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Candling and field atlas of early egg development in Common Eiders *Somateria mollissima* in the central Baltic

Ovoskopija in terenski atlas zgodnjega razvoja pri gagah *Somateria mollissima* v Osrednjem Baltiku

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Here we present the results of candling 258 eggs from 50 nests of Common Eiders *Somateria mollissima* in a colony in the central Baltic. Of these, 223 (86%) had a developing foetus. Among the 35 (14%) failed eggs, 15 were unfertilized and 20 contained a dead embryo. The prevalence of failed eggs is similar to the average proportion of failed eggs reported previously by the Christiansø Scientific Field Station during 1998–2014. The reason for the high percentage of failed eggs is unknown; however, low pre-incubation body mass and energetic stress is likely to be the main factor. In addition, we incubated 8 eggs in the laboratory from day 0 to hatch in order to follow the development and foetal morphology. This resulted in a field atlas from which it is possible to estimate date of incubation start using candling in early incubation (days 1–12). The atlas is a new possibility for field biologists to estimate the first day of incubation of breeding eiders and the prevalence of unfertilized and rotten eggs, which is important for studying their biology and population dynamics.

Key words: Common Eider, Baltic, candling, fertilization, starvation, stress

Ključne besede: gaga, Baltik, ovoskopija, oploditev, stradanje, stres

1. Introduction

The Christiansø archipelago in the southern part of the Baltic Proper holds the second largest Danish colony of breeding Common Eiders *Somateria molissima* (Christensen & Bregnballe 2011). It consists of 1,500–1,700 pairs and has been monitored since 1950 (Lyngs 1992, 2009, 2014). This long-term monitoring revealed that during 1970–1990, the population increased from approximately 1,000 to approximately 3,000 breeding pairs, while from 1990 until today the population has declined by approximately 50% (Lyngs 2009, 2014). The reasons for this is unknown, however, limited access to food in wintering areas and the breeding location, infectious diseases, blooming toxic algae and failed eggs have been suggested as some of the main reasons (Buchman 2010, Camphuysen *et al.* 2002, Christensen *et al.* 2008, Larsson *et al.* 2014, Laursen & Møller 2014).

In order to understand the fluctuations in the number of breeding eiders and clutch size, we established a research programme running in 2015–18. Briefly, the study showed that incubating females underwent extreme physiological stress over the approximately 26-day incubation period. The weight loss for a certain proportion of the colony in some years is borderline to cachexia and breeding failure (Garbus 2016, Garbus *et al.* 2018). As part of this, the prevalence of failed eggs has been studied, since earlier indications suggest that this could be a potential problem for the colony and population dynamics (Lyngs 2009, 2014).

Here, we present the results of the controlled study of candling incubating eider eggs and provide the first field atlas of developmental stages in Common Eiders. Eggs were monitored and candled, and controlled incubation in the laboratory was conducted. The present publication is of value to field studies that measure fertility ratios and estimations of date of incubation start in breeding eiders.

2. Materials and methods

During April-May 2015, 258 eggs and egg membranes from 50 nests of incubating habituated eiders were monitored at Christiansø located northeast of Bornholm in the Baltic Proper (55°19’N 15°11’E, Figure 1). All 258 eggs were candled to investigate the frequency of failed eggs and the relation to the health of female eiders. An oviscope was constructed from a flashlight (Cree XPE High Power LED 3 W 150 Lumen) modified with a rubber cup on the top (Figure 2). The flashlight had a sufficient brightness to examine the eider eggs. The entire candling procedure was performed in darkness using a mackintosh covered cardboard box. Using nitrile examination gloves, the base of the egg was held between thumb and forefinger, placed directly against the light and tilted slightly to one side while rotating the egg. The candling was conducted from day 8 to 12 of the incubation stage as recommended for ducks having long incubation periods (Ernst *et al.* 2004). The 10-second candling is considered to pose no risk to the further egg development.

Birds were kept in a cotton bag during all procedures to reduce stress. In cases where all eggs were removed from the nest due to complete failure, artificial replacement eggs were used to keep birds at their nests and thereby reduce stress levels. All nests were checked daily and visually inspected at distance.

Eggs were divided into two groups: active eggs and failed eggs (Hemmings *et al.* 2011). Failed eggs were further divided into infertile eggs or dead embryos. Information on the methods and their interpretations is described in Ernst *et al.* (2004).

