EGG FORMATION IN CASSIN'S AUKLET

LEE B. ASTHEIMER[1]

Department of Avian Sciences, University of California, Davis, California 95616 USA

ABSTRACT.—Female Cassin's Auklets (*Ptychoramphus aleuticus*) were given oral doses of lipophilic and proteophilic dyes during their prelaying period. Yolk ring structure in freshly laid eggs was analyzed to determine the timing of yolk deposition using the dye-stained layer as a date marker within the yolk. Additional information from necropsies of five females killed at specific intervals during the formation of a replacement egg indicated that only a single follicle enlarges in the ovary during a reproductive event and this yolk requires about 8 days to complete rapid yolk deposition (RYD). Once RYD is completed, the egg is not laid for a period of 4.2 ± 1.0 days, an interval termed the lag period (Astheimer et al. 1979). Albumen synthesis appears to begin at, or just before, completion of the yolk and continues until approximately 1 day before oviposition. During this time, however, the yolk remains in the follicle. Replacement eggs are laid 14.5 ± 1.7 days following egg removal or natural loss. Combining the above information with egg composition data, the daily deposition of lipid and protein from the female into the egg was computed. Evidence from the size distribution of small (<5 mm) primordial follicles suggests that the auklet ovary undergoes a cycle of follicular growth and atresia before a single follicle is selected for RYD. Based on these findings, and the correlation between initiation of spring egg laying and decreased sea surface temperatures for Farallon Island auklets, I suggest that the onset of RYD is modulated by environmental conditions. *Received 26 August 1985, accepted 17 February 1986.*

THE process and timing of events leading to egg production are integral components of the reproductive cycle, but generally have been neglected in quantitative assessments of the energy costs of reproduction. Recent studies have focused on the temporal aspects of egg formation in seabirds and the consequent energetic cost to the female (Grau 1982, 1984; Grau and Astheimer 1982; Astheimer and Grau 1985; Astheimer 1985). Theoretically, the prelaying period is energetically more demanding for species that lay larger eggs relative to their body mass because females must direct a greater proportion of dietary or stored nutrients to the egg. In addition, for many colonial seabirds, intraspecific competition for nest sites or mates necessitates remaining close to the colony and may restrict foraging range and, indirectly, food availability during this period. The daily energy cost of egg production could be reduced by extending the period of egg formation; however, this must be weighed against the effects of carrying extra mass on locomotory costs.

Most avian biologists would agree that the timing of hatching relative to food availability is critical to nestling survival and thus constitutes a strong selective pressure. However, because periods of incubation and of egg formation are relatively fixed for a given species (Astheimer 1985, Astheimer and Grau 1985, Grau 1984), hatching dates are inextricably linked to the initiation of the clutch in the ovary (with the notable exception of a few Procellariiformes; Boersma and Wheelwright 1979). Although environmental factors are known to influence the timing of recrudescence, endocrine activity, and courtship behavior, it is the beginning of yolk deposition in follicles selected for growth (the follicular hierarchy) that marks the definitive initiation of the clutch and that, barring yolk resorption, will determine the hatching date. The relationship of this process of rapid yolk deposition (RYD) to environmental stimuli has not been investigated in nondomestic avian species.

In this paper I report on studies of egg formation in Cassin's Auklet (*Ptychoramphus aleuticus*) conducted on Southeast Farallon Island (SEFI), California between 1978 and 1982. Cassin's Auklets are small, nocturnal alcids that nest in burrows and rock crevices on islands

off the Pacific coast, from Buldir Island in the Aleutian Island chain, Alaska (52°N), to Guadalupe Island, Baja California, Mexico (26°N; Harrison 1983). The Farallon Islands (34°N), 43 km west of San Francisco, support an auklet breeding colony, estimated at 105,000 birds (Manuwal 1972), that is unusual in its apparent year-round residency in the waters surrounding these islands (Manuwal 1974b).

Although equipped with a pair of lateral incubation patches, Cassin's Auklets lay only a single-egg clutch (Manuwal 1974a). Double brooding commonly occurs in years when first broods are begun early in the season (Boekelheide 1980, Manuwal 1979). The auklets begin excavating burrows and engaging in preliminary courtship activity in early January, and the first eggs usually are laid in March (Manuwal 1979, pers. obs.). Laying synchrony is variable in the Farallon population; in some years laying extends over a 4-month period (Manuwal 1972, Boekelheide pers. comm.). The proximate stimuli that initiate a reproductive effort remain unclear, although there appears to be a highly significant correlation between the onset and synchrony of laying with a decrease in sea surface temperature, itself an indicator of oceanic productivity [Point Reyes Bird Observatory (PRBO) unpubl. data; see Discussion]. I attempted to relate egg laying and the timing of egg formation to sea surface temperatures for the years 1979–1982.

MATERIALS AND METHODS

Two hundred and seventy wooden nest boxes designed for auklets were installed on SEFI in the fall of 1977 (Ainley et al. 1981; nest-box design in Ainley et al. 1979). I had access to these boxes thereafter, and by 1979 most were occupied by mated pairs.

To study the time sequence of yolk deposition, I fed doses of lipophilic dye to prelaying females. This dye is incorporated into yolk lipoproteins, synthesized in the liver, and deposited on the developing follicle in a discrete layer (Gilbert 1971, Grau 1976). Because dye uptake and lipid deposition is rapid when a bird is forming a yolk, the dye effectively marks the date of dosing as a layer within the yolk structure.

In a preliminary study, 9 females were fed a nonrepeating sequence of different dye capsules (25 mg each) to ascertain whether yolk rings in auklet eggs exhibit a circadian periodicity in deposition, as they do in poultry (Riddle 1911, Grau 1976). In eggs from domestic chickens (*Gallus domesticus*) and Japanese

Quail (*Coturnix c. japonica*), each pair of light- and dark-staining rings (as enhanced by treatment with potassium dichromate) is deposited over a 24-h period (Warren and Scott 1935, Opel 1966).

Combinations of the following four dyes were administered at 40-mg doses in 1979: lipophilic dyes Sudan Black B, Sudan IV (red), and Victoria Blue and proteophilic Rhodamine B (magenta). The latter binds to both yolk and albumen proteins and, although a pattern of albumen deposition is not retained in the laid egg, I anticipated that Rhodamine B would bind to albumen being synthesized in the magnum portion of the oviduct on the day the dye was administered. In 1981 only Sudan Black and Rhodamine were given; the dose of the former was doubled to 80 mg. Except for birds treated in the 1978 nonrepeating sequence experiment, no bird was treated more frequently than 5-day intervals.

