Research Article

Journal of Applied Toxicology

Received: 17 April 2009, Revised: 1 June 2009, Accepted: 2 June 2009, Published online in Wiley InterScience

(www.interscience.wiley.com) DOI 10.1002/jat.1458

The physiological stress response and oxidative stress biomarkers in rainbow trout and brook trout from selenium-impacted streams in a coal mining region

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ABSTRACT: Selenium (Se) is an essential element that can be toxic at concentrations slightly greater than those required for homeostasis. The main chronic toxic effects of Se in fish are teratogenic deformities, but Se can also activate the physiological stress response and redox cycle with reduced glutathione causing oxidative damage. Rainbow trout, *Oncorhynchus mykiss*, appear to be more sensitive to Se than brook trout, *Salvelinus fontinalis*. The objective of this study was to compare the physiological stress response (plasma cortisol, glucose, triiodothyronine, thyroxine, gill Na^+/K^+ ATPase, cortisol secretory capacity, K and liver somatic index) and oxidative stress biomarkers (liver GSH, GPx, lipid peroxidation, vitamin A and vitamin E) in rainbow trout (RNTR) and brook trout (BKTR) collected from reference and Se-exposed streams. The physiological stress response was not impaired (cortisol secretory capacity unchanged); although there were species differences in plasma cortisol and plasma glucose levels. Liver GSH, GPx and vitamin levels were higher in RNTR than BKTR, but lipid peroxidation levels were not different. The elevated GSH reserves may make RNTR more sensitive to Se-induced lipid peroxidation, but this may be offset by the RNTR's higher antioxidant (GPx and vitamin) levels. Species-specific biochemical differences may mediate differences in Se sensitivity and be used in aquatic Se risk assessments. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: rainbow trout; brook trout; selenium; stress response; oxidative stress; vitamin A; vitamin E; cortisol; reduced glutathione; lipid peroxidation

INTRODUCTION

Selenium (Se), an essential element, is a constituent of several key enzymes including the antioxidants glutathione peroxidase and thioredoxin reductase, as well as deiodinase, an enzyme involved in thyroid hormone activation (Köhrle *et al.*, 2005). At concentrations in excess of the homeostatic requirements, Se bioaccumulates in aquatic food chains causing toxicity symptoms in fish and aquatic birds (Hamilton, 2004). Selenium is naturally elevated in soils derived from black shales and phosphate rocks (Haygarth, 1994). Anthropogenic activities, such as coal mining, can release previously unavailable Se into aquatic environments (Lemly, 1999). Elevated Se has been documented on the northeastern slopes of the Canadian Rocky Mountains in fish (Palace *et al.*, 2004a; Holm *et al.*, 2005), invertebrates (Wayland and Crosley, 2006) and water (Holm *et al.*, 2005; Wayland and Crosley, 2006) of streams impacted by coal mining. The current energy crisis has increased the amount of coal mined and burned for power. Thus, there is an urgent need for data concerning the effects of Se released by these activities on aquatic biota.

Vulnerability to Se differs among fish species. Se-linked teratogenesis has been documented in rainbow trout (RNTR), *Oncorhychus mykiss,* (Holm *et al.*, 2005), cutthroat trout, *Oncorhynchus clarki* (Rudolph *et al.*, 2008), channel catfish, *Ictalurus punctatus*, green sunfish, *Lepomis cyanellus*, mosquito fish, *Notropis lutrensis* (Lemly, 2002), razorback sucker, *Xyrauchen texanus* (Hamilton *et al.*, 2005a), white sucker, *Catostomus commersoni* (de Rosemond *et al.*, 2005) and the Sacramento splittail, *Pogonichthys macrolepidotus* (Teh *et al.*, 2004), but not in brook trout (BKTR), *Salvelinus fontinalis* (Holm *et al.*, 2005), and the fathead minnow, *Pimephales promelas* (Halter *et al.*, 1980). Selenium decreased the growth rate of fathead minnows (Dobbs *et al.*, 1996) and RNTR (Hilton *et al.*, 1980), but not bluegill (Lohner *et al.*, 2001). Other physiological systems, such as the physiological stress response, are also influenced by elevated Se (Miller *et al.*, 2007).

The physiological stress response (PSR) enables fish to respond appropriately to stressors and maintain homeostasis (Carr and Norris, 2006). Little is known about the effect of Se on the PSR in fish, but Se activated the PSR in rats (Potmis *et al.*, 1993), eiders (Wayland *et al.*, 2002), and RNTR (Miller *et al.*, 2007) by increasing stress hormone levels. Contaminants can also impair the PSR (Bisson and Hontela, 2002) by damaging components within the signaling pathway through mechanisms such as oxidative stress (Dorval and Hontela, 2003).

