

Indications of phenotypic plasticity in moulting birds: captive geese reveal adaptive changes in mineralisation of their long bones during wing moult

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Abstract Bone is continually undergoing cycles of apposition and resorption referred to as adaptive remodeling. We tested the hypothesis that captive moulting Barnacle Geese (*Branta leucopsis*) would show adaptive bone mineralisation during the flightless period of their annual flight feather moult, despite having never flown. The three leg bones showed selective changes in mineralisation in terms of mass and mineral content, while the wing bones did not change in mass or mineral content. The tibia/fibula was the only bone to also undergo significant changes in mass, increasing as moult progressed then decreasing significantly towards the end of moult. This was not a response to changing body mass. Instead, we propose that this is a response to the requirement for increased strength brought about by the significant increase in the force producing

muscles that attach to the tibia. The femur and tarsometatarsus showed the opposite trend, with mineral content decreasing significantly during mid-moult before increasing again at the end. These changes were also independent of changing body mass, suggesting instead that the calcium, or rather calcium derivatives, were mobilised for feather regrowth. This study demonstrates significant and selective adaptive natural changes in bone mass and mineralization that have not been previously demonstrated. That they should also occur in captive birds which show a decrease in locomotion during the wing moult period, suggests a high endogenous capacity for these changes.

Keywords Bone mineralisation · Barnacle Goose · Bone changes · Mineral content · Moult

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Zusammenfassung Knochengewebe macht fortwährende Zyklen von Aufbau und Abbau durch, die als adaptive Remodellierung bezeichnet werden. Wir haben die Hypothese getestet, dass in Gefangenschaft gehaltene mausernde Weißwangengänse (*Branta leucopsis*) während der flugunfähigen Phase ihrer jährlichen Flugfedermauser adaptive Knochenmineralisation zeigen, obwohl sie niemals geflogen sind. Die drei Beinknochen zeigten selektive Veränderungen der Mineralisation bezüglich Masse und Mineralgehalt, während sich Masse und Mineralgehalt der Flügelknochen nicht änderten. Der Unterschenkelknochen war der einzige Knochen, der auch eine signifikante Änderung seiner Gesamtmasse erfuhr, die im Verlauf der Mauser zunächst anstieg und dann zum Ende der Mauser hin abnahm. Dies war nicht auf Veränderungen der Körpermasse zurückzuführen. Stattdessen schlagen wir vor, dass es damit zusammenhängt, dass dieser Knochen stabiler werden muss, da sich die am Schienbein ansetzenden Kraft produzierenden Muskeln vergrößern. Oberschenkel

und Tarsometatarsus offenbarten den entgegengesetzten Trend—der Mineralgehalt nahm in der Mitte der Mauserperiode signifikant ab und dann gegen Ende wieder zu. Diese Veränderungen waren ebenfalls unabhängig von Änderungen der Körpermasse und deuten stattdessen darauf hin, dass Kalzium oder eher Kalziumderivate für das Federwachstum mobilisiert wurden. Diese Studie zeigt signifikante und selektive adaptive natürliche Veränderungen von Knochenmasse und -mineralisation, die zuvor nicht nachgewiesen worden sind. Dass diese Veränderungen auch bei in Gefangenschaft gehaltenen Vögeln auftreten, die sich während der Flügelmauser weniger fortbewegen, deutet auf eine hohe endogene Kapazität für diese Veränderungen hin.

Introduction

Living bones are continually undergoing processes of apposition and resorption that are primarily driven by changes in the mechanical load placed upon them. This is frequently referred to as adaptive remodelling (Cowin 1998). Bone retains this ability to model and remodel throughout a major portion of an animal's life cycle (Biewener and Bertram 1994). This adaptive remodelling is regulated by the activities of osteoblasts, osteoclasts and osteocytes, which sense their mechanical environment and regulate deposition or resorption of the bone matrix accordingly (Pead et al. 1988; Skerry et al. 1989; Antalíková et al. 2001). Mechanical stimuli due to muscle contraction, resulting from both static and dynamic loading components, play an important role in the remodelling of mature bone (e.g. Ferretti et al. 2003; see also Fritton et al. 2000; Reich et al. 2005). Skeletal unloading as a result of paralysis or extended periods of weightlessness typically leads to rapid loss of bone mass, while exercise stimulates a site-specific increase, although exceptions do occur (Wallace et al. 2007). Mineral composition of the bone, however, has not been found to alter significantly in the same manner as mass (Biewener and Bertram 1994). Studies have shown that different bones appear to exhibit different sensitivities to applied mechanical loading, and other factors such as gender will affect how bone responds to changes in the mechanical environment (Habib and Ruff 2007, Antalíková et al. 2001). Importantly, most studies investigating the effects of increased exercise and/or weightlessness have been conducted using artificial changes in activity, as opposed to studying natural, non-manipulated systems.

