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YOLK FORMATION IN SOME CHARADRIIFORM BIRDS

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By counting and measuring the major ova of breeding birds at autopsy and combining these data with time intervals between ovipositions, rough estimates have been made of the time required to form yolk in some non-captive birds (King 1973). Direct studies have been made in domestic fowl (*Gallus gallus var. domesticus;* Gilbert 1972), turkeys (*Meleagris gallopavo*; Bacon and Cherms 1968), and Common Quail (*Coturnix coturnix*; Bacon and Koontz 1971), by feeding the birds a capsule containing dye each day, and counting dye rings in the yolks after the eggs have been hardcooked. Recently developed methods of fixing and staining eggs have revealed differences in yolk deposited during day and night, thus permitting another estimation of the number of days during which yolk was deposited, and without direct contact with the female (Grau 1976). In eggs from chickens and quail that had been fed dyes, yolk that stained darkly with dichromate was shown to be deposited during the active daytime feeding periods, while pale-staining yolk was deposited during the night. Thus, pairs of light and dark rings, which together take a day to be deposited, may be counted to estimate time of yolk formation.

In the present study we have applied the yolk ring method of estimating the number of days during which the bulk of the yolk is deposited around the central white core (Grau 1976) to the eggs of some shorebirds, gulls, terns and alcids.

MATERIALS AND METHODS

Eggs were collected near Old Chevak on the Clarence Rhode National Wildlife Range (Alaska) in 1975, on Southeast Farallon Island (California) in 1976 and 1977, in Canada near Lakes Huron and Ontario and from the Bay of Fundy area, New Brunswick in 1977, and from Middleton Island in the Gulf of Alaska in 1978.

Dates of peak arrival of birds and of first egg-laying of Chevak, Alaska species were recorded. Most eggs were gathered before incubation began, and when possible, the order of laying of each egg in the clutch was noted.

Eggs of Great Black-backed Gull, Herring Gull, Ring-billed Gull, and Black Guillemot were collected in Canada; eggs of Western Gull, Pigeon Guillemot, and Cassin's Auklet on the Farallon Islands; and eggs of Glaucous-winged Gull, Black-legged Kittiwake, Common Murre, and Tufted Puffin on Middleton Island, Alaska. The remainder of the eggs came from the area near Chevak. Linear measurements were made only on Alaskan and Canadian eggs.

Eggs from Alaska and the Farallon Islands were shipped to Davis, California in boxes filled with blocks of foam rubber packing material in which cylindrical holes were cut to keep each egg at least 4 cm from the sides and from other eggs. The temperature of the eggs was not controlled until eggs arrived at the laboratory. They were degassed in a vacuum chamber for 16 h to avoid air bubbles in the final preparation, frozen in air at −20°C, fixed in 4% formalin for 16 h at 65°C, cut in half, and one half stained by 6% potassium dichromate for 16 h at 65°C (Grau 1976). The Canadian eggs were degassed, frozen, fixed in formalin, and then transported to Davis and stained. Unstained yolk halves were retained for comparison with stained halves.

Profiles were drawn of the ring sequences of stained yolks. Dark-staining material was presented as peaks and pale-staining material as valleys. Differences in peak heights were ignored, but variation along the horizontal axis was used to estimate time. Several attempts were made to obtain more accurate profiles through the use of a transmission densitometer applied to photographic negatives or to color transparencies. These were not as useful in interpreting observations as hand-drawn profiles. The peak-valley pairs—each representing one day—were counted to determine the total number of days required for yolk formation. Observations were also made of unstained yolk halves, with particular notice taken of patterns of naturally occurring pigments or variation in the texture of yolk comprising a ring.

RESULTS

The stained slices of yolks from different species differed greatly in appearance. However, all showed alternating pale and dark rings (Fig. 1a) that are characteristic of yolk formation over a 24-hour period (Grau 1976). The periods of rapid yolk deposition and mean egg dimensions are given in Table 1. In general, smaller birds had shorter periods of rapid yolk formation.