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Table 1. Selenium water levels, and muscle Se content of brook trout (BKTR) and rainbow trout (RNTR) from reference and Se-contaminated sites near Hinton, Alberta, Canada (mean of 2005 and 2006)

Site	Site Type	Water Se (μg l^{-1})[a]	Sample size		Muscle Se (μg g^{-1})[b]	
			RNTR	BKTR	RNTR	BKTR
Deerlick Creek	Reference	0.19	20	–	0.62 ± 0.05	–
Wampus Creek	Reference	–	12	–	0.74 ± 0.06	–
Cold Creek	Reference	0.15 ± 0.04	–	27	–	0.32 ± 0.01
Gregg River	Low Se	3.85 ± 1.58	18	24	1.75 ± 0.14	1.35 ± 0.10
Luscar Creek	High Se	24.59 ± 5.71	14	27	3.29 ± 0.60	4.40 ± 0.33

[a]Water samples were not collected from Wampus Creek and only collected from Deerlick Creek in 2005.
[b]Wet weight; RNTR muscle moisuture = 78.70 + 0.12%; BKTR muscle moisture = 78.04 + 0.47%.

Oxidative stress occurs when reactive oxygen species (ROS) overwhelm cellular antioxidants and damage lipids, proteins and DNA, resulting in aging and disease. Reactive oxygen species are by-products of cellular processes such as cellular respiration, but exposure to contaminants, such as Se, can also increase their production (Kelly *et al.*, 1998; Palace *et al.*, 2004b). Antioxidants and oxidative stress damage indicators may be used as biomarkers of contaminant exposure. Lipid peroxidation (LPO) measures lipid damage from oxidative stress while activities of antioxidants such as Se-dependant glutathione peroxidase (GPx) and levels of non-enzymatic antioxidants vitamins A and E may provide a measure of the capacity to prevent peroxidation (Kelly *et al.*, 1998). Species-specific differences in antioxidant status may explain species-specific vulnerability to contaminants such as selenium that induce oxidative stress.

The objective of this field study was to determine if species-specific differences in sensitivity to Se exist in the PSR and oxidative stress biomarkers of RNTR and BKTR from a coal mining area near Hinton, AB. This area contains the only native RNTR population on the east side of the Rocky Mountains, the Athabasca rainbow trout (Carl *et al.*, 1994). In contrast, BKTR in this area are an introduced species from eastern Canada (Nelson and Paetz, 1992) and have been expanding their range within the native RNTR habitat. Documenting the species-specific sensitivities of RNTR and BKTR will help fisheries managers make appropriate decisions here, and in other areas where both species are exposed to elevated Se levels.

METHODS

Chemicals

Sucrose, EDTA, imidazole-HCl, NaCl, KCl, oubain, Na_2ATP, $MgCl_2{\cdot}6H_2O$, $(NH_4)_6Mo_7O_{24}{\cdot}4H2O$, $FeSO_4{\cdot}7H2O$, H_2SO_4, potassium phosphate (KH_2PO_4), Tris–HCl, 2-mercaptoethanol, metaphosphoric acid, butylated hydroxytoluene (BHT), porcine adrenocorticotropic hormone (ACTH) I-39, minimal essential medium (MEM), bovine serum albumin, $NaHCO_3$, Bradford reagent, HPLC-grade dicholoromethane, ethyl acetate, hexane, acetonitrile and methanol as well as the authentic standards tocopherol, tocopherol acetate, retinol and retinol palmitate were purchased from Sigma–Aldrich (Oakville, Ontario, Canada). Dehydroretinol was a gift from Hoffman LaRoche (Basel, Switzerland). Retinyl esters were synthesized as previously described (Palace *et al.*, 1999). MS-222 was purchased from MPBiomedicals (Solon, Ohio, USA). Ultra pure nitric acid was purchased from Fischer Scientific (Ottawa, Ontario, Canada). GOD-PAP reagent was purchased from Roche Diagnostic (Laval, Québec).

Study Sites and Fish Collection

Animal-use protocols have been approved by the University of Lethbridge Animal Care Committee in accordance with national guidelines. Post-spawning adult and immature RNTR, *Oncorhychus mykiss*, and BKTR, *Salvelinus fontinalis*, were collected from the northeastern slopes of the Rocky Mountains near Hinton, Alberta, Canada (latitude 53.40; longitude –117.58), a region with active open pit coal mines. Fish were captured with a Smith-Root LR-24 electroshocker from reference and Se-contaminated streams over three days. RNTR were captured in early June 2005 and 2006 from Wampus Creek (reference), Deerlick Creek (reference), the Gregg River (low Se) and Luscar Creek (high Se) (Table 1). BKTR were captured in late October 2005 and 2006 from Cold Creek (reference), the Gregg River (low Se), and Luscar Creek (high Se). Water samples were collected for Se analysis.

