GROWTH AND DEVELOPMENT OF KNOWN-AGE RING-BILLED GULL EMBRYOS

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This paper describes growth and development of Ring-billed Gull (*Larus delawarensis*) embryos. It provides a basis for estimating the age of eggs at previously unvisited colonies. The data also supply a way to determine, within a colony, the location of early and later nesting pairs by comparing, during the same sampling time, relative ages of eggs located in different parts of a colony.

STUDY AREA AND METHODS

There are no descriptions of embryo growth and development of Ring-billed Gulls in the literature. Dawkins et al. (1965) compared increase in body weight and development of California Gull (*L. californicus*) and Domestic Chicken (*Gallus gallus*) embryos using known-age eggs. Drent (1970), using known-age embryos plus data from Paludan (1951) and Harris (1964) formulated a logarithmic body weight curve for the Herring Gull (*L. argentatus*). Maunder and Threlfall (1972) described the growth and development of various parts of Black-legged Kittiwake (*Rissa tridactyla*) embryos. Gilbertson and Hale (1974) used Maunder and Threlfall's (1972:800) body weight curve for the Black-legged Kittiwake embryos to age those of the Herring Gull. We consider such inter-specific comparisons weak because different species do not necessarily show the same developmental characteristics at equivalent age. We decided not to use the egg flotation technique devised by Westerkov (1950) because the results vary depending on egg size and the age of the egg when incubation starts. Additionally, Schreiber (1970) noted that addled and infertile eggs of Western Gulls (*L. occidentalis*) show essentially the same flotation as viable eggs during the early stages of development.

We conducted this study in 1975 at a colony of approximately 800 pairs of Ring-billed Gulls on Granite Island (48°43'N, 88°29'W), Black Bay, northern Lake Superior, Ontario. The island is a strongly undulating granite outcrop 402 m by 201 m with a summit 30 m above the surrounding water. Soil and vegetation occur in depressions of the rock surface. Each spring Ring-billed Gulls nest in the depressions especially near the summit away from wave action and possible flooding. Dominant plants in the depressions are Kentucky bluegrass (*Poa pratensis*), rough cinquefoil (*Potentilla norvegica*), and red raspberry (*Rubus strigosus*). The remainder of the island is densely forested with balsam fir (*Abies balsamea*), white cedar (*Thuja occidentalis*), and white birch (*Betula papyrifera*).

On 16 May we marked, with a black felt pen, 31 1-egg clutches and 5 2-egg clutches in the center and 19 1-egg clutches and 13 2-egg clutches on the periphery of the Granite Island colony to determine if equivalent age embryos showed equal development in the two areas. Central and peripheral clutches were designated respectively as those near the geometric center of the colony and those forming the outside border (Dexheimer and Southern 1974). Only nests which subsequently contained 3 eggs formed our sample so that we eliminated potential variation in development because of different clutch sizes. Our final sample was 29 central and 21 peripheral nests.

