

## THE AMERICAN DIPPER AS A BIOINDICATOR OF SELENIUM CONTAMINATION IN A COAL MINE-AFFECTED STREAM IN WEST-CENTRAL ALBERTA, CANADA

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**Abstract.** Elevated levels of selenium have been found in water and aquatic biota downstream from two open-pit coal mines in the Rocky Mountain foothills of Alberta. Birds are particularly sensitive to excessive dietary selenium. However, there is relatively little information on selenium accumulation in birds' eggs on fast-flowing mountain streams. We determined levels of selenium in water samples, caddisfly larvae and eggs of American dippers (*Cinclus mexicanus*) nesting on the Gregg River, downstream from the mines, and on reference streams in the same general vicinity. Selenium levels (mean, 95% confidence limits) in water samples and caddisflies collected from sites near dipper nests on the Gregg River (water: 4.26, 1.90–9.56  $\mu\text{g L}^{-1}$ ; caddisflies: 8.43, 7.51–9.46  $\mu\text{g g dry wt}^{-1}$ ) were greater than those collected from sites near nests on reference rivers (water: 0.38, 0.21–0.71  $\mu\text{g L}^{-1}$ ; caddisflies: 4.65, 4.35–4.97  $\mu\text{g g dry wt}^{-1}$ ). The mean ( $\pm 1\text{SE}$ ) selenium level in dipper eggs from the Gregg River ( $6.3 \pm 0.2 \mu\text{g g}^{-1} \text{dry wt}$ ) was significantly higher than it was in eggs from reference streams ( $4.9 \pm 0.2 \mu\text{g g}^{-1} \text{dry wt}$ ). Concentrations of selenium in eggs were significantly correlated with those in water samples ( $r = 0.45$ ). The maximum selenium level in eggs from the Gregg River ( $9.0 \mu\text{g g}^{-1}$ ) may have been high enough to warrant concern from an ecotoxicological perspective. The American dipper can serve as a useful bioindicator of selenium contamination in mountainous, lotic ecosystems.

**Keywords:** selenium, coal mining, Alberta, birds, American dippers

### 1. Introduction

Coal is mined in the Rocky Mountains of Alberta and British Columbia, mainly at a small number of large open-pit mines (Ryan and Dittrick, 2000; Alberta Energy, 2005). At open-pit mines, large quantities of soil and rock are disturbed by various mining activities that can expose the overburden to air and surface waters. Waste materials are generally deposited on the surface in tailings piles, ponds, landfills, dumps and rock drains. Surface waters may drain from these deposits into nearby aquatic ecosystems and eventually into streams that drain the local watershed (Hamilton and Buhl, 2004).

There is concern that open-pit coal mines in the Rocky Mountains of Canada can mobilize selenium into fast-flowing streams in greater quantities than would normally occur in a natural setting (Casey and Siwik, 2000; McDonald and Stroscher, 2000).

Lemly (1999) proposed that selenium should not bioaccumulate to as significant a degree in fast-flowing streams as in lentic ecosystems. In support of his proposal, Adams *et al.* (2000) found that selenium bioaccumulation factors in fish from lotic environments were about ten-fold lower than those in fish from lentic environments and that fish tissue selenium concentrations remained relatively low and constant across a range of water concentrations up to  $13 \mu\text{g L}^{-1}$ . In addition, Orr *et al.* (2005) have recently shown that biota in coal mine-affected lentic systems accumulated more selenium than those in coal mine-affected lotic systems. However, contrasting with these results and fueling concern about potential effects of coal mining on selenium mobilization and bioaccumulation, are studies that reported increased levels of selenium in fish at sites downstream from coal mines in the Rocky Mountains of Canada (Kennedy *et al.*, 2000; Palace *et al.*, 2004; Holm *et al.*, 2005). One of those studies reported a significant, selenium-dependent increase in deformities in trout fry hatched from fertilized eggs of fish from coal mine-affected streams (Holm *et al.*, 2005), heightening to an even greater extent the level of concern about possible coal mining effects.