After electroshocking, fish were kept in secure enclosures overnight *in situ*. The next morning, the fish were transported (up to 0.5 h) to the sampling station and kept in oxygenated bins until sampling the same morning. Fish were anesthetised with MS-222 (0.1 g L^{-1}), blood sampled from the caudal blood vessels and sacrificed by spinal transection. Plasma for cortisol, glucose, triiodothyronine (T3) and thyroxine (T4) analyses was recovered (blood centrifuged for 5 min at 16 000 *g*) and flash frozen in liquid nitrogen. Carcasses were kept on ice (maximum 4 h) until dissection. Fork length, weight, sex, maturity and liver weight were recorded. Condition factor [K = (weight × 100)/length^3] and liver somatic index [LSI = (liver weight/body weight) × 100] were calculated. Livers [GPx, reduced glutathione (GSH), LPO, vitamins A and E] and gills (Na^+/K^+ ATPase activity) were removed and flash frozen in liquid nitrogen. The head kidney was also removed for the adrenocortical challenge (see below). Muscle samples for Se analysis were taken from the left side of the fish under the dorsal fin.

Selenium Analyses

Unfiltered water samples were acidified with 0.05% ultra pure nitric acid and analyzed for total Se. Total Se was measured by inductively coupled plasma–mass spectrometry (ICP-MS) on an Elan DRC-II ICP-MS with CH_4 as the reaction gas, as previously described (Miller *et al.*, 2009). Muscle samples were shipped frozen for total Se analysis (hydride generation–atomic absorp-

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tion; detection limit, 0.05 μg g^{-1}) as previously described (Miller *et al.*, 2009).

Biochemical Analyses

Cortisol (07-221102), total T3 (06B-254215) and total T4 (06B-254011) were measured with radioimmunoassay kits from Medicorp, Montréal, Québec, Canada. Assay characteristics including intra- and inter-assay variability were assessed with internal standards as previously described (Levesque *et al.*, 2003). Glucose was determined by incubating (60 min, 23 °C) the sample with GOD-PAP reagent (Roche Diagnostic, Laval, Québec) and measuring the absorbance at 510 nm.

Na^+/K^+-ATPase activity in a gill homogenate was measured by liberating PO_4 from a hydrolysis reaction with ATPase as previously described (Miller *et al.*, 2007). Na^+/K^+ ATPase activity is expressed as U per mg protein and one unit is one μmole liberated PO_4. Protein was determined by incubating (5 min, 23 °C) the sample with Bradford Reagent and measuring the absorbance at 595 nm.

Liver homogenates were prepared in a 50 mM potassium phosphate buffer (GSH, GPx and LPO) and a 50 mM Tris–HCl buffer (GPx) as previously described (Miller *et al.*, 2007). GSH was assayed within an hour, and supernatants for GPx, and LPO analysis were kept at –80 °C for a maximum of 2 days before analysis. Total cellular GPx was determined by measuring the decrease in absorbance (340 nm) due to the decline in nicotinamide adenine dinucleotide phosphate (NADPH) at 23–25 °C as previously described (Miller *et al.*, 2007). GPx activity was expressed as mU mg^{-1} protein and one mU is defined as 1 nmol of NADPH consumed per minute. Liver GSH was determined using the Bioxytech GSH 400 kit (no. 21011), Montréal, Québec, Canada. GSH forms a thioether that reacts with a reagent forming a thiol (400 nm). GSH is expressed as μmol GSH per mg protein. Liver LPO was determined using the Bioxytech LPO-596 kit (no. 21012), Montréal, Québec, Canada. The kit measured the reaction of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HA), end products of the LPO process, with *n*-methyl-2-phenylindole at 45 °C and 586 nm. LPO is expressed as U mg^{-1} protein, where one unit is 1 μM MDA and 4-HA. Vitamins A (retinol, dehydroretinol and total retinyl esters) and E (tocopherol) were analyzed in the liver using an HPLC method previously described (Palace and Brown, 1994) and modified (Palace *et al.*, 1998).

Adrenocortical Challenge

The ability of fish to secrete cortisol was tested by stimulating cortisol production with ACTH in a coarse head kidney homogenate as described previously (Levesque *et al.*, 2003). The homogenate was pre-incubated for 2 h to remove any cortisol secreted during the confinement and handling stress, then the incubation medium was replaced with either MEM (basal cortisol secretion) or 2 units of ACTH in MEM (stimulated cortisol secretion). The ability of the head kidney to secrete cortisol is expressed as the change in cortisol secretion (Δ ng ml^{-1}) after stimulation with 2 units of ACTH (stimulated cortisol – control cortisol).

