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## Timing of Mineral Sequestration in Leg Bones of White-tailed Ptarmigan

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**ABSTRACT.**—Birds are unique among vertebrates in that they protect their eggs with rigid, calcium-rich shells. Thus, for a short period of time during the annual reproductive cycle, birds experience extraordinarily high demands for calcium. Two strategies appear to exist for meeting those temporally high demands. Some birds apparently seek out calcium-rich foods immediately prior to and during egg laying whereas others may store calcium in their skeletons over a much longer period of time, mobilizing those reserves only when they are needed for production of eggshells. In this study, we used dual energy, X-ray absorptiometry and inductively coupled plasma mass spectroscopy to monitor annual

shifts in bone mineral content in the legs of White-tailed Ptarmigans (*Lagopus leucurus*). The study organisms were known to live on calcium-poor soils. Despite an apparent shortage of calcium in their diets, the test subjects stored substantial amounts of calcium in their leg bones in months prior to reproduction. Those stores were subsequently depleted during the egg-laying period. We suggest ability to store calcium in the skeleton may afford this species more flexibility in selecting suitable breeding habitats than would be possible otherwise.

In many avian species, eggs are laid at a rate of one per day. Calcium-rich eggshells are formed, one at a time, in the final 24 h before each egg is laid (Romanoff and Romanoff 1949, Simkiss 1961, 1967). The question asked by many researchers in recent years has been, how do birds—particularly those living on calcium-poor soils—ingest enough calcium in the final hours before an egg is laid to meet the extraordinarily high

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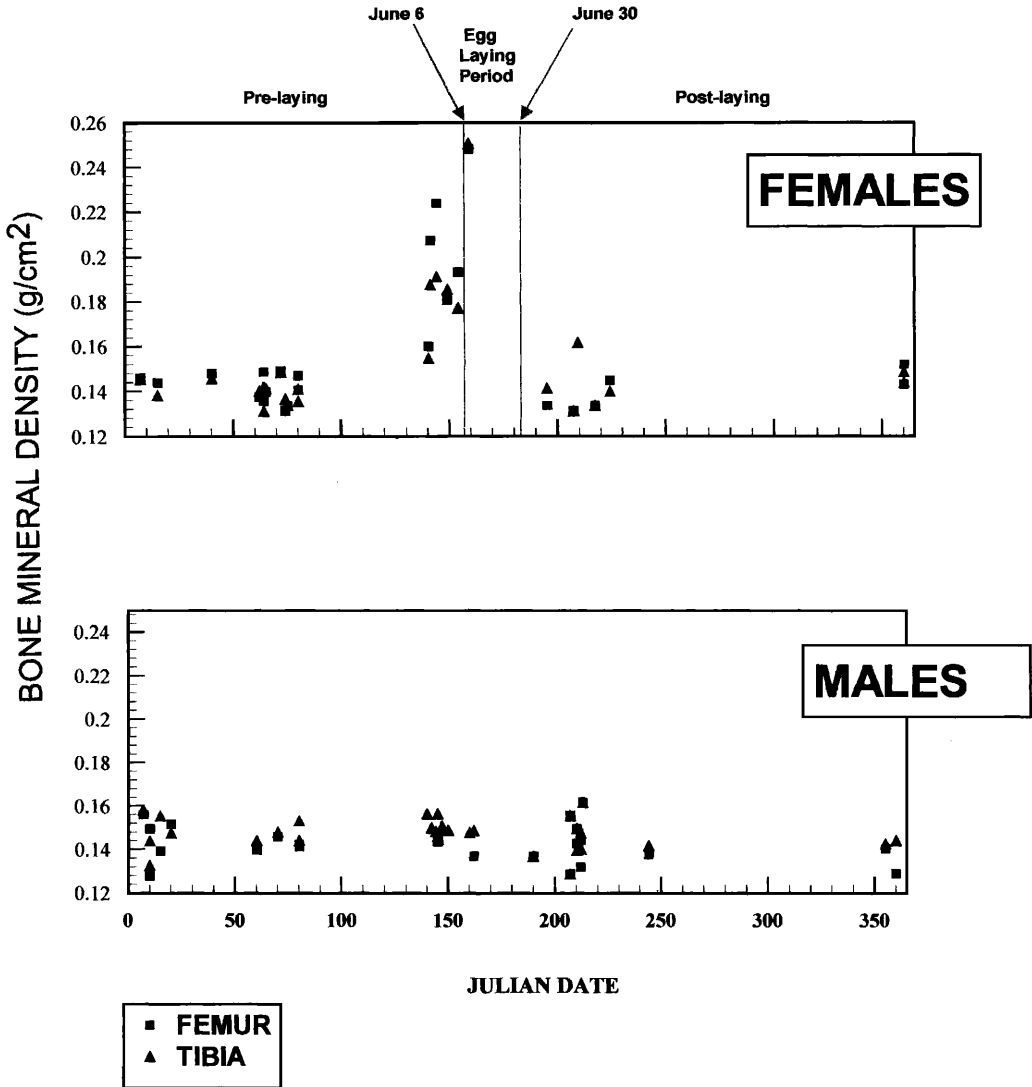


