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Abstract.—Estimates of wildlife population viability through measurements of contaminant stressors, such as water mercury concentrations, were modified with variables specific to New York's Adirondack Park to develop a wildlife criterion value for the Common Loon (*Gavia immer*). Biotic and abiotic samples were collected for mercury analysis on 44 Adirondack lakes over a 2-year period (2003 to 2004). From 1998 to 2007, Common Loon blood samples were collected for mercury analysis from the 44 lakes, loon feather samples from 40 lakes, and nonviable eggs from 29 lakes. It was determined that 2.00 ng Hg/L or less in the water was small enough to prevent male Adirondack Common Loons from accumulating mercury in levels high enough to impact reproductive success and behavior, while a water sample of 1.69 ng Hg/L or less was small enough to not cause impacts to female Common Loons. These wildlife criterion values are greater than the wildlife criterion value of 1.30 ng Hg/L applied to avian species by the Great Lakes Water Quality Initiative. The Common Loon-based wildlife criterion value provides a valuable estimate of the mercury thresholds associated with biotic impacts due to mercury contamination in aquatic ecosystems, enabling legislators to integrate these standards into policies that better protect environmental quality. Based on the water samples collected, it was estimated that the wildlife criterion value accurately predicted the protection of 61% of female and 73% of male Adirondack loons. More rigorous sampling of the abiotic compartment over a wider temporal and spatial scale is necessary to fully understand how water quality parameters relate to Common Loon reproductive success. *Received 21 January 2013, accepted 16 April 2013.*

Key words.—Adirondack Park, Common Loon, *Gavia immer*, mercury, wildlife criterion value.

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Long-term studies of biotic mercury levels, particularly those of high-trophic level species such as the Common Loon (*Gavia immer*), provide an invaluable foundation of baseline scientific data for the development of wildlife criterion values (WCV; Nichols *et al.* 1999) designed to quantify environmental mercury (Hg) levels with detectable wildlife impacts. Ultimately, these values contribute ecotoxicological risk assessment information and serve as recommended guidelines for policy makers in the development of legislation relating to the environmental risks that mercury deposition poses to wildlife and aquatic ecosystems.

Mercury risk assessments in the northeastern USA have shown that the Common Loon (loon) is a suitable bioindicator of aquatic mercury toxicity based on its ecology, the logistics of studying this species, and the high value the general public places on these iconic birds (Evers 2006). Subsequently, many studies have been conducted on this species that document the numerous neurotoxic, physiological

and reproductive impacts associated with elevated mercury concentrations (Nocera and Taylor 1998; Olsen *et al.* 2000; Evers *et al.* 2008; Scheuhammer *et al.* 2008).

Current pollution prevention programs are designed to monitor, reduce and remove known environmental toxins, such as mercury, from the environment in an effort to improve and protect the ecological integrity of vital natural resources. Recently, stringent regulations of mercury emissions from coal-fired power plants have been implemented in New England and New York, and the U.S. Environmental Protection Agency finalized the federally mandated Mercury and Air Toxics Standards (MATS) Rule in December 2011, which requires all coal-fired power plants in the United States to adopt best available pollution control technologies by 2016 (U.S. Environmental Protection Agency 2011). Additionally, a national standardized biotic mercury monitoring program has been proposed that would greatly inform Federal and State mercury-related policies, provide data for predictive ecological models, and characterize the biological effects in the United States from the redistribution of anthropogenic mercury on the landscape (Mason *et al.* 2005; Evers *et al.* 2011). This proposed mercury monitoring program would also ensure that recently implemented regional and Federal regulations are effective at preventing local biological mercury hotspots (Evers *et al.* 2007) and reducing biotic impacts, such as the documented decreased reproductive success in the Adirondack Common Loon as a consequence of mercury exposure (Schoch *et al.* 2014).

