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MINERAL RETENTION, MEDULLARY BONE FORMATION, AND REPRODUCTION IN THE WHITE-TAILED PTARMIGAN (*LAGOPUS LEUCURUS*): A CRITIQUE OF LARISON ET AL. (2001)

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FOR MANY YEARS, calcium has received little attention in studies of the nutritional requirements for successful reproduction in free-living birds even though it constitutes 98% of the dry mass of the avian eggshell (Romanoff and Romanoff 1949). Despite the importance of calcium in avian reproduction (see Reynolds and Perrins 2003), ornithologists have been more concerned with macronutrient requirements (i.e. fat, protein, carbohydrate) for successful reproduction in birds (see review in Carey 1996); only in the past few decades has there been interest in how birds meet the high demands for calcium during reproduction (e.g. Simkiss 1967, Hurwitz 1978, Ankney and Scott 1980). Furthermore, only in the last two decades has the focus shifted to investigation of micro- (e.g. essential amino acids, vitamins, and minerals) as well as macronutrient limitations on breeding performance of birds (for accounts and tests of calcium-limited reproduction, see Drent and Woldendorp 1989, Graveland et al. 1994).

Recently Larison et al. (2001) described a study of White-tailed Ptarmigans (*Lagopus leucurus*) breeding in Colorado where dietary calcium availability was low. (Note that although the authors use “mineral” in the title of their paper, calcium appears to be the focus of their study and the main thrust of their discussions.) By radiotracking birds, they were able to define the laying periods for the 1998 and 1999 breeding seasons. They collected males and females during prelaying, laying, and postlaying and dissected femora and tibiotarsi from each bird. Using X-ray absorptiometry (DXA) to examine bone-mineral density of right bones and in-

ductively coupled plasma mass spectrometry (ICP-MS) to analyze chemical composition of left bones, they suggested that females (but not males) stored calcium prior to the onset of laying. Furthermore, those stores were depleted during egg formation. The authors concluded that long-term calcium storage may afford White-tailed Ptarmigans the opportunity to select breeding habitats where dietary calcium availability is low. The authors also inferred that by storing calcium months in advance of reproduction, this species has reduced its reliance on exogenous calcium during egg-laying, thereby gaining access to a wider selection of suitable breeding habitat.

At first glance, these findings are exciting because gallinaceous birds in particular are sensitive to dietary calcium availability, and their distributions may reflect availability of sufficient exogenous calcium for their reproductive requirements (Dale 1955, Wilson 1959). During egg-laying, birds increase consumption of calcareous grit to obtain sufficient calcium for eggshell formation (Sadler 1961). Although Larison et al. (2001) provided no details of calcium availability within their study area for comparison with other studies of birds breeding in calcium-poor areas (e.g. Graveland 1995), their choice of study species and study area were appropriate to test predictions of long-term calcium storage for birds that “live where calcium is hard to find....” Furthermore, they employed rigorous and sensitive laboratory methods. The study’s main shortcomings lie in methodological flaws that make their conclusions questionable.

Central to their study was the classification of collected females as “prelaying”. The authors

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monitored timing of breeding for birds carrying radio transmitters during the two years of study. Presumably, birds were then categorized as prelaying or laying according to when they were sacrificed in relation to the laying period of the radiotagged birds the authors observed. Because a destructive technique was used to examine the skeletal structure of birds, breeding status could have been determined more accurately from examination of the carcasses. At the simplest level, birds could have been categorized as reproductive or nonreproductive on the basis of the extent of recrudescence of reproductive tissues (e.g. Krementz and Ankney 1995). Furthermore, for birds collected a few days before the first egg was laid and after the clutch was completed, unequivocal determination of laying date would have been possible through measurement of the size of pre- and postovulatory follicles. Sizes of pre- and postovulatory follicles of the study species can be measured at various times before and after egg-laying onset, respectively. Subsequently, the size of the largest yolk follicle and the largest postovulatory (regressing) follicle would have allowed prediction of clutch initiation dates for hens sacrificed during prelaying, laying, and postlaying (for details see Jones and Ward 1976).