Because Cassin's Auklets are monomorphic in plumage characteristics, gender recognition was difficult. Therefore, in 1978 and 1979, to ensure dosing the females, both members of pairs were given dye capsules. In 1981 and 1982 bill depth at the anterior border of the nares was measured to distinguish gender, following Nelson (1981), who determined bill-depth ranges for male and female auklets from salvaged carcasses. In 1981 only the smaller-billed member of a pair, presumed to be female, was dosed.

Dyes, contained in number 0 gelatin capsules, were administered orally. Birds were removed from their nest boxes at night, and moistened capsules were placed well down their throats, followed by sufficient water to induce swallowing. Treated individuals were marked on the breast with picric acid (1979) or banded with a number 3 U.S. Fish and Wildlife Service stainless steel band. Birds were normally returned to their nest boxes within 3–5 min of capture. Regurgitation of capsules sometimes occurred, and occasionally red- or blue-stained vegetation was found surrounding a box the day following dosing; eggs were not collected from these birds.

Transit time for passage of visible signs of dye in the excreta was 4–6 h for three birds retained overnight, with a large portion of the dose expelled in the first hour. Transit of the dye through the gut may have been accelerated in birds with empty stomachs, which may have resulted in poor dye absorption. To improve dye uptake in 1981, capsules contained a larger dose of dye and 30–40 mg of dried egg yolk.

All boxes (240 in 1979, 125 in 1981) were checked daily beginning in early March. Dosing was begun after the first egg in the colony was found, on the assumption that a large proportion of the females of pairs found in boxes at that time could be expected to lay within 2 weeks. All dosing was done at night, between 2100 and 0200. In 1979, 224 birds (100 pairs and 24 singles) were given dye, while in 1981 84 females were dosed. During 1981, females that did

not lay within 2 weeks of dosing were given a second dye capsule. After dosing, boxes were checked daily for the presence of an egg and for parent identification (dyed breast or band number). When the parent was recognized as a dosed bird or its mate, the egg was removed, weighed, measured, and stored at 5°C. Seventy-two eggs were collected in 1979 and 65 in 1981.

In the laboratory eggs were placed under vacuum to remove air bubbles in the yolk and frozen at −20°C for 48 h. After fixation in 4% formalin at 65°C for 18 h, yolks were weighed and halved in the plane perpendicular to the chalazae. One half was immersed in 6% potassium dichromate at 65°C for 18 h to enhance the definition of light and dark rings. Central slices were cut from each half, ring pairs were counted on the dichromate-stained slices, and the position of the dye ring was determined. Using the dye ring as a marker for dose date, the dates of initiation and completion of RYD and the difference (number of days) between the completion of RYD and oviposition (i.e. the lag period) were determined.

During 1981, daily checks of nest boxes from which the first egg had been removed continued through July to determine the timing and frequency of replacement laying. All replacement eggs were measured, and the identity of both parents was verified. A second replacement egg (third egg) was laid by 14 females. The regularity of replacement laying permitted me to devise a schedule to kill a small number of females at selected intervals during formation of their replacement egg. Five females were killed between 11 and 15 days after their first egg had been removed to document the size and location of the yolk during the lag period. These auklets were weighed and killed quickly by cervical dislocation. Oviducts and ovaries were removed, photographed, and placed in 10% buffered formalin, with the exception of the enlarged follicles, which were treated as above and analyzed for yolk ring structure. Small ovarian follicles (0.5–4 mm), separated from the ovarian tissue, were counted and ranked according to their diameter. Pre- and postovulatory oviducal tissues were prepared for histological sections.

Egg composition was determined for 43 eggs, with albumen mass computed as the difference between fresh-egg contents and yolk mass. Fixed yolks were separated from adherent albumen with absorbent tissue and weighed to the nearest 0.01 g. Previous comparisons of frozen, fixed, and boiled domestic turkey (*Meleagris gallopavo*) egg components showed no significant differences in the yolk mass obtained by each method (Goldfarb unpubl. data). Albumen was washed from the shells with warm water, and shells, with membranes, were air dried at 60°C and weighed to 0.01 g. The caloric value of 4 auklet yolks was determined using a Parr Adiabatic Calorimeter with benzoic acid as a standard.
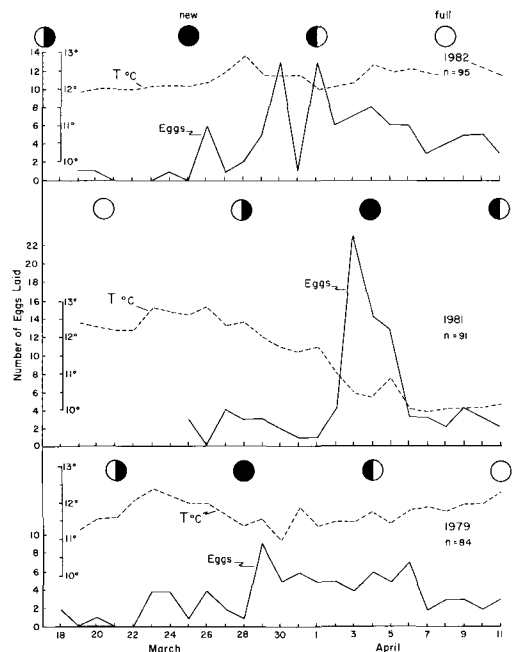

Fig. 1. The pattern of egg laying (solid line) in nest boxes on Southeast Farallon Island (SEFI) in 1979, 1981, and 1982. Inset scale, 10–13°C for sea surface temperatures (broken line) taken daily at SEFI (PRBO unpubl. data). Phases of the moon are indicated.

The daily increment in yolk mass was calculated for 9 eggs on the basis of total yolk mass and the sequential difference between increasing radii defined by the yolk rings (Astheimer and Grau 1985). This method assumes yolk deposition occurs on a spherical surface, although the yolk rings may become compressed or appear irregular following freezing, fixation, and slicing.

Statistical tests follow Zar (1974) and were performed using a statistical package (STATPAK, Northwest Analytical); sample means are reported ±1 SE.

