Aquatic Toxicology 105 (2011) 643–651

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Attenuation of the cortisol response to stress in female rainbow trout chronically exposed to dietary selenomethionine

Steve Wiseman[a,*], Jith K. Thomas[a], Landon McPhee[a], Olesya Hursky[a], Jason C. Raine[a], Michael Pietrock[a,b], John P. Giesy[a,b,c,d,e,f,g], Markus Hecker[a,h], David M. Janz[a,b]

[a] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5B3
[b] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5B3
[c] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[d] Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong SAR, China
[e] School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China
[f] Department of Zoology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
[g] State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University, Nanjing, China
[h] School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5CB

ARTICLE INFO

Article history:
Received 28 July 2011
Received in revised form 2 September 2011
Accepted 8 September 2011

Keywords:
Stressor
MC2R
Cortisone
Stress response
PBR
Corticosteroidogenesis
Selenium
Cortisol

ABSTRACT

Selenomethionine (Se-Met) is the major dietary form of selenium (Se). While Se is a required nutrient, it can also influence the physiological stress response because it stimulates greater concentrations of cortisol in blood plasma of exposed fish. However, little is known about the effects of exposure to Se on the ability to cope with a secondary stressor. In the current study, female rainbow trout were exposed to an environmentally relevant dietary concentration (8.47 mg Se/kg dry mass (dm)) of Se-Met for 126 d, after which time fish were subjected to a 3-min handling stressor and sampled at 2 h and 24 h post-stressor exposure. Concentrations of cortisol, cortisone, glucose, and lactate in blood plasma and concentrations of glycogen and triglycerides in liver and muscle were determined. Abundances of transcripts of proteins involved in corticosteroidogenesis were determined using quantitative RT-PCR. Concentrations of cortisol were significantly greater in blood plasma of trout exposed to Se-Met, relative to control trout sampled prior to the handling stressor. A typical response of cortisol to the handling stressor was observed in the control trout. However, trout exposed to Se-Met were unable to mount a cortisol response to the handling stressor. Concentrations of cortisone, the inactive metabolite of cortisol, were significantly greater following the handling stressor in trout exposed to Se-Met. In trout exposed to Se-Met, transcript abundance of melanocortin 2 receptor (*mc2r*) and peripheral benzodiazepine receptor (*pbr*) were greater, which is consistent with the conclusion that synthesis of cortisol was greater. However, abundances of transcripts of cytochrome P450 side-chain cleavage (*p450scc*) and cytochrome P450 11B1 (*cyp11b1*) were not significantly different between controls and Se-Met exposed trout. Exposure to Se-Met affected accumulation and tissue partitioning of glycogen and triglycerides in liver and muscle as concentrations of these energy reserves were greater in muscle, but not liver. Concentrations of glycogen and triglycerides in muscle, but not in liver, were lesser following the handling stressor suggesting that the muscle energy reserves are an important source of energy required for recovery from the handling stressor. The results of the study demonstrate that chronic exposure to dietary Se-Met elicits a stress response, but prevents a cortisol response to a secondary handling stressor, most likely due to cortisol inactivation. Moreover, exposure to Se-Met has effects on concentrations of energy reserves that are important for providing the energy necessary to cope with a secondary stressor.

1. Introduction

Stressors of natural and anthropogenic origins threaten organism health by disrupting homeostasis. Organisms have evolved a suite of mechanisms, collectively termed the integrated stress response, that are crucial for helping the organism regain homeostasis upon exposure to stressors (Wendelaar Bonga, 1997; Sapolsky et al., 2000). During the primary phase of the responses of

* Corresponding author at: Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK, Canada, S7N 5B3. Tel.: +1 306 966 4912; fax: +1 306 970 4796.
E-mail address: steve.wiseman@usask.ca (S. Wiseman).

0166-445X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2011.09.002

teleosts to a stressor, neuroendocrine factors stimulate chromaffin cells and the hypothalamic–pituitary–interrenal (HPI) axis, which results in release of catecholamines and glucocorticoid hormones, respectively. The release of catecholamines results in shorter-term metabolic adjustments such as the stimulation of glucose production by glycogenolysis, whereas cortisol results in longer-term homeostatic adjustments including stimulation of gluconeogenesis (Wendelaar Bonga, 1997; Mommsen et al., 1999). The secondary phase of the stress response is characterized by an increase in circulating concentrations of glucose and lactate in blood plasma, which is largely dependent upon the actions of the neuroendocrine system involvement in the primary response (Mommsen et al., 1999).