Bird eggs are exposed to selenium by maternal transfer of organoselenium accumulated by the female through her diet (Ohlendorf, 1996). Bird eggs are sensitive to selenium toxicity (Ohlendorf *et al.*, 1986; Heinz *et al.*, 1989; Skorupa and Ohendorf, 1991; Adams *et al.*, 1998). Therefore, elevated levels of selenium in streams draining coal mines could impair the reproductive success of species of aquatic birds attempting to breed there. Such species include the American dipper (*Cinclus mexicanus*), the spotted sandpiper (*Actitis macularia*) and the harlequin duck (*Histrionicus histrionicus*), a species designated as 'sensitive' in the province of Alberta because of concern about possible long-term population declines (MacCallum, 2001).

The American dipper is an aquatic bird that occurs on fast-flowing, clear streams (Kingery, 1996). It feeds mainly on immature aquatic insects, which serve as a dietary source of selenium. During the breeding season, dippers establish territories along streams in close proximity to their nests (Kingery, 1996). Its animal diet and territoriality make the American dipper a potentially useful avian bioindicator species for contaminants that are transferred through stream food chains. The closely-related Eurasian dipper (*C. cinclus*) has been used as an indicator of stream quality in Europe for many years (Ormerod and Tyler, 1987, 1990; O'Halloran *et al.*, 2003). In North America, the use of dippers as indicators of stream quality is a more recent phenomenon (Strom *et al.*, 2002; Feck and Hall, 2004; Morrissey *et al.*, 2004, 2005). Harding *et al.* (2005) examined dipper reproductive performance and selenium levels in dipper eggs on streams located downstream from coal mines in south-eastern British Columbia. They reported that selenium levels were not higher in dipper eggs from nests on coal mine-affected streams than in eggs from nests on reference streams, a phenomenon they attributed, in part, to the low bioaccumulation rates inherent in lotic ecosystems.

In this study, we explored the potential usefulness of the American dipper as an avian bioindicator species in mountainous stream ecosystems with elevated selenium levels. In particular, we examined selenium levels in eggs of American dippers on a mine-affected stream and on reference streams and contrasted our results with those of Harding *et al.* (2005). We have also compared selenium levels in water samples and caddisfly larvae collected near dipper nests on mine-affected and reference streams. Caddisflies were used as indicators of selenium levels in the diet of dippers because they have been shown to be an important component of their diets (Ealey, 1977; Ormerod, 1985; Ormerod and Tyler, 1991; Kingery, 1996; Feck and Hall, 2004). Finally, by comparing our data to toxicity thresholds for birds, we have assessed whether the selenium levels may have been high enough to warrant concern from a toxicological perspective.

## 2. Materials and Methods

### 2.1. STUDY AREA

The study was done from 2001–2004 on first- to third-order streams in the vicinity of Hinton, Alberta. The Gregg River receives drainage water from two adjacent, open-pit coal mines, one of which had closed in 2000 and was being remediated and the other which was still operating. Reference sites were located on the upper McLeod River, Whitehorse Creek, Wildhay River, North and South Berland Rivers and the South Sulphur River. The upper McLeod River served as the main reference river for this study. When this study was done, there were no operating mines but some small coal mines had operated within the watershed until the early 1950s. Because of low concentrations of selenium in water and sediment samples taken from the upper McLeod River, we judged it to be an adequate reference site for this study (Casey and Siwik, 2000; Wayland and Crosley, *in press*). The other reference sites were pristine with no known point sources of pollution. The Gregg River is a major tributary of the McLeod but all samples collected on the latter river came from sites located >20 km upstream from the confluence. Whitehorse Creek joins the McLeod in the upper reaches of the latter stream. All rivers in this study are part of the Athabasca River drainage, but the Wildhay, North and South Berland Rivers and the South Sulphur are not directly connected to the McLeod or the Gregg Rivers.

