BLOOD-LEAD AND ALAD ACTIVITY LEVELS OF COOPER’S HAWKS (ACCIPITER COOPERI) MIGRATING THROUGH THE SOUTHERN ROCKY MOUNTAINS

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ABSTRACT.—Predatory or scavenging raptors can be exposed to lead contamination through the ingestion of hunter-injured or killed game species that contain residual lead bullets, pellets, or fragments thereof, or prey contaminated by lead from other anthropogenic sources. We studied the incidence of lead exposure in Cooper’s Hawks (Accipiter cooperi) by sampling southward and northward migrating populations. Cooper’s Hawks have been regularly captured for biological data collection at two long-term monitoring and banding stations in north-central New Mexico. We identified blood-lead concentrations, erythrocyte ALAD activity, and hematocrit levels in fall migrating adults and juveniles (N = 45 and 15, respectively), and spring migrating adults (N = 38; no juveniles were captured in spring). Blood-lead concentrations of spring migrating adults (\( \bar{x} = 0.063 \pm SE 0.011 \) \( \mu g/g \)) were significantly greater than both fall adults and juveniles (0.032 ± 0.003 \( \mu g/g \) and 0.028 ± 0.004 \( \mu g/g \), respectively). Blood-lead concentrations did not reach levels sufficient to inhibit erythrocyte ALAD activity or depress hematocrit levels. ALAD activity appeared to be age-dependent, however, as activity in fall juveniles (74.9 ± 2.2 units) was significantly greater than in both fall and spring adults (63.0 ± 2.9 units and 54.0 ± 3.1 units, respectively). Hematocrit values indicated no detectable differences between migration season or age. Our findings suggested that Cooper’s Hawks were exposed to higher environmental levels of lead in their winter range than they were in the breeding range, though not at concentrations known to cause detrimental health effects.

KEY WORDS: Cooper’s Hawk; Accipiter cooperi; lead; ALAD; migration; toxicology.

PLOMO SANGUINEO Y NIVELES DE ACTIVIDAD ALAD EN HALCONES DE COOPER MIGRATORIOS (ACCIPITER COOPERI) EN EL SUR DE LAS MONTAÑAS ROCOSAS

RESUMEN.—Las rapaces de predadoras o carroñeras pueden estar expuestas a contaminación con Plomo a través de la ingestión de especies de caza, heridas o matadas, que contienen plomo residual de balas, perdigones, fragmentos de los mismos, o presas contaminadas con plomo a partir de otras fuentes antropogénicas. Estudiamos la potencialidad a la exposición al plomo en los gavilanes de Cooper (Accipiter cooperi) tomando muestras de sus poblaciones migratorias hacia el sur y hacia el norte. Los Halcones de Cooper han sido capturados regularmente para colectar datos biológicos en dos estaciones de monitoreo y marcaje a largo plazo en el Norte-centro de Nuevo México. Las concentraciones de plomo sanguíneo, la actividad ALAD de los eritrocitos, y los niveles de hematocrito fueron identificados en adultos y juveniles emigrantes de otoño (N = 45 y 15, respectivamente), y adultos migrantes de primavera (N = 38; ningún juvenil fue capturado en la sesión de primavera). Las concentraciones de plomo en la sangre de los adultos migratorios de primavera (\( \bar{x} = 0.063 \pm ES 0.011 \) \( \mu g/g \)) fue incre-
The toxicological risks to waterfowl from spent lead shot used in hunting are well documented (Bellrose 1959, Stout and Cornwell 1976, Zwank et al. 1985, Sanderson and Bellrose 1986), and have led to the elimination of lead-based ammunition used for waterfowl hunting in United States and Canada. Investigations have also assessed the dangers of lead-based ammunition in upland game and mammal hunting. A risk assessment of lead effects in upland-bird species concluded that lead is likely to accumulate in birds following accidental ingestion of lead shot (Kendall et al. 1996). Lead artifacts and sinkers have been ingested as grit in high hunting-use and high fishing-use areas (Burger et al. 1997, Lewis et al. 2001), resulting in prolonged release of lead within the gizzard. Game birds may also be injured or killed during hunting attempts, but remain uncollected in the field. Subsequently, scavengers and raptors may be exposed to lead contamination through ingestion of these incapacitated birds.

