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USING POST-OVULATORY FOLLICLES TO DETERMINE LAYING HISTORIES OF AMERICAN COOTS: IMPLICATIONS FOR NUTRIENT-RESERVE STUDIES

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Abstract.—Post-ovulatory follicles (POFs) are often used to estimate egg production of collected birds, especially in studies of nutrient-reserve dynamics of laying waterfowl. We assessed the reliability of this technique in American Coots (*Fulica americana*) by conducting macroscopic and microscopic POF counts and comparing these estimates with egg counts obtained through frequent nest monitoring. Macroscopic POF counts were unreliable, but microscopic counts were significantly correlated with known clutch size ($r = 0.74$, $n = 12$, $P = 0.006$). On average, microscopic POF counts differed from known clutch size by -0.42 (SD = 1.51), which represented approximately -4% bias and 15% measurement error. Errors in POF counts were likely due to rapid regression of POFs or to mis-identification of atretic follicles. We used simulation modeling to assess the effects of such errors on estimates of nutrient commitment to clutch formation. Our simulations showed that small errors in POF counts are unlikely to have any serious effects; investigators concerned with low power and precision could benefit most by obtaining adequate sample sizes.

UTILIZACIÓN DE FOLÍCULOS POSTOVULACIÓN PARA DETERMINAR LA HISTORIA DE PUESTA DE INDIVIDUOS DE *FULICA AMERICANA:* IMPLICACIONES PARA ESTUDIOS DE RESERVA DE NUTRIENTES

Sinopsis.—Los folículos postovulación (FPO) se utilizan comunmente para estimar la producción de huevos en aves que se coleccionan, particularmente en estudios de dinámica de reserva de nutrientes de aves acuáticas. Utilizando a *Fulica americana,* examinamos la confiabilidad de esta técnica. Para esto, se hicieron conteos macroscópicos y microscópicos de FPO y se compararon los estimados con la cantidad de huevos puestos en nidos monitoreados. Los conteos macroscópicos resultaron ser poco confiables. No obstante, los microscópicos se correlacionaron significativamente con el tamaño de las camadas ($r = 0.74$, $n = 12$, $P = 0.006$). Los conteos microscópicos de FPO divergieron en promedio de los números obtenidos en camadas por -0.42 (DS = 1.51), lo que representa un sesgo de -4% y 15% en error de medidas. Los errores en el conteo de FPO se debieron a la rápida regresión de los FPO o a la identificacion equivocada de algunos tipos de folículos. Utilizamos modelos de simulación para determinar los efectos de tales errores en el estimado de la obligación de nutrientes para la formación de la camada. Las simulaciones mostraron que errores pequeños en el estimado de FPO no tienen gran relevancia. Aquellos investigadores preocupados con el poco poder y presición en la toma de este tipo de datos podrían beneficiarse utilizando muestras de tamaño adecuado.

Numerous investigators have used post-ovulatory follicles (POFs) to determine laying histories of collected birds (reviewed by Semel and Sherman 1991). Presence or absence of one or more POFs has been used to indicate whether or not a given female initiated a laying sequence during the most recent breeding season (Hannon 1981), whereas actual counts

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of POFs have been used to indicate how many eggs were laid (Buss et al. 1951, Payne 1973, Scott and Ankney 1983). This latter technique is especially common in studies of nutrient-reserve dynamics of laying waterfowl, where counts of POFs are used to estimate nutrient commitment to clutch formation (e.g., Alisauskas and Ankney 1994, Ankney and Afton 1988).