RESULTS

Laying was initiated in nest boxes 1 week later in 1981 than in 1979 and 1982 (Fig. 1). The laying pattern of treated birds closely paralleled that of untreated birds that resided in undisturbed nest boxes and burrows checked less frequently (PRBO unpubl. data). The mean male bill depth of 10.83 ± 0.04 mm differed significantly from the female mean of 9.59 ± 0.05 mm in a paired Student's *t*-test of 99 breeding pairs ($t = 21.60$, $P < 0.001$). These results, from live
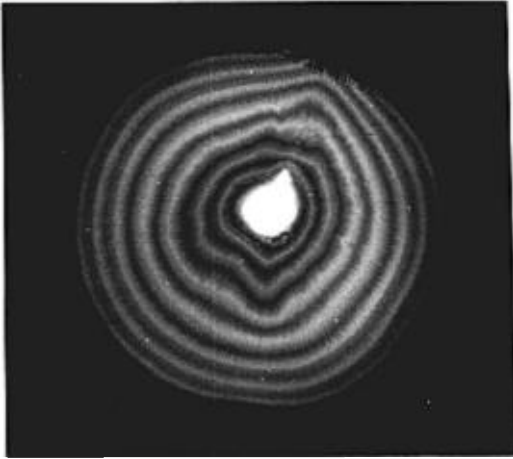
Fig. 2. A central section through the yolk of a Cassin's Auklet egg stained with potassium dichromate. Eight pairs of light- and dark-staining rings can be identified.

mating pairs, are consistent with those obtained by Nelson (1981) from salvaged carcasses.

The 1978 nonrepeating dye sequence experiment (Table 1) demonstrated that in the 4 eggs (of 9) that contained dye rings, each pair of light and dark yolk rings represented approximately 24 h of deposition in auklets, thus confirming the circadian periodicity of yolk deposition and agreeing with previous studies of domestic birds (Grau 1976) and Emus (*Dromaius novaehollandiae*; Hirsch and Grau 1981). The poor uptake of dye, resulting in faint dye rings, however, prompted an increase in dye dosage from 25 to 40 mg in 1979.

Fresh Cassin's Auklet yolks varied in color from yellow to red-orange, the latter indicative of deposition of dietary carotenoids, presumably from euphausiids, the dominant prey item during most breeding seasons (Manuwal 1972, pers. obs.). The central slices of yolk stained with potassium dichromate showed clear light- and dark-staining layers (Fig. 2). Analysis of 94 yolks revealed a mean of 8.1 pairs of yolk rings (range = 6–9; Table 2), which indicates that 7–9 days were required for the completion of RYD. The same length of time was necessary for formation of the yolks of replacement eggs (8.0 days; Table 2).

Most of the contents of dye capsules fed to auklets was excreted as a single bolus; appar-

TABLE 1. Yolk ring analysis from eggs of Cassin's Auklets fed a nonrepeating sequence of dyes.

Bird/ yolk no.	Dose sequence[a]										Yolk ring pattern[b]								
	April			May							Inner ring								Outer ring
	28	29	30	1	2	3	4	5	6	7									
1	B			R							0	0	B	0	0	0	R	0	0
2		B		R							0	0	0	B	B	B	0	B	0
3			B	R							0	B	0	0	R	R	0	0	0
4	B							B		B	B	0	0	0	0	0	0	0	0

[a] B = dose of 25 mg Sudan Black B, R = dose of 25 mg Sudan IV (red).
[b] B = black dye ring, R = red dye ring, 0 = no dye ring present.

ently only a small percentage of the dose was absorbed by plasma yolk lipids. Faint, but definite, dye rings were distinguished in 20 (27.8%) eggs collected in 1979 and 34 (52.0%) in 1981. This allowed determination of the lag period, which averaged 4.2 ± 0.19 days in 1981 (range = 2.5–6 days). In 1979 this period was somewhat longer and more variable (4.6 ± 0.49 days, range = 3–9 days).

The phenomenon of the lag period gives rise to questions concerning the events occurring during this period. By feeding female auklets the proteophilic dye Rhodamine B, I hoped to determine whether albumen was synthesized during a particular segment of the lag period, that is, between 1 and 5 days before the auklets laid. Of the eggs laid by birds dosed with Rhodamine during their lag period, 39% (25 of 64) had magenta-stained albumen. An additional 20.4% of the 1981 eggs laid by dosed females contained albumen stained a fluorescent chartreuse green. Assuming that the intensity of albumen pigmentation can be correlated with the amount of Rhodamine deposited in the albumen, and that Rhodamine deposition can be used as a qualitative estimate of albumen synthesis, it appears that the largest amount of albumen is synthesized in the early half of the lag period (Fig. 3). The occurrence of green albumen in eggs from birds dosed 3–9 days before laying (Fig. 3) may be due to deposition of a metabolic by-product of Rhodamine B, since samples of both wet and dry Rhodamine- and green-stained albumen fluoresce intensely under UV light (254 nm), while dried white albumen from eggs laid by untreated birds does not. The bright chartreuse pigment was unique to eggs from Rhodamine-treated birds and also occurred in similarly treated Adélie Penguin (*Pygoscelis adeliae*) eggs (Astheimer and Grau 1985).
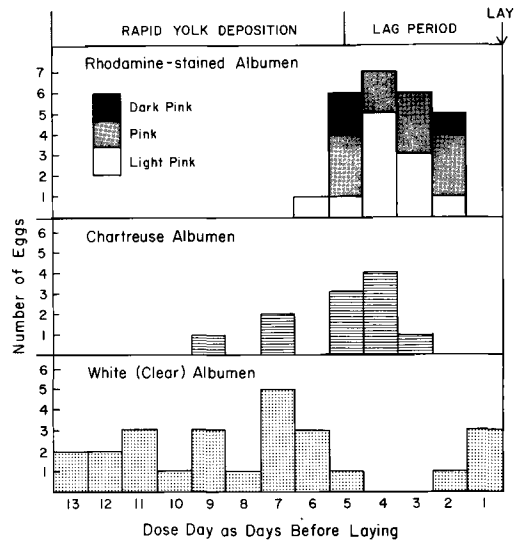

Fig. 3.   The distribution of albumen color in eggs laid by birds treated with 25 mg of Rhodamine B 1–15 days before laying.

