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Environmental Contaminants in Wild Martens (*Martes americana*) and Wolverines (*Gulo luscus*)

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While monitoring contaminants in osprey (*Pandion halieatus* L.) upstream and downstream from pulp mills along the Columbia River and Thompson Rivers (a tributary to the Fraser River) of British Columbia, an unusual pattern of organochlorine contamination was observed in their eggs that could not be accounted for by known local sources of contamination (Whitehead et al. 1993). To test the theory of a non-local source of higher chlorinated dioxins and furans, and to determine if there were any effects of these or other contaminants on aquatic mustelids, Harding et al. (1998) and Harding et al. (1999) analysed contaminants in 2 resident piscivores: mink (*Mustela vison* Schreber) and river otter (*Lontra canadensis* Schreber). To help identify the source of contaminants, whether through atmospheric deposition or liquid effluents, martens (*Martes americana* Turton), and wolverines (*Gulo gulo* L.) were also collected. Both of these mustelids are entirely terrestrial in habitat and prey selection.

Martens are terrestrial/arboreal, preferring mature conifer or mixed wood forests. Average home range size is about 3 km^2 and 1 km^2 for males and females, respectively (Strickland and Douglas 1987). They are opportunistic feeders, varying their diet seasonally with availability of prey; rodents are preferred (Strickland and Douglas 1987).

The wolverine is the largest terrestrial mustelid. The males of the subspecies in this study area (*G. g. luscus*) weigh about 15 kg (Banfield 1974), about twice that of river otters. Krebs and Lewis (1999) found home ranges of wolverine males ($\bar{x}$=1005 km^2) in British Columbia to be significantly larger than females ($\bar{x}$=310 km^2). Wolverines are omnivorous, eating a variety of roots and berries and scavenging and preying on bird nestlings and eggs and small and large mammals.

MATERIALS AND METHODS

Skinned mink, marten and river otter carcasses were collected from commercial trappers during the winters of 1994–1995 (Nov.–Mar.) and 1995–1996 (Nov.–Jan.). The wolverine specimens were similarly collected by the British Columbia Ministry of Environment, Lands and Parks during the winter of 1997. Liver samples were placed in 250 ml hexane-washed jars, frozen and shipped to the

laboratory at the Pacific Wildlife Research Center for dissection and sample preparation. One upper and 1 lower canine tooth of the wolverines were removed for dental cementum analysis of age. Insufficient tissue was available in the marten livers for metals analysis; however, to obtain heavy metal concentrations in martens and provide a comparison between marten and mink kidneys (Harding et al. 1998), all of the marten kidneys were submitted for metal analysis.

Wolverine and marten livers were analyzed individually for organics and metals at the Environment Canada's Pacific Environmental Science Center laboratory in North Vancouver, British Columbia The samples were analyzed by GC/MS for the pesticides aldrin, dieldrin, endosulfan I and II, endrin, heptachlor, heptachlor epoxide, p,p=-DDD, p,p=-DDE, p,p=-DDT and the PCBs. PCBs were reported as Aroclor mixtures 1221, 1232, 1242, 1016, 1254, 1260 and 1262. Livers were homogenized, acetone-extracted and partitioned with 2% NaCl. Extracts were then treated, as necessary, with florisil, concentrated sulfuric acid, activated copper and/or mercury to remove interferences. Glass columns prepared with glass wool, 2% deactivated florisil and heat-treated sodium sulfate were washed with 50 ml hexane. A 2 ml sample extract was loaded, and the first and second fractions were eluted with 35 ml hexane and 150 ml 1:1 hexane/dichloromethane (DCM), respectively. The first fraction contained the PCB, p,p=-DDE, aldrin and heptachlor, while the second fraction contained the remainder of the pesticides. Fractions were roto-evaporated, then quantitated using a HP-5890 series II high resolution gas chromatograph with electron capture detection. Method blanks were included with each run and 20% of the samples were split and analyzed as duplicates. Discrepancies of greater than 20% among duplicates would have been considered acceptable. External 0.1 and 1.0 μg/g PCB standards were analyzed twice during each sample run. Instruments were calibrated at 3 concentrations using pesticide mixture and Aroclor 1260 standards. Average recoveries were 89%, with coefficients of variation of 1.4-12.7%. Minimum detectable concentrations were 0.01 μg/g wet weight for PCB, and 0.002 μg/g wet weight for pesticides.

