

# MERCURY CONCENTRATIONS IN FISH-EATING BIRDS FROM THE PINCHI LAKE AREA IN RELATION TO PRODUCTIVITY AND REPRODUCTIVE SUCCESS

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## ABSTRACT

Bald eagles and red-necked grebes were monitored from 2000 - 2002 on several lakes along the Pinchi fault, an area with a known source of geologic mercury and previous Hg mining (on Pinchi Lake), in an effort to discern whether increased Hg concentrations were affecting reproductive success and productivity. To determine whether or not Hg levels were elevated, fish tissues (rainbow trout and northern pikeminnow) were collected, eagles breeding on 5 lakes (Pinchi, Tezzeron, Stuart, Great Beaver and Fraser) were sampled for blood- and feather-Hg concentrations, and eggs were collected from red-necked grebes. Concentrations of Hg in all grebe eggs, while highest in eggs collected from Pinchi Lake, were below 0.5 µg/g wet wt., often cited as the lowest observed adverse effect level for Hg developmental toxicity in birds. Reproductive success and average productivity of the bald eagles over the 3-year period were 62% and 0.98 chicks/territory on Pinchi Lake compared to 64% and 1.17 chicks/territory on all other study lakes combined. Significant relationships were found between adult and chick blood Hg levels from the same nests as well as chick blood and feather Hg concentrations. Despite the apparently elevated Hg levels found in adult eagles from Pinchi Lake (mean blood Hg = 6.54 µg/g wet wt.), the birds appeared to be in excellent condition. The adult eagle with the highest level of Hg in blood (9.44 µg/g wet wt.) successfully raised two eaglets in each of the summers of 2001 and 2002, making it one of the most productive birds in the study area. Thus, while dietary Hg exposure is greater in eagles and grebes from Pinchi Lake, it does not appear to adversely affect reproductive success or productivity. This study illustrates how reproductive monitoring can be used to verify or refute conclusions that might be drawn from findings suggesting a possible contaminant effect on local wildlife.

## INTRODUCTION

It is well-known that mercury (Hg) originating from specific anthropogenic sources (e.g. chlor-alkali plants) has, in the past, contributed significantly to the contamination of aquatic organisms, resulting in toxicity to piscivorous birds (Barr, 1986; Becker and Bigham, 1995). However, few studies have addressed the extent to which inorganic Hg from local geologic sources is methylated and incorporated into the food chain. An area along the Pinchi fault in central British Columbia is a known source of geologic Hg in the form of cinnabar (HgS). Various piscivorous birds breed in the area including bald eagles (*Haliaeetus leucocephalus*), red-necked grebes (*Podiceps grisgenae*), osprey (*Pandion haliaetus*) and common loons (*Gavia immer*). Previous studies (e.g., Reid and Morley, 1975; Cook, 1996) had shown that higher levels of Hg exist in fish and sediments from Pinchi Lake, while lower amounts exist in

nearby lakes such as Tezzeron. This area provided an ideal location to study the possible toxic effects of natural and mining-related Hg releases on local piscivorous breeding bird populations because lakes with a range of sediment Hg concentrations were all present in close proximity.

Few studies have focused on the accumulation and effects of methylmercury (meHg) on free-living populations of bald eagles or red-necked grebes. Both red-necked grebes and bald eagles feed primarily on fish during the breeding season when an adequate supply is present (Buehler, 2000; Stout and Nuechterlein, 1999), and almost all of the Hg found in fish muscle (94 - 99%) is in the methylated form (Scheuhammer et al., 1998). Therefore, piscivorous birds can be at high risk of meHg exposure because of their dependence on a fish diet. This study examined Hg in blood and feathers of adult bald eagles and eaglets, Hg in red-necked grebe eggs, and assessed the effects of increased Hg on nesting success and productivity in an area influenced by natural and Hg-mining related wastes. Further, this study demonstrated how field monitoring can provide valuable information for determining potential risks to wildlife.

## METHODS

### Study Area

The study area (Figure 1) was chosen based on its proximity to a well-known source of natural Hg. Pinchi Lake was specifically chosen as the treatment lake for this study because of past Hg-contamination associated with Hg-mining operations located adjacent to the lake (primarily from 1940-1944). Tezzeron was selected as a naturally-influenced control lake because of its location over the Pinchi fault. The southeastern portion of Stuart Lake was examined because of its location downstream of Pinchi Lake, although any influence was expected to be negligible. Great Beaver and Fraser Lakes were chosen as pristine control lakes because they are hydrologically separated from the other study lakes and were not known to be influenced by Hg.