The main objective of this study was to use a long-term mercury dataset to determine a recommended water mercury level that would protect the Adirondack Common Loon population from the risk of behavioral and reproductive impacts due to environmental mercury pollution. Ecological risk was assessed using a formula for a WCV that provides a water column mercury value that is protective of wildlife at the population level. The WCV estimates wildlife population viability through measurements of contaminant stressors, such as surface water mercury concentrations (Nichols *et al.* 1999). Development of the WCV requires knowledge of mercury concentrations that are hazardous to loons at the population level (i.e., test dose), as well as the bioaccumulation factor at two trophic levels (i.e., mercury increase from unfiltered water to perch; Nichols *et al.* 1999). Ultimately, the development of a loon-based WCV provides much-needed information for policy makers to better assess and regulate the threats of anthropogenic mercury to aquatic ecosystems.

Methods

Study Area

Aquatic food web biotic and abiotic samples were collected on 44 lakes within the Adirondack Park over a 2-year period from 16 July 2003 to 28 August 2003 and from 16 July 2004 to 15 September 2004. Sediment, water, zooplankton, crayfish, and prey fish samples were collected within loon territories. Common Loon tissue (blood, feather, and/or egg) samples were collected from the 44 lakes during the breeding season from 1998-2007 (Fig. 1).

Sample Collection and Laboratory Analysis

Forty-nine water mercury samples were collected from all 44 lakes using a standard two-person "clean hands-dirty hands" protocol (U.S. Environmental Protection Agency 2001). Zooplankton samples were collected from 43 lakes via tow-nets for taxonomy identification, biomass determination, and mercury analysis according to the protocol developed for the Regional Environmental Monitoring and Assessment Program's assessment of mercury in Vermont and New Hampshire lakes (Chen *et al.* 2000). Crayfish samples were collected from 26 lakes via minnow traps, visual scans, and night-lighting techniques. Crayfish were identified to species (*Orconectes limosus*, *O. propinquus*, *Procambarus acutus*, and *Cambarus robustus*). Sediment sample locations were determined by the locations where crayfish were collected. Sediment cores for mercury analysis were collected from 32 lakes using a clean hands-dirty hands protocol (U.S. Environmental Protection Agency 2001).

A composite of fish in each of four size-classes (small: 5-10 cm, medium: 10-15 cm, large: 15-20 cm, and extra large: 20-25 cm) was collected on each lake using hook and line gear, trap nets, seine nets, and gill nets. Fish species were not combined in the composite samples for mercury analysis. Fish were identified to species (yellow perch, *Perca flavescens*; banded killifish, *Fundulus diaphanous*; blacknose dace, *Rhinichthys atratulus*; brown bullhead, *Ameiurus nebulosus*; brown trout, *Salmo trutta*; common shiner, *Luxilus cornutus*; creek chub, *Semotilus atromaculatus*; fallfish, *Semotilus corporalis*; golden shiner, *Notemigonus crysoleucas*; hornyhead chub, *Nocomis bigut-*

