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### Lead effects on body composition and organ size of wintering canvasbacks *Aythya valisineria* in Louisiana

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We tested whether lead exposure, as evidenced by liver lead concentration, affected body composition and organ sizes of canvasback ducks *Aythya valisineria* in Louisiana during winter 1987-88. After adjusting for body size, sex, age, and site and month of collection, we found decreases in ingesta-free body mass; breast, leg, and body protein; body fat; intestine length; and liver and gizzard masses associated with increased liver lead concentrations. There were no apparent associations between liver lead concentrations and testes and body ash masses, or caecal length. We used the concentration of 26.7 ppm of liver lead on a dry matter (dm) basis as indicative of lead toxicosis. We predicted that a canvasback with 26.7 ppm dm liver lead would weigh 209 g less and have 105 g less fat than an unexposed individual. Whereas many lead exposed canvasbacks may survive through winter, their subsequent survival, ability to reproduce and perform other annual cycle events may be compromised. We recommend management to make lead unavailable to waterfowl at major concentration areas and periodic monitoring of lead contamination in waterfowl populations.

*Key words:* Anatidae, *Aythya valisineria*, condition, ducks, lead toxicosis

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Despite a ban on use of lead shot by waterfowl hunters in the United States (U.S. Department of Interior 1988), ingestion of spent lead shotgun pellets (hereafter referred to as lead shot) and, to a lesser extent, lead fishing sinkers by free-living waterfowl is common (reviewed in Pain 1992) and may remain so for many years (Bellrose 1959, Longcore, Corr & Spencer 1982, Peters & Afton 1993). Ingestion of lead shot can be lethal (Friend 1987). Non-lethal exposure can produce sickened birds having compromised immune systems (Hunter & Woebeser 1979), gastrointestinal dysfunction (Lawler, Duke & Redig 1991), and increased risk of hunting or natural mortality because of decreased wariness (Bellrose 1959, Heitmeyer, Fredrickson & Humburg 1993). Both controlled dosing and observational studies have shown that many wild ducks survive bouts of lead exposure (Franson, Haramis, Perry & Moore 1986, Mautino & Bell 1986, Hohman, Moore & Franson 1995); however, birds that survive lead exposure may have reduced body mass (Sanderson & Bellrose 1986), reduced pectoral muscle (Clemens, Krook, Aronson & Stevens 1975), impaired hemopoiesis (Dieter & Finley 1978, Dieter 1979), and delayed testicular development (Jeske & Thul 1986). Hence, assessing the full impact of environmental pollutants such as lead on waterfowl populations is underestimated by counts of dead and sickened birds.

A substantial portion of canvasbacks wintering and staging in the Mississippi Flyway has elevated lead levels (Hohman, Pritchert, Pace, Woolington & Helm 1990, Havera, Whitton & Shealy 1992, Custer & Hohman 1994). For example, at Catahoula Lake, which has attracted more wintering canvasbacks than any other site in North America, 40% of canvasbacks had elevated lead in liver concentrations ( $>6.7$  ppm dm) and 33% had concentrations consistent with lead intoxication ( $>26.7$  ppm dm) during winter 1987-88 (Friend 1985, Custer & Hohman 1994). Body mass of canvasbacks that had ingested lead shot was reduced (Hohman et al. 1990). Because relative body mass can affect survival (Haramis, Nichols, Pollock & Hines 1986, Hepp, Blohm, Reynolds, Hines & Nichols 1986, but see Kremetz, Hinds, Corr & Owen 1989), Hohman et al. (1990) speculated that overwinter and annual survival rates of birds receiving sublethal lead exposure may be reduced. Indeed, overwinter survival (57-58%) of Catahoula canvasbacks with blood lead concentrations  $>0.2$  ppm wet mass basis (wm), was lower than the 82-92% survival of canvasbacks with background blood lead

during the three winters of 1991-1994 (Hohman et al. 1995).

