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Short communication

A test of an osteologically based age determination technique in the Double-crested Cormorant *Phalacrocorax auritus*

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The ability to determine the age of birds accurately from their skeletons would be useful not only for population biology and wildlife management research but for avian palaeontological and archaeological analyses as well. While accurate osteologically based age determination techniques are available for some amphibians and reptiles (Peabody 1961, Reid 1981), fishes (e.g. Bagenal 1974, Summerfelt & Hall 1987) and mammals (e.g. Morris 1972, Grue & Jensen 1979, Lieberman 1994), none is well established for birds. It is thus normally possible only to determine whether bird bones belonged to nestlings, fledglings or adults based on the degree of bone development, cranial pneumatization or morphometrics (e.g. McNeil & Burton 1972, Jannett 1983, Sugimori *et al.* 1985, Siegel-Causey 1989). However, a fine-grained age determination technique for birds, based on analyses of annual rings registered in the circumferential lamellae of different bones, has produced some promising results (e.g. Van Soest & Van Utrecht 1971, Stone & Morris 1981, Koubek & Hrabe 1984, Klomp & Furness 1992; but see also Nelson 1976, Schaaf 1979, Nelson & Bookhout 1980). Most recently, Klomp and Furness (1992) demonstrated, with a sample of 16 known-aged birds, that the number of endosteal lamellae revealed in transverse thin sections of the tibiotarsus corresponded very tightly with the age in years of the birds in four of the five species examined. The technique was tested using naive observers who read photographs of 1-mm-thick polished transverse sections, taken through a transmitted light microscope. Previous work has also documented the presence of rings in the circumferential lamellae in either the periosteum or endosteum in a number of different skeletal elements from many other bird species (Van Soest & Van Utrecht 1971, Schaaf 1979, Nelson & Bookhout 1980, Stone & Morris 1981, Koubek & Hrabe 1984).

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Although this technique proved remarkably successful for most of the species Klomp and Furness examined, given the small samples analysed for each species and the inaccurate results obtained for one of them, it is clear that the technique must be tested for any given species prior to practical applications. Here we provide such a test for the Double-crested Cormorant *Phalacrocorax auritus*.

METHODS

Twenty-one known-aged Double-crested Cormorants, all banded as chicks, were used in this study. These birds were among a sample of 376 cormorants collected in 1995 from the Les Cheneaux Islands of Lake Huron, northern Michigan. The birds, ranging in age from 1 to 9 years old, were collected by the Michigan Department of Natural Resources for dietary studies. We had access to complete skeletons of five of these birds as well as small longbone fragments, from elements unknown, for the remaining 16. The complete specimens used in this study are held at the Utah Museum of Natural History: the specimens that provided the fragmented longbones are at the University of Michigan, Museum of Zoology.

The methods we used to prepare transverse sections are similar to those described by Klomp and Furness (1992), although we found an embedding procedure produced slides with excellent clarity in far less time. Using a Multi-Pro Dremmel tool, we first cut 5-mm transverse sections from each bone. After sectioning, we dehydrated the specimens for 2 days in successively higher concentrations of ethanol (beginning with 70% and ending with 100%). The sections were then bathed in xylene for 8 h. The sections were then embedded with a solution of 85% methyl methacrylate, 15% dibutyl phthalate and 2 g (per 100 mL of solution) of benzoyl peroxide. After hardening, the embedded specimens were cut into 1-mm-thick sections using a diamond-edged lapidary saw (Buehler Isomet Low-speed); they were then mounted onto slides with a permabond adhesive. After drying, the slides were ground and polished for several minutes using a Buehler grinder/polisher. The slides were then rinsed and dried. Experiments with staining the sections did not improve their clarity.

From our sample of five complete skeletons, we prepared thin-sections derived from the proximal, mid and distal-diaphyses of the right femur, humerus and tibiotarsus for a total of nine sections per bird. Single thin-sections were prepared from the 16 indeterminate longbone fragments.