### 2.2. SAMPLE COLLECTIONS

Nests were visited from late May until early July each year. In most instances, a single egg, selected at random, was taken from each nest and placed on ice in a cooler. In six instances, two eggs were taken from nests and treated as above. From 2001–2003, stream water samples were collected in clean, one-time use, metal-free high-density polypropylene bottles at mid-stream sites adjacent to nests from which

eggs were taken. Samples were kept cool on ice or in a refrigerator until they reached the laboratory. Caddisfly larvae (Hydropsychidae) were collected by kick-netting into a dip net at distances up to approximately 100 m upstream and downstream from dipper nests. Within 1 hr of collection, larvae were removed from the net using Teflon-coated forceps, rinsed thoroughly in stream water and placed in acid-washed vials on dry ice. At the end of each day, samples were transferred to a freezer. On the Gregg River, all samples were collected at distances  $\leq$  15 km from the mines.

### 2.3. SELENIUM ANALYSIS

Eggs and caddisfly larvae were analyzed by the Alberta Research Council. Samples were weighed and then oven-dried at 95 °C for 4 hr. After drying, they were re-weighed to determine moisture content. A 0.5 g portion of the dried sample was weighed in each digestion liner and 5 mL of nitric acid was added. The microwave digestion was carried out in closed vessels at controlled temperature (165 °C) and controlled pressure (220 PSI), using a QWAVE-1000 microwave sample preparation system (Questron, Mercerville, NJ, USA). Digested solutions were then diluted with distilled, de-ionized water and analyzed by inductively-coupled plasma-mass spectrometry using a Perkin-Elmer Elan 5000 ICP quadrupole mass spectrometer (Thornhill, ON, Canada). In the ICP-MS analysis, values of samples and standards were reagent blank subtracted. Indium was used as an internal standard. External calibration curves were plotted linearly through zero for each isotope. Correction equations were applied to values to account for interference from chloride (Wu *et al.*, 1997).

The quality of the distilled, de-ionized water was pre-checked by ICP-MS scanning; blank samples including laboratory reagent blanks and digestion blanks were analyzed together with samples; samples of NIST standard reference material (oyster tissue NIST 1566a, whole egg powder NIST 8415) were microwave digested and analyzed together with the samples. Also, two eggs from each of three nests were analyzed to provide a measure of within-nest variability and two samples of caddisflies collected at each of two sites were analyzed to provide a measure of within-site variability. The relative standard deviation of replicate analyses of the same sample was  $7.2 \pm 5.8\%$  (mean  $\pm$  1SD). Recovery was  $98 \pm 8.3\%$  from spiked samples,  $98.9 \pm 4.9\%$  from NIST 1566a (oyster tissue) and  $98.3 \pm 2.7\%$  from NIST 1845 (whole egg powder). Within-site variability in selenium levels, measured as the relative standard deviation of analyses of two samples from the same nest or stream site, ranged from 1.5–16.5% (median = 8.4%) for dipper eggs and from 4.4–12.0% for caddisflies.

Water samples were analyzed at Environment Canada's National Laboratory for Environmental Testing. Methods and instrumentation changed once during the course of this study. In 2001 and 2002, selenium was analyzed as follows. Samples were shaken and 100 mL aliquots were transferred to an Erlenmeyer digestion flask. Potassium persulphate and hydrochloric acid were added, and the samples were

digested on a hotplate to oxidize all selenium species to selenate. After reducing the sample volume to approximately 10 mL, the sample was removed from the hotplate and allowed to cool. The digest was then transferred to a volumetric cylinder, 7 mL of hydrochloric acid was added, and the volume was brought to 20 mL with de-ionized water. This solution was re-transferred to an Erlenmeyer flask and re-heated to 90 °C to convert selenate to selenite. The digest was then cooled and subjected to hydride generation in an automated continuous flow system. Hydrides of selenium were formed by the action of sodium borohydride in an acidic medium, and were analyzed by inductively coupled plasma – optical emission spectrometry (ICP-OES, IRIS, Thermo Jarrel Ash, Franklin, MA). In 2003, samples were acidified with nitric acid and placed in a sealed container in a convection oven. The samples were allowed to digest in-bottle for 16 hours at 60 °C, and then allowed to cool to room temperature. Selenium was analyzed by inductively coupled plasma – sector field mass spectrometry (ICP – SFMS, Element II, ThermoFinnigan, Bremen, Germany) Standard reference materials and spiked samples were analyzed. Relative standard deviations ranged from 6.2–6.9%, based on duplicate analysis of spiked samples, and from 0–34% (median = 1.8%), based on analysis of duplicate or triplicate field samples. Recovery was 118% from the standard reference material (water, NIST 1643a) and ranged from 100–106% from spiked samples. In 2001 and 2002, the detection limit was  $0.1 \mu\text{g L}^{-1}$  and in 2003 it was  $0.05 \mu\text{g L}^{-1}$ .

