

# ACCUMULATION OF SELENIUM AND LACK OF EFFECTS ON AMERICAN DIPPERS AND SPOTTED SANDPIPERS

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## ABSTRACT

We compared selenium concentrations in the eggs of 2 riverine waterbirds, American dippers (*Cinclus mexicanus*) and spotted sandpipers (*Actitis macularia*), with measures of productivity: clutch size, brood size, and health status of eggs and young. In American dippers, the mean egg selenium concentration from the exposed areas was slightly higher (1.08 µg/g ww) than in the reference areas (0.95 µg/g ww), but this difference was not significant. For spotted sandpipers, the mean egg selenium concentration in the exposed areas was  $2.2 \pm 0.5$  µg/g ww and in the reference areas,  $1.2 \pm 0.14$  µg/g ww. This difference was significant, and was close to published toxicity thresholds. There were no significant differences between reference areas and exposed groups for clutch size, hatching success, or fledging success in either species. There was also no correlation between selenium concentrations and eggs laid, eggs hatched or hatchlings fledged in either species. There was no significant difference in health status of eggs (viable, inviable, or dead embryo) between the exposed and reference sites, and no relationship between selenium concentrations and the eggs' health status. Although selenium was being taken up in spotted sandpipers, it was below levels that would affect reproduction or productivity.

## INTRODUCTION

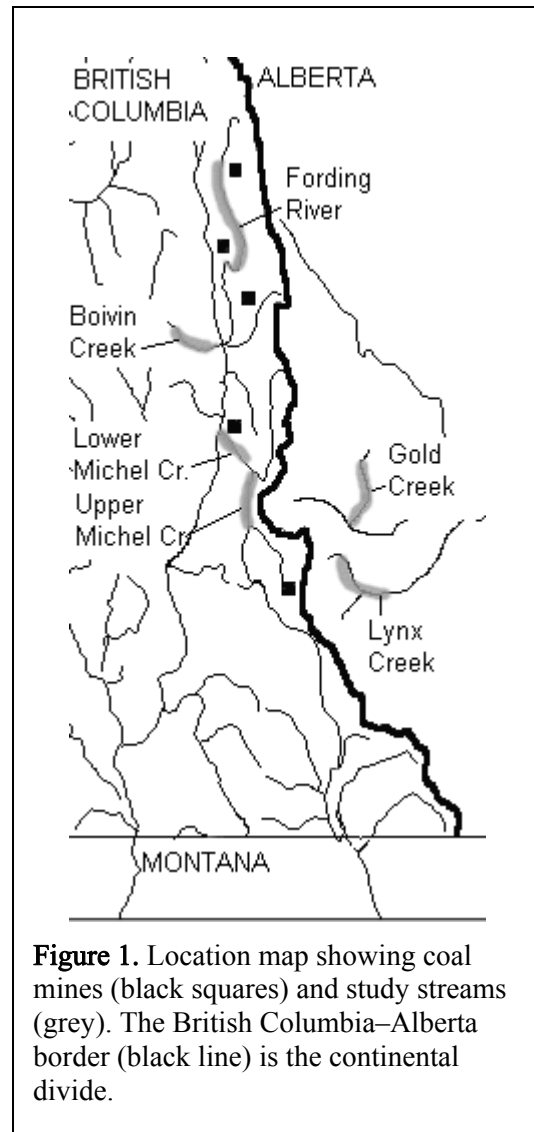
The discovery of elevated selenium concentrations in the Elk River (**Figure 1**) below several coal mines (McDonald and Stroscher 1998) raised concern because selenium can be toxic to birds and fish at relatively low concentrations. Selenium concentrations as high as 37 µg/L have been recorded in tributary streams; in the Elk River itself, selenium concentrations above the coal mines are generally no more than 1.0 µg/L, while those below the coal mines were as high as 7.4 µg/L (McDonald and Stroscher 1998, Kennedy *et al.* 2000, McDonald and Stroscher 2000, EVS

Environment Consultants 2003). Adams *et al.* (1998) suggest that reproductive effects in birds may occur above water-borne selenium concentrations of 6.8 µg/L.