For metals and selenium analysis, kidneys from martens and livers from wolverines were excised with hexane-washed utensils, weighed, and stored frozen in hexane-washed, heat-treated jars until metals analyses were conducted. Tissues were freeze-dried and digested in acid (in 3 stages, 65% HNO3, 97% H2SO4 and 37% HCl) before analysis. Metals and selenium were analyzed using inductively coupled argon-plasma emission spectroscopy (ICP), graphite furnace (GF: HGA-300 graphite furnace and a Perkin-Elmer 3030b spectrometer) spectrometry for high resolution cadmium and lead and atomic absorption spectroscopy (Perkin-Elmer 3030-AAS) for mercury. Detection limits were 0.02, 0.2 and 0.01 μg/g dry weight for cadmium, lead and mercury, respectively. Detection limits for ICP data ranged between 0.08 and 4 μg/g dry weight.

Lobster and dogfish tissues were used as reference standards. One marten kidney was split and analyzed as a duplicate to measure laboratory variability. Duplicates

revealed <10% variability. In all other cases, graphite furnace results were used for lead and cadmium over ICP results.

One–way analyses of variance (ANOVA) were used with the data for mink and otters reported previously (Harding et al. 1998; Harding et al. 1999) to check for differences among sites and species. Also, associations among the many heavy metals and trace organics were evaluated using Pearson correlation matrices, independently for each species. All statistical evaluations of metals were conducted on dry weight values using SYSTAT™ 5.0 (Wilkinson 1990). Non-detections were expressed as half of the detection limit for the preceding analyses. Graphs were made using Grapher™ (Golden Software Inc., Golden, Colorado).

RESULTS AND DISCUSSION

In all, 5 martens and 13 wolverines were collected, in addition to the 26 mink and 32 otters previously reported (Harding et al. 1998; Harding et al. 1999). Of the wolverines, 5 (38.4%) were female and 8 (61.5%) were male and their ages ranged from 0.5 to 10.5 years. One of the females and 1 of the males could not be aged. For 2 of the wolverines, too little liver tissue was provided for chemical analysis. Of the 5 martens, 4 were males. Females of both species were lighter than males, although differences in body length were unpronounced. Results were analysed together with the 26 mink and 32 river otters reported previously (Harding et al. 1998; Harding et al. 1999).

In marten livers, no organochlorine residues were detected, in contrast to some other wild marten populations in which moderate concentrations were reported (Steeves et al. 1991). In wolverine livers, no organochlorine residues were detected except for trace amounts of DDE (0.07 to 0.098 μg/g wet weight) in 4 of 11 specimens (the detection limit was 0.002 μg/g wet weight).

The absence of any organochlorine pesticides in martens and the absence or low levels in wolverines shows that these terrestrial mustelids have far less exposure to synthetic chemicals than their aquatic counterparts (Harding et al. 1999). This is not surprising, considering their capture in wilderness areas and the lesser biomagnification mechanisms in terrestrial prey, compared to aquatic. Few studies of organochlorine concentrations in wolverines have been reported. Hoekstra et al. (2003) found a wider range and but lower concentrations of organochlorine contaminants and pesticides in 12 wolverine liver samples from the Kugluktuk area west of Great Bear Lake in Nunavut (their higher resolution method produced lower detection limits). For example, they found a mean of 0.0011 μg/g wet weight (range of 0.00014 to 0.0602 μg/g wet weight) of total DDT, DDE and DDD, compared to a maximum of 0.098 μg/g DDE in this study. Poole et al. (1998) found similar means of 0.0095 μg/g DDT, as well as 0.073 μg/g sum (43 congeners) PCB in mink from the same region of the Northwest Territories. In both studies, the authors attributed the source to atmospheric transport.

