Condor 84:172–177

EGG FORMATION IN FIORDLAND CRESTED PENGUINS (*EUDYPTES PACHYRHYNCHUS*)

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ABSTRACT.—Female Fiordland Crested Penguins (*Eudyptes pachyrhynchus*) lay a clutch of two eggs, the second being significantly larger than the first. To investigate this disparity, I gave experimental birds a single oral dose of 300 mg Sudan black B dye. This was deposited in their yolks over a period of several days, and helped to reveal the timing of egg formation events. The yolks were pale yellow and lacked marked structure except for the dye band. Staining the yolks with dichromate revealed faint daily rings. For both first and second eggs, mean time for yolk formation was 16 days (range: 14–18 days). The larger, second eggs contained higher proportions of albumen and lower proportions of yolk than first eggs; shell proportions were constant. During the female's fasting period of egg formation, the amount of nutrients transferred from tissues to eggs was not large compared with body reserves. The mean interval between date of yolk completion and laying was seven days (range: four to nine days). The relative positions of the dye bands showed that the first yolk grew for several days before the second yolk began to grow.

In contrast to other marine birds, the two eggs laid by crested penguins (*Eudyptes* spp.) differ significantly in size: the second is markedly larger than the first. Even if both eggs hatch, only one chick is reared (Warham 1975). In the Fiordland Crested Penguin (*E. pachyrhynchus*) birds arrive at the nesting sites (South Island, New Zealand) in late June, at the beginning of austral winter (Warham 1974), and lay eggs from 26 July to 14 August, with a peak on 6 August. Thus, after a fairly long "courtship," their egg-laying period is short and rather synchronous among pairs. The adults do not feed from the time they arrive ashore until after the second egg is laid, 30 to 40 days later. The second egg is about 20% larger than the first, and is laid four days later (range 2–6). Full incubation is begun by the male after the laying of the second egg, and the chicks hatch 31–36 days later. There is no regularity as to which egg hatches first.

In this study I sought to learn more about the significance of size disparity in the two eggs, by determining: (1) yolk formation times; (2) whether the two yolks begin to grow at the same time, or one begins to grow four days before the other; (3) the factors responsible for differences in egg size; and (4) whether the times elapsing between completion of yolk formation and laying differ for first and second eggs.

METHODS

In order to provide a time marker in yolk formation, I used the dye-feeding technique of Gilbert (1972), followed by dichromate staining of yolk (Grau 1976) to reveal the total time of yolk formation. In this combined method, the dye ring in the yolk indicated, within a few hours, the time when the dye capsule was given. The daily variation in yolk structure that became apparent after dichromate staining enabled me to count days until yolk formation ceased, and thus determine the date of yolk completion.

On 23 July 1979, 40 penguins nesting at Jackson Head, South Westland, New Zealand (44°S, 169°15′E) were caught with the use of hand nets and wire hooks, and fed doses of dye. Each bird was given two No. 0 gelatin capsules, each containing a mixture of 150 mg Sudan black B and 450 mg dried chicken egg yolk. Dosed birds were marked on the breast with a solution of either picric acid or rhodamine B. When both members of a pair were present, only the smaller-billed, the female, was dosed. Those birds not clearly paired were also dosed, even though some were probably males.

When the area was next visited on 30 July, I found seven eggs, and collected six. In order to minimize adverse effects on reproduction in the colony, I collected only one egg from each nest. Further observations and collections were made on 31 July and 2, 3, and 5 August. Other days were omitted to minimize disturbance.

Eggs were kept at temperatures below 10°C and at high humidity until they were taken to the laboratory on 6 August, where they were weighed, photographed, degree of shell staining noted, degassed in vacuum overnight, and