Statistical Analyses

Data was analyzed using JMP IN 5.1.2. (1989–2002 SAS Institute Inc.). All tests used $\alpha = 0.05$, and data were transformed (log or Box–Cox transformation) to respect normality. An analysis of covariance was used to compare the effect of Se on the PSR and oxidative stress parameters in BKTR and RNTR. Species, muscle Se levels (an indicator of Se exposure), site type (an indicator of other stressors), length, sex and year were considered as covariates. Only the covariates that contributed to a significant amount of the variation in the parameter were included in the final model. A factor contributed to a significant amount of the variation when the *P*-value for that factor in the ANCOVA test was less than $\alpha = 0.05$. The assumption of homogenous slopes was tested and all models except total retinyl esters passed.

RESULTS

Total water Se concentrations were highest at Luscar Creek, followed by the Gregg River and then the reference sites (Table 1). Muscle Se concentrations followed a similar pattern in both BKTR and RNTR with the highest Se concentrations occurring in fish from Luscar Creek (Table 1). At each site, muscle Se levels were similar between the two species.

Models including the covariates that describe a significant ($P < 0.05$) amount of variation in the PSR parameters and the transformations used are given in Table 2. Plasma cortisol levels were significantly influenced by species, sex and site type, but not muscle Se levels (Table 2). BKTR plasma cortisol levels were elevated at both Se exposed sites; this trend was less evident in RNTR (Table 3). The ability to secrete cortisol (adrenocortical challenge) was not significantly influenced by any of the parameters tested (Table 2; data not shown). Plasma glucose levels were significantly ($P < 0.05$) influenced by species and site type and fork length (Table 2). Plasma glucose levels were lower in BKTR than RNTR (Table 3). Gill Na^+/K^+-ATPase activities were significantly ($P < 0.05$) influenced by sex and year (Table 2) with higher gill Na^+/K^+ ATPase activities in the year 2006 (2005 = 0.48 ± 0.02 U mg^{-1} protein; 2006 = 0.68 ± 0.03 U mg^{-1} protein) and in male fish (immature = 0.44 ± 0.05 U mg^{-1} protein; female = 0.52 ± 0.04 U mg^{-1} protein; male = 0.63 ± 0.03 U mg^{-1} protein). The plasma thyroid hormones (T3 and T4), were significantly ($P < 0.05$) influenced by species, year, and length. Plasma T4 levels were also influenced by site type (Table 2). Plasma T3, and especially T4, were lower in RNTR captured in Se exposed streams, but were similar among sites in BKTR (Table 3).

Condition factor was significantly influenced by species, site type and length (Table 2). It was elevated in both RNTR and BKTR caught in Se exposed streams (Table 3). The liver somatic index was significantly influenced by muscle Se levels and sex (Table 2). Liver somatic index increased with muscle Se levels and the females had higher liver somatic indices than the males and immature fish [Fig. 1(B)].

Models including the covariates that describe a significant ($P < 0.05$) amount of variation in the oxidative stress parameters and the transformations used are given in Table 2. Liver GSH levels were significantly influenced by muscle Se levels and species (Table 2). RNTR had higher GSH levels than BKTR and, in both species, liver GSH levels declined with increasing muscle Se levels [Fig. 1(A)]. Liver GPx activities were significantly influenced by species and year (Table 2). Activities were higher in 2005 than 2006, and higher in RNTR than BKTR (Fig. 2). Lipid peroxidation levels in the liver were significantly influenced by sex, site type and fork length (Table 2). Liver LPO levels increased with length, but fish captured in reference sites had higher levels than those captured in the Se-exposed sites (Fig. 3). Liver tocopherol (vitamin

Table 2. Analysis of covariance models that describe a significant amount of variation in PSR and oxidative stress parameters of rainbow trout and brook trout from reference and Se-contaminated streams in 2005 and 2006 near Hinton, Alberta, Canada

Parameter	Data transformation[a]	Adjusted r^2	Model
Physiological stress response			
Plasma cortisol	0.4	0.2906	Species + sex + site type
Plasma glucose	–0.8	0.1465	Species + site type + length
Gill Na^+/K^+-ATPase	–	0.2350	Sex + year
Plasma T3	0.6	0.1975	Species + year + length
Plasma T4	0.6	0.3431	Species + site type + year + length
Adrenocortical challenge	0.4	–	No significant model
Condition factor	1.4	0.2538	Species + site type + length
Liver somatic index	log	0.0661	Selenium + sex
Hepatic oxidative stress parameters			
Reduced glutathione	–	0.4795	Selenium + species
Glutathione peroxidase	log	0.1087	Species + year
Lipid peroxidation	0.2	0.3508	Sex + site type + length
Tocopherol	log	0.4073	Species + Se
Dehydroretinol	log	0.5860	Species + sex + year
Retinol	log	0.3987	Species + sex
Total retinyl esters	0.4	0.1998	Species + Se + species*Se

[a]The number indicates the λ value used in the Box–Cox transformation.