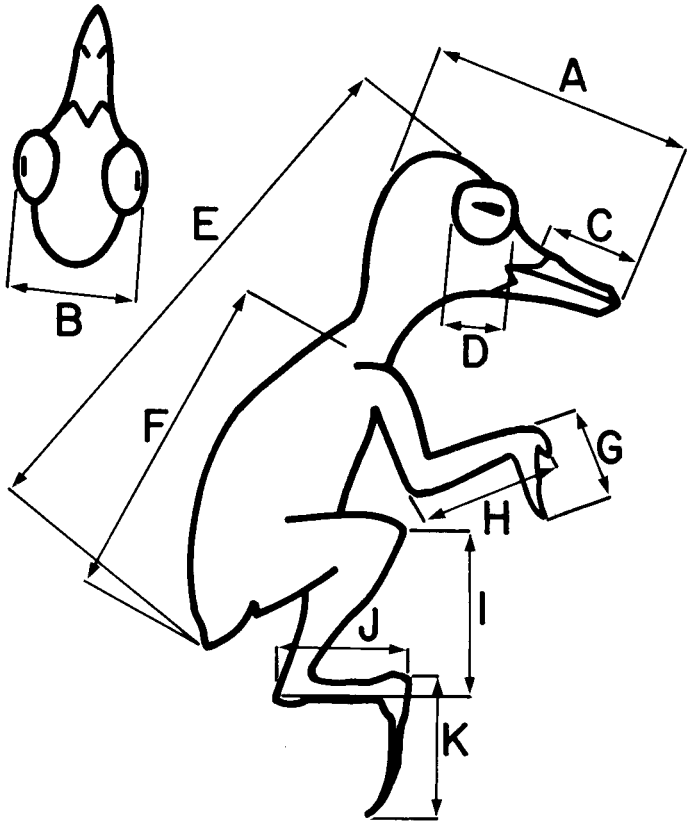

FIG. 1. Measurements of Ring-billed Gull embryos. A, head length; B, head width; C, culmen; D, eye diameter; E, total length; F, back length; G, hand; H, forearm; I, tibia; J, tarsus; K, midtoe. Modified from Maunder and Threlfall (1972).

The age of eggs found in the study nests on our first visit was determined by assuming a 1.9 day interval between laying of successive eggs in Ring-billed Gulls (Vermeer 1970: 20). For example, if a 1-egg clutch on 16 May contained 2 eggs on 17 May, we assumed the first egg was laid on 15 May. If a second egg was not in the nest on 17 May but was by 18 May, we assumed the first egg was laid on 16 May. We considered the longest egg in the 2-egg clutches marked on 16 May the first laid (Vermeer 1969, Ryder 1975). Because we assumed the 1.9 day interval between successive eggs, we aged embryos to an accuracy of ±24 h and grouped embryos according to age into 3-day intervals.

On 17 May we collected 2 clutches each from the center and periphery of the colony in which the second egg was freshly laid and 2 clutches from each area in which the third egg was fresh. From these, we determined if any development occurs in first and second eggs by the time the third egg is laid. Sampling for the remainder of the study involved taking individual first, second, and third eggs of known age from different nests in each

TABLE 1

EXTREMES IN EARLY DEVELOPMENT OF KNOWN-AGE RING-BILLED GULL EMBRYOS FROM THE CENTER AND PERIPHERY OF THE GRANITE ISLAND COLONY, 1975

Age (days)	Development	
	Center	Periphery
1	No development to blastodisc with diameter 0.66 cm.	No development.
3	Head fold stage of primitive streak to embryo with 30 somites, heart beating, area vasculosa developed.	No development to primitive streak.
5	Embryo with 18 somites to an embryo 0.82 cm in length, wing and leg buds visible.	Embryo with 14 somites to 16 somites.
6	Embryo 0.79 cm in length, slightly prominent midbrain to embryo 1.0 cm in length, leg bud 0.30 cm and wing bud 0.42 cm, choroid fissure visible.	Embryo with 23 somites to embryo 0.99 cm in length, prominent midbrain, choroid fissure visible.
7–9	Embryo 0.93–1.56 cm in length, area vasculosa 4.52 cm at sinus terminalis, body wt. 0.25–0.40 g, wing bud 0.36–0.43 cm, leg bud 0.32–0.41 cm, well defined choroid fissure.	Embryo 0.78–1.54 cm in length, area vasculosa 3.74–6.10 cm at sinus terminalis, body weight 0.30–0.50 g, wing bud 0.39–0.46 cm, leg bud 0.28–0.53 cm, well defined choroid fissure.

sampling period so that each of the eggs collected was of equal age per sampling day. Each egg removed from a nest was replaced by an unmarked one in an attempt to eliminate any growth changes in the remaining study eggs which might have resulted from reduced attentiveness by the parents because of a smaller clutch (see Beer 1965).