Cyclic osteoporosis has been predicted to occur in moulting birds (Meister 1951; Murphy and King 1992) to provide a source of calcium or calcium derivatives for elsewhere in the body. This can include the feather constituent chondroitin sulphate for replacement of feathers

(Meister 1951) and phosphorous for muscle hypertrophy (Hanson and Jones 1976). Cyclic osteoporosis can also occur in female birds prior to egg production, to provide a calcium source for the eggshell (e.g. Raveling et al. 1978; Larison et al. 2001). Waterfowl undergo an annual simultaneous flight feather moult, which renders them flightless for approximately 3–4 weeks (Owen and Ogilvie 1979). During this period, many changes are observed both in the bird's behaviour and physiology (e.g. Hohman et al. 1992). For example, birds will often become inactive and devote less time to foraging and maintenance such as preening (e.g. Canvasbacks, *Aythya valisineria*, Thompson 1992; Harlequin Ducks, *Histrionicus histrionicus*, Adams et al. 2000). In addition, some species switch from diurnal to nocturnal foraging (e.g. Kahlert et al. 1996), increase vigilance bouts (e.g. Kahlert 2003) and/or reduce vocalisations to avoid detection by predators (e.g. Bailey 1981). Many species of wild moulting waterfowl have also been recorded losing significant amounts of body mass during the flightless period of moult (e.g. Fox and Kahlert 2005) and undergo significant increases in metabolic rate (e.g. Guozhen and Hongfa 1986). During this period, wild moulting waterfowl become totally reliant on leg-powered locomotion to escape predators and to forage. As a result, the locomotor muscles show cycles of atrophy and hypertrophy consistent, to some extent, with the changes in usage of the muscles. Portugal et al. (2009a), recently demonstrated that these changes in body mass, metabolic rate and musculature also occur in captive moulting waterfowl (Barnacle Geese, *Branta leucopsis*, B.), despite the fact that the birds had never flown, were safe from predators, had a constant food source and had always relied only on leg-powered locomotion. In particular, the muscle groups in the legs that are responsible for force production (ankle plantarflexors, hip extensors and adductors) showed a significant increase in mass at the onset of the flightless period of moult, suggesting that the legs had become better adapted for rapid running and/or swimming (Portugal et al. 2009a). These findings are consistent with the suggestion of Dietz et al. (2000) that these muscular changes in birds can be partially anticipatory, and that exercise is not essential to build muscle mass in birds. This suggests a partially endogenous origin for the control of these changes, while also providing evidence it is possible to use captive birds for studying physiological changes during moult that would not be possible in wild birds (see also Portugal et al. 2007, 2009a, b, 2010).

In this preliminary study, we tested the hypothesis that the apposition and resorption of the leg and wing bones of captive geese is also under endogenous control and the mass and mineral content of bones would change in response to the flightless stage of moult, despite the fact that these animals had never flown. Any such changes in bone mass and mineral content must either be (1) common

to both leg and wing bones as a result of cyclic osteoporosis during moult, or (2) consistent with maintenance and/or enhancement of terrestrial locomotion, in the same manner as the leg muscles.

Materials and methods

Birds and sampling

A captive population of 39 Barnacle Geese obtained as 3-week-old goslings was maintained under natural light in large outdoor aviaries at the University of Birmingham. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK) which has held a self-sustaining captive population of this species since 1982. The geese were fed with a 50:50 diet (Lilico, Surrey, UK) of mixed poultry corn (4% fat, 12% protein and 71% carbohydrate) and poultry growers pellets (3% fat, 16% protein and 61% carbohydrate), and water was available ad libitum. The aviary (30 × 25 m) consisted of 3 large pools, areas of shrub and trees, and open grass. Birds were sampled from 10 July (1 week prior to the commencement of wing moult) to 3 November (when wing moult had been completed for 2 months) 2006, with extra sampling periods at the end of July and beginning of August during peak wing moult (see “Results” for sample sizes). At the time of sampling, all birds were at least 2 years old, and had never bred. Each bird was anesthetized with 5% isoflurane gas mixed with a 4:1 ratio of air and oxygen, and then injected with a lethal dose of pentobarbitone (140 mg/kg) into the intertarsal vein. Final body mass was measured (± 5 g) before the carcasses were double-bagged and frozen at -20°C until the dissections were performed.