No additional information about prelaying females was provided. For example, information about the ages of prelaying birds and, for nonyearlings, details of clutch sizes in previous breeding seasons would have allowed the reader to hazard an educated guess about the likelihood of birds breeding and their clutch sizes. The main drawback with sampling a population in that manner is that there is no guarantee that birds would have bred had they not been collected. Of course, for females collected during egg-laying and postlaying periods, the authors could have quantified the reproductive investment of birds from the number of eggs already laid or from the size of completed clutches. However, subsequently plotting bone mineral density against Julian date (see fig. 1 in Larison et al. 2001) is not as informative as plotting a temporal axis showing days since clutch completion or since laying onset for each collected bird. Placing the majority of collected birds in a time frame relative to egg-laying would have been possible with examination of reproductive tissues (see above). To reach the conclusion that calcium storage occurred

"months prior to reproduction," the authors were faced with an impossible task of showing that females would have bred months after they were collected. Such long-term calcium storage in the skeleton could only be proven through repeated sampling of birds across pre- and postlaying periods with a nondestructive technique capable of fine-scale resolution of skeletal structure. To my knowledge, such an analytical tool is not currently available.

The authors discussed bone mineral densities of females "before, during, and after egg-laying...." However, from consulting figure 1 in Larison et al. (2001), only one female was collected during egg-laying. To show that mineral sequestration in long bones of females met the "extraordinarily high demands for calcium" during reproduction, the authors needed to demonstrate that such accumulation of calcium occurred in birds that were laying at the time of collection. Presenting data from a single laying bird is inadequate and unacceptable.

Erroneous conclusions of this study resulted from imprecise "prelaying" classification arising from the aforementioned methodological failings. I question whether Larison et al. (2001) report long-term storage of calcium in this species; instead, I suggest that their findings were consistent with the formation (and subsequent destruction) of medullary bone, a specialized type of bone that forms in the medullary cavities of long bones (e.g. femur, tibiotarsus). Medullary bone has been found in a number of avian species to date (e.g. Mallard [*Anas platyrhynchos*], Landauer et al. 1941; chicken [*Gallus domesticus*], Common 1933; Northern Bobwhite [*Colinus virginianus*], Ringeon 1940; Japanese Quail [*Coturnix c. japonica*], Yamamoto et al. 2001; Spruce Grouse [*Dendragapus canadensis*], Reynolds 1993; Rock Dove [*Columba livia*], Kyes and Potter 1934; Canary [*Serinus canaria*], Bloom et al. 1958; House Sparrow [*Passer domesticus*], Kirschbaum et al. 1939; Zebra Finch [*Taeniopygia guttata*], Reynolds 1998). This specialized bone is a labile store of calcium that is deposited through the action of estrogen and testosterone approximately 10–14 days prior to the onset of laying (Johnson 2000). Future research will probably demonstrate that medullary bone forms in the majority of breeding birds with the exception of those lacking pneumatized skeletons (e.g. Brown Kiwi [*Apteryx australis*], Welty and Baptista 1988). Medullary bone supplies

calcium to the shell gland when dietary sources are inadequate to provide sufficient calcium for complete calcification of the eggshell (Simkiss 1961). Serological and radiological evidence (Etches 1987, Reynolds 1997) supports a diurnal pattern of bone formation and destruction that furnishes high calcium demands of the female during egg formation. Following clutch completion, medullary bone is completely resorbed over the course of approximately a week in the Rock Dove (Bloom et al. 1941). The White-tailed Ptarmigan in Colorado has a first clutch size of four to eight eggs (Braun et al. 1993), and, if eggs are laid on consecutive days, medullary bone could potentially persist in its long bones for at least 29 days (i.e. 14 days prelaying, eight days laying, and seven days postlaying) before it is completely resorbed.

Four prelaying birds showing elevated bone mineral densities were collected approximately 3, 9, 13, and 16 days before the start of the defined egg-laying period, as estimated from figure 1 of Larison et al. (2001). Given the proximity of the collection dates to the start of the defined laying period, and, despite no knowledge of potential laying dates, it is likely that sampled birds possessed medullary bone deposits in preparation for egg-laying. Larison et al. (2001) report "a dramatic shift in bone structure" several weeks before egg-laying began. That is also consistent with deposition of medullary bone. Whereas the hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) crystalline structure of medullary bone is similar to that of cortical (structural) bone, medullary bone is more heavily calcified with a hydroxyapatite to collagen ratio double that of cortical bone (Ascenzi et al. 1963). That was reflected in bone mineral densities of prelaying ptarmigans being 70% higher than those of nonlaying birds. Associated with medullary bone formation would be an increase in skeletal phosphorus levels (see chemical formula for hydroxyapatite above). The authors found that this occurred in females collected during prelaying. Similarly, ICP-MS indicated that the majority of the increase in bone-mineral density was explained by an increase in skeletal calcium levels (see table 2 and figure 2 in Larison et al. 2001).