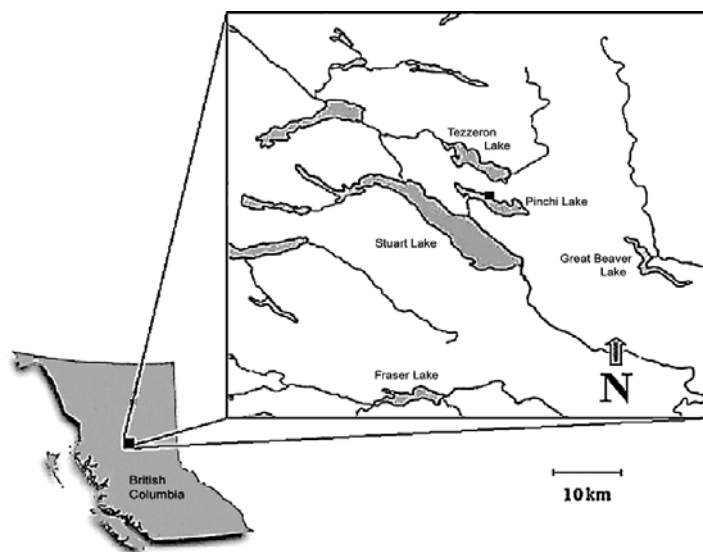


Figure 1: Study Area

## Sampling

Adult eagles were captured in mid-June of 2001 and 2002, when they were actively hunting to feed growing chicks, using a floating fish set as described by Cain and Hodges (1989). After capture, each bird was weighed using a portable Pesola® scale attached to a tensor bandage enclosing the talons. The #2 secondary feather from each wing was cut below the base of the vane and retained in Ziploc® bag for later Hg analysis. Approximately 10 ml of blood were drawn from the brachial vein using a 22-gauge needle, immediately transferred to a sterile heparinized vacutainer, and kept for later Hg analyses. Measurements of hallux claw length and bill depth were taken using calipers and used to determine the sex of adult birds (Bortolotti, 1984). Each bird was then fitted with a US Fish and Wildlife Service numbered band and released.

Eaglets were sampled during the last week of June to the first week of July 2000-2002, inclusive. Eaglets tended to be at least 8 weeks old when sampled, with some close to fledging. A qualified tree climber ascended to each nest and lowered the eaglets to the ground for sampling. Blood samples (approximately 10 ml) were taken using a 21-gauge needle, and birds were weighed and banded as above. Body feathers for Hg analysis were plucked from the chest region and stored in Ziploc® bags.

Red-necked grebe eggs (a single egg from each nest having more than one egg) were collected in 2000 and 2001, immediately following initiation of nesting in late May/early June. Only the first egg laid was collected as this should represent the highest concentration of mercury in the brood. Eggs were collected from all study lakes except Stuart Lake, where no grebe nests were found. Eggs were frozen whole in Ziploc® bags prior to analysis. In the lab, eggs were thawed, and contents removed and homogenized. A portion of each homogenate was weighed, freeze-dried for a minimum of 36 hr, and reweighed. Moisture content was calculated, and the dried material was analyzed for total Hg, as described below.

## Mercury Analysis

All samples were analyzed for total Hg at the National Wildlife Research Centre in Hull, Quebec. Blood and eggs collected in 2000 were digested and analyzed by continuous-flow cold vapor atomic absorption spectrophotometry (CVAAS) according to Scheuhammer and Bond (1991). Blood and eggs collected in 2001 and 2002, were analyzed directly for total Hg using an automated mercury analyzer (AMA-254) as described by Weech et al. (2004). Feathers were washed prior to analysis by shaking in acetone for 1 min, in dilute (1%) Triton-X 100 for another minute, followed by thorough rinsing in double-deionized water, and allowed to air-dry overnight. Each eaglet feather sample consisted of a pool of approximately five breast feathers/bird that were washed and digested together. To enable digestion of the entire adult feather, each feather was cut into 10 or 11, 1-inch pieces. After digestion, samples from 2000 were analyzed by CVAAS, and samples from 2001 and 2002 were analyzed using an AMA-254.