A 5-point classification moult score system was used to assign the stage of moult in the birds (see Portugal et al. 2007 for more details). Briefly, moult score was defined as (1) pre-wing moult, (2) primaries and secondaries remain, new primary pin visible, (3) all primaries and secondaries missing, (4) new primaries visible well beyond primary coverts and secondaries visible beyond secondary coverts (small flight feathers covering the primaries and secondaries), and (5) post-wing-moult. Approximate (± 1 day) durations of moult scores 2, 3 and 4 were 7, 11 and 12 days respectively.

Bone preparation

One wing and one leg from each bird were cleaned of flesh, muscle and connective tissue by a colony of flesh-eating beetles (*Dermestes maculatus*) at the Natural History Museum, Tring, UK. Three long bones from the leg (femur, tibia/fibula and tarsometatarsus) and wing (ulna, radius and

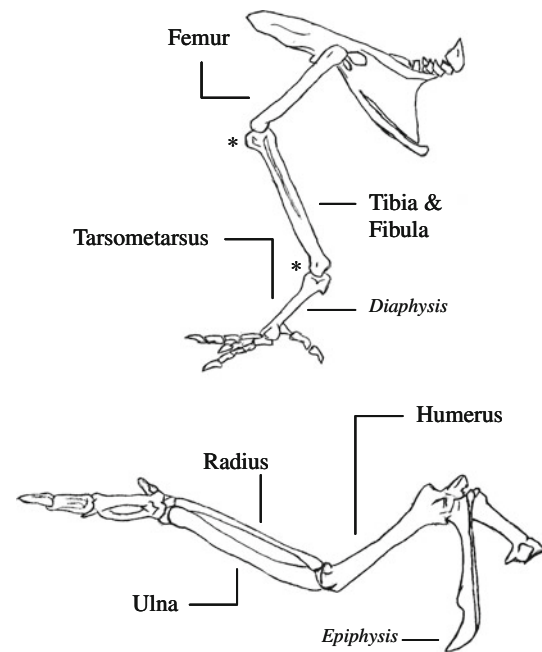


Fig. 1 View of the three leg (*top*) and three wing (*bottom*) bones used in the study. The *asterisks* on the tibia/fibula on the leg show the approximate main origin and insertion points of the ankle plantar-flexors leg muscles in Barnacle Geese, *Branta leucopsis*

humerus) were identified and used for analysis (Fig. 1). Each bone was weighed and the length measured, then cut into segments using a dentist’s drill (Milnes, Surrey, UK). The diaphysis was cut transversely, and longitudinal cuts were made into the epiphysis to allow the marrow to be removed. After removing the marrow, the bones were rinsed in warm water, and allowed to dry for 24 h at room temperature. The washed and dried bone segments (of the whole bone) were then placed into an oven to dry to constant mass at 90°C . Following this, each bone was weighed on a precision electronic balance (Sartorius, Goettingen, Germany), placed in a small ceramic crucible and then the two were weighed together. The bones and crucible were placed in a muffle furnace (AAF 1100; Carbolite, Hope, UK), for 30 h at 650°C . Immediately upon removal from the furnace, the ashed bone and crucible were placed into a desiccator to cool, before being weighed again.

Statistical analysis

Data were analysed in Minitab (MiniTab, State College, PA, USA) using generalised linear models (GLM) with Tukey post-hoc testing. All tests are considered significant at the level $\alpha = 0.05$. Values given are means \pm SEM. The percent mineral content by mass of each bone was determined as post-ashed mass/pre-ashed mass $\times 100$. These values were not arc-signed transformed for analysis, as all percentage values were normally distributed between 35

and 75%. Models of best-fit to describe wet mass, dry mass and % mineral content were derived by firstly including all possible main effects and all possible second degree interactions in the model. These main effects were moult score, body mass and sex for % mineral content, with bone length added for wet and dry mass analyses. Each non-significant term was then removed, iteratively, by backwards elimination. Non-significant main effects were retained in the model if the variable was included as a statistically significant interaction.

