

AN EPIZOOTIC OF COMMON LOONS IN COASTAL WATERS OF NORTH CAROLINA:  
CONCENTRATIONS OF ELEMENTAL CONTAMINANTS AND RESULTS OF  
NECROPSIES

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**Abstract**—A 1993 die-off of common loons (*Gavia immer*) in the coastal waters of North Carolina was investigated with emphasis on comparing mercury, selenium, arsenic, and lead between birds from the epizootic and reference specimens. Die-off specimens were emaciated but contained no ingested foreign bodies and no lesions suggestive of infectious disease. Results of bacteriology, virology, parasitology, and botulism testing were unremarkable. The geometric mean concentrations (wet weight) of liver mercury (10.9 ppm), and arsenic (0.96 ppm) did not differ between specimens from the die-off and reference loons from the same area that died of other causes. The geometric mean liver selenium concentration of die-off specimens (10.4 ppm) was significantly higher than that of reference loons. Liver lead concentrations were <0.20 ppm in all but one sample (5.83 ppm). The geometric mean mercury concentration in the primary remiges of die-off specimens (5.44 ppm dry weight) was significantly lower than in reference birds. Liver mercury significantly correlated with liver selenium on a molar concentration basis. We interpret the range of liver mercury concentrations in birds from the epizootic, similar liver mercury concentrations in reference loons, and higher mercury concentrations in reference loon feathers as evidence that factors other than mercury were primarily responsible for the emaciation diagnosed as the cause of mortality.

**Keywords**—Common loon *Gavia immer* Mercury Selenium Epizootic

## INTRODUCTION

The history of common loon (*Gavia immer*) mortality along the Atlantic and Gulf Coasts has been associated with unknown causes of emaciation [1,2]. In addition to hypotheses on food limitation, migration and molt stress, parasitism, and storm-related trauma, mercury toxicity has been discussed as a potential causative or contributing factor in some of these events [2–4]. In North Carolina, significant loon mortality has been documented; the etiology of these die-offs is of interest because North Carolina's coastal waters are a major wintering area for this species [5,6].

Following severe storms in March and April 1993, common loons were found dead or dying on barrier island beaches in southeastern North Carolina (between latitudes 34°30'N and 35°00'N). Loons were retrieved between March 17 and April 21, with the majority of the dead or dying birds observed in mid April. Total mortality was documented to exceed 200 and estimated at 500 to 700; only common loons died in large numbers, but mortality included northern gannets (*Sula bassanus*) [1]. We investigated this epornitic with an emphasis on comparing elemental contaminant concentrations in specimens from the die-off to reference specimens.

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## METHODS

*Carcass retrieval and examination*

Examination of 10 debilitated loons at a wildlife rehabilitation clinic included measurement of packed red blood cell volume (as a percentage of total blood volume) and plasma protein (with a refractometer). Five loons dying in rehabilitation were saved for necropsy, as were 25 dead loons collected from the field. Necropsies were conducted by the National Wildlife Health Center, Madison, Wisconsin, USA, according to published procedures [7]. Gross internal and external examinations were conducted on each carcass to identify wounds, traumatic injuries, and lesions of organ systems suggestive of diseases and to assess body condition based on fat reserves and pectoral muscle development.

Samples of liver and intestine from six loons and air sacs from two were tested for bacteria by inoculation onto 5% sheep red blood agar and eosin–methylene blue plates (Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 72 h. Bacterial isolates were characterized by the API-20E system (Analytab Products, Plainview, NY, USA). Serum from heart blood of four birds was tested for avian botulism types C and E with the mouse protection test [8]. Virus isolation attempts from samples of liver and intestine, from four and nine loons, respectively, were carried out in cell cultures and embryonating eggs [9,10]. Tissues for fungal isolation attempts were inoculated onto Sabouraud's dextrose starch agar and incubated at 36°C for 72 h. Tissues, including brain, liver, kidney, lung, heart, and intestine, were collected from seven loons for histopathology. Tissues were fixed in 10% neutral buffered

formalin, embedded in paraffin, sectioned at 5  $\mu\text{m}$  for light microscopy, and stained with hematoxylin and eosin.

#### Analytical chemistry

During necropsy of 23 adult loons, a portion of each liver was removed and placed in individual acid-washed glass jars with Teflon<sup>®</sup>-lined lids. The primary flight feathers from one wing were removed with shears (the calamus was severed near the skin surface, allowing recovery of almost the entire feather) and stored in plastic bags. Before analysis, primary remiges were vigorously washed with distilled, deionized water to remove external debris. One or more of the primary remiges were analyzed at random from each bird. Samples were analyzed by Hazleton Laboratories America (HAZL), Madison, Wisconsin, USA. Aliquants of samples for mercury analysis were digested with sulfuric acid and nitric acid [11]; mercury determination was performed by cold-vapor reduction atomic absorption spectrophotometry [12]. Aliquants for analysis of arsenic, selenium, and lead were prepared by nitric acid digestion and analyzed by graphite furnace atomic absorption spectrophotometry [13].