After about 20 h of studying and counting lamellae from these sections using a polarizing light microscope under 200× magnification, two of us (J.M.B. and D.C.R.) performed blind tests. Following the approach of Klomp and Furness (1992), we counted the maximum number of endosteal lamellae present on each section. This was most easily accomplished by counting the rows of nuclei within

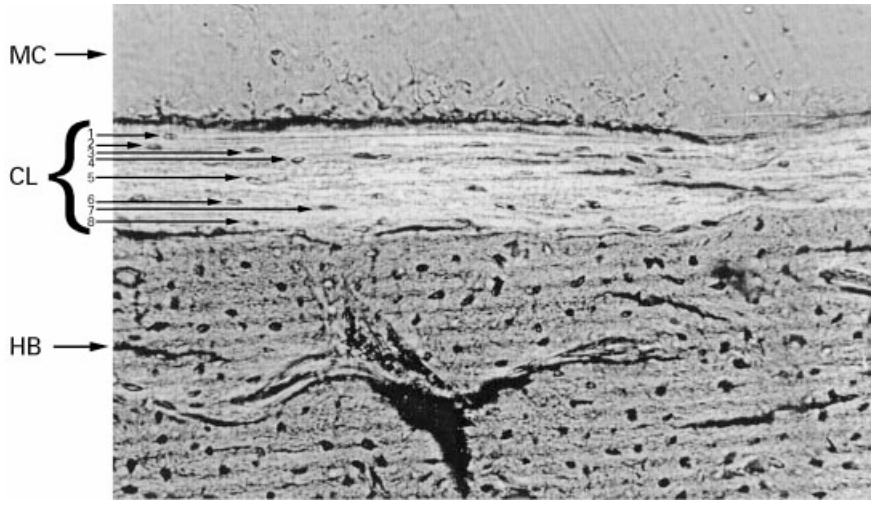

Figure 1. Photograph of a segment of a transverse longbone section taken from a 2-year-old Double-crested Cormorant, showing the medullary cavity (MC), the circumferential lamellae (CL) of the endosteum, and Haversion bone (HB). Both observers counted eight lamellae for this specimen.

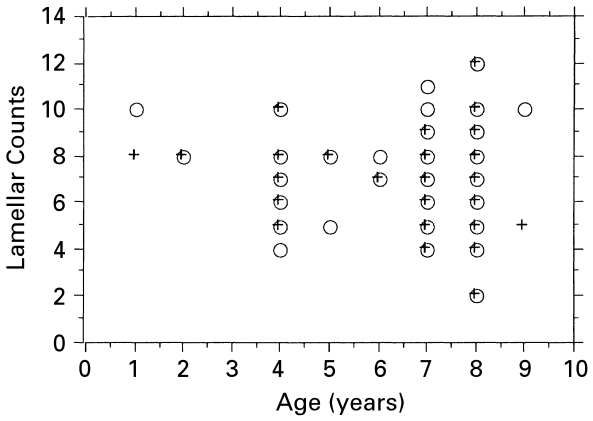

Figure 2. Scattergram of endosteal lamellar counts by age (+, observer 1; ○, observer 2).

the endosteum. Each observer examined the entire circumference of the endosteum before selecting the clearest portion to count lamellae.

RESULTS AND DISCUSSION

Table 1 presents the results of our blind tests on the counts of endosteal lamellae. A photograph of the thin-section from a 2-year-old bird showing eight lamellae is provided in Fig. 1. No relationship was indicated between endosteal lamellar counts and the true age of birds for either observer in this sample (Fig. 2; Observer 1: $r^2 < 0.01$, $F = 0.27$, $df = 1,53$, $P = 0.60$; Observer 2: $r^2 < 0.01$, $F = 0.02$, $df = 1,53$, $P = 0.89$). Similar results were obtained by using the mean of the counts derived from the two observers and when males and females were analysed separately. In addition, no significant relationships were found between the true age of birds and endosteal lamellar counts when different elements and portions of elements were analysed separately or when only single sections were included from each of the five complete individuals.

While lamellar counts do not correlate at all with age in these birds, there was usually close agreement in the counts between the two observers. Specifically, the mean difference in lamellar counts between observers did not differ significantly from zero (mean difference = 0.291, $df = 54$, paired $t = 1.029$, $P = 0.31$). The observers' counts differed by two or less in 78% of the sections and three or less in 93% of them. This suggests that the observers were usually seeing close to the same number of lamellae. The greater discrepancies between the two observers may have resulted from counts being taken at different points along the same section as lamella number appeared to vary within some of the sections. But even when the cases in which the two observers' counts differed by three or more ($n = 12$) were excluded from the analysis, there was still no relationship between lamellar counts and the true age of birds for either observer (Observer 1: $r^2 < 0.01$, $F = 0.29$, $df = 1,42$, $P = 0.60$; Observer 2: $r^2 < 0.02$, $F = 0.70$, $df = 1,42$, $P = 0.41$).

