COMMON LOONS (*Gavia immer*) NESTING ON LOW pH LAKES IN NORTHERN WISCONSIN HAVE ELEVATED BLOOD MERCURY CONTENT

M. W. MEYER[1], D. C. EVERS[2], T. DAULTON[3], and W. E. BRASELTON[4]

[1] *Bureau of Research, Wisconsin Department of Natural Resources, 1350 Femrite Drive, Monona, Wisconsin 53716,* [2] *Biodiversity, Inc., Paradise, MI 49768,* [3] *Loonwatch, Sigurd Olson Environmental Institute, Ashland, WI 54806,* [4] *Animal Health Diagnostic Laboratory, P.O. Box 30076, Lansing, MI 48909*

Abstract. The Wisconsin Department of Natural Resources conducted a pilot study during the summer of 1991 to determine the extent of mercury (Hg) exposure in common loons (Gavia immer) breeding in Wisconsin. Loons are at risk to elevated Hg exposure in Wisconsin because they often nest on acidified, low alkalinity lakes. Fish from these lakes bioaccumulate MeHg to a greater extent than biota from neutral pH lakes. Using nightlighting techniques, 35 adult loons were captured on 20 northern Wisconsin lakes (pH=5.0-8.7) in 1991. Blood and feather samples were collected for Hg analysis. The mean Hg content of blood cells collected from adult loons on low pH lakes ($pH \leq 6.3$) was significantly greater than the Hg content of adult loons collected on neutral/alkaline pH lakes ($pH \geq 7.0$) ($F=19.87$, $P<0.001$). There was a highly significant negative linear relationship between adult loon blood cell Hg concentrations and lake pH ($r^2=0.38$, $F=15.27$, $P<0.001$); indicating loons nesting on low pH lakes receive greater Hg exposure than loons nesting on neutral pH lakes. The relationship was greater amongst adult males ($r^2=0.56$) than amongst adult females ($r^2=0.36$). Because of this documented exposure, an additional 330 loons were captured 1992-94 on 73 lakes in northern Wisconsin. The Hg exposure of adult and juvenile common loons is being quantified. Individual loons were fitted with unique color-coded leg bands, and the 1992-96 reproductive performance, annual return rates, and nesting behavior of adult loons with the known Hg exposure is currently being assessed.

1. Introduction

Most point sources of mercury (Hg) contamination have been identified and controlled in the United States, however Hg contamination persists. Elevated concentrations of Hg continue to be documented in aquatic biota from remote regions of Scandinavia and North America; the most seriously impacted U.S. and Canadian lakes are found in Minnesota, Michigan, Wisconsin, Florida, Ontario, and Quebec (Swain *et al.*, 1992). Many lakes in these states contain fish with Hg levels that pose health risks for human consumption. A mass balance study of Hg in aquatic systems indicates that significant portion of the Hg found in these fish is of atmospheric and anthropogenic origin (Rada *et al.*, 1989). An analysis of lake sediment cores in Minnesota and Wisconsin indicates that the current rate of Hg deposition is 3 to 4x greater than it was in the 1800s (Swain *et al.*, 1992); important deposition sources are fossil fuel combustion, municipal waste incineration, and industrial processes. Aquatic biota from low pH, low alkalinity lakes receiving increased Hg deposition are more likely to bioaccumulate Hg than biota from neutral/alkaline pH lakes. This is reflected in the strong inverse relationship between the pH and alkalinity of lakes in northern Wisconsin and the Hg content of piscivorous fish (Cope *et al.*, 1990; Lathrop *et al.*, 1989). A complex suite of factors contribute to Hg biomagnification in the food web of low pH lakes. Most important are a net increase in the amount of Hg methylated by sediment bacteria (the form of Hg which is toxic and bioaccumulates) and an increased permeability of fish tissue to MeHg absorption (Winfrey and Rudd, 1990). Because of the highly toxic effects of MeHg, concern has been expressed that piscivorous wildlife inhabiting low pH lakes may be exposed to toxic levels of MeHg in their prey (Wiener, 1987; Scheuhammer, 1991).

Water, Air, and Soil Pollution **80**: 871–880, 1995.