FIG. 1. Bone-mineral densities in male and female White-tailed Ptarmigan in Colorado. The first egg was laid in the study area on days 157 and 161 in 1998 and 1999, respectively. The last egg was laid on days 181 and 178, also in 1998 and 1999. "Egg-laying period" was therefore defined as days 157 to 181.

demands of eggshell formation? Apparently a number of bird species do seek out calcium rich foods in the days immediately preceding the laying of an egg (Graveland and Berends 1997, Nisbet 1997). But as early as 1954, Taylor and Moore questioned whether birds were physiologically capable of processing calcium at a sufficiently high rate to meet the demand. They observed (1954:112), "In the laying hen the rate of calcium deposition in the shell is often greater than the rate of absorption from the intestine. . .". A number of researchers have since suggested that chickens and pigeons, at least, may avoid that bottleneck by storing calcium as medullary bone in the weeks, or months prior

to the breeding season (Simkiss 1961, 1967; Taylor 1970, Gilbert 1983, Hurwitz 1987). It has been suggested those birds mobilize skeletal reserves to meet the temporally high demands of eggshell formation. More recently, however, other researchers reported no calcium storage prior to the laying of eggs in Tree Swallows (*Tachycineta bicolor*) and Brown-headed Cowbirds (*Molothrus ater*; Pahl et al. 1997). Thus, there appear to be two strategies of resource use in birds. Some birds may accumulate calcium over the weeks or months prior to reproduction and then mobilize those resources during egg-laying, whereas others must consume sufficient resources on a daily basis to meet temporally high de-

mands (Drent and Daan 1980, Winkler and Allen 1996, Pahl et al. 1997).

The extent to which birds store energy and nutrients needed for egg production prior to the breeding season has important implications for understanding the relationship between reproductive success and environmental conditions. An ability to store calcium over long periods of time before reproduction might provide some added flexibility in selection of suitable breeding habitat. Such an ability might permit a bird to breed in calcium-poor habitats not otherwise suitable.

Alpine environments are structurally simple with relatively few plant species (Martin 2001). Many such areas, including the site used in this study, are also calcium-poor (Church et al. 1997). White-tailed Ptarmigan (*Lagopus leucurus*) live in those areas and are known to feed on calcium-poor foods (Braun et al. 1993, Larison 2001). The objective of this study was to provide a temporally explicit analysis of the timing of calcium sequestration in that species. Our hypothesis was that female ptarmigan—possibly because they live where calcium is hard to find—would store calcium in their skeletons in the months prior to laying eggs. We also expected that stored calcium would be depleted as eggs were laid. Bone-calcium levels might, therefore, be seen to cycle annually. Further, we hypothesized that males would show no such annual cycling. This paper results from a broader study of fitness in White-tailed Ptarmigan in Colorado (Larison 2001).