Both local and atmospheric sources of organic contaminants are likely for the specimens in this study. At the peak of production in 1969, over 900,000 kg of DDT were produced in Canada (Statistics Canada 1971) and used in agricultural regions such as the lower Fraser River valley and the southern portion of the Columbia River valley. About 90,000 kg of DDT were also used for forest pest control in British Columbia from the 1940s through the 1960s (Nigam 1975). Harris et al. (2000) found that DDT and its metabolites have persisted in soils of the Okanagan River valley (a fruit-growing region, part of the Columbia River basin west of the study area) for 20 years after the cessation of use, and that they were transferred to birds through soil invertebrates. However, Donald et al. (1993) found DDT and DDE in lake trout from the Rocky Mountains and attributed the source to long range atmospheric transport from agricultural and industrial regions in Asia. The DDE in wolverines is therefore probably from a combination of current atmospheric sources and past local use. The lower concentrations found in terrestrial compared to aquatic mustelids suggest contrasting uptake pathways, as was found in the European pine marten (*Martes martes* L.) (Bremle et al. 1997).

Concentrations of trace metals and the metalloid selenium in marten kidneys and wolverine livers are given in Table 1. The concentrations were generally low and within the range of values reported elsewhere for mink and river otters (see review by Harding et al. 1998).

Among these results and those reported previously for mink and otters (Harding et al. 1998), there was some variation in mercury in mustelid tissues, with means of 0.18 μg/g to 4.6 μg/g dry weight in livers, but the wolverines had the lowest (and least variable) concentrations. Marten kidneys had mean mercury concentrations of 1.02 μg/g dry weight, or 0.31 μg/g wet weight. Only the mink from the upper Fraser River had mercury as low, but this group was also the most variable, with some values up to 9.0 μg/g dry weight. Wolverine livers had the lowest mean mercury concentration, 0.18 μg/g dry weight, or 0.053 μg/g wet weight. By contrast, in eastern Canada, mink livers and kidneys had higher mean mercury concentrations of 64 μg/g wet weight and 34 μg/g wet weight, respectively (Fortin et al. 2001), reflecting both their aquatic uptake pathways and the geochemistry and industrial history of that region. Mean cadmium concentrations in mink, marten and wolverine livers were all <7.0 μg/g dry weight and not significantly different from each other.

Mink (Harding et al. 1998) and marten kidneys had significantly different concentrations of cadmium ($p=0.032$), magnesium ($p=0.011$), manganese ($p=0.015$), mercury ($p=0.007$), potassium ($p=0.03$), strontium ($p=0.003$) and zinc ($p=0.03$). Mink had higher concentrations of the heavy metals, cadmium, mercury and strontium, while marten had higher concentrations of magnesium, manganese, potassium and zinc. Lead was no different among the 2 species, while cadmium was higher in Kootenay River mink but not Fraser River mink, and mercury was

Table 1. Metals and selenium concentrations (μg/g) in martens and wolverines.

Element	Marten Kidneys (n=5)		Wolverine Livers (n=11)	
	dry wt. (mean±SD)	wet wt. (mean)	dry wt. (mean±SD)	wet wt. (mean)
Aluminum	11.3±5.5	3.4	11.0±11	3.14
Antimony	2.6±0.60	0.79	2.0±0.34	0.724
Barium	0.11±0.07	0.033	0.3±0.2	0.081
Beryllium	0.05±0.01	0.015	0.08±0.01	0.023
Cadmium	0.82±0.06	0.25	0.29±0.11	0.086
Calcium	374±31	114	330±37.9	99.8
Cobalt	<0.4	n.a.	0.4±0.08	0.116
Chromium	<0.4	n.a.	0.5±0.16	0.138
Copper	12.7±1.2	3.9	45.2±5.84	13.8
Iron	777±92	236	1049±114	314
Lead	0.53±0.43	0.16	0.18±0.05	0.054
Magnesium	720±69	219	620±21.6	188
Manganese	4.32±0.49	1.3	9.43±0.88	2.87
Mercury	1.02±0.46	0.31	0.18±0.09	0.053
Molybdenum	0.62±0.14	0.19	2.0±0.1	0.69
Nickel	1.2±0.20	0.36	2.0±1	0.662
Potassium	10471±691	3183	8074±201	2452
Selenium	<4	n.a.	6.2±0.8	1.72
Sodium	5374±368	1634	3890±142	1178
Strontium	0.13±0.03	0.040	0.1±0.03	0.042
Tin	3.4±0.60	1.0	<4	n.a.
Titanium	0.30±0.20	0.091	1.1±0.21	0.322
Zinc	85±10	26	127±9.5	38.4

lower in martens (martens were only obtained from 1 region, the upper Columbia River).