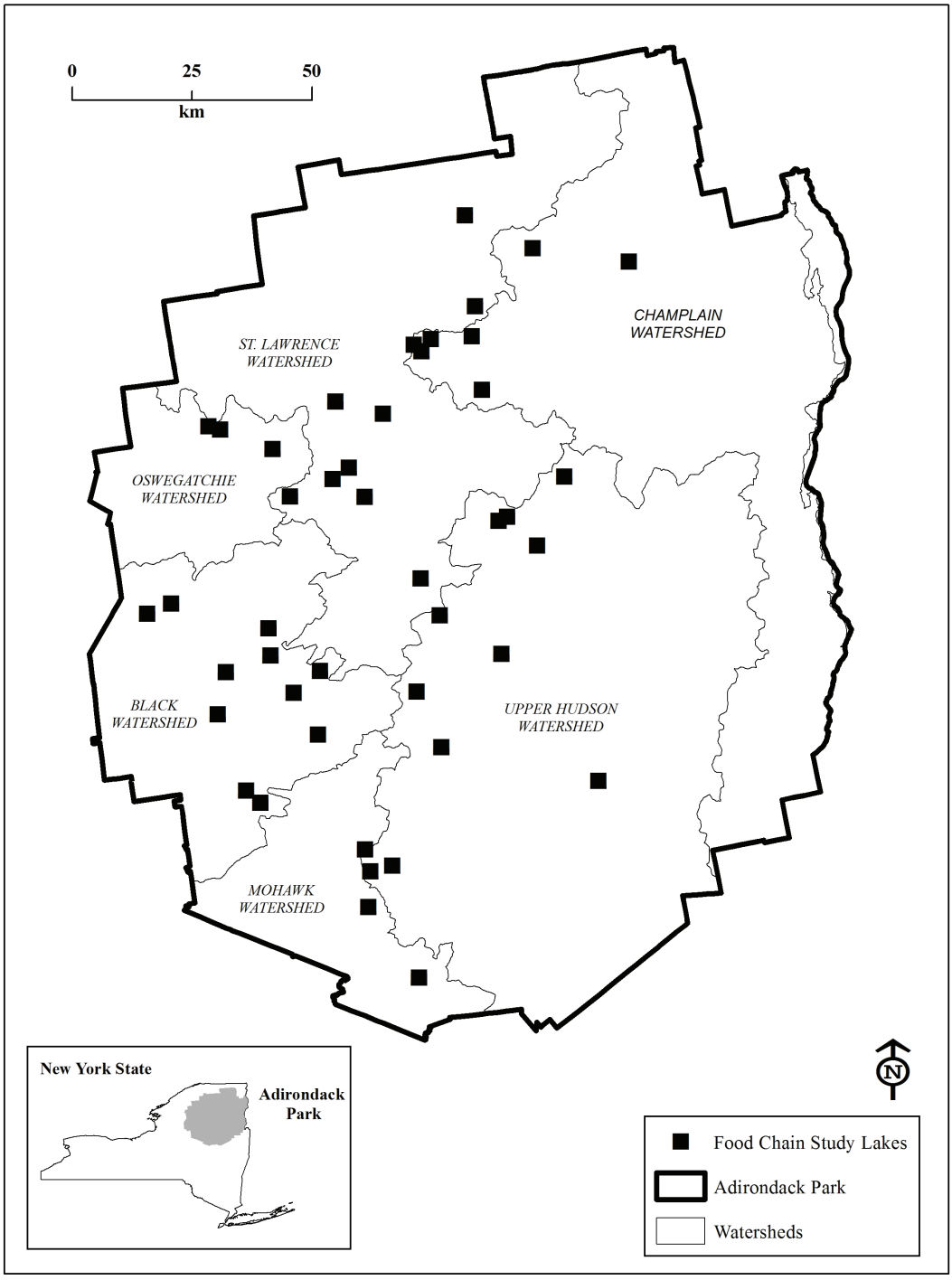


Figure 1. Study lakes (2003-2004) located within New York's Adirondack Park.

tatus; lake chub, *Couesius plumbeus*; largemouth bass, *Micropterus salmoides*; northern redbelly dace, *Phoxinus eos*; pumpkinseed, *Lepomis gibbosus*; rainbow smelt, *Osmerus mordax*; redear sunfish, *Lepomis microlophus*; rock bass, *Ambloplites rupestris*; smallmouth bass, *Micropterus dolomieu*; walleye, *Sander vitreus*). Otoliths and scales were collected from fish larger than 15 cm in length to aid in aging the size classes.

From 1998-2007, Common Loon samples were collected for mercury analysis: blood samples from all 44 lakes, loon feather samples from 40 lakes, and nonviable eggs from 29 lakes. Common Loons were captured using night-lighting and playback techniques (Evers 2001). Established tissue sample collection protocols (Evers *et al.* 1998, 2003, 2005) were followed. Blood samples were non-lethally collected from the tibiotarsal vein of 137 loons for evaluation of short-term mercury accumulation. Feather samples were collected from 124 Common Loons to provide an indication of long-term mercury accumulation. Feather samples included two central tail feathers and the second secondary feather from each wing. Seventy-two abandoned non-viable loon eggs were opportunistically collected over the 10-year period and processed following standardized protocols (Evers *et al.* 2003).