#### 2.4. STATISTICS

When two or more samples were collected from a given nest or site in a given year, their values were averaged and average values were used in subsequent analyses. Egg samples collected from the same nest in different years were treated as independent samples even though it is possible the same female used the nest in successive years. Data were checked for normality and homogeneity of variances and, where necessary, were log-transformed prior to analysis. PROC GLM (SAS Institute, 1999) was used to do analyses of variance in a randomized complete block design to test the effects of year and site type (coal mine-affected stream site or reference site) on selenium levels in water, caddisflies and eggs. PROC CORR (SAS Institute, 1988) was used to examine the relationship between selenium levels in water and those in eggs.

### 3. Results

Mean  $\pm$  1SE concentrations of selenium in eggs from nests on the Gregg River, a stream that drains two open-pit coal mines, ranged from  $4.4 \pm 0.58$ – $7.14 \pm 0.82 \mu\text{g g}^{-1}$ , dry wt. In reference streams, corresponding values ranged from  $2.6$ – $6.4 \pm 0.67$  (Table I). Mean  $\pm$  1SE concentrations of selenium in caddisflies collected near Gregg River nests ranged from  $7.23 \pm 0.62$ – $9.19 \mu\text{g g}$  dry wt $^{-1}$  whereas those collected near nests on reference streams ranged from

TABLE I

Mean  $\pm$  1SE (n) selenium concentrations in water samples ( $\mu\text{g L}^{-1}$ ), caddisfly larvae and American Dipper eggs ( $\mu\text{g g}^{-1}$ , dry wt) collected in reference streams (REF) and the Gregg River (CMA), a stream that drains two open-pit coal mines in the Rocky Mountain foothills

Year	Water Samples		Caddisfly Larvae		Dipper Eggs	
	REF	CMA	REF	CMA	REF	CMA
2001	0.54 $\pm$ 0.07 (7)	2.72 $\pm$ 1.09 (3)	4.67 $\pm$ 0.59 (6)	9.14 (1)	5.51 $\pm$ 0.33 (12)	6.58 $\pm$ 0.67 (3)
2002	0.38 $\pm$ 0.09 (5)	5.78 $\pm$ 1.22 (5)	4.11 $\pm$ 0.25 (3)	7.23 $\pm$ 0.62 (4)	5.06 $\pm$ 0.52 (5)	6.97 $\pm$ 0.52 (5)
2003	0.37 $\pm$ 0.11 (3)	6.36 $\pm$ 0.1 (2)	5.46 $\pm$ 0.02 (2)	9.19 (1)	6.40 $\pm$ 0.67 (3)	7.14 $\pm$ 0.82 (2)
2004	–	–	–	–	2.55 (1)	4.40 $\pm$ 0.58 (4)

4.11  $\pm$  0.25–5.46  $\pm$  0.02  $\mu\text{g g dry wt}^{-1}$ . Ranges of mean values in water samples were as follows: 2.72  $\pm$  1.09–6.36  $\pm$  0.1  $\mu\text{g L}^{-1}$  for sampling locations near nests on the Gregg River and 0.37  $\pm$  0.11–0.54  $\pm$  0.07  $\mu\text{g L}^{-1}$  for sampling locations near nests on reference streams.