The low pH, low alkalinity lakes of northern Wisconsin are important nesting habitat of the common loon (Gavia immer) (Blair, 1990; WDNR unpubl. data). Loons are top predators on these lakes, consuming primarily 10 to 250 g fish, thus are at risk to increased Hg exposure. There is evidence that increased Hg exposure can reduce common loon reproduction. Impaired common loon productivity was related to the consumption of Hg-contaminated prey in Ontario, Canada (Fimreite, 1974; Barr, 1986). Fimreite (1974) observed that common loon chicks were absent along Hg-contaminated reaches of that river system, while Barr (1986) found that loons which nested on Hg-contaminated English River system lakes had lower nest success than did loons nesting on lakes with low levels of Hg in prey items. Barr (1986) indicated that reductions in egg laying and territorial fidelity were associated with mean prey (10 to 250g fish) Hg concentrations of 0.3 to 0.4 ug/g fresh weight (fw).

There is evidence that Wisconsin loons may consume prey with levels of Hg which could impair reproduction. Loons typically consume fish which weigh 10 to 250 grams (Barr, 1986). At least two fish within this size class were analyzed for Hg by the Wisconsin Department of Natural Resources (WDNR) Fish Contaminant monitoring program on seventy five lakes in northern Wisconsin. Fish from 36% of those surveyed (27 lakes) had Hg concentrations in excess of 0.3 ug/g (fw) (WDNR unpubl. data), the level associated with loon reproductive impairment in Ontario (Barr, 1986). In addition, the total Hg content (fw) of livers of 3 loons collected on low pH lakes (pH <6.3) in northern Wisconsin [31 ug Hg/g (fw), Minonk Lake, Vilas Co; 90 ug Hg/g (fw), North Bass L., Iron Co.; and 33 ug Hg/g (fw), Duck L., Iron Co. (Belant and Anderson, 1990)] all exceeded the mean Hg content of livers from loons collected on the contaminated waters of the English River system in Ontario [total liver Hg = 29.7 ± 12.4 ug Hg/g (fw); Barr 1986].

In 1991, the WDNR Bureau of Research, Sigurd Olson Environmental Institute, and Whitefish Point Bird Observatory undertook a pilot study to investigate whether fish Hg contamination poses a health risk to loons nesting in Wisconsin. Common loon Hg exposure was measured on 20 northern Wisconsin lakes (pH 5.0 to 8.7) by capturing common loons and measuring the Hg content feather and blood samples.

2. Materials and Methods

The Wisconsin LoonWatch volunteer loon monitoring network and the Wisconsin DNR Master Waterbody Database were used to identify 80 study lakes (40 "high Hg", low pH lakes and 40 "low Hg" neutral/alkaline pH lakes) in Ashland, Bayfield, Iron, Vilas, Oneida, and Forest counties in northern Wisconsin. This regions represents the "core" of Wisconsin's breeding loon population estimated at approximately 2900 adults in 1990 (Dunn, 1992). Lakes were selected which had a recent history of resident loons and had a broad range of pH (4.8 to 9.2) and alkalinity (-9 to 950 ueq/L). The study lakes were selected to block for variables such as lake size, presence/absence of islands, and shoreline development.

Study lakes were surveyed by boat 1 to 3 weeks after they were ice-free to determine whether loon breeding pair were in residence (late April to mid May). Study lakes with resident loon pair were then revisited mid June to early July to identify which territorial pair had chicks. During the month of July we captured adults and loon chicks on the study lakes. A 6-member capture

crew used 2 sport boats powered with 6 hp gas motors, spotlights, and tape-recordings of loon calls to lure the loon family to the capture boats. The loons were then netted with a salmon landing net, restrained, and transported to shore. Adult and chick secondary feathers and blood were sampled for Hg analysis. Blood was obtained using 22 gauge needles and 10 cm^3 syringes on the adults, 5 cm^3 syringes with 25 gauge needles for the chicks; 10 cm^3 was drawn from the brachial vein of the adults, 3 cm^3 from the brachial vein of the chicks (>3 weeks old). Blood was placed in orange top vacutainers, allowed to clot, then centrifuged at 4500 rpm for 10 minutes. The serum was separated from the clotted material (red blood cells and fibrin) and both portions were frozen. Captured loons were fitted with USFWS stainless steel or aluminum leg bands and were individually marked with color-coded plastic "wrap-around" leg bands glued with an acetone-based adhesive cement.

