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DETERMINING CLUTCH SIZE AND LAYING DATES USING OVARIAN FOLLICLES

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Abstract.—Ovarian follicles of Eastern and Western Meadowlarks (*Sturnella magna* and *S. neglecta*) and Hermit and Townsend's Warblers (*Dendroica occidentalis* and *D. townsendi*) grow and regress sufficiently rapidly for laying intervals to be distinguished. We describe how to deduce clutch size from counts of ovarian follicles and to estimate laying dates from curves describing the growth of preovulatory and regression of postovulatory follicles. Our estimates of clutch size for warblers and meadowlarks were not different from those published in the literature or obtained from museum records. For birds collected during or near laying, dates of egg laying could be estimated over a 5–6-day period for warblers and a 7–9-day period for meadowlarks. Our records increased the number of clutch initiation dates for Hermit Warblers by 54%. The window of time during which clutch size can be deduced from ovarian follicles depends on: (1) how quickly follicles grow and regress, (2) how long postovulatory follicles can be identified, and (3) clutch size. This window of time is fairly narrow for both species because their follicles grow and regress quickly and because they lay relatively large clutches. Nonetheless, the clutch sizes we deduced from ovarian follicles for Hermit Warbler increased the number of records by 48%. We encourage researchers and collectors to record ovarian follicle measurements for all female specimens, and recommend a standard format for recording follicle size measurements on museum labels.

DETERMINANDO EL TAMAÑO DE CAMADA Y LAS FECHAS DE ECLOSIÓN USANDO LOS FOLÍCULOS DE OVARIO.

Sinopsis.—Los folículos de ovario de *Sturnella magna* y *S. neglecta* y de *Dendroica occidentalis* y *D. towsendi* crecen y se regresan lo suficientemente rápido como para que los intérvalos de oclosión se distingan. Describimos la forma de distinguir el tamaño de la camada de conteos de los folículos del ovario y cómo estimar las fechas de eclosión de curvas que describen el crecimiento preovulatorio como la regresión postovulatoria de los folículos. Nuestros estimados para el tamaño de camada de las especies antes mencionadas no resultan diferentes de esos reportados en la literatura u obtenidos de registros de museos. Para aves colectadas durante o previo a ovopositar, las fechas para ovopositar pueden estimarse durante un período de 5 a 6 días en *Dendroica* y durante un período de 7 a 9 días en *Sturnella.* Nuestros registros aumentaron el número de iniciación de fechas de camadas de *Dendroica occidentalis* por 54%. La ventana de tiempo durante la cual el tamaño de camada puede deducirse de los folículos de ovarío depende de tres variables: (1) cuán rapidamente crecen y regresan los folículos, (2) por cuanto tiempo puede identificarse los foliculos postovulatorios, y (3) tamaño de camada. Esta ventana de tiempo es relativamente estrecha para ambas especies debido a que sus folículos crecen y recrecen rápidamente y porque depositan camadas relativamente grandes. Sin embargo, los tamaños de camada que dedujimos de los folículos de ovario de *Dendroica occidentalis* aumentó el número de registros por 45%. Invitamos a investigadores y a colectores a que registren medidas de folículos de ovarios para

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todos los especímenes femeninos, y recomendamos un formato básico para registrar medidas de tamaño de folículo en etiquetas de museos.

Preovulatory follicles contain ova that are enlarging for ovulation, and postovulatory follicles represent yolks of eggs that are either present in the oviduct or already laid. These follicles grow and regress quickly enough so that follicles differing in age by one laying interval are readily distinguishable near the time of ovulation (Payne 1966). Consequently, curves representing the growth and regression of pre- and postovulatory follicles can be used to estimate laying dates for birds with enlarged ovarian follicles.

Counts of postovulatory follicles have been used to estimate clutch size and laying rates by birds (Payne 1977, Scott 1978, Houston et al. 1983, Scott and Ankney 1983, Ankney and Afton 1988, Arnold et al. 1997). Such counts have often been made with serially sectioned ovaries because magnification simplifies the identification of postovulatory follicles and extends the time period during which they can be aged (Payne 1966). For species that lay small clutches there is generally good agreement between the number of postovulatory follicles and the number of eggs that a female lays (Davis 1958, Kabat et al. 1948, Payne 1973). For birds laying larger clutches the method is less reliable (Arnold et al. 1997). After laying has begun, enlarged preovulatory follicles can be used in combination with postovulatory follicles to make reliable estimates of clutch size (Payne 1969, Jones and Ward 1976).