The biotic and abiotic samples collected during the summers of 2003-2004 were submitted for laboratory analysis of mercury concentrations. Total mercury was analyzed via oxidation, purge and trap, and cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran model 2600) based on U.S. Environmental Protection Agency (2002) method 1631, revision E. Methylmercury was analyzed via distillation, aqueous ethylation, purge and trap, desorption, and CVAFS based on U.S. Environmental Protection Agency (2001) method 1631. All samples were analyzed for total mercury. Methylmercury was analyzed in water, sediment, and zooplankton. All biotic mercury and methylmercury concentrations are expressed in µg/g on a wet weight (ww) basis. Laboratory protocols for analyzing total mercury in Common Loon tissues followed Evers *et al.* (2003) for eggs and Evers *et al.* (1998) for blood and feathers (except for feathers collected after 2002). Analyses for methylmercury in loon tissues were not conducted, as more than 95% of total mercury in the blood is in the methyl form (Wolfe *et al.* 2007), thus loon total mercury concentrations are reflective of their methylmercury levels.

Statistical Analysis

Loon unit calculations. Since the Adirondack Common Loon mercury data come from multiple tissues, comparisons of results obtained between locations and years can be difficult to conduct or assess. To best evaluate and use existing data from various biotic compartments, mercury concentrations require a single common unit. Thus, a Common Loon dataset from New York (1998-2008, $n = 381$) was compiled to address this issue. Subsets of the data, in which there were multiple mercury data points from a single territory and year, were used to develop relationships between mercury in different tissues. These models were then applied to the larger dataset to present data from all tissue types, territories and years, in a common unit, designated as the female loon unit (FLU) (Evers *et al.* 2011). Egg mercury levels are correlated with female mercury exposure, as female loons depurate mercury into their eggs (Evers *et al.* 2003). Juvenile loon mercury levels, likewise, could be assumed to be highly correlated with female mercury concentrations, as they tend to eat prey of similar size as the females. There is no clear link between egg mercury or juvenile blood mercury with male blood mercury. Therefore, all male blood mercury, juvenile blood mercury, and egg mercury were each separately regressed with female blood mercury to convert all tissues to FLUs. Female adult blood levels were also converted into male loon units (MLUs), as male loons on the breeding grounds tend to have higher mercury levels than females regardless of body weight, presumably due to the depuration of female body mercury into eggs. Thus, presentation of mercury data in FLUs presents a different picture than in MLUs. FLUs are a more universal unit since they include egg and juvenile data and they represent the expected or observed blood mercury of adult females. As male mercury exposure is generally higher than for females, even in the same locations and years, examination of the data in the form of MLUs is useful for predicting male exposure in the region.

Wildlife criterion value. Similar to research conducted on the Common Loon population in Maine (Evers *et al.* 2005), the WCV formula developed by Nichols *et al.* (1999) was modified with newly acquired information from recent studies to develop a New York-based WCV. The original WCV had several limitations that were improved upon in this study, including additional exposure parameters and updated values based on recent, long-term Common Loon studies. Three standard matrices were used to develop the WCV, including loon blood, feather, and egg mercury levels (Evers *et al.* 2004).

Due to differences in body weight, dietary fractions, and ingestion rates, a separate WCV was calculated for both male and female loons in the Adirondack Park using the following formula (Nichols *et al.* 1999):

$$WCV = \frac{(TD \times [1/\{UF_L \times UF_A \times UF_S\}]) \times WT_A}{W_A + ([FD_3 \times F_A \times BCF_3] + [FD_4 \times F_A \times BCF_4])}$$

where TD represents the tested dose from toxicity studies with wildlife species (ug Hg/kg body weight/day). The UF_L value is the uncertainty factor between the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL). The UF_A represents the uncertainty factor between species and UF_S is the uncertainty factor between subchronic and chronic levels of impacts. Values for WT_A reflect the average species weight (kg) and W_A is an estimate of the average daily volume of water consumed (L/day). FD_3 and FD_4 values represent a fraction of diet from trophic level 3 (insect prey specialist fish) and trophic level 4 (fish prey specialist fish), respectively, and F_A is the average daily mass of food consumed (kg/day). The values for BCF_3 and BCF_4 are estimates of aquatic life bioconcentration factors (L/kg of Hg in fish / Hg in water) for trophic level 3 and trophic level 4, respectively.