For quality assurance, certified reference materials (Dogfish liver - DOLT-2 and Dogfish muscle - DORM-2) from the National Research Council of Canada (Ottawa, ON, Canada), and a number of duplicate samples were analyzed in conjunction with blood and feather Hg samples. All certified

reference materials were recovered at  $\pm 10\%$  of the certified value (96.5 – 109.6% for DOLT-2; 95.4 – 110.0% for DORM-2), regardless of the method of Hg analysis used.

### Eagle Reproductive Success

Just after ice-off (beginning of May) in 2000 and 2001, eagle nests were located by surveying shorelines by boat, with the aid of a 1996 British Columbia Ministry of the Environment helicopter survey of active and old eagle and osprey nests. In 2002, a new helicopter survey of the study area was conducted to ensure that no nests were missed in previous surveys. All active nesting territories were monitored throughout the breeding season. Productivity and reproductive success were determined by climbing all nest trees at the end of June/beginning of July when chicks were approximately eight weeks old.

### Statistical Analyses

Paired *t*-tests were used to determine whether there was a significant difference in blood and feather Hg concentrations in sibling eaglets. To determine whether adult eagles or eaglets from Pinchi Lake had higher blood and feather Hg concentrations than eagles from the other study lakes, a one-way analysis of variance (ANOVA) followed by Tukey's test for mean separation was used. A two-tailed *t*-test was used to test for a difference in blood-Hg concentrations between male and female adult eagles from the reference lakes (Tezzeron, Stuart and Fraser). Pearson correlations were used to test for relationships between blood and feather Hg for both adult eagles and eaglets. A two-way ANOVA using year and lake as factors was used to test whether reproductive success and productivity differed significantly across all study lakes. The relationship between average Hg concentrations in eaglet blood and eagle productivity for each lake and year was tested using Pearson correlation. All statistical analyses were performed using SigmaStat for Windows (Version 2.03S, Jandel Scientific, San Rafael, CA, USA).

## **RESULTS AND DISCUSSION**

### Red-necked Grebes

Over the 2000 and 2001 field seasons, 6 eggs were collected from Pinchi Lake, 5 each from Tezzeron and Great Beaver, and 8 from Fraser Lake. Average moisture content of all eggs was 78.7%. Mercury concentrations (mean  $\pm$  SD  $\mu\text{g/g}$  dry wt.) in eggs obtained from Pinchi =  $1.17 \pm 0.48$ , Tezzeron =  $0.83 \pm 0.17$ , Great Beaver =  $0.70 \pm 0.27$  and Fraser =  $0.47 \pm 0.12$ . Egg-Hg levels varied significantly among lakes ( $p = 0.001$ ), with multiple comparison tests showing that eggs from Pinchi Lake had higher mean Hg concentrations than eggs from all other lakes combined, and also from Fraser Lake individually; however other differences between lakes were not significant.

Mercury concentrations in grebe eggs from all 5 study lakes were below the lowest observed adverse effect level (LOAEL) found in previous egg-Hg studies ( $0.5 \mu\text{g/g}$  wet wt., or  $2.5 \mu\text{g/g}$  dry wt. based on 80% moisture content), and forage fish of the size consumed by red-necked grebes were below the Hg threshold effects level ( $0.3 \mu\text{g/g}$  wet wt.; Barr, 1986) determined for fish-eating aquatic birds (Weech et al., 2004). In addition, grebe numbers on Pinchi Lake were comparable to both Tezzeron and Great Beaver Lakes. Fraser Lake, which had considerably more sheltered and shallow, weedy habitat compared

to the other study lakes also had a much larger grebe population. Because of the necessity of creating floating, yet anchored nests, habitat appeared to be the limiting factor for red-necked grebes. Nesting was semi-colonial on the study lakes, with nests often no more than a few metres apart. The floating nests were very susceptible to destruction by wave action, which appeared to be the main cause of nest failure. Only two of the more than 100 nests found over the course of the study had been obviously depredated, with eggshell remnants still present in the nest. Based on these observations, it did not appear that Hg was a factor in choice of nesting location or nesting success of red-necked grebes in the study area.