Studies of the amount and distribution of body constituents have increased our understanding of the annual cycle in the nutritional needs and stresses on waterfowl. For example, canvasbacks acquire energy and nutrients during winter to offset costs (e.g. moult, migration, and courtship) incurred during spring and acquisition and loss occur differentially among tissues (Hohman 1993). Thus, the impact of lead exposure on wintering waterfowl may lead to important changes in the relative distribution as well as amounts of fat and protein stores. We undertook this study to further examine dynamics of weight loss associated with lead contamination in waterfowl. Specifically, we tested relationships between liver lead and individual body components (body, breast, and leg protein, body fat, and body ash) and size of organs (intestinal and caecal lengths, and testes, liver, and gizzard masses).

## Methods

This study was conducted at Catahoula Lake in central Louisiana ( $31^{\circ}15'N$ ,  $92^{\circ}00'W$ ) and at the Mississippi River Delta in coastal southeastern Louisiana ( $29^{\circ}15'N$ ,  $89^{\circ}5'W$ ) (see Hohman et al. 1990 for site descriptions). Sixty-eight to 74% of all canvasbacks observed on monthly statewide surveys in winter 1987-88 were recorded at these two sites (USGS/BRD files, National Wetlands Research Center, Lafayette, La.). Maximum numbers of canvasbacks counted were 58,000 on Catahoula Lake and 14,000 on the Mississippi River Delta.

Catahoula Lake, averaging about 30,000 hunter days per year, is one of the most intensively hunted waterfowl areas in Louisiana (Wills & Glasgow 1965, Wycoff, Wills & Glasgow 1971, Shealy 1982). About 500 permanent hides or blinds are maintained on the lake with additional temporary hides on the brushy perimeter or floating on open water. Use of shotshells containing lead was completely prohibited on Catahoula Lake in winter 1988-89, but lead shot remains readily available to waterfowl (Peters 1992, Peters & Afton 1993). Use of lead shot was prohibited at the Mississippi River Delta in winter 1987-88.

We shot canvasbacks from flocks of  $\geq 3$  birds between the 10th and 20th of each month from November 1987 through February 1988. Birds were collected at night from boats equipped with spotlights, or



Table 1. Sample sizes from canvasback duck population strata designated for comparison of effects of liver lead on body composition in Louisiana during the winter (November - February) of 1987-88.

Month	Location	Female		Male	
		AHY <sup>1</sup>	HY <sup>2</sup>	AHY <sup>1</sup>	HY <sup>2</sup>
November	Catahoula	5	5		
	Mississippi River Delta	5	5		
December	Catahoula	7	8	8	6
	Mississippi River Delta	5	5	5	5
January	Catahoula	5	5		
	Mississippi River Delta	5	5		
February	Catahoula	7	8	7	6
	Mississippi River Delta	5	5	5	5

<sup>1</sup> AHY = After hatch year (>1 year old);

<sup>2</sup> HY = hatch year (<1 year old)

during daytime from natural blinds following behavioural observation. By observing behaviour and avoiding birds in groups of <3, we eliminated the collection of obviously lead-sickened birds (Friend 1987). We measured ( $\pm 0.1$  mm except where noted) bill length from the commissural point to tip of nail, maximum bill width distal to nares, keel length, tarsal bone length, body length measured from the tip of the bill to the base of the middle rectrix, with bird held on its back ( $\pm 0.5$  cm), culmen height and length, head width and length, and body mass ( $\pm 5$  g). Birds were considered hatch-year (immature) or after-hatch-year (adult) based on plumage (Haramis, Derleth & McAuley 1982) or cloacal characteristics (Hochbaum 1942). Carcasses were placed on wet ice in the field and later frozen ( $< -20^{\circ}\text{C}$ ). Within three months of freezing, gizzard (emptied), liver, omental fat, reproductive organs, and right leg (all muscles having either origin or insertion on the femur or tibiotarsus) and breast muscles were excised and weighed ( $\pm 0.01$  g). Intestine length and combined length of caeca were measured ( $\pm 1$  mm). Contents of the digestive tract were removed, weighed ( $\pm 0.001$  g), and subtracted from body mass to determine ingesta-free body mass (hereafter body mass). As detailed in Hohman (1993), proximate analyses were performed on canvasback carcasses to determine total body fat and protein, ash, and leg muscle and breast muscle protein. Estimates of total fat and total protein did not include composition of livers, which were analysed separately for trace elements.