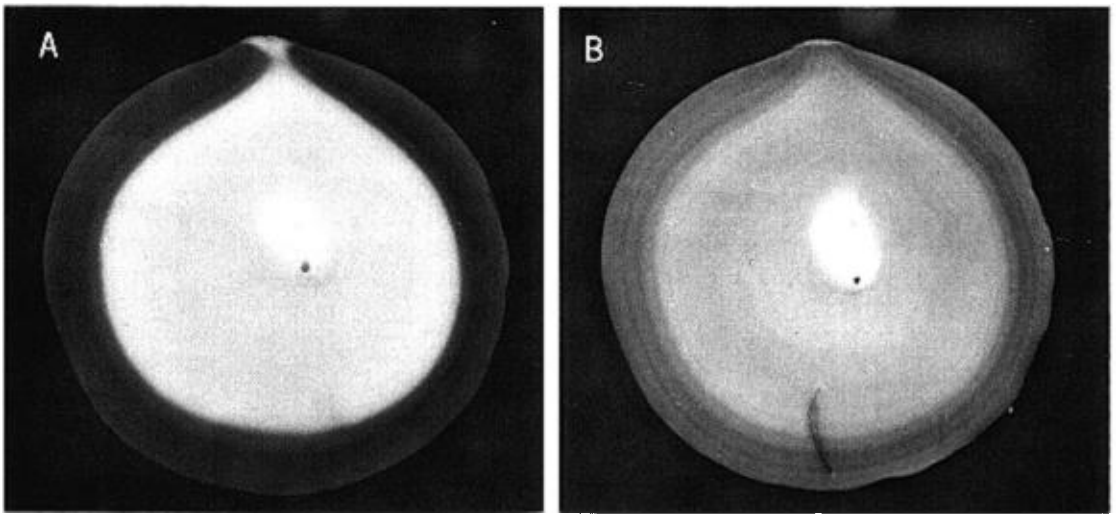

FIGURE 1. Slices of yolk from the egg of a Fiordland Crested Penguin that was given Sudan black B dye on 23 July, 12 days before laying (3 August). (A) Slice of unstained yolk showing a dye band 4.7 mm thick. The blastoderm area was not dyed. Total diameter 37.4 mm. (B) Slice of yolk stained with dichromate. Rings were difficult to see even in this egg, in which they showed more clearly than in most of the eggs in this study. The split in the yolk is an artifact of fixation. The dye band extends to 27 July. The total number of rings was 16, of which 11 were deposited before the dye was given. The time from first ring deposition to laying was 23 days.

frozen at −20°C. Shells were removed, dried in air at 65°C, and weighed. Yolks were fixed in 4% formalin at 65°C for 16 h, weighed, and cut in half. One half was put into 6% potassium dichromate for 16 h at 65°C. Slices were made, washed in water, and photographed. Rings of pale- and dark-staining yolk were counted, the positions of the dye bands were noted and the bands measured; yolk diameter was also determined. Albumen weight was calculated as the difference between the total fresh weight and the combined weight of shell and fixed yolk. Thickness of dry shell was measured at several places with vernier calipers.

Samples of yolk and albumen from two frozen first eggs of clutches were analyzed to determine dry matter and lipid as follows: yolk was dried to constant weight in an oven at 65°C and the residue was extracted with acetone followed by hexane-acetone (5:1). Albumen was dried similarly, but was not extracted.

In order to calculate the daily nutrient deposition, I considered the nonlipid dry matter to be protein containing gross energy of 5.65 kcal per g, and the lipid 9.5 kcal per g (Ricklefs 1977). Using an enlarged photograph of a dichromate-stained section of yolk from a representative first egg, I measured each ring, and calculated the actual radius. Then, assuming that the yolk was a sphere, I calculated the daily yolk volume increments, omitting the small density correction between volume and weight. A representative second egg was similarly treated. Amounts of protein, lipid, and total energy added to the yolk each day were then calculated. I assumed that albumen deposition proceeded over a four-day period, beginning the day after yolk completion.

RESULTS

Yolks of all eggs were uniformly pale yellow with little structural variation, except for four eggs, which had a pale pink area 8 mm in diameter immediately surrounding the center, and the 12 eggs that contained a blue band of dye extending to the yolk margin (Fig. 1). The dye bands varied in thickness from 0.5 to 6.0 mm.