After removal of 86 eggs from banded auklets in 1981, 78 (90.7%) of these females laid a replacement egg, usually in the same or a nearby nest box with the same mate (Astheimer unpubl. data). The mean time between the removal of the first egg and laying of the replacement egg (i.e. the relaying interval) was 14.5 days (Table 3). In 6 instances, not included in the above mean, the elapsed time was 24–30 days, or approximately double the expected relaying interval; several of these cases involved nest-box switches or disturbances by researchers. The relaying interval corresponds well with the combined time required for RYD (8–9 days) and the observed lag period (4–6 days). The additional 1–4 days may be necessary for hormonal changes required to reinitiate the pro-

TABLE 2.   Comparison of first and replacement eggs in Cassin's Auklets.

	First egg			Replacement egg				
	Mean	SE	*n*	Mean	SE	*n*	*t*-stat[a]	
Rapid yolk deposition (days)	8.12	0.06	94	8.01	0.26	6	0.463	NS
Relaying interval[b] (days)	14.45	0.21	70	15.77	0.31	14	2.386	*
Length (mm)	46.64	0.26	42	46.56	0.23	42	0.114	NS
Width (mm)	33.36	0.17	42	33.31	0.19	42	0.136	NS

[a] Paired *t*-tests between first and replacement egg data. * = $P < 0.05$, NS = not significant.
[b] First-egg relay interval is between 1st and 2nd eggs; replacement relay interval is between 2nd and 3rd eggs.

TABLE 3. Characteristics of egg formation in Cassin's Auklets.

	Bird identification							
	Birds killed for necropsy					Salvaged carcasses		
	A	B	C	D	E	F	G	H
Female mass (g)	209	203	196	—	245	—	—	—
Hematocrit (%)	49.3	49.0	49.4	41.7	41.4	—	—	—
Serum Ca (mg/dl)	21.4	30.0	16.8	11.0	—	—	—	—
Days between egg removal and death	11.5	12	14	15	15	—	—	—
Stage of egg formation[a]	OF	OF	OF	OF	SH	OF	POV	POV
No. of follicles in RYD	1	1	1	1	0	1	0	0
No. of yolk rings	7	7–8	8.5	8	8	—	—	—
Yolk mass (g)	6.52	7.98	8.62	7.75	10.18	—	—	—
Yolk diameter (mm)	22.8	23.5	24.0	22.8	24.5	19.0	—	—
Oviduct								
Length (mm)	—	234.9	270.5	292.0	—	182	37.6	78.0
Mass (g)	—	9.45	10.55	14.25	—	8.7	0.4	0.9
Magnum width (mm)	12.0	9.7	13.5	11.2	—	8.8	2.4	4.2
Follicular hierarchy (no. of follicles <4 mm)								
Size class								
>3 mm	0	0	0	0	1	1	1	0
3 mm	1	1	1	1	2	1	4	3
2–3 mm	10	3	7	7	1	5	5	6
1–2 mm	17	6	4	30	5	6	11	9
<1 mm	42	9	43	32	26	21	0	27

[a] OF = ovum in follicle, POV = postovulatory follicle, SH = shelled egg in uterus.

cess of egg formation (see Discussion). Surprisingly, removal of an egg at various times during the 37–42-day auklet incubation cycle does not significantly affect the relaying interval, although replacement time tends to be slightly shorter (14.8 ± 0.67 days, $n = 5$) when removal occurs in the last half of incubation than in the first half (15.8 ± 0.30 days, $n = 7$, excluding eggs removed within 1 day of laying). In 14 incidents where the replacement egg was removed, a second replacement was laid, with a somewhat longer relaying interval (Table 2). Paired measurements of length, width, and RYD periods of first and second eggs laid by the same female were compared and did not differ significantly (Table 2).

Results from necropsies of 5 females killed during the last third of their relaying interval and of 3 salvaged carcasses showed either one enlarged ovum contained in a follicle or one postovulatory follicle (Table 3). The timing of formation of the replacement egg relative to the day of removal of the first egg is presented in Fig. 4. These results indicate that the completed yolk remains in the follicle until its re-

lease, about 24 h before laying (Fig. 5). Note (Fig. 5) the completed yolk and the albumen-replete magnum of bird B (5A) and the shelled egg in the uterus and the albumen-depleted magnum of bird E (5B). Follicular retention of the ovum is substantiated by birds C and D (Table 3).

Based on the number of yolk rings present in the single enlarged ova of birds A–D, RYD was completed at the time of sacrifice, with the possible exception of bird A, whose yolk had only 7 pairs of yolk rings. The masses, however, are 0.4 g (4.5%) to 2.5 g (27%) less than the mean yolk mass of freshly laid eggs (Table 4). This discrepancy may be explained by the process of plumping, the influx of water into albumen just before shell deposition. During the passage of the egg through the oviduct, the yolk may also experience an increase in water content during plumping. Draper (1966) found that in chickens the water content of oviposited yolk was 8% greater than that of oviducal yolk ($n = 10$). It is also possible that in the smallest of these yolks (A and D) the last layer of yolk was not completed.
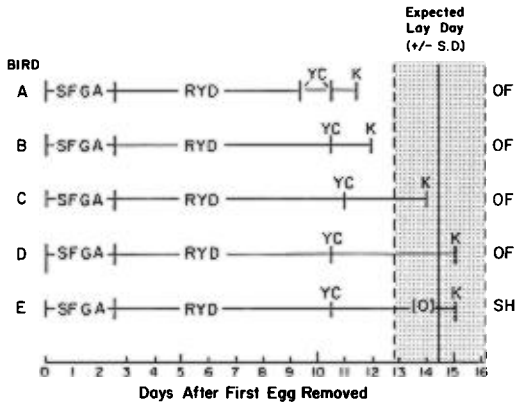
Fig. 4. The timing of egg formation for 5 auklets necropsied during the formation of a replacement egg, where the first egg was removed on day 0. SFGA = estimated period of small follicle growth and atresia cycle (length of this period estimated from that in chickens; see text), RYD = rapid yolk deposition, YC = yolk completion, K = bird killed, OF = enlarged ovum in follicle, SH = shelled egg in uterus, (O) = estimated day of ovulation. Note: 2.5 days are allowed after egg removal for the enlargement of the primordial follicles (see Discussion).

Composition of 43 eggs is presented in Table 4. The mean caloric value of auklet yolk was 7.35 ± 0.007 kcal/g dry weight ($n = 4$).