Selenium concentrations in eggs from nests on the Gregg River were significantly different than those from nests on reference streams ( $P = 0.02$ ), the former averaging about 1.3 times greater than the latter (Figure 1). In addition selenium concentrations in eggs differed significantly among years ( $P = 0.008$ ) and were lower in 2004 than in earlier years (Table I). Selenium concentrations in caddisfly larvae collected near Gregg River nests were significantly different from those in larvae collected near reference site nests ( $P = 0.008$ ), the former averaging about 1.8 times greater than the latter (Figure 1). Similarly, selenium levels in water samples collected near Gregg River nests were significantly different than those collected near reference site nests ( $P = 0.03$ ), the former averaging about 11 times greater than the latter (Figure 1).

Selenium levels in eggs were positively correlated with log-transformed selenium levels in water ( $r = 0.45$ ,  $P = 0.026$ , Figure 2).

The maximum selenium level in eggs was 9.0  $\mu\text{g g}^{-1}$ . Approximately 11% of all eggs contained  $\geq 8 \mu\text{g g}^{-1}$  while a further 29% contained from 6–8  $\mu\text{g g}^{-1}$ .

#### 4. Discussion

##### 4.1. SELENIUM ACCUMULATION IN BIOTA

In reference streams, selenium levels in water were always less than 1  $\mu\text{g L}^{-1}$  and were very similar to levels reported from uncontaminated, non-seleniferous

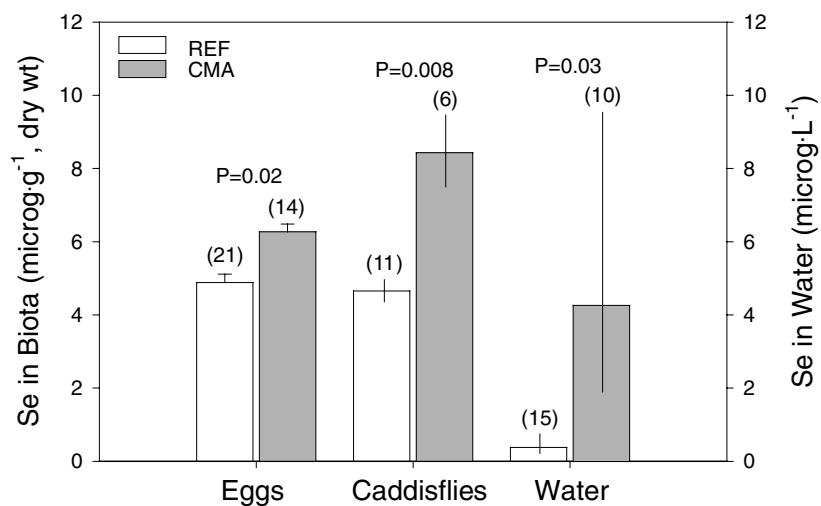


Figure 1. Least-squared mean selenium concentrations in American dipper eggs, caddisfly larvae and water samples collected from nests (Eggs) or at stream sites near nests (Caddisflies and Water) on the coal mine-affected Gregg River and on nearby reference streams. In legend, REF refers to reference sites and CMA refers to sites on the coal mine-affected Gregg River. Numbers above bars refer to sample sizes. Lines above and/or within bars represent the standard errors (Eggs) or the 95% confidence intervals (Caddisflies and Water). Also shown are the  $P$  values for the differences between coal mine-affected and reference sites.