We opened eggs by the procedures outlined in Rugh (1962). This involved cutting around the widest diameter of the egg and emptying the entire contents into a petri dish without breaking the yolk. During the early stages of development we retained the embryo in the yolk and measured the diameter of the blastodisc and area vasculosa, number of somites, and other general developmental characteristics using a Wild M5 dissecting microscope. We removed embryos older than 6 days from the yolk and immediately weighed them to the nearest 0.1 g on a triple beam balance and measured them to 0.01 mm with vernier calipers. Embryos were preserved in 10% neutral buffered formalin. The measurements taken are illustrated in Fig. 1. Later development is defined here as that shown by an embryo 10 or more days old. By this time all of the various body parts are easily visible and can be measured accurately.

RESULTS

Early development (1–9 days).—The aging of these embryos presented problems because of considerable variation in development among embryos

TABLE 2

INTRA-CLUTCH VARIATION IN EARLY DEVELOPMENT OF RING-BILLED GULL EMBRYOS FROM THE CENTER AND PERIPHERY OF THE GRANITE ISLAND COLONY, 1975

Egg No.	Age (days)	Development	
		Center	Periphery
1	3	11 somites.	Embryonic shield stage.
2	1	No development.	No development.
1	3	Primitive streak stage.	No development.
2	1	No development.	No development.
1	5	18–19 somites; area vasculosa developing.	14 somites; initiation of area vasculosa.
2	3	7 somites; optic vesicle visible.	Head fold stage of primitive streak.
3	1	No development.	No development.
1	5	Embryo 0.82 cm in length. Limb buds visible.	16 somites, head turned.
2	3	Area vasculosa well developed. Heart visible and beating.	Head fold stage of primitive streak.
3	1	Diameter of blastodisc 0.66 cm.	No development.

of the same age. Table 1 details our results of embryos from the center and periphery of the colony to 9 days of age. Embryos from the center of the colony were slightly advanced to those of equal age from the periphery (Tables 1 and 2).

It was clear from our collections of complete clutches on 17 May that some development occurred in first and second eggs before the third egg was laid (Table 2). In all clutches the first egg showed more development than the second and the second more than the third. Similar variation in first, second, and third eggs of Herring Gulls was reported by Parsons (1972). The significance of this result is that based on the apparent differential development among eggs of a single clutch, individual eggs should be aged according to the day each was laid and not from the day on which the clutch was completed (see Drent 1970:80 and Parsons 1972:540).

Later development (10 days to hatching).—Figure 2 illustrates typical Ring-billed Gull embryos in each of the 3-day groupings. Embryos collected from the center were slightly, but not significantly ($P > 0.05$) larger than their peripheral counterparts. Consequently, we grouped all embryos from both

