3)	34.72	35.03	37.45	26.37	33.84	36.00	1.65
Ciconiiformes (4)	35.01	35.12	37.64	30.43	34.12	36.02	1.27
Pelecaniformes (6)	35.91	35.95	38.08	31.71	35.39	36.55	0.96
Accipitriformes (3)	36.03	36.24	37.83	31.99	35.34	36.83	1.04
Columbiformes (5)	36.87	36.83	40.24	33.21	36.08	37.73	1.27

9.4. Contacts and data sharing

9.4.1. Behavioral Ecology Research Group (BERG)

We are a research team at the Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, who have created this guide. We have longstanding experience in monitoring animal behaviour, biologging and the study of incubation in birds. On our group's website, you will find information on our current projects, publications, the software tools we are developing, and contact details for individual team members.

If required, we can assist you in selecting suitable dataloggers for incubation monitoring, designing methodology for data collection, resolving technical issues during installation, and processing, analysing and interpreting the data obtained. If you are interested in collaboration, consultation or participation in shared research activities, we recommend using the contact details provided on the research group's website: www.fzp.czu.cz/en/r-9409-science-research/r-9674-leading-research-groups/r-14317-behavioral-ecology-research-group/r-14324-team-news

9.4.2. Incubase platform

This web platform is designed for the management, sharing and analysis of data related to the artificial incubation of bird eggs. It enables the recording of individual eggs, monitoring their development during incubation, and comparing one's own results with data from other users. The platform provides recommendations for optimising incubation conditions and facilitates the sharing of experiences amongst breeders, zoos, wildlife rescue centres and research institutions.

The platform also includes Inkupedia, an encyclopaedia of bird incubation which serves as an open database of information on the incubation of bird species. For a number of species, you will find photographs of the eggs, their dimensions and weight, the incubation period and the timing of egg-laying. The database is constantly being expanded to include new species and new records obtained from collaborating institutions.

The platform is available at: www.incubase.fzp.czu.cz/

9.4.3. Manufacturers of recommended dataloggers

AXY5 XS

Manufacturer: TechnoSmArt Europe s.r.l. (Italy)

The AXY5 XS miniature biologging datalogger is a commercially available device designed for long-term recording of temperature and movement. Manufacturer's website: www.technosmart.eu/axy-5-xs/

MSR145

Manufacturer: MSR Electronics GmbH (Switzerland)

MThe MSR145 is a commercially available multi-channel datalogger capable of simultaneously recording temperature and relative humidity, with a large memory capacity and long battery life. Manufacturer's website: www.msr.ch

Cameras

For long-term video recording of the incubation process, commercially available Wi-Fi cameras, such as the Wyze Cam (Wyze Labs, Inc., Washington), were tested as part of the development of this methodology. The camera allows long-term continuous recording, can be powered by a power bank, and stores data on a memory card. The choice of a specific model should be adapted to the requirements of the monitored environment, particularly the availability of electricity, Wi-Fi and the required image quality. Manufacturer's website: www.wyze.com.

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