Feather and blood samples were analyzed at the Animal Health Diagnostic Laboratory at Michigan State University. Feathers, serum, and clot material (blood cells and fibrin) collected in 1991 were analyzed for trace metal and mineral content using inductively coupled argon plasma (ICP) emission spectroscopy (Jarrell Ash Polyscan 61E ICP and Jarrell Ash Model 955). We chose ICP analysis for screening samples as this study was exploratory and ICP allowed for simultaneous analysis of Hg, calcium (Ca), and selenium (Se) in a cost effective manner. While the ICP mercury detection limits were high (1 ug/g Hg), this level was known to be capable of detecting Hg in adult tissues. Feathers were washed with acetone 3 times, rinsed with doubly distilled water once, and finally rinsed once with acetone. Following washing the feather was cut into several pieces, the basal portion of the shaft below the vein was discarded. Before ICP analysis, approximately 1 g (fw) of feather or serum clot were digested with 2 ml HNO_3 in a 95°C oven overnight. Following digestion, samples were quantitatively transferred to 10 ml class A volumetric flasks containing 100 ug/g yttrium, brought to volume with water, inverted several times, then analyzed.

Chick blood samples collected in 1991 were below ICP detection levels, thus an additional 20 loon chicks were captured on 20 Wisconsin lakes in 1992. The Hg concentrations of chick whole blood samples (1 to 3 cm^3) was determined by cold vapor atomic absorption spectrophotometry (DL=0.01 ug Hg/g; Hazelton Environmental Services, Madison, WI). Whole blood was preserved with 10% formalin (1:20 ratio of formalin to blood) and refrigerated until analyzed.

The pH and acid neutralizing capacity (alkalinity) of water samples from the study lakes were also determined. Water samples were collected in clean polyethylene sample bottles with air displacement caps. Samples were refrigerated until analysis (within 4 days of collection). Alkalinity was determined using the gran titration method.

The SAS General Linear Model (PROC GLM, SAS 1982) was used to determine if the mean tissue levels of Hg differed between male and female adult loons and between adult loons sampled on low vs. neutral/alkaline pH lakes.

3. Results and Discussion

Blood and feather samples were collected from 35 adults and 30 chicks captured on 20 lakes in northern Wisconsin in 1991. The pH and alkalinity of the lakes loons were captured on ranged from 5.0 to 8.7 and -9.4 to 889.4 ueq/L respectively and were highly correlated (r=0.98, Table

I). Loon capture success was excellent (88% for adults, 95% for chicks) and injury was minimal (one adult was treated for a minor foot injury and was released; it was reobserved swimming and foraging normally one month later). In addition, 28 of the 30 captured chicks were reobserved 4 to 5 weeks after capture (93%), indicating that handling mortality was slight or none.

Table I

Water chemistry of Wisconsin lakes where loons were captured 1991.

LAKE	COUNTY	pH	ALKALINITY (ueq/L)
Wharton	Vilas	5.0	-5.4
Nineweb	Vilas	5.1	-9.4
Pincherry	Vilas	5.1	-7.0
Wabasso	Vilas	5.5	5.2
L. Bass	Vilas	5.6	5.4
Lumen	Oneida	6.0	8.9
Sugar Maple	Vilas	6.1	13.9
Imogene	Vilas	6.2	21.7
French	Iron	6.3	34.1
Bills	Vilas	6.4	24.8
Jag Lake	Vilas	6.6	34.5
Washburn	Oneida	6.7	46.6
Indian	Vilas	7.0	98.0
Razorback	Vilas	7.5	329.1
Whitefish	Oneida	7.8	637.7
Trude	Iron	7.8	616.2
Muskellunge	Oneida	8.3	544.0
L. Bearskin	Oneida	8.4	715.6
Catherine	Iron	8.4	820.0
Wilson	Iron	8.7	889.4

The samples of adult feathers and blood cells all had detectable levels of Hg, however serum did not. Hg bonds to sulphur within proteins such as keratin and cell membranes and thus is concentrated in feathers and blood cells (Crewther *et al.*, 1965). All loon chick blood samples were below ICP Hg detection limits. The mean Hg content of blood cells collected from adult loons on low pH lakes ($pH \leq 6.3$) was significantly greater than the clot Hg content of adult loons collected on neutral/alkaline pH lakes ($pH \geq 7.0$) (Table II; $F = 19.87$, $P < 0.001$). The mean male and female serum clot Hg levels were not statistically different (Table II; $F = 2.41$, $P = 0.14$). The linear relationship between adult loon blood cell Hg and lake pH was also highly significant