The mean number of rings in dichromate-stained yolks was 15.9 for first and 16.4 for second eggs (Table 2); the mean for all eggs was 16.1. In birds that feed daily, yolk ring structure is clear (Roudybush et al. 1979); however in this species, which fasts throughout egg formation, it was difficult to count the rings because some yolks appeared almost homogeneous. Because of this uncertainty, differences between first and second eggs cannot be considered significant. Date of yolk completion was determined by counting rings only in the blue-dyed band (Fig. 2).

The total time for egg formation could be determined only when dye was present, even if the date of laying and the number of rings were known, because the time between yolk completion and laying could not be determined without being able to assign a date to one of the rings.

The mean interval between yolk completion and egg laying was 6.8 days. In first eggs it was

TABLE 1. Times of yolk and total egg formation in Fiordland Crested Penguins. Dye was fed 23 July. Means exclude numbers marked (?). Clutch position of eggs marked (*) was assumed on the basis of their larger size. Ring numbers refer to yolk material that is stained darkly by dichromate. It is assumed that one dark ring is deposited each day.

Clutch position	Dye band thickness, mm	Yolk diameter, mm	Rings in dye band, number	Date yolk completed	Date laid	Time between yolk completion and laying, days	Total rings, number	Time for egg formation, days
First eggs, dye present	2.0	34.9	4	26 July	31 July	5	14	19
	5.3	33.6	6	28 July	5 Aug	7	14	21
	5.0	35.7	7	29 July	2 Aug	4	15	19
	5.9	33.2	7	29 July	4 Aug	6	15	21
	5.3	36.0	8	30 July	4 Aug	6	18	24
	3.7	36.3	5	27 July	3 Aug	8	18	26
	2.2	35.0	3	25 July	2 Aug	9	18 (?)	27 (?)
	2.5	35.0	4	26 July	3 Aug	8	11 (?)	19 (?)
					Means	6.6	15.7	21.7
First eggs, dye absent							14	
							15	
							15	
							16	
							18	
							18	
							16.0	
					First eggs, mean		15.9	
Second eggs, dye present	4.7	37.4	5	27 July	3 Aug	7	16	23
	2.6	36.7	4	26 July	3 Aug	8	17	25
	3.6	37.8	5	27 July	2 Aug	6	18	24
	0.4	35.3	1	23 July	31 July	8	18	25
					Means	7.1	17.3	24.3
Second eggs, dye absent							14*	
							16*	
							16 (?)	
					Second eggs, mean		16.4	
Not known							16	
							16	
					Means, all eggs 6.8		16.1	22.7

6.6 days, with a range of four to nine. (Two of these eggs were omitted because only the outer rings were sufficiently distinct to be counted.) In the second eggs the mean for four eggs was 7.1 days, with a range of six to eight days.

The weights, dimensions, and gross compositions of eight first and seven second eggs

TABLE 2. Composition of eggs of Fiordland Crested Penguins. All first eggs and four of the second eggs contained dye. Figures are mean values ± standard deviations.

Number	This study		Warham (1974)	
	First eggs 8	Second eggs 7[b]	Small eggs (*n*)	Large eggs (*n*)
** Length, mm	68.2 ± 1.3	73.0 ± 3.2	68.0 ± 2.6 (134)	71.2 ± 2.3 (121)
**Breadth, mm	50.8 ± 1.5	54.5 ± 1.5	51.9 ± 1.9 (134)	55.0 ± 1.8 (121)
**Weight, g	96.8 ± 7.7	120.3 ± 9.4	99.9 ± 7.8 (66)	120.3 ± 8.6 (52)
**Shell weight, g	10.5 ± 1.6	13.4 ± 1.5		
**Contents weight,[a] g	86.2 ± 6.5	106.9 ± 8.1		
*Yolk weight, g	24.5 ± 2.5	28.0 ± 1.9		
**Albumen weight,[a] g	61.7 ± 4.4	78.9 ± 6.3		
Shell thickness, mm	0.50 ± 0.07	0.54 ± 0.04		
Shell, % of total	10.8 ± 1.1	11.1 ± 0.6		
**Yolk, % of total	25.3 ± 1.4	23.3 ± 0.4		
**Albumen, % of total	63.8 ± 1.4	65.6 ± 0.7		

[a] Calculated by difference.
[b] Two of these eggs were not known to be second eggs, but were assumed to be because each was the larger of a pair.
* Difference between first and second eggs statistically significant ($P < 0.05$, *t*-test).
** Difference significant ($P < 0.01$).