Although the POF technique has been widely used, there have been relatively few attempts to validate this technique using birds with known laying histories (Ankney 1974, Davis 1958, Kabat et al. 1948, Kennedy et al. 1989, Payne 1973). Payne (1973) achieved nearly perfect concordance between clutch size and POF counts among Red-winged and Tricolored Blackbirds (*Agelaius phoeniceus* and *A. tricolor*). Ankney (1974) examined POFs of post-laying Lesser Snow Geese (*Chen caerulescens caerulescens*) and estimated clutch size correctly for 67 of 75 females (88%); the eight discrepancies (12%) were well within the expected range of errors due to partial clutch predation or brood parasitism (i.e., errors in clutch size determination), and need not imply errors in POF counts. Kabat et al. (1948) studied captive Ring-necked Pheasants (*Phasianus colchicus*), and although only one of 38 POF counts matched exactly with known laying histories, there was a significant correlation between the two measurements ($r = 0.56$, $P = 0.0003$; raw data reanalyzed by us). Kabat et al. (1948) examined POFs from birds that had laid large numbers of eggs (range 15–76) and had been killed up to 350 days post-laying, so we were not surprised that their counts lacked precision (see Payne 1973). Davis (1958) and Kennedy et al. (1989) studied box-nesting European Starlings (*Sturnus vulgaris*) and obtained very low concordance between POF counts and clutch size, although the imprecision of their counts may have been partly attributable to high levels of intraspecific nest parasitism.

Arnold (1990) investigated nutrient dynamics of clutch formation in American Coots (*Fulica americana*) during 1987–1988 and found that macroscopic counts of POFs were uncorrelated with clutch sizes ($r = -0.13$, $n = 29$, $P = 0.51$). Although Arnold believed that this discrepancy was caused by rapid regression of POFs, there were a number of factors that could have invalidated his estimates of clutch size, such as parasitic egg laying, partial clutch loss, renesting, or continuation nesting. Most of these errors can be corrected for by monitoring nests frequently and comparing egg characteristics among clutches (Arnold 1993, Lyon 1993), but these techniques were still being developed in 1987–1988 when Arnold first utilized POF counts. Our objective in this study was to provide a more rigorous assessment of POF counts in American Coots.

METHODS

In 1991, we collected 12 coots from 8 wetlands located near Minnedosa, Manitoba (50°10'N, 99°47'W). Nest searches had been conducted every 4 d and previously discovered nests had been revisited every 1–2 d, so we had accurate laying histories for these nests. Because these coots were either trapped on their nests (Weller 1957) or shot as they swam from their nests during early morning hours (0500–0650 h), all 12 birds could be positively associated with specific nests. Seven coots still had developing follicles and five had completed their clutches within 0–3 d. Ovaries were removed within 2 h of collection and stored in individual glass jars containing a 10% solution of formalin. After ovaries were fixed, Arnold examined intact ovaries and counted numbers of POFs that were visible without magnification; we refer to these as macroscopic counts. Macroscopic counts were obtained for 7 of the 12 ovaries; the remaining five females could be identified as postlayers based on size and appearance of the ovary and oviduct, but POFs were no longer discernable. Thompson later examined each ovary macroscopically and removed all obvious POFs. Ovaries were then reexamined using a 6.4–16× dissecting microscope to detect smaller follicles. Suspicious follicles were examined with a blunt probe to determine if there was an epithelial opening in the stigma region (Scott and Ankney 1983). Open follicles containing yolky residue were assumed to represent burst atretic follicles and were not counted (Davis 1942). We refer to Thompson's counts as microscopic counts. In both cases, counts of POFs were conducted without knowledge of nesting information.

Nesting histories for collected coots were reconstructed based on nest records and eggshell characteristics. Each egg was individually numbered with a permanent black marker so that the disappearance of individual eggs from the clutch could be detected. While nests were being monitored, parasitism could be identified based on supernormal laying rates (>1 egg/d). Parasitic eggs could also be recognized based on eggshell characteristics (see Lyon 1993: Fig. 1), and these same characteristics could be used to match parasitic eggs with the coots that laid them (most parastic eggs are produced by coots that also nest normally, usually in adjacent territories; Lyon 1993). In blind recognition experiments, Arnold (1990: Appendix 2) correctly identified 94 of 100 parasitic eggs and matched these eggs to the proper parasites in 77 of 100 trials. Renests and continuation nests were identified based on several factors, including temporal and spatial proximity of nests, egg size, and most importantly, eggshell characteristics (Arnold 1993). We define apparent clutch size as the number of eggs that were in the nest on the day each female was collected (including any oviducal egg a female may have been carrying when she was collected). We define known clutch size as the total number of eggs a female was known to produce; this could differ from apparent clutch size due to parasitic egg laying, partial clutch loss, continuation nesting, or renesting.