DISCUSSION

Yolk formation by Cassin's Auklets is a rapid process, particularly when compared with Procellariiformes of similar size. For example, the Fairy Prion (*Pachyptila turtur*) also lays a one-egg clutch, but requires twice as long as a Cassin's Auklet to form a yolk of comparable mass (16.7 days to produce a yolk of 9.09 g, $n = 22$; Fig. 6). When synthesis is expressed as average total yolk mass/mean total days of yolk deposition as a crude index for species comparison, however, yolk formation in Cassin's Auklets is somewhat slower than in other alcids for which data are available: Cassin's Auklet, 1.13 g/day; Common Murre (*Uria aalge*), 2.34 g/day; Tufted Puffin (*Fratercula cirrhata*), 3.13 g/day; Ancient Murrelet (*Synthliboramphus antiquus*), 1.94 g/day; and Pigeon Guillemot (*Cepphus columba*), 1.19 g/day (Grau et al. 1978, Roudybush et al. 1979, Grau and Astheimer 1982, Astheimer unpubl. data.). The latter two species, which exhibit rel-
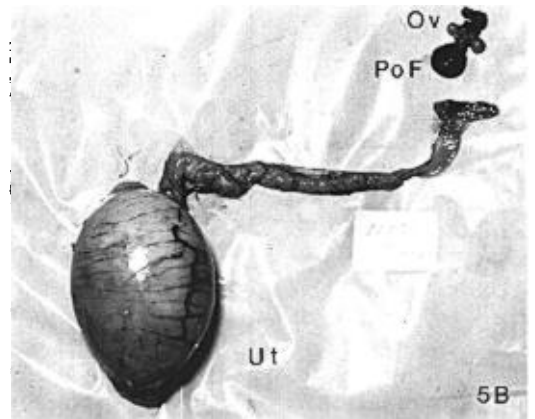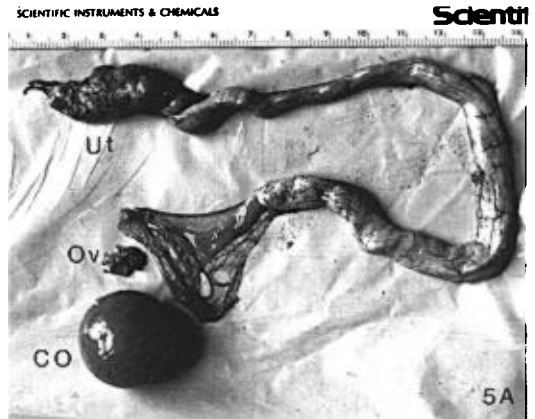

Fig. 5. Oviducts and ovaries from Cassin's Auklets killed 12 (5A) and 15 (5B) days after removal of their first egg. In 5A the single completed ovum (CO) is held in the follicle and the oviduct is enlarged. The yolk of the shelled egg in the uterus (Ut) of 5B was ovulated from the single postovulatory follicle (PoF) apparent in the ovary (Ov).

atively slow rates of deposition, both lay a two-egg clutch. This index ignores other important intraspecific differences such as female body mass, relative yolk mass, and the actual rate of yolk deposition.

The total time required for a female Cassin's Auklet to form its single egg was estimated at 15 days (range = 12.5–17.5 days). This includes 2.5 days growth of the small follicles, 8.1 days RYD on the primary follicle, and 4.2 days lag period. The time required for small follicular growth is not known empirically and was estimated here (Gilbert et al. 1983; see below). The assumption that the presence of Rhoda-

TABLE 4. Cassin's Auklet egg composition ($n = 43$).

	Mean	SE	% FNBM[a]
Fresh mass	28.19	0.34	16.59
Shell			
Mass	1.91	0.03	
%	6.77	0.07	
Yolk			
Mass	9.04	0.12	5.32
%	32.31	0.41	
Albumen			
Mass	17.23	0.28	10.13
%	61.03	1.47	

[a] FNBM = female nonbreeding body mass = 170 g (Fry et al. unpubl. data).

Fig. 6. Cumulative yolk growth in Cassin's Auklet and the Fairy Prion.

mine-stained albumen in a laid egg indicates the albumen was being synthesized on the dose day may not be completely justified, because this water-soluble dye is pervasive and stains all body tissues. Excretion of Rhodamine may be dependent on the strength of its binding to protein or on the rate of protein turnover. The distribution of albumen color and dye intensity in relation to the timing of egg formation, however, reveals a pattern that suggests that albumen is synthesized 2–5 days before laying, an interval that corresponds surprisingly well to the lag period (Fig. 3). The constancy in the duration of both the RYD and lag periods between years, and the predictable replacement interval, attest to the determinant nature of the process of egg formation.

From determinations of the mean daily increment in yolk mass ($n = 9$), I calculated the amount of lipid and protein (Fig. 7, top) and the corresponding energy content (Fig. 7, bottom) of material deposited on the yolk. All albumen dry matter (12% of wet mass) was considered to be protein, containing gross energy of 5.65 kcal/g (Ricklefs 1977), while the gross energy of auklet yolk was determined empirically to be 7.35 kcal/g dry yolk mass (dry mass being 48.5% of wet yolk mass). The mass of albumen synthesized daily during the lag period (days 9–12 of egg formation; Fig. 7, bottom) was assumed to be constant because, although it appears to be greatest early in the lag period (days 9 and 10; Fig. 3), no quantitative estimates of daily albumen synthesis were made. Under this assumption, the total albumen protein content was divided evenly over the lag period and was estimated to be 0.52
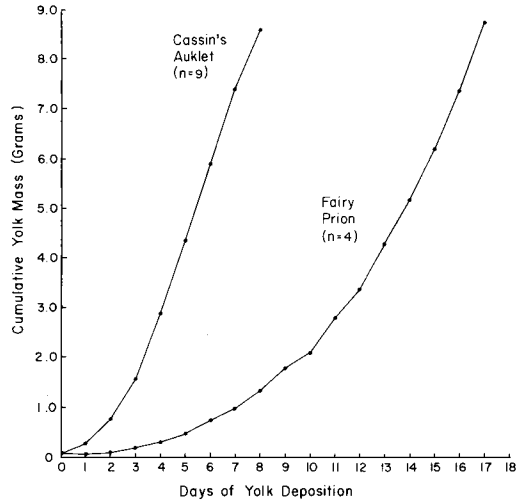
g/day, 1.6 times that deposited during maximal yolk deposition (about 0.33 g/day on days 4–7 in Fig. 7).