Table II

Feather and clotted blood cell Hg concentrations [(N) Mean (ug/g fw) ± 1SD] of Wisconsin adult common loons

		LOW pH LAKES (pH<6.3)	NEUTRAL pH LAKES (pH≥7.0)
FEATHERS	Adult males	(5) 15 ± 3	(7) 11 ± 3
	Adult females	(7) 9 ± 3	(4) 7 ± 1
	All adults	(12) 12 ± 4	(11) 9 ± 3
BLOOD CELLS	Adult males	(5) 6 ± 2	(7) 2 ± 1
	Adult females	(7) 4 ± 2	(4) 1 ± 1
	All adults	(12) 5 ± 2	(11) 2 ± 1

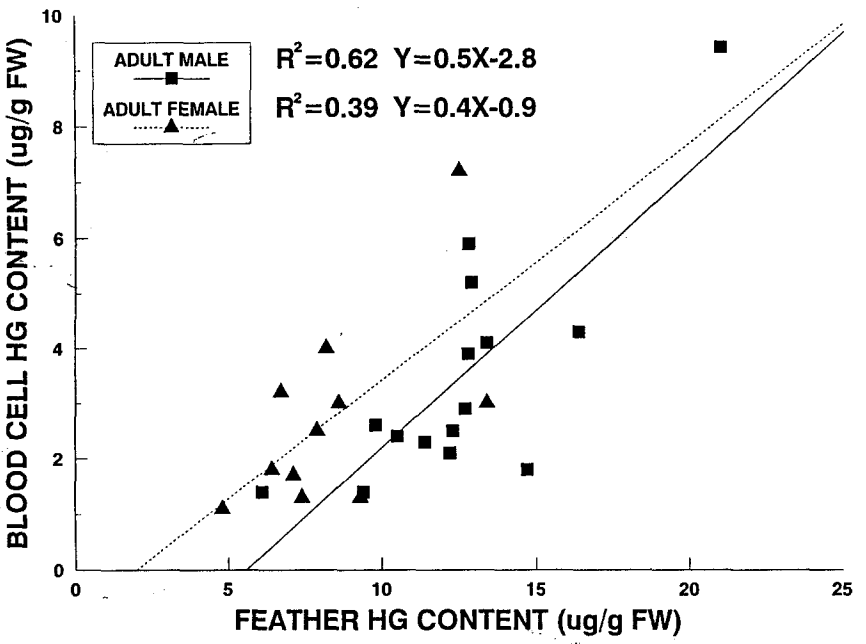


Fig. 1. Wisconsin 1991 adult common loon blood cell Hg content (fw) vs. lake pH

(Figure 1, $r^2=0.38$, $F=15.27$, $P<0.001$); and the relationship was greater amongst adult males ($r^2=0.56$) than amongst adult females ($r^2=0.36$).

Mean feather Hg concentrations were not significantly different between adult loons captured on low vs. neutral pH lakes (Table II; $F=4.02$, $P>0.05$), however males had significantly greater levels than did females (Table II; $F=10.25$, $P<0.01$). The overall linear relationship between adult feather Hg content and lake pH was not significant (Figure 2, $r^2=0.08$; $P>0.05$); however the relationship between adult male feather Hg content and lake pH (Figure 2, $r^2=0.42$; $P<0.05$) was significant.