To evaluate how accurate the WCV is at predicting protection of Common Loons in the Adirondack Park, the WCV values were compared to the water mercury concentrations obtained from our sampling effort. The WCV can be considered accurate for all instances where the water mercury level was below the WCV value and the loon mercury concentration (FLU or MLU) was be-

low the threshold for effect (< 3 ppm, wet weight (ww)) and all instances where the water mercury level was above the WCV value and the loon mercury concentration was above the threshold for effects.

RESULTS

Recommended Water Mercury Level to Protect the Adirondack Common Loon Population

Exposure parameters determined for the WCV are based on the following:

Tested dose (TD). The tested dose from toxicity studies with wildlife species is calculated using the mercury levels in prey items known to cause effects and the ingestion rate of the species being investigated. A prey fish mercury level of 0.16 µg/kg has been documented to be a relevant level for impacts associated with Common Loon reproduction (Burgess and Meyer 2008; Evers *et al.* 2008). To calculate the tested dose for Adirondack Park loons, the ingestion rate was multiplied by the fish mercury level of 0.16 µg/kg.

Uncertainty factors (UF_L, UF_A, UF_S). The uncertainty factors identified by Nichols *et al.* (1999) were not quantified specifically for the Adirondack Park. Instead, estimated values from the Great Lakes Water Quality Initiative were used. The uncertainty factor between species (UF_A) is 3, the uncertainty factor between sub-chronic and chronic levels of impacts (UF_S) is 1, and the uncertainty factor between the LOAEL and the NOAEL (UF_L) is 2.

Body weight (WT_A). The average body weight for female Common Loons in the Adirondack Park was 4.31 kg (n = 94, SD = 0.358) and the average body weight for males was 5.59 kg (n = 101, SD = 0.392).

Water consumed (W_A). Average daily volume of water (W_A = 0.12 L/d) consumed is based on the generic value for Common Loons given in Nichols *et al.* (1999).

Diet fraction (FD_3, FD_4). The fraction of diet coming from either trophic level 3 (insect prey specialist) or 4 (fish prey specialist) fish is based on Barr (1996), who found that juvenile loons in Ontario, Canada, consumed 83% trophic level 3 fish and 17% trophic level 4 fish. Considering that juveniles are not yet at their full body weight, the amount of fish consumed in each size class is adjusted to represent the size classes used in this study. Adirondack Park female loons (mean = 4.31 kg, n = 94, SD = 0.358) are calculated to be 15% larger than the juveniles Barr (1996) observed (mean = 3.68 kg, n = 7, SD = 0.73), and so consume 15% less trophic level 3 fish (71% trophic level 3 and 29% trophic level 4). Adirondack Park male loons (mean = 5.59 kg, n = 101, SD = 0.392) are 34% larger than the juveniles (mean = 3.68 kg, n = 7, SD = 0.73; Barr 1996), and so likely consume 34% less trophic level 3 fish (55% trophic level 3 and 45% trophic level 4).

Ingestion rate (F_A). Ingestion rate is the average daily mass of food consumed by Common Loons, which varies between males and females due to variation in body size. Ingestion rate was calculated as 20% of the average body weight, which equals 1.118 kg/day for males and 0.862 kg/day for females.

Bioconcentration factor (BCF_3, BCF_4). Mercury concentrations within the food web varied by many orders of magnitude between water and loon samples. The average mercury concentrations followed the predicted pattern of biomagnification through the food web, with an increase in mercury as it moved from lower trophic levels (mean water total Hg = 1.73 ppt, n = 44 lakes, SD = 0.92; zooplankton = 0.006 µg/g, n = 40, SD = 0.004; and crayfish = 0.047 µg/g, n = 26, SD = 0.020) to higher trophic levels (small to medium sized fish = 0.096 µg/g, n = 44, SD = 0.055; large to extra-large fish = 0.167 µg/g, n = 42, SD = 0.085; and loons: adult female blood = 1.72 µg/g, n = 36, SD = 1.040; adult male blood = 2.16 µg/g, n = 37, SD = 1.028). Trophic level 4 fish had higher average mercury values than trophic level 3 fish. Mercury data collected between 2003 and 2004 for prey items within different trophic levels of the aquatic food web indicate mean BCFs of 56,000 for trophic level 3 fish and 97,000 for trophic level 4 fish, based on the relationship of total mercury in unfiltered water with total mercury in fish (Fig. 2).