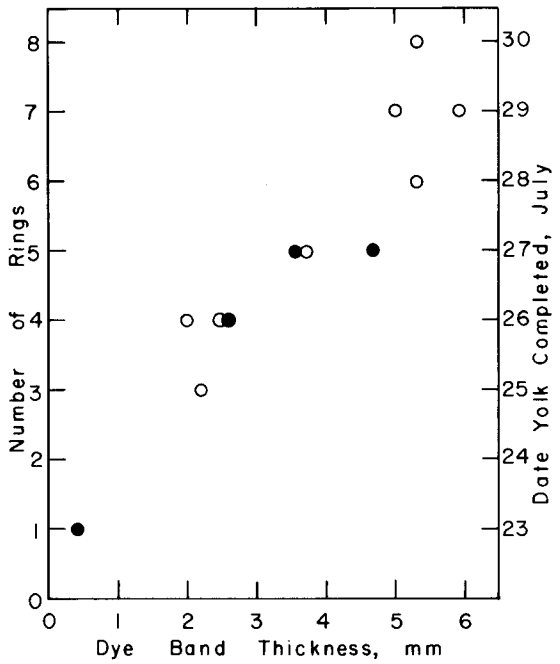

FIGURE 2. Numbers of dichromate-stained rings in dye bands of yolks after dosing penguins with Sudan black B. Dates when yolk deposition was completed are estimated from ring counts and the date when birds were dosed (23 July). Open circles are first eggs; closed circles are second eggs.

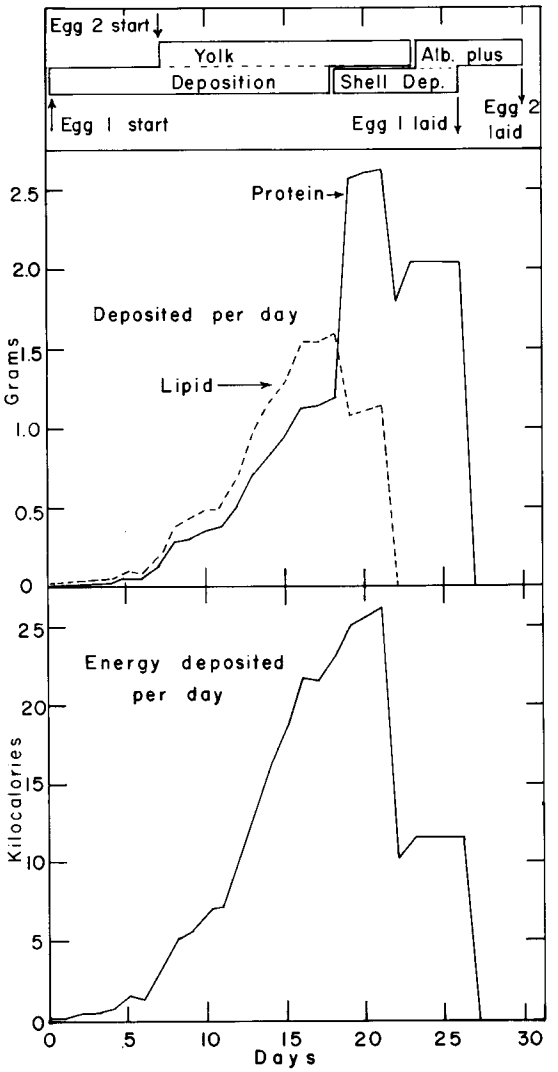

FIGURE 3. Estimated amounts of protein, lipid, and energy deposited daily in the two eggs laid by a Fiordland Crested Penguin. See text for details.

are given in Table 2. As noted by Warham (1974; see also Table 2), second eggs are larger; in the present study they weighed 24% more than first eggs. The yolks of second eggs weighed only 14% more than first eggs, compared with the albumens, which weighed 28.5% more. Yolk comprised 25.3% of first eggs, and 23.3% of second eggs. Thus second eggs contained a slightly higher proportion of albumen than first eggs. The mean difference in weight between the first and second eggs was 23.6 g, 73% of which was albumen, 15% yolk, and 12% shell.

