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Selenium Bioaccumulation in Stocked Fish as an Indicator of Fishery Potential in Pit Lakes on Reclaimed Coal Mines in Alberta, Canada

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Abstract Pit lakes are a common reclamation strategy for open pit mines; however, there is a concern about their water quality and suitability as fish habitat because they are often contaminated by metals or metalloids. This study assessed the exposure of fish and invertebrates to selenium (Se) and other metals and metalloids in pit lakes formed by open pit coal mining in Tertiary (thermal coal) and in Cretaceous (metallurgical coal) bedrock. Juvenile hatchery rainbow trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, were stocked into two thermal coal pit lakes (water Se < 2 μg/L, low water Se) and two metallurgical coal pit lakes (water Se > 15 μg/L, high water Se). Se accumulation in stocked fish and concentrations in invertebrates were characterized over a period of 2 years. In the metallurgical pits, invertebrates had higher Se concentrations and fish accumulated Se to higher levels (exceeding USEPA tissue Se guidelines) than biota in the thermal pits. Rainbow and brook trout accumulated similar concentrations of Se in their muscle and exhibited a similar relationship between whole-body and muscle Se concentrations. These results may be used by resource managers to assess compliance with whole-body tissue Se guidelines and to determine if pit lakes in coal mining areas pose a significant Se risk to wildlife or human health. The high Se exposure in metallurgical coal pits indicates that under the current mining and reclamation strategy, these lakes are not suitable for management as recreational "put and take" fisheries.

Keywords Toxic thresholds · Trout · Metallurgical coal · Thermal coal · Tissue burdens · Aquatic

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Introduction

Open pit mining has become increasingly common as excavation technology improves, leaving behind a worldwide legacy of empty mine pits (Hildmann and Wunsche 1996; Axler and others 1998; Kumar and others 2009) which often extend below the water table, forming lakes. Such mine pit lakes are an integral component of the environmental management strategy for surface mine reclamation (Schultze and others 2010). These lakes are usually very deep with limited littoral zone development, and since catchments tend to be small and wind sheltered (Huber and others 2008), the water column remains poorly mixed and often permanently stratified (Hrdinka and Sobr 2010). While mine pit lakes constitute a significant water resource in some areas, especially in arid and semi-arid regions where natural water bodies are scarce (Kumar and others 2009), their utility is often limited by issues of water quality. Thus, depending on the geochemical conditions, lake water can be contaminated with metals, metalloids, or salts, and can be strongly acidic or alkaline. This poses significant contamination risks to surface and groundwater resources, as well as major remediation challenges (Castro and Moore 2000; Dessouki and others 2005; Neil and others 2009).

Water quality in mine pit lakes generally improves with time depending on the geochemical (Eary 1999), microbial, and biological processes involved (Ronicke and others 2010), but their use as a reclamation strategy requires that they be a stable integrated component of the landscape, posing no health risks to humans or wildlife, and have sufficient integrity to support self-sustaining communities of algae, invertebrates, fish, and other wildlife. Although, ideally, this management practice should be compatible with a wide range of uses including recreational fishing or aquaculture (Axler and others 1998; Mallo and others 2010; McNaughton and Lee 2010), toxic metals and metalloids can bioaccumulate in tissue of fish and other biota (Friedrich and Halden 2011), and potentially pose health risks to humans and wildlife. The creation of mine pit lakes is a common practice; however, their general suitability for fish, and the quality of water with respect to both productivity and potential toxicants, is controversial.

Alberta relies heavily on coal reserves for power production, and the majority of the province's thermal coal is obtained from surface pits in tertiary sandstones. Westward, in the foothills of the Rocky Mountains as slope and elevations increase, these tertiary deposits have eroded exposing older coal seams in Cretaceous age shales that are being surface mined for metallurgical grade coal (Langenburg and others 1989; MacDonald and others 1989). These Cretaceous shales tend to be sulfur (S) and selenium (Se) rich due to intense volcanism associated with orogenic activity in the western mountain ranges during this period. The thermal grade coal mined from the more recent tertiary deposits, tend to be lower in S and Se content (Langenburg and others 1989; MacDonald and others 1989). The presence of Se in mine drainage can pose a significant management challenge since this element, which can be highly toxic to fish (Janz and others 2010), is easily leached from rocks, leading to significant cumulative effects in the downstream drainage system. Although some lakes formed in Se-rich mine pits may be suitable for stocking fish (i.e., fish survive and grow), they may pose a significant problem for managers because the Se that bioaccumulates in their tissues may exceed guidelines for human consumption and pose a hazard to wild vertebrate predators (Luoma and Presser 2009).

Although an element essential for biological function, Se becomes toxic at concentrations only slightly greater than those required; teratogenic deformities and embryonic death are the major toxic effects in oviparous vertebrates exposed to excess Se (Janz and others 2010). The rate of teratogenesis is positively correlated with egg Se burdens in cutthroat trout, bluegill sunfish, rainbow trout, and northern pike (Janz and others 2010). Species-specific sensitivity to Se has been documented in fish (Holm and others 2005; Miller and Hontela 2011). Reproductive effect thresholds range from 16 to 40 µg/g dry weight (dw) Se in the egg or ovary, and there is some evidence that rainbow trout, *Oncorhynchus mykiss*, may be more sensitive to the effects of Se than other salmonids such as cutthroat trout, *Oncorhynchus clarkii*, or brook trout, *Salvelinus fontinalis* (Holm and others 2005; Rudolph and others 2008; Miller and Hontela 2011), although the relative sensitivities to dietary Se have not been compared. The importance of differences in accumulation rates, tissue partitioning, and/or metabolic pathways in species-specific sensitivity to Se has not been investigated thus far. Se bioaccumulates through the food chain, but the greatest accumulation occurs during uptake by primary producers which concentrate Se from the water 10^2 to 10^6 times, while invertebrates and fish have bioaccumulation factors of only 0.6–23 and 1–3, respectively (Luoma and Presser 2009; Presser and Luoma 2010; Stewart and others 2010). The current understanding of Se distribution and its accumulation in invertebrates and fish in mine pit lakes is limited, but most evidence suggests that animals, including fish, are exposed primarily through diet (Luoma and Presser 2009; Stewart and others 2010).

The goal of the present study was to characterize and compare the exposure to Se, and other metals and metalloids in fish and invertebrates from two lakes in coal pits excavated from tertiary bedrock, where Se water concentrations are low (East of Robb, AB) and two pits excavated from Cretaceous bedrock (South of Hinton, AB) where Se water concentrations are higher. Since pit lakes vary in nutrient richness, one lake with high and one lake with low total phosphorus concentration was sampled in each region. Field studies of resident biota, particularly of fish, may be complicated by movement in and out of the impacted system, lack of information on the organism's exposure history, and confounding factors such as age of the fish or temperature fluctuations. To avoid these confounding factors, hatchery-reared juvenile rainbow trout and brook trout of the same age were stocked into pit lakes and sampled over a period of 2 years.