The higher concentrations of mercury in mink may reflect their aquatic prey because of methylation of mercury in sediments and uptake in aquatic biota, as opposed to the terrestrial prey of martens. Northern squawfish (*Ptychocheilus oregonensis*) and walleye (*Stizostedion vitreum*) from the lower Columbia had means of 0.48 - 0.62 μg/g wet weight mercury and 0.21 - 0.40 μg/g wet weight mercury, respectively (Smith 1987), while whitefish (*Prosopium williamsoni*) from the lower Columbia had significantly elevated mercury concentrations (mean of 0.069 μg/g wet weight) (Nener et al. 1995).

Although cadmium toxicity is not well documented in mustelids, studies of other mammals suggest that renal dysfunction occurs at kidney concentrations around 40 to 200 μg/g wet weight (Friberg et al. 1974). Wren (1985) found 58 μg/g wet weight in the kidney tissue of a mercury-poisoned wild river otter.

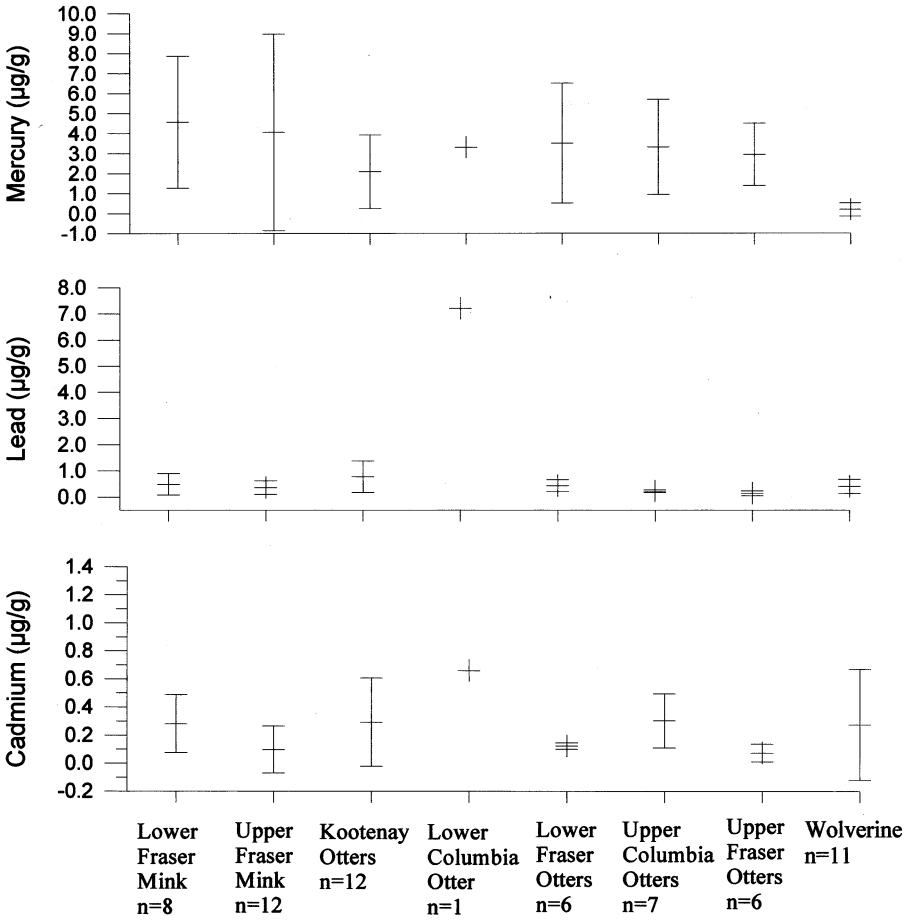

Figure 1. Mercury, lead and cadmium concentrations (µg/g dry weight) in wolverine livers compared to mink and otter data from Harding et al. (1998).

Kidney concentrations of cadmium, iron, magnesium and manganese, and liver concentrations of cadmium all ranged higher than those detected in ranch mink during a dietary background study (Stejskal et al. 1989), but values are still likely not sufficient to produce toxic effects in affected martens.

As with the results for aquatic mustelids (Harding et al. 1998; Harding et al. 1999), the results for these 2 terrestrial mustelids raise little concern for the effects of contaminants on these essentially wilderness populations. They may be considered as background levels (Harding 2002).

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