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Element Patterns in Feathers of Nestling Black-Crowned Night-Herons, *Nycticorax nycticorax* L., from Four Colonies in Delaware, Maryland, and Minnesota

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Abstract The pattern of elements in nestling black-crowned night-heron feathers from a rural Minnesota colony differed from colonies in industrialized regions of Maryland and Delaware. Except for chromium, however, the differences did not reflect the elements associated with waters and sediments of the Maryland and Delaware colonies. Therefore, elements in water and sediment do not necessarily bioaccumulate in night-heron feathers in relation to potential exposure. Although trace element patterns in feathers indicated differences among geographical locations, they did not separate all locations well and their usefulness as an indicator of natal colony location may be limited.

Keywords Elements · Feathers · Metals · Black-crowned night-heron

Element concentrations in feathers have been used extensively to evaluate trace element contamination in birds (Burger 1994). In addition to contaminant biomonitoring, element concentrations in feathers have been evaluated as a method to identify local populations and geographic birth sites of first-year breeders (Edwards and Smith 1984; Donovan et al. 2006). However, element patterns in feathers within a site can differ among avian species (Donovan et al. 2006; Custer et al. 2007). Elements in the nestlings (and later their feathers) can originate from the egg or from the diet (Becker et al. 1994). As nestlings grow and new feathers develop, the influence of diet on element concentrations in feathers becomes more pronounced. The diet reflects mineral patterns of the region and elements in the atmosphere. Some of these elements may be more readily incorporated into bird tissues including feathers than others. As nestlings grow significant exogenous exposure can also occur (Weyers et al. 1988).

Element data were reported in feathers of nestling black-crowned night-herons (*Nycticorax nycticorax*) from four colonies: Agassiz National Wildlife Refuge (Agassiz) in Minnesota (Custer et al. 2007), Pea Patch Island in Delaware, and Baltimore Harbor, and Holland Island in Maryland (Golden et al. 2003). Based on univariate analysis, concentrations of Al, Ba, Fe, Mg, Mn, and Pb in feathers were greater at Pea Patch Island than Baltimore Harbor or Holland Island (Golden et al. 2003). Our objective was to determine, based on a multivariate analysis, if patterns of elements in feathers of nestling black-crowned night-herons differed among the four colonies and to identify the elements that contributed to any colony differences. Because of elevated concentrations of Cd, Cu, Cr, Hg, Mn, Pb, and Zn in water and sediments in the industrialized waters near Baltimore Harbor and Pea Patch Island (reviewed in McGee et al. 1999; Sutton et al. 1996) and because elemental patterns in feathers should reflect regional differences in geochemistry, we hypothesized that element patterns in nestling black-crowned night-heron feathers from the rural Agassiz colony should differ from the Baltimore Harbor and Pea Patch Island colonies. We also hypothesized that the differences reported in element concentrations in black-crowned night-heron feathers among Pea Patch Island, Baltimore Harbor, and Holland

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Island (Golden et al. 2003) would also be observed in a multivariate analysis. Finally, we tested the hypothesis that trace element patterns in feathers of black-crowned night-heron nestlings were sufficiently distinct to indicate geographic locations.

Materials and Methods

Feather samples were collected in 1998 from Pea Patch Island, Baltimore Harbor and Holland Island and in 1999 from Agassiz. Details of feather collection and element analysis for the Pea Patch Island, Baltimore Harbor, and Holland Island colonies are presented in Golden et al. (2003); the Agassiz colony information is presented in Custer et al. (2007). For Pea Patch Island, Baltimore Harbor, and Holland Island samples, body feathers (0.1–0.5 g) were clipped from 14- to 16-day-old black-crowned night-heron nestlings. For the Agassiz samples, all feathers from one side of the body were removed from 10-day-old black-crowned night-heron nestlings. For purposes of this study, we assume that element concentrations in black-crowned night-heron body feathers are comparable to a composite of all feathers from one side of the body. Element concentrations can vary among feather types and among feather structures (Burger 1994). However, we found no information comparing element concentrations in individual feather types to composites of all body feathers. In both studies, the feathers were analyzed for 19 elements (Al, As, Ba, B, Be, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn) by Research Triangle Institute (Research Triangle Park, NC). Feathers were not washed prior to analysis. We assume that using unwashed feathers did not bias our results because the nestling feathers had limited time exposure (<16 days) to exogenous contaminants. Feathers of young birds would be expected to have the least amount of external contamination (Burger 1994). Additionally, feathers of 30-day-old blackbirds (*Turdus merula*) did not demonstrate significant exogenous contamination of Pb, Cd, or Zn (Weyers et al. 1988).