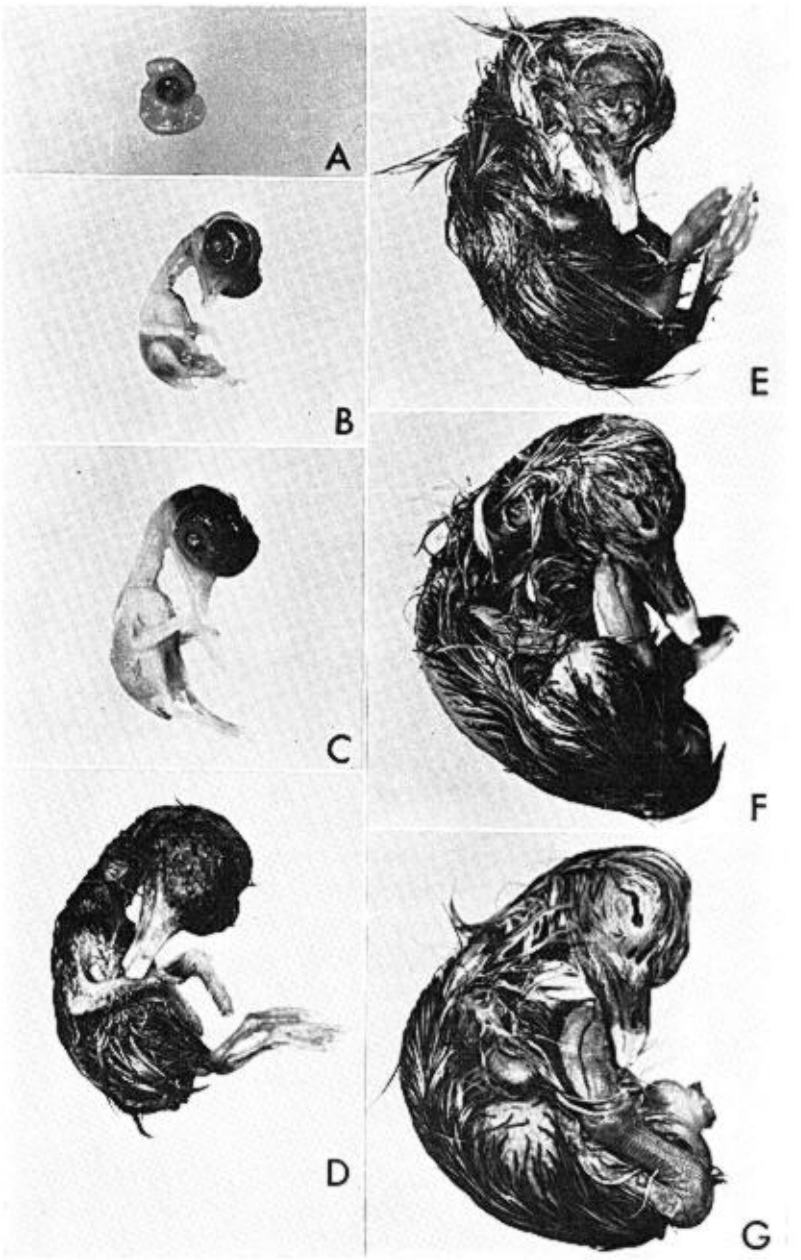

FIG. 2. A series of known-age Ring-billed Gull embryos, Granite Island, 1975. A, 7–9 days; B, 10–12 days; C, 13–15 days; D, 16–18 days; E, 19–21 days; F, 22–24 days; G, 25–27 days (pipping, note intact yolk sac). Embryos shown actual size.

