Wilson Bull., 98(4), 1986, pp. 516–525

POTENTIAL USE OF FEATHER CHEMISTRY AS AN INDICATOR OF RELATIVE GROWTH

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ABSTRACT.—We explored the potential for using the elemental composition of feathers as an indicator of intraspecific variation in postnatal development. Instrumental neutron activation analysis (INAA) was used to determine the chemical profile of back and tail feathers of Bald Eagle (*Haliaeetus leucocephalus*) nestlings from Saskatchewan. Growth variables included parameters of Gompertz equations for weight, the rate of body feather development, the rate and timing of rectrix growth, and the age at which the nestlings made their first flight from the nest. There were significant correlation and multiple regression analyses involving growth parameters and the concentrations of sodium, chlorine, magnesium, manganese, calcium, and aluminum, but not bromine. Results often differed between the sexes and between the 2 types of feathers. Eaglets with rapid growth generally had higher concentrations of elements than birds with slower growth. Use of INAA may alleviate some of the problems associated with traditional means of collecting data on avian growth. *Received 29 July 1985, accepted 31 March 1986.*

Assessment of intraspecific variation in avian growth is frequently a laborious exercise requiring repeated visits to many nests to weigh and measure nestlings. A study of large nidicolous birds such as eagles (Accipitridae), for example, may require in excess of 100 days in the field. In addition, traditional means of data collection may not be suitable for all species. There are some birds, by virtue of their sensitivity to investigator-induced disturbance (e.g., Fetterolf 1983) or inaccessibility of nests (e.g., on sea cliffs), for which it is unwise or difficult to quantify postnatal development. When such difficulties prevail, a biochemical parameter that is easily measured and indicative of growth rate would be valuable.

In this paper we explore the possibility that the elemental composition of feathers can be used as an indicator of the relative growth of Bald Eagle (*Haliaeetus leucocephalus*) nestlings. Feather chemistry has been investigated as a means of identifying the geographic origins of migrating and wintering populations of birds (e.g., Hanson and Jones 1976, Parrish et al. 1983), and as an indicator of environmental pollution (e.g., Berg et al. 1966, Rose and Parker 1982). Several different analytical techniques have been used to determine the concentrations of various elements (e.g., Kelsall et al. 1975, Kelsall and Burton 1977, Rose and Parker 1982); we employed instrumental neutron activation analysis (INAA). By our meth-

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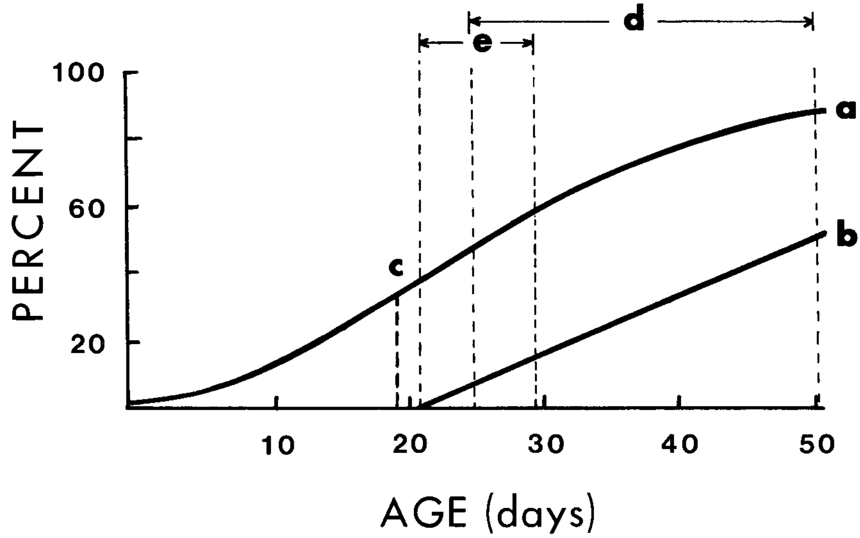

FIG. 1. Development of nestling Bald Eagles (sexes averaged) in relation to age prior to the time of feather collection (at about 50 days old): (a) weight growth as a percent of asymptotic size, (b) central rectrix growth as a percent of final size, (c) inflection point of the weight growth curve, (d) approximate time span from unsheathing to clipping of the back feathers (actual emergence from the skin is somewhat earlier), (e) approximate period of growth represented by the rectrix sample.

od, feathers are irradiated with neutrons from a nuclear reactor. These neutrons interact with the nuclei of the atoms in the feathers, and in doing so create radioisotopes. Gamma rays, with energies specific to each element, are emitted during the radioactive decay of the feather, and are measured by a spectrometer. Estimates of the concentrations of several elements are made simultaneously.