Selenium that has bioaccumulated into tissues can have a wide variety of toxic effects to birds, including effects on metabolism, growth, and reproduction. Selenium in the eggs of wild water birds is often strongly correlated with the concentrations in the water where they live (Ohlendorf *et al.* 1993, Adams *et al.* 1998, Skorupa 1998). It affects reproduction in birds through mortality of the egg or hatchling and through teratogenesis. Teratogenic deformities can include anophthalmia (no eyes), spinal and bill deformations, defects in internal organs, and histological abnormalities. The objective of this study was to determine if selenium was affecting the productivity of two waterbirds in the Elk River Valley which are exposed to selenium via their diet: American dippers (*Cinclus mexicanus*) and spotted sandpipers (*Actitis macularia*).

### **Materials and Methods**

Based on previous data on selenium concentrations in water, sediments, and benthic macroinvertebrates, we selected the portions of the Fording River and two tributaries (Line and lower Michel Creeks) that receive coalmine runoff as exposed locations. There were five reference areas: Boivin Creek, upper Elk River, Lynx Creek, upper Michel Creek, and Gold Creek. These reference areas were located upstream or out of the drainage basin of any mine runoff, except for upper Michel Creek, which was far enough downstream and sufficiently diluted with clean tributaries that its selenium concentrations were also background. Gold and Lynx creeks are on the Alberta side of the Continental divide. Since Gold, Lynx, and Boivin creeks had not been sampled previously, we took samples of water and benthic invertebrates at the beginning of the study in May 2002, as well as a water sample from the upper Elk River. The water samples from these reference area creeks confirmed that they represented background selenium concentrations (all concentrations  $\leq 0.1$  µg/L Se). The benthos samples from Gold and Lynx creeks were in the range of previous reference area selenium concentrations (2.3 to 6.6 mg/kg dw), but those from Boivin Creek, on the west side of the Elk Valley where there are no coal mines, had 12.8 mg/kg Se dw.



**Figure 1.** Location map showing coal mines (black squares) and study streams (grey). The British Columbia-Alberta border (black line) is the continental divide.

Selenium concentrations in riffle benthos (mainly Ephemeroptera [mayflies], Diptera [flies], Perlodid stoneflies, and Hydropsychid caddisflies) in the Elk River Valley ranged from 1.5 to 10.7 µg/g dw overall; from 1.5 to 6.84 µg/g dw in upriver reference sites and 3.1 to 10.7 µg/g dry weight at sites below mine runoff (McDonald and Stroscher 1998, EVS Environment Consultants 2002).

During March and April 2002, we monitored courtship and nesting behaviour to locate nests. When egg-laying was complete for each nest and the female began incubating, we counted the eggs and collected one egg from each nest for chemical analysis, up to the number allowed by our federal permit (seven eggs each in exposed and reference locations for each species). However, at one nest each from an exposed and a reference area, we collected all eggs to test for within-clutch variability of selenium concentrations. Tissue collection and storage procedures followed Canadian Wildlife Service (1992, 1997) protocols.

While the altricial American dipper nestlings were still in the nest and being fed by the adults, from 1 to 10 d after hatching, we counted and checked them for mortality and teratogenic effects. When the nestlings began to appear outside the nests, we used binoculars or a 20x spotting scope to identify any obvious teratogenic effects or mortality. We continued these observations until the young were fledged and left the nest area. The productivity data for American dippers included the number of eggs laid, the number that hatched, and the number that successfully fledged.

The precocious spotted sandpipers, however, left the nest soon after hatching, so that the number successfully fledging either could not be counted, or could not be definitely associated with specific nests. Therefore, the productivity data for spotted sandpipers included only the number of eggs laid and the number that hatched.

We weighed and measured each egg and opened it to examine the embryo (if any) for general condition and viability. If no embryo was present, we considered it infertile. If an embryo was present, we examined the eyes, bill, wings, and legs to determine the presence of gross teratogenic effects or other abnormalities. If an embryo was present but dead, we recorded it as inviable. After examination, we placed the eggshell and contents into acid-washed sample jars and shipped them to the laboratory.

Eggs were analyzed for arsenic, mercury, selenium, and vanadium: they were homogenized and digested with nitric acid followed by repeated additions of hydrogen peroxide. Instrument analysis was by ICP/MS (Inductively coupled plasma – mass spectrometry; EPA Method 6020). (As, Vn and Hg were also analysed but are not reported here.) Micro-digestion techniques were used to produce acceptable detection limits for the small aliquot sizes. We assessed quality control, in addition to the laboratory's internal standards, by analyzing 2 pairs of duplicate samples of sandpiper eggs, with a difference of 5% for 1 and 11% for the other. Percent moisture was not included in the analysis for all of the eggs because of the small aliquot size, but 2

American dipper eggs collected separately for this purpose had 87.1% and 86.7% moisture (mean=86.9%), from which we calculated dry weights.