#### Reference specimens

To help interpret residue chemistry results, loons dying of other causes were collected as reference samples. Reference specimens were considered suitable only if the cause of death was known, blood protein and hematocrit were normal, or adequate musculature and fat deposits were present. Ten adult common loons were collected in 1993 and 1994 from the same geographic region as the 1993 die-off; the majority came from a wildlife rehabilitation center where they had been euthanized because of traumatic injuries. The reference birds were of approximately normal weights and apparently healthy until they died or were euthanized. Livers and primary remiges from reference specimens were collected in the same manner as samples from the die-off specimens. Preparation of these samples for analyses followed the procedure described for the die-off samples, but primary remiges from each specimen were pooled and homogenized rather than analyzed individually. Reference specimens were analyzed by Research Triangle Institute (RTI), Durham, North Carolina, USA, using methods similar to those used by HAZL [12,13]; RTI prepared samples by closed-container microwave digestion.

Methods used by both laboratories included analyses of blanks, spiked samples, standard reference material (SRM), and duplicates for all analytes. Blanks for the mercury determinations were  $<0.01$  ppm (lower limit of detection) for both laboratories; blanks for selenium and arsenic determinations were  $<0.10$  ppm for both laboratories. Blanks for lead were  $<0.10$  ppm at HAZL and  $<0.15$  ppm at RTI. The relative percent deviations of duplicate analyses for all analytes were within acceptable ranges for method precision. Percent recoveries of mercury, selenium, and arsenic in SRM samples by HAZL (93.5, 104, and 102, respectively) and RTI (98.4, 95.4, and 85.4, respectively) indicate acceptable method accuracy for all parameters and directly comparable performance between laboratories. Reported concentrations are not corrected for percent recoveries. All concentrations in livers are reported as parts per million wet weight; because moisture content of these samples averaged 75%, dry weight values can be approximated by multiplying wet weight concentrations by a factor of 4. All feather mercury concentrations are reported as parts per million dry weight.

Table 1. Comparison of carcass weights and contaminant concentrations between common loons from the North Carolina 1993 die-off and reference specimens

Parameter	Sample population	n	Geometric mean	Range
Liver (ppm wet wt.)				
Mercury	1993 Die-off	23	10.9	2.08–84.3
	Reference	10	8.25	1.10–36.6
Selenium	1993 Die-off	15	10.4	4.08–15.9
	Reference	10	6.80*	1.37–21.3
Arsenic	1993 Die-off	15	0.96	0.23–3.68
	Reference	10	0.63	$<0.10$ –2.12
Lead	1993 Die-off	15	$<0.10$	$<0.10$ –5.83
	Reference	10	$<0.15^a$	$<0.15$
Feather (ppm dry wt.)				
Mercury	1993 Die-off	23	5.44	2.38–12.2
	Reference	10	9.66*	5.87–15.6
Carcass weight (g)				
Females	1993 Die-off	15	2,280	1,790–3,040
	Reference	5	3,000*	2,400–4,400
Males	1993 Die-off	8	2,470	2,110–2,780
	Reference	4	3,030*	2,500–3,500

<sup>a</sup> Because all reference specimen lead values were below detection, no ANOVA was conducted.

\* Reference specimens differ significantly from die-off specimens at  $p \leq 0.05$  using SAS general linear model procedure ANOVA.

#### Statistical analyses

Contaminant concentrations and the ratio of liver selenium to liver mercury (on a molar basis) between die-off and reference birds were examined by ANOVA; the SAS general linear model procedure [14] was used because sample sizes were not balanced. The ANOVA used dry-weight contaminant data because of potential desiccation of the specimens [15,16]. Log-transformations of the contaminant variables were used because several of these were not normally distributed; transformations resulted in normally distributed data for all parameters except selenium in the die-off specimens. The SAS correlation coefficient procedure was used to assess the degree of correlation between liver mercury and carcass weight, liver selenium, and feather mercury. The level of significance for all comparisons was  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

#### General condition and significant necropsy findings

Debilitated loons transported to a wildlife rehabilitation clinic did not respond to treatment; they were weak, anemic (hematocrit, 8–25%), and hypoproteinemic (plasma protein concentration,  $<1$ –2.0 g/dl). The normal range for hematocrit is 35 to 55%, and plasma protein normally ranges from 3.5 to 5.5 g/dl [17]. Nearly all loons examined from the die-off were either adult birds or the oldest subadult age class. They were in wing molt or had recently completed it and were likely without flight mobility or, potentially, physically depleted after the flightless molt period. Only adults were included among the specimens submitted for chemical analyses. All reference loons were adults, and their molt status ranged from not yet initiated ( $n = 2$ ) to partial or complete replacement of primaries ( $n = 8$ ). Loons from the die-off weighed approx. 20% less than the reference specimens, a difference that was significant (Table 1).