MATERIALS AND METHODS

Study subjects.—Feathers of wild nestling Bald Eagles were collected at Besnard Lake, north-central Saskatchewan (55°20′N, 106°00′W). Nestlings were of known age, and sex was determined by size (Bortolotti 1984a). Back feathers were collected in 1980, 1981, and 1982 in mid-July when the chicks were between 45 and 55 days old ($\bar{x}$ = 50.3). The feathers, clipped at random from the upper-middle area of the back just above the skin, had unsheathed about 20 days earlier (Bortolotti 1984b) and were approximately 40 to 50 mm long. The distal 55 mm of one of the outer rectrices was also collected from each nestling in 1981 and 1982. Based on the rate of elongation of the central rectrix (Bortolotti 1984a), the sample represented about 7 or 8 days of growth (Fig. 1). Feathers were also available from 4 female and 2 male Bald Eagles (>2 years old) live-trapped on Besnard Lake between 1980 and 1982.

TABLE 1

CONCENTRATIONS OF ELEMENTS IN THE BACK AND TAIL FEATHERS OF NESTLING BALD EAGLES

Element	Back[a]		Tail[b]	
	$\bar{x}$	SD	$\bar{x}$	SD
Br	59.8	15.49	27.8	9.48
Mn	7.67	4.085	70.6	38.03
Al	17.8	11.06	118	119.4
Na	535	284.7	640	242.9
Cl	4390	1465	2540	1167
Mg	—[c]	—	521	219.7
Ca	—[c]	—	2230	766

[a] N = 46.
[b] N = 31.
[c] Percent error too large for reliable estimates.

Males and females were analyzed separately because Bald Eagles are sexually dimorphic in size and several growth parameters (Bortolotti 1984b). Further details on the study area and aspects of the biology of this population of eagles are described in Gerrard et al. (1983) and Bortolotti (1984a, b).

Sample preparation and irradiation technique.—Feathers were cleaned by manual agitation in 50 ml of ether for 3 min. Each sample was air-dried and placed in a polyethylene irradiation vial that had been rinsed previously with distilled water and dried. All samples were washed and irradiated within 3 to 4 months of collection. Further details are given in Bortolotti and Barlow (1985).

The SLOWPOKE reactor at the University of Toronto was used for all irradiations. Feathers were irradiated by a neutron flux between 1×10^{11} and 1×10^{12} n/cm^2·sec for 5 min. After a delay period of 4 min following irradiation, the gamma rays were counted for 5 min using one spectrometer (Canberra GE[Li] detector; 8100 series, 4096 channel Canberra analyzer; teletype printer). The intensities of the gamma-ray peaks were determined by automatic analyzer integration of manually selected spectral regions. Using this methodology, we irradiated and spectrometrically analyzed 10 feathers/h.

Detection and statistical analysis of elemental composition.—Eleven elements were detected by the analytical procedure used: iodine (I), bromine (Br), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), vanadium (V), aluminum (Al), chlorine (Cl), calcium (Ca), and sulfur (S). In each sample, the Mg concentration was reduced by 16% of the Al concentration to compensate for the fact that ^{27}Mg is formed when ^{27}Al is bombarded with fast neutrons (R. G. V. Hancock, pers. comm.).

The precision with which concentrations could be estimated was not equal among elements or for the same element between the 2 types of feathers (Bortolotti and Barlow 1985). Only those concentrations that could be determined with a high degree of precision (<10% error; Bortolotti and Barlow 1985) were statistically examined. Data were transformed where appropriate to satisfy assumptions of parametric statistical tests.