E) and total retinyl esters (storage form of vitamin A) were significantly influenced by species and Se (Table 2). Tocopherol levels were greater in RNTR than BKTR; in both species liver tocopherol decreased with increasing Se exposure [Fig. 4(A)]. Total retinyl esters in the liver of RNTR decreased with increasing Se exposure in RNTR, but Se exposure did not influence liver total retinyl esters in BKTR [Fig. 4(B)]. Liver retinol levels (vitamin A1) were significantly influenced by species and sex while liver dehydroretinol (vitamin A2) levels were influenced by species, sex and year (Table 2). Liver retinol levels were higher in RNTR than BKTR, and were highest in the males of both species (Table 4). Dehydroretinol levels were also greater in RNTR than BKTR, but immature RNTR and male BKTR had the highest levels (Table 4).

DISCUSSION

In this study, fish were free to move in and out of Se-impacted streams, as suggested by earlier studies at these sites (Palace *et al.*, 2007). To control for this movement, muscle Se levels, not site type, were used to define exposure. Muscle Se levels reflect a fish's recent exposure as fish accumulate Se from water and food, and may depurate a significant amount when they move to cleaner environments (Hamilton *et al.*, 2005b). Water and muscle Se levels measured were similar to those documented in previous studies (Holm *et al.*, 2005; Wayland and Crosley, 2006) and muscle Se levels in RNTR and BKTR caught at the same sites were not different. Thus any differences observed in physiological and biochemical endpoints are not necessarily linked to differences in exposure but rather to species-specific differences, including differences in sensitivity to Se.

Plasma cortisol secretion after a confinement challenge was influenced by species, sex and site type, but not muscle Se levels. Similarly *in vitro* cortisol secretion in response to ACTH (the adrenocortical challenge) was not significantly influenced by muscle Se levels. Thus, the PSR of RNTR and BKTR caught in Se-contaminated and reference streams were not impaired. While acute exposure to waterborne Se can activate the PSR of RNTR, there is no evidence that longer exposures to Se impair it (Miller *et al.*, 2007). Other work has shown that RNTR raise their plasma cortisol levels higher than BKTR in response to a confinement stress (Benfey and Biron, 2000) suggesting BKTR are less sensitive to stressors than RNTR. A similar trend was observed in the present study, although it was not as definite. RNTR from the reference and low Se-contaminated streams, but not high Se-contaminated streams raised their cortisol levels higher than BKTR in response to the confinement challenge. This difference may indicate that BKTR are less susceptible to stressors than RNTR but may also relate to species differences in tissue sensitivity or hormone metabolism.

Plasma glucose levels and gill Na^+/K^+-ATPase activities are typically elevated with increased cortisol secretion (Shrimpton and McCormick, 1999; Carr and Norris, 2006). Plasma glucose levels of BKTR and RNTR were not influenced by Se exposure, but they were influenced by species, site type and fork length. BKTR had lower plasma glucose levels than RNTR, as previously documented (Benfey and Biron, 2000). Gill Na^+/K^+-ATPase activities were influenced by year and sex, but not muscle Se levels. This was expected as Se did not significantly influence plasma cortisol levels and cortisol increases gill Na^+/K^+-ATPase activity (Shrimpton and McCormick, 1999). Plasma thyroid hormones (T3 and T4) levels are also influenced by cortisol (Brown *et al.*, 1991). Cortisol can increase the conversion of T4 to T3 and increase the clearance of T3 from the plasma (Brown *et al.*, 1991). Exposure to chemical stressors may increase plasma T3 and T4 levels and subchronic Se exposure increased the plasma T3 and T4 levels of juvenile RNTR (Miller *et al.*, 2007). Our models suggest that plasma thyroid hormones were influenced by fork length and fish species. Larger RNTR had greater T3 and T4 levels, but this relationship was not evident in the BKTR. Thyroid hormone levels in

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Table 3. The effect of site type and species on selected PSR parameters in RNTR and BKTR from reference and Se impacted streams

	Plasma cortisol (ng ml^{-1})		Plasma glucose (mg ml^{-1})		Plasma T3 (ng ml^{-1})		Plasma T4 (ng ml^{-1})		Condition factor	
	RNTR	BKTR	RNTR	BKTR	RNTR	BKTR	RNTR	BKTR	RNTR	BKTR
Reference	188.5 ± 17.3	91.4 ± 13.8	1.12 ± 0.11	0.88 ± 0.03	5.38 ± 0.63	3.25 ± 0.41	19.6 ± 1.70	8.3 ± 1.13	1.04 ± 0.03	0.92 ± 0.02
Low Se exposure	201.6 ± 19.2	162.0 ± 21.3	1.39 ± 0.08	0.87 ± 0.04	4.82 ± 0.60	2.73 ± 0.35	21.9 ± 2.15	12.2 ± 1.18	1.08 ± 0.02	1.08 ± 0.02
High Se exposure	128.6 ± 12.6	140.8 ± 15.6	1.17 ± 0.12	0.99 ± 0.04	3.55 ± 0.54	4.50 ± 0.47	13.1 ± 1.4	10.8 ± 1.05	1.14 ± 0.02	1.06 ± 0.03