All specimens from the die-off were emaciated, with no apparent fat reserves and reduced pectoral muscle develop-

ment. No lead shot, sinkers, or other foreign bodies were found in the gastrointestinal tracts. The histopathologic lesions that have been described in the brain and spinal cord of birds with mercury toxicity [18,19] were not seen. However, autolysis prevented an adequate histopathologic evaluation of tissues. Histopathologic lesions consistent with parasitism were noted in each of the seven loons from which tissues were examined. Renal tubular nephrosis or necrosis and hepatic necrosis were found in the kidneys and livers respectively, of four of seven loons. These lesions can result from a variety of etiologies, including exposure to toxicants, such as metals. Indeed, kidney lesions have been associated with high tissue concentrations of cadmium and mercury in birds [20]. *Escherichia coli*, *Enterococcus* sp., and *Streptococcus* sp. were isolated from the intestine of three, two, and one loon, respectively. These bacteria were not thought to have contributed significantly to mortality. No viruses or fungi were isolated, and avian botulism toxin was not detected.

#### Elemental contaminants

Geometric mean contaminant residues of the die-off and reference specimens are given in Table 1. Liver mercury concentrations exceeded 30 ppm, a value that has been associated with mercury toxicity in birds [21], in 13% of the loons from the die-off. Overall, the concentrations of mercury in livers varied widely, from 2.08 to 84.3 ppm. This lack of uniformly elevated liver mercury residues was not an artifact of age, sex, or extent of disease progression.

Liver mercury concentrations in the die-off specimens did not differ from reference specimens (Table 1). Although liver methylmercury concentrations would increase the toxicological relevance of this comparison, others have demonstrated a negative correlation between the percentage of organic mercury and total mercury and a predominance of inorganic mercury in livers of several species of birds [21–24].

Apparently healthy seabirds often have liver mercury concentrations that exceed values shown to be toxic to birds associated predominantly with freshwater environments [21–24]. Although common loons breed in freshwater environments, they winter in marine and estuarine ecosystems and are common winter residents in coastal North Carolina from November through April each year [6,25].

The liver mercury concentrations reported here for wintering adult loons are similar to those reported in assessments of adults from the breeding grounds [26–28] and are approximately one-half of concentrations reported for emaciated loons from a 1983 coastal Florida die-off [2]. Similar studies of loons on the breeding and wintering grounds have generally found higher concentrations of mercury in emaciated or diseased loons than in apparently healthy loons [2,26–28]. These differences may indicate a mercury-related effect on the diseased loons; they may also be an artifact of emaciation acting to concentrate mercury burdens in remaining tissues. From limited data on weights of presumed healthy wintering loons, it is possible that our reference specimens are slightly underweight; the mean weight of reference specimens in this assessment is approx. 15% less than that of loons previously collected from the North Carolina coast [29]. This difference has minimal significance relative to the utility of the reference specimens in comparisons with emaciated loons from the 1993 die-off because (1) weights were significantly different between these groups and (2) the key attribute of the reference birds was that they were not part of the 1993 die-off. Nev-