The yolks of the two eggs analyzed contained 49.1 and 48.3% dry matter, 28.5 and 27.8% lipid, and 20.5 and 20.5% nonlipid dry matter. The albumen of these same eggs contained 10.3% and 10.9% dry matter.

An estimate of the amounts of nutrients deposited daily in the two eggs was plotted against time (Fig. 3). The daily increments of lipid, protein, and energy rose slowly for the first 10 days, then increased sharply for the next 10 days. When yolk formation was completed, lipid deposition ceased, but deposition of energetic nutrients, particularly protein, remained high for several more days before egg formation was completed. Nutrients deposited each day were calculated solely from yolk volumes up to day 16. After that, albumen nutrients were extrapolated from data on laying hens (Gilbert 1972), and may be less accurate.

DISCUSSION

The methods I used here have not previously been applied to penguins, but have been used to study egg formation in other birds. In order to apply them, I made four assumptions:

(1) *The dye is an accurate time marker.* Strong evidence from autopsy experiments with domestic birds supports this assumption (Gilbert 1972), but no data exist for free-living birds. In chickens (*Gallus gallus, var. domesticus*), approximately four hours elapsed between the feeding of 10 mg Sudan black B dye and the appearance of a readily visible amount of dye in the yolk. It seems likely that the large dose of dye given in the present study would

be deposited in the yolk during the first few hours after dosing.

(2) *Feeding the dye did not affect the formation of yolk or other egg components, or the laying of the egg.* The dye rings were found at different distances from the margins in the different eggs (Fig. 2); in other words, the yolks were in different stages of completion when the dye was fed, hence different times elapsed from the date of the dye marker until the date of yolk completion. I saw no evidence of abnormal behavioral changes, nest desertions, premature oviposition, or unusual time lapses between first and second eggs of a clutch.

(3) *Pairs of light and dark rings represent daily variation in yolk structure.* Experimental data from studies with chickens and Japanese Quail (*Coturnix coturnix japonica*) strongly support this assumption (Grau 1976). Data obtained from free-living birds that have been fed dyes (Grau et al. 1978, Astheimer et al. 1979), or from birds that have ingested natural pigments (Roudybush et al. 1979), are also consistent with this assumption. In the present study, the thickness of the dye band was directly related to the number of rings laid down after the dye was fed (Fig. 2). The best way to check this assumption in free-living birds would be to feed minimum doses of different dyes at known intervals of two or more days and count the number of rings between the doses. This has been done in experiments with two captive Emus (*Dromaius novahollandiae*, in which daily rings of dichromate-stained yolk were visible between dye rings (Hirsch and Grau 1981), and in free-living Cassin's Auklets (*Ptychoramphus aleuticus*; Grau et al. 1978, Astheimer et al. 1979). Unfortunately, it was not feasible to perform this experiment as part of the present study.

(4) *Estimates of time of laying are accurate.* Data on time of laying would have been more accurate had the nests been checked every day. However, I feared that daily disturbance would adversely affect laying, so I visited nests mainly on alternate days and used the extent of shell staining as a guide in estimating laying time of some eggs.

In all of the 12 eggs that contained dye, the times of yolk formation and of total egg formation were similar, with little difference between first and second eggs of the clutch. It was far more difficult to count the rings of these eggs than those of some other seabirds (Roudybush et al. 1979), yet when the rings of the same yolks were recounted several weeks apart, the counts were consistent.

The probable period for yolk formation in this species is 16 days (Table 1). The numbers of rings within the dye bands and the band thicknesses, combined with the observations of laying dates, indicate that approximately seven days elapsed between the completion of yolk formation and laying. This same delay was found in both first and second eggs. When combined with the observation that yolk formation took essentially the same time for both eggs, it indicates that the second yolk began to grow about four days after the first. This egg was laid some 23 days later, also about four days after the first. The lag of seven days between yolk completion and laying is long compared with domestic fowl (Gilbert 1972), Turkeys (*Meleagris gallopavo*; Bacon and Cherms 1968), and Japanese Quail (Bacon and Koontz 1971), in which the interval is rarely longer than one day.