In teleost fish, biosynthesis and release of cortisol is regulated by the HPI-axis (Mommsen et al., 1999; Wendelaar Bonga, 1997). In response to a stressor, corticosteroid releasing factor (CRF) is released from the hypothalamus and stimulates release of adrencorticotropic hormone (ACTH) from the anterior pituitary. ACTH binds to the melanocortin 2 receptor (MC2R) in steroidogenic interrenal cells of the head kidney, and activates corticosteroidogenesis (Aluru and Vijayan, 2008). Cortisol biosynthesis is initiated when cholesterol is transported from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR; Stocco et al., 2005) and the peripheral-type benzodiazepine receptor (PBR; Papadopoulos, 2004). The rate-limiting step in cortisol synthesis is the conversion of cholesterol to pregnenolone by cytochrome P450 side chain cleavage (P450scc). The terminal step in this process is the conversion of 11-deoxycortisol to cortisol by cytochrome P45011β1 (CYP11B1), commonly termed 11-β-hydroxylase (Payne and Hales, 2004).

In all vertebrates, including fish, selenium (Se) is an essential micronutrient. With the exception of higher plants and yeast, Se is an integral component of selenoproteins found in all organisms (Hesketh, 2008, reviewed in Janz, 2011). Selenium exists as inorganic (selenite, selenate) and organic (seleno-amino acids and selenoproteins) forms, with the seleno-amino acid selenomethionine (Se-Met) being the predominant dietary source of Se to fish (Fan et al., 2002). Dietary concentrations of 0.1–0.5 μg/g dry mass (dm) are required to maintain normal Se-dependent physiological processes, including growth. However, when dietary concentrations exceed 3.0 μg/g dm there is the potential for rapid concentration-dependent bioaccumulation to toxic concentrations (Lemly, 1997, reviewed in Janz et al., 2010). Elevated concentrations of Se can lead to a suite of adverse biological effects, including developmental deformities, impaired growth, and mortalities (Woock et al., 1987; Muscatello et al., 2006). Recently, it has been demonstrated that elevated concentrations of Se can also affect the endocrine system of fish. Specifically, chronic dietary exposure to Se-Met resulted in increased blood plasma concentrations of the sex steroid hormones estradiol (E2) and testosterone (T) of female rainbow trout (*Oncorhynchus mykiss*) (Wiseman et al., 2011).

Evidence to date suggests that Se may also influence the physiological stress response because it can stimulate greater concentrations of cortisol in exposed organisms. Whole body concentrations of cortisol in zebrafish exposed to 26.6 μg/g dm of dietary Se-Met were greater than those in unexposed zebrafish (Thomas and Janz, 2011). Concentrations of cortisol in blood plasma of rainbow trout exposed to 2.52 or 3.60 mg/L waterborne sodium selenite (Na_2SeO_3) for 96 h, or 0.36 mg/L sodium selenite for 30 d were greater than those in control trout that were not exposed to Se (Miller et al., 2007). Concentrations of cortisol were greater in brook trout (*Salvelinus fontinalis*) from Se-impacted streams than did those of trout from a reference site (Miller et al., 2009). Although exposure to Se has the potential to modulate cortisol dynamics in fish, the effect of dietary exposure to Se-Met on the integrated stress response to a secondary stressor has not been investigated. Therefore, in this study we investigated the potential impact of environmentally relevant concentrations of dietary Se-Met on the stress response of female rainbow trout. Concentrations of Se mimicked those measured in fish and invertebrates collected from Se-impacted sites (Lemly, 1997; Fan et al., 2002; Hamilton, 2004; Muscatello et al., 2006). After chronic exposure to Se-Met, trout were subjected to a handling stressor and the physiological stress response to this secondary stressor was investigated.