From 402 canvasbacks collected, we chose a sample of 137 livers for lead concentration analyses at the Environmental Trace Substances Research Center, Columbia, Missouri (Table 1). The sample was composed of 120 livers chosen at random within strata characterized by sex, age, month and location of capture plus 17 livers taken from birds found to have

at least one ingested lead shot pellet in their gizzard. Livers were freeze-dried, weighed, and homogenized in a blender. Subsamples of freeze-dried livers were digested in nitric-perchloric acid and analysed for lead by inductively coupled plasma analysis (ICP). Samples with liver lead concentrations (LEAD) below the detection limit of ICP (4 ppm dm) were analysed by graphite furnace. Nominal limit of detection for lead was 0.08 ppm dm.

We tested for significant trends between LEAD and total fat, total protein, leg protein, breast protein, ash, intestine length, caeca length, and gizzard, liver, and testes masses of canvasbacks corresponding to their LEAD by using analysis of covariance models with Type III sums of squares (PROC GLM, SAS Instit., Inc. 1988) in a means model parameterization (Searle 1987: 384-456). First, we subjected the correlation matrix of nine measurements (culmen, tarsus, keel, bill, body, and head lengths; bill and head widths; and culmen height) of the full set of 402 collected birds to principal component analysis (PROC PRINCOMP, SAS for calculations). The first principal component accounted for 61% of the variance in the original measures, and described positive covariation among all measures, and factor loading ranged from 0.27 to 0.37. Following Ankney & Alisauskas (1991: 801), we used scores along the first principal component as a measure of body size (SIZE) and, hence, as a covariate in analyses of factors affecting canvasback body composition and organ sizes. Our models took the form:

$$Y_{ij} = \text{STRATUM}_i + \alpha \text{SIZE}_{ij} + \beta (\text{LEAD}_{ij})$$

or

$$Y_{ij} = \text{STRATUM}_i + \alpha \text{SIZE}_{ij} + \beta \log_e(\text{LEAD}_{ij})$$

where  $Y_{ij}$  was a measure of body constituent or organ size for the  $j^{\text{th}}$  bird within the  $i^{\text{th}}$  STRATUM;



Table 2. Evidence relating body composition and organ size declines to liver lead concentration in canvasback ducks wintering in Louisiana during 1987-88. Values from analysis of variance tables for regression models in which STRATA were used as categorical predictors, BODYSIZE was a continuous covariate, and either LOG<sub>e</sub> LIVER LEAD or LIVER LEAD concentration was a continuous predictor. Tests were based on Type III sums of squares.

Response variable	STRATA (P)	BODYSIZE (P)	Coefficient	P
<i>LOG<sub>e</sub> LIVER LEAD</i>				
Ingesta-free body mass	<0.0001	<0.0001	-34.5	<0.0001
Breast protein	0.0191	0.0002	-0.513	0.0006
Leg protein	0.0022	<0.0001	-0.203	<0.0001
Total protein	0.1259	0.0009	-3.79	0.0005
Fat	0.0006	0.1873	-17.4	<0.0001
Gizzard mass	<0.0001	0.3941	-2.47	<0.0001
Liver mass	0.3783	0.2018	-0.168	0.6936
Ash	0.8375	0.0076	-0.323	0.4267
Intestine length	<0.0001	0.0910	0.568	0.3642
Cecae length	0.2329	0.1212	0.997	0.5643
Testes mass <sup>1</sup>	0.1514	0.5656	-0.011	0.0427
<i>LIVER LEAD</i>				
Liver mass	0.0610	0.2730	-0.0589	0.0125
Intestine length	<0.0001	0.1321	-0.0767	0.0272