Here we describe a macroscopic approach to estimating clutch size and laying dates using counts of pre- and postovulatory follicles (Payne 1969) and curves of follicular growth and regression. We present the first such curves for Hermit (*Dendroica occidentalis*) and Townsend's (*D. townsendi*) Warblers and Eastern (*Sturnella magna*) and Western (*S. neglecta*) Meadowlarks and explain how such curves may be used to extrapolate laying dates. Finally, we recommend a standard format for recording follicle-size measurements and condition of the ovary on museum labels. Because breeding phenologies are poorly known for many species, these methods should provide valuable estimates of clutch size and timing of breeding for species that lay mid- to small-sized clutches and whose nests are rarely found.

METHODS

Measurements of postovulatory follicles or enlarged ova were obtained for 164 meadowlarks and 30 warblers collected for studies on hybridization (Rohwer 1972, Rohwer and Wood 1998). The meadowlarks were collected in the central and southern Great Plains and the desert grasslands of the southwestern United States from 1968 to 1970. The warblers were collected in Washington and Oregon from 1986 to 1995. Ovaries and oviducts of fresh or frozen birds were examined macroscopically. We measured to the nearest half millimeter the diameters of developing preovulatory follicles (which are spherical) and the diameter of regressing postovulatory follicles (which are roughly circular when flattened), and we described the condition of oviducal eggs (see Lewin 1963:262).

Warblers and meadowlarks arrive on the breeding grounds in the spring with a downy ventrum and with ova that are less than 1 mm and 2 mm in diameter, respectively (pers. obs.). Ova grow quickly just prior to laying and the brood patch defeathers during laying. Postovulatory follicles regress rapidly and the brood patch becomes edematous approximately when the penultimate egg is laid.

Estimating clutch size.—We estimated clutch size by counting the number of pre- and postovulatory follicles for females collected during or shortly after laying. We used only ovaries in good condition and we distinguished postovulatory follicles from atretic follicles (Kennedy et al. 1989, Arnold et al. 1997). Postovulatory follicles are irregularly shaped and appear as elongated, flattened and folded structures, with a slit (stigma) at the site of ovulation that remains visible for several days after ovulation (Davis 1942b, Payne 1966). In contrast, atretic follicles are smooth, opaque yellow, rounded and lack a slit (Davis 1942b, Erpino 1969, Payne 1966). We also attempted to distinguished burst atretic follicles from postovulatory follicles. Burst atretic follicles superficially resemble postovulatory follicles but can usually be distinguished by the presence of yolk or yolk residue either in or around the follicle (Davis 1942a, 1958). We used a blunt probe and a dissecting microscope or 10× hand lens to distinguish follicles.

Caution should be exercised when developing ova are used to estimate clutch size because some ova may not ovulate (Jones and Ward 1976). Therefore, we used preovulatory follicles in estimates of clutch size only for individuals that had ovulated at least three eggs. By using all postovulatory follicles that could be identified in an ovary, clutch size could be estimated for a period of 3–5 d for laying warblers and meadowlarks. Although more classes of pre- and postovulatory follicles could be distinguished for meadowlarks than for warblers, the window of time during which clutch size could be estimated was not longer for meadowlarks because they often lay larger clutches than warblers.

Estimating laying dates.—For females with enlarged follicles, curves of follicular growth and regression can be used to estimate laying dates. The only specimens that can be used to establish these curves are those having an oviducal egg. To establish the average rate at which preovulatory follicles develop, we compared the smallest developing follicle to the next largest (one laying interval further developed), and so on up to the largest follicle, which represents the next ovum to be ovulated. The same comparison was made for postovulatory follicles; the largest was compared to the next largest (one laying interval older) and so on down to the smallest identifiable postovulatory follicle.

Knowledge of the laying interval is critical to estimating laying dates because the stages of follicular growth and regression are separated by one laying interval. The laying interval for Townsend's Warblers and for Eastern and Western Meadowlarks is 1-d (Lanyon 1994, 1995; Matsuoka et al. 1997). The laying interval is not known for Hermit Warblers, but other congeners have 1-d intervals (Mayfield 1960, Nolan 1978, Morse 1993, Holmes 1994).