The authors failed to discuss their findings in relation to medullary bone formation, instead referring to medullary bone and its role in providing calcium for egg formation in the

introductory text. Unfortunately, they chose to relate medullary bone to reproductive function in the chicken. That is understandable given the detailed knowledge of calcium requirements for egg formation in that species. Whereas the chicken is a good model for medullary bone function (i.e. timing of skeletal calcium mobilization with respect to egg formation), it is unrepresentative when addressing timing of medullary bone formation in relation to the onset of egg-laying. For chickens that can lay up to 250 eggs in a given year, medullary bone is continuously present (Dacke et al. 1993). It is therefore inappropriate of the authors to suggest that chickens store calcium as medullary bone "weeks, or months prior to the breeding season" unless they are specifically referring to pullets. Furthermore, it is impossible to define the breeding season for a continuous layer maintained on a long-day light schedule.

The authors also apply the above statement to Rock Doves, but it is known that medullary bone forms only ~10 days prior to the onset of egg-laying (Bloom et al. 1941). It would have been preferable to introduce the concept of medullary bone in relating the calcium logistics of a free-living species where there is detailed knowledge. For example, it is known that laying House Sparrows deposit ingested calcium as medullary bone used for overnight calcification of the egg (Krementz and Ankney 1995) in the same way as chickens. As Larison et al. (2001) propounded in their study, Krementz and Ankney (1995) found that total body calcium in the House Sparrow increased prior to egg production, remained high during laying, and declined only after clutch completion.

It is important to make a distinction between short- (weeks) and long-term (months) storage of calcium because, of the species studied to date, most are incapable of long-term calcium storage. Instead, females increase consumption of calcium in the few days prior to the onset of laying (Graveland and Berends 1997). High dietary calcium availability is fundamental for viable egg formation in most species and has been well-demonstrated in the Brown-headed Cowbird (*Molothrus ater*), an indeterminate layer. Holford and Roby (1993) found that captive birds could lay 67 eggs in as many days. Removal of calcium from the diet of captive birds resulted in 61% fewer eggs being laid than when *ad libitum* calcium was available.

That the White-tailed Ptarmigan is capable of storing calcium as skeletal deposits "in months prior to reproduction" would indeed be interesting and it would join a small and exclusive group of species that exhibit this ability. For example, female Red Knots (*Calidris canutus*) stored calcium a few weeks before egg-laying and their skeletal masses increased by 30–50% during this period (Piersma et al. 1996). Females can therefore lay two of their four-egg clutches before ingesting any calcium. Early-breeding Ruddy Ducks (*Oxyura jamaicensis*) depleted endogenous reserves of calcium with mineral reserves declining by 0.08 g for every gram of eggshell deposited for successive eggs in a clutch (Alisauskas and Ankney 1994). That was not the case for later-breeding birds that relied much more on exogenous calcium. Many Arctic-nesting goose species start egg-laying within a few days of arrival on their breeding grounds (Raveling 1978) before they can have accumulated significant calcium from dietary sources. For those species, endogenous reserves of nutrients, including calcium, might be the primary determinant of clutch size (Ryder 1970, Ankney and MacInnes 1978).

As the nutritional strategies of breeding birds are investigated further, many species may be discovered that rely heavily upon skeletal reserves of calcium deposited months in advance of egg-laying for their reproductive requirements. However, the findings presented by Larison et al. (2001) do not substantiate the addition of the White-tailed Ptarmigan to that currently small group of species. Instead, the authors have documented the occurrence of medullary bone, albeit with sophisticated analytical techniques. That said, Larison et al. (2001) is a valuable study and should stimulate further research to study how this species breeds in areas of Colorado where calcium availability is low. Breeding birds demonstrate proficiencies for acquiring sufficient calcium from their breeding habitats for successful reproduction, even under conditions of low calcium availability (e.g. Blue Tit [*Parus caeruleus*], Ramsay and Houston 1999; Zebra Finch, Reynolds 2001). Foraging strategies for and the utilization of exogenous calcium during breeding should be investigated further for White-tailed Ptarmigans to obtain evidence of calcium-limited reproduction. Only then will it be worth undertaking extensive sampling of free-living females throughout winter, spring,

and early summer to test whether this species is truly capable of long-term calcium storage.

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