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SHORT NOTE

Timing of egg formation in the little penguin (*Eudyptula minor*)

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In the late 1970s and 1980s Professor C.R. (Dick) Grau and colleagues at the University of California, Davis, studied the timing of egg formation in a selection of sea birds and related this to the sequence of breeding events prior to laying. The results are summarised in Grau (1984; see table 3). They included 3 penguin species from different genera: the Fiordland crested penguin (*Eudyptes pachyrhynchus*; Grau 1982), Adelie penguin (*Pygoscelis adeliae*; Astheimer & Grau 1985), and the white-flippered form of the little penguin (*Eudyptula minor*). The results of the little penguin study were not published at the time. This note gives an outline of that study and a summary of its results.

Egg formation comprises a period of yolk deposition followed by a 'lag' period of up to several days during which ovulation occurs and albumen and shell are added. In seabirds, egg yolk is laid down in concentric layers in a daily pattern of alternate rings that differ in transparency (Grau 1984). These rings can be observed in transverse sections of the yolk of unincubated eggs that have been stained to better show their layered structure (Grau 1976). By counting the number of rings, the time taken for yolk deposition can be estimated. The lag time between the completion of yolk deposition and the egg being laid can also be estimated using the dye-feeding technique to place a date marker in the yolk (Gilbert 1972).

To estimate the timing of egg formation freshly-laid eggs were obtained from a white-flippered penguin colony on Onawe Peninsula (43° 46' S, 172° 55' E) at the head of Akaroa Harbour, Banks Peninsula. On 12 September 1979 the birds present in 17 burrows were each given a gelatine capsule containing 75 mg Sudan black B mixed in dried chicken egg yolk. Eggs were laid in 10 of these burrows 5 to 13 days after dosing. Two A eggs (first laid) and 8 B eggs (second laid) were collected from different clutches. These were degassed, frozen for 24 hours, and fixed in formalin for 16 hours. Each yolk was cut in half transversely and the radial width of the Sudan

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** Deceased

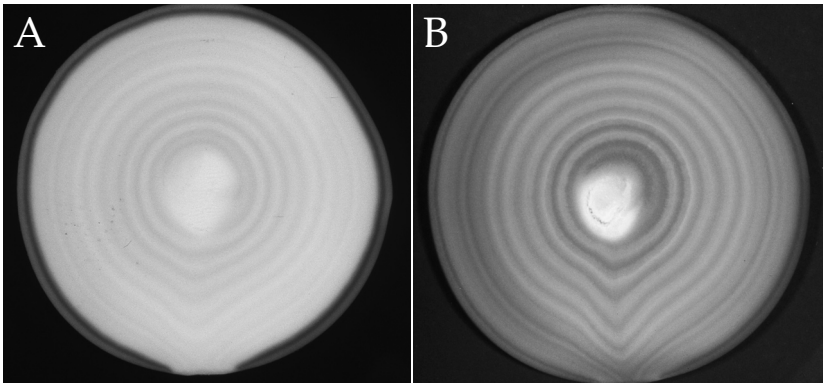

Fig. 1. Transverse section through the yolk of egg number 2 in Table 1 showing the concentric pattern of yolk rings: A, unstained with a Sudan black dye band spanning the 2 outer yolk rings; and B, after being stained with potassium dichromate to darken the parts of the yolk laid down during the day. The outer yolk rings are typically narrower and less clear.

black-dyed outer layers measured. The exposed surfaces were then stained with 6% potassium dichromate for 16 hours to accentuate the daily pattern of deposition in the yolks (*cf.* Figs. 1 A & B). This darkened the more transparent rings, which are those laid down during the day (Grau 1984). It was assumed that Sudan black dye was deposited in the yolk on the day the bird was dosed, and that the eggs were laid on the day they were found in the nest, although this could have happened up to 24 hours earlier.

The results are presented in Table 1 for each of the 9 eggs which had a Sudan black dye band in their yolk. The number of dark yolk rings in these eggs varied from 13-15 and the following lag period took from 3-5 days. Together these show that yolk formation in the little penguin starts on average 18 (range 17-20) days before the egg is laid.

The other penguin species studied appear to take a little longer to produce their eggs than little penguins. In the Adelie penguin, yolk formation takes on average 15 days with a lag period of 6 days (Astheimer & Grau 1985), and for the Fiordland crested penguin it is 16 and 7 days, respectively (Grau 1982). These results are remarkably similar considering the 3 species are from different genera. The biggest difference is in the length of their lag periods which are consistent for the respective species both between and within clutches. It is not clear what contributes to the relatively long lag periods in seabirds. The obvious possibilities are a delay in ovulation, prolonged formation of the egg after ovulation, or its retention in the oviduct for a period. For penguins the evidence favours delayed ovulation (Astheimer & Grau 1985).

During the weeks prior to laying, little penguins of breeding age alternate periods ashore at their nest sites with periods at sea. This is a regular feature of their behaviour which resembles the pre-laying presence and exodus common in petrels (Procellariiformes; Warham 1990). On Phillip Island, Australia, the numbers of little penguins ashore peak around 28-21 and 7-1 days before the first egg is laid (Reilly & Cullen 1981) with females being absent from the colony for an average of 10.6 (range 6-17) days during the intervening period (Chiaradia & Kerry 1999). For the white-flippered penguin the exodus of females starts about 20 days before the first egg is laid, which is just before yolk formation begins. They are absent from their nest sites until about 8 days before laying after which they spend progressively more time ashore alternating this with days at sea. The females then spend at least 1 day at sea between laying their first and second egg after which the male takes the first incubation shift (C.N. Challies, *unpubl. data*).

While egg formation in little penguins occurs mainly when the female is at sea, and presumably feeding, this is not the case with all penguins. In most species the females return to their nest sites before

Table 1. Timing of egg formation in the little penguin. Number of yolk rings is the number of dark stained rings in the yolk; lag time is the number of days between the date the Sudan black was given and the egg was laid less the number of yolk rings covered by the dye band; and egg formation is the number of days from the initiation of yolk formation until the egg was laid (*i.e.,* number of yolk rings plus lag time). Egg numbers 1 & 2 were A eggs, while the others were B eggs.

Egg number	Number of yolk rings	'Lag' time (days)	Egg formation (days)
1	14	3	17
2	13	5	18
3	14	3	17
4	15	3	18
5	14	4	18
6	15	5	20
7	14	5	19
8	13	5	18
9	13	5	18
Means	13.9	4.2	18.1

yolk formation is complete (Ancel *et al*. 2013), and rely on stored energy for varying periods prior to and during laying. Adelie penguins for example return to their nest sites during yolk formation and fast for *c*.14 days (Astheimer & Grau 1985), while Fiordland crested penguins arrive before yolk formation starts and fast for *c*.30 days (Grau 1982; Warham 1974).

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