Non-metric multi-dimensional scaling plots (MDS), (Kruskal 1964) were constructed to display patterns of element concentrations among sample groups. Analysis of similarity tests (ANOSIM procedure, PRIMER v6) were used to test hypotheses regarding the pattern of elements in feathers among colonies (Clarke and Warwick 2001). This initial multivariate test was used to protect against Type I errors which can occur when many univariate tests are run on a single data set. Analysis of similarity is a multivariate analogue of analysis of variance; it is built on a simple non-parametric permutation procedure and applied to the rank similarity matrix underlying the ordination of samples. The test statistic, "R," may vary from -1 to $+1$. An R value close to $+1$ indicates that there are very clear differences in patterns among the groups being tested. A value near zero means that the distribution of patterns is as similar among the groups as within the groups. An R is considered significant based on its p-value (e.g. $p < 0.05$). Because R can be significantly different from zero yet inconsequentially small, the size of R indicates the degree of difference. Clear differences in patterns are evident when R is ≥ 0.4. There is some support for pattern differences when R is ≥ 0.3 to <0.4, and patterns barely differ when R is <0.3 (adapted from Clarke and Warwick 2001). Variables were included in the analyses if $\geq 50\%$ of samples had detectable values; one-half the detection limit was assigned to samples below the detection limit for Ba (19 of 38 not detected), Cd (18 not detected), Cr (11 not detected), and Pb (7 not detected). Element data were log-transformed prior to ANOSIM analysis. Bray–Curtis resemblance matrices were used. When pattern differences were identified, the similarity percentage (SIMPER procedure, PRIMER v6) subroutine was used to identify which elements contributed to the differences.

Results and Discussion

Thirteen of the 19 elements analyzed were detected in $>50\%$ of black-crowned night-heron feathers and were included in the analysis (Table 1). The log-transformed concentration pattern of these 13 elements differed significantly among colonies ($R = 0.36$, Table 2). Except for the comparison of Holland Island and Pea Patch Island ($R = 0.08$, $p = 0.11$), all colony pair wise comparisons of element patterns in feathers differed significantly from one another (Table 2). An MDS plot (Fig. 1) suggested a left to right distribution in the pattern of elements from Baltimore Harbor to Pea Patch Island on the horizontal axis; Holland Island and Agassiz feather samples overlapped in the middle. In contrast, Agassiz samples appeared to be separated from the other three colonies on the vertical axis of the MDS plot.

Element concentrations of Al, Fe, Mn, and Sr were major contributors to the dissimilarity (accounting for 59%) between Baltimore Harbor and Pea Patch Island (SIMPER subroutine, data not shown). Feathers from Pea Patch Island had higher concentrations of all of these elements compared to feathers from Baltimore Harbor (Table 1). These patterns are visually obvious in the increasing concentrations from left to right on the horizontal axis of the bubble diagrams for these three elements (Fig. 2).

