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 standardized 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 is often 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 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 domesticus*), game species (e.g. *Zenaida macroura, Colinus virginianus, etc.*), waterfowl (e.g. *Aythya americana*) and some species popular in the pet trade (e.g. *Amazona amazonica*), current methods of candling eggs for determining incubation stage for species used in field research are less well established. (Brach et al., 1982; Delany et al., 1999; Hanson and Kossack 1957; Weller 1956)

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 (Boersma et al., 2023; Cockburn et al., 2016; Colombelli-Négrel et al., 2022; Feeney et al., 2013; Mulder and Magrath, 1994; Peters et al., 2002) 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 hand (Delany et al., 1999, Lokemoen 1996). The method we propose minimizes the likelihood of damage to the eggs while in the hand, and reduces the time of candling compared to 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 near-passerine species.

Methods

Study species

The Superb Fairy-wren (*Malurus cyaneus)* is a cooperatively breeding passerine endemic to Australia. They construct easily accessible nests, generally less than two meters above the ground, and lay one egg on consecutive days until the clutch is complete (typically 3–4 eggs). Incubation begins when 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, however, additional analysis of embryo development in wild bird populations is needed to confirm this (Hemmings and Birkhead 2016).

Study site

This study was conducted on the western side of Lake Samsonvale (27°16′S, 152°51′E), in South-east 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 (*M. lamberti*) Fairy-wren populations (Baldassarre et al., 2014, Boersma et al., 2023, Welklin et al., 2021). The field site is characterized by eucalypt woodland with partial remnant dry tropical forest. Extensive invasive species are present and dominate the understory, especially *Lantana camara*.

Constructing a candling box

Using a soldering iron, we created a peer-hole in a plastic, lidded box (19x26x15 cm). This enabled a cellular 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 median axis parallel to the surface of the candler (Fig. 1).

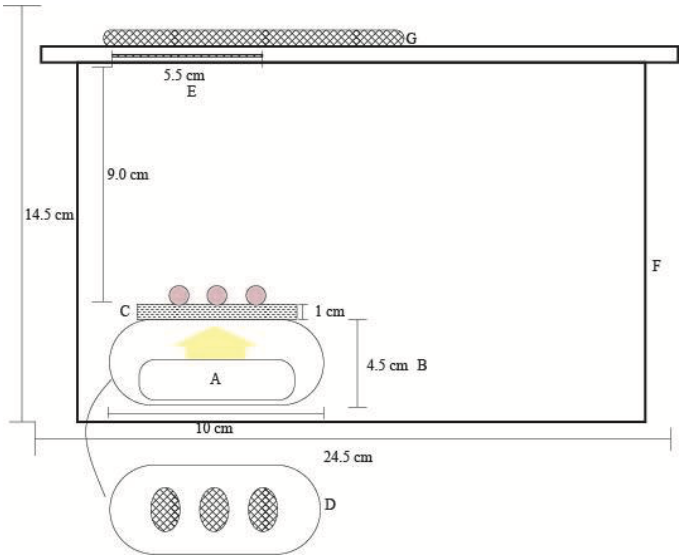

Figure 1. Candling box schematic. A, light source (200 lumens); B, container for light source with soldered-in peer holes on upper surface; C, foam for placing eggs; D, view from above of the candling device B; E, peer-hole for cell phone camera; F, IKEA *sockerbit* box; G, cell phone resting on lid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

Candling eggs

Eggs from a single clutch were placed on top of the light container within the candling box with a closed lid, placed on the ground and standardized with a level, and a cellphone rested on top of the lid with the camera aligned to the peerhole. For the eggs used in this study, the age of the eggs was 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 six hours after first light at the study site, until the tenth day of incubation. Eggs were rotated and photographed to examine variation in viewable egg contents within and between days (Fig. 1).

Results

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

Table 1. Descriptions of the daily developmental progress of the embryos in Fig 1.

Day 1	The egg yolk is suspended and concentrated, contrasting with the clear albumen. A gradient of dark orange to yellow extends radially from the center of the yolk, and there is no vascularization. The air cell is relatively small, and when rotated the yolk is free-floating.
Day 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. Vascularization is not apparent.
Day 3	Vascularization is clearly visible, with an **early-stage embryo forming** and a visible heartbeat. The vascularization is visible but not yet very red in color. One large blood vessel is clearly visible, extending outwards from the embryo.
Day 4	The growing embryo is clearly red with vascularization, 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.
Day 5	The egg on this day appears mostly red, with moderate vascularization 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.
Day 6	The embryo at this stage makes up an estimated one-third of the egg, nearly diffuse, and the albumen is not heavily vascularized.
Day 7	This stage can be characterized by extensive vascularization, 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.
Day 8	This is the first day that the air cell is noticeably larger, and is 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 tip of the egg. The egg is still red in color.
Day 9	The developing embryo is more than half of the egg, with little movement, and an air cell making up nearly one-fourth of the egg. Differentiating this stage with Day 8 is difficult, but the **lack of movement and larger air cell can aid in determining stage**.
Day 10	The developing embryo takes up nearly all visible space in the egg that is not the 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 vascularized with a **distinct red edge**.