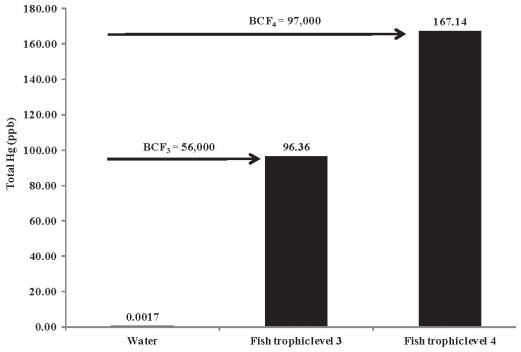


Figure 2. Bioconcentration factors for fish in trophic level 3 and fish in trophic level 4, compared to unfiltered water.

Using the algorithm based on Nichols *et al.* (1999) and the mercury exposure parameters (Table 1), a WCV for male loons was calculated as 2.002 ng Hg/L using the following equation:

$$WCV_{male} = \frac{\{0.179 \times [1/(2 \times 3 \times 1)]\} \times 5.59}{0.12 + [(0.55 \times 1.118 \times 56{,}000) + (0.45 \times 1.118 \times 97{,}000)]}$$

$$WCV_{male} = 2.002 \text{ ng Hg/L}$$

Similarly, a WCV of 1.693 ng Hg/L was calculated for female Common Loons using the following equation:

$$WCV_{female} = \frac{\{0.138 \times [1/(2 \times 3 \times 1)]\} \times 4.31}{0.12 + [(0.71 \times 0.862 \times 56{,}000) + (0.29 \times 0.862 \times 97{,}000)]}$$

$$WCV_{female} = 1.693 \text{ ng Hg/L}$$

Accuracy of the WCV within the Adirondack Park

The WCV estimates were compared to the water mercury concentrations for the study lakes to assess the accuracy of the WCV for predicting the protection of Adirondack Common Loons. The WCVs were found to predict loon risk accurately for 61% of female and 73% of male loons in the Adirondack Park (Figs. 3a and 3b).

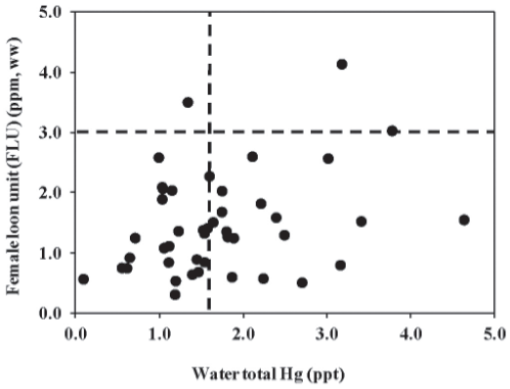
DISCUSSION

The results of these calculations indicate that water levels equal to or less than 2.002 ng Hg/L are protective of male loons, and that those equal to or less than 1.693 ng Hg/L are protective of female Common Loons at the population level within the Adirondack Park. Both of these WCVs are greater than the WCV of 1.30 ng Hg/L that the Great Lakes Water Quality Initiative uses for avian species (Evers *et al.* 2004). While differences may exist between the WCVs calculated for the Adirondack Park and the Great Lakes regions, two areas significantly impacted by elevated mercury deposition, these estimates serve as valuable guidelines for policy makers looking to make more informed decisions about the regulation of environmental mercury contamination.

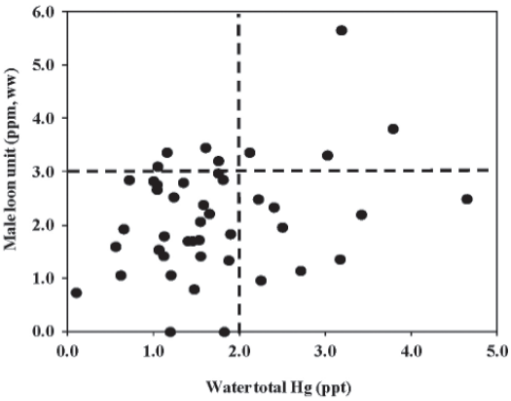
WCV estimates for water mercury were also compared to known water mercury concentrations that were sampled within

Table 1. Sex-specific variables used to calculate the wildlife criterion value for the Adirondack Common Loon (*Gavia immer*).