Results

Sample sizes for the five stages of moult were 7, 7, 10, 6 and 9, respectively. Neither sex or body mass were significant factors in any of the models. Of the six bones, only the tibia/fibula underwent significant changes in both wet (GLM, $F_{4,35} = 3.63$, $P = 0.001$) and dry (GLM, $F_{4,35} = 3.49$, $P = 0.002$, Fig. 2) mass. Post-hoc testing showed

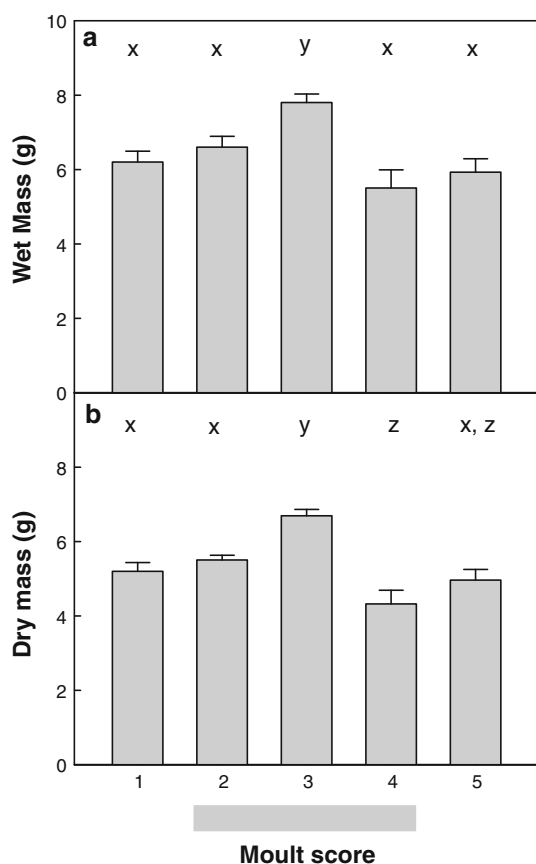


Fig. 2 Changes in wet (a) and dry (b) mean mass (\pm SEM) in the tibia/fibula leg bone during wing moult of Barnacle Geese. Post-hoc testing revealed significant differences between moult score categories. Bars not sharing the same number were significantly different. The shaded horizontal bar indicates moult scores during the wing moult period (July–August)

there to be significant increases in wet and dry mass between moult scores 1 and 3 before returning to pre-moult level at moult score 4 (Fig. 2).

The % mineral content of the femur (GLM, $F_{4,35} = 9.66$, $P < 0.001$, Fig. 3a), tarsometarsus (GLM, $F_{4,35} = 2.72$, $P = 0.045$, Fig. 3c) and tibia/fibula (GLM, $F_{4,35} = 3.95$, $P < 0.01$, Fig. 3b) changed significantly during the study period but did not follow the same pattern of change (Fig. 3a–c).

