Australian Field Ornithology 2024, **41**, 150–154
http://dx.doi.org/10.20938/afo41150154

A photographic guide for determining egg incubation stage in the Superb Fairy-wren *Malurus cyaneus*

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Abstract. When monitoring the nesting biology of wild birds, nests are often found after the eggs have been laid and incubation has commenced. Candling – the use of a bright light to illuminate egg contents – is a useful method for estimating embryo development and incubation stage. This information is used to estimate when incubation started and predict when eggs will hatch. As the focus of several long-term monitoring studies, a guide for assessing the stage of embryo development in the Superb Fairy-wren *Malurus cyaneus* will enable a standardised estimation of egg development for nests found after laying has been completed. Here, we present a photographic guide for determining embryo development using candling in the Superb Fairy-wren. In the process of developing this guide, we also address the need for a cost-effective and accessible method for candling eggs in the field with a portable candling device.

Introduction

Determining when eggs are laid can be an important aim of field-based avian population monitoring projects (Double *et al*. 2005; Boersma *et al*. 2023). Ideally, a nest is found while it is being built, so that the exact lay date(s) can be determined during subsequent monitoring. However, nests are often found after the eggs are laid and incubation has begun. An estimate of when incubation commenced can be calculated using data on a species' average incubation period if the egg hatch date is determined. An alternative approach is to use a bright light to illuminate egg contents and estimate the stage of embryonic development (hereafter 'candling'). This method can be used when the nest is found, thereby ensuring that lay date estimates can be obtained immediately (Lokemoen & Koford 1996). This can be especially useful as it ensures estimates can be gained even if subsequent nest failure occurs and hatch date cannot be determined. Although egg development is well documented in chickens *Gallus gallus domesticus*, game species (e.g. Mourning Dove *Zenaida macroura,* Northern Bobwhite *Colinus virginians*), waterfowl (e.g. Redhead *Aythya americana*) and some species popular in the pet trade (e.g. Orange-winged Amazon *Amazona amazonica*), current methods of candling eggs for determining incubation stage for species used in field research are less well established (Weller 1956; Hanson & Kossack 1957; Brach *et al.* 1982; Delany *et al*. 1999).

Here, we present a photographic guide for assessing embryo development using candling methods in the Superb Fairy-wren *Malurus cyaneus*, a species that is the focus of several long-term population monitoring studies (Mulder & Magrath 1994; Peters *et al*. 2002; Feeney *et al.* 2013; Cockburn *et al*. 2016; Colombelli-Négrel *et al.* 2022) but for which no such resource has been published. Additionally, we outline a method that we developed to candle passerine eggs using readily available materials that can be easily modified for use in other species. Current in-field candling methods for passerines consist of handheld illumination with a flashlight and tube, or using the sun to illuminate the developing egg while in the hand (Lokemoen & Koford 1996; Delany *et al.* 1999). The method we propose minimises the likelihood of damage to the eggs while in the hand, may reduce the time of candling compared with other methods and, most importantly, the candling device can be used in the field. Several eggs can be candled and photographed at once, allowing for the potential of a photographic library of stages of embryonic development of other passerine and closely related species.

Methods

Study species

The Superb Fairy-wren is a cooperatively breeding passerine endemic to Australia. It constructs easily accessible nests, generally <2 m above the ground, and lays one egg on consecutive days until the clutch is complete (typically 3–4 eggs). Incubation begins when