RESULTS AND DISCUSSION

Apparent clutch size was the same as known clutch size for 9 of 12 coots (Table 1). For the remaining three coots, apparent clutch size was biased low due to continuation nesting or renesting (i.e., these three coots had laid 2–6 eggs in a previous nest bowl). We did not detect any

TABLE 1. Estimates of egg production in American Coots as determined from counts of macro- and microscopic postovulatory follicles (POFs) and nest visits. Apparent clutch size includes all eggs in the current nest bowl, whereas known clutch size accounts for renesting, parasitism, and partial clutch loss (see text).

	POF counts		Clutch size		
Coot	Macro[a]	Micro	Apparent	Known	Comments
9133	5	5	5	5	
9142	8	9	8	8	
9149	—	7	10	10	Possibly from adj. 9-egg nest
9150	5	11	12	12	
9151	—	11	11	11	
9153	5	12	11	11	1 "double POF" counted by JT
9154	8	11	5	11	Continuation nester: 6 + 5 eggs
9155	8	12	10	10	
9156	—	9	10	10	
9160	4	12	6	12	Continuation nester: 6 + 6 eggs
9161	—	9	10	12	Renester: 2 + 10 eggs
9162	—	9	10	10	3 "unusual follicles" not counted
Mean	6.1	9.8	9.0	10.2	

[a] A dash implies that no POFs could be discerned by macroscopic counts; however, these birds could still be identified as post-layers by the condition of their ovary and oviduct.

parasitism or partial clutch loss at these 12 nests. Parasitism was detected at 16 of 132 completed coot nests (12.1%) in 1991.

There was no correlation between known clutch size and POF counts when ovaries were examined macroscopically ($r = -0.14$, $n = 7$, $P = 0.76$), consistent with Arnold's (1990) earlier findings. Although two macroscopic counts were identical to known clutch size, the remaining five POF counts were gross underestimates (Table 1).

Microscopic POF counts were more precise than macroscopic counts (Table 1), and counts were obtained for all 12 ovaries. Although microscopic counts matched known clutch size for only four of 12 coots, the correlation between the two measurements was highly significant ($r = 0.74$, $P = 0.006$) and provided a better estimate of actual laying histories than did apparent clutch size ($r = 0.45$, $P = 0.14$); this poor correlation between known and apparent clutch size was caused by three coots that had laid eggs in previous nests. Five microscopic counts differed from known clutch size by one egg, one differed by two eggs, and two differed by three eggs. The mean deviation from known clutch size was −0.42 (SD = 1.51), which did not differ from zero ($P = 0.36$); three POF counts were too high, and five were too low (Table 1). Expressed as a proportion of known clutch size, bias of microscopic POF counts was −4.1% (−0.42/10.2) and the coefficient of variation was 14.8% (1.51/10.2).

Our sample of coot ovaries probably represented a worst case scenario for assessing the reliability of POF counts, at least with respect to waterfowl studies. The mean clutch size in our sample was 10.2 eggs, which is substantially larger than the mean number of POFs in most duck studies (ca. 4–5; Alisauskas and Ankney 1994, Ankney and Afton 1988). Only two coots had laid less than 10 eggs (5 and 8), and for both of these birds our macroscopic POF counts were correct. Large clutch sizes can exacerbate problems associated with POF regression, especially since POFs appear to regress more rapidly in coots than in any of the 11 species of waterfowl that we have so far examined. Dry mass of coot POFs had declined to ≤ 0.02 g within 3 d of laying (T. W. Arnold, unpubl. data), whereas dry mass of POFs in ducks typically exceeds 0.10 g for at least 5 d (Esler 1994). Coots initiate incubation during early laying (i.e., eggs 3–6; T. W. Arnold, unpubl. data), whereas waterfowl delay incubation until late laying (Afton and Paulus 1992), suggesting a possible causal relationship between incubation onset (i.e., rising prolactin levels) and POF regression. Rapid regression of POFs was probably responsible for most of our underestimates of clutch size. Overestimates of clutch size may have been caused by misidentification of burst atretic follicles, which can be easily confused with POFs (Davis 1942). Most post-laying coots had one or more atretic follicles (T. W. Arnold and C. D. Ankney, unpubl. data), whereas atretic follicles are much less common among post-laying waterfowl (C. D. Ankney and J. E. Thompson, unpubl. data).