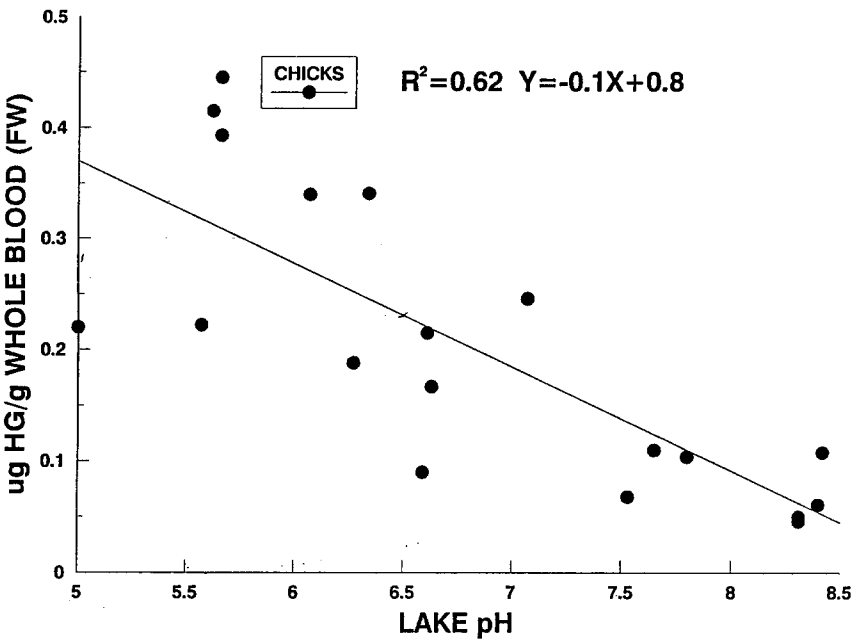
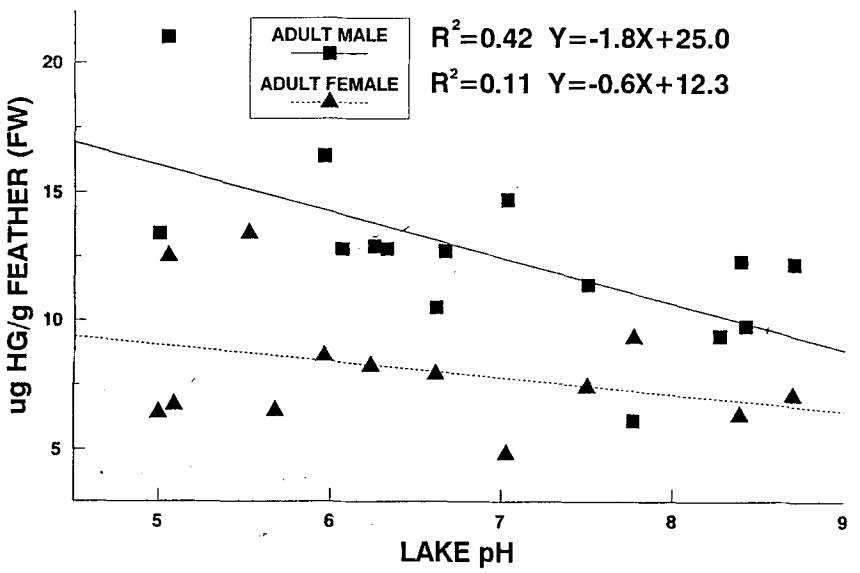


Fig. 2. Wisconsin 1991 adult common loon feather Hg content (fw) vs. lake pH

Feather Hg content represents the body burden of Hg at the time of feather formation; loons molt while on their wintering grounds along the Atlantic coast and Gulf of Mexico (Furness *et al.*, 1986). Therefore, the Hg content of adult feathers collected in the summer represents the loon's Hg burden while it regrew its flight feathers on the wintering grounds, and appears less related to the water chemistry of the lake they nested on. Blood Hg levels more closely reflect the Hg status of loons at the time they are captured. Therefore, the elevated levels of Hg in the blood cells of adult loons nesting on low pH lakes in Wisconsin indicates that they are exposed to greater levels of Hg in their prey than are loon nesting on neutral/alkaline pH lakes. The lesser feather Hg levels in adult females may indicate that they metabolize and/or excrete Hg more efficiently than do males thus have a lower body burden at the time of feather formation. Alternatively,

females are 15 to 20% smaller than males, thus may feed on smaller fish and have lower Hg exposure.

An additional 24 chick common loon blood samples were collected in 1992. All samples tested were above instrument detection levels (DL = 0.01 ug Hg/g). A highly significant linear relationship was found between chick whole blood Hg concentrations (fw) and lake pH (Figure 3, $r^2=0.62$, $P<0.01$). It was anticipated that chick Hg exposure would be most closely associated with the water chemistry of the lake they were captured on because chicks usually receive all their prey from the nest lake while adults may forage on additional lakes.