2. Materials and methods

2.1. Chemicals

Seleno-L-methionine (purity > 98%) was purchased from Sigma–Aldrich (Oakville, ON, Canada). Cortisol-d_4 and cortisone-d_3 were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

2.2. Preparation of Se-Met laced trout food

A Se stock solution of 1 g/L nominal concentration was prepared by dissolving 250 mg seleno-L-methionine (Sigma–Aldrich) in 100 mL of nanopure water. A 15 mL aliquot of this stock solution was diluted in 375 mL of nanopure water and this solution was mixed with 1 kg of crushed commercial trout pellet (Martin Classic Sinking Fish Feed, Martin Mills Inc., Elmira, ON, Canada). The resulting "paste" was processed in a noodle maker, frozen at −70 °C, and broken into pieces of approximately 5 mm^3.

To determine the concentration of Se in control and spiked food, samples of each diet were lyophilized and homogenized by use of a mortar and pestle. An aliquant of 100 mg of homogenized food was cold-digested in Teflon vials by use of 5 mL of ultra-pure nitric acid and 1.5 mL hydrogen peroxide. After digestion, samples were concentrated on a hot plate (<75 °C) and reconstituted in 5 mL of 2% ultra-pure nitric acid. Reconstituted samples were stored at 4 °C until analysis. Total concentrations of Se were determined by use of inductively coupled plasma mass spectrometry (ICP-MS) at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK, Canada). A limit of quantification (LOQ) of 0.5 μg Se/g dm food was determined from method blanks. Recovery of Se was determined using certified reference material (TORT-2, lobster hepatopancreas, NRC, Ottawa, ON, Canada). The average concentration of Se in the spiked food was 8.47 μg/g dm. The average concentration of Se in the untreated pellets used to feed the control fish was 2.03 μg/g dm.

2.3. Experimental protocol

Experimental protocols with live fish were conducted in the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan's Toxicology Centre, which is an approved animal use facility. This work was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Female rainbow trout were approximately 1.5 yr of age and were randomly selected from an in house stock reared from eggs obtained from a commercial supplier (Troutlodge, Sumner, WA, USA). Prior to this study trout were reared in 1666 L tanks supplied with running water at approximately 6 °C and maintained under a 12L:12D photoperiod. Trout were fed to satiety once daily with a commercial trout feed (Martin Classic Sinking Fish Feed).

For the experiment 120 trout were randomly assigned to six 719-L tanks supplied with continuous running water at a flow rate of 4 L/min and maintained at approximately 6 °C under a 12L:12D photoperiod. Three of the tanks were randomly designated for control trout and three tanks were randomly designated for Se-Met exposed trout. The mean weight of trout at initiation of the exposure was 147.6 ± 15.5 g. During the exposure trout were fed

approximately 1.5% bodyweight of control or Se-Met laced feed 6 days per week, split between 2 daily feedings to ensure complete consumption of the food. The duration of the exposure was 126 days.

Following the exposure period trout were subjected to a standardised handling disturbance in which trout were netted and held out of the water for 3 min. Fish were collected for quantification of parameters prior to the handling disturbance and then 2 or 24 h after the stress of handling was applied. Sampling was performed by quickly netting individual trout and immediately anaesthetizing with 150 mg/L MS-222, which induced anesthesia within 2 min. Fish mass (g) and length (cm) were recorded for calculation of condition factor (k). Blood was collected from the caudal vein using heparinized syringes and stored at 4 °C overnight to ensure complete clotting and then centrifuged at 2000 × g for 15 min at 4 °C. Samples of plasma were frozen for subsequent quantification of corticosteroids, lactate and glucose. Head kidney tissue was excised and stored at −80 °C until needed for analysis of target gene transcript abundances. Livers were massed for calculation of the liver-somatic index (HSI), and then frozen at −80 °C until later analysis of glycogen, triglycerides, and total concentration of Se. Muscle tissue was excised from the area posterior to the dorsal fin and frozen at −80 °C until later analysis of glycogen and triglycerides.

2.4. Quantification of selenium in liver

Total concentrations of Se in liver were determined by use of ICP-MS at the Toxicology Centre (University of Saskatchewan) exactly as described above for the determination of concentrations of Se in food. A LOQ of 0.5 μg Se/g dm tissue was determined from method blanks. Mean moisture content of liver, calculated as the difference in mass between fresh tissue and lyophilized tissue, was 74.5 ± 1.16%.