In addition to this, newly laid eggs in 2016 (n=4) and 2017 (n=4) were brought for incubation in the laboratory. The eggs were incubated on days 1–28 and the hatched ducklings were brought back to the nest and taken over by either the mother or a nanny. The nests were visually observed, and all ducklings were successfully accepted by their mothers or nannies. An America motor incubator (America A/S, Thisted, Denmark) was used for incubation. A thermometer and hygrometer were placed inside the incubator in order to monitor and adjust temperature and humidity. The settings used were 38.5°C on the first 21 days with a humidity of 55%. In the last 4–5 days of incubation, a temperature of 38.9°C was applied. From day 8, the eggs were humidified daily with a spray containing water at 38.5 °C. The eggs were turned four times daily (8.00, 12.00, 18.00 and 22.00). All eggs were photographed daily at 13.00 and simultaneously cooled during transport (1–2 minutes). Around the time of hatching, humidity was adjusted to 70%. For photodocumentation of egg development, a Cree XPE High Power LED 3 W 150 lumen was used for early development and an EC4S NITECORE® LED Flashlight 2150 Lumen for late development. Lumen settings varied from 1000–2150. Both flashlights were modified with rubber cubs placed in a holder fitting the morphology of the eggs. Pictures were taken with a Nikon D3S using a 60 mm macro lens (f/9, 1250 ISO, 1 sec exposure) in a partly dark room.

For basic training, the candling procedure was tested at the Department of Disease Biology, University of Copenhagen in March 2015 on day 10 of incubation. Candling of 200 chicken eggs showed fourteen unfertilized and seven dead embryos adding up to 10% failed eggs. No false negatives were observed.

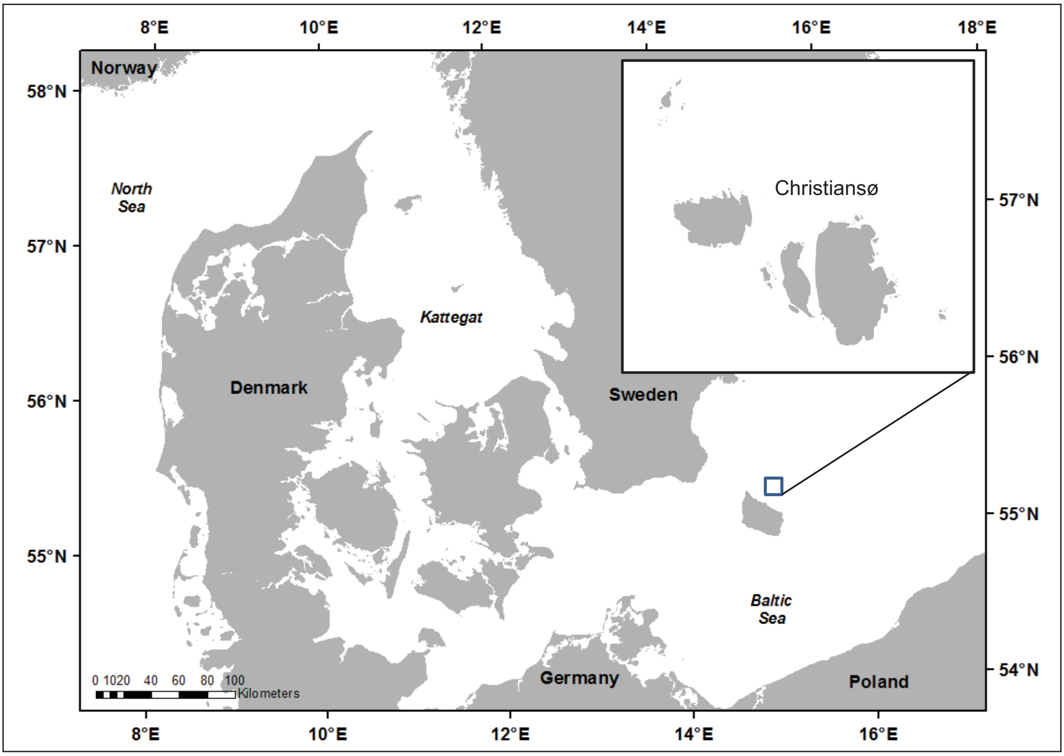

Figure 1. Map of the archipelago Christiansø study area

Slika 1: Zemljevid preučevanega območja v arhipelagu Christiansø

Figure 2. Egg candling using a modified Cree XPE High Power LED® as oviscope inside a cardboard box

Slika 2: Ovoskopija z uporabo modificiranega orodja Cree XPE High Power LED® kot oviskopa v kartonski škatli

3. Results and discussion

3.1. Candling and hatching

The candling and egg membrane counting showed that 223 (86%) eggs had a developing foetus. Among the 35 (14%) failed eggs, fifteen were unfertilized (6%) and twenty contained dead embryos (8%). This percentage of failed eggs (14%) is the same as the average proportion of failed eggs found by the Christiansø Scientific Field Station during 1998-2014 (14%; range 8–23%) (Lyngs 2014).