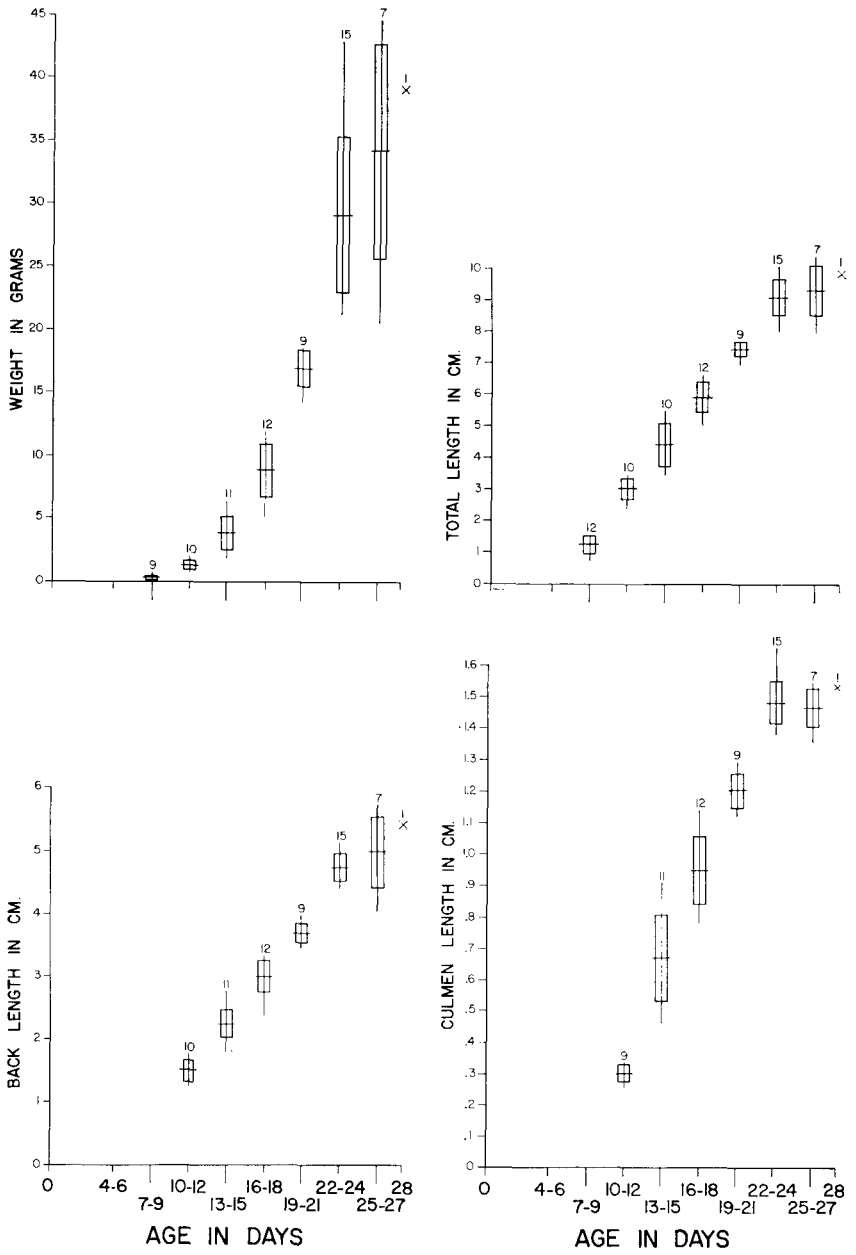

FIG. 3. Increase in weight, total length, back length, and culmen length of Ring-billed Gull embryos, Granite Island, 1975. Vertical line is range; horizontal line is mean; rectangles enclose $\overline{X} \pm 1$ SD, and number above is sample size.