Methods

Study Sites

Natural lakes in the western portion of Alberta are moderately alkaline (50–200 mg/L $CaCO_3$, pH 7.5–8.3) with conductivity 100–250 µS, and ion chemistry dominated by Ca and HCO_3 (Ca $\gg$ Na, Mg, K, and $HCO_3 \gg SO_4 > Cl$) (Mitchell and Prepas 1990). The chemistry of pit lakes more closely resembles groundwater than lakes in the region. Pit lakes in the study region are typically more alkaline, pH (8.03–8.62) and conductivity (360–1,800 μS) are higher, and similar to natural lakes, anions $HCO_3 > SO_4 > Cl$; however, Na is usually >Ca (Table S1). Among the pit lakes of the Alberta foothills, the thermal coal pits excavated from Tertiary age sandstones near Robb (Fig. 1) tended to be shallower, and more closely resembled natural lakes in their chemical make-up than the metallurgical grade coal pits from the older Cretaceous age shales south of Hinton (Table S1). The metallurgical pit lakes tended to be more alkaline, higher in conductivity and pH, and more dominated by Na over Ca, than the thermal coal pits. In addition, both SO_4 and Se levels are higher in the metallurgical pit lakes than the thermal pit lakes (Table S1).

For this study, pit lakes formed by open pit coal mining were chosen based on the type of mine (metallurgical, thermal), access, and size. Pit 24 and Pit 44, located on an open pit thermal coal mine near Robb, AB, Canada (53.1°N, 117.5°W, 1,300–1,500 m elevation, Fig. 1), were characterized by low water Se and are referred to as low (L) Se pit lakes. Luscar Lake and Pit C4, excavated in metallurgical coal mines near Hinton, AB, Canada (53.0 N, 116.7°W, 1,400–1,700 m elevation), were characterized by high water Se and are referred to as high (H) Se pit lakes. Although all four lakes are oligotrophic (Table 1), two pit lakes with higher total phosphorus are referred to as high nutrient (LH, low Se and high nutrient; HH, high Se and high nutrient) and two lakes with ultra low total phosphorus are referred to as low nutrient (LL, low Se and low nutrient; HL, high Se and low nutrient). None of the pit lakes had surficial outflow or inflow, and were closed to recreational fisheries. Morphometric and limnological characteristics of these lakes are described in Table 1. Thus Pit 44 is designated LL, Pit 24 is LH, Pit C4 is HL, and Luscar Lake is designated HH.

Water Quality

Water temperature, oxygen, and conductivity were measured at 0, 5, and 10 m (YSI, model 85), along with Secchi disk transparency and surface pH (VWR, model SP21) at

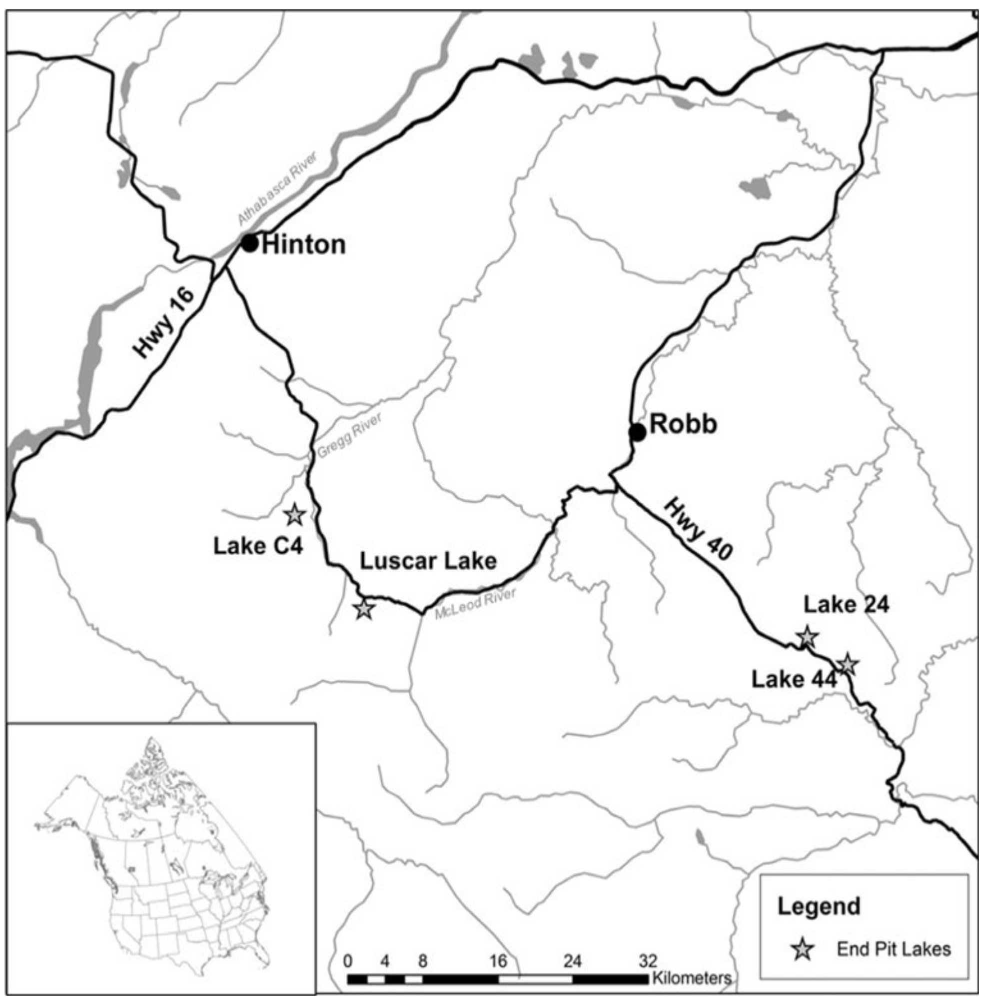

Fig.1 Locations of pit lakes experimentally stocked with rainbow trout and brook trout in coal mining region near Hinton, Alberta, Canada. Low water Se lakes: Pit 44 (LL) and Pit 24 (LH); high water Se pit lakes: Pit C4 (HL) and Luscar Lake (HH)

Table 1 Surface water quality data (mean ± SE), Se accumulation, and morphometric characteristics of the pit lakes stocked with rainbow trout and brook trout in north-eastern Alberta, Canada