Five elements (Al, Cr, Mg, Sr, and Fe) were major contributors to the dissimilarity of Agassiz from the other colonies (data not shown). Chromium and Sr had lower concentrations at Agassiz than the other colonies (Table 1) and these differences are visually obvious on the vertical

Table 1 Metal and selenium geometric mean concentrations (μg/g dry weight) in feathers of nestling black-crowned night-herons

Element	Geometric mean (μg/g dry weight) and 95% confidence limits			
	Maryland 1998		Delaware 1998	Minnesota 1999
	Baltimore Harbor N = 12	Holland Island N = 9	Pea Patch Island N = 12	Agassiz N = 5
Al	9.18 (6.82–12.3)	42.3 (28.4–63.0)	78.85 (37.9–164.3)	18.95 (11.9–30.2)
Ba	0.52 (0.36–0.75)	0.60 (0.42–0.86)	1.51 (0.91–2.50)	<0.9
Cd	0.016 (0.008–0.03)	0.012 (0.007–0.019)	0.029 (0.011–0.08)	0.15 (0.12–0.19)
Cr	3.28 (2.40–4.48)	3.16 (1.63–6.12)	2.48 (1.29–4.76)	<0.9
Cu	6.05 (4.56–8.03)	7.90 (6.72–9.29)	6.63 (5.64–7.80)	6.21 (5.29–7.28)
Fe	36.4 (23.5–56.5)	63.3 (48.4–82.8)	154.4 (93.1–255.9)	45.4 (41.3–49.9)
Hg	0.805 (0.597–1.09)	0.81 (0.67–0.98)	1.25 (0.81–1.91)	1.69 (1.46–1.95)
Mg	218.9 (159.3–300.7)	331.9 (281.4–391.5)	359.0 (309.4–416.5)	704.7 (670.1–741.1)
Mn	2.28 (1.61–3.24)	3.11 (1.64–5.91)	7.59 (4.90–11.7)	3.80 (2.97–4.85)
Pb	0.32 (0.18–0.59)	0.11 (0.07–0.18)	0.41 (0.21–0.79)	<0.9
Se	2.18 (1.76–2.69)	2.11 (1.91–2.33)	2.16 (1.88–2.47)	2.10 (1.85–2.37)
Sr	4.56 (2.37–8.76)	5.11 (3.85–6.79)	7.67 (5.25–11.2)	1.13 (0.95–1.34)
Zn	127.4 (102.1–159.1)	155.1 (143.1–168.1)	168.8 (149.8–190.1)	241.4 (225.7–258.3)

N = the number of nestling samples. Barium, Cr, and lead were not detected in any of the five samples from Agassiz; the detection limit was 0.9 μg/g dry weight

Table 2 Analysis of similarity results for element patterns among black-crowned night-heron feathers from Baltimore Harbor, Holland Island, Pea Patch Island in 1998 and Agassiz in 1999

Comparison	*R* statistic	*P*-value
One-way analysis		
Global	0.36	0.001
Baltimore Harbor vs. Holland Island	0.29	0.007
Baltimore Harbor vs. Pea Patch Island	0.44	0.001
Baltimore Harbor vs. Agassiz	0.48	0.003
Holland Island vs. Pea Patch Island	0.08	0.110
Holland Island vs. Agassiz	0.77	0.001
Pea Patch Island vs. Agassiz	0.43	0.009

Elements analyzed included Al, Ba, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Pb, Se, Sr, and Zn

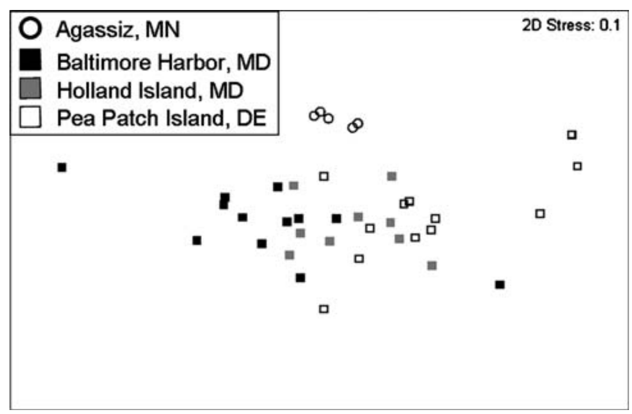

Fig. 1 Non-metric multi-dimensional scaling (MDS) plot ordination of Bray–Curtis similarities from log-transformed black-crowned night-heron feather element data for four locations. Elements included in the analysis were Al, Ba, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Pb, Se, Sr, and Zn. Note that the axes of MDS plots are without units