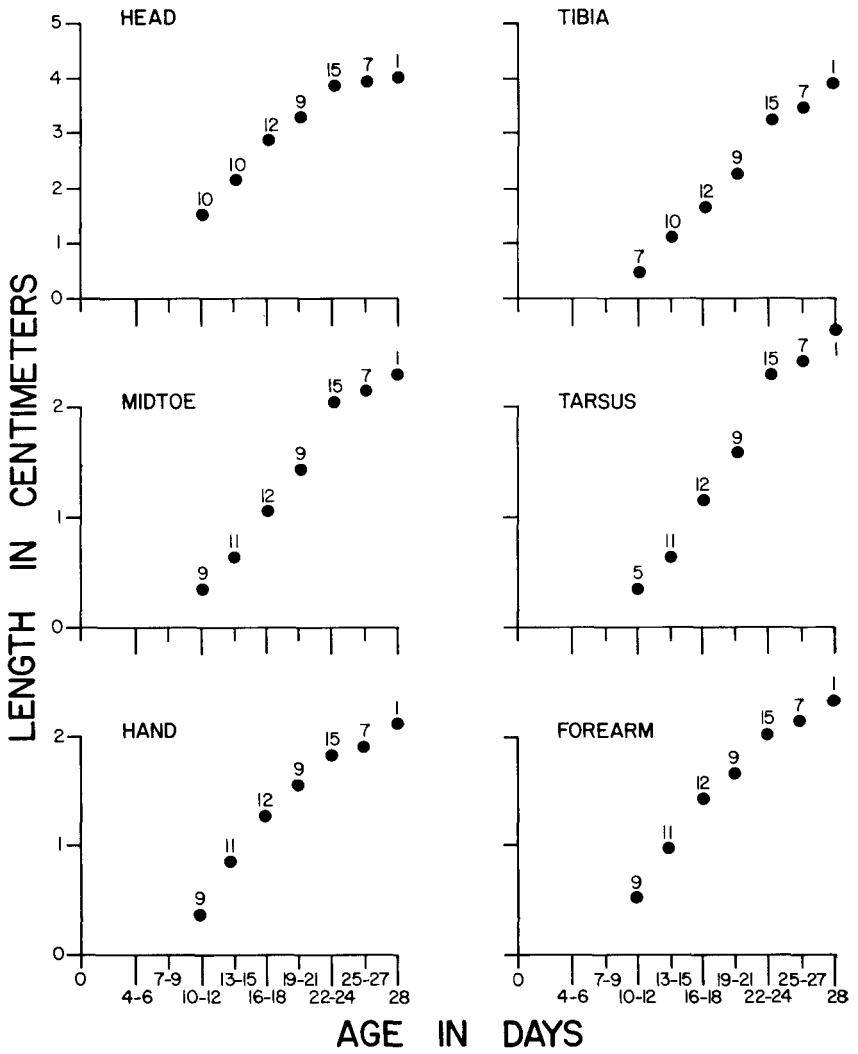

FIG. 4. Growth of body parts of Ring-billed Gull embryos, Granite Island, 1975. Number above each dot is sample size.

areas to determine aging parameters useful to the field investigator. Figure 3 shows growth in terms of body weight, total length, back length, and culmen length. No overlap at 1 standard deviation occurred in any of the age group measurements except those recorded near the end of incubation. Some of the chicks in the 22–24-day and 25–27-day groups were in the process of pipping

TABLE 3

FIELD CHART FOR AGING RING-BILLED GULL EMBRYOS

Age (days)	Characteristics
1– 3	No development through primitive streak stage to presence of somites, heart beating, blood vessels on area vasculosa.
4– 6	Embryo usually $\leqq$1.0 cm in length; wing and leg buds appearing; midbrain becoming prominent, eye pigmented; choroid fissure visible.
7– 9	Embryo 1–2 cm in length; less than 1 g body wt.
10–12	Embryo 2–4 cm in length; 1–2 g body wt. Choroid fissure complete.
13–15	Embryo 4–5 cm in length; 2–6 g body wt.; pterylae visible; feathering on spinal and caudal tracts.
16–18	Embryo 5–7 cm in length; 6–13 g body wt. All pterylae feathered; dorsal pterylae well feathered.
19–21	Embryo 7–8 cm in length; 13–20 g body wt. All pterylae feathered; pigment appearing in upper and lower mandibles, claws, and legs.
22–24	Embryo $>$8 cm in length; $>$20 g body wt.; mandibles, claws, and feet well pigmented; yolk compacted when close to hatching.

and of similar size. The point at 28 days in Figs. 3 and 4 represents 1 peripheral embryo which, along with others, tended to take longer to start pipping compared to embryos from the center.

Figure 4 presents growth curves for various body parts. They are not as useful by themselves as an aging tool because of overlap at 1 standard deviation in most cases. However, they do provide aid to the field researcher as a supplement to data in Fig. 3.

Table 3 summarizes the results. Based on 2 easily obtained measurements, weight and length of body, one can estimate the age of an embryo to within 3 days. Supplementary information such as degree of feathering and pigmentation also aid in embryo age determination.

DISCUSSION

Data in this report provide the basic information required to age embryos of Ring-billed Gulls to within 3 days. Of interest are the statistically insignificant differences between equivalent age embryos from the center and periphery of the colony. The general lack of differences in development characteristics and size of newly hatched chicks from the 2 areas suggests the young have approximately the same chance of survival. Dexheimer and Southern

(1974) found no significant difference in fledging success between central and peripheral Ring-billed Gull chicks on an island similar to our study area where flooding posed no problem. They did find significant differences in fledging success on an island where peripheral chicks were exposed to wave action and excessive wetting causing death.

SUMMARY

Characteristics for aging Ring-billed Gull embryos to within 3 days are given. Body weight, total length, back length, and culmen length from 10 days after laying to hatching were the most accurate aging parameters. No overlap at 1 standard deviation occurred in any of the above characters in 3-day intervals.

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