2.5. Quantification of corticosteroids in blood plasma

Concentrations of cortisol and cortisone in blood plasma were determined as described previously with a few modifications (Chang et al., 2009, 2010). Briefly, surrogate deuterium-labeled standards were spiked into 450 μL of plasma and the samples were extracted two times with 2.5 volumes of diethyl ether by vortex-mixing for 1 min followed by centrifugation at 8000 × g for 5 min. The water phase was discarded, the solvent phase was evaporated under nitrogen, and the dried residue was dissolved in 200 μL methanol before LC–MS/MS analysis.

Separation and quantification of hormones was conducted by use of an Agilent 1200 series high pressure liquid chromatography (HPLC) system (Santa Clara, CA, USA) connected to an API 3000 triple-quadrupole tandem mass spectrometer (MS/MS) system (PE Sciex, Concord, ON, Canada). Chromatography was performed using 0.1% formic acid (solvent C) and methanol (solvent D) with a gradient elution of C:D = 65:35 (0–2 min), 45:55 (2–10 min), 0:100 (10–18 min) at a flow rate of 250 μL/min. Extracts were separated at room temperature on a Betasil C18 column (100 mm × 2.1 mm, 5 μm particle size; Thermo, Waltham, MA, USA) before MS/MS analysis. To correct for any losses of analytes during sample preparation, and to compensate for variations in instrument performance between injections, labeled surrogate standards were used, based on the methods described by Chang et al. (2010). Cortisol-d_4 was used for cortisol and cortisone-d_3 was used for cortisone. Quantitative results for each of the analytes were calculated using the peak area ratios of the analyte to its surrogate in blood plasma samples and the corresponding calibration curve. The linear calibration range studied was 0.5–1000 ng/mL with coefficients of determination greater than 0.99. The LOQ for both cortisol and cortisone was 0.5 ng/mL. Where hormone concentrations were less than the LOQ a value of one half the LOQ was used for statistical purposes. Quantification of analytes in the blood plasma was conducted by use of the AB Sciex Analyst 1.4.1 software (Applied Bioscience, Foster City, CA, USA).

2.6. Quantification of glucose and lactate in blood plasma

Concentrations of glucose in blood plasma were determined according to the method described by Goertzen (2011). Concentrations of lactate in blood plasma were determined by use of a commercially available kit according to the manufacturer's instructions (Eton Bioscience Inc., San Diego, CA).

2.7. Quantification of triglycerides in liver and muscle

Concentrations of triglycerides in muscle and liver were determined using a commercially available kit (Sigma–Aldrich, Oakville, ON, CA) developed by McGowan et al. (1983) and validated for measuring triglycerides in fish samples (Weber et al., 2003). A glycerol solution was used to develop the standard curve.

2.8. Quantification of glycogen in liver and muscle

Concentrations of glycogen in muscle and liver were determined by use of a modified version of the methods of Gómez-Lechón et al. (1996). Reagents for the glycogen assay reagents were purchased from Sigma–Aldrich. The standard curve was created using purified Type IX bovine liver glycogen.

2.9. Real-time PCR

Total RNA was extracted from approximately 30 mg of head kidney or liver tissue using the RNeasy Plus Mini Kit (Qiagen, Mississauga, ON, CA) according to the manufacturer's protocol. Purified RNA was quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Welmington, DE, USA). RNA integrity was checked on a 1% denaturing formaldehyde-agarose gel with ethidium bromide and visualized under ultraviolet (UV) light on a VersaDoc 4000MP imaging system (Bio-Rad, Mississauga, ON, CA). The purified RNA samples were stored at −80 °C until analysis. First-strand cDNA synthesis was performed using an iScriptTM cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions using 1 μg total RNA. The cDNA samples were stored at −80 °C until further analysis.