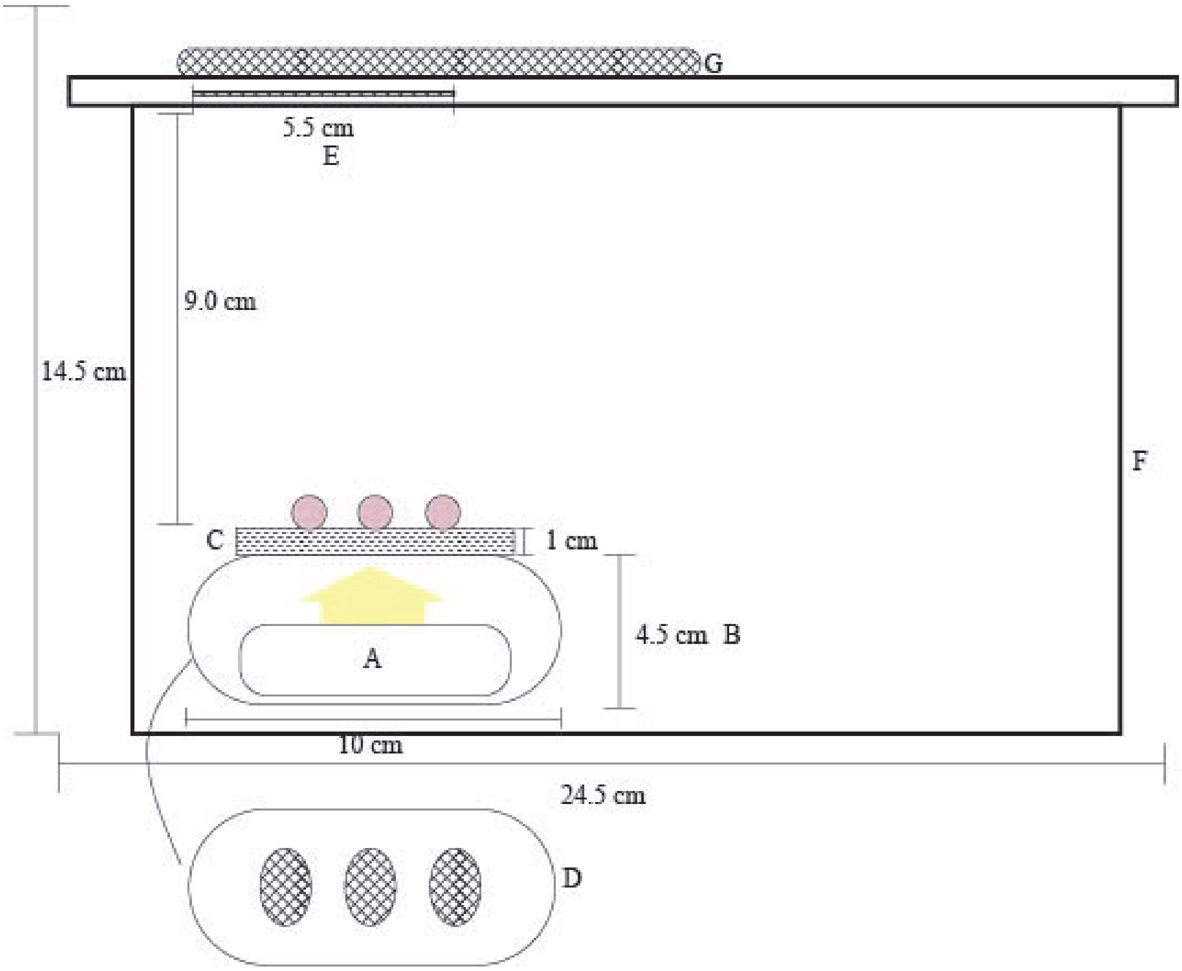

Figure 1. Candling box schematic. A, light source (200 lumens); B, container for light source with soldered-in peer-holes on upper surface; C, foam for placing eggs; D, view from above of the candling device B; E, peer-hole for mobile phone camera; F, IKEA sockerbit box; G, mobile phone resting on lid with camera through soldered peer-hole. Eggs shown resting on the surface of the candling device.

the last egg is laid (Rowley 1964). It is presumed that development of eggs in this species aligns closely with that of other passerine species, but additional analysis of embryo development in wild bird populations is needed to confirm this (Hemmings & Birkhead 2016).

Study site

This study was conducted on the western side of Lake Samsonvale (27°16′S, 152°51′E), in south-eastern Queensland, Australia, between August and December 2023. Superb Fairy-wren breeding biology is monitored at this site alongside closely related Red-backed *M. melanocephalus* and Variegated Fairy-wren *M. lamberti* populations (Baldassarre *et al*. 2014; Welklin *et al*. 2021; Boersma *et al.* 2023). The study site is characterised by eucalypt woodland with partial remnant dry tropical forest. Extensive areas of invasive plant species dominate the understorey, especially Common Lantana *Lantana camara*.

Constructing a candling box

Using a soldering iron, we created a peer-hole in a plastic, lidded box (19 × 26 × 15 cm). This enabled a mobile phone to be mounted on top of the box lid, allowing the lens of the phone camera to view into the box through the peer-hole. Within the box, a light container was constructed using a smaller plastic container which housed a light source of 200 lumens and was lined with cotton and foam. Three holes in the lid of the smaller container allowed light to shine through and illuminate the contents of the developing eggs which rested on a piece of foam above the holes with the medial axis parallel to the surface of the candler (Figure 1).

