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Concentrations of Metals in Feathers and Blood of Nestling Black-Crowned Night-Herons (*Nycticorax nycticorax*) in Chesapeake and Delaware Bays

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Destruction and degradation of wetland habitat has been a contributing factor to the population decline of Atlantic coast wading bird colonies for several decades. Pea Patch Island in Delaware Bay, the largest heronry north of Florida, has exhibited a population reduction from approximately 12,250 breeding pairs of wading birds in 1993 to only 3,886 pairs in 2000 (Love 2001). One of Maryland's largest Black-crowned Night-Heron (*Nycticorax nycticorax;* hereafter BCNH) colonies, located at Fort Carroll in Baltimore Harbor, suffered a similar decrease in nesting birds during the same period. The number of BCNH pairs breeding in this colony dropped by approximately 75% between 1995 and 1998 (Rattner et al. 2001). Wading birds at both of these sites are exposed to industrial or agricultural contaminants.

Recent studies have examined organic contaminant exposure and reproductive success of wading birds at Pea Patch Island and Baltimore Harbor (Parsons et al. 2000; Parsons et al. 2001; Rattner et al. 2000; Rattner et al. 2001). However, there is relatively little information on exposure of wading birds to metals in these regions. Concentrations of several elements (Ag, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) in water and sediments in Delaware Bay or Baltimore Harbor exceed levels associated with adverse effects in invertebrates and fish (reviewed in McGee et al. 1999; Sutton et al. 1996). As industrial and agricultural activities continue to encroach on areas that support heron colonies, there is a need to monitor contaminant exposure and potential effects on the health of these species. Because of their widespread distribution and feeding habits, BCNHs have been used extensively as a representative species in estuarine biomonitoring projects (e.g., Kushlan 1993; Rattner et al. 2000; 2001). Collection of blood and feather samples provides a nonlethal method to quantify inorganic contaminant exposure of birds, while having a minimal impact on the population being studied. Herein we report concentrations of metal, metalloid, and trace element exposure in heron nestlings from colonies in Chesapeake and Delaware Bays.

MATERIALS AND METHODS

In May and June of 1998, blood, feathers, and regurgitated food boluses were collected from nestling BCNHs residing in three heronries in Delaware and

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Chesapeake Bays. Study sites included Pea Patch Island, Delaware (N39°35', W75°34'), Fort Carroll in Baltimore Harbor, Maryland (N39°12'51", W76°31'08"), and Holland Island, Maryland (N38°07', W76°05'), a remote, undeveloped site in the southern Chesapeake Bay (reference site).

Feathers and blood were collected from 12 randomly sampled 14 to 16 day old BCNHs (1/nest) each at Pea Patch Island and Fort Carroll, and from 9 at Holland Island. Nestling from Pea Patch Island and Fort Carroll were of known age, and nestlings from Holland Island were aged based on culmen length (Custer and Peterson 1991). Body feathers (0.1-0.5 g) were clipped from nestlings and stored in Whirlpak bags at room temperature. Whole blood (3-5 mL) was drawn via jugular venipuncture into a heparinized syringe and stored frozen in polypropylene cryotubes at -10°C. Regurgitated food boluses were opportunistically collected during handling and stored at -10°C in chemically clean jars (ICHEM Research, New Castle, DE).

All samples were analyzed for 19 metals, metalloids, and trace elements by Research Triangle Institute (Research Triangle Park, NC) and results were quality assured by the Patuxent Analytical Control Facility (Laurel, MD). Feathers were not washed prior to analysis and concentrations may therefore be slightly elevated from exogenous sources. However, for nestlings of this age, the duration of this exposure is extremely limited. The emergence of pinfeathers on BCNHs occurs between days 10 and 12 (McVaugh 1972), resulting in minimal external exposure before sample collection. Considering the short exposure time and the presence of metal residues in food samples, it is assumed that the primary contributor to metal content remains dietary despite the lack of washing.

All samples were digested in a warm nitric acid solution, with the addition of hydrogen peroxide, followed by microwave concentration, and redilution in deionized water. Al, As, B, Ba, Be, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sr, V, and Zn were analyzed by ICP-mass spectroscopy using Leeman Labs Spec I sequential or ES2000 simultaneous spectrometer (USEPA, 1994). Se was analyzed using graphite furnace atomic absorption using a Perkin-Elmer Zeeman 3030 or 4100 ZL atomic absorption spectrometer. Concentrations of Hg were determined by cold vapor atomic absorption using a Leeman PS200 Hg Analyzer and $SnCl_4$ as the reducing agent. The lower limit of detection ranged from 0.004 ug/g dw Hg to 0.9 ug/g Cr in feathers, 0.0003 ug/g ww Hg to 1.3 ug/g Fe and Mg in blood, and 0.002 ug/g dw Hg to 10.1 ug/g Fe and Mg in regurgitated food samples.