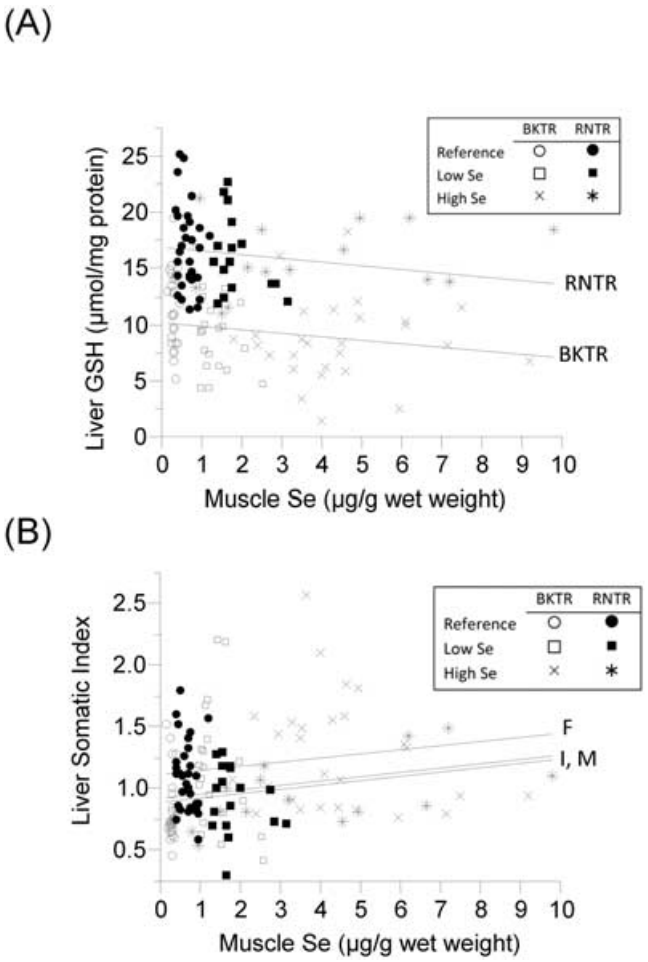

Figure 1. Relationship between muscle Se levels and liver glutathione (A) and liver somatic index (B) of rainbow trout and brook trout caught in reference and Se contaminated streams in 2005 & 2006. The lines represent the significant models described in Table 2.

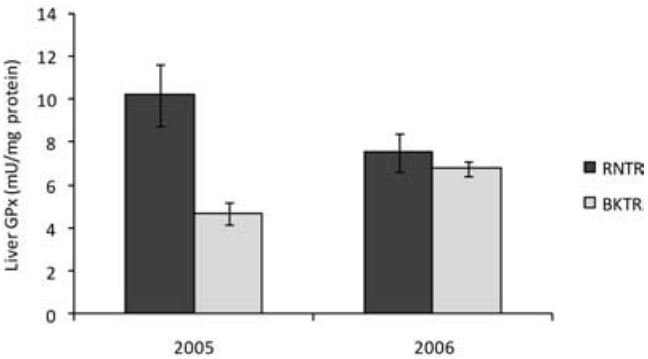

Figure 2. Liver glutathione peroxidase activities of rainbow trout and brook trout from coal mining impacted streams and reference sites in 2005 and 2006.

fish may be linked to growth (Nankervis and Southgate, 2006), but the species-specific relationships between growth and thyroid function have not yet been fully elucidated.

Condition factor and liver somatic index are well-established indicators of tertiary stress responses and changes may result from altered allocation of energy reserves. Condition factor was

significantly influenced by species, site type and length, while the liver somatic index was influenced by muscle Se levels and sex. Condition factor appeared to be elevated in the Se exposed sites, and the liver somatic index increased with muscle Se levels. Generally, with exposure to a contaminant, condition factor (Laflamme *et al.*, 2000) and sometimes liver somatic index (Cleveland *et al.*, 1993) decrease as the fish use their energy to deal with the contaminant, rather than allocating energy for growth and energy storage. Condition factor and LSI may be elevated in fish from contaminated systems if the contaminant alters the food web by eliminating competitors or increases prey abundance (Campbell *et al.*, 2003). Future studies will characterize the food webs in the reference and Se-contaminated streams.