To the best of our best knowledge, no other large-scale investigations on failed eider eggs have been conducted, making it difficult to compare with the proportion of failed eggs in other colonies. However, the loss of incubated eggs from modern, high-hatching chicken strains, stored under optimal conditions, should be no more than 10%.

Losses in waterfowl may be slightly higher (Ernst *et al.* 2004). The reasons for the relatively large proportion of failed eggs in the Christiansø colony is unknown, however, low initial body weight and energetic stress is likely to be the main reason (Garbus 2016, Garbus *et al.* 2018, Hemmings *et al.* 2011). Nutritional deficiencies, early infections and contaminant exposure constitute the common cause of dead-in-shell embryos in chickens, which is a supporting weight of evidence for similar cause and effects in the present investigation of the Christiansø breeding colony (Alcorn 2008, Hoffman 1990).

3.2. Candling atlas

Figure 3 shows the atlas of the day 1–12 stages of incubating Christiansø eider foetus. It is seen that the centrally located embryo takes form as a small-condensed area forming into a red / dark area. From the embryo, blood vessels radiate to the extra-embryonic membrane. The simple circulatory system evolves into a more advanced system and the embryo takes gradually more space of the egg. After day 12, the examination of the egg is complicated or no longer suitable as a measure of foetal age as the foetus gets darker and takes up more space. During the last period of the egg development, the foetus is only visible as a dark shadow with a prominent air sac. In addition, Figure 4 shows examples of dead and infertile eggs, respectively, at Christiansø. A blood ring is seen in the dead egg, and the infertile egg appears clear and transparent with no development.

3.3. Future considerations

The atlas and candling method provide field biologists with a tool to estimate first day of incubation of breeding eiders and prevalence of unfertilized and rotten eggs, which is important for studying their biology and population dynamics (Robertson & Cooke 1993) including what is known about males during the winter season (Garbus *et al.* In press).

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Povzetek

Avtorji članka predstavljajo rezultate ovoskopije 258 jajc iz 50 gnezd gage *Somateria mollissima* v koloniji na Osrednjem Baltiku. Od teh jih je 223 (86 %) vsebovalo razvijajoče se zarodke. Med 35 (14 %) propadlimi jajci je bilo 14 neoplojenih, 20 pa jih je vsebovalo mrtve zarodke. Prevladujoče število propadlih jajc je podobno povprečnem deležu propadlih jajc, o katerem so v obdobju 1998–2014 poročali z Znanstvene terenske postaje v arhipelagu Christiansø. Razlog za ta visoki odstotek propadlih jajc sicer ni znan, vendar avtorji domnevajo, da gre glavni faktor za takšno stanje po vsej verjetnosti pripisati telesni masi pred valjenjem in energetskemu stresu. Da bi sledili razvoju in zarodkovni morfologiji, so se avtorji odločili za inkubacijo osmih jajc v laboratoriju od dneva 0. Rezultat je bil terenski atlas, iz katerega je mogoče oceniti datum začetka inkubacije z uporabo ovoskopije med zgodnjo inkubacijo (dnevi 1–12). Atlas ponuja terenskim biologom novo možnost ocenjevanja prvega dne valjenja gnezdečih gag in prevladujoče število neoplojenih in gnilih jajc, kar je pomembno za preučevanje njihove biologije in populacijske dinamike.