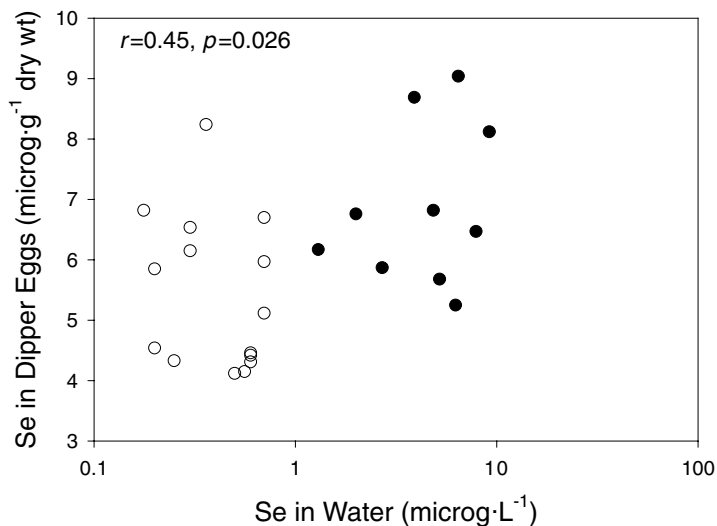


Figure 2. Relationship between selenium concentrations in water and those in American Dipper eggs from nests on the coal mine-affected Gregg River and on nearby reference rivers. Open and shaded circles represent reference and coal mine-affected sites, respectively.

watersheds (Maier and Knight, 1994; USEPA, 2004). Selenium levels in water on the Gregg River averaged about  $5 \mu\text{g L}^{-1}$ . These levels were similar to those found in some moderately-contaminated aquatic ecosystems but were substantially lower than those in highly-contaminated agricultural areas in the western USA (Skorupa, 1998; Seiler *et al.*, 2003; Hamilton and Buhl, 2004). They were also lower than the range of mean values ( $8\text{--}34 \mu\text{g L}^{-1}$ ) reported in streams that drain open-pit coal mines in southeastern British Columbia (Harding *et al.*, 2005).

Selenium levels were higher in caddisfly larvae collected near nest sites on the coal mine-affected Gregg River than they were in larvae collected near reference site nests. Caddisflies are often the most abundant group of insects in the diet of breeding dippers, although their abundance is sometimes exceeded by that of mayflies (Ealey, 1977; Ormerod, 1985; Ormerod and Tyler, 1991; Feck and Hall, 2004). Recently, salmon fry have also been reported to be important in the diet of breeding dippers in southwestern British Columbia (Morrissey *et al.*, 2004). However, salmon do not occur in Alberta and fish were not reported to be part of the diet of dippers nesting near Rocky Mountain streams similar to those in this study (Ealey, 1977). In our study area, caddisflies contained lower concentrations of selenium than mayflies but higher concentrations than stoneflies (Wayland and Crosley, in press). In addition, selenium levels in caddisflies on coal mine-affected streams in our study area were higher than those in caddisflies from reference streams, whereas selenium levels in mayflies and stoneflies did not differ between mine-affected and reference streams (Wayland and Crosley, in press). The importance of caddisflies in the diet of dippers as documented in the scientific literature coupled with the higher levels of selenium in caddisflies and American dipper eggs in the coal mine-affected Gregg River suggests that caddisflies were an important source of dietary selenium in dippers.

Selenium levels in eggs of American dippers and other aquatic birds from uncontaminated areas typically range from  $1\text{--}3 \mu\text{g g}^{-1}$  dry wt (Skorupa and Ohlendorf, 1991; Morrissey *et al.*, 2004), a range of concentrations that is lower than that found in dipper eggs from our reference sites. The finding of slightly elevated selenium levels in dipper eggs from reference sites in this study is consistent with the higher than expected selenium levels in caddisflies (medians from  $3.9\text{--}6.1 \mu\text{g g}^{-1}$ ) and mayflies (medians from  $7.1\text{--}9.3$ ) from several of the same reference streams reported in another study (Wayland and Crosley, in press). Caddisflies and mayflies are often important foods for dippers (Ealey, 1977; Ormerod, 1985; Ormerod and Tyler, 1991; Feck and Hall, 2004) and may have been an important source of dietary selenium in this study.