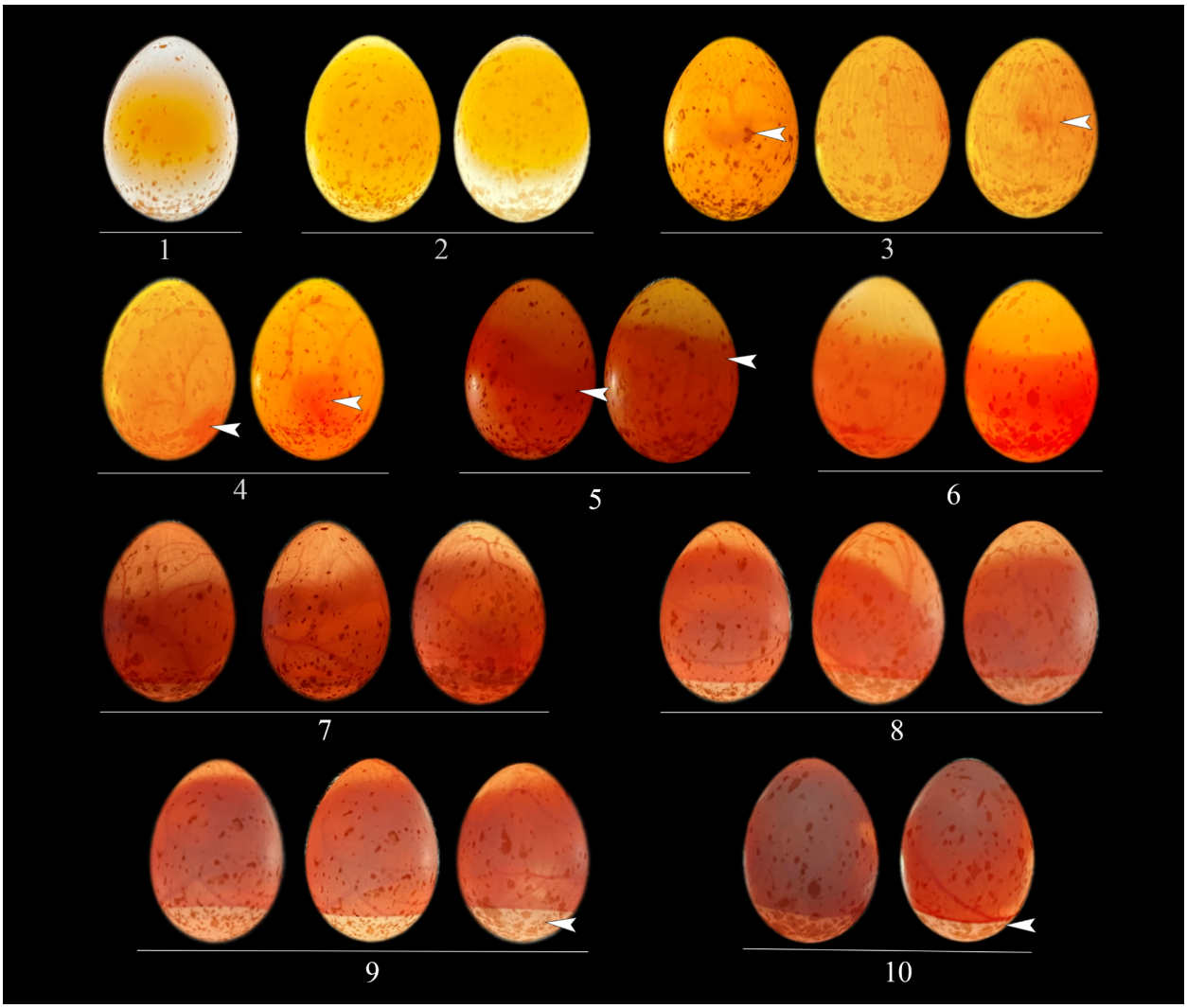

Figure 2. Daily photographs of actively incubated Superb Fairy-wren eggs. Numbers on each photo indicate the day of incubation (i.e., 1 is the first day of incubation). Arrows indicate identifiable characteristics of the development stage, which are in bold in the descriptions of the incubation stage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

Discussion

Here, we provide a photographic guide for determining incubation stage of actively incubated eggs of Superb Fairy-wrens, and outline the candling method 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 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. Furthermore, differences in development during incubation can vary among individuals because of weather events, microclimatic variation, or maternal or parental investment in dual-incubating species (Martin and Schwabl, 2007). We therefore emphasize that this guide is intended to be used to help obtain an estimate, and the use of additional estimate techniques (e.g. back calculating lay date from hatch date) are likely to provide the most robust estimates. 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.

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