Concentrations of elements were tested for homogeneity of variance, and values $\log_{10}$-transformed to stabilize variances. A value of one-half the lower limit of detection was assigned to samples with undetectable concentrations, if detectable quantities were present in more than half of the samples from a study site. Means were compared among sites using one-way analysis of variance and the Newman-Keuls multiple comparison test. Element concentrations between tissues were examined for linear relationships using Pearson product-moment correlation.

RESULTS AND DISCUSSION

Concentrations of metals in feathers reflect levels in blood at the time of feather formation, either from current dietary exposure or mobilization from internal organs (Burger 1993). As heavy metals are poorly transferred from the hen into the egg (with the exception of organic forms, such as methylmercury), concentrations in nestling feathers are presumably reflective of post-hatching dietary exposure. Though concentrations of certain elements have been shown to increase with time as a result of external contamination (Weyers et al. 1988), feathers from juvenile birds tend to receive their greatest contribution from internal sources (Armiard-Triquet et al. 1991). In this present study, metal concentrations in feathers were generally low compared to published values, although several site differences were apparent (Table 1). For nestlings at Pea Patch Island, concentrations of Al, Ba, Fe, Mg, Mn, and Pb were significantly greater ($p<0.05$) than those at either Baltimore Harbor or Holland Island. At Baltimore Harbor, concentrations of most elements in feathers of BCNH nestlings were equivalent to or lower than the reference site, with the sole exception of Pb. Concentrations of known toxicants Hg, Cd, and Se did not differ among sites ($p>0.05$).

Mean Pb concentrations at both Pea Patch Island and Baltimore Harbor (0.41 and 0.32 ug/g, respectively) were greater than the Holland Island reference site, though similar to or less than those of juvenile birds from several other studies in the United States (Burger 1993), including those from fledgling BCNHs in the Agassiz National Wildlife Refuge in rural northwestern Minnesota (Burger and Gochfeld 1996). However, Pb concentrations in a few samples at both sites were quite high (extreme values: 3.77 and 6.47 ug/g), and 7 of 12 individual values at Pea Patch Island and 4 of 12 at Baltimore Harbor exceeded the reference site mean by more than two standard deviations (Fig. 1). As herons can feed at locations up to 8 km from the breeding colony (Erwin et al. 1991), it is not unusual to see individual variation in the extent of contaminant exposure. Adverse effect threshold levels have not been established for Pb in feathers, although controlled studies have shown that early Pb exposure in the Herring Gull (*Larus argentatus*) and Common Tern (*Sterna hirundo*) can result in behavioral alterations that affect survival (Burger and Gochfeld 2000). In addition, poor survival of Pb-exposed nestling Little Blue Herons (*Egretta caerulea*) has been noted in colonies exhibiting otherwise normal reproductive success (Spahn and Sherry 1999).

Feather concentrations of Mn at Pea Patch Island were greater than at Baltimore Harbor and Holland Island, and were generally higher than found in other juvenile birds at Pea Patch Island, (Cattle Egrets, *Bubulcus ibis,* 3.4 ug Mn/g), and elsewhere in the United States (Burger 1993). Eggs of BCNHs collected at Pea Patch Island in 1997 also exhibited elevated concentrations of Mn (Rattner et al. 2000). Though an essential element, elevated levels of Mn have been shown to cause behavioral effects in birds (Laskey and Edens 1985; Burger and Gochfeld 1995). Insufficient data currently exist to predict hazardous levels of Mn in

Table 1. Metal concentrations (ug/g dry weight) in feathers of nestling BCNHs.

