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Chronic Lead Exposure, Body Condition, and Testis Mass in Wild Mallard Ducks

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Spent lead shot can be ingested by birds and may cause acute lead poisoning and death (US Fish and Wildlife Service [USFWS] 1988). With the continent-wide ban prohibiting the use of lead shot for the harvesting of waterfowl in North America (USFWS 1988; Environment Canada, August 19, 1997, News Release), a significant source of new lead has been prevented from entering the environment. However, no restrictions exist prohibiting the use of lead shot for hunting upland game birds (e.g., grouse) and small mammals (e.g., rabbits). In addition, it has been estimated that it takes between 100 to 300 years for the complete transformation of lead pellets already in the environment (Jorgensen and Willems 1987). Taking these factors into account, a real need exists to study the lasting effects of lead in the environment and its chronic effects on organisms, such as birds.

Lead is a non-essential, toxic metal that is detrimental to living organisms exposed to it. For example, it has been reported for several bird species that body weight gains can be suppressed through ingestion of dietary lead (Morgan et al. 1975; Edens et al. 1976; cf. Finley and Dieter 1978) and dosing with lead shot (Sanderson et al. 1981). Hohman et al. (1990) has found in canvasbacks (*Aythya valisineria*) that after accounting for confounding factors such as, age, monthly variation, and body size, birds with lead shot in their gizzards had body mass values significantly reduced (10%) compared to unexposed birds. Thus, Hohman et al. (1990) postulated that waterfowl exposed to sublethal levels of lead (i.e., chronic exposure), may weigh less than unexposed birds. This is a noteworthy point because body mass (corrected for body size) is an indicator of the general condition of a bird and body condition is important for survival and reproduction (Tsuji et al. 1994).

Although numerous studies have documented detrimental effects of lead exposure on the reproductive success of female birds (Edens et al. 1976; see Scheuhammer 1987, for a review), relatively little is known with respect to lead exposure and male reproductive potential/success in birds, except for a few studies (e.g., Morgan et al. 1975; Edens et al. 1976; Kendall and Scanlon 1981; Sanderson et al. 1981). In the Sanderson et al. (1981) study, active spermatogenesis was not seen histologically in any wild mallards (*Anas platyrhynchos*) dosed with shot containing lead. Further, Kendall and Scanlon (1981) report that testes weights were significantly lower in

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lead-in-water treated ringed turtle doves (*Streptopelia risoria*) compared to controls. Similarly, reduced testicular weights in lead-treated bird groups compared to controls have been reported for Japanese quail (*Coturnix coturnix japonica*; Morgan et al. 1975; Edens et al. 1976). These studies suggest that lead exposure may have a detrimental effect on the reproductive potential of exposed male birds.

Since male birds do not possess accessory reproductive organs (Lake 1971), reproductive potential relates to three factors: sperm availability, quality, and quantity. In order to ensure fertilization, gametes must be available at the appropriate time when the female is receptive and fertile, of good quality, and in sufficient quantity. In birds, an increase in sperm number per inseminate has been related to an increase in fertility (Wall and Jones 1977). Also, larger testes have been shown to produce greater volumes of semen with more sperm per ejaculate (Brillard and deReviers 1985). Moreover, fertility in birds has been shown to be decreased when the number of spermatozoa per inseminate falls below one million sperm per insemination (Parker et al. 1942). Taking these studies into account, it becomes apparent that if lead is affecting testicular size in birds, reproductive potential of exposed males would be affected detrimentally. In this paper, we examined morphological variation in male, wild mallard ducks and relate body condition and reproductive potential (testis mass) to lead concentration in bone.

MATERIALS AND METHODS

Wing bones (radii) and livers were "salvaged" from 39 male, wild mallard ducks harvested for food during the period 1995 - 1998 (late April - early May), by First Nation Cree near Fort Albany, Ontario, Canada (52.15 N; 81.35 W). Following methodology similar to Dickson and Scheuhammer (1993), whole radii were excised using a stainless steel blade, cleaned of associated tissue, sealed in individually marked, plastic zip-lock bags, and stored frozen until further processing. Damaged wing bones were not used in the study. Livers (whole when available) were stored and processed in a similar manner as described above.