*Methods.*—The study area was in the upper Animas River watershed near Silverton, Colorado (40°26'N, 105°45'W). We put radio transmitters on females in the study area and monitored breeding success between May 1998 and December 1999. During that period, the first eggs were laid on 6 June 1998 and 10 June 1999. The last eggs were laid on 30 and 27 June, respectively. Thus, we defined the "egg-laying period" as 6 to 30 June (Julian dates 157 and 181). We collected a total of 56 birds (25 females and 31 males) before, during, and after the egg-laying period ( $n = 19, 18, 19$ , respectively). From each bird, femurs and tibiotarsii were excised immediately after returning from the field. The bones of the right leg of each bird were placed in 10% saline solution and frozen in preparation for bone-mineral density studies. The left set of leg bones was cleaned by dermestid beetles and air-dried in preparation for chemical analysis.

Structural and chemical compositions of leg bones were analyzed using dual energy, X-ray absorptiometry (DXA), and inductively coupled plasma mass spectroscopy (ICP-MS). DXA analyzes structure. Mass spectrometry is used to determine chemical content. Although the DXA instrument system is capable of providing highly accurate, site-specific density data, it cannot distinguish between the various minerals present in the bone. Mass spectrometry, whereas incapable of providing site-specific data,

can distinguish between mineral elements. Together, those instruments permitted us to observe changes in bone structure and content over time.

We used a Hologic QDR-4500 to measure bone mineral content (grams) and bone mineral density (grams per square centimeter) in the leg bones of each bird collected. This instrument is a fan-beam densitometer that measures photo transmission. We used the Hologic small animal software program 6.1 optimized for adult rats weighing between 100 and 750 g. Daily quality control was performed on the instrument and all bones were scanned and analyzed by the same researcher. Global scans were made of each bone. Independent readings were also taken from a 1 cm<sup>2</sup> area at the geometric center and at the distal and proximal ends of each bone. Standardized scan widths and lengths were established and used with all bones. Bone-mineral (BMD) density and content were measured simultaneously. Reproducibility has been shown to be excellent for that instrument (Mitlak et al. 1994, Makan et al. 1997). We measured reproducibility by first choosing four test bones and then scanning each of those bones several times. We calculated a coefficient of variation ( $CV = SD/mean$ ) of BMD measurements of the four bones. The CV of that instrument in those tests of ptarmigan bones was <1%.

Lyophilized samples were crushed in acid-washed crucibles and covered with 20% trace metals grade nitric acid for two hours. Samples were heated to 450°C in a muffle furnace overnight. Ashed samples (0.2 g) were digested in a 2 mL concentrated nitric, 1 mL concentrated hydrochloric, and 2 mL concentrated hydrofluoric acids (after Briggs and Meier 1999). A 1:10 dilution of the sample was then made using 1% nitric acid. This sample was analyzed using an ICP-MS (Perkins Elmer Elan 6000). A dual detector calibration and autolens adjustment was performed prior to machine use, following manufacturer's specifications. Two calibration standards and a 1% nitric acid blank were used with each batch of samples.

*Results.*—Several weeks prior to the egg-laying period, female ptarmigan experienced a dramatic shift in bone structure and chemical composition. DXA showed bone mineral densities were dramatically elevated in all hens examined as early as 19 May (Fig. 1). BMD levels were 70% higher in prelaying birds, having risen from an annual average (excluding the breeding season) of 0.1399 to a high of 0.2485 g/cm<sup>2</sup> during the weeks just prior to egg-laying. Immediately prior to the egg-laying period, mean global BMDs were 31% higher in femurs and 34% higher in tibiotarsii (Table 1). Mass spectroscopy showed the majority of this change in BMD was due to an increase in bone calcium levels (Table 2). A smaller rise in bone phosphorus levels also occurred. Sodium and potassium levels declined as hens approached the egg-laying period. There was a slight, temporary depression in bone calcium and phosphorus levels in postlaying

TABLE 1. BMD levels in leg bones of female White-tailed Ptarmigan collected before and after egg-laying period (grams per square centimeter).