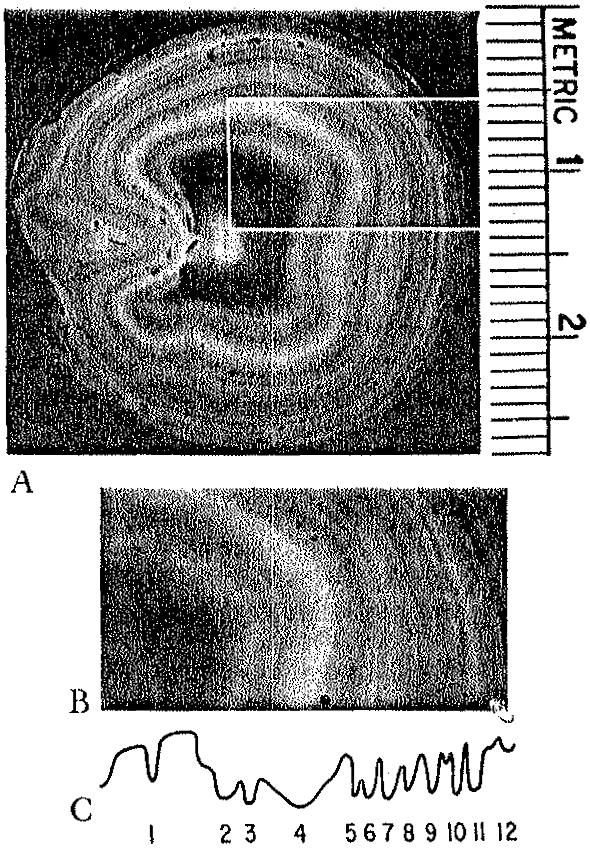

FIGURE 1. A. Photograph of a cross section of a dichromate-stained Bar-tailed Godwit yolk. This yolk required 12 days for formation. The curving of the rings toward the center of the yolk on the left side of the photograph was caused by the neck of the latebra—a structure resulting from non-uniform yolk deposition in the region of the blastodisc. Spots on the yolk are gas bubbles. This particular egg was not degassed completely. B. An enlargement of the outlined part of A showing part of the yolk from the center (left side of the photograph) to the outside. C. The hand-drawn profile represents dark-staining yolk as peaks and pale-staining yolk as valleys. Numbers indicate the days of yolk formation.

The yolks of many shorebirds and seabirds were found to have rings of highly pigmented yolk. Colors ranged from yellow through orange to red.

In order to illustrate the variations in ring appearances and the profiles that were derived from observing slices of stained yolks, Figure 1b includes a photograph of a slice of Bartailed Godwit yolk and the profile that represents it.

Under some conditions, it is possible to deduce the sequence of laying of several eggs of a clutch. In Figures 2, 3, 4 and 5, profiles of the yolks of a Mew Gull, a Western Gull, a Bar-tailed Godwit and a Western Sandpiper are presented in the order in which the eggs were laid. The order of laying of the Mew Gull and Western Gull eggs

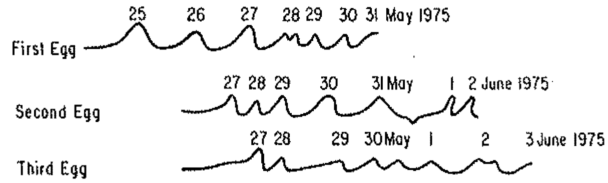

FIGURE 2. Yolk profiles of three Mew Gull eggs found in the nest on June 1, 3 and 4, leaving two days between the laying of the first and second eggs. Mew Gulls generally required seven days for yolk formation. However, delay in the onset of yolk formation of egg 2 and simultaneous onset of yolk deposition of eggs 2 and 3 may have caused a delay in ovulation of yolk 3. Although yolk 3 had been undergoing rapid deposition long enough to be ovulated, it was held and yolk deposition continued uninterrupted for an extra day.

was known from daily observation, whereas the godwit and sandpiper eggs had already been laid when the nests were found. Roudybush and Grau (unpubl. data) have verified the order in which eggs were laid, deduced from ring structure, in known sequences of eggs of geese, quail, chickens, ducks, gulls, and shorebirds.