The basal metabolic rate (BMR) of an auklet-size nonpasserine (170 g nonbreeding female mass; Fry et al. unpubl. data) was estimated to be 22.7 kcal/day (allometric equation for non-passerines, Kendeigh et al. 1977). Assuming a conversion efficiency of 77% (Brody 1945) for synthesis of egg lipids and proteins, the peak daily cost of egg production during RYD was estimated to be 7.27 kcal/day, or 32% of estimated BMR, of which only about 2.4 kcal (10.6% of estimated BMR) constitute yolk protein production. During the lag phase, when only albumen proteins are being synthesized, the average daily cost was estimated as 3.86 kcal/day, or 17% of BMR. The extent to which synthesis of particular yolk or albumen proteins may limit egg formation is unknown, but there may be an overall energetic benefit in reducing daily protein demand by extending albumen synthesis over the 4-day lag period.

The observed lag period was much longer for Cassin's Auklets than the 24–26 h observed in domestic hens (Warren and Scott 1935), Japanese Quail (Opel 1966), and usually in the domestic turkey (Wolford et al. 1964). Bacon and Cherms (1968), however, reported that turkeys have sporadic "rest" periods of 2–4 days between an expected ovulation and laying. Lag periods of predictable length occur in other

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species, notably a lag of 5–7 days in Adélie Penguins (Astheimer and Grau 1985), 7 days in Fiordland Crested Penguins (*Eudyptes pachyrhynchus*; Grau 1982), 4 days in White Flippered Penguins (*E. minor albosignata*; Grau 1984), and 10 days in Northern Royal Albatrosses (*Diomedea epomophora sanfordi*; Grau unpubl. data) and in Emus (Hirsch and Grau 1981).

The functional significance of the lag period is elusive. If this 4-day period allows the female auklet flexibility in the time of oviposition, greater variability in its length would be expected. Instead, egg formation appears to be of fixed duration; once rapid yolk deposition has begun, the options are limited either to continuing through to laying or to resorbing the yolk, thus abandoning that potential clutch. In Cassin's Auklet, the completed yolk is held, apparently quiescent, in the follicle during most of the lag period. Yolk resorption can occur during this time, but after ovulation resorption from the oviduct is not possible unless the yolk is extruded into the body cavity. Resorption of a completed yolk could occur during the lag period in birds that lack sufficient protein to complete albumen synthesis. Without evidence for yolk resorption in natural populations, however, the role of nutritional status as a selective pressure in the evolution or maintenance of the lag period remains speculative.

Gilbert et al. (1983) found that numerous small follicles (1–5 mm in diameter) in the ovaries of domestic hens undergo enlargement, but within 2–5 days most of these "primordial" follicles become atretic, leaving a few of the largest the potential to undergo RYD. This cycle of atresia results in a single small follicle entering the follicular hierarchy daily during the reproductive cycle of the domestic hen. Such prebreeding "priming" of the ovary may be a physiological necessity in wild birds that allows for ovarian production of estrogens early in the breeding season and a readiness to respond to appropriate environmental stimuli (proximate factors). Evidence for a similar cycle was observed in the ovaries of female auklets killed while producing an egg (birds A–E, Table 3), where a distinct reduction in the number of small follicles of increasing diameter classes was seen. Because RYD occurs on only one follicle in these auklets (i.e. the follicular hierarchy contains only one follicle), the remaining large primordial follicles may become
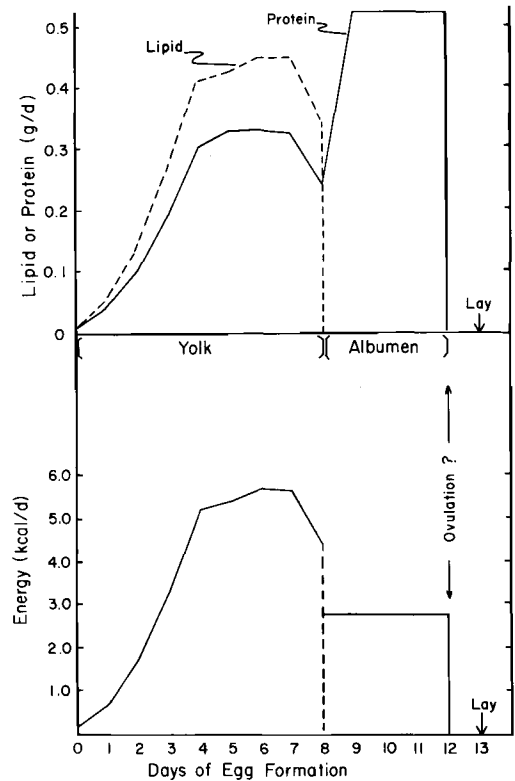

Fig. 7. Mean lipid and protein deposition in Cassin's Auklet eggs ($n = 7$; top) and corresponding energy deposited as egg components ($n = 7$; bottom).

atretic shortly after the single yolk is completed. Evidence for the latter hypothesis is implied by the well-vascularized, "non-atretic" condition (Gilbert et al. 1983) of small follicles in birds A and F (Table 3), each of which had one nearly completed yolk. In contrast, small follicles in birds B–E had either blood pools within the follicle or no follicular vascularization, both indicative of atresia; all four of these birds had either a completed yolk in the follicle or a shelled egg in the uterus.

The predictable nature of the relaying interval is of interest when considering the timing of follicular development and egg formation. The time necessary for reducing (via atresia) a cohort of 20–30 1-mm follicles to a few 5-mm follicles appears to be 2–5 days in domestic hens (Perry et al. 1983). After removal or loss of a first egg, Cassin's Auklets probably require a similar period to produce a single 4–5-mm yolk

that will complete RYD and become the replacement egg. Based on the relaying interval and the time required for yolk deposition and the lag period, the period of early follicular development was estimated to be 2–3 days (Fig. 4). The capacity to relay also emphasizes the flexible nature of the reproductive effort in this species. This plasticity, alluded to by Manuwal (1979), allows the initiation of a reproductive bout within days of appropriate environmental cues. Alternatively, abandonment of eggs, as a response to actual or predicted resource limitations or to mate loss, can occur with impunity, as the birds are able to repeat the process rapidly.