<sup>1</sup>A better model for Testes mass includes Month (P = 0.0015), Age (P = 0.036), and LOG<sub>e</sub> LIVER LEAD (Coefficient = -0.0056 ± 0.0025; P = 0.0269)

STRATUM<sub>i</sub> referring to the *i*<sup>th</sup> grouping of birds according to sex, age, month, and location at capture. By evaluating both LEAD and log<sub>e</sub>(LEAD) as covariate predictors we increased the likelihood of detecting a significant relationship when a non-linear relationship was present. Using log<sub>e</sub>(LEAD) would place more emphasis on changes occurring at small values of LEAD (<2 ppm dm) where our data were concentrated. We assigned a value of 0.04 ppm dm LEAD (1/2 the minimum detectable limit) to birds with LEAD below detectable levels. In all cases, we tested for heterogeneity of slopes among STRATA for both SIZE and LEAD (Littell, Freund & Spector 1991). We inspected plots of residuals to examine adherence to model assumptions (Cook & Weisberg 1982).

Although we avoided collecting obviously sickened ducks, we were concerned that our results might be unduly influenced by heavily exposed birds if any had LEAD > 26.7 ppm dm (converted from 8 ppm wm), a level considered to indicate lead toxicosis when supported by pathology (Friend 1985). Hence, we repeated our analyses on those body components affected by lead after excluding birds with LEAD > 26.7 ppm dm.

## Results

LEAD above the nominal detectable level of 0.08 ppm occurred in 130 of 137 canvasbacks (range: 0.09-130 ppm). LEAD varied significantly among strata (P < 0.001), but did not vary with SIZE within a stratum (P = 0.9103). Custer & Hohman (1994) provided a detailed description of the variability of liver lead among strata for our sample. In summary,

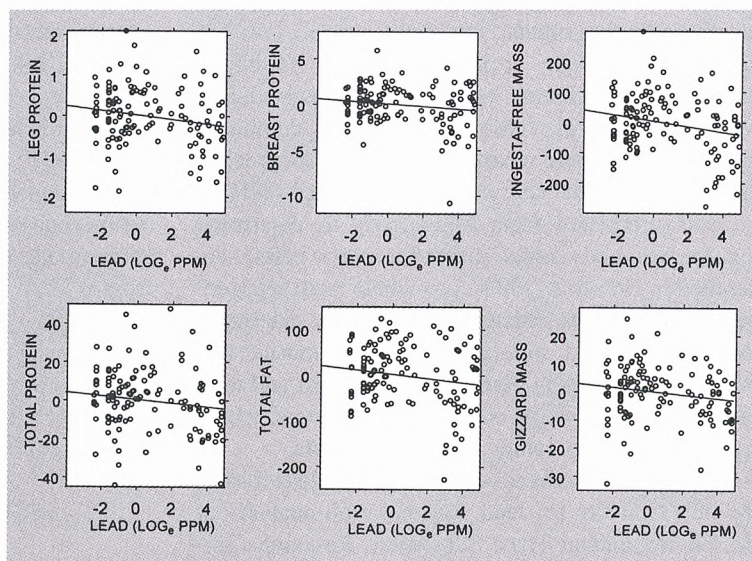


Figure 1. Relationship between liver lead concentration and body components of 137 canvasback ducks collected in Louisiana during winter 1987-88. Observed liver lead concentrations (log<sub>e</sub> ppm) are plotted against body constituents (g) adjusted for body size and stratum (sex, age, month, site of collection) along with least-square regression lines.