. Fig 3. Wisconsin 1992 common loon chick whole blood Hg concentrations (fw) vs. lake pH

The linear relationship between 1991 adult common loon blood cell Hg and feather Hg concentrations was significant (Figure 4, $r^2=0.46$, $P<0.05$), with the relationship greater for adult males ($r^2=0.62$) than for adult females ($r^2=0.39$). Though the relationship is statistically significant, the relation is relatively weak for a feather Hg vs. blood Hg comparison. This is not surprising as the loon's winter diet (common loons winter along the U.S. Atlantic and Gulf of Mexico coastline) is obviously different from the diet on the breeding grounds in Wisconsin; size of prey items may also vary. The Hg content of fish (the primary prey item of loons) is known to be species and size specific thus exposure levels likely differ between sites.

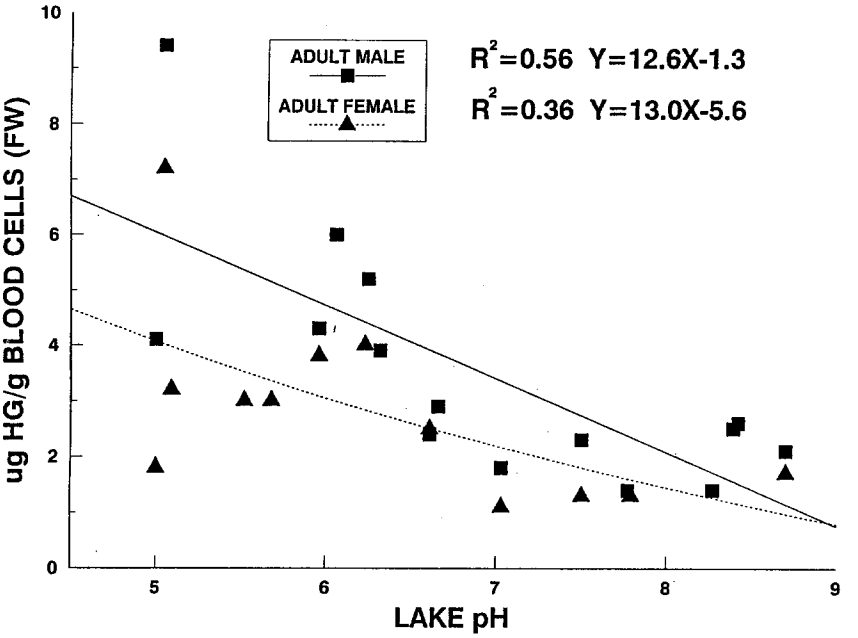


Fig. 4. Wisconsin 1991 adult common loon blood cell Hg content vs. feather Hg content

Serum clot selenium (Se) levels were also greater in adults captured on low pH lakes (Table III; $F=5.18$, $P<0.05$). As Se is known to ameliorate the toxic effects of mercury (Cuvin-Aralar and Furness, 1991) its increased concentration in these samples may indicate that loons possess a mechanism which reduces the toxicity risk. However, the linear relationship between blood cell Hg vs. blood cell Se concentrations (fw) was not significant ($r^2=0.11$, $P>0.05$) and while the blood cell Hg:Se ratio is approximately 1:1 on neutral/alkaline lakes it approaches 2:1 on acidified lakes (Tables I and II).

It has been hypothesized that the availability of nutritionally essential minerals such as calcium (Ca) may be reduced in acidified habitats (Scheuhammer, 1991). The mean serum clot Ca content of adult loons captured on low pH lakes was significantly less (Table III; $F=10.09$, $p<0.005$) than the clot Ca content of adult loons sampled on neutral pH lakes. Male and female Ca levels were similar ($P>0.10$). It has been hypothesized that a scarcity of Ca-rich aquatic invertebrates may occur in acidified habitats and result in reduced avian production (Scheuhammer, 1991). The lesser Ca levels in the loon tissue collected from low pH lakes does not necessarily indicate that those loons experience a deficiency, however the fact that there is less Ca in blood cells is of interest.