After thawing, samples were oven dried to constant weight, at 70°C for 96 hrs. For radii, one-cm-long pieces were cut from the central portion, along the longitudinal axis. These pieces were then cut into smaller pieces using a stainless steel instrument. Liver samples were ground in a spice mill with stainless steel blades. For each 0.10g of material (bone or liver) sampled, 0.5mL of trace-metal grade nitric acid was added. For livers, all samples were approximately 0.50g; while for bone, between 0.3 and 1.0g of each sample was digested. These mixtures were digested for 24 hrs at room temperature, followed by digestion at 100°C for 6 hrs. Following digestion, resultant mixtures were diluted with distilled deionized water to a final acid concentration of 25% v/v and then filtered through Whatman 42 ashless filter paper. Lead concentrations were determined using a Perkin Elmer Model 460 atomic absorption spectrometer (bone - graphite furnace, detection limit [dl] of 0.10μg/g dry weight

[dw]; liver - flame, dl of 1.0μg/g dw). All samples had triplicate readings taken and % residual standard deviation of all triplicate readings was 7%.

For bone lead analysis, a multi-element reference standard (US National Institute of Standards and Technology [USNIST], Standard Reference Material 1643c) was used in calibration. Repeated readings were recorded until absorbance values for a sample were within ±10% of each other. Two blank samples (2.5mL of nitric acid; final volume of 10mL) and a bonemeal reference standard (USNIST 1486) were included with each digest. No lead was detected in any of the blanks (i.e. concentrations were <dl) and bonemeal reference standards were on average within 5% of the expected value. Ten duplicate bone samples (26% of the total sample) were analyzed, being on average within 15% of the expected value.

For liver lead analysis, calibration standards were dilutions of Delta Scientific high-purity multi-element standard (100±0.5mg/L) made to the same chemical matrix as the samples. Two blank samples and a bovine liver reference standard (USNIST 1577b) were included with each digest. All lead levels in blanks were <dl and bovine liver reference standards were on average within 5% of expected value. Eleven duplicate samples (28% of the total sample) were analyzed; no deviation from previous values were noted.

Males with bone lead concentrations ≥ 10μg/g dw were considered to have elevated concentrations of lead. Dickson and Scheuhammer (1993) considered the value 10μg/g as a threshold defining elevated lead concentrations in wing bones (radii) for dabbling ducks, based on their study of 8,692 immature birds collected in Canada, and the existing experimental evidence. Thus, males were assigned to one of two categories: background level, <10μg/g, n=32; and elevated level, ≥ 10μg/g, n=7.

Two morphological variables were measured on each specimen: body mass (ingesta-free), wet weight, measured to the nearest 0.1g on a triple-beam balance; and tarsometatarsus length, the bone measurement from the tip of the intercondylar prominence to trochlea for digit III, measured to the nearest 0.05cm with vernier calipers. A subset of 31 individuals had testis mass measured, to the nearest 0.1g on a triple-beam balance. The right testis (when available) was measured because mallards deviate from most other species of birds where the left testis is larger than the right (Johnson 1961).

Data were transformed to natural logarithms to equalize the variance of the data. Variation in character means between males with elevated lead concentrations and males with background levels of lead was assessed multivariately by single-classification multivariate analysis of variance (MANOVA) and univariately by single classification analysis of variance (ANOVA) for each character. Bivariate linear regression analysis was used to examine the relationship between body mass and body size (tarsometatarsus is often used as a body size indicator in birds; Tsuji et al. 1994), and testis mass and body mass among individuals.

RESULTS AND DISCUSSION

No liver analysed sample had a lead concentration ≥ 1.0µg/g dw (dl). If a liver sample had been found to contain a lead concentration ≥20µg/g, this individual would have been considered to be suffering from acute lead poisoning and removed from the present study. The reason for removing such an individual from the study relates to the fact that acute high-level lead exposure in birds has been shown to rapidly increase lead levels in both liver and bone. Thus, by determining liver-lead concentrations in our specimens, we allowed for the differentiation between two routes of exposure known to result in elevated lead concentrations in bone: acute high-level lead exposure, and chronic low-level lead exposure (Finley and Dieter 1978). Since no elevations for lead were detected in liver samples, elevations in bone lead were considered chronic in nature. We assume that birds exposed to acute high-levels of lead do not survive.

Of 39 birds examined, 7 (18%) were found to have elevated bone-lead concentrations, while 32 (82%) were at background levels. Similarly, Dickson and Scheuhammer (1993) reported that of 8,692 dabbling birds collected in Canada, approximately 17% of wing bones were considered elevated for lead (≥ 10µg/g dw). It appears that chronic lead exposure in our sample is representative of the general Canadian population of dabbling ducks.

Table 1. Morphometric characters and ANOVA between male mallards with elevated levels of lead (≥ 10µg/g dw) in their wing bones and those at background levels (<10µg/g).