Harding *et al.* (2005) reported that selenium levels in eggs of dippers from coal mine-affected sites in southeastern British Columbia, Canada, where mean aqueous selenium concentrations ranged from  $8\text{--}34 \mu\text{g L}^{-1}$ , did not differ from levels in eggs at nearby reference sites where mean aqueous selenium levels were generally  $\leq 1.0 \mu\text{g L}^{-1}$ . They further indicated small sample sizes ( $n = 11$  at reference sites and  $10$  at coal mine-affected sites) and consequent low statistical power may



have precluded distinguishing between coal mine-affected and reference areas. In contrast, with only slightly larger samples sizes and at lower aqueous selenium levels, we have shown that selenium levels in dipper eggs from a coal mine-affected stream averaged 30% higher than those in eggs from nearby streams in Alberta.

Selenium levels in stream water at the nest sites were correlated with those in dipper eggs, although the *r*-value was quite low, probably because of the complex processes controlling bioaccumulation of selenium (Lemly, 1999). Adams *et al.* (1998) reported that relationships between selenium in water and eggs are site-specific, a phenomenon they attributed, in part, to among-site variation in diet. Dippers feed on a wide variety of aquatic insects and it is likely that the composition of their diet is influenced by the availability of different types of insects within their territories (Kingery, 1996). In our study area, mayflies accumulated greater amounts of selenium than caddisflies which, in turn, accumulated greater amounts than stoneflies (Wayland and Crosley, in press). Thus dietary differences among female dippers in our study area likely contributed to among-site variation in selenium levels in their eggs. Such differences also probably weakened the relationship between selenium levels in water and eggs. Another potentially important consideration that may have weakened the relationship between selenium concentrations in water and eggs relates to territory size and the potential for variability in selenium loadings to different branches of a stream within a given territory. Territories average about 1 km of stream length but may range up to 4 km (Kingery, 1996). Within that distance in our study area, small streams often intersected with the stream on which the nest was located. We were unable to determine if dipper territories are likely to include multiple branches of the same stream system. If they are likely to include multiple branches and if selenium loading differs among those stream branches, then there could be substantial variation in dietary exposure to selenium within the same territory. Our strategy of collecting a single water sample adjacent to the nest site would not have adequately represented such variability. Such a scenario would also weaken the relationship between selenium levels in eggs and water samples.

Adams *et al.* (2000) reported that in lotic ecosystems, selenium levels in fish tissue remained fairly low and constant up to a water concentration of about  $13 \mu\text{g L}^{-1}$ . Furthermore, Harding *et al.* (2005) hypothesized that the low biological transformation and uptake rate of selenium in lotic ecosystems would limit its uptake into bird eggs as well. In contrast, we found that selenium levels in dipper eggs were positively correlated with those in water over a range of  $<1\text{--}9.2 \mu\text{g L}^{-1}$ . Furthermore, selenium concentrations in dipper eggs were clearly elevated at coal mine-affected sites where water selenium concentrations ranged from  $1.3\text{--}9.2 \mu\text{g L}^{-1}$ . Our results suggest that in fast-flowing, stream ecosystems, even modest increases in aqueous selenium concentrations could result in increased selenium exposure in aquatic birds such as dippers.

#### 4.2. TOXICITY THRESHOLDS

Contaminant residues in sensitive tissues are often used to make inferences about the potential risk they pose to the organisms within whose tissues the contaminants were measured (Keith, 1996). Egg hatchability and its converse, egg inviability, are considered to be sensitive endpoints for selenium toxicity in birds while teratogenicity and deformities are less sensitive endpoints (Skorupa, 1998; Ohlendorf, 2003). A number of threshold concentrations in birds' eggs associated with egg inviability and teratogenicity have been proposed. Selenium-induced egg inviability and teratogenicity vary widely among different species of birds (Ohlendorf *et al.*, 1986; Skorupa and Ohlendorf, 1991; Ohlendorf, 2003) so that thresholds derived from data on one species may not be applicable to another. Whether dippers are less, more or equally as sensitive to selenium as the species used to derive toxicity thresholds is not known.