Investigations of expelled castings collected from two wild raptor populations in Spain found lead shot artifacts in 6% and 11% of those collected (Mateo et al. 2001). Prevalence as high as 70% was reported in Bald Eagle (Haliaeetus leucocephalus) castings in Utah (Platt 1976). Both of these cases indicate exposure from an array of food items. Lead toxicosis and exposure to lead shot have been recognized in a variety of wild raptors including eagles, goshawks, vultures, and kites (Garner 1991, Miller et al. 1998, Platt et al. 1999, Wayland et al. 1999, Mateo et al. 2001). Documented lead-artifact-induced mortalities of Golden Eagles (Aquila chrysaetos), Red-tailed (Buteo jamaicensis) and Rough-legged hawks (Buteo lagopus), and Peregrine (Falco peregrinus) and Prairie falcons (Falco mexicanus), species not known to rely heavily on waterfowl for prey items, further imply a secondary risk of lead toxicosis from upland-bird and mammal prey (Locke and Friend 1992). Lead poisoning resulting from the ingestion of lead fragments in consumed carcasses likely contributed to the historic decline of the California Condor (Gymnogyps californianus; Pattee et al. 1990, Meretsky et al. 2000). More recently, lead contamination has impacted the condor reintroduction program with the deaths of at least four birds in northern Arizona (Cilek et al. 2000) caused ostensibly by lead-artifact ingestion.

Lead toxicity induces effects on vascular, nervous, renal, immune, reproductive, and hematopoietic systems, as well as behavioral abnormalities (Eisler 1988, Burger 1995). At lower tissue concentrations, several biomarkers have been used to assess sublethal effects. Delta-aminolevulinic-acid dehydratase (ALAD) is a widely studied, heine-related enzyme that is altered by lead contamination at low exposure levels. This zinc-dependent enzyme is easily inhibited by lead substitution and has been extensively characterized as a sensitive indicator of low-level lead exposure (Hoffinan et al. 1981, Goering et al. 1986, Scheuhammer 1987, Pain 1996, McBride 2003). With increased exposure and ALAD inhibition, detrimental health effects may occur with decreases in hemoglobin production and erythrocyte concentrations. Blood-sample collection and analysis, thus, provides a sensitive, non-lethal means for monitoring health effects and lead-body burden.

Cooper’s Hawks (Accipiter cooperii) are a medium-sized raptor, breeding throughout much of the United States, southern Canada, and northern Mexico. Inhabiting primarily coniferous and mixed forests (Rosenfield and Bielefeldt 1993), they can become habituated to human disturbance and may use urban and suburban areas that provide appropriate habitat (Stahlecker and Beach 1979, Boal and Mannan 1999). Cooper’s Hawks routinely feed on avian prey as a primary food
source, with small game birds such as dove (e.g., *Zenaida* spp.) and quail (e.g., *Colinus virginianus*) being potential prey (Rosenfield and Bielefeldt 1993, Boal 1997). This places the hawks at risk of ingesting spent-lead shot imbedded in injured game birds. Consequently, the Cooper’s Hawk is a species of primary concern for lead exposure due to its predation on upland game birds (Kendall et al. 1996).

Migratory Cooper’s Hawks are regularly captured for biological data collection each yr at two long-term monitoring and banding stations in north-central New Mexico (Hoffman et al. 2002). We investigated lead exposure and related health effects in the breeding and wintering ranges of migratory Cooper’s Hawks by sampling their southward and northward migrating populations, respectively, at these sites.

**STUDY AREA AND METHODS**

Cooper’s Hawks were trapped during migration at two relatively close research sites in the Cibola National Forest of north-central New Mexico. Blood sampling occurred during normal processing of captured birds for banding and data collection. Samples were collected at the Manzano Mountains station (34°42.25’N, 106°24.67’W) during fall migration from 15 September–21 October 2001 and at the Sandia Mountains station (35°05.21’N, 106°25.93’W) during spring migration from 11–14 April 2002. These two sites are located ca. 40 km apart along the same migratory-flight path, and have been situated to better sample migrants during each respective season. Samples were taken from all birds captured, regardless of gender or age class.