We compared the number of eggs laid per nesting pair, number of eggs hatched per clutch, and number of chicks fledged (American dippers only) per nest between reference and exposed areas using correspondence analysis of chi-square ( $X^2$ ) contingency tables. For descriptive statistics of clutch and (American dippers only) brood size, we adjusted the number of nestlings and fledglings in each nest to account for the eggs that we removed by adding the number that would have hatched, based on the proportion that did hatch. In spotted sandpipers, we encountered some infertile and inviable eggs and tested them for significance between exposed and reference areas. Nests that were destroyed by floods or snow were not included in subsequent productivity calculations.

## RESULTS

### American Dippers

In 45 territories monitored, 36 American dipper pairs built nests. However, snow and floods destroyed 9 nests, leaving 27 active first nests. One pair re-nested after fledging a full clutch, bringing the total number of active nests from which we collected eggs and made productivity observations to 28. In addition to the 14 eggs collected (7 each in reference and exposed areas) as per the federal scientific permit and the study design, 9 eggs were collected that failed to hatch, or that were left after nest abandonment. The total American dipper egg sample size was 23: 13 from reference streams and 10 from exposed streams.

Summary results of chemical analysis for mean egg selenium and productivity observations are illustrated in **Figure 2** and summarized in **Table 1**.

**Table 1.** Selenium concentrations ( $\mu\text{g/g}$ ) and productivity in American dippers and spotted sandpipers (mean  $\pm$  SD).

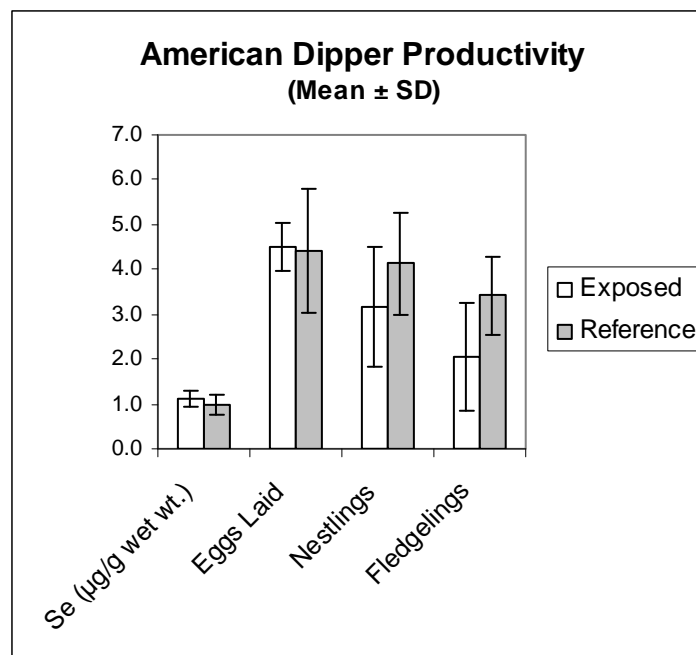
	<i>Eggs: (n) Mean <math>\pm</math> SD</i>		<i>Nests: (n) Mean <math>\pm</math> SD</i>		
	Se wet wt.	Se dry wt.	Eggs Laid	Nestlings	Fledglings
<i>American dippers</i>					
Reference	(13) $0.99 \pm 0.2$	7.59	(14) $4.42 \pm 1.4$	$4.13 \pm 1.1$	$3.43 \pm 0.9$
Exposed	(10) $1.11 \pm 0.2$	8.47	(14) $4.50 \pm 0.5$	$3.16 \pm 1.3$	$2.05 \pm 1.2$
<i>Spotted sandpipers</i>					
Reference	(14) $1.1 \pm 0.2$	8.52	(31) $4.0 \pm 0.0$	$3.8 \pm 0.6$	NA
Exposed	(26) $2.3 \pm 0.8$	17.2	(32) $3.8 \pm 0.5$	$2.8 \pm 1.4$	NA

NA=Not Applicable, see text.

Overall, the mean egg selenium concentration from the exposed areas was slightly higher ( $1.08 \mu\text{g/g ww}$ ) than in the reference areas ( $0.95 \mu\text{g/g ww}$ ), but was not significantly different ( $p=0.77$ ).