In this study, the mean selenium level in dipper eggs from the Gregg River equaled the lowest published mean egg selenium toxicity threshold of  $6\text{--}7\ \mu\text{g g}^{-1}$  (Skorupa *et al.*, 1998). It was only slightly lower than  $8\ \mu\text{g g}^{-1}$ , another oft-cited mean egg toxicity threshold (Ohlendorf and Santolo, 1994; Seiler *et al.*, 2003). However, it was lower than mean egg selenium thresholds (12.5, 13, 16, and  $12\text{--}15\ \mu\text{g g}^{-1}$ ) based on experimental studies with captive mallards (Fairbrother *et al.*, 1999; Adams *et al.*, 2003; Ohlendorf, 2003; Brix *et al.*, 2005).

More than 50% of the eggs had selenium levels greater than the lowest threshold level for individual eggs ( $5\ \mu\text{g g}^{-1}$  Ohlendorf *et al.*, 1986) while the maximum selenium level ( $9.0\ \mu\text{g g}^{-1}$ ) approached the second lowest proposed toxicity threshold for individual eggs ( $10\ \mu\text{g g}^{-1}$ , Skorupa and Ohlendorf, 1991). Levels of selenium in all eggs were lower than the  $3\ \mu\text{g g wet wt}^{-1}$  threshold (approximately  $14\ \mu\text{g g dry wt}^{-1}$ , based on a moisture content of 78.4% in the dipper eggs analyzed in this study) associated with reproductive problems, primarily hatching failure and embryonic deformities in individual bird eggs (Heinz, 1996)

Harding *et al.* (2005) reported that 79% of dipper eggs incubated to full-term hatched in dipper nests on coal mine-affected streams in southeastern British Columbia whereas 94% of eggs from reference streams hatched. This difference was nearly significant ( $P = 0.056$ ). Eggs that failed to hatch were described as infertile or inviable but these two causes of egg failure were not distinguished from one another. Obvious deformities in embryos and chicks were not observed and the authors concluded that reproductive success of dippers was not adversely affected by exposure to selenium at coal mine-affected sites. In that study, dipper eggs on coal mine-affected streams contained  $1.10 \pm 0.06\ \mu\text{g g wet wt}^{-1}$  (mean  $\pm$  1SE) whereas those on reference streams contained  $0.96 \pm 0.06\ \mu\text{g g}^{-1}$ . In order to compare selenium levels in eggs collected in this study to Harding *et al.*'s (2005) results, we used moisture contents, which were known for each egg, to convert our dry weight-based selenium concentrations to wet weight-based concentrations. Mean  $\pm$  1SE moisture content in our eggs was  $78.4 \pm 0.9\%$ , which is very close to the mean values

(75.7–76.8%) for dipper eggs reported by Morrissey *et al.* (2004). Eggs from the coal mine-affected Gregg River contained  $1.35 \pm 0.10 \mu\text{g g wet wt}^{-1}$  whereas those from reference sites contained  $1.10 \pm 0.06$ , levels that are slightly higher than those reported by Harding *et al.* (2005).

Compared to deformities and teratogenesis, egg hatchability is a more sensitive marker of selenium-induced effects on avian reproductive success (Ohlendorf, 2003). Thus, we believe it is imprudent to interpret the absence of deformities in Harding *et al.*'s (2005) study as evidence of a lack of effects of selenium on reproductive success of dippers. Furthermore, the near-significant, 15% reduction in hatchability of dipper eggs on coal mine-affected streams reported by Harding *et al.* (2005) coupled with the slightly higher levels of selenium in eggs in this study and their proximity to some of the lower toxicity thresholds lead us to conclude that there is a possibility that selenium exposure could reduce reproductive success of dippers on the coal mine-affected Gregg River.

In conclusion, we found elevated selenium levels in eggs of American dippers on a coal mine-affected stream in which aqueous levels of selenium were only moderately elevated. Our results are inconsistent with the contention that the low biological transformation and uptake rate inherent in lotic ecosystems should preclude increased accumulation of selenium in moderately-contaminated fast-flowing streams. Finally, levels of selenium in dipper eggs may have approached levels of concern from an ecotoxicological perspective, suggesting the need for further field studies to assess hatchability and reproductive success of dippers on streams with elevated selenium levels.

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