While Klomp and Furness (1992) showed that the relationship between endosteal lamellar counts and the true ages of birds was extremely tight for Great Skua *Catharacta skua*, Redshank *Tringa totanus*, Northern Fulmar *Fulmarus glacialis* and European Shag *Phalacrocorax aristotelis*, the technique failed to provide accurate results for their sample of Brünnich's Guillemots *Uria lomvia*. The results from our analysis of Double-crested

Table 1. The number of endosteal circumferential lamellae as counted by two observers for known-aged Double-crested Cormorants.

Age (years)	Lamellar counts Observer 1	Observer 2	Sex	Element	Portion*
4	7	6	–	Fragment	–
8	7	6	F	Fragment	–
4	6	6	M	Fragment	–
4	5	4	F	Fragment	–
1	8	10	F	Fragment	–
4	8	6	–	Fragment	–
5	8	8	–	Fragment	–
5	8	5	F	Fragment	–
7	4	8	M	Fragment	–
2	8	8	M	Fragment	–
6	7	8	M	Fragment	–
6	7	7	M	Fragment	–
8	2	2	M	Fragment	–
9	5	10	M	Fragment	–
4	8	10	F	Femur	d
8	7	6	F	Femur	d
7	7	9	F	Femur	d
8	4	8	F	Femur	d
8	8	10	F	Femur	m
7	8	7	F	Femur	m
7	8	9	F	Femur	m
8	12	9	F	Femur	m
4	8	8	F	Femur	m
8	8	7	F	Femur	p
8	7	6	F	Femur	p
7	7	10	F	Femur	p
7	5	4	F	Femur	p
4	7	5	F	Femur	p
8	5	5	F	Humerus	d
8	4	6	F	Humerus	d
7	6	8	F	Humerus	d
8	6	4	F	Humerus	m
7	8	6	F	Humerus	m
7	8	9	F	Humerus	m
8	8	6	F	Humerus	m
4	8	8	F	Humerus	m
8	9	7	F	Humerus	m
7	7	5	F	Humerus	p
8	10	8	F	Humerus	p
8	10	12	F	Humerus	p
7	9	5	F	Humerus	p
8	7	10	F	Humerus	p
4	6	7	F	Humerus	p
7	8	8	F	Tibiotarsus	d
7	8	7	F	Tibiotarsus	d
4	7	5	F	Tibiotarsus	d
8	7	5	F	Tibiotarsus	d
8	8	5	F	Tibiotarsus	m
7	8	11	F	Tibiotarsus	m
4	6	4	F	Tibiotarsus	m
7	7	5	F	Tibiotarsus	m
8	8	5	F	Tibiotarsus	p
8	8	5	F	Tibiotarsus	p
4	10	8	F	Tibiotarsus	p
7	9	7	F	Tibiotarsus	p

*p = proximal, m = midshaft, d = distal, – = indeterminate.

Cormorants suggest that the technique is inaccurate for this species also.

Annual cycles in climate, diet, reproduction and migration have each been proposed as possible factors resulting in the layered structure of bone and dental tissue in vertebrates (e.g. Peabody 1961, Bagenal 1974, Lieberman 1994). While layering of the endosteal circumferential lamellae is clearly evident in both Brünnich's Guillemots and Double-crested Cormorants, both species that are migratory annual breeders exhibiting seasonal changes in diet (Johnsgard 1993, Gaston & Jones 1998 p. 154), lamellar counts do not correlate with age.

The resorption or remodelling of bone (see Miller 1992) might be a factor influencing birds to exhibit fewer lamellae than their age in years. However, almost all younger cormorants (1–4 years) in our sample exhibited far more lamellae than their age in years (Fig. 1; Table 1). The lamellar counts for Brünnich's Guillemots are also higher than the age of the birds in almost every case. Future research is clearly required to determine the precise biological basis of layering in circumferential lamellae and the ecological variables that affect it. Such research could reveal why endosteal lamellar counts appear to be highly correlated with age in some species but not in others.

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