Character	Elevated $\bar{x}$±sd range (n)	Background $\bar{x}$±sd range (n)	F[a]
Body mass, g	1175.1±108.0 1030.0-1299.0 (7)	1233.7±144.4 964.1-1520.0 (32)	.77[ns]
Tarsometatarsus length, cm	4.72±0.14 4.60-4.94 (7)	4.62±0.19 4.20-5.00 (32)	1.79[ns]
Testis mass, g	9.1±3.4 6.6-15.6 (6)	9.1±2.6 5.3-14.8 (25)	.02[ns]

[a]Significance of F: [ns], P≥0.19

MANOVA of the three measured characters indicated no significant difference (F approximation of Wilk's lambda=0.869, d.f.=3 and 27, P<0.276) between males with elevated bone-lead levels and those at background levels. ANOVA of each character (so as to include all individuals, n=39) revealed no significant differences in the three characters measured for the two groups (Table 1). Similarly, Finley and Dieter (1978) found no significant difference in body mass of lead pellet-dosed mallards ($\bar{x}$=1132g) and controls ($\bar{x}$=1136g). By contrast, in the study by Sanderson et al. (1981), it was reported that the average loss in body weight, per duck, per day was directly related to the average amount of lead administered per individual and the average amount of lead eroded from dosed pellets. However, more important than absolute body size is condition of an individual; individuals may be disproportionately large or small, as will be explained when discussing results from the linear regression analysis. In the present study, no significant differences between the two groups were also found for tarsometatarsus length and testis mass. Since samples were collected during late April to early May, the part of the breeding season when male mallards possess full sexual capacity (Johnson 1961) and greatest number of sperm per ejaculate (Watanabe 1961), any meaningful differences should have been apparent at this time. In addition, testicular weights in the present study were within the normal size range for wild mallards, shown histologically to possess full reproductive capacity (range: 3.27-15.99g; Johnson 1961).

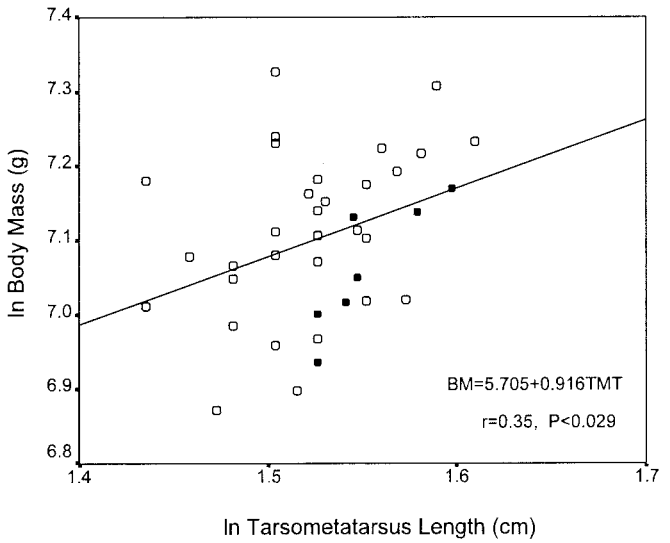

Figure 1. Relationship between the natural log of body mass and tarsometatarsus length of 39 male mallard ducks having differing bone lead concentrations. Males with elevated bone-lead levels (≥10μg/g dw) are represented by solid squares, while birds at background lead levels (<1μg/g dw) are represented by open squares.

No significant relationship was found between testis mass and body mass (r=-0.24, P=0.202). In other bird families that have been studied (e.g., Phasianidae: Parker et al. 1942; Tsuji et al. 1992), body mass has been positively associated with testis mass.

A significant relationship between body mass and body size (P<0.029) was found (Figure 1). Length of tarsometatarsus was used as a measure of body size in comparing variation in body mass of individuals with elevated bone lead levels and males at background levels. Linear regression analysis revealed that 12.3% (r=0.35) of the variation in body mass was attributable to variation in tarsometatarsus length (Figure 1). Among background-level males, 46.9% had values of body mass less than those predicted by the regression equation, while 71.4% (5 of 7) of elevated males had values of body mass lower than predicted. ANOVA of residual variation between elevated and background-level males was not significant (F=3.56, P=0.067). Thus, males with elevated lead levels were not significantly lighter for their body size compared to background-level males, although a tendency towards a decrease in body condition was evident for males with elevated lead levels (Figure 1).

In overview, no decrease in reproductive potential, as measured by testis mass, was noted for the birds sampled with elevated bone-lead levels. However, our study cannot preclude the possibility that sperm production and viability were impaired due to chronic lead exposure. Although our data are suggestive that males with elevated lead levels are disproportionately light (i.e., a decrease in body condition) compared to predicted values, no significant difference between background-level and elevated males was noted. Perhaps, with a larger sample size, a significant difference in body condition between the two groups of males may become evident. Lastly, it should be noted that in the present study only one metal (lead) was examined; thus, the effects of other toxic metals cannot be discounted (Scheuhammer 1987).

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