for the 120 randomly sampled livers, geometric mean concentrations ranged from 0.15 ppm for female birds at Mississippi River Delta during January to 47.2 ppm for males at Catahoula Lake during December. Regardless of variability in LEAD among strata, linear relationships of either LEAD or SIZE with measurements of body composition and organ size, when evident, were consistent among strata (i.e. linear trends were homogeneous among strata,  $P > 0.25$ ). Body mass, breast protein, leg protein, total protein, fat, intestine length, liver mass and gizzard mass were negatively related to LEAD, but we found no relationship between LEAD and variation in ASH, caeca length, and testes masses (Table 2). Except for liver mass and intestine length,  $\log_e(\text{LEAD})$  was as good or a better (based on  $R^2$ ) predictor of change than LEAD. For liver mass and intestine length, higher LEAD was significantly related to smaller size of these organs but  $\log_e(\text{LEAD})$  was not.

Our sample contained 31 birds that had LEAD  $> 26.7$  ppm dm. Indeed, 12 birds had LEAD  $> 85.2$  ppm dm, which was the mean lead concentration found by Zwank, Wright, Shealy & Newsom (1985) from 22 sick, dying, or dead canvasbacks that they collected at Catahoula Lake during winter 1977-78. For analyses which excluded 31 birds with LEAD  $> 26.7$  ppm dm, all except total protein ( $P = 0.590$ ), liver mass ( $P = 0.132$ ), and intestine length ( $P = 0.644$ ) still had significant negative linear relationships with LEAD. Based on scatterplots of residuals from models that excluded LEAD, influence of LEAD was most apparent at concentrations above 12-15 ppm dm (Fig. 1).

## Discussion

Emaciation (i.e. depleted body fat and atrophied

breast muscles) is consistent with lead toxicosis in waterfowl (Friend 1987). Reduced body mass also accompanies sublethal exposures of waterfowl to lead (Sanderson & Bellrose 1986, Hohman et al. 1990, Havera et al. 1992). We found high LEAD associated with reduced body mass among wintering canvasbacks with no other readily apparent clinical signs of lead toxicosis such as reluctance to fly, 'roof-shaped' wing position, and seeking seclusion (Friend 1987). A bird with LEAD of 26.7 ppm dm would incur an estimated 209 g decline in body mass when compared with an unexposed bird (LEAD = 0.08 ppm dm; Table 3). This difference is 74% greater than previously estimated by Hohman et al. (1990) based on presence or absence of ingested lead shot in the gizzard. Our findings demonstrated that mass loss was due to substantial losses of body protein (total protein, leg protein, breast protein, gizzard mass) and fat.

Causes for body mass declines in lead-exposed waterfowl are complex. Anorexia is characteristic of some birds with elevated lead levels (Clemens et al. 1975). Indeed, if leg protein was related to level of swimming or diving activity when foraging as suggested by Hohman (1993), then reduced leg protein in lead-exposed canvasbacks may indicate that they fed less than unexposed birds. Similarly, if breast protein and gizzard mass were related to use, declines in masses of these tissues may indicate lead-induced reductions in flight and digestive activities, respectively. Lead-exposed birds, however, are not always anorexic (Cook & Trainer 1966). Mass loss may be an outcome of reduced digestive efficiency from impaired neural function or digestive motility (Cory-Slechta, Garman & Siedman 1980) or simply from lysis of muscle tissue by lead (Clemens et al. 1975). Regardless of whether birds were digestively impaired or anorexic, reduced stores of fat and pro-

Table 3. Predicted effects of selected concentrations of liver lead on body composition of wintering canvasbacks. Predictions are based on regression models that used LOG<sub>e</sub> LIVER LEAD, BODYSIZE and STRATA (sex, age, month, site of collection) to predict component sizes of birds collected in two study areas of Louisiana during 1987-88.