Bone	Before (May and June)		After (July through April)		Difference (%)
	Mean (g/cm <sup>2</sup> )	SE	Mean (g/cm <sup>2</sup> )	SE	
<b>Femur</b>					
Global scan	0.1998	0.006	0.1373	0.001	31
Center point	0.2149	0.013	0.1617	0.005	25
Distal end	0.1725	0.013	0.1128	0.006	35
<b>Tibiotarsus</b>					
Global scan	0.2110	0.005	0.1402	0.003	34
Center point	0.2369	0.009	0.1546	0.004	35
Proximal end	0.2467	0.023	0.1128	0.011	54

hens but, by the end of the breeding season, those levels had returned to pre-April concentrations.

There was a clear difference in the temporal dynamics of bone structure and composition between males and females (Fig. 1). Whereas bone mineral densities and calcium content of female leg bones spiked in the weeks prior to the egg-laying period, there was no change in males. BMD and calcium levels were generally higher in males than in females during non-breeding portions of the year but during the breeding season, levels were highest among females (Fig. 2).

*Discussion.*— Differences reported in BMDs and bone mineral levels before, during, and after egg-laying suggest the structure and chemical composition of leg bones cycle annually in female White-tailed Ptarmigan but remain constant in males. That is consistent with the hypothesis that female White-tailed Ptarmigan store minerals in their leg bones prior to the onset of the egg-laying period. Further, it is apparent that storage begins at least several weeks prior to this period. That BMD and BMC levels returned to pre-April levels immediately after the egg-laying period, is consistent with the hypothesis that female ptarmigan used skeletal calcium for construction of eggshells. We did not specifically track minerals through the anatomy of the ptarmigan, but the timing of uptake and subsequent depletion of skeletal calcium strongly suggests a connection between bone minerals and eggshell formation.

Ptarmigan generally remain on the ground whenever possible and walk rather than fly. That may ex-

plain why this species is able to store large quantities of calcium in its leg bones whereas some passerines apparently cannot. The extra weight associated with storage of minerals in leg bones is likely less energetically expensive for grouse than it would be for passerines.

A number of researchers have demonstrated a seasonal, rapid mass-gain in female ptarmigan during the months (March–April) immediately before the breeding season (Braun et al. 1993). Others have found seasonal changes in mass of reproductive organs did not account for the overall increase in body mass (Hannon et al. 1979, Hannon and Roland 1984). The observed rapid rate of mineral sequestration recorded in this study might, in part, explain observed increases in female mass.

It has long been thought that calcium availability may limit bird distribution. Accordingly, a calcium storage mechanism might permit more flexibility in territory selection in ptarmigan. That alpine environments frequently tend to be calcium-poor (Church et al. 1997, Larison 2001) raises the question of whether persistence in such areas is linked to the ptarmigan's ability to sequester calcium. Mineral sequestration—particularly if it occurs prebreeding, preselection of territory, or both—could also explain some of the lengthy dispersal movements that female ptarmigan make in late spring before they settle on territories (Martin et al. 2000).

This study demonstrates that a tool designed for studies of human health can be useful in the study

TABLE 2. Bone mineral levels in leg bones of female White-tailed Ptarmigan collected immediately before and after the egg-laying period percentage.