The order of laying of eggs in a clutch was deduced by selecting characteristic rings in sections of the eggs, and counting the days between these rings and the outer margin of the yolk. Recognizable yolk rings depos-

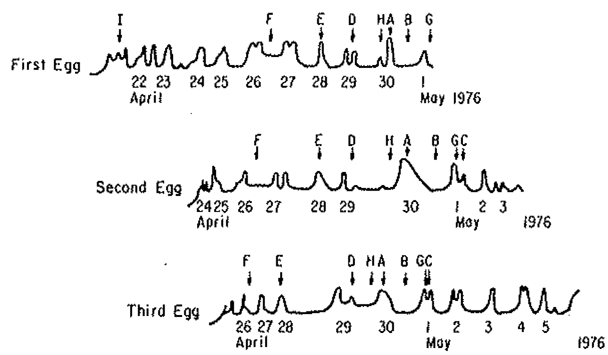

FIGURE 3. Yolk profiles of three eggs of a Western Gull, which were laid and collected on 2, 4 and 6 May 1976. Working separately, without reference to the known order of laying two authors were able to ascertain the order in which the eggs were laid. Some of the characteristic rings indicated on these profiles by letters were found on either unstained or ferrocyanide-stained yolk slices. They were marked on the dichromate-stained yolk profiles to show the relative position of all characteristic rings found in these yolks: A) Slaty grey dichromate-stained ring. B) Light brown dichromate-staining ring. C) Double peak deposited on one day; two similar very fine rings on dichromate-stained slices. This peak pair was not as symmetrical in the second egg as in the third, and was missing in the first egg. D) Dichromate-stained double peak similar to C. E) Distinct slate-colored dichromate peak. F) Broad yellow ring on unstained slice. G) Distinct bright yellow ring on unstained slice. H) Area of large yolk spheres observed on ferrocyanide-stained yolk. I) Dark center characteristic of the first egg laid.

TABLE 1. Time required for the rapid phase of yolk formation, and egg size, in some seabirds and shorebirds.

Species	Time for yolk formation (days)	Clutch size	Egg size[2] (mm)
SCOLOPACIDAE			
Bar-tailed Godwit (*Limosa lapponica*)	8–12 (8)[1]	4	56.1 × 39.0
Ruddy Turnstone (*Arenaria interpres*)	5–6 (4)	4	40.6 × 30.2
Western Sandpiper (*Calidris mauri*)	5–8 (9)	4	32.1 × 22.0
PHALAROPODIDAE			
Red Phalarope (*Phalaropus fulicarius*)	4–5 (5)	4	31.4 × 22.2
Northern Phalarope (*Lobipes lobatus*)	6–7 (7)	4	28.4 × 20.2
LARIDAE			
Glaucous Gull (*Larus hyperboreus*)	12 (2)	3	72.9 × 53.0
Great Black-backed Gull (*L. marinus*)	13 (1)	3	85.0 × 54.3
Glaucous-winged Gull (*L. glaucescens*)	12 (2)	3	
Western Gull (*L. occidentalis*)	10–11 (8)	3	
Herring Gull (*L. argentatus*)	11–13 (17)	3	70.9 × 49.9
Ring-billed Gull (*L. delawarensis*)	12 (1)	3	60.0 × 43.6
Mew Gull (*L. canus*)	5–8 (6)	3	60.0 × 42.5
Black-legged Kittiwake (*Rissa tridactyla*)	9 (3)	2	
Sabine's Gull (*Xema sabini*)	7–8 (4)	2	34.8 × 28.8
Arctic Tern (*Sterna paradisaea*)	6 (2)	2	38.6 × 29.6
ALCIDAE			
Common Murre (*Uria aalge*)	12–18 (7)	1	
Black Guillemot (*Cepphus grylle*)	8 (1)	2	
Pigeon Guillemot (*C. columba*)	10 (2)	2	
Cassin's Auklet (*Ptychoramphus aleuticus*)	8 (4)	1	
Tufted Puffin (*Lunda cirrhata*)	12–13 (2)	1	

[1] The number of eggs examined.
[2] The mean length and width.

ited simultaneously in several consecutive yolks of a clutch are defined as characteristic rings. Yolk properties that were useful in identifying characteristic rings were variation in pigmentation, sensitivity to staining, and variation in texture. In the earliest laid egg the characteristic ring was nearest the margin.