	Pea Patch Island N=12	Baltimore Harbor N=12	Holland Island N=9
Al	78.85^{A} (13.9-775)	9.18^{B} (5.16-28.1)	42.3^{A} (14.1-90.6)
As	0.19 (ND-0.512)	0.25 (0.029-1.26)	0.41 (0.187-1.76)
B	0.71 (ND-6.04)	0.41 (ND-1.44)	0.19 (ND-0.862)
Ba	1.51^{A} (0.340-8.85)	0.52^{B} (0.292-3.08)	0.60^{B} (0.279-1.47)
Cd	----- (ND-1.20)	0.016 (ND-0.152)	----- (ND-0.038)
Cr	2.49 (ND-68.5)	3.28 (1.18-10.4)	3.17 (ND-10.9)
Cu	6.63 (3.77-12.3)	6.05 (1.97-12.3)	7.90 (5.33-12.3)
Fe	154.3^{A} (42.8-779)	36.4^{B} (12.2-294)	63.29^{B} (37.4-135)
Hg	1.25 (0.501-5.18)	0.805 (0.177-1.38)	0.81 (0.439-1.12)
Mg	359.0^{A} (237-589)	218.9^{B} (65.5-754)	331.9^{A} (209-423)
Mn	7.59^{A} (3.10-25.5)	2.28^{B} (1.32-12.0)	3.11^{B} (0.866-14.1)
Ni	0.26 (ND-0.701)	0.27 (0.134-0.937)	0.20 (ND-0.423)
Pb	0.41^{A} (0.086-3.77)	0.32^{A} (0.094-6.47)	0.11^{B} (ND-0.322)
Se	2.16 (1.44-3.57)	2.18 (0.91-3.48)	2.11 (1.65-2.53)
Sr	7.67 (3.16-27.0)	4.56 (1.51-106)	5.11 (2.81-9.33)
Zn	168.8 (128-264)	127.4 (40.3-173)	155.1 (123-176)

Values are geometric mean (extremes); ND = not detected; --- = no mean calculated as contaminant was detected in fewer than half of the samples.
Means with similar letters are not significantly different using the Newman-Keuls multiple comparison test (α=0.05).

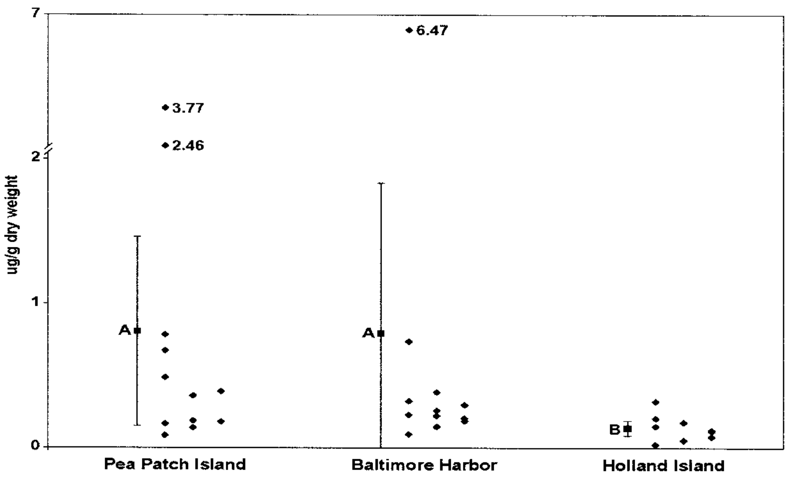

Figure 1. Lead concentrations (mean ± SD and individual observations) in nestling BCNH feathers. Sites with similar letters are not significantly different using the Newman-Keuls multiple comparison test (α=0.05).

feathers, though the approval of methylcyclopentadienyl manganese tricarbonyl (MMT) as a fuel additive by the U.S. Environmental Protection Agency in 1995 (60 FR 36414, July 17, 1995) and the potential for a possible increase in airborne Mn levels call for increased monitoring of this element. In Canada, where MMT has been in use for decades, increased levels of Mn in liver and feces of Rock Doves (*Columba livia*) in urban areas correlated with increased atmospheric Mn concentrations (Loranger et al. 1994).

Nestling feathers collected at Pea Patch Island also contained elevated concentrations of Al, Ba, Fe, and Mg, though comparative data and information on the toxicological significance for these elements in feathers are extremely limited. Data on Al concentrations in heron feathers are lacking, though values are greater than those found in nestling Bald Eagle (*Haliaeetus leucocephalus*) feathers in Canada (8.5-27.8 ug/g) (Bortolotti and Barlow 1988). Controlled studies have shown adverse effects of Al on avian growth and survival, and Al has been suggested as a possible causative factor of defective eggshell formation (reviewed by Sparling 1996). Mercury, which has been well-characterized in feathers, was detected at concentrations below the threshold (5-40 ug/g dw) associated with adverse effects (Eisler 2000).