Migrating birds were trapped using captive lure birds and an assortment of bow-nets, dhoo-gaza traps, and mist nets (Hoffman et al. 2002). Blood samples were collected nonlethally by brachial veni-puncture, and separated into aliquots of ca. 150 μl for ALAD activity determinations, with the remaining sample stored in chemically clean vials for metal analysis. Samples were packed in ice and returned to lab facilities where they were stored at −80°C until analysis. Packed cell volumes (PCVs) of the samples were determined at the time of collection using 100 μl microhematocrit capillary tubes.

Sample preparation for lead analysis used a modification of U.S. Environmental Protection Agency (1996) method No. 3050B. Blood samples were placed in Teflon beakers and digested with 5 ml nitric acid on 120°C hot plates. Upon complete digestion, the nitric acid was evaporated to ca. 1–2 ml. Aliquots of 1.5 ml hydrogen peroxide (30%) were added and the samples were heated until fully reacted. Samples were transferred to 10 ml volumetric flasks and brought up to final volume using ultra-pure (MilliQ®, Millipore, Billerica, MA U.S.A.) water.

All samples were analyzed for lead utilizing a Perkin Elmer® AAnalyst 600 atomic absorption spectrophotometer with graphite furnace (GFAA) and all data captured by Perkin Elmer® AAWinLab (version 3.71) instrument control software. Five-point calibration curves were developed using traceable NIST standards. The method-detection limit for lead in blood samples, on a wet-weight basis, was 0.054 μg Pb/g blood. Samples falling below the detection limit (BDL) were assigned a value of one-half the detection limit (0.017 μg/g).

Erythrocyte ALAD enzyme activity was measured using a modification of Pain (1987). Three 25 μl aliquots of previously frozen whole blood were each added to 725 μl ultra-pure water prior to incubation. The assay was started by the addition of 500 μl of 8-aminolevulinic acid (ALA; 100 mM final concentration, or FC) in sodium phosphate buffer (pH 6.4), and incubated in the 38°C water bath for 1 hr. Enzyme activity was halted with the addition of 500 μl of trichloroacetic acid solution (612 mM FC). Precipitated proteins were separated by centrifugation (2000 rpm for 10 min) and 100 μl aliquots of the supernatant solution containing the reaction product, porphobilinogen, were pipetted into the appropriate wells of a 96-well microtiter plate. Ehrlich’s indicator reagent (100 μl) was then added to each well, which was then covered and analyzed immediately. Absorbance was read every min for 10 min, at 555 nm (kinetic mode) using a SPECTROMax Plus 96-well spectrophotometric plate reader controlled by Molecular Devices® SOFTmax Pro software (Molecular Devices Corp., Sunnyvale, CA U.S.A.). The maximum value (background corrected) was selected for use in the activity determination. Enzyme activity was calculated using the equation:

\[ \text{Activity} = \frac{(11,580 \times \text{Max. Absorbance}_{550})}{\text{Hematocrit}} \]

where 11,580 is a conversion factor based on the molar-extinction coefficient specific to the porphobilinogen/p-dimethylaminobenzaldehyde complex (62,000 M⁻¹ cm⁻¹), as well as stoichiometric calculations and dilution factors. Max. Absorbance₅₅₀ was measured in absorbance units and Hematocrit was expressed as a percent. ALAD activity units were expressed as nmoles ALA × min⁻¹ × ml RBC⁻¹.

Statistical analyses were performed using SigmaStat for Windows (Jandel Corporation, SPSS Inc., Chicago, IL U.S.A.). Hematocrit values and ALAD activity were analyzed for significant differences between ages and between migration seasons using a one-way analysis of variance (ANOVA). Blood-lead concentrations were not normally distributed due to numerous samples assigned the BDL value of one-half the detection limit. Therefore, data sets were tested using a Kruskal-Wallis analysis of ranks test. No sex-dependent differences were identified for any of the tested parameters \((P > 0.28)\); thus, genders were combined for the final statistical analyses. Linear regression equations were calculated to examine the relationships between detectable blood-lead concentrations and ALAD activity. For all tests, results were considered significant if \(P \leq 0.05\).