We conclude that POFs in coots degenerate too quickly to allow laying histories to be determined by macroscopic counts. Microscopic counts can, however, still provide a reasonably accurate estimate of egg production. Moreover, microscopic POF counts are more accurate than estimates of apparent clutch size based on infrequent nest visits, because infrequent visits cannot detect parasitism or renesting. However, we would not use POF counts to provide an index of parasitism rates in coots because our data would have erroneously suggested that 8 of 12 coots had parasitized or been parasitized, whereas frequent nest visits detected no parasitism for these 12 birds.

We used a simulation model to assess the effect of imprecision in POF counts on estimates of nutrient use during clutch formation. Most recent studies of nutrient dynamics during clutch formation have regressed stored nutrient reserves (i.e., carcass fat) against nutrients committed to eggs and follicles (i.e., reproductive fat), where the absolute value of the slope of this regression estimates the proportion of clutch nutrients obtained from stored reserves (Ankney and Alisauskas 1991). For our simulation, we assumed that coots would obtain 80% of their clutch lipids from stored reserves (i.e., $b = -0.80$), that among-female variation in nutrient reserves was moderate (i.e. $\mathrm{CV}[Y] = 30\%$), and that measurement error ($\mathrm{ME}[X]$) was 0 or 15%. We used three different sample sizes of laying females: 100, 50, or 20. Parameter values for $\mathrm{CV}[Y]$, b, and n are appropriate for coots (Alisauskas and Ankney 1985) and are also typical of previous studies involving waterfowl (Ankney and Alisauskas 1991), whereas $\mathrm{ME}[X]$ equaled 0 or the 15% observed in our study. For each value of $\mathrm{ME}[X]$ and n, we conducted 1000 Monte Carlo simulations using SAS (SAS Institute Inc. 1985) to determine what proportion of simulations were significant at $P \leq 0.05$ (i.e., statistical power) and the mean value of b_1 over all 1000 simulations (i.e., bias). With $n = 20$ birds, measurement error did not affect power (power was 40.7% with 15% ME and 40.5% with 0% ME), although it caused slopes to be underestimated by about 5% ($b = -0.76$ vs. the true value of -0.80). Increasing sample sizes to 50 or 100 raised power to 74.8 and 95.9%, respectively, but did not eliminate the slight bias in b.

We conclude that investigators can reduce measurement error substantially by viewing ovaries under a dissecting microscope and exploring suspicious follicles with a blunt probe. However, further reduction of measurement error requires that investigators use multiple nest visits to verify individual laying histories. Because minor levels of measurement error in POF counts will have little effect on studies of nutrient reserve dynamics in laying females, investigators concerned about power and precision would be better off obtaining larger samples of laying birds ($n = 50$ to 100), especially if relationships are expected to be weak. If measurement error in X is extreme (i.e., >30%), use of nutrient reserves will be underestimated, but even this bias is not extreme (T. W. Arnold, unpubl. data).

The POF technique has been used most extensively in nutrient-reserve studies of waterfowl, where we suspect that measurement error is less than 10%, although it would be worth verifying this suspicion on a known sample of birds (i.e., captive ducks housed in individual pens). Some measurement error is inevitable because ducks are routinely collected by shooting, and ovarian damage often occurs (e.g., 3 of the 12 coots in our study had badly shot-damaged ovaries). Measurement error in X can also arise from using species-specific averages for egg composition, rather than actual egg composition from individual females (Alisauskas and Ankney 1994). Hence, we suspect that our simulation results based on moderate measurement error in X would be applicable to most studies of nutrient-reserve dynamics (i.e., whenever laying histories or egg composition are not measured directly for each collected female). Although investigators should always strive to increase accuracy and precision, our simulations demonstrate that nutrient-reserve studies are extremely robust to moderate amounts of measurement error.

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