The physiological or cognitive ability of birds to assess environmental stimuli early in the breeding season that may facilitate successful chick rearing later is especially important where such stimuli are temporally unpredictable. The Farallon Island population of Cassin's Auklets are resident in the waters off central California year-round and breed earlier than other seabirds nesting there. It is possible that climatic trends indicative of sea conditions during the breeding season would be evident to the birds as early as December or January. One possible stimulus for the Farallon auklets is upwelling of deep, cold, nutrient-rich water along the north and central California coast in early spring (Sverdrup et al. 1942, Ainley and Lewis 1974). The onset, extent, and duration of upwelling, however, vary annually. A decrease in sea surface temperatures is one indication of local upwelling, which auklets and other seabirds might respond to in assessing potential ocean productivity near a breeding colony. This hypothesis is supported by the correlation of early laying initiation in years where sea surface temperatures averaged below 12°C during January and February (1979 and 1982; PRBO unpubl. data). In contrast, 1981 sea surface temperatures remained relatively high (12–14°C) until mid-March, and peak auklet laying was delayed until early April. This apparent relationship, however, requires analysis of many more years of data to interpret any causal relation between sea surface temperatures and auklet laying.

When upwelling occurs early in spring, auklets may be able to predict an assured food supply for feeding their chicks, and thus lay early. In such years many auklets attempt a second brood, and those with greatest success are those pairs that fledge their first chick early in the season (Boekelheide 1980). Thus, there is a definite advantage to laying early, if an adequate food supply can be anticipated.

For auklets to respond successfully to proximate effectors, such as water temperature or phytoplankton density, such cues must predict suitable conditions at least 13 days in advance of laying to accommodate the time required for egg formation. It is interesting, but probably coincidental, that the 12–15-day period required for egg formation matches half a lunar cycle. Specifically, if egg formation began during a full moon, that egg would be laid during a new moon. Peak laying dates for Cassin's Auklets on Southeast Farallon Island vary annually, and are sometimes correlated with a new moon (1979 and 1981, Fig. 1). Auklets may use lunar cues in the absence of decreasing ocean temperatures (e.g. 1981, Fig. 1) or possibly in late spring when breeding Western Gulls (*Larus occidentalis*) are more restricted in their feeding range and a potential threat (Manuwal 1972), but lunar phase is not an important determinant in laying replacement eggs. In fact, replacement eggs seem to be produced as rapidly as physiologically possible, a response that may reflect experimental manipulation in the present context.

One interpretation of the stimuli responsible for initiating egg production in auklets may be as follows: Ovarian (and testicular?) activity begins in response to increasing day length, even as early as January when auklets begin cleaning out nest burrows and pairs occasionally are found together. If the limited cycle of follicular growth and atresia begins at this time, and if sea conditions, as a secondary modulator, are positive, formation of the single egg may begin shortly thereafter, permitting auklets to lay as early as the first week of March (Manuwal 1972). If, as in 1981, upwelling is not anticipated, follicular cycling may continue until signs of upwelling appear.

cation toward the final draft. The Farallon Islands are maintained under the joint administration of the U.S. Fish and Wildlife Service and the U.S. Coast Guard, and are under the stewardship of Point Reyes Bird Observatory (PRBO). I gratefully acknowledge the advice, assistance, and logistic support of the biologists and staff of PRBO, especially R. J. Boekelheide and T. McElroy. I appreciate the assistance in the field by volunteers J. Nusbaum, P. Abbott, and K. Schafer and thank PRBO, S. Goldfarb, and T. E. Roudybush for kindly allowing use of their unpublished findings. Funding was provided in part through a Frank M. Chapman Fund award, a Sigma Xi Grant-in-aid of research, a University of California, Davis, Jastro-Shields award to Astheimer, and a contract from the U.S. Minerals Management Services (formerly Bureau of Land Management) to C. R. Grau. Special thanks go to the Farallon patrol skippers of the San Francisco Oceanic Society for their seamanship and hospitality. This is contribution No. 284 of Point Reyes Bird Observatory.

LITERATURE CITED

AINLEY, D. G., C. R. GRAU, & S. H. MORRELL. 1979. Influence of petroleum on egg formation and embryonic development in seabirds. Final Report, Res. Unit. 423. OCSEAP. U.S. Dept. Interior, BLM, through the U.S. Dept. Commerce NOAA.

——, ——, T. E. ROUDYBUSH, S. H. MORRELL, & J. M. UTTS. 1981. Petroleum ingestion reduces reproduction in Cassin's Auklets. Marine Poll. Bull. 12: 314–317.

——, & T. J. LEWIS. 1974. The history of the Farallon Island marine bird populations. 1854–1972. Condor 76: 432–446.

ASTHEIMER, L. B. 1985. Long-laying intervals: a possible mechanism. Auk 102: 401–405.

——, & C. R. GRAU. 1985. The timing and energetic consequences of egg formation in Adélie Penguins. Condor 87: 256–267.

——, T. E. ROUDYBUSH, & C. R. GRAU. 1979. Egg formation in the Cassin's Auklet. Pacific Seabird Group Bull. 6: 29.

BACON, W. L., & F. L. CHERMS. 1968. Ovarian follicular growth and maturation in domestic turkey. Poultry Sci. 47: 1303–1314.

BOEKELHEIDE, R. J. 1980. Double brooding in 1980: was it worth it? Abstract. 1980 Amer. Ornithol. Union Meeting, Ft. Collins, Colorado.

BOERSMA, P. D., & N. WHEELWRIGHT. 1979. Egg neglect in the Procellariiformes: reproductive adaptations in the Fork-tailed Storm-petrel. Condor 81: 157–165.

BRODY, S. 1945. Bioenergetics and growth. New York, Hafner.

DRAPER, M. H. 1966. The accumulation of water and electrolytes in the egg of the hen. Pp. 63–74 in

Physiology of the domestic fowl (C. Horton-Smith and E. C. Amoroso, Eds.). Edinburgh, Oliver and Boyd.

GILBERT, A. S. 1971. The activity of the ovary in relation to egg production. Pp. 3–21 in Egg formation and production (B. M. Freeman and P. E. Lake, Eds.). Edinburgh, British Poultry Sci. Ltd.

——, M. M. PERRY, D. WADDINGTON, & M. A. HARDIE. 1983. Role of atresia in establishing the follicular hierarchy in the ovary of the domestic hen (Gallus domesticus). J. Reprod. Fert. 69: 221–227.