	LL (Pit 44)	LH (Pit 24)	HL (Pit C4)	HH (Luscar L.)	*F* statistics, *P* values
Total Se (μg/L water)	2.00 ± 0.17^{B}	0.44 ± 0.08^{A}	16.7 ± 1.3^{C}	50.5 ± 10.1^{D}	$F_{3,16} = 163.71$, $P < 0.0001$
Selenite (% of total)	<DL	100^{B}	8.85 ± 3.04^{A}	4.62 ± 1.24^{A}	$F_{2,96} = 196.31$; $P = 0.0001$
Selenate (% of total)	100 ± 0.00	<DL	89.68 ± 5.53	92.68 ± 3.64	$F_{2,13}$=1.54; $P = 0.2580$
Trophic status	Ultraoligotrophic	Oligotrophic	Ultraoligotrophic	Oligotrophic	
Invertebrate biomass (mg/m^3)	Low 6.54 ± 3.7^{AB}	High 59.52 ± 29.1^{B}	Low 1.71 ± 0.9^{A}	High 51.57 ± 16.5^{B}	$F_{3,16} = 9.16$; $P = 0.0010$
Total Se benthic invertebrates (μg/g dw)	6.55 ± 2.67^{AB}	4.51 ± 0.91^{A}	8.54 ± 2.72^{AB}	13.18 ± 2.67^{B}	$F_{3,27} = 4.12$; $P = 0.0158$
Total Se fish after 24 months (μg/g dw)	12.8 ± 1.8^{A}	11.0 ± 0.81^{A}	28.5 ± 3.1^{B}	31.3 ± 3.4^{B}	$F_{3,65} = 5.1$; $P < 0.001$
Fish Se BAF[a] after 24 months	6,400	25,000	1,710	620	
Benthic invertebrate Se BAF	3,300	11,340	625	375	
Oxygen (mg/L)	8.7 ± 0.6	9.0 ± 0.4	9.0 ± 0.2	8.8 ± 0.8	$F_{3,15} = 0.11$; $P = 0.9501$
Conductivity (μS)	363 ± 17^{AB}	292 ± 15^{A}	666 ± 87^{BC}	803 ± 146^{C}	$F_{3,15} = 9.71$; $P = 0.0008$
Water temperature (°C)[b]	$f = 6.1 \pm 1.4$	$f = 6.3 \pm 0.6$	$f = 3.8 \pm 1.7$	$f = 4.4$	$F_{3,3} = 0.88$; $P = 0.5407$
	$s = 12.1 \pm 1.1$	$s = 13.8 \pm 1.4$	$s = 12.8 \pm 1.5$	$s = 12.2 \pm 1.0$	$F_{3,8} = 0.36$; $P = 0.7849$
pH	8.00 ± 0.20	7.86 ± 0.24	7.97 ± 0.22	7.68 ± 0.28	$F_{3,16} = 0.37$; $P = 0.3699$
Secchi disk depth (m)	2.7 ± 0.6^{A}	6.3 ± 0.8^{B}	2.2 ± 0.3^{A}	1.8 ± 0.4^{A}	$F_{3,16} = 13.22$; $P < 0.0001$
Water hardness (mg/L $CaCO_3$)[c]	72	66	175	112	
Nitrates (mg/L N)[c]	<0.02	<0.02	5.1	4.3	
Nitrites (mg/L N)[c]	<0.02	<0.02	<0.02	<0.02	
Sulfates (mg/L)[c]	67	44	202	211	
Total phosphorus (μg/L)[c]	2.0	7.5	2.0	6.0	
Area (Ha)	8.06	5.00	5.41	10.80	
Max. length (m)	688	492	450	560	
Max. width (m)	132	141	153	340	
Max. depth (m)	24		37	7	
Shoreline length (m)	1,700	1,111	1,143	1,568	
Shoreline development[d]	1.7	1.4	1.4	1.3	
% Area < 3 m	25.3		7.0	65.0	
Elevation (m)	1,336	1,371	1,560	1,696	

Morphometric data sources Stemo (2005); Elk Valley Coal (2006)

DL (detection limits) were 0.1, 0.22, 0.36 μg/L for total Se, selenite, and selenate respectively

[a] Bioaccumulation factor BAF = [Se] in fish and invertebrate tissue/[Se] in water

[b] *f* indicates the fall water temperature and s the summer water temperature. Different letters indicate a significant difference among sites (1-way ANOVA, $P < 0.05$); No effect of species was observed (2-way ANOVA)

[c] Only collected during June 2009 (24 month Se exposure)

[d] Shoreline development = ratio of shore line length to the circumference of a circle with an area equal to that of the lake

each site during all sampling periods (0, 5, 12, 17, and 24 months after stocking of the fish). Surface water samples were collected during each sampling period and frozen without filtering for total Se and Se-speciation analyses. Total Se (dissolved and particulate fraction) was measured (detection limit = 0.1 μg/L) by inductively coupled plasma-mass spectrometry (ICP-MS) (Miller and others 2009) at the Ultra-Clean Trace Elements Laboratory (UCTEL) at the University of Manitoba. Selenate (detection limit = 0.36 μg/L) and selenite (detection limit = 0.22 μg/L) were measured with a non-metallic liquid chromatograph interfaced with an ICP-MS as previously described (Hu and others 2009). Additional surface water samples were collected at 24 months after stocking for analyses of nutrient and trace metal concentrations (49 parameters; Table 2) using standard methods (Eaton and others 2005).

Invertebrates

Pelagic invertebrate samples were collected using a plankton tow net. At each site and sampling period (0, 5, 12, 17, and 24 months after stocking), three 25 m surface tows and three 10 m vertical tows were completed, samples were frozen for later identification to order and Se analyses, and biomass (mg dw/m^3) was calculated. During the 24 month sampling period, kick net samples were also used to collect littoral invertebrate samples from each pit lake. Invertebrates were sorted, identified to order (Clifford 1991), and freeze dried for later total Se analyses. Invertebrate diversity was defined as the order richness (number of orders) in littoral and pelagic invertebrate samples.

Fish

Animal-use protocols were approved by the Animal Care Committee of the University of Lethbridge in accordance with national guidelines. Juvenile rainbow trout (weight = 10.64 ± 2.2 g) and brook trout (weight = 3.13 ± 0.4 g) were obtained from the Allison Creek Brood Trout station (Blairmore, AB, Canada) and the Sam Livingston Fish Hatchery (Calgary, AB, Canada), respectively. At the hatcheries, fish were raised in water containing 0.47 ± 0.11 μg/L Se and fed a diet containing 1.86 ± 0.66 μg/g dw Se. Both species (100 fish/hectare) were stocked into the four pit lakes in June of 2007 and 2008.

Rainbow trout and brook trout were collected at 5, 12, 17, and 24 months after the initial stocking in June 2007. Fish were captured using 1 h gill net sets (22–62 mm mesh) and euthanized with clove oil (160 ppm, emulsified in ethanol). Fish were kept on ice during transportation to the field laboratory. Dissections began 2.5–3.0 h after the last net was retrieved to standardize the travel times from field sites. At the field laboratory, fork lengths and body mass were collected and stomach contents (for diet composition and Se analysis), and muscle samples (for Se analysis and moisture content) were removed and frozen for later analyses. During each sampling time, one average body mass fish of each species from each pit lake was used for whole-body Se analyses. A muscle sample and the gonads were removed from this fish and frozen, with the carcass, for later Se analyses. The whole-body Se concentrations include the gonad and muscle samples. All the rainbow trout sampled for the whole body, muscle, and gonad Se were sexually immature and gonads could not be sexed. Brook trout were sexually immature in the spring and in gonadal recrudescence in the fall.