**RESULTS**

We collected 98 blood samples over the two migratory seasons. We collected 60 samples during fall migration: 15 from juveniles, 45 from adults...
We collected 38 samples from adults during spring migration. We were unable to capture any juveniles during the spring collection period. Fall juvenile lead concentrations varied from 0.017 (half detection limit) to 0.071 μg/g (x̄ = 0.028 ± SE 0.004 μg/g, median = 0.017 μg/g). Only six of 15 samples were above detectable limits (x̄ = 0.045 ± 0.005 μg/g). Fall adult lead concentrations varied from 0.017 to 0.112 μg/g (x̄ = 0.032 ± 0.003 μg/g, median = 0.017 μg/g). Nineteen of 45 values were above detectable limits (x̄ = 0.052 ± 0.004 μg/g). Spring adult values (N = 38) varied from 0.017 (half detection limit) to 0.356 μg/g (x̄ = 0.063 ± 0.011 μg/g, median = 0.047 μg/g). Twenty-five of 38 values were above detectable limits (x̄ = 0.086 ± 0.015 μg/g). Blood-lead levels showed significant variation between migration season and age (P = 0.005), with higher concentrations in spring adults than either fall adults or fall juveniles.

Due to storage and volume constraints, only 47 of 98 (46%) blood samples were analyzed for erythrocyte ALAD enzyme activity (Fig. 1B). Fall juvenile samples (N = 9) showed activities ranging from 67.2-84.2 units (x̄ = 74.9 ± 2.2 units). Fall adult samples (N = 20) showed activities ranging from 43.8-100 units (x̄ = 63.0 ± 2.9 units). Spring adult samples varied from 16.4-67.1 units (x̄ = 54.0 ± 3.1 units). Enzyme activity appeared to be age-dependent; fall juvenile values were significantly higher than fall adult and spring adult values (P = 0.001). Further, spring adult ALAD enzyme activity was reduced 28% and 14% when compared to fall juvenile and fall adult samples, respectively.

We assessed packed cell volumes for 58 of 98 (59%) blood samples (Fig. 1C). Fall juvenile values (N = 10) varied from 42-48% (x̄ = 45.9% ± 0.6). Fall adult hematocrit values (N = 20) varied from 41–50% (x̄ = 46.4% ± 0.5). Values for spring adult samples (N = 28) ranged from 37–56% (x̄ = 47.6% ± 1.0). Packed cell volumes did not vary significantly between seasons or ages.

In examining the relationship between blood-lead concentration and health effects, individual ALAD activity was regressed as a function of corresponding blood-lead concentration. However, only 2% of the variability in enzyme activity could be explained by the concentration of lead in the blood (r² = 0.02).

**DISCUSSION**

Blood-lead levels in Falconiformes are considered “subclinical” at levels between 0.2 and 1.5 μg/g. (Fig. 1A).
The majority of Cooper’s Hawks tested demonstrated little evidence of significant lead intoxication, these three individuals exhibited sufficiently increased blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts. Mallards (Anas platyrhynchos) dosed with a single No. 4 shot exhibited similar blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts. Mallards (Anas platyrhynchos) dosed with a single No. 4 shot exhibited similar blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts. Mallards (Anas platyrhynchos) dosed with a single No. 4 shot exhibited similar blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts. Mallards (Anas platyrhynchos) dosed with a single No. 4 shot exhibited similar blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts. Mallards (Anas platyrhynchos) dosed with a single No. 4 shot exhibited similar blood-lead concentrations that suggested exposure to either contaminated food items or ingestion of lead artifacts.
ever, no seasonal differences were identified in this study.

This study is the first effort in an investigation of secondary exposure to lead-based ammunition and lead-based fishing sinkers in raptors, and will be followed by further assessments of other migratory and nonmigratory raptors from other major U.S. migratory flyways. In a similar investigation of migrating Sharp-shinned Hawks (Accipiter striatus) in the eastern United States (Pennsylvania and New Jersey), liver-lead concentrations from 19 individuals ($\chi = 0.007 \mu g/g$ wet weight) were found to be within background levels, indicating little accumulation risk for this related species (Bohall-Wood et al. 1996). Though migratory Cooper’s Hawks do not appear to be at significant risk from lead-artifact ingestion in the Rocky Mountain Flyway, hunting practices and density vary widely in the U.S. and the potential for increased exposure resulting from greater hunting and fishing pressures in other regions of the country exists. Additionally, individuals experiencing higher exposure levels may be precluded from successful migration, and may not be identified in studies such as these. Further investigation of a greater variety of raptor species is required before an assessment of the true risks to raptors will be possible.

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LITERATURE CITED