### Mercury in Eaglets

A total of 43 eaglets were sampled for blood and 41 for feathers, from a total of 25 different nests during 2000 – 2002 (Table 1). Sibling eaglets did not differ significantly with respect to Hg concentrations in blood ( $p = 0.447$ ) or feathers ( $p = 0.892$ ). However, Hg concentrations in eaglet blood from the different study lakes differed significantly ( $p < 0.001$ ). Specifically, eaglets from Pinchi Lake had significantly higher blood Hg levels compared to eaglets from each of Stuart, Great Beaver and Fraser Lakes. Blood Hg levels in eaglets from Pinchi and Tezzeron Lakes did not differ significantly ( $p = 0.359$ ). Eaglets from Pinchi Lake had significantly higher Hg concentrations in feathers ( $p < 0.001$ ) compared to eaglets from all other study lakes, including Tezzeron. A highly significant positive correlation was found between blood and feather Hg concentrations in eaglets ( $r = 0.755$ ,  $p < 0.0001$ ). Also, blood mercury levels in eaglets were highly correlated with their parents ( $r = 0.913$ ,  $p = 0.004$ ), with eaglets averaging about 14-times lower blood-Hg concentrations (Figure 2).

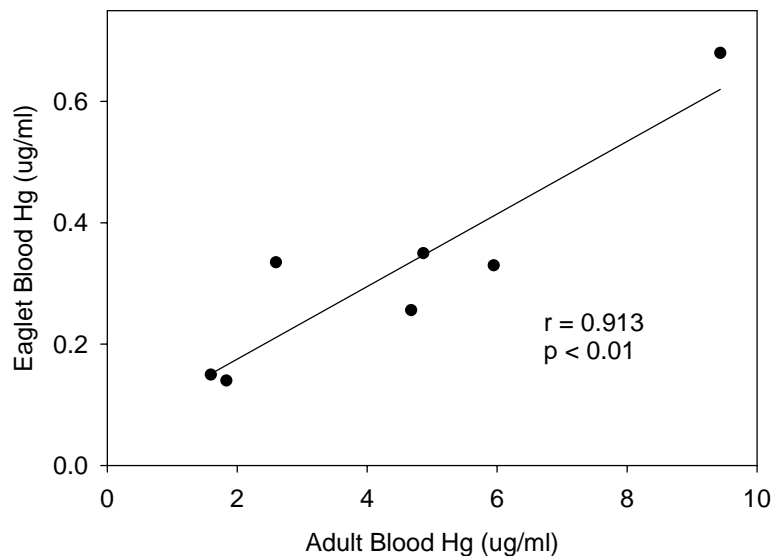


Figure 2: Relationship between blood-Hg concentrations in adult bald eagles and their chicks. Sibling blood-Hg concentrations were averaged for the comparison

The difference in magnitude of blood Hg concentrations between chicks and adults is somewhat surprising given that adult eagles and their chicks tend to feed from the same food items. However, the lower blood Hg concentrations in chicks can be explained by removal through deposition into growing

full body plumage during the first few months of age in comparison to the slower six-month staged molt of adults. Deposition of meHg into growing feathers is an important removal mechanism for a substantial portion of the body burden of meHg, particularly in growing chicks (Becker et al., 1994). The relationship shown in Figure 2 also demonstrates that eaglets can potentially be used to estimate Hg levels in adults, given the relative difficulty of capturing adult eagles.

Mercury concentrations in blood and feathers of eaglets from Pinchi Lake were similar to those reported for eaglets on lakes in Maine, USA (Welch, 1994). Maine was one of the few states where Hg in bald eagle eggs frequently exceeded 0.3 µg/g, whereas concentrations were < 0.2 µg/g in most other sampling locations in the US (Wiemeyer et al., 1984; Wiemeyer et al., 1993); thus Hg exposure for eagles on some lakes in Maine, like those on Pinchi Lake, may be atypically high.



The correlation between mercury concentrations in blood and feathers of eaglets in this study is similar to relationships previously reported for eaglets and other fish-eating birds, although the magnitude of the relationship often differs. While bald eagle chicks from central Florida had approximately 25 times more Hg in feathers than in blood (Wood et al., 1996), eaglets from Maine had approximately 44 times greater Hg in feathers than in blood (Welch, 1994) despite similar mean blood-Hg levels (0.13 µg/g wet wt. and 0.12 µg/g wet wt., respectively). Eaglets from our study were similar to eaglets from central Florida, having approximately 27 times more Hg in feathers than in blood. While Hg concentrations in blood and feathers are highly correlated in growing young of various fish-eating bird species, relationships vary considerably among species and geographical location, and are likely influenced by differences in diet, growth rates (of whole body and feathers), and degree of Hg exposure.