Growth variables.—Individual Gompertz growth equations (Ricklefs 1967) were fitted to weight data on 26 males and 20 females collected from 1980 to 1982 to determine the growth parameters *a* (asymptotic size), *K* (a constant proportional to the overall growth rate), and *t* (age at which the inflection point of the curve occurs). In addition, linear

TABLE 2

CORRELATIONS BETWEEN CONCENTRATIONS OF ELEMENTS IN BACK FEATHERS AND GROWTH PARAMETERS OF NESTLING BALD EAGLES

Growth parameter	Sex	N	Element Br	Mn	Na	Al	Cl
Asymptotic weight (*a*)	M	26	0.007[a]	−0.116	0.070	−0.096	−0.017
	F	20	−0.025	0.003	−0.044	−0.051	0.019
Weight growth rate (*K*)	M	26	0.134	0.314	0.085	−0.073	0.218
	F	20	−0.092	0.486[b]	−0.087	0.480[b]	−0.224
Weight curve inflection (*t*)	M	26	−0.330	−0.076	−0.241	0.265	−0.411[b]
	F	20	0.111	−0.057	0.021	−0.066	−0.006
Fledging age	M	25	−0.387	−0.105	−0.438[b]	0.427[b]	−0.489[b]
	F	18	−0.140	−0.083	−0.132	0.102	−0.147

[a] Correlation coefficient (*r*).
[b] $P < 0.05$.

regressions of age on central rectrix length were done to establish the rate of rectrix growth (the slope of the line) and the age when the feather emerged from the body (the intercept) for each eaglet. Although it was not the central rectrix that was chemically analyzed, presumably its growth is representative of all the feathers of the tail. The age at which the birds first flew from the nest (fledged) was determined from daily visits to the nests. Fledging age is a good indicator of overall development (Bortolotti 1984b). Eaglets of similar age can be strikingly different in appearance because of different degrees of body feathering. Nestlings were classified as "early" developers if the age at which feathers unsheathed was less than or equal to 26 days for the humeral tract, 30 days for head and back feathers, and 43 days for tarsal feathers; otherwise they were referred to as "late" developers (Bortolotti 1984b). As not every measure of growth was available for every bird, sample sizes vary slightly.

RESULTS

Elements whose concentrations were determined with a high degree of precision were Mn, Al, Cl, Br, and Na in back feathers; and Mn, Al, Cl, Br, Na, Ca, and Mg in tail feathers (Table 1).

There were statistically significant correlations between growth parameters and concentrations of elements in both back (Table 2) and tail (Table 3) feathers. Multiple linear regression models were used to see if the entire chemical profile of feathers could be used as a predictor of growth. When back and tail feathers were analyzed separately, fledging age of females could be predicted by the elemental composition of tail feathers ($F = 8.39$, df = 7,4, $R^2 = 0.936$, $P < 0.05$, with significant sources of variation being Mg, Mn, and Ca concentrations), and the fledging age of males could be predicted by the elemental composition of back feathers ($F = 3.61$, df = 5,19, $R^2 = 0.487$, $P < 0.02$, with significant sources of variation

TABLE 3

CORRELATIONS BETWEEN CONCENTRATIONS OF ELEMENTS IN TAIL FEATHERS AND GROWTH PARAMETERS OF NESTLING BALD EAGLES

Growth parameter	Sex	N	Element Br	Mg	Mn	Na	Al	Cl	Ca
Asymptotic weight	M	17	0.480^{a}	−0.355	−0.360	0.552^{b}	−0.190	0.605^{c}	-0.673^{c}
(*a*)	F	14	−0.015	−0.140	−0.128	−0.036	−0.252	0.012	0.027
Weight growth	M	17	−0.189	0.343	0.442	−0.225	−0.181	−0.343	0.166
rate (*K*)	F	14	−0.289	0.612^{b}	0.625^{b}	0.535^{b}	0.421	−0.144	0.471
Weight curve	M	17	0.248	−0.356	−0.407	0.188	0.118	0.425	−0.210
inflection (*t*)	F	14	−0.068	−0.301	−0.257	−0.213	−0.091	−0.097	−0.268
Fledging age	M	16	0.080	−0.262	−0.166	−0.126	0.095	0.063	−0.245
	F	12	−0.191	−0.329	−0.451	−0.258	0.271	−0.255	−0.461
Age rectrix first	M	14	0.513	-0.582^{b}	-0.546^{b}	0.322	−0.059	0.624^{b}	−0.497
emerged	F	12	0.379	-0.716^{c}	-0.768^{c}	−0.541	−0.398	0.092	-0.709^{c}
Rate of rectrix	M	14	0.098	0.069	−0.085	−0.085	−0.332	−0.011	0.183
growth	F	12	−0.568	0.420	0.511	0.240	0.373	−0.342	0.208

[a] Correlation coefficient (*r*).

[b] $P < 0.05$.

[c] $P < 0.01$.