Calculating laying dates for birds collected with an egg in the oviduct is straightforward. The largest postovulatory follicle represents the ovum in the oviduct that would have been laid the day after collection; the second largest postovulatory follicle represents the egg that was laid on the day of collection, and so forth. Conversely, the largest preovulatory follicle represents the egg that would have been laid two days after the date of collection (assuming it would ovulate); the second largest represents the egg that would have been laid three days after collection, and so forth. Laying dates can also be estimated for birds without an egg in the oviduct and with one or more enlarged follicle(s). This is done by lining up the enlarged follicle(s) with the follicle sizes in Figure 1 (see results), and extrapolating from the date of collection as shown on the lower most axis. Obviously estimates of laying dates will be wrong for ova that would have been resorbed. Estimates will also be wrong if the follicles were incorrectly assigned to dates, but the stages of follicular growth and regression are so distinct that such estimates are unlikely to be off by more than 1 d (Fig. 1).

RESULTS

Clutch size.—For Townsend's Warblers the mean clutch size estimated from counts of pre- and postovulatory follicles was 4.8 (n = 5, mode = 5, range = 4–5); all Hermit Warblers laid 4 egg clutches (n = 8). These clutch sizes are similar to means obtained from egg sets collected in the same geographic region (Townsend's: mean = 4.9, n = 10, mode = 5, range = 4–5, Fisher's exact P = 1.0; Hermit: mean = 4.3, n = 8, mode = 4, range = 4–5, Fisher's exact P = 0.2).

For Eastern Meadowlarks the mean clutch size estimated from pre- and postovulatory follicles was 4.7 (n = 44, mode = 4, range = 2–7); for Western Meadowlarks the mean was 4.9 (n = 23, mode = 5, range = 3–7). Eastern Meadowlarks in Kansas lay a mean of 5.2 eggs (n = 26, range = 4–7; Johnston 1964), which is almost identical to the mean of 5.04 (n = 27, mode = 6, range = 3–7) estimated for the laying females we collected in Kansas. There are no published clutch records for Western Meadowlarks near our collecting areas (Lanyon 1994); however, two nests located by Rohwer in these same areas contained five eggs each, similar to our estimate. For one Western Meadowlark collected approximately 3 d after completing her clutch, there was an exact match between numbers of postovulatory follicles and number of eggs in the nest.

Estimating laying dates.—To establish the curves that describe follicular growth and regression, we could only use the 14 warblers and 58 meadowlarks that had eggs in their oviducts when they were collected (Fig. 1). We found no difference in mean follicle size between either pair of species for any of the 1-d laying intervals that had sample sizes greater than