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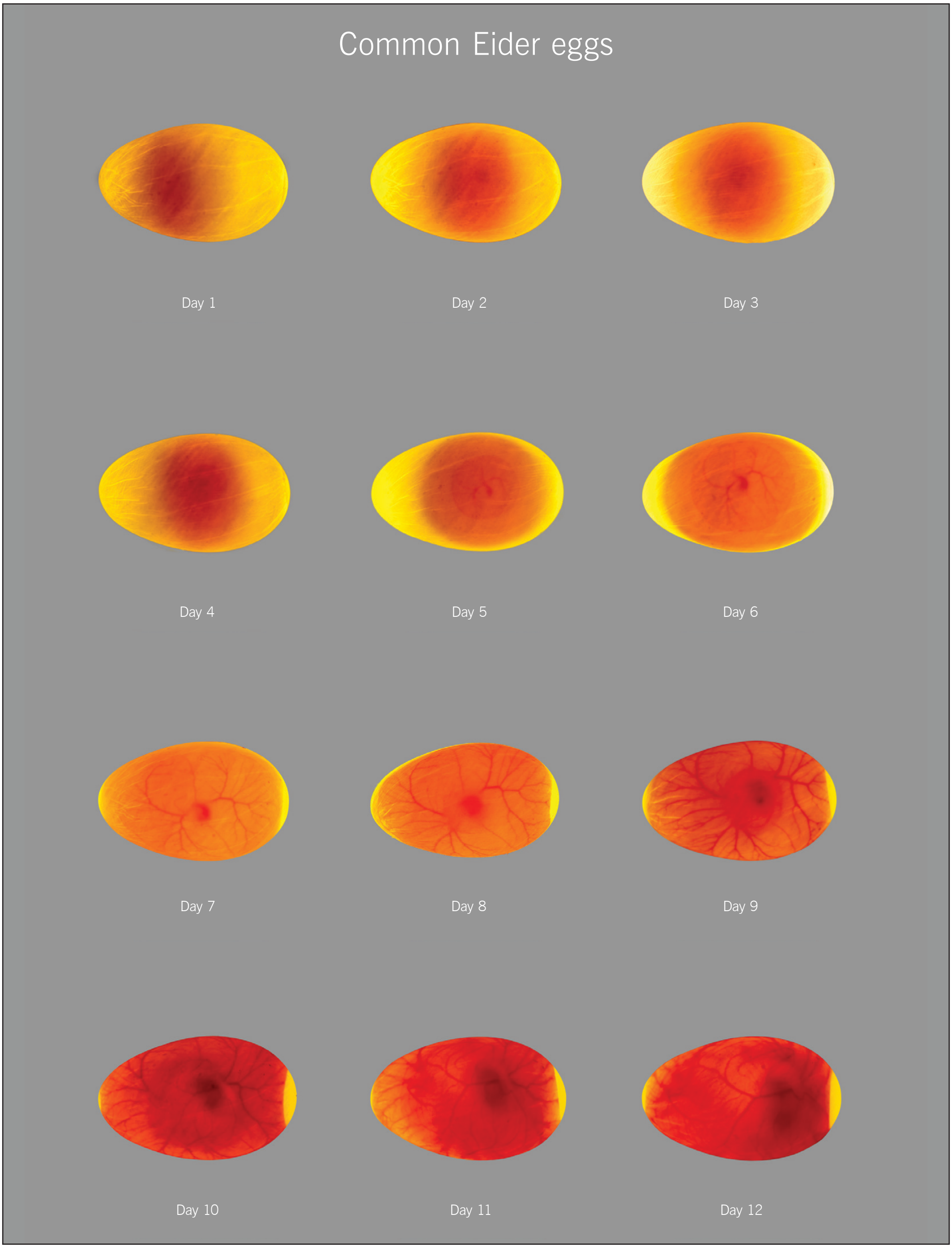
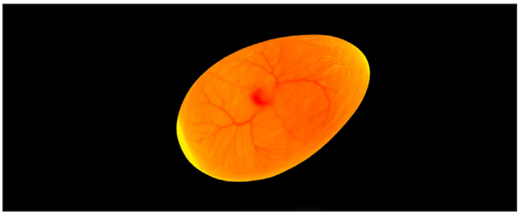
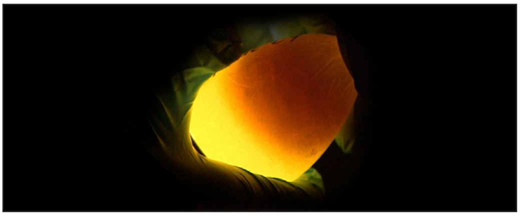


Figure 3: Stages of egg incubation on days 1–12 at Christiansø

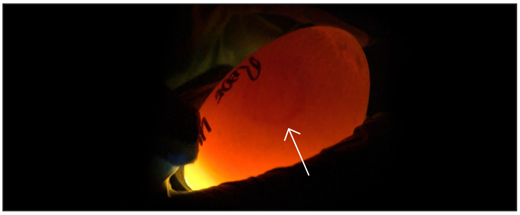
Slika 3: Faze valjenja v dnevih 1–12 v arhipelagu Christiansø



The active egg (day 7) Note the circulatory system



The unfertilized egg Note the clear yolk



The dead embryo The blood rings observed indicates death of embryo

Figure 4: From top to bottom: active egg (day 7), unfertilized egg, egg containing dead embryo at Christiansø. Note the circulatory system in the active egg and that the infertile egg appears clear and transparent with no development, while the beginning of a blood ring is seen in the dead egg (arrow).

Slika 4: Od zgoraj navzdol: aktivno jajce (7. dan), neoplojeno jajce, jajce z mrtvim zarodkom. Glej obtočni sistem v aktivnem jajcu in kako je neplodno jajce videti čisto in prozorno brez znakov razvoja, medtem ko je v mrtvem jajcu opaziti začetek oblikovanja krvnega prstana (puščica).

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