When a characteristic ring was deposited, each yolk being formed received characteristic ring material as part of the outer ring of the follicle. Because each follicle was at a different stage of development, the distance from the characteristic ring to the outer margin of the yolk differed among yolks. For example, if a bird laid four eggs over a four-day period, and seven days were required for rapid yolk formation, plus one day for albumen and shell formation, then the bird must have started forming yolks on days 1, 2, 3, and 4 to be able to lay eggs on days 9, 10, 11, and 12. If a characteristic ring were deposited on day 6, yolk A would have shown that characteristic ring as occurring one day before ovulation; yolk B, two days before ovulation; yolk C, three days and yolk D, four days. By noting how many days before ovulation the same characteristic ring occurred in each of the different eggs of the same clutch, it was possible to deduce the sequence in which the eggs were laid.

An example is presented in Figure 4, which shows profiles of three eggs from the nest of a Bar-tailed Godwit. A ring with a recognizable color or structure was chosen as the characteristic ring. Its position with respect to the outer margin and to other rings was determined. By using several such characteristic rings at different distances from the margin, the likelihood of deducing the correct sequence was enhanced. Details of a few characteristic ring types are illustrated in Figure 3. These methods were applied to the eggs illustrated in Figure 4, and to other clutches not presented. In cases where the actual order of laying was known, placing the eggs in order using the sequence deduced from the characteristic rings agreed with the known order. This has been accomplished in several clutches of eggs of Western Gulls, Mew Gulls, Ruddy Turnstones, and three species of geese. This has also been done to a limited extent in clutches of eggs in which some eggs were laid before the nest was discovered but in which eggs were still being laid. Species in which this has been done include Red and Northern phalaropes and Western Sandpipers.

Characteristic rings were also useful in deducing probable intervals between ovipositions. A delay of a day in the initiation

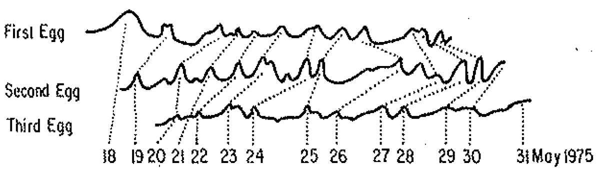

FIGURE 4. Yolk profiles of three Bar-tailed Godwit eggs which had been laid before the nest was discovered. By examination of these stained slices and others that are not stained, the sequence of laying was deduced (see text). The widths of the rings on 24–28 May 1975 in the three eggs were variable, and the profiles appear confusing. Direct observation of ring properties (which are not apparent in yolk profiles) such as color, texture and yolk granule size, however, left little doubt that the sequence was correct.

TABLE 2. Peak arrival and first egg laying with respect to time required for the rapid phase of yolk formation (1975).

Species	Peak arrival	First laying	Difference (days)	Time of yolk formation
Glaucous Gull	9 May	10 June	32	12
Bar-tailed Godwit	10 May	30 May	20	8–12
Ruddy Turnstone	11 May	30 May	19	5–6
Western Sandpiper	10 May	29 May	19	5–8
Mew Gull	10 May	1 June	22	5–8
Sabine's Gull	10 May	1 June	22	8
Arctic Tern	10 May	12 June	33	6
Northern Phalarope	13 May	1 June	18	6–7
Red Phalarope	26 May	3 June	8	4–5

of rapid yolk deposition is the most likely reason for a delay in oviposition. Firm evidence on this point is available only from observations in domestic fowl (Gilbert 1972).

Table 2 shows that for all species presented, the time of yolk formation is less than the time between peak arrival and first egg laying.

DISCUSSION

The internal yolk structures of the charadriiform eggs available for this study were similar to those of gallinaceous birds and geese (Grau 1976). There was considerable variation in the red, orange, and yellow colors in the rings of various eggs as well as variation in color and clarity of rings stained by dichromate. As far as could be told from internal evidence of ring appearance in eggs of a clutch in relation to day of oviposition, a pair of rings (one pale-staining and one dark-staining with dichromate) was deposited each day. Presumably in species which breed at high latitudes these rings relate more to activity than to photoperiod.