### Mercury in Adult Bald Eagles

Thirteen adult eagles were sampled during the study (five in 2001, eight in 2002; including one bird from Stuart Lake that was caught in both 2001 and 2002). Of the 13 birds caught, three were from Pinchi, two from Tezzeron, three from Fraser and five from Stuart. Mercury concentrations in blood or feathers were not significantly influenced by the sex of the bird (blood  $p = 0.687$ , feathers  $p = 0.636$ ). Adult eagles from Pinchi Lake had significantly higher concentrations of Hg in blood than adult eagles from Fraser and Stuart Lakes ( $p = 0.022$ ), but not from Tezzeron Lake ( $p = 0.342$ ; Table 1). In feathers, adults from Pinchi Lake had significantly higher Hg concentrations compared to adults from Fraser Lake only ( $p = 0.032$ ; Table 1). No correlation was found between blood and feather Hg concentrations in adult birds.



Table 1: Mercury concentrations [ $\mu\text{g/g}$  wet wt.; mean  $\pm$  SD and (range)] in blood and feathers of adult bald eagles and eaglets from the Pinchi Lake area. (2000 – 2002).

	Adult Eagles			Eaglets		
	<i>n</i>	Hg in blood	Hg in feathers	<i>n</i>	Hg in blood	Hg in feathers
Pinchi L.	3	6.5 $\pm$ 2.5 (4.7 - 9.4)	40 $\pm$ 22 (24 - 65)	12	0.57 $\pm$ 0.16 (0.37 - 0.79)	18 $\pm$ 6.1 (10 - 28)
Tezzeron L.	2	4.1 $\pm$ 0.9 (3.5 - 4.7)	13 $\pm$ 2.2 (11 - 14)	5	0.42 $\pm$ 0.16 (0.26 - 0.60)	9.5 $\pm$ 1.1 (8.1 - 11)
Stuart L.	5	2.9 $\pm$ 1.2 (1.6 - 4.9)	14 $\pm$ 5.3 (10 - 22)	14	0.27 $\pm$ 0.09 (0.14 - 0.44)	6.2 $\pm$ 1.5 (3.6 - 9.3)
Fraser L.	3	2.0 $\pm$ 0.3 (1.8 - 2.3)	9.3 $\pm$ 1.0 (8.1 - 10)	6	0.20 $\pm$ 0.05 (0.12 - 0.25)	5.5 $\pm$ 1.9 (3.9 - 6.9)
Great Beaver L.	0	--	--	6	0.20 $\pm$ 0.03 (0.15 - 0.24)	7.1 $\pm$ 2.8 (5.1 - 12.5)

The average and maximum concentrations of Hg in blood of adult eagles from Pinchi Lake (6.5 and 9.4  $\mu\text{g/g}$  wet wt., respectively) were higher than for eagles from nearby Stuart and Fraser Lakes, and numerous other locations in BC (Weech, 2003). There are few other reports of blood-Hg concentrations  $>6$   $\mu\text{g/g}$  in free-living birds. In Montana, two sub-adult eagles had 7.0 and 9.5  $\mu\text{g/g}$  wet wt. Hg in blood (Wiemeyer et al., 1989); and some common loons from the Canadian maritimes have been found with blood-Hg concentrations up to 7.8  $\mu\text{g/g}$  wet wt. (Evers et al., 1998). Several species of water birds nesting on the Lower Carson River, Nevada, where thousands of tons of Hg were released into the watershed during the late 1800s as a by-product of gold and silver ore refining, had highly elevated Hg concentrations in blood. The highest blood concentrations (11.6 – 22.0  $\mu\text{g/g}$  wet wt.) were in adult double-crested cormorants (*Phalacrocorax auritus*), but concentrations were also elevated in many snowy egrets (*Egretta thula*) (2.8 – 10.3  $\mu\text{g/g}$  wet wt.) and black-crowned night herons (*Nycticorax nycticorax*) (3.2 – 14.2  $\mu\text{g/g}$  wet wt.) (Henny et al., 2002). In non-contaminated environments, mean Hg concentrations in adult eagle blood are generally  $<3$   $\mu\text{g/g}$ . Mean Hg concentrations in blood of adult eagles captured in Oregon and Montana were 2.3 and 2.0  $\mu\text{g/g}$  wet wt., respectively (Wiemeyer et al., 1989), concentrations comparable to those of adult eagles from Pinchi-area reference lakes. Similarly, Hg concentrations in the blood of 15 adult bald eagles wintering in the Klamath Basin of Oregon/California, averaged 2.29  $\mu\text{g/g}$  wet wt. (Frenzel and Anthony, 1989); and Anthony et al. (1993) reported an average 3.07  $\mu\text{g/g}$  wet wt. Hg in blood of adult bald eagles from the Columbia River estuary, Oregon (range = 1.30-4.10  $\mu\text{g/g}$  wet wt.). Clearly, Hg exposure in eagles nesting on Pinchi Lake is high compared to adult eagles from most other locations in North America for which data are available.