The antioxidant defenses, GSH, GPx, vitamin E (tocopherol), and vitamin A (retinol, dehydroretinol and total retinyl esters) were significantly influenced by species. RNTR had higher GSH level, GPx activities and vitamin levels than BKTR. GPx and GSH were elevated above levels previously measured in laboratory studies (Hilton *et al.*, 1980; Orun *et al.*, 2005). Reduced glutathione levels were also influenced by muscle Se levels. In both species, liver GSH reserves declined with increasing muscle Se levels. A similar result was observed in a laboratory study of juvenile RNTR (Miller *et al.*, 2007), but this is a first field study to report species-specific effects of Se on GSH levels in wild rainbow and brook trout. Reduced glutathione can cycle with methylselenol, a derivative of selenomethionine, and produce a Se radical that can cause oxidative damage (Spallholz *et al.*, 2004). This may explain the decline in liver GSH reserves observed in fish with greater Se

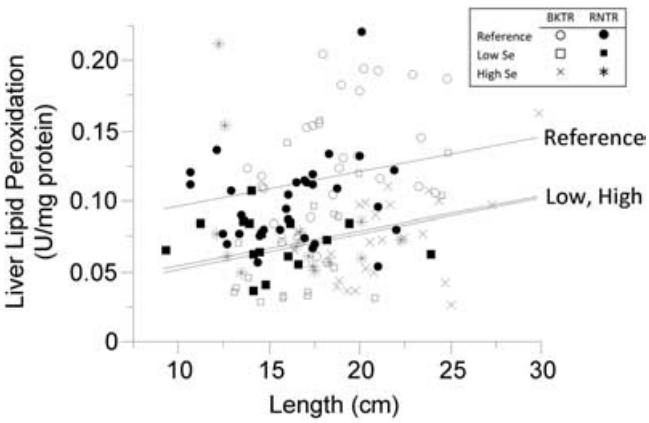

Figure 3. Relationship between liver lipid peroxidation, fork length and site type for rainbow trout and brook trout caught in reference and Se contaminated streams in 2005 & 2006. The lines represent the significant model described in Table 2.

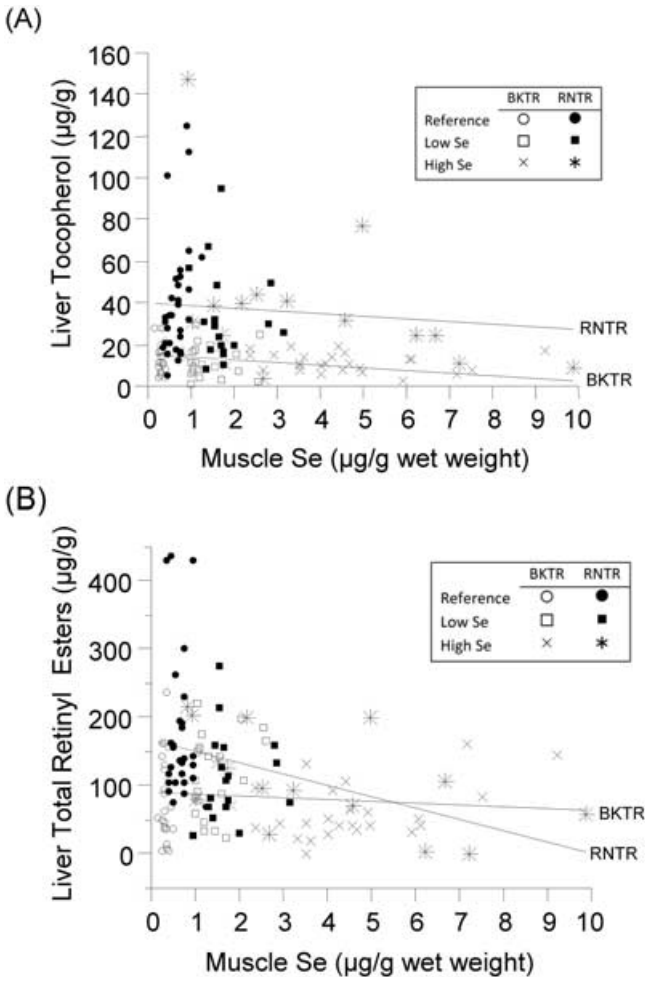

Figure 4. Relationship between muscle Se levels and liver tocopherol (A) and total retinyl esters (B) of rainbow trout and brook trout caught in reference and Se contaminated streams in 2005 & 2006. The lines represent the significant models described in Table 2.