Of 9 elements consistently detected in blood, differences among sites were only found for Hg and Se (Table 2). Concentrations of Hg were greater at Pea Patch Island than other sites. Values were generally lower than those found in other studies of nestling fish-eating birds (e.g., Great Egret, *Ardea alba*, Sepulveda et al. 1999; Great Blue Heron, *A. herodias*, Wolfe and Norman 1998), but were similar to values in juvenile Common Loons (*Gavia immer*) in the Great Lakes and western regions (Evers et al. 1998). Great Blue Heron nestlings in Clear

Lake, California, did not experience growth rate and reproductive success impairment, despite Hg concentrations in blood that were nearly an order of magnitude greater than those at Pea Patch Island (Wolfe and Norman 1998). Concentrations of Se in blood were lower at Pea Patch Island than other sites. Little data exist on Se blood levels for nestling birds, however, as Se has been shown to have protective properties against Hg toxicity, it is interesting to note that these low levels of Se were detected at the same site of elevated blood Hg concentrations. Other metals did not vary among sites or were not detected in blood samples.

Table 2. Metal concentrations (ug/g wet weight) in blood of nestling BCNHs.

	Pea Patch Island N=12	Baltimore Harbor N=12	Holland Island N=9
As	0.111 (0.0289-0.857)	0.072 (ND-0.294)	0.128 (0.0367-0.220)
Cd	0.102 (ND-0.0304)	--- (ND-0.0190)	0.011 (ND-0.0237)
Cu	0.425 (0.178-0.825)	0.408 (0.319-0.608)	0.379 (0.242-0.639)
Fe	352.4 (266-470)	349.6 (290-448)	412.5 (243-590)
Hg	0.138^{A} (0.0624-0.29)	0.080^{B} (0.0434-0.172)	0.0759^{B} (0.04-0.229)
Mg	94.48 (68.5-124)	94.6 (71.2-114)	99.39 (66.4-135)
Mn	0.0306 (0.0118-0.0573)	0.023 (0.0114-0.0482)	0.0220 (ND-0.0682)
Se	0.347^{B} (0.231-0.487)	0.539^{A} (0.396-0.926)	0.499^{A} (0.335-0.814)
Zn	4.32 (2.82-5.52)	4.37 (3.70-5.07)	4.36 (2.97-5.60)

Values are geometric mean (extremes); ND = not detected; --- = no mean calculated as contaminant was detected in fewer than half of the samples. Means with similar letters are not significantly different using the Newman-Keuls multiple comparison test (α=0.05).

The numbers of regurgitated food boluses (containing small fish and/or crustaceans) from Pea Patch Island (N=3), Baltimore Harbor (N=7), and Holland Island (N=2) were too small for statistical comparison. Detectable quantities of metals (except B and Be) were found in most samples. Extreme concentrations at Pea Patch Island and Baltimore Harbor appeared elevated for Al (442 and 714 ug/g dw), Mn (152 and 98.5 ug/g), Pb (1.54 and 7.82 ug/g), Sr (534 and 382 ug/g), and Zn (181 and 213 ug/g), though no geographic differences were observed between Sr and Zn levels in either feathers or blood.

Mercury was the only metal for which blood and feather concentrations were correlated ($r=0.39$, $p<0.05$), though the relationship was weaker than that found for nestling Great Egret scapular feathers (Sepulveda et al. 1999), juvenile Common Loon secondary feathers (Evers et al. 1998), and remiges of Hg-dosed juvenile Great Skua (*Catharacta skua*) (Bearhop et al. 2000). Body feathers, collected in the present study, tend to show the least variation among feather types and appear to be the best single indicator of Hg in the plumage of a bird (Burger 1993). However, in a controlled dosing study of juvenile Great Skua, considerable variation was found in the ability of individuals to transfer and excrete Hg into feathers (Bearhop et al. 2000). A similar situation may exist for BCNH nestlings.

In conclusion, nestling BCNHs from Pea Patch Island and Baltimore Harbor generally exhibited low to moderate concentrations of elements in feathers and blood. Based on these results, metal pollution does not appear to be an immediate threat to these BCNH colonies. In 1998, reproductive success at Baltimore Harbor was found to be sufficient to sustain a stable population (Rattner et al. 2001). Hatching success of BCNHs at Pea Patch Island, where metal concentrations tended to be greater, was lower than that found in other Atlantic Coast colonies, though the main impediment to nestling survival at this site was attributed to nestling mortality from predation (Parsons et al. 2001). In addition, the BCNH population at Pea Patch Island has remained fairly stable compared to other species at this mixed rookery between 1993 and 1998 (Love 2001). BCNHs may be particularly well-adapted to survive in industrialized areas (Rattner et al. 2000). Based on the present findings, future study may wish to focus on Pb, Mn, and Hg exposure, and their potential effects in wading birds.

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