Color variations in yolks are presumed to result from the ingestion of food containing natural pigments (Fox 1976). Some fat-soluble dyes (Denton 1940) as well as naturally occurring pigments (Grimbleby and Black 1952) have been shown to be readily deposited in yolk when they are fed to birds who are forming yolk. The time required for absorption and deposition of pigments such as Sudan IV is approximately four hours in quail (Bacon and Koontz 1971), and chickens (Gilbert 1972). The composition of shorebird and seabird yolk pigments is not known, but astaxanthin and related carotenoids (Fox 1976) are the most likely materials. These red pigments are normal components of crustacea, some other invertebrates, and some fish. Fox (1976) found no astaxanthin in yolks of flamingos fed astaxanthin, but Johnson, Lewis and Grau (personal observ.) found that astaxanthin is deposited in the yolks of chickens.

Even though the source of yolk pigment is not known in these samples, some estimate can be made of the time of day when the pigments were consumed. If the dark and pale rings of dichromate-stained yolk halves, corresponding to day and night, are compared to the unstained halves of the same yolks, the pigments are generally present in dark-staining rings and often only in the beginning of these rings (Fig. 6). This suggests that most natural pigments were consumed in the early morning, because that is the time when dark-staining rings are deposited in eggs of chickens and quail (Grau 1976).

These methods of studying egg formation, applied here to some seabirds and shorebirds, provide for the first time estimates of the time required for the rapid period of yolk deposition. The indirect estimates summarized by King (1973) on the Herring Gull, the only species in which comparisons with the present work are possible, suggested a shorter time than found by counting rings.

The validity of applying techniques such as ours, developed first with captive or do-

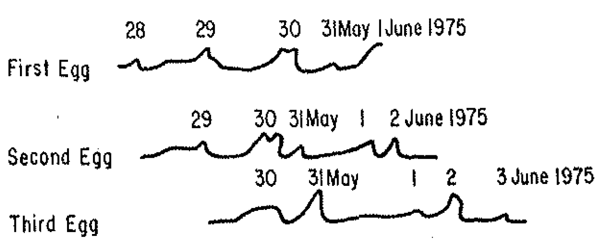

FIGURE 5. Yolk profiles of three Western Sandpiper eggs found in one nest. The sequence was deduced from appearance of characteristic rings. The double bump on May 30th served as the initial characteristic ring which could be recognized in other yolks.

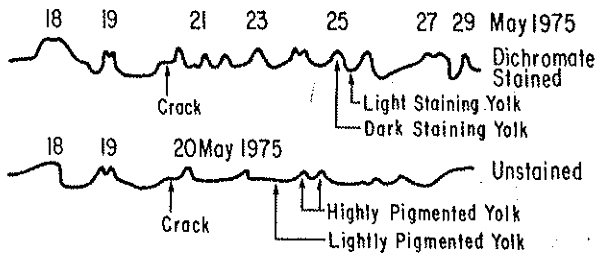

FIGURE 6. Yolk profiles of a single Bar-tailed Godwit egg. Above is the dichromate-stained half of the yolk and below is the unstained half. The arrows indicate a crack that was found after the yolk was cut in half; this crack extended into both halves of the yolk and was used as an additional reference mark. Note correlations between some of the pigmented areas and peaks on the dichromate-stained half. Because darkly staining material (dichromate stain) is laid down during the day, it is concluded that most of the natural pigments of this yolk were ingested during the early morning.

mestic birds, to wild birds must be questioned until corroborative data can be obtained by using independent measurements such as feeding dyes in daily doses.

In attempting to relate time of arrival on the breeding ground, or environmental events at the nesting site, to structure or composition of yolk rings, assumptions have been made of the intervals between completion of yolk formation and oviposition, and of the continuous nature of yolk deposition. In domestic birds that lay an egg daily in a large clutch, the timing of events has been well documented (Gilbert 1971). In chickens, for example, ovulation usually occurs within a few hours after yolk formation is complete, and some 30 min after oviposition of the previous egg. Formation of albumen, shell membranes, and shell takes approximately 24 h, 20 h of which is taken up by shell deposition. No comparable information is available for wild birds, but it is likely that similar times are required in birds that lay an egg each day until the clutch of several eggs is complete. Gilbert (1970) reported that in about 20% of the first-laid of a sequence of chicken eggs, yolk deposition ceased 24 h before ovulation. We have some preliminary evidence from some nongallinaceous birds that the period between completion of yolk deposition and oviposition may be several days instead of the single day assumed here.