Mineral	Before (May and June)			After (July through April)			P-value
	Mean (%)	n	SE	Mean (%)	n	SE	
Ca	42.18	5	0.52	38.12	14	0.31	0.001
K	0.20	5	0.02	0.22	14	0.01	0.28
P	21.80	5	0.46	20.43	14	0.28	0.02
Na	0.73	5	0.05	0.92	14	0.03	0.01

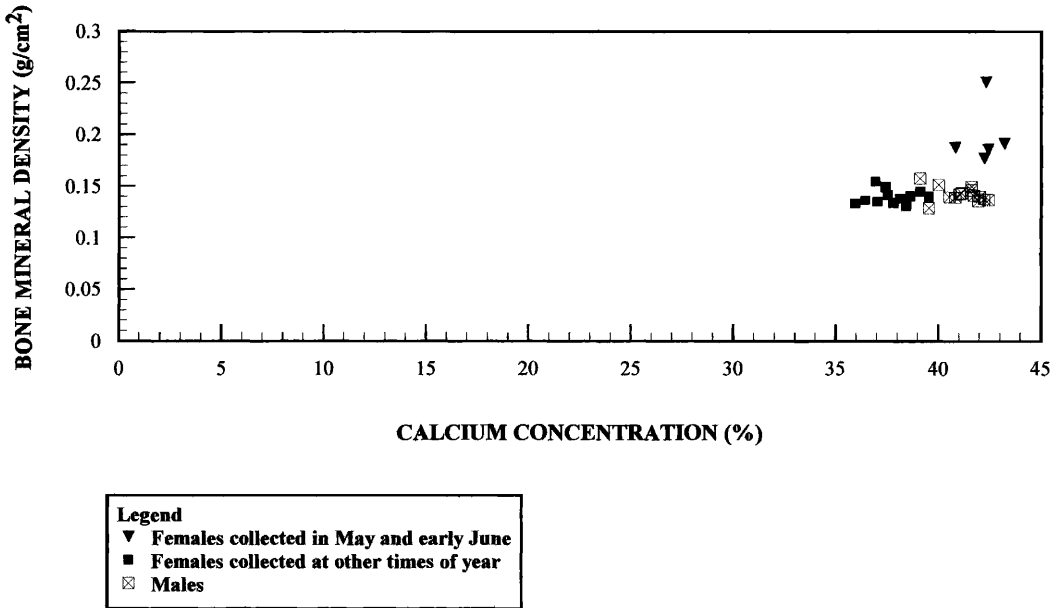


FIG. 2. Bone-mineral density and bone calcium content in right femur of White-tailed Ptarmigan in Colorado.

of wildlife. DXA made it possible for us to view, with exceptional precision and clarity, aspects of avian physiology that were until now difficult to observe directly. In addition, the combination of DXA and ICP-MS permitted us to not only measure small changes in skeletal structure but also to determine which minerals may be responsible for those changes.

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## Experimental Support for a New Drift Block Design to Assess Seabird Mortality from Oil Pollution

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**ABSTRACT.**—Seabird mortality from large oil spills and chronic oil pollution is often significant. Total mortality estimates are derived from counts of dead birds that wash ashore and are corrected for numbers lost at sea. Past attempts to estimate proportion of birds that die at sea and wash ashore have included several experiments using carcasses and different types of wooden drift blocks. Results varied greatly depending on environmental conditions and distance from shore where blocks or carcasses were released. Wind seemed to be the predominant factor determining movement over large distances, whereas tidal currents influenced deposition on specific

beaches. Determining timing and location of arrival of dead birds on beaches are crucial for accurate mortality estimates. Drift experiments using beached birds that have already drifted at sea for an undetermined length of time are inaccurate due to natural buoyancy loss and decomposition. To determine accuracy of drift block designs used in the past, we compared drift characteristics and patterns between four drift block designs and fresh murre (*Uria* spp.) carcasses. Our experiments showed that drift blocks used in the past have none of the drift characteristics of dead seabirds, because they have much larger areas exposed to wind and hence drift much faster and farther than murre carcasses. Past mortality estimates using those blocks are therefore doubtful. The drift block design that most accurately mim-

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