Variable	Male	Female
TD: Tested dose (µg/day)	0.179	0.138
UF_L: Uncertainty factor between the lowest observed adverse effect level (LOAEL) and the no observed adverse effects level (NOAEL)	2	2
UF_A: Uncertainty factor between species	3	3
UF_S: Uncertainty factor between sub-chronic and chronic levels of impacts	1	1
WT_A: Body weight (kg)	5.59	4.31
W_A: Water consumed (L/day)	0.12	0.12
FD_3: Diet fraction from trophic level 3 fish	0.55	0.71
BCF_3: Bioconcentration factor for trophic level 3 fish	56,000	56,000
FD_4: Diet fraction from trophic level 4 fish	0.45	0.29
F_A: Ingestion rate (kg/day)	1.118	0.862
BCF_4: Bioconcentration factor for trophic level 4 fish	97,000	97,000



	Water below WCV (< 1.7 ppt)	Water above WCV (> 1.7 ppt)
Female loon blood Hg above threshold for effects (> 3 ppm)	2%	5%
Female loon blood Hg below threshold for effects (< 3 ppm)	56%	37%



	Water below WCV (< 2.0 ppt)	Water above WCV (> 2.0 ppt)
Male loon blood Hg above threshold for effects (> 3 ppm)	9%	9%
Male loon blood Hg below threshold for effects (< 3 ppm)	64%	18%

Figure 3. Relationship between wildlife criterion value and water mercury concentrations for female (3a) and male (3b) Common Loons in the Adirondack Park, New York. Fifty-six percent of female loons and 64% of male loons had blood Hg < 3 ppm and water Hg below WCV of 1.7 ppt (female) and 2 ppt (male), while 37% of female loons and 18% of male loons had blood Hg < 3 ppm and water Hg above WCV of 1.7 ppt (female) and 2 ppt (male). Two percent of female loons and 9% of male loons had blood Hg > 3 ppm and water Hg below WCV of 1.7 ppt (female) and 2 ppt (male), while 5% of female and 9% of male loons had blood Hg < 3 ppm and water Hg above WCV of 1.7 ppt (female) and 2 ppt (male).

the Adirondack Park as part of a simultaneous, collaborative study and it was determined that roughly two-thirds of individuals were accurately classified based on the water mercury concentrations for the respective study lake. Water quality data were collected within a relatively short time frame, which standardizes the mercury comparison between lakes, but may not account for a large amount of yearly, monthly, and daily variation in water mercury that naturally occurs in lakes. Although the water mercury concentrations sampled are likely a small snapshot of mercury within the lakes, it is important to note that the WCV formula is calculated independent of actual water mercury values within the study area. Therefore, the WCV is likely accurate and serves as a solid foundation for future ecotoxicology and mercury studies related to the Common Loon, and additional testing of water mercury within the study lakes would further improve the regional dataset and contribute to more robust correlations among the WCV, loon blood mercury levels, and water mercury concentrations.

In summary, the development of WCVs, based on the synthesis of relevant wildlife studies and current scientific literature, provides an invaluable estimate of the mercury thresholds associated with biotic impacts and allows legislators to incorporate these standards into policies aimed at protecting vital natural values such as air quality, water quality and wildlife health.

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Mercury analyses of the abiotic and biotic tissues collected in this study were conducted by the Animal Health Diagnostics Laboratory, University of Pennsylvania (loon blood), Trace Element Research Laboratory, Texas A & M (crayfish, fish, loon blood and loon eggs), Harvard School of Public Health (2003-2007 loon feathers), University of Connecticut's Center of Environmental Sciences and Engineering Metals Laboratory (2006-2007 loon feathers), Dartmouth College (zooplankton), and Center for Environmental Systems Engineering Laboratory at Syracuse University (water, sediment, and zooplankton).

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