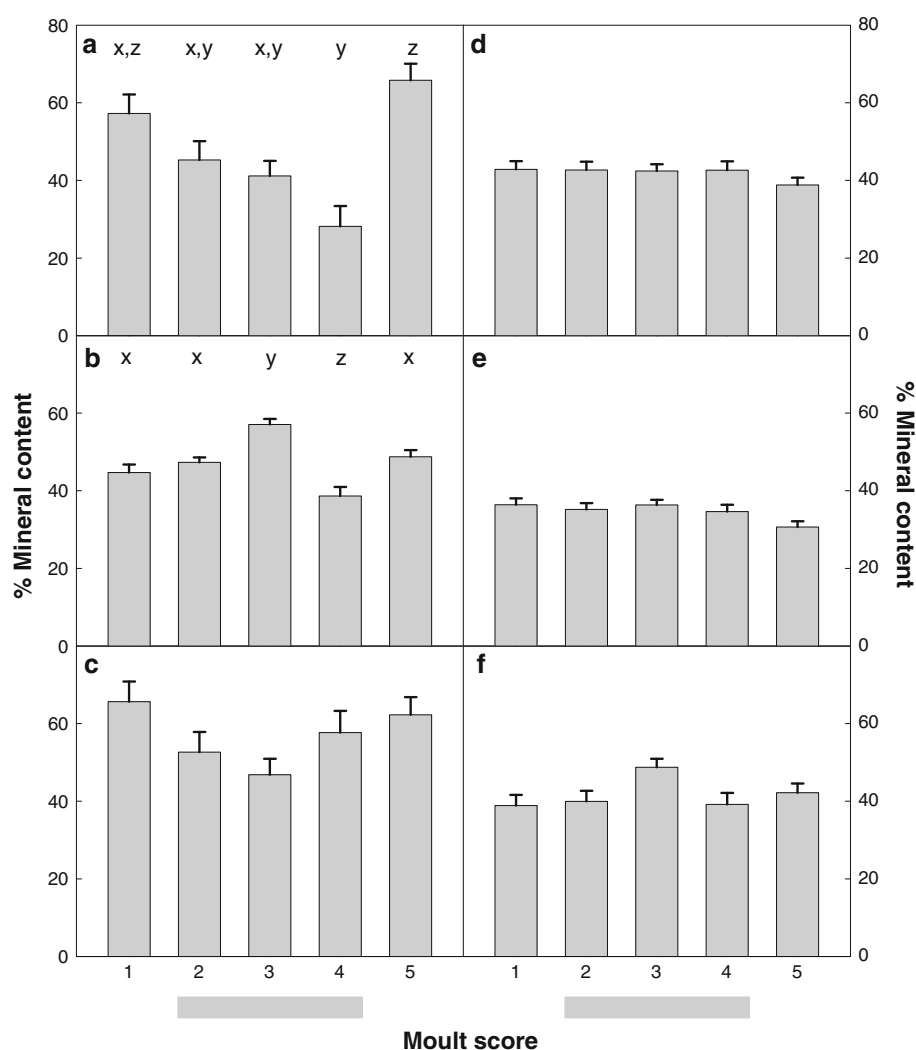
Post-hoc testing revealed that % mineral content in the femur underwent a significant reduction between moult scores 1 and 4, decreasing from 57.29 (± 4.8)% at moult score 1–28.14 (± 5.2)% at moult score 4. The % mineral content of the tibia/fibula (Fig. 3b) increased between moult scores 2 and 3, peaking at 54.06 (± 2.4)% at moult score 3, before decreasing significantly at moult score 4–38.64 (± 2.3)%. Post-hoc testing of the tarsometarsus revealed no significant differences between moult scores but Tukey's test revealed the overall significant difference was likely to be the result of the apparent decrease in % mineral content between moult scores 1 and 3 ($P = 0.052$).

None of the wing bones underwent any significant changes in mass or % mineral content during the study period (Fig. 3d–f).

Discussion

During the flightless stage of wing moult, the leg bones underwent significant changes in their mass and mineral composition, while no significant changes were observed in the wing bones. Sibonga et al. (2000) found that simple immobilisation had no effect on the mass or mineral composition of bone in rats, and only extra mechanical loading resulted in a remodelling response. This may partially explain the lack of significant change in the mass and mineral content of the wing bones in the geese; lack of use was not sufficient to cause a significant decrease in bone mass or mineral content, and it has been previously reported that minimal activity per day is sufficient to maintain bone strength in birds, without a requirement for intense exercise (Fritton et al. 2000). However, it has also been found that decreased loading results in significant decreases in bone mass (Biewener and Bertram 1994; Bloomfield et al. 2002). During wing moult, the flightless geese are at risk from predation and also find their foraging opportunities severely restricted. There is, therefore, a benefit to minimising the duration of the flightless period (Owen and Ogilvie 1979; Portugal et al. 2007). The lack of significant change in the wing bones of the captive geese would suggest the nutritional benefits involved in redistributing calcium from the wing bones to elsewhere is outweighed by the requirement for the wings to be ready to

Fig. 3 Change in mean percentage mineral content (\pm SEM) of **a** femur, **b** tibia/fibula, **c** tarsometatarsus, **d** ulna, **e** radius and **f** humerus during wing moult of Barnacle Geese. The calcium content of the leg bones (femur, tibia/fibula and tarsometatarsus) changed significantly during wing moult but the calcium content of the wing bones (ulna, radius and humerus) did not. Post-hoc testing revealed significant differences between moult score categories. Bars not sharing the same number were significantly different. The shaded horizontal bar indicates moult scores during the wing moult period (July–August)



return to flight at the earliest possible opportunity. The wing bones would also be a poor source of calcium in comparison to other parts of the skeletal system, such as the breastbone and pelvis which contain proportionally more calcium than the wing bones (Meister 1951). Further study on the whole skeletal system before, during and after moult would identify the primary sites where cyclic osteoporosis occurs.