On a dry weight basis, based on a mean percent moisture of 86.9% in a subsample, exposed and reference area means were 8.0  $\mu\text{g/g}$  and 7.4  $\mu\text{g/g}$  dw, respectively.

American dipper clutch sizes averaged  $4.5 \pm 0.5$  eggs per nest in the exposed areas and hatching success (eggs hatched:eggs laid) was 0.70 (**Figure 2**). In the reference areas, clutch sizes averaged  $4.4 \pm 1.4$  and hatching success was 0.93. All of the American dipper pairs that bred produced either 4 or 5 eggs except for 1 pair in a reference area that re-nested after producing 3 fledglings and then produced 3 more. Of the eggs that hatched in the exposed areas, 73% (19 of 26) fledged. In the reference areas, 78% (36 of 46) of the eggs that hatched, fledged. There were no significant differences between reference areas and exposed groups for clutch size ( $X^2=0.768$ ,  $p=0.681$ ), hatching success ( $X^2=0.033$ ,  $p=0.855$ ) or fledging success ( $X^2=0.033$ ,  $p=0.855$ ). There was no correlation between selenium concentrations and eggs laid, eggs hatched or hatchlings fledged (linear regression,  $p<0.05$ ).



**Figure 2.** American dipper productivity and mean egg selenium concentrations (mean  $\pm$  SD). Left axis is mean number for eggs laid, number of nestlings and number of fledglings and  $\mu\text{g/g}$  wet weight for selenium.

We saw no obvious abnormalities that might suggest teratogenic effects in the embryos, or in the juveniles, and no dead embryos. There was no difference in egg size between exposed and reference areas. Because of the small numbers of eggs in this dataset, selenium concentration with egg weight was examined as a simple correlation for each group (reference and exposed); the  $r$  values were very low, 0.21 and 0.02 respectively, neither of which is significant at  $p=0.10$ .

The range of selenium concentrations in the 4 infertile eggs spanned the range for dipper eggs from lowest (0.6  $\mu\text{g/g}$  ww) to highest (1.4  $\mu\text{g/g}$  ww). The frequencies of viable vs. inviable eggs

were identical (not counting 2 broken eggs). Therefore, there was no effect of selenium concentration on egg size or health.

### **Selenium in Spotted Sandpiper Eggs and Effects on Productivity**

In 82 territories that we monitored, we found 43 nests representing all of the study creeks except Line Creek. In addition to the 14 eggs collected (7 each in reference and exposed areas) as per the federal scientific permit, we collected 26 eggs that failed to hatch or that were left after nest abandonment. Most (19) of these were on lower Michel Creek (one of the three exposure locations). The total sample size was 40 sandpiper eggs, 14 from reference areas and 26 from exposed areas.

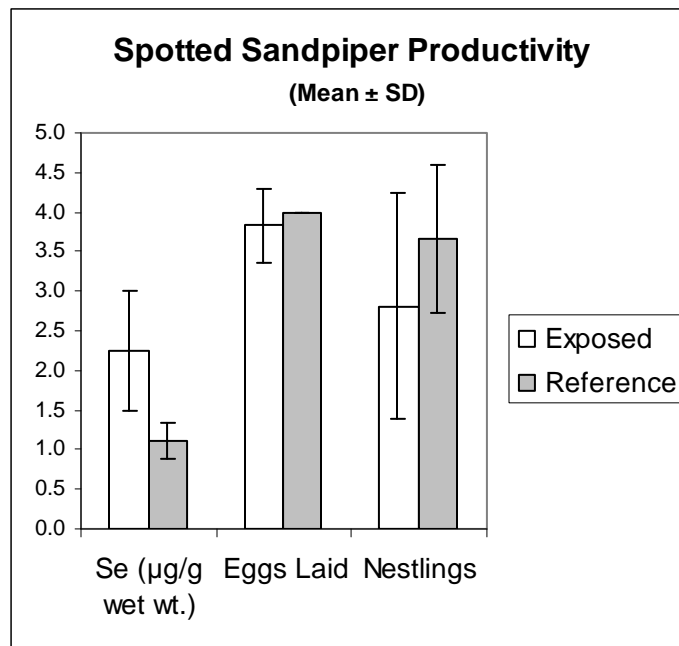
The mean egg selenium concentration in spotted sandpipers from the exposed areas ( $2.2 \pm 0.5$   $\mu\text{g/g}$  ww) was about twice that of American dippers. The mean selenium concentration in the reference areas was  $1.2 \pm 0.14$   $\mu\text{g/g}$  ww. On a dry weight basis, based on the same percent moisture 86.9% that we found in American dipper eggs, spotted sandpipers in exposed and reference areas had mean egg selenium concentrations of 16.8 and 8.3  $\mu\text{g/g}$  dw, respectively.