Table III

Clotted blood cell Se and Ca concentrations [(N) Mean (ug/g fw) ± 1SD] of Wisconsin adult common loons

		LOW pH LAKES (pH<6.3)	NEUTRAL pH LAKES (pH≥7.0)
SELENIUM	Adult males	(7) 3 ± 1	(7) 2 ± 1
	Adult females	(6) 4 ± 2	(4) 2 ± 1
	All adults	(13) 3 ± 2	(11) 2 ± 1
CALCIUM	Adult males	(7) 23 ± 6	(7) 31 ± 9
	Adult females	(6) 16 ± 7	(4) 28 ± 6
	All adults	(13) 20 ± 8	(11) 30 ± 8

4. Conclusion

Adult common loons and chicks captured on Wisconsin lakes with pH ~ 5.3 had elevated Hg concentrations in blood samples. The level of Hg exposure of chicks was more closely related to lake pH than was adult exposure; perhaps reflecting different feeding habits. There is an indication that calcium may also be limited in biota from acidic lakes (loon blood Ca levels were less on acidic lakes); but blood Se levels were elevated, perhaps sparing some of the toxic effects of mercury on low pH lakes. The finding that loons nesting on low pH lakes in Wisconsin have greater Hg exposure justified an expanded investigation into the effect of the Hg exposure on common loon productivity and survival in Wisconsin. WDNR Bureau of Research and Biodiversity, Inc. captured, sampled, and banded an additional 330 adult and chick common loons on 73 Wisconsin lakes 1992-94. Whole blood samples from chicks and adult loons are being analyzed by the US Fish and Wildlife Service and adult feathers are being analyzed at the Animal Health Diagnostic Laboratory at Michigan State University. In addition, the reproductive performance of color-marked loons on the 73 study lakes is being documented 1992-96; field technicians determine annual adult return rates, locate nests, count eggs and chicks, and determine fledging rates. We will also measure whether reduced Ca or increased aluminum concentrations occur in loon tissues on acidified lakes; these compounds may also cause reproductive anomalies in acidified habitats (Scheuhammer, 1991). This data will allow for an assessment of the impact of Hg exposure on common loon nest success in Wisconsin.

ACKNOWLEDGEMENTS

The 1991 loon capture team included Mary Derr, Paul Dziepak, Brian Johnson, and Jean Thomson. Jeff Wilson, Greg Kessler, and Bruce Bacon, WDNR Mercer, provided boats, motors, vehicles, lodging, and logistical support for both the productivity and loon capture portion of the study. Sample submission was supervised by Dr. R. Poppenga, Animal Health Diagnostic Laboratory, MSU; sample preparation was performed by Jean Thomson. Numerous WDNR staff volunteered to assist in loon capture and productivity surveys and are gratefully acknowledged. Financial support for this research was received from the North American Loon Fund, the Sigurd Olson Environmental Institute, the Wisconsin DNR Bureau of Research, and the Wisconsin Acid Deposition Research Council.

REFERENCES

Barr, J.F.: 1986, *Can. Wildl. Serv. Occasional Pap. No. 56* 23p.
Belant, J. & Anderson, R. K.: 1990, *Passenger Pigeon* **52**, 307-310.
Blair, R.B.: 1990, *Passenger Pigeon* **52**, 119-126.
Cope, W.G., Wiener, J.G., and Rada, R.G.: 1990, *Environ. Toxicol. Chem.* **9**, 931-940.
Crewther, W.B., Fraser, R.D., Lennox, F.G., Lindley, H.: 1965, *Adv. Prot. Chem.* **20**, 191-346.
Cuvin-Aralar, L.A., & Furness, R.W.: 1991, *Ecotox. Env. Safety* **21**, 348-364.
Dunn, T.: 1992, *Passenger Pigeon* **54**, 19-29.
Fimreite, N.: 1974, *J. Wildl. Manage.* **38**, 120-131.
Furness, R.W., Muirhead, S.J., Woodburn, M.: 1986, *Mar. Pollut. Bull.* **17**, 27-30.
Lathrop, R.C., Noonan, K.C., Guenther, P.M., Brasino, T.L., Rasmussen, P.W.: 1989. *Tech. Bull. No. 163. Wis. Dept. Nat. Resour.*, Madison, WI. 40 pp.
Rada, R.G., J.G. Wiener, M.R. Winfrey, D.E. Powell.: 1989, *Arch. Environ. Contam. Toxicol.* **18**, 175-181.
Scheuhammer, A.M.: 1991, *Environ. Poll.* **71**, 329-375.
Swain, E.B., Engstrom, D.R., Brigham, M.E., Henning, T.A., Brezonik, P.L.: 1992, *Science* **257**, 784-787.
Wiener, J.G.: 1987, *Trans. 52nd N.A. Wild. & Nat. Res. Conf.*, 645-657.
Winfrey, M.R. & J.W. Rudd.: 1990, *Environ. Toxicol. & Chem.* **9**, 853-869.