Stomach contents were sorted and identified to order (Clifford 1991). For each fish, the total volume (mL) of the stomach content and the volume (mL) of each invertebrate order were determined using a 10 mL graduated cylinder. Samples from each order were pooled based on site and sampling period, freeze dried, and weighed. Stomach contents were pooled for Se analyses into the following groups: predators (Hydrachnidia, Odonata, Hemiptera, Hirudinea, and Nematoda), detritivores (Ephemeroptera and Amphipoda), filter feeders and herbivores (Cladocera, Copepoda, Gastropoda, and Pelecypoda), miscellaneous (fish eggs, rocks, plants), terrestrial (Hymenoptera, Neuroptera, Orthoptera, and Lepidoptera), and omnivores (Trichoptera, Coleoptera, and Diptera).

Tissue Se Analyses and Accumulation Indices

Total Se was measured in freeze dried fish muscle tissue and whole-body samples, stomach contents, gonads, and invertebrates, by hydride generation-atomic absorption spectrometry (detection limit = 0.05 μg/g), as previously described (Miller and others 2009). Se bioaccumulation factors (BAF = [Se] in tissue/[Se] in water) and Se Trophic Transfer Factors (TTF = [Se] in fish muscle/[Se] in diet), as described in other studies (DeForest and others 2007; Presser and Luoma 2010) were calculated for rainbow trout and brook trout stocked into the pit lakes and sampled after 24 months.

Statistical Analyses

All statistical analyses were performed using the JMP 7.0.2 software package. Surface water quality data, diversity, invertebrate Se concentrations, diet Se concentrations, and muscle Se concentrations were compared using a one-way ANOVA for site and a post hoc Tukey–Kramer HSD. A nested ANOVA was used to investigate the effect of species on diet composition at each site. Analyses of covariance (ANCOVA) were used to investigate the effect of species and months after stocking (continuous variable) on

Table 2 Trace metals and ion concentrations in the surface water of the four pit lakes in June 2009 (24 month sampling)

Parameter	LL	LH	HL	HH
Ions (mg/L)				
Calcium	18.3	14.0	29.6	17.9
Bicarbonates	210	179	260	444
Bromides	<0.1	<0.1	<0.1	<0.1
Carbonates	0	3	0	9
Chlorides	0.4	0.3	0.9	0.7
Fluorides	0.15	0.09	0.16	0.36
Iron	<0.03	<0.03	<0.03	<0.03
Magnesium	6.4	7.6	24.6	16.3
Manganese	<0.01	<0.01	<0.01	<0.01
$NO_3 + NO_2$ as N	<0.02	<0.02	5.1	4.3
Potassium	4.3	3.5	2.8	2.2
Sodium	85	63	135	251
Trace metals (µg/L)				
Aluminum	29.1	10.8	12.0	63.2
Antimony	<0.3	<0.3	2.4	2.5
Arsenic	<0.04	<0.04	<0.04	<0.04
Barium	75.5	56.5	54.3	69.5
Beryllium	<0.05	<0.05	<0.05	<0.05
Boron	42.1	15.6	40.6	26.7
Cadmium	<0.05	<0.05	<0.05	<0.05
Chromium	<0.1	<0.1	<0.1	0.1
Cobalt	<0.1	<0.1	0.1	0.2
Copper	0.4	0.5	3.5	0.8
Lead	<0.1	<0.1	<0.1	<0.1
Mercury	<0.05	<0.05	<0.05	<0.05
Molybdenum	11.2	0.1	14.4	21.5
Nickle	1.2	0.6	10.9	2.6
Silver	<0.04	<0.04	<0.04	<0.04
Strontium	635	326	1,356	894
Tellurium	<0.07	<0.07	<0.07	<0.07
Thallium	<0.03	<0.03	<0.03	<0.03
Thorium	<0.03	<0.03	<0.03	<0.03
Tin	<0.02	<0.02	<0.02	<0.02
Titanium	0.4	0.1	0.1	0.4
Tungsten	0.1	0.07	0.1	0.2
Uranium	2.1	0.9	5.0	5.3
Vanadium	0.1	0.2	0.1	0.3
Zinc	<0.2	<0.2	1	0.3
Zirconium	<0.01	<0.01	<0.1	0.1

muscle Se concentrations. Two-way ANOVAs were used to investigate the effect of species and site on condition factor and specific growth rate. ANCOVA and linear regression analyses were used to investigate the relationships between whole-body Se concentrations, and gonad and muscle Se concentrations in both rainbow trout and brook trout. All analyses used $\alpha = 0.05$.

Results

Water Quality

Total water Se concentrations were lower in Pit 44 (LL) and Pit 24 (LH) than in Pit C4 (HL) and Luscar Lake (HH) ($P < 0.05$; Table 1). Selenate was the most abundant form of Se present in water column of LL, HL, and HH, but selenite represented 100, 8.85, and 4.62 % of Se in LH, HL, and HH, respectively ($P < 0.05$, Table 1). Surface oxygen concentrations, pH, and water temperature were similar between all four pit lakes (Table 1). Surface conductivity was lower ($P < 0.05$) in the low Se lakes (LL and LH) than in high Se lakes (HL and HH), and water transparency (Secchi disk depth) was the highest in LH ($P < 0.05$; Table 1). Water hardness and sulfates were greater in HL and HH than LL and LH during June 2009 (Table 1). Nitrites were below detection limits at all sites, and nitrates were only detectable in HL and HH. Phosphorus concentrations were low in LL and HL, and slightly higher in LH and HH in June 2009. The cation regime was $Na^+ > Ca^+ > Mg^+ > K^+$ for all of the lakes and the anion regime was $HCO_3^- > SO_4^- > Cl^-$ for all of the lakes (Table 2). With the exception of Se, none of the trace elements measured were above the CCME Canadian Water Quality Guidelines for the protection of aquatic life or the USEPA's Aquatic Life Criteria (Table 2).

Invertebrates

LH and HH both had higher total invertebrate biomass in the water column than LL and HL (Fig. 2a or Table 1; $P < 0.05$); thus, LH and HH were classified as "nutrient-rich" lakes, and LL and HL as "nutrient-poor" lakes. There was a significant positive linear relationship between invertebrate biomass and phosphorus concentrations in the pit lakes ($R^2 = 0.98$; Biomass $= 10.55 \times$ (phosphorus) $-$ 16.37; $F_{1,2} = 121$; $P = 0.008$). Invertebrate tow samples also identified slightly different pelagic community assemblages at each pit lake (Fig. 2a). Pelagic diversity (number of orders; $F_{3,16} = 4.5$; $P = 0.02$) was the greatest in LH (7 ± 1) and HH (7 ± 1), followed by HL (4 ± 1) and LL (3 ± 1). Kick samples collected during June 2009 also showed different littoral community assemblages present in each of the pit lakes (Fig. 2b). Littoral diversity was 11 in LH and LL, 9 in HH, and 8 in HL.