The ANOVA result regarding replication was that the random factor of eggs coming from the same nest was not significant ( $p=0.372$ ), and therefore all the eggs were treated in the same way in the analysis without regard to their having nestmates or not. The weight of eggs also was not a factor affecting selenium concentration in the subsample of replicates, as we found no significant correlation between egg weight and selenium concentration. This result suggested that the amount of selenium in an egg was primarily determined by other factors, such as exposure to selenium or random variation. After excluding any problems with unequal replication and covariance on weight, the main treatment effect, we then examined the reference sites *vs.* exposed sites, and found the difference highly significant at  $p=0.001$ : exposed sites had higher selenium concentrations.

Spotted sandpipers' clutch sizes averaged  $3.8 \pm 0.5$  eggs per nest in the exposed areas and hatching success was 74% (**Figure 3**). In the reference areas, clutch sizes averaged  $4.0 \pm 0.0$  and hatching success was 91%. Almost all spotted sandpiper pairs (96%) produced 4 eggs; 1 pair produced 1 egg and 3 pairs produced 3 eggs. Since spotted sandpipers are precocious and leave the nests to forage with the adults soon after hatching, we could rarely keep the broods under observation until they were fully fledged. Consequently, we do not report fledging success; however, all of the broods that we were able to find and associate with nests that we had observed had the same number of fledglings as there had been hatchlings.

There were no significant differences between reference and exposed groups for clutch size ( $X^2=4.011$ ,  $p=0.135$ ) or hatching success ( $X^2=1.231$ ,  $p=0.267$ ). However, the differences in clutch sizes between reference and exposed areas was close to significant, because 4 nests in the exposed group had fewer eggs and there was total uniformity among the clutches in the reference

sites. There was no correlation between selenium concentration and either clutch or brood size (linear regression,  $p < 0.05$ ).



**Figure 3.** Spotted sandpiper productivity and mean egg selenium concentrations (mean  $\pm$  SD). Left axis is mean number of eggs laid and number of nestlings and  $\mu\text{g/g ww}$  for selenium.

We saw no visible, physical abnormalities in either the embryos or the juveniles, but we found 2 dead embryos in eggs from a nest on exposed lower Michel Creek. To evaluate the difference in selenium concentrations between eggs by health status, we divided the samples into four categories of health (excluding 2 damaged eggs): Viable, Damaged, Infertile or Dead. There was no significant difference in health status between the exposed and reference sites ( $p = 0.42$ ).

## DISCUSSION

The mean egg selenium concentrations in American dippers were higher in this study (7.6 to 8.5  $\mu\text{g/g dw}$ ) than those that Morrisey *et al.* (2003) found in coastal streams, which were less than 3.0  $\mu\text{g/g dry weight}$ . Wayland (2003 in preparation) found mean selenium concentrations at coal mine-affected sites in Alberta similar to those in this study, and higher in exposed than reference areas (6.75 and 5.30  $\mu\text{g/g dw}$ , respectively). Taken together, these results suggest that American dippers in the Elk Valley are accumulating selenium, but that the moderate and variable concentrations in their prey throughout the region preclude differences between exposed and reference areas. The higher concentrations in spotted sandpiper eggs from exposed areas suggest an association with mine runoff. The differences in uptake between American dippers and spotted sandpipers may relate to differences in their prey (the sandpipers take smaller invertebrates, and more Dipterans, from the water's edge, rather than feeding on riffle benthos), or metabolism.

Skorupa and Ohlendorf (1991), in a synthesis of toxicity data, suggested a toxicity threshold in birds' eggs of about 8.0 µg/g dw. Fairbrother *et al.* (1999, 2000), reviewing their own and other studies, suggested a mean egg selenium threshold (endpoint=chick mortality) in the range of 12 to 15 µg/g dw for mallards. Based on the Fairbrother *et al.* (1999, 2000) and Adams *et al.* (1998) mallard toxicity models, spotted sandpipers' egg selenium concentrations at the exposed sites would be about at or a little above the thresholds, and below them at the reference sites. American dipper would be below the lower threshold at all sites. This is consistent with our data that showed differences in productivity of spotted sandpipers between exposed and reference areas to be close to the level of statistical significance, while for American dipper, the differences were well below significance levels.