Table 4. Liver dehydroretinol and retinol levels (mean + SE) in RNTR and BKTR from Se contaminated and reference streams

	Dehydroretinol (µg g^{-1})[a]		Retinol[a,b] (µg g^{-1})	
Sex	RNTR	BKTR	RNTR	BKTR
Male	21.21 ± 3.82	5.25 ± 0.77	26.86 ± 4.29	7.23 ± 0.77
Female	13.83 ± 2.34	2.59 ± 0.31	15.93 ± 3.12	3.56 ± 0.39
Immature	24.09 ± 14.59	2.96 ± 0.43	6.56 ± 1.82	5.95 ± 2.37

[a]Mean of 2005 and 2006 samples.
[b]RNTR and BKTR caught at all sites in 2005 (RNTR = 24.93 + 4.41; BKTR 6.67 + 0.77) had higher retinol levels than those caught in 2006 (RNTR = 15.68 + 1.94; BKTR = 4.48 + 0.50). No significant differences between fish from reference and Se-contaminated streams were detected; samples were pooled.

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loads. Excess GSH in the presence of Se can create reactive oxygen species, and if antioxidant defenses are inadequate, cells may be damaged. RNTR have more GSH available to cycle with Se than BKTR. This may make them more vulnerable to Se-induced oxidative damage or lipid peroxidation.

Lipid peroxidation levels were not influenced by Se exposure or species, but by site type, sex and length. Similarly, the LPO levels of RNTR were not altered by sodium selenite exposure in the laboratory (Orun *et al.*, 2005; Miller *et al.*, 2007). This suggests that the antioxidant defenses of these fish were adequate and any oxidative radicals formed by Se-GSH interactions did not cause excessive damage. RNTR may be more vulnerable to Se-induced oxidative damage, but they also had higher antioxidants (GPx, vitamin E and vitamin A) than BKTR. The vitamins and GPx scavenge ROS and protect cell membranes from lipid peroxidation (Kelly *et al.*, 1998); thus RNTR have more protection from ROS induced damage than BKTR. RNTR liver vitamin E levels and the storage form of vitamin A (retinyl esters) levels decreased with increasing Se exposure, supporting the hypothesis that vitamins A and E protect against Se-induced oxidative stress and become depleted during chronic exposures. BKTR vitamin E levels also decreased with increasing Se exposure, but the retinyl esters (storage form of vitamin A) did not. If there is less GSH available in BKTR to cycle with Se and create ROS, then BKTR will require fewer antioxidants and vitamin A stores should not deplete as fast. Oxidative damage has also been linked to the aging process. Lipid peroxidation levels were slightly (but not significantly) elevated in 3+ year bullhead and RNTR than 1+ year fish (Otto and Moon, 1996), suggesting that the increase in LPO levels observed in this study with fork length may be due to the aging process. However, this hypothesis requires further laboratory testing.

The major chronic toxic effects of Se are Se-induced reproductive deformities and the subsequent decline in reproductive success (Hamilton, 2004). Rainbow trout exhibit these deformities at a higher rate than brook trout when exposed to similar amounts of Se in the field (Holm *et al.*, 2005). In fish, liver stores of vitamin A and E are mobilized to the ovary during gonad development. Once in the gonad, they can act as non-enzymatic antioxidants and decrease deformity rates (for a review see Palace and Werner, 2006). In the present study, RNTR had higher liver vitamin E and A levels than BKTR, but both tocopherol and retinal esters decreased with increasing Se exposure. The rate of decrease in total retinal esters was greater for RNTR than BKTR; therefore, RNTR exposed to high levels of Se may have fewer retinal esters (vitamin A) available to mobilize to their eggs than BKTR. If RNTR exposed to Se mobilize less vitamin A to their eggs than BKTR, RNTR may be more susceptible to Se-induced oxidative stress during embryo development. This mechanism may operate additively with the one previously described by Palace *et al.* (2004b) to cause a greater Se-induced deformity rate in RNTR than BKTR.

In conclusion, chronic exposure to environmental Se did not activate or impair the PSR in RNTR or BKTR and Se did not influence cortisol secretion, glycemia or thyroid status. However, there were species differences in some of the PSR parameters, including plasma cortisol and glucose. Rainbow trout may be more sensitive to Se-induced oxidative damage, as they have larger GSH reserves, but this may be offset by their higher antioxidant levels. Selenium depleted liver vitamin stores more rapidly in RNTR than BKTR, and this may contribute to the species specific Se-induced teratogenesis. More research is needed to link cellular (biochemical) species-specific effects of Se to the population (competition) effects in RNTR and BKTR.

Acknowledgments

We would like to thank R. Flitton, W. Warnock, I. Harper, C. Friesen, K. Wautier, G. Sterling and R. Hawryluk for their assistance in the field and the laboratory. We would also like to thank S. Mittermuller for the muscle Se analysis as well as Dr F. Wang and D. Armstrong (University of Manitoba) for the water Se analysis. This project was funded by the Natural Sciences and Engineering Research Council's (NSERC) Metals In The Human Environment-Strategic Network (MITHE-SN, Project A6) and an NSERC post graduate scholarship (PGSM-302510-2004) to L. L. Miller.

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