Benthic invertebrates from HH had significantly higher concentrations of Se than those collected from LH (Table 1), while the concentrations in invertebrates collected from LL and HL were intermediate, not significantly different from the other pit lakes. Detritivores and miscellaneous items from the stomachs of fish caught in HH had significantly higher Se content than those from lakes LL and LH

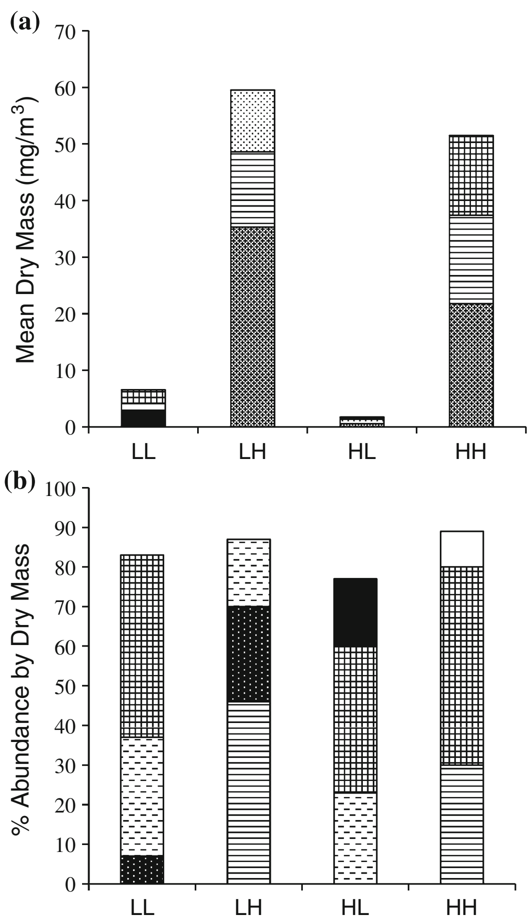

Fig. 2 **a** Invertebrate biomass (mg/m^3) collected during pelagic invertebrate tows and **b** benthic invertebrate community composition (% of dw; kick samples) in June 2009. *Bar shading* indentifies the three most abundant orders at each site. Data shown is an average of all sampling times. Cladocera ( ), Gastropoda ( ), Copepoda ( ), Diptera ( ), Pelecypoda ( ), Ephemeroptera ( ), Odonata ( ), and Trichoptera ( )

(Table 3). No other significant differences in Se concentration of stomach contents were observed (Table 3). Benthic invertebrates (all orders) from the low Se pit lakes (LL and LH) had significantly greater BAF than those from the high Se pit lakes (HL and HH; Table 1).

Fish

Although omnivores were a large part of all fish diets, slight differences in invertebrate prey in stomach contents of rainbow trout and brook trout were observed (Table 3). Rainbow trout consumed significantly less detritivores and more miscellaneous food items than brook trout in LL, but no other species differences were observed (Table 3).

Fish muscle Se concentrations (Fig. 3) were significantly different among sites. Muscle Se concentrations in the hatchery rainbow trout (1.25 ± 0.62 µg/g dw) and brook trout (0.60 ± 0.07 µg/g dw) on Day 0 were similar ($F_{1,54} = 0.94$; $P = 0.33$), and were significantly lower than those in fish sampled after stocking ($F_{4,478} = 273$; $P < 0.0001$). Fish from LH (mean of all sampling times = 6.87 ± 0.37 µg Se/g dw for brook trout; no rainbow trout were caught) and LL (7.42 ± 0.43 µg Se/g dw) had significantly lower muscle Se concentrations than those from HL (20.25 ± 0.60 µg Se/g dw) and HH (27.12 ± 0.87 µg Se/g dw). Fish from HH had significantly higher muscle Se concentrations than fish caught in all the other pit lakes. In LH, brook trout muscle Se concentrations increased with exposure duration (Fig. 3). In LL, brook trout accumulated more Se in their muscle than rainbow trout, but this difference was not apparent until 24 months of Se exposure (Fig. 3). In HL, there was no significant difference in the muscle Se concentrations between the two species; however, muscle Se concentrations increased with time (Fig. 3). In HH, brook trout muscle Se concentrations were greater than rainbow trout concentrations after 5 months of exposure; however, this difference was not evident after 12 months of Se exposure (Fig. 3).

There was no significant effect of fish species on Se BAF; however, there was an effect of site (2-way ANOVA; Table 1). Fish from LH had greater Se BAF than fish from LL, HL, or HH. There was no significant effect of species or site on the Se TTFs ($F_{6,20} = 0.85$; $P = 0.55$; mean of all fish = 2.11 ± 0.20).

There was a significant positive relationship between muscle Se concentrations and whole-body Se concentrations in both rainbow trout (mean body mass at 24 months = 159.9±15.3 g, fork length 22.6 ± 0.78 cm) and brook trout (mean body mass at 24 months = 143.3±12.0 g, fork length 22.0 ± 0.52 cm) stocked into the pit lakes and sampled over a 24 month period ($P < 0.0001$; $R^2 = 0.65$; Fig. 4a) and there was no significant difference ($P = 0.37$) in this relationship between the two species. There was a positive relationship linking immature rainbow trout gonad Se concentrations (GSI < 0.5 %) to muscle Se concentrations ($P = 0.06$, $R^2 = 0.47$; Fig. 4b), and a significant positive relationship between brook trout female ovary (mean GSI = 0.35 ± 0.04 % in spring, and 3.5 ± 0.7 % in the fall) and muscle Se concentrations ($P < 0.0001$, $R^2 = 0.9$). Female brook trout ovary Se levels were 23.55 ± 5.47, 12.24 ± 2.53, 44.93 ± 0.53, and 85.27 ± 12.05 µg/g dw for Lakes LL, LH, HL, and HH, respectively. The relationship between testis and muscle Se concentrations was not statistically significant for the males ($P = 0.89$, $R^2 = 0.007$; Fig. 4b).