GRAU, C. R. 1976. Ring structure of the avian egg yolk. Poultry Sci. 55: 1418–1422.

——. 1982. Egg formation in Fiordland Crested Penguins (Eudyptes pachyrhynchus). Condor 84: 172–177.

——. 1984. Egg formation. Pp. 33–57 in Seabird energetics (G. C. Whittow and H. Rahn, Eds.). New York, Plenum Press.

——, & L. B. ASTHEIMER. 1982. A comparison of egg formation in seabird groups. Pacific Seabird Group Bull. 9: 65.

——, T. E. ROUDYBUSH, S. H. MORRELL, & D. G. AINLEY. 1978. Relation of yolk deposition to oviposition in Cassin's Auklet (Ptychoramphus aleuticus). Abstr., Pacific Seabird Group Bull. 5: 71.

HARRISON, P. 1983. Seabirds: an identification guide. Beckenham, England, Croom Helm Ltd.

HIRSCH, K. V., & C. R. GRAU. 1981. Yolk formation and oviposition in captive Emus. Condor 83: 381–382.

KENDEIGH, S. C., V. R. DOL'NIK, & V. M. GAVRILOV. 1977. Avian energetics. Pp. 127–204 in Granivorous birds in ecosystems (J. Pinowski and S. C. Kendeigh, Eds.). Cambridge, England, Cambridge Univ. Press.

MANUWAL, D. A. 1972. Population ecology of Cassin's Auklet on Southeast Farallon Island, California. Unpublished Ph.D. dissertation, Los Angeles, Univ. California.

——. 1974a. The incubation patches of Cassin's Auklets. Condor 76: 481–484.

——. 1974b. The natural history of Cassin's Auklet (Ptychoramphus aleuticus). Condor 76: 421–431.

——. 1979. Reproductive commitment and success of Cassin's Auklet. Condor 81: 111–121.

NELSON, D. A. 1981. Sexual differences in measurements of Cassin's Auklets. J. Field Ornithol. 52: 233–234.

OPEL, H. 1966. The timing of oviposition and ovulation in the quail (Coturnix coturnix japonica). Brit. Poultry Sci. 7: 29–38.

PERRY, M. M., D. WADDINGTON, A. B. GILBERT, & M. A. HARDIE. 1983. Growth rates of the small yolky follicles in the ovary of the domestic fowl. IRCS Med. Sci. 11: 979–980.

RICKLEFS, R. E. 1977. Composition of the eggs of several bird species. Auk 94: 350–356.

RIDDLE, O. 1911. On the formation, significance and chemistry of the white and yellow yolk of ova. J. Morphol. 22: 455–490.

ROUDYBUSH, T. E., C. R. GRAU, M. R. PETERSON, D. G. AINLEY, K. V. HIRSCH, A. P. GILMAN, & S. M. PATTEN. 1979. Yolk formation in some charadriiform birds. Condor 81: 293–298.

SVERDRUP, H. U., M. W. JOHNSON, & R. H. FLEMING.

1942. The oceans, their physics, chemistry, and general biology. New York, Prentice-Hall, Inc.

WARREN, D. C., & H. M. SCOTT. 1935. The time factor in egg formation. Poultry Sci. 14: 195–207.

WOLFORD, J. H., R. K. RINGER, & T. H. COLMAN. 1964. Lag time interval between successive eggs and oviposition time in the turkey. Poultry Sci. 43: 612–615.

ZAR, J. H. 1974. Biostatistical analysis. Englewood Cliffs, New Jersey, Prentice-Hall, Inc.

100 Years Ago in The Auk

From "On an old portrait of Audubon, painted by himself, and a word about some of his early drawings" by R. W. Shufeldt (1886, Auk 3: 417–420):

[Shufeldt had come into possession of three drawings, which he described as follows.]

"They are each and all drawn by a combination of crayon and water-colors upon a thin and *not expensive* kind of drawing-paper, now brittle and soiled by age. Audubon had evidently numbered these drawings of his, and these numbers are 44, 77, and 96, a European Magpie, a Coot, and a Green Woodpecker, respectively . . . .

"As I have said, the earliest of these drawings is the one of the Magpie—and let us look at it for a moment. It is life size, as they all three are, and the bird is represented standing on the ground, being drawn lengthwise on the paper. The execution is quite crude, though the naturalist 'sticks out' in it, for notwithstanding the somewhat awkward position the bird is in, there is life in it. The ground is simply a wash of pale green and brown, while over on one side of the paper he has 'tried his brush,' having made some rough concentric circles with paint dabs about them. Beneath this drawing we find written in lead-pencil in two lines, 'La Pie, Buffon,' 'Pye, Piot Magpye, Pianet, english,' and over to the left-hand corner, 'No. 44.'

"The second picture is that of a Coot, and is a marked improvement upon the Magpie. Far more pains have been taken with the feet, legs, bill, and eye, though little has been gained in the natural attitude of the bird. It is also represented standing up on the dry ground, which is here of a pale violet wash, unbroken by anything in the shape of stones or vegetation. Except very faintly in the wing, no attempt has been made to individualize the feathers, the entire body being of a dead black, worked in either by burnt cork or crayon. Beneath this figure has been written in lead-pencil, but gone over again by the same hand in ink, 'La foulque *ou* La Morelle— Buffon, Riviere Loire Joselle—' 'English—the Coot,—'

"As is usually the case among juvenile artists, both this bird and the Magpie are represented upon direct lateral view, and no evidence has yet appeared to hint to us of the wonderful power Audubon eventually came to possess in figuring his birds in their every attitude.

"There is a peculiar pleasure that takes possession of us as we turn to the third and last of these figures, the one representing the Green Woodpecker (*Gecinus viridis*). It is a wonderful improvement, in every particular, upon both of the others. The details of the plumage and other structures are brought out with great delicacy, and refinement of touch; while the attitude of the bird, an old male, is even better than many of those published in his famous work. The colors are soft and have been so handled, as to lend to the plumage a very flossy and natural appearance, while the old trunk, upon the side of which the bird is represented, presents several evidences of an increase of the power to paint such objects. We find written in lead pencil beneath this figure, in two lines, and in rather a Frenchy hand, 'Le Pic vert, Buffon,' 'the Green Woodpecker—British Zoology.' "