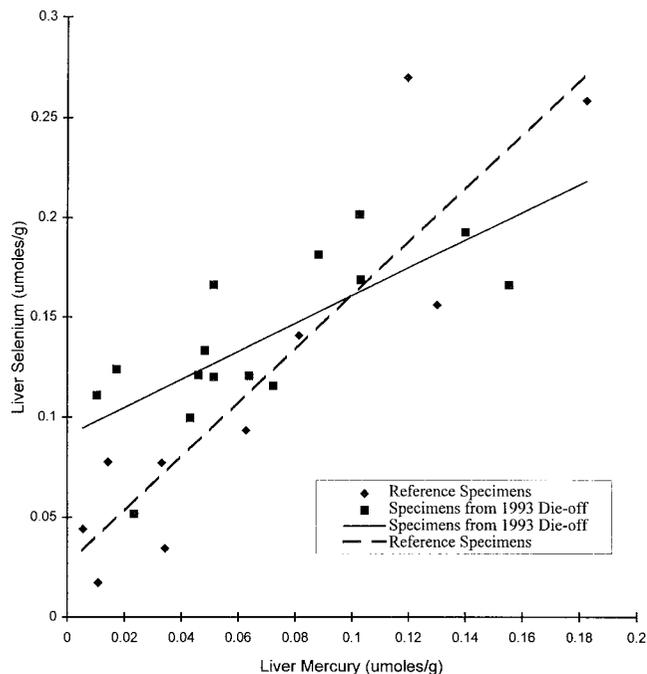


Fig. 1. Mercury and selenium concentrations in livers of common loons from eastern North Carolina: comparison of specimens from the 1993 die-off and reference specimens. Liver mercury was significantly positively correlated with liver selenium on a molar concentration basis for the die-off specimens ( $r = 0.74$ ,  $p = 0.0015$ ), reference specimens ( $r = 0.91$ ,  $p = 0.0003$ ), and combined samples ( $r = 0.82$ ,  $p = 0.0001$ ). The average molar ratio of selenium to mercury in liver was 2.85:1 and did not differ between die-off and reference loons.

ertheless, the reference loons in this assessment should be regarded as "presumed healthy," based on the necropsies, rather than "normal."

Liver mercury concentrations were not significantly correlated with carcass weight. Log-transformed concentrations of liver mercury were significantly positively correlated with log-transformed concentrations of feather mercury for the die-off specimens ( $r = 0.42$ ,  $p = 0.0441$ ) and reference specimens ( $r = 0.70$ ,  $p = 0.0254$ ) but not the combined samples. Liver mercury was significantly positively correlated with liver selenium on a molar concentration basis for the die-off specimens ( $r = 0.74$ ,  $p = 0.0015$ ), reference specimens ( $r = 0.91$ ,  $p = 0.0003$ ), and the combined samples ( $r = 0.82$ ,  $p = 0.0001$ ) (Fig. 1). The average molar ratio of selenium to mercury in liver was 2.85:1 and did not differ between loons from the die-off and reference specimens. Dietary selenium can decrease the toxicity of mercury in birds [30,31]. Selenium has the potential to protect against the toxicity of mercury in these birds.

Mercury was found at significantly higher concentrations in primary remiges of the reference birds in this assessment (Table 1). All mercury stored in feathers is in the methyl form [32]. Feather mercury concentrations are reflective of methylmercury circulating in the blood at the time of feather growth [33]; the blood methylmercury concentration is a function of the quantity of mercury in the diet and the amount of methylmercury stored in soft body tissues between molts [32,34]. Because the majority of the emaciated adult loons were completing, or had just completed, molt and feather replacement, mercury concentrations in their primaries should reflect physiological conditions near the onset of mortality. The higher

concentrations of mercury in the feathers of the apparently healthy reference birds may reflect normal physiological processing of the body's mercury burden.

Elemental contaminant concentrations in feathers are often difficult to interpret because of differences in feather age and replacement sequence. Furthermore, the analytical laboratories handled feather samples from reference specimens (composite of primaries) and die-off specimens (randomly selected primaries) differently. Common loons undergo a simultaneous molt of their primaries between late January and March [1,29,35]. Because of this simultaneous molt and replacement pattern, we expect insignificant differences in the mercury concentration among primaries of individual specimens from the die-off and believe the data from the die-off and reference specimens are comparable.

One loon from the die-off had a liver lead concentration of 5.83 ppm; no other specimens had concentrations that exceeded 0.20 ppm. Although the 5.83 ppm of lead in liver could be consistent with a diagnosis of lead poisoning, no necropsy results are available from that specimen, precluding a definitive diagnosis. The lack of liver lead exceeding 0.20 ppm in any of the other 14 specimens examined indicates that lead poisoning, a documented cause of loon mortality on the breeding grounds [4,28,36], was not a significant mortality factor with the emaciated loons we examined.

The etiology of the 1993 epizootic is unknown; however, the combined effects of molt stress, possible food limitation, parasitism, inability to fly to more efficient foraging areas, and storm-related trauma appear to be viable explanations for the observed emaciation [1,2]. Although the breeding biology of the common loon is well understood, comparatively few studies of their nonbreeding biology have been conducted [37]. Of particular importance is the need for detailed assessments of normal mortality patterns, impacts of storms, precise timing of molt, impacts of molt on physiology, mobility during molt, prey preferences, and prey abundance. Work to elucidate these factors is required to improve our understanding of the causes of mortality on the wintering grounds.

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