Teratogenesis was a less sensitive endpoint than mortality (Fairbrother *et al.* 1994), and an EC<sub>10</sub> threshold for teratogenesis of 20 µg/g dw selenium has been proposed (Adams *et al.* 1998). All of our bird eggs' selenium concentrations were below that threshold, and we did not observe any deformations in embryos from the 63 eggs that we examined, nor in the 251 chicks that we observed.

Spotted sandpipers had a relatively high rate of infertility/inviability of eggs, but this was not related to selenium concentrations and was not different between exposed and reference areas. The most likely explanation is disruption of nesting success by flooding, which caused delayed nesting and a high incidence of nest abandonment. It is also possible that the highway and industrial activity along lower Michel Creek could have contributed to a relatively high incidence of nest abandonment in that area.

These results beg the question: why were no effects of selenium demonstrated, when the concentrations in water were above provincial (2 µg/L – Nagpal and Howell 2001) and U.S. guidelines (U.S. Environmental Protection Agency 1998), and above concentrations at which effects could occur (Adams *et al.* 1998)? This situation may be similar to that of the Kennecott Utah Copper mine in Utah, U.S.A. There, selenium in the diets of American avocets (*Americana recurvirostra*), black-necked stilts (*Himantopus mexicanus*), coots (*Fulica americana*), and several other waterfowl and shorebird species exceeded levels reported in other studies as associated with reproductive impairment (Ecological Planning and Toxicology Inc and Parametrix Inc 1997). However, as in the Elk River valley, there was no difference in reproductive success at that site compared to the same species using nearby reference sites.

One reason might be the mechanism of bioaccumulation. Selenium occurs in water as dissolved selenite and selenate and as particulate matter (in plankton, suspended organic detritus, elemental selenium and selenite adsorbed on clay particles). Organic selenides occur in a variety of poorly known compounds including selenomethionine, which is highly biologically reactive. It is generally agreed that ingestion is by far the most important uptake route in consumer organisms, that organic detritus is a key pathway, and that bacterial conversion of selenium to organic forms, which occurs mostly in lentic (standing or stagnant water, such as ponds and swamps) systems, is a critical transformation process (U.S. Environmental Protection Agency 1998).



Other reasons for the lack of effects at these exposures might have been the presence of other elements such as boron, arsenic and mercury that are known to act antagonistically with selenium in waterfowl, ameliorating adverse effects; or differences in diet or condition (Hoffman *et al.* 1992, Stanley *et al.* 1994, Stanley *et al.* 1996, Hoffman and Heinz 1998, Adams *et al.* 2003). However, arsenic and mercury were only present at trace concentrations in American dipper and spotted sandpiper eggs in this study (arsenic was below detection limits in most eggs), and were not correlated with selenium concentrations. Thus, antagonistic effects from other elements do not appear to be responsible for the observed lack of adverse effects.

Most of the field studies of selenium toxicity in waterfowl and waterbirds to date have been conducted in lentic systems. Lotic (rapidly flowing) systems, such as those of the study area, have not been well studied. Mechanisms that facilitate transformation of selenium into bioavailable forms and uptake in aquatic systems – uptake of selenium by rooted plants and oxidization by photosynthesis and uptake by bottom-dwelling invertebrates and detritus-feeding fish and wildlife – are prominent in lentic systems, but scarce in lotic systems (Lemly and Smith 1987, Lemly 1999). Lemly (1999) found that 90% of the total selenium in an aquatic system may be in the upper few centimetres of sediment and overlying detritus. The streams sampled for this study, however, arise from glaciers and permanent snowfields and run at high velocity over clean boulders and gravel. Gradients ranged from a -0.6% (Fording River) to -4.1% (Boivin Creek). These are lotic streams that have little sediment and short residence times, in contrast to the lentic systems that comprise the bulk of selenium studies. We hypothesize that the lack of effects in the two waterbirds in this study, despite the high selenium concentrations, is likely due to their lotic environment's low biological transformation and uptake rates.

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