axis of the respective bubble diagrams (Fig. 2). Magnesium exhibited higher concentrations at Agassiz than the other colonies (Table 1, Fig. 2). Iron and Al concentrations at Agassiz were intermediate to concentrations at the other colonies (Table 1).

The differences in the element pattern in feathers among black-crowned night-heron colonies support our earlier hypothesis that rural Agassiz should differ from industrialized Baltimore Harbor and Pea Patch Island. However, only Cr concentrations fit the predicted pattern of higher element concentrations in feathers from industrialized areas. This is somewhat surprising in light of elevated concentrations of other elements (e.g. Cd, Cu, Hg, Mn, Pb, Zn) in water and sediments of Baltimore Harbor or Pea Patch Island (reviewed in McGee et al. 1999; Sutton et al. 1996). Chromium concentrations were higher in feathers from Baltimore Harbor and Pea Patch Island than Agassiz (Table 1) and Cr was a major element contributing to the dissimilarity between Agassiz and each of the other colonies (Fig. 2). However, Cu and Mn concentrations were intermediate at Agassiz compared to the other colonies and Cd, Hg, and Zn were qualitatively higher at Agassiz.

The differences in the element patterns between the younger nestlings (10-days-of-age) at Agassiz and the older nestlings (14- to 16-days-of-age) at the other three colonies were probably not related to age. Lead concentrations in feathers of blackbird nestlings did not significantly increase from 8- to 30-days-of-age and Cd and Zn concentrations

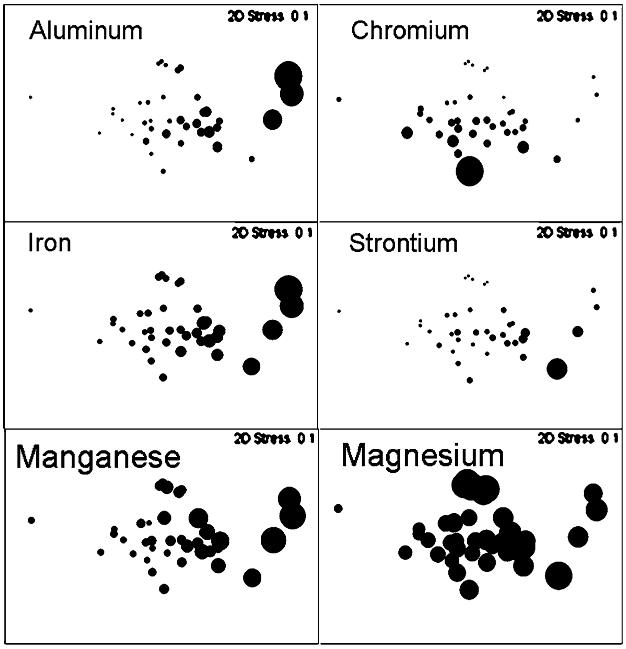

Fig. 2 Non-metric multi-dimensional scaling (MDS) plot ordination from Fig. 1, but with superimposed circles of increasing size, representing increasing concentrations of each element (Al, Fe, Mn, Cr, Sr, and Mg). Note that the axes of MDS plots are without units

did not significantly increase from 8- to 154-days-of-age (Weyers et al. 1988).

The toxicological significance of the elemental concentrations in the nestling feathers at these four colonies has been discussed earlier (Golden et al. 2003; Custer et al. 2007). In summary, element concentrations in black-crowned night-heron nestling feathers at all colonies were generally low to moderate and do not seem to threaten these populations.