Discussion

The objective of the study was to characterize the Se exposure regime (water, invertebrates, and fish) in pit lakes

Table 3 Selenium concentrations[a] and composition (mean ± SE) of stomach contents of rainbow trout and brook trout collected from the pit lakes

Group	LL		LH		HL		HH	
	Se (µg/g dw)	Composition (%)	Se (µg/g dw)	Composition (%)	Se (µg/g dw)	Composition (%)	Se (µg/g dw)	Composition (%)
Detritivores	5.31 ± 0.85^{A}	RT: $6.30 \pm 1.60^{A,*}$	4.84 ± 0.79^{A}	–	11.68 ± 0.91^{AB}	RT: 6.59 ± 1.35^{A}	17.92 ± 4.20^{B}	RT: 18.26 ± 2.46^{A}
		BT: 24.70 ± 3.51^{A}		BT: 35.2 ± 4.56^{A}		BT: 5.71 ± 1.84^{A}		BT: 27.60 ± 4.45^{A}
Herbivores and filter feeders	6.06 ± 1.09	RT: 14.79 ± 4.23	9.16 ± 4.83	–	2.72	RT: 7.82 ± 2.40	9.20 ± 1.86	RT: 16.77 ± 3.50
		BT: 17.99 ± 3.99		BT: 24.50 ± 4.15		BT: 6.80 ± 2.16		BT: 4.63 ± 2.07
Miscellaneous	1.45 ± 0.31^{A}	RT: $26.93 \pm 3.88^{A,*}$	35.87	–	3.80 ± 0.76^{AB}	RT: 16.77 ± 3.01^{A}	11.47 ± 3.22^{B}	RT: 12.58 ± 2.49^{A}
		BT: 8.41 ± 1.50^{B}		BT: 2.63 ± 0.76^{A}		BT: 14.22 ± 2.52^{AB}		BT: 5.12 ± 2.54^{AB}
Omnivores	4.51 ± 0.97	RT: 28.48 ± 3.33	5.74 ± 1.18	–	13.08 ± 1.76	RT: 50.97 ± 4.00	17.13 ± 8.38	RT: 41.62 ± 3.75
		BT: 31.97 ± 3.67		BT: 25.90 ± 3.83		BT: 50.84 ± 3.86		BT: 45.20 ± 5.77
Predators	1.24 ± 0.84	RT: 4.55 ± 2.81^{A}	4.90 ± 0.84	–	18.83 ± 14.56	RT: 3.38 ± 0.85^{A}	11.63 ± 0.29	RT: 2.69 ± 1.59^{A}
		BT: 6.57 ± 1.72^{A}		BT: 8.47 ± 2.36^{A}		BT: 11.12 ± 2.43^{A}		BT: 5.27 ± 2.21^{A}
Terrestrial	–	RT: 0.61 ± 0.37	–	–	2.71 ± 0.85	RT: 3.31 ± 1.07	2.47	RT: 0.63 ± 0.53
		BT: 1.71 ± 1.21		BT: 1.70 ± 0.69		BT: 5.42 ± 1.88		BT: 0.09 ± 0.09

* Indicates a significant fish species difference at a site for a group (nested ANOVA species within pit lake); predators: $F_{3,426} = 3.20$, $P = 0.0234$; detritivores: $F_{3,426} = 4.12$, $P = 0.0067$; filter feeders and herbivores: $F_{3,426} = 1.89$, $P = 0.1299$; terrestrial: $F_{3,426} = 0.74$, $P = 0.5274$; omnivores: $F_{3,426} = 0.11$; $P = 0.9567$; miscellaneous: $F_{3,426} = 6.58$, $P = 0.0002$

[a] Stomach contents of both species were pooled into groups before Se analyses. Different letters indicate a significant difference between sites for each group (1-way ANOVA): detritivores: $F_{3,9} = 9.15$; $P = 0.0043$; miscellaneous items: $F_{3,9} = 24.15$; $P = <0.0001$; omnivores: $F_{3,10} = 2.16$; $P = 0.1557$; predators: $F_{3,4} = 0.46$; $P = 0.7230$; terrestrial: $F_{1,1} = 0.03$; $P = 0.8971$; herbivores: $F_{3,5} = 0.80$; $P = 0.5437$)

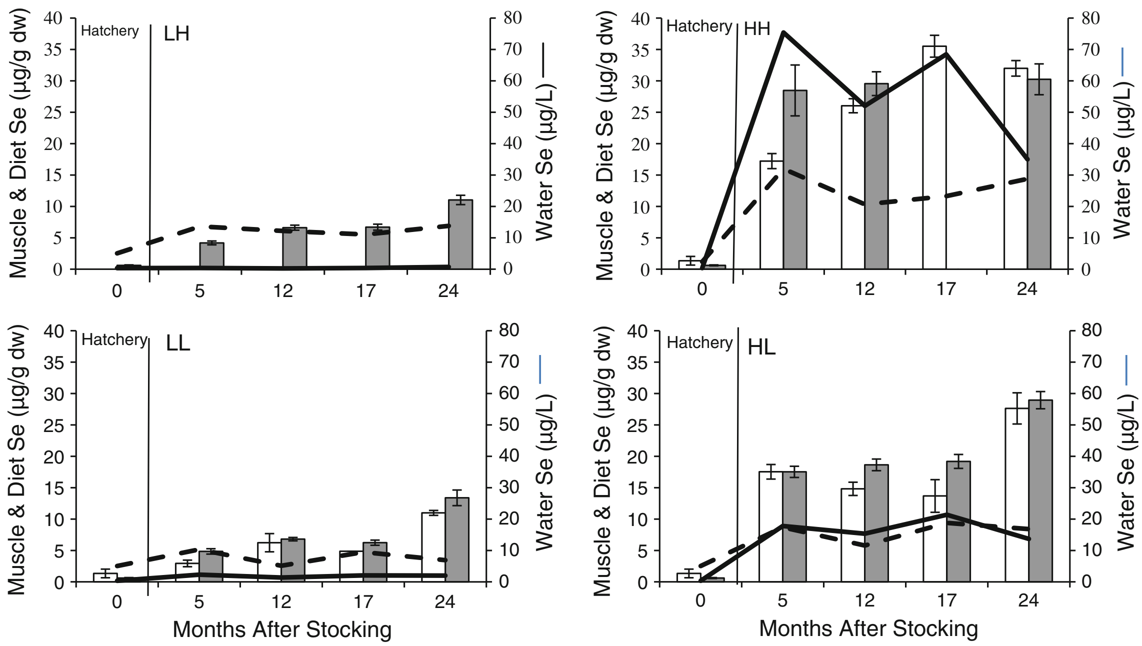

Fig. 3 Selenium concentrations of water (*solid line*), stomach contents (*dashed line*), rainbow trout (mean ± SE) muscle (*open bars*) and brook trout muscle (*shaded bars*) from the pit lakes at 0, 5, 12, 17, and 24 months after stocking. ANCOVAs testing muscle Se concentrations in fish for each lake were significant (LL: $R^2 = 0.39$, $F_{3,109} = 24.38$, $P < 0.0001$; LH: $R^2 = 0.46$, $F_{3,73} = 60.46$, $P < 0.0001$; HL: $R^2 = 0.21$, $F_{3,131} = 12.55$, $P < 0.0001$; HH: $R^2 = 0.28$, $F_{3,100} = 14.30$, $P < 0.0001$). The months after stocking term was significant for each lake ($P < 0.0001$). The species term was significant for LL ($P = 0.0386$) and HH ($P = 0.0162$), but not HL ($P = 0.0845$). There was a significant interaction between species and months after stocking for LL ($P = 0.0495$) and HH ($P = 0.0020$), but not SL ($P = 0.3367$). Muscle moisture was 78.15 ± 0.15 and 78.44 ± 0.11 % for rainbow trout and brook trout, respectively