It is at present impossible to account for the lag between the completion of yolk formation and laying. One possible reason is that the completed yolk may remain in the follicle for several days before ovulation while tissue breakdown is proceeding to release the amino acids needed for albumen formation. Then, once the mature ovum is released, fertilization and deposition of the albumen and shell proceed relatively rapidly, as in the domestic fowl (Warren and Scott 1935). Another possibility is that ovulation occurs soon after yolk completion and that albumen and shell deposition follow slowly over several days. Actual timing, however, may fall between these two extremes. Warham (1974) noted that copulation in this species "appears to be restricted to the few days before laying."

Yolk formation begins during the second week in July, shortly after the birds came ashore. From arrival until the second egg is laid and feeding is resumed 30 to 40 days later, females lose 34% of their original 4,100 g and males lose 26% of their 4,500 g (Warham 1974). The extra 250 g lost by females is approximately equal to the 230 g of the two eggs combined. Amounts of tissues that would yield the principal egg components were calculated assuming that muscle tissue contains 25.2% protein and 5.6% lipid, based on data from Canada Geese (*Branta canadensis*; Raveling 1979); that adipose tissue contains 4% protein, based on composition of separable animal fat (Watt and Merrill 1963), and 84% lipid, based on data from passerines (Johnston 1970); and that medullary bone contains 19.3% calcium, based on data from pigeons (Ascenzi et al. 1963). From these assumptions, the data of Table 2, and the analysis of penguin eggs presented above, the equivalent of 100 g muscle tissue, 11 g adipose tissue and 50 g bone were needed to provide the following nutrients to the total mass of two eggs: 25.4

g protein; 14.8 g lipid, 284 kcal energy, and 9.6 g calcium.

Penguins that do not eat during the egg formation period provide a striking contrast to the examples of birds cited by King (1973) and by Ricklefs (1974), in which food limitations reduce or halt egg production. The heavy demands that egg formation places on the nutrients stored during migration of wild geese (Raveling 1979) far exceed those for the two eggs laid by crested penguins (Warham 1974). The penguins of the present study need, for the early stages of egg formation, relatively small amounts of tissue reserves. Even during the two-week period before laying, the amounts of nutrients transferred daily from tissues to eggs are not large, and the total egg weight represents only 6% of the female's weight when she starts breeding.

The disparity in size between the first and second eggs of the clutch in eudyptids has been envisaged by Warham (1975:221) "as ensuring that when two eggs hatch only one chick survives after the first few days, but that if one egg is lost before hatching, the other, if still available, can take its place effectively." The larger, second egg of the clutch contains a slightly higher proportion of albumen and a lower proportion of yolk than the first, and produces a larger chick; however, size differences are apparently not important in relation to hatching (Warham 1975).

ACKNOWLEDGMENTS

This research was done during a sabbatical leave of absence at the University of Canterbury, Christchurch, New Zealand. Valuable field assistance was given by Graham J. Wilson, Paul Ensor, and Lois S. Grau. The advice of John Warham and the hospitality of Professor W. C. Clark and the Department of Zoology are gratefully acknowledged. David G. Ainley provided helpful suggestions for improving the manuscript.

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Condor 84:177

RECENT PUBLICATION

International Vertebrate Morphologists Directory 1980.— Compiled and edited by G. Fleischer. 1981. H.-R. Duncker, Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen. 182 p. Paper cover. Source: the publisher, Aulweg 123, D-6300 Giessen, West Germany. This is a directory to 646 vertebrate morphologists from 36 countries, those who responded to Professor Duncker's first solicitation for entries. Each entry gives the scientist's name, address, and specialty. Following this catalog list are indexes according to vertebrate group, organ system, method, and country. The directory should foster communication among fellow-specialists (this reviewer was unaware of some of his listed colleagues) and assist non-morphologists in finding such people when their expertise is needed. Duncker encourages unlisted morphologists to make themselves known to him so that a fuller edition can be published.