Candling eggs

Eggs from a single clutch were placed on top of the light source within the candling box. The closed box was made level on the ground, and a mobile phone rested on top of the lid with its camera aligned to the peer-hole (Figure 1). For the eggs used in this study, the age of the eggs was

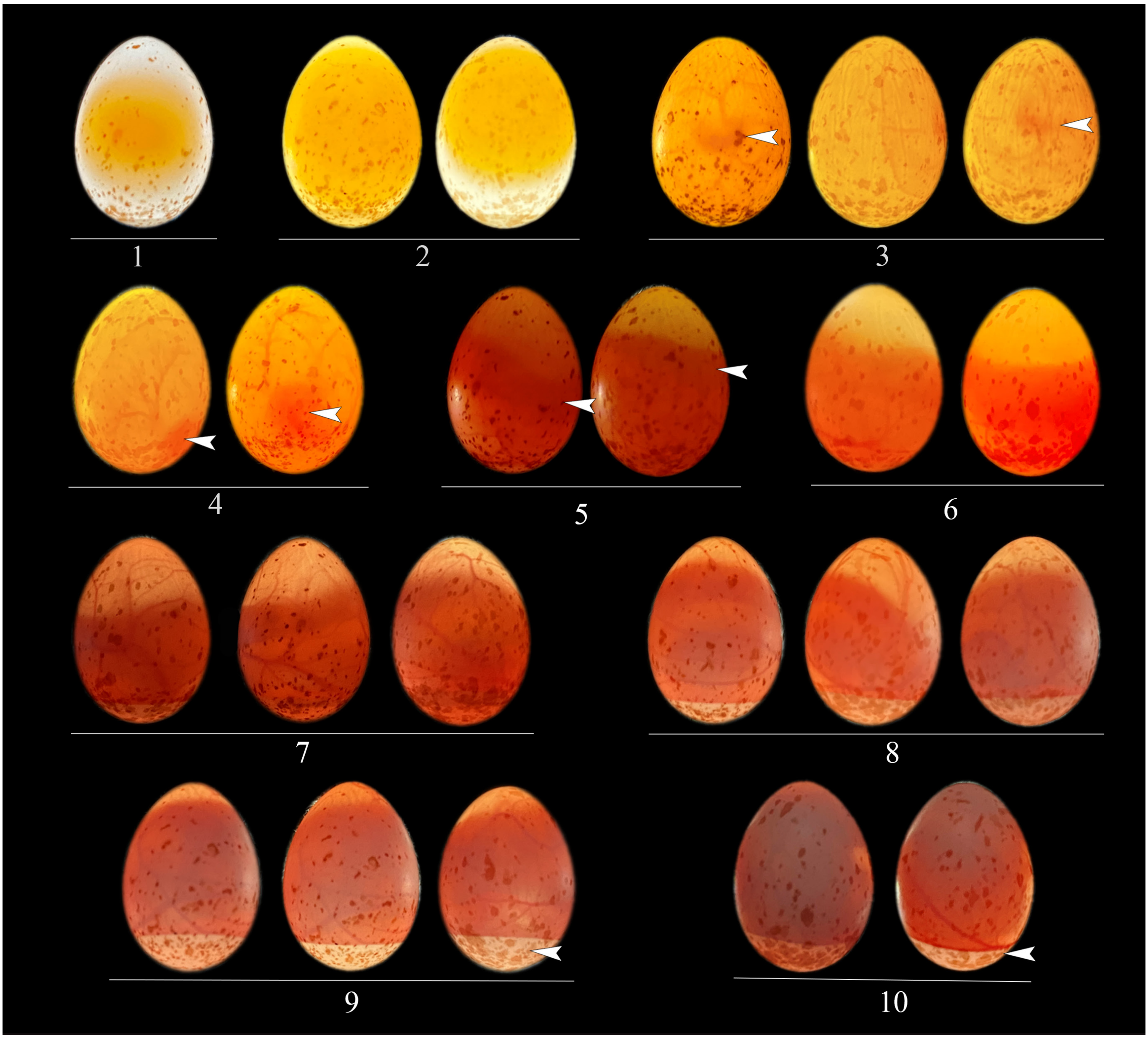

Figure 2. Daily photographs of actively incubated Superb Fairy-wren eggs. Numbers on each photograph indicate the day of incubation (i.e. 1 is the first day of incubation). Arrows indicate identifiable characteristics of the development stage, which are in bold in the descriptions of the incubation stage in Table 1.