formed by coal mining and to address water quality and potential habitat suitability concerns of these water bodies, which are becoming a common feature on the post-mining landscapes. The two pit lakes excavated from Tertiary bedrock (thermal coal) had low water Se concentrations (0.44 and 2 µg/L), while the two pit lakes excavated from Cretaceous shale (metallurgical coal) had higher Se (16 and 50 µg/L) and greatly exceeded the Canadian water quality guideline of 1 µg/L. Concentrations of Se in invertebrates and fish reflected the water Se, with rapid bioaccumulation evident in fish at 5 months after stocking and BAFs inversely related to water Se concentrations as previously reported (McGeer and others 2003; DeForest and others 2007). In all the lakes, fish tissue Se burdens increased with time and were significantly higher in the pit lakes with the higher water Se. The USEPA has proposed a whole-body criterion of 7.91 µg/g dw for long-term reproductive effects in fish (USEPA 2004) while Holm and others (2005) indicated a threshold value for rainbow trout deformities between 8 and 10 µg/g (wet weight) for egg Se and 1.8 µg/g for muscle. Using the whole-body: muscle regression equations (similar for rainbow trout and brook trout) developed in this study, the muscle threshold for long-term effects corresponding to this proposed criterion was 7.09 µg/g dw for rainbow trout and 6.34 µg/g dw for brook trout. All of the fish from the high Se lakes exceeded this criterion and fish from the low Se pit lakes also exceeded this criterion after 2 years of exposure. In this study, the Se accumulation did not differ between rainbow and brook trout.

The water quality of the pit lakes selected for this study was representative of the two types of pit lakes in this coal mining area (Table S1), the metallurgical coal pits and thermal coal pits. Besides higher Se and SO_4 concentrations, metallurgical mine pits have higher conductivity, nitrates and Na, and are often deeper. Pit lakes often have elevated concentrations of arsenic and Se, ranging from 0.1 to 3 mg/L in alkaline waters (Eary 1999). In the pit lakes studied here, arsenic was below detection limits; however, Se was elevated in two of the lakes (HL and HH). The Se present in the water column was primarily selenate (89–100 % of total Se), except in one of the low (0.44 µg/L) Se lake (LH) where 100 % of total Se was selenite. The parent rock at these lakes consists predominantly of Cretaceous shale (Langenburg and others 1989), a rock that releases selenate and small amounts of selenite when weathered (Kharaka and others 2001). Conversely, the parent rock at lakes LL and LH originates from the Tertiary period and has a much lower Se content (MacDonald and others 1989). The water Se concentrations in pit lakes LL, HL, and HH exceeded the Canadian Water Quality Guideline for the Protection of Aquatic Life (1 µg/L);

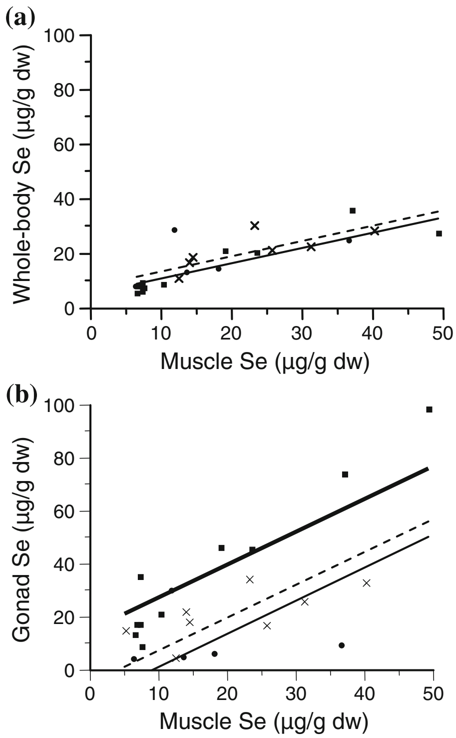

Fig. 4 Relationship between **a** whole-body and muscle Se concentrations for rainbow trout (immature, *dashed line* and *crosses*), whole-body Se = 0.4781(muscle Se) + 9.6710, and brook trout (female, *bold line* and *filled square*; male, *solid line* and *filled circle*), whole-body Se = 0.5766(muscle Se) + 5.0798, collected throughout a 24 month period, and **b** gonad and muscle Se concentrations in immature rainbow trout [gonad Se = 0.5802(muscle Se) + 8.5300] and in female brook trout [ovary Se = 1.8898(muscle Se) + 3.8514]

however, no other trace element or water quality parameter exceeded these guidelines. Sulfate concentrations, although not exceeding guidelines, were elevated in the high Se pit lakes.

The lakes in this study were oligotrophic to ultra-oligotrophic, with low phosphorus concentrations. The pelagic invertebrate biomass was greater in the pit lakes with more phosphorus (LH and HH), regardless of Se concentrations. Pelagic diversity also increased with productivity; however, littoral diversity did not follow this pattern. Instead, littoral diversity was highest in the low Se pit lakes and lowest in the high Se pit lakes. Sensitivity of aquatic invertebrates to Se follows a general taxonomic pattern and sensitivity (based on lethal response to acute water borne Se exposure) from the most sensitive to the least, is as follows: amphipods > dipterans > gastropods > Hirudinea (deBruyn and Chapman 2007). In our study, invertebrate community composition did not reflect the sensitivity pattern described above, but instead appeared to be driven by succession, littoral zone availability and most likely colonization. The ultra-oligotrophic lakes are younger than the other two pit lakes and may still be in the early stages of succession. These younger ultra-oligotrophic pit lakes had more abundant Diptera populations than the older oligotrophic pit lakes, regardless of Se concentration. Changes from a chironomid (Diptera) dominated community to a more natural and diverse community has also been documented in another pit lake as it aged (Wolanski 1999).

Se concentrations were generally greatest in invertebrates collected from the high Se pit lakes. Lemly suggested a dietary threshold concentration of ≤3 µg/g dw (Lemly 1993) to protect fish populations from the toxic effects of Se. In all the pit lakes studied, invertebrate body burdens and stomach contents of fish sampled exceed that dietary threshold, suggesting Se-induced effects on fish may occur. However, insects from reference streams in west-central Alberta had Se burdens of 4–7 µg/g dw (Wayland and Crosley 2006; Andrahennadi and others 2007), also exceeding Lemly's proposed threshold to protect fish populations. Fish collected from these same streams did not exhibit high rates of teratogenesis (Holm and others 2005); thus, the Se body burdens of aquatic invertebrates from the low Se pit lakes appear to be within the normal range for the area and Lemly's dietary threshold may be too conservative.

The diet of rainbow trout and brook trout varied with species and lake, however no clear patterns linking stomach content composition and muscle Se concentrations in rainbow trout or brook trout were evident in the present study. Moreover, since different invertebrate groups in the same lake did not have significantly different Se concentrations, dietary differences among fish species or among lakes should not greatly affect Se bioaccumulation by fish. Similar to the invertebrate Se burdens, fish muscle Se concentrations were higher in the high Se lakes than in the low Se lakes. Moreover, fish muscle Se concentrations increased with exposure duration in lakes LH and HL, and similar but non-significant trends were observed in fish from Lake LL, suggesting that even after a 2 year exposure, Se accumulation in trout from the pit lakes may not have reached equilibrium.