Number 2 secondary feathers from adult eagles were chosen for Hg analysis because the same feathers have often been used to estimate Hg exposure in another large piscivorous bird, the common loon (Evers et al. 1998). However, bald eagles undergo their yearly molt on their summer breeding territories, whereas secondaries obtained from loons during summer sampling would have been grown on their wintering grounds. Therefore, adult eagle feather-Hg concentrations may be more indicative of meHg exposure on breeding lakes than is the case for loons. Unlike eaglets, no correlation was found between

adult blood and feather Hg concentrations; however this was most likely due to the small sample size, as the relationship was only marginally insignificant ( $p = 0.087$ ).

### Bald Eagle Productivity and Reproductive Success

From 2000 to 2002 inclusive, 62% of active territories (i.e., where pairs of adult eagles were observed near a nest at the beginning of May) on Pinchi Lake successfully produced eight-week old eaglets, compared to 64% of active territories on all reference lakes combined. There was no significant difference among lakes with respect to overall reproductive success ( $p = 0.95$ ) or productivity ( $p = 0.48$ ). Productivity, as measured by the number of eaglets produced per active territory found in May, averaged 0.98 on Pinchi Lake compared to 1.17 on reference lakes combined. Approximately 27% of all eaglets produced over the 3-year period were raised on Pinchi Lake. In addition, there was no significant relationship ( $r = -0.254$ ,  $p = 0.403$ ) between Hg concentrations in blood of eaglets and eagle productivity.

Methylmercury is a potent reproductive toxicant. Approximately  $0.3 \mu\text{g/g}$  Hg wet wt. in prey fish was previously shown to result in decreased reproductive success in wild common loons (fewer eggs laid, decreased hatching success, and reduced nest and territory-site fidelity; Barr 1986). Heinz (1974) found reduced egg laying and hatching success in mallards experimentally exposed to  $3 \mu\text{g/g}$  dry wt. dietary meHg (the equivalent of approximately  $0.7 \mu\text{g/g}$  wet wt. in fish muscle). Heinz (1979) also reported that female mallards fed  $0.5 \mu\text{g/g}$  meHg in their diet laid fewer eggs than control birds, and also laid significantly more eggs outside their nest boxes, therefore producing fewer chicks. Dietary concentrations of meHg known to cause reproductive impairment are typically one fifth of the level that would be required to cause significant neurological defects in adult birds (Scheuhammer, 1988). However, in the present study, no indication of low reproductive success or productivity was observed for the small sample of eagles nesting on Pinchi Lake, even at blood-Hg concentrations approaching  $10 \mu\text{g/ml}$ . Despite elevated Hg levels in adult eagles breeding on Pinchi Lake, birds appeared to be in excellent body condition, with no evidence of meHg toxicity such as abnormal behavior or lack of coordination. The most highly exposed adult raised two chicks in both 2001 and 2002 making it one of the most productive birds in the study area. Overall productivity of bald eagles from the study area was indicative of a healthy population producing  $> 0.7$  young/occupied territory, a value associated with a self-sustaining population (Sprunt et al., 1973). Reproductive success and productivity on Pinchi and surrounding lakes was similar to that of a population of reference bald eagles nesting on Adak and Tanaga Islands in the Western Andreanof Islands of the Aleutian Archipelago, Alaska, where productivity averaged approximately one chick/occupied territory (Anthony et al., 1999); and to reference populations of bald eagles nesting in the lower Fraser River Valley, BC, and around southeastern Vancouver Island, BC, where productivity averaged 1.2 and 0.95 young/occupied territory, respectively (Elliott et al., 1998).



## CONCLUSIONS

Based on the above study of fish-eating birds from the Pinchi Lake area, Hg appears to be having no obvious adverse effects on reproduction. Red-necked grebe eggs showed Hg concentrations below the LOAEL for reproductive effects from all study lakes, and bald eagles showed similar reproductive success and productivity to reference populations, despite elevated levels of Hg in tissues of Pinchi Lake birds. As shown, concurrent tissue sampling and population monitoring techniques can provide a useful means to substantiate findings that might suggest a possible contaminant effect on local wildlife.

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