Results from the multivariate analysis were similar to earlier univariate analyses among the three east coast colonies (Golden et al. 2003). The multivariate analysis SIMPER routine suggested that Baltimore Harbor and Pea Patch Island differed because of Al, Fe, Mn, and Sr concentrations. Geometric means of the top three of these elements (Al, Fe, and Mn) also differed between Pea Patch Island and Baltimore Harbor; Pea Patch Island had higher concentrations of all three elements (Golden et al. 2003).

Although trace element patterns in black-crowned night-heron feathers separated most geographical locations they did not separate all locations well. Element patterns were distinguished for five of the six pair wise tests for differences among the four sites. However, the element pattern analysis of feathers from Holland Island did not differ from Pea Patch Island. Natal locations of cavity nesting songbirds were also not identified with a high degree of accuracy on the basis of element patterns in feathers (Donovan et al. 2006).

In conclusion, based on a multivariate analysis, the pattern of elements in nestling black-crowned night-heron feathers from the rural Agassiz colony differed as hypothesized from industrialized Baltimore Harbor and Pea Patch Island. Except for Cr, however, the differences did not reflect the elements associated with waters and sediments of the three east coast colonies. Therefore, elements in water and sediment do not necessarily bioaccumulate in night-heron tissues in relation to potential exposure. Additionally, the multivariate analysis of the three east coast colonies supported the results of earlier univariate analyses. Finally, our results suggest that although trace element patterns in feathers can identify differences among geographical locations they do not separate all locations well and their usefulness as an indicator of natal colony location may be limited.

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References

Becker PH, Henning D, Furness RW (1994) Differences in mercury contamination and elimination during feather development in gull and tern broods. Arch Environ Contam Toxicol 27:162–167. doi:10.1007/BF00214258

Burger J (1994) Metals in avian feathers: bioindicators of environmental pollution. Rev Environ Toxicol 5:203–311

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. Plymouth Marine Laboratories, Plymouth, UK

Custer TW, Custer CM, Eichhorst BA, Warburton D (2007) Selenium and metal concentrations in waterbird eggs and chicks at Agassiz National Wildlife Refuge, Minnesota. Arch Environ Contam Toxicol 53:103–109. doi:10.1007/s00244-006-0139-7

Donovan T, Buzas J, Jones P, Gibbs HL (2006) Tracking dispersal in birds: assessing the potential of elemental markers. Auk 123:500–511. doi:10.1642/0004-8038(2006)123[500:TDIBAT]2.0.CO;2

Edwards WR, Smith KE (1984) Exploratory experiments on the stability of mineral profiles of feathers. J Wildl Manage 48:853–866. doi:10.2307/3801432

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29:1–27. doi:10.1007/BF02289565

Golden NH, Rattner BA, McGowan PC, Parsons KC, Ottinger MA (2003) Concentrations of metals in feathers and blood of nestling black-crowned night-herons (*Nycticorax nycticorax*) in Chesapeake and Delaware Bays. Bull Environ Contam Toxicol 70:385–393. doi:10.1007/s00128-002-0203-6

McGee BL, Fisher DJ, Yonkos LT, Ziegler GG (1999) Assessment of sediment contamination, acute toxicity, and population viability of the estuarine amphipod *Leptocheirus plumulosus* in Baltimore

Harbor, Maryland, USA. Environ Toxicol Chem 18:2151–2160. doi:10.1897/1551-5028(1999)018<2151:AOSCAT>2.3.CO;2
Sutton CC, O'Herron JCII, Zappalorti RT (1996) The scientific characterization of the Delaware Estuary. Delaware Estuary Program (DRBC Project No. 321; HA File No 93.21), 200 pp
Weyers BE, Gluck E, Stoeppler M (1988) Investigation of the significance of heavy metal contents of blackbird feathers. Sci Total Environ 77:61–67. doi:10.1016/0048-9697(88)90315-4