Component	(1) Minimum (g) (0.08 ppm)	(2) Median (g) (0.74 ppm)	(3) At toxicosis (g) (26.7 ppm)	Loss <sup>a</sup> (g) (1) - (3)	Loss <sup>b</sup> (g) (2) - (3)
Ingesta-free body mass	90.7	10.8	-117.9	209	128.7 (10.5%)
Breast protein	1.3	0.15	-1.69	2.99	1.85 (7.2%)
Leg protein	0.53	0.06	-0.68	1.21	0.75 (7.3%)
Total protein	10.1	1.2	-13.1	23.2	14.3 (6.6%)
Fat	45.7	5.5	-59.5	105.2	64.9 (31.0%)
Gizzard mass	6.47	0.77	-8.41	14.9	9.18 (6.6%)

<sup>a</sup>Loss in predicted constituent mass between minimum observable liver lead exposure (1) and at toxicosis (3).

<sup>b</sup>Loss in predicted constituent mass between observed median liver lead exposure (2) and at toxicosis (3).



tein which are catabolized for body maintenance during periods of negative energy balance (Robbins 1983), mean reduced body mass associated with elevated risk of mortality (Haramis et al. 1986). Fat was negatively associated with LEAD at all concentrations, whereas total protein was not related to LEAD among birds with < 26.7 ppm LEAD. Thus, low fat reserves occurred at much lower levels of LEAD exposure and fat losses were probably substantial before birds lost much protein.

Lead-exposed canvasbacks incurred substantial reductions in fat. For example, we estimated that canvasbacks with 26.7 ppm dm LEAD had 105 g (52%) less fat than unexposed birds. Assuming that

a) basal metabolic rate (BMR) is related to body mass ( $BMR = 75 \times (\text{body mass [kg]})^{0.72}$ ; Owen & Reinecke 1979),

b) daily energy expenditure (DEE) for canvasbacks resting in water is 2-times BMR (Takekawa 1987),

c) catabolized fat yields 39.6 kJ/g (Ricklefs 1974), and

d) conversion efficiency of fat to energy is 100%, then catabolism of 105 g of fat yields 10.1-12.5 times BMR or 5.1-6.3 times DEE for canvasbacks ranging in body mass from 1,086-1,459 g (body mass of canvasbacks wintering at Catahoula Lake, Louisiana, in winter 1987-88; W.L. Hohman, unpubl. data). In terms of migration distances ( $M_L [\text{kJ bird}^{-1} \text{ km}^{-1}] = 0.1055 (\text{body mass [g]})^{0.47}$ ; Kendeigh, Dol'nik & Gavrillov 1977), 105 g fat (4,150 kJ) represented the energy required by a 1,252 g bird (mean body mass of a canvasback in late winter, W.L. Hohman, unpubl. data) to migrate 1,378 km. This is about 85% of the distance (1,600 km) between the Gulf of Mexico and the southern part of the canvasback breeding range in northern prairies of the United States (Serie & Sharp 1989).

Timing of exposure could have a large influence on the biological effects of lead on wintering canvasbacks. Body mass and fat of canvasbacks wintering in Louisiana are lowest in November and December (Hohman 1993). Exposure to lead in those months might substantially deplete fat reserves to lethal levels. Indeed, LEAD was elevated in both males and females at Catahoula Lake during December (Custer & Hohman 1994). Whereas birds may survive lead exposure in other months, reduced fat may affect timing and performance of subsequent events in the annual cycle. For example, if variation in fat levels regulates population turnover at migratory stopover

sites as suggested by Serie & Sharp (1989), then onset of spring migration may be delayed in lead-exposed birds compared to unexposed birds. Reproductive performance of canvasbacks may also be affected by exposure to lead in winter, if time of arrival on spring-staging and breeding areas (i.e. sites where birds initiate courtship and pair-bond formation (Weller 1965)) influences reproductive success (cf. Saylor & Afton 1981) and migration chronology. Additional research is needed to determine whether or not lead effects persist beyond winter and thereby influence annual survival rates and reproductive performance.

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