TABLE 4

RELATIVE MAGNITUDE OF CONCENTRATIONS OF ELEMENTS IN RELATION TO ATTRIBUTES OF GROWTH OF NESTLING BALD EAGLES

Attribute	Relative magnitude of concentrations High	Low
Large body size	Na, Cl	Ca
Fast weight growth	Mg, Mn, Na, Al	
Early rapid weight growth	Cl	
Early fledging	Na, Cl	Al
Early rectrix growth	Mn, Mg, Ca	Cl
Early body feather development	Mn, Mg	

being Mn concentrations). When the concentrations of back and tail feathers were used together, models predicting the *a* ($F = 82.60$, df $= 10,2$, $R^2 = 0.998$, $P < 0.02$, all variables contributing significantly) and *K* ($F = 21.76$, df $= 10,2$, $R^2 = 0.991$, $P < 0.05$, with significant sources of variation being Na, Al, Cl, and Ca concentrations in tail feathers) parameters of the weight growth equations of females were statistically significant.

The difference in feather chemistry between birds with early and late plumage development was tested with an analysis of variance (ANOVA) using sex and relative development as factors. Early and late birds were significantly different in the concentration of Mn in tail ($F = 4.39$, df $= 1,28$, $P < 0.05$) and back ($F = 4.61$, df $= 1,32$, $P < 0.05$) feathers, and nearly so for Mg ($F = 3.76$, df $= 1,28$, $P = 0.06$) in tail feathers.

Because sibling competition influences nestling growth (Bortolotti 1984b; 1986a, b) we examined the chemical profile of first-hatched vs second-hatched young within each brood. There were no significant ($P > 0.05$) differences in ANOVAs examining the effect of hatching sequence on the concentrations of elements. Similarly, Sign tests (Siegel 1956) of the difference between siblings were nonsignificant.

Birds with attributes generally considered to be desirable and indicative of good growth, such as early weight and feather development, rapid weight gain, and large body size, had higher concentrations of most elements than did birds with poor growth (Table 4). Mn, Mg, and Cl were the most consistent correlates of good development.

DISCUSSION

The elemental composition of feathers is presumably influenced by the intake and absorption of elements into the bird and by various physiological processes. There also is evidence that at least some concentrations of elements may be altered by environmental factors external to the bird after the feathers have matured (Rose and Parker 1982, Edwards and Smith 1984). Adsorption of ions may involve bonding on an ion-exchange basis. Other data suggest that feather chemistry may be relatively stable (Hanson and Jones 1976, Parrish et al. 1983, Bortolotti and Barlow 1985). Although the concentrations of some elements of some feathers potentially may be altered (e.g., by heavy metal pollution; Rose and Parker 1982), such chemical instability may not preclude the use of the elemental analysis of feathers as a research tool. Individual chemical identities of birds, even once modified, may still be obvious (Edwards and Smith 1984). In addition, when correlative evidence is sought, as it was in this study, absolute concentrations of elements may be of secondary importance. It does not seem plausible that an external source of contamination could bias our results to create several spurious correlations between mineral concentrations and various independent growth variables, especially as all of our birds were sampled from one lake in a consistent manner.

Several lines of evidence suggest that the chemical profiles of feathers are associated with biological functions within the bird. Most elements we detected showed some statistically significant relationship with one or more measure of growth (Table 4). Br, the only element not demonstrated to have any such relationship, has not been conclusively shown to perform any essential function in animals (Linder 1985). In their study of seasonal fluctuations in mineral content of Alaskan moose (*Alces alces*) hair, Flynn and Franzmann (1974) and Franzmann et al. (1975) found that the peaks of element concentrations coincided with the peak of physical condition. In particular, Mn and Mg fluctuated greatly and appeared to be related to seasonal differences in nutrition (Flynn and Franzmann 1974). Mn and Mg concentrations in feathers were associated with the weight, rectrix, and body feather growth of eagles (Table 4). In one respect, it is surprising that there are consistent significant correlations for an element among growth variables because most measures of development used in this paper are independent of each other. There is no correlation between the rate of weight and feather development (Bortolotti 1984b), or between fledging age and weight growth rate (Bortolotti 1986a).