known, with incubation beginning on the third day of laying a three-egg clutch. Eggs were taken out of the focal nest, placed within the box, candled and photographed daily, within the first 6 hours after first light at the study site, until the tenth day of incubation. Eggs were rotated and photographed to examine variation in visible egg contents within and between days

Results

We used the candling box to illuminate Superb Fairy-wren eggs in order to evaluate incubation stage and visualise embryo development over 10 days of incubation. Photographs revealed daily advancement of embryo development (Figure 2) and allowed for verbal descriptions of embryo development across days (Table 1).

Discussion

Here, we provide a photographic guide for determining incubation stage of actively incubated eggs of Superb Fairy-wrens, and outline the candling method that we used to capture these images. In-field candling methods are useful for future projects when nests are found mid-incubation, and photographic guides can be helpful in aiding determination of incubation stage. Differences in the stage of development between days can be subtle (Lokemoen & Koford 1996), and future studies are necessary to contribute to a photographic library of egg development in other species, particularly those not closely related to these *Malurus* wrens. This study continuously monitored three eggs from the same clutch that were laid and incubated by one female. We therefore emphasise that this guide is intended to be used to help obtain an estimate, and the use

Table 1. Descriptions of the daily developmental progress of the Superb Fairy-wren embryos in Figure 1.

Day	*Observations*
1	The egg yolk is suspended and concentrated, contrasting with the clear albumen. A gradient of dark orange to yellow extends radially from the centre of the yolk, and there is no vascularisation. The air cell is relatively small, and when rotated the yolk is free-floating.
2	The egg yolk is diffuse and initially appears to cover most of the egg's interior and, upon rotation, covers roughly half of the contents of the egg. Vascularisation is not apparent.
3	Vascularisation is clearly visible, with an **early-stage embryo forming** and a visible heartbeat. The vascularisation is visible but not yet very red in color. One large blood vessel is clearly visible, extending outwards from the embryo.
4	The growing embryo is clearly red, with vascularisation extending to the edges of the egg. This stage is similar to Day 3 of incubation, with a **larger embryo** and several distinct blood vessels rather than a single branching vessel.
5	The egg appears mostly red, with moderate vascularisation and a distinct difference between albumen, embryo, and air cell. The **embryo itself appears as a band** diagonal across the egg, encompassing one-third of the egg.
6	The embryo takes up an estimated one-third of the egg, nearly diffuse, and the albumen is not heavily vascularised.
7	This stage shows extensive vascularisation, a visible embryo that takes up an estimated one-third of the egg, and a small air cell. The embryo is mobile within the egg.
8	This is the first day that the air cell is noticeably larger, and takes up roughly one-fifth of the egg. The **mobile embryo** takes up more than half of the egg. The blood vessels are defined and most noticeable towards the narrow end of the egg. The egg is still red.
9	The developing embryo takes up more than half of the egg and shows little movement, and the air cell takes up nearly one-quarter of the egg. Differentiating this stage from Day 8 is difficult, but the **lack of movement and larger air cell can aid in determining the stage**.
10	The developing embryo takes up nearly all visible space in the egg that is not air cell. The air cell is beginning to become asymmetric, and takes up roughly one-fifth of the egg. The edge of the contents of the egg are vascularised with a **distinct red edge**.

of additional estimation techniques (e.g. back calculating lay date from hatch date) are likely to provide the most robust estimates. Future studies would benefit from extending this by monitoring eggs from different individuals to determine the extent of variation in development, as well as monitoring how development changes as conditions change (e.g. microclimatic variation, or maternal or parental investment in dual-incubating species: Martin & Schwabl 2007). With this photographic guide and details of our candling method, in-field determination of incubation stage of eggs in *Malurus* species is achievable and may extend to other passerine species once additional photographic evidence of egg development is made available.

Acknowledgements

We thank Seqwater for access to the field site and support of our research. This research was conducted with approval from Griffith University Animal Ethics Unit (ENV/06/23/AEC), the Queensland Department of Environment and Science (P-PTUKI-100283615) and Seqwater (NPD20250326). It was supported by Birds Queensland (53420), the Wildlife Research and Education Institute, and the Doñana Biological Station.

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Received 31 January 2024, accepted 23 September 2024, published online 28 November 2024