Although no data on leg bone mass (g) or ash content exists from wild Barnacle Geese, data from other wild goose species confirms our pre-moult and post-moult values were typical for geese in general. Ankney (1979) reported a dry mass range of the femur, tibia and tarsometatarsus combined of 19.3 ± 0.5 – 29.4 ± 1.0 g (c. 8.1–12.1 g/kg) for wild Lesser Snow Geese, *Chen caerulescens caerulescens*, compared to a range of 11.9 ± 1.1 – 16.7 ± 0.7 g (c. 5.4–7.6 g/kg) in the captive Barnacle Geese used in the present study. Snow geese are significantly larger than Barnacle Geese (e.g. mean body mass of 2,050 and 2,500 g for wild Barnacle and Snow Geese, respectively; Kear

2005), so a slightly heavier bone mass would be expected. However, the approximate doubling in Snow Geese skeletal mass is considerably higher than would be expected from mean body mass, and the near isometric scaling relationship between skeletal mass and body mass in birds. This, however, may be a product of the Barnacle Geese in the present study being captive, and it is possible that wild Barnacle Geese would have higher dry masses of both the tibia and the tarsometatarsus. Raveling et al. (1978) found an ash content value of 53.4 and 54.4% for the femur and tibiotarsus in wild cackling Canada Geese, *Branta canadensis minima*, and McAlister (1983) reported a mean ash content of 62% in the femur for wild Greylag Geese, *Anser anser*. Mean ash content values of 57 and 58% were reported for the tibia bone from captive Emus, *Dromaius novaehollandiae* and chickens, *Gallus gallus domesticus*, respectively (Main and Biewener 2007; Newman and Leeson, 1998). This compares to 56% (mean) in the tibia in the captive Barnacle Geese, and 60% for the femur. In comparison, for a flying bird (Barn Owl, *Tyto alba*), the tibia and femur

combined had an approximate mineral content of 61.5%, while the tarsometatarsus was c. 35%.

The femur and tarsometatarsus both underwent significant decreases in mineral content during peak wing moult, while the tibia/fibula increased in wet and dry mass and mineral content. This is despite all three bones being in the same limb. Similar results have been observed in chickens, where only the tibia/fibula showed an increase in mass in response to exercise, and the femur and tarsus did not (Biewener and Bertram 1994). The different leg bones show varying levels of sensitivity to the perceived change in mechanical load, and in this case in particular, change in muscle contraction. The largest functional muscle groups (both in terms of mass and number of muscles) are the ankle plantarflexors (gastrocnemius medialis and lateralis), hip extensors and adductors (see Portugal et al. 2009a for full list), the majority of which both originate and insert on the tibia (ankle plantarflexors), or partially insert (hip extensors and extensors) (see Fig. 1). Furthermore, this group shows the largest increase in mass during wing moult (Portugal et al. 2009a). The mechanical demands on the tibia bone during wing moult are, therefore, greater than those for the tarsometatarsus and femur. The femur and tarsometatarsus bear their greatest loads during take-off and landing, and the main muscles responsible for this are attached to these bones (Rubenson et al. 2006). The decrease in mineral content observed in the femur and tarsometatarsus could therefore be a response to a lack of use of these muscles during the flightless wing moult.

The changes observed in the present study were not the result of an overall change in mechanical loading due to varying body mass, nor a response to a change in behaviour. Instead, the present results suggest that changes in bone mass and mineral composition in the leg bones of moulting waterfowl are likely to be a balance between the maintenance or enhancement of an escape response capacity and a requirement for the redistribution of calcium. Where possible, wild moulting waterfowl may use cyclic osteoporosis of some bones (e.g. femur, tarsometatarsus), to provide a source of calcium for elsewhere in the body (Meister 1951; Murphy and King 1992). In particular, chondroitin sulphate, a calcium derivative, is vital for feather morphogenesis (Pays et al. 1997). In contrast, other bones (e.g. tibia/fibula) may increase in mass and mineral content due to a significant increase in the mass of the force producing muscles that are attached to them.

This study demonstrates significant changes in mineralization levels that have not been previously reported. Of particular significance is the occurrence of these changes in a naturally occurring biological system, where changes in mechanical load and limb usage have not been artificially imposed on the animal. Furthermore, their presence in captive animals provides evidence that the control

mechanism for these changes is partially independent of the exogenous factors to which wild moulting birds would be exposed. Instead, we propose that the changes are in anticipation of the requirement for escape locomotion that ultimately does not manifest itself in a captive environment.

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