It is possible that the trends reported here are not particularly strong because our sample included only apparently healthy chicks that survived to fledge. We did not examine the feathers of obviously stressed nestlings. An indication that feather chemistry may be related to physical condition came from data on a Bald Eagle that was captured at Besnard Lake in 1982. This bird was missing all the phalanges on one tarsus; very likely the result of being caught in a trap set for furbearers (Bortolotti, pers. obs.). It was emaciated and could not fly. The back feathers of this bird had the lowest concentrations of Mn, Al, and Ca, and its tail feathers had the lowest concentrations of Mg and Cl of any feathers of eagles (not nestlings) analyzed from Besnard Lake. Data on nestling feathers are consistent with this observation; attributes of good growth were positively associated with most elements (Table 4). Hanson and Jones (1968) reported that captive Canada Geese (*Branta canadensis*) on experimental rations had abnormally developed primary feathers with much lower concentrations of most minerals compared to wild geese; however, the elements examined were not specified.

Bortolotti and Barlow (1985) show that feathers are not just sinks for the excretion of mineral surpluses as had been proposed by Hanson and Jones (1976). Rather the chemical profile is controlled to some degree by properties of growing feathers. Minerals, however, could be incorporated into feathers in a variety of ways. Concentrations would be inversely related to growth rate if deposition was time-dependent, i.e., if elements were incorporated into the feather at a rate per unit time as the feather grew. If such were the case, birds with similar plasma concentrations of elements could differ in feather chemistry because of differences in feather growth rate. For a given length of feather (as used in this study), a larger amount of an element would be deposited in a slow-growing feather. Results in Table 3 relating the rate of elongation of the central rectrix to concentrations in tail feathers fail to support the hypothesis of time-dependent deposition. Therefore, perhaps qualitative differences in growth and physical condition correspond with quantitative differences in mineral composition of the plasma and consequently the feathers. Controlled experiments are needed to investigate properly any causal associations between developmental processes and the chemical profile of feathers.

One might expect that feather chemistry would only be indicative of events that occurred during the period of growth represented by the portion of feather sampled (Fig. 1). Correlation and multiple regression analyses, however, suggest that fledging age may be predicted by feather chemistry. Male and female Bald Eagles fledge at a mean age of 78 and 82 days, respectively (Bortolotti, 1986b), considerably after the feathers used in this study were grown. This suggests that fledging age may be influenced by factors operating during an early stage of development.

A comparison of the data for back and tail feathers shows that they differ in their relationships to growth parameters (Table 2 and 3). These differences might be expected because the two types of feathers differ chemically (Table 1; see Bortolotti and Barlow 1985), and in the degree of variation among individual birds (Bortolotti and Barlow, unpubl. data). Similarly, there is also little uniformity between the correlations of growth parameters and concentrations for males and females except for the age at which the rectrices emerged. There is no a priori reason to expect the sexes to have the same relationship between feather chemistry and growth, as males and females differ markedly in development (Bortolotti 1984b).

Given the preliminary nature of our results, and what little is known about feather chemistry, we believe that the elemental analysis of feathers is currently more valuable as a research or management tool than as a means of studying avian physiology. INAA has many desirable attributes as an analytical technique. It is highly sensitive, and a multitude of elements in trace concentrations can be detected simultaneously (Kruger 1971). Unlike many other methods, little preparation of the sample is necessary; there is no need for wet digestions, ashing, etc. INAA is nondestructive so samples may be repeatedly irradiated if necessary to test laundering methods, assess precision, or just reexamine samples that are outliers and thus may be errors. The birds used in the study need not be sacrificed, nor even harmed, for small masses (e.g., 0.003 g; Bortolotti and Barlow 1985) can be used in INAA. It is unlikely that the flying ability of most species would be impaired by the clipping of a small portion of one feather.

ACKNOWLEDGMENTS

This study would not have been possible without the generous assistance of R. G. V. Hancock at the SLOWPOKE reactor. The technical assistance of S. Aufreiter was also extremely helpful. N. J. Flood, V. Honeyman, and J. Staniforth assisted in collecting feathers. D. W. A. Whitfield and J. M. Gerrard were invaluable colleagues in the field. A. Harmata and J. M. Gerrard supplied feathers from four eagles live-trapped on Besnard Lake in 1982. G. H. Parker, J. P. Kelsall, H. A. Trueman, B. Naylor, and L. Bendell-Young improved the manuscript with their comments. Fieldwork was financially supported by the World Wildlife Fund (Canada), the National Wildlife Federation, and the Natural Sciences and Engineering Research Council grant A3472 to J. C. Barlow. The SLOWPOKE facilities were supported by a NSERC infrastructure grant. To all these individuals and institutions we express our most sincere gratitude.

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