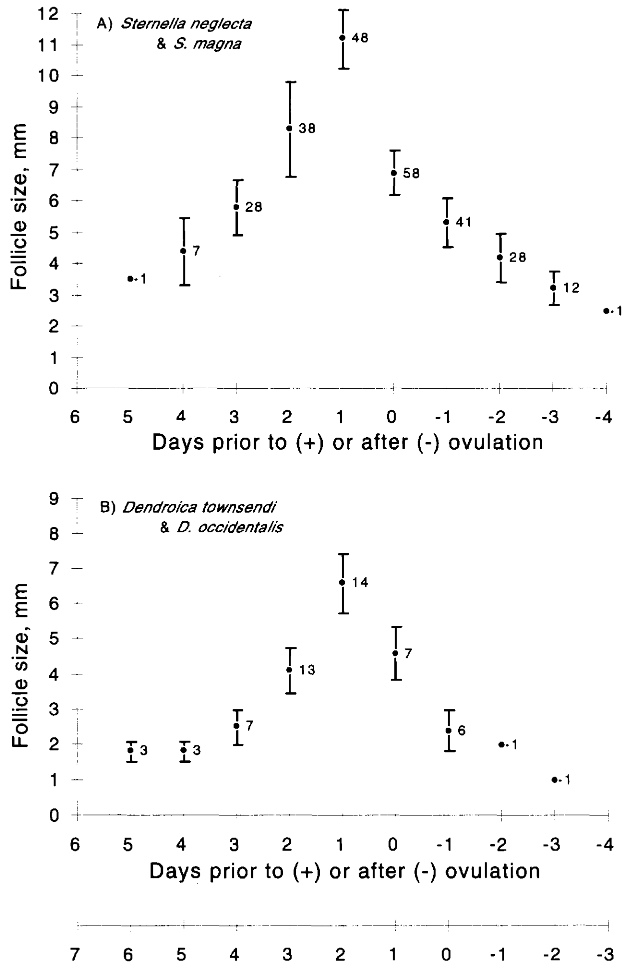

FIGURE 1. Mean size of enlarging preovulatory follicles and regressing postovulatory follicles for Eastern and Western Meadowlarks (a) and Hermit and Townsend's Warblers (b). On the connected x-axes, day 0 represents the day of collection, positive days represent preovulatory follicles, and negative days represent postovulatory follicles. The egg in the oviduct came from the postovulatory follicle for day 0. The supplemental x-axis gives the number of days that must be added to or subtracted from the collection date to assign laying dates to enlarged follicles. Error bars indicate standard deviations and numbers sample size.

one (*t*-tests; all *P*s > 0.1). We therefore combined follicular measurements into a single curve for the two warblers and the two meadowlarks (Fig. 1).

For females collected with an egg in the oviduct, the sizes of pre- and postovulatory follicles always could be ranked without ambiguity into size classes representing the 1-d intervals of laying. The pooled data were somewhat more variable because females may be collected at various times during the day, relative to the time that they ovulated. Nonetheless, 1-d differences in preovulatory follicle sizes were significant starting 3 d before ovulation for warblers and 4 d before ovulation for meadowlarks (t-tests; Ps < 0.01). One-day differences in postovulatory follicle sizes were significant at 1 d after ovulation for warblers and up to 3 d after ovulation for meadowlarks (t-tests; Ps < 0.01). These results show that there is at least a 4 d window for warblers and a 7 d window for meadowlarks during which enlarged pre- and postovulatory follicles can be used to estimate laying dates (Fig. 1). Note also that follicle size classes are distinct for both warblers and meadowlarks for an additional day before and after ovulation (Fig. 1); these differences would surely be significant with larger samples and should be used in estimates of laying dates.

DISCUSSION

Counts of ovarian follicles provided reasonable estimates of clutch size for Hermit and Townsend's Warblers and for Eastern and Western Meadowlarks. Ovarian follicles in these species grow and regress rapidly enough that laying intervals can be distinguished. We develop curves describing the growth and regression of pre- and postovulatory follicles for these species. We also describe how these curves can be used to estimate laying dates for birds collected with enlarged ovarian follicles.

Utility of the method.—Currently, timing of breeding, clutch size, and even breeding ranges for many species are poorly known, especially for species whose nests are rarely found. Therefore, estimates of clutch size and laying dates obtained from macroscopic examination of the ovaries can provide information valuable to a diversity of projects (e.g., Kennedy et al. 1989, Jackson 1993).

The period of time during which laying dates can be estimated from follicles varies inversely with yolk size. Eggs with small yolks come from small follicles, and small follicles persist as recognizable structures for less time than large follicles. Thus, the window of time during which laying intervals can be distinguished by differences in follicle size is relatively short for species that lay small eggs. Pre- and postovulatory follicles may be distinguished for a longer period of time in species that lay large eggs (Meyer et al. 1947, Kabat et al. 1948, Lewin 1963, Hannon 1981). Semel and Sherman (1991) provided a table of the length of time postovulatory follicles persist as distinguishable structures for various birds.

Estimating clutch size for species that produce large clutches of small eggs presents a similar problem. Obviously clutch size cannot be estimated when it is larger than the sum of enlarged preovulatory follicles and of recognizable postovulatory follicles. Fortunately, many tropical species lay small clutches and it is for these species that estimates of clutch size are most needed.

Our data has added considerably to the information available on clutch size and laying dates for the species we examined. For Hermit Warblers, we increased the number of clutches of known size by 48% and the number of clutch initiation dates by 54% (Pearson 1997). These figures highlight the value of such estimates for species whose nests are rarely found.

Recommendations.—We urge collectors to record the measurement of each enlarging ovum and of each regressing postovulatory follicle for their specimens. A good label for a laying female might read as follows: "soft-shelled egg in oviduct; 3 ruptured follicles: 10 (= egg in oviduct), 7, and 4.5 mm; enlarged ova: 12, 7.5 mm; other ova 1.5 mm or smaller; oviduct thick and glandular, 8 mm at cloaca; brood patch partially defeathered, not edematous." Such information should be recorded for every female examined near laying. Immediate research value cannot predict the future importance of specimens (Remsen 1995); thus, collectors should record as much data as possible for every specimen they prepare.

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