In order to compare fish tissue Se measurements to published guidelines, which are based on whole body (USEPA 2004) and gonad (DeForest and others 2012) Se concentrations, we developed regressions predicting whole-body and gonad Se from muscle Se concentrations. The exceedance of the USEPA whole-body criterion of 7.91 µg Se/g dw for long-term reproductive effects in fish (USEPA 2004) in the high Se pit lakes suggests that long-term reproductive effects could occur in these lentic systems. Similarly, the ovary Se concentrations of brook trout from the high Se lakes exceeds DeForest and others (2012) suggested egg and ovary Se guideline of 20 µg/g dw, indicating the potential for reproductive effects; however, such effects were outside the scope of this experiment since our fish were stocked as very young individuals and no spawning habitat was available. Similar Se exposure regimes in Luscar creek, a lotic system (Holm and others 2005), resulted in larval deformities in rainbow trout.

The development of a muscle Se: whole-body Se regression creates an opportunity for non-lethal monitoring activities, through use of muscle biopsies (e.g., Palace and others 2004). In west-central Alberta, native rainbow trout populations in streams impacted by coal mines are declining, while the introduced brook trout inhabiting the same streams are increasing (Rasmussen and Taylor 2007). The regression equations developed here could be used by fisheries managers to predict whole-body Se concentrations (and thus potential effects) from muscle concentrations, monitoring the Se concentrations without further negatively impacting native rainbow trout populations. The gonad: muscle tissue Se relationship for brook trout observed in the present study is similar to those observed previously for this species (Holm and others 2005), suggesting that species relationships are fairly constant across sites. This relationship is different from those that have been previously observed in rainbow trout; however, fish movement in and out of contaminated areas may account for this difference. Rainbow trout have higher ovary Se burdens for similar muscle concentrations than brook trout (deBruyn and others 2008; Holm and others 2005). In fact, rainbow trout appear to be the fish species with the highest egg Se burden for a given Se exposure (deBruyn and others 2008) and there is some evidence that it may be highly sensitive to Se-induced teratogenesis (DeForest 2008). The rainbow trout captured from the pit lakes in this study were not mature; thus, egg Se regressions are not available.

The pattern of Se bioaccumulation varied among the four lakes but in a manner that reflected the factors known to influence this process. Mean tissue body burdens in fish and in invertebrates from the low Se lakes (LL with water Se of 0.44 μg/L and LH with 2.0 μg/L), were not different between the two lakes despite a ~4-fold difference in water Se concentrations. This is consistent with known patterns of Se uptake by algae at low water Se concentrations; under these conditions, algal body burdens are insensitive to external concentrations, and BAF's are high (10^3–10^4), as reported in other studies (Baines and Fisher 2001; Morlon and others 2006). In the high Se lakes (HL and HH), the tissue body burdens of fish and invertebrates reflect the water concentrations and are highest in Lake HH, where water concentrations are very high (50 μg/L). BAF's in these high Se lakes are lower (10^2–10^3), which likely reflects, at least in part, the 3–5 fold higher sulfate levels in these lakes. Se bioaccumulation by algae and invertebrates is known to be strongly reduced by ion competition with sulfate (Hansen and others 1993; Janz and others 2010; Fournier and others 2010). Although fish and invertebrate tissue Se concentrations were highest in Lake HH, the BAF's in this lake were the lowest of the four lakes studied, and this likely reflected the combined effects of high sulfate and high food abundance. Growth dilution resulting from higher phosphorus levels in growth media has been shown to reduce the bioaccumulation of water borne Se by algae (Riedel and Sanders 1996); and enhanced feeding and growth in mayfly larvae has been shown to reduce their accumulation of dietary Se (Conley and others 2011). Although there were other chemical (conductivity, nitrates, Na) and physical (depth) differences between the high and low Se lakes, effect of these factors on Se bioaccumulation is unlikely.

This study generated a unique data set to characterize the Se status of biota in coal mining pit lakes, including resident invertebrates as well as experimentally stocked fish. Even though all the pit lakes were located on reclaimed mining sites, a significant difference in Se concentrations in water, invertebrates and fish were observed between the two sets of lakes, providing a system for comparisons of the metallurgical and thermal coal mining pit lakes. Whole-body Se: muscle Se relationships were developed for rainbow trout and brook trout, to facilitate non-lethal monitoring of Se exposure and comparison to whole-body tissue guidelines. Fish from the high Se pit lakes accumulated Se above the proposed USEPA criterion for effects.

Although pit lakes in coal mines can be suitable for stocking of trout, regulatory agencies such as the Alberta Fish and Wildlife are sometimes reluctant to manage these as recreational "put and take" fisheries. Risk assessments carried out by the Health Surveillance Branch (Alberta Health and Wellness 2000) consider the Se levels in fish muscle from high Se metallurgical mine pit lakes to pose a significant health risk to people that consumed significant quantities of these fish. Thus, at very least, strict consumption guidelines would be required. These contaminated fish would likely also pose significant hazards to wild vertebrate predators, as No Effects Hazard Concentration (NEHC) for bald eagles diet was exceeded by fish in the high Se lakes (NEHC = 4.0 μg/g ww; 16.0 μg/g dw, Hink and others 2009). The corresponding criterion for mink (NEHC = 1.1 μg/g ww, 4.4 μg/g dw; Hink and others 2009) was exceeded by fish from all of our lakes. It is of interest to note that many pit lakes have shallow shores and are accessible to mammalian wildlife.

An additional management issue in regard to the high Se pit lakes is their potential impact on wild fish populations. While none of the pit lakes in our study had surficial outlets or inlets, several lakes in both mining regions do discharge through fish-bearing streams and in the past wild Athabasca rainbow trout have colonized some of the pit lakes (Casey and Siwik 2000; Elk Valley Coal 2006) and bioaccumulated sufficient Se in their body tissues to make successful reproduction unlikely (Casey and Siwik 2000; DeForest and others 2012). In addition to the hazard that such surficially connected lakes pose as "attractive" habitat to wild fish populations, the water draining from some of these lakes represents a significant input of Se to downstream rivers and may pose a significant cumulative effects threat (Luoma and Presser 2009; Presser and Luoma 2010). Indeed, Alberta Environment (unpublished data) has monitored Se levels in the Gregg River, which receives mine drainage, and in the mainstem of the McLeod River into which it drains, and observed Se levels gradually increasing over the last decade. Thus, with an increasing mine footprint in the watershed, the entire McLeod system could be at risk of crossing Se fish reproduction thresholds in the future. Even lakes that do not presently drain into streams, either because pits are not full or because of natural or artificial barriers, could begin to drain under altered hydrological conditions and pose hazards such as those discussed above. The results of our study indicate that under the current mining and reclamation strategy, the metallurgical coal pits are not suitable for management as recreational "put and take" fisheries.

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