Once initiated, the major part of yolk formation is a continuous process in chickens (Gilbert 1972), and presumably in other birds. We know of no evidence that yolk deposition may stop for one or more days, and then be resumed, but this possibility cannot be disregarded.

Table 1 demonstrates variation among species in the time required to form yolks. In general within families, smaller birds required less time, but notable exceptions exist. For example, the Mew Gull, a relatively large larid of those sampled, formed eggs rapidly. Nor did the relationship between egg size and yolk formation time hold true when birds of different families were compared. For example the Bar-tailed Godwit required as much time to form yolks as did the large gulls. There was no clearly direct relationship to clutch or egg size (Table 1).

The only relationship to the time between arrival and laying (Table 2), was that it always equaled or exceeded the time of yolk formation. This means that all yolk formation can occur on the breeding grounds. This observation is similar to that with Cackling Geese (*Branta canadensis minima*) reported by Raveling (1978). The ability to determine the amount of time required for egg formation, as described in this paper, may ultimately help us to understand better the ecology and energetics of avian reproduction.

SUMMARY

Variation in appearance of yolk rings was used to determine duration of the rapid yolk formation period in several shorebirds and seabirds. After freezing, yolks were fixed in formalin, and unstained and dichromate-stained slices were examined. The range in days required to deposit the yolk was estimated by counting pairs of pale and dark rings, which represent one day per pair. With some exceptions, yolk formation in small alcids required from 8 to 10 days; in large gulls from 10 to 13 days; in small gulls from 5 to 8 days; and in small shorebirds from 4 to 7 days. This type of analysis can also yield information on the sequence of egg laying in a clutch and the timing of deposition of natural pigments in the yolk.

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RECENT PUBLICATIONS

Shorebirds in Marine Environments.—Edited by Frank A. Pitelka. 1979. Studies in Avian Biology No. 2, Cooper Ornithological Society. 262 p. Paper cover. $8.90. Available: see back cover. Here are the papers that were given at a 1977 symposium sponsored by the Pacific Seabird Group. Fifteen papers deal with aspects of distribution, migration, or conservation, and nine deal with ecology or behavior. Most of the studies concern shorebirds on the Pacific coast of the Americas. An introduction by Pitelka sets the scene, while summarizing remarks by J. R. Jehl, Jr. and J. A. Wiens bring out the major points and suggest directions for future work. Maps, graphs, photographs, and lists of references. This well-edited collection will be valuable not only to researchers but also to those who are trying to preserve coastal habitats and their wildlife.

The Role of Insectivorous Birds in Forest Ecosystems.—Edited by J. D. Dickson, R. N. Conner, R. R. Fleet, J. C. Kroll, and J. A. Jackson. 1979. Academic Press, New York. 381 p. $24.00. The 20 papers in this volume were presented at a symposium held in July, 1978 in Nacogdoches, Texas. Rapid publication was accomplished by reproducing the printing plates directly from typescript. The articles deal with censusing techniques, foraging, habitat use, population dynamics, and the effects of insectivorous birds in forest ecosystems. Concluding remarks by Stanley H. Anderson tie the papers together. Graphs, photographs, and lists of references. These papers will be of interest to researchers studying the ecology of forest birds.

Greenshanks.—Desmond and Maimie Nethersole-Thompson. 1979. Buteo Books, Vermillion, South Dakota. 275 p. $27.50. Few birds, particularly shorebirds, have received such long, intensive, and affectionate study as has the Greenshank from Desmond Nethersole-Thompson. His work on this species—the European counterpart of the Greater Yellowlegs—began in 1932 and led to his first book, *The Greenshank* (Collins, London, 1959). This new book is the culmination of fresh research begun in 1961, in the highlands of northwestern Scotland. Collaborative fieldwork by all members of the family has yielded new information and insights into the biology of these birds. Yet the book is not provincial, the authors' findings being integrated with others from elsewhere in the species' range. Many tables and appendixes report additional data. David Parmelee has written a gracious and personal preface, and several other workers have contributed special sections. The bird and its environment are shown in many drawings and four lovely color plates by Donald Watson, and in pictures by several photographers. Maps, graphs, sonagrams, selected bibliography, and index. Altogether, this is an exemplary account of a single species and an essential book for shorebird specialists.