Quantitative real-time PCR was performed in 96-well PCR plates by use of an ABI 7300 Real-Time PCR System (Applied Biosystems). The sequences of the gene-specific PCR primers are shown (Table 1). A separate 45 μL PCR reaction mixture consisting of Power SYBR Green master mix (Applied Biosystems), cDNA, gene-specific primers, and nuclease free water was prepared for each cDNA sample and primer pair. A final reaction volume of 20 μL was transferred to each well and reactions were performed in duplicate. The PCR reaction mixture was denatured at 95 °C for 10 min before the first PCR cycle. The thermal cycle profile was as follows: denature for 10 s at 95 °C and extension for 1 min at 60 °C for a total of 40 PCR cycles. Target gene transcript abundance was quantified according to the Mean Normalized Expression (MNE) method of Simon (2003).

2.10. Statistical analysis

The normality of each dataset was assessed using the Kolomogrov–Smirnov one-sample test and homogeneity of variance was determined using Levene's test. Data were log transformed when necessary to improve homogeneity of variances.

Table 1
Sequences, annealing temperatures, and corresponding target gene Genbank accession numbers of oligonucleotide primers used in semi-quantitative real-time PCR.

Target transcript	Accession #	Sequence (5′–3′)	Annealing temperature
β-actin	AF157514	F: AGAGCTACGAGCTGCCTGAC R: GCAAGACTCCATACCGAGGA	60
pbr	AY029216	F: AGCCTACCAAGCTCCGTGTA R: CTAGATGAGGCAGGGCAGTC	60
star	AB047032	F: TTCGTTAGTGTTCGCTGTGC R: CCGTTCTCTGCCCTAACAAC	60
p450scc	S57305	F: AACGCTGAGGCTTCATCCAGTT R: ACCAGAGTCCCACAAGGTATGT	60
cyp11b1	NM_001124264	F: CCAGGAGAATGTGGTGTCCT R: CCTCCTTCTTGGTCTTGCTG	60
mc2r	EU119870	F: TCACGCTCACCATGCTATTC R: GGAACAGAGAGCGGTAGCAC	60

Non-transformed data are shown in all figures. The effects of Se-Met, time, and any interaction between these factors, on changes in concentrations of corticosteroids, glucose, and lactate in blood plasma, as well as concentrations of glycogen and triglycerides in muscle and liver, were determined by two-way ANOVA. When interaction effects between time post-stressor exposure and Se-Met treatment were observed, a one-way ANOVA was used to compare the effect of time within each treatment group followed by a Tukey's post-hoc test, where appropriate. The effect of Se-Met exposure at each time point was assessed by a two-sample *t*-test. All statistical analyses were performed with SPSS version 11.0 (SPSS, Chicago, IL, USA) and data were expressed as mean ± S.E.M. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Concentrations of selenium in liver

Three individuals from the control and Se-Met exposure groups were randomly selected for quantification of Se in liver tissue. The concentration of Se (36.0 ± 0.05 μg/g dm) in liver of trout that were fed the diet supplemented with Se-Met was significantly greater ($p < 0.05$) than the concentration in liver of control trout (3.80 ± 0.94 μg/g dm).

3.2. Growth and somatic indices

There was no mortality of trout fed either the control diet or the diet augmented with Se-Met. Trout fed Se-Met in the diet had a significantly greater ($p < 0.05$) body mass but were not longer (fork length) than controls. The mean condition factor of trout fed Se-Met was significantly greater ($p < 0.05$) than that of the controls. The mass of the liver and HSI were also significantly greater ($p < 0.05$) in trout fed Se-Met when compared to the controls (Table 2).

3.3. Concentrations of corticosteroids in blood plasma

Concentrations of cortisol in blood plasma were significantly greater ($p < 0.05$) in trout exposed to dietary Se-Met sampled prior to the handling stressor compared to control trout sampled prior to the handling stressor (Fig. 1A). Concentrations of cortisol in blood plasma from control trout were significantly greater ($p < 0.05$) 2 h post-stressor compared to concentrations from trout sampled pre-stressor and concentrations at 24 h post-stressor exposure. There was no significant difference between concentrations of cortisol before the stressor was applied and 24 h post-stressor in control trout. Concentrations of cortisol in blood plasma from trout exposed to Se-Met were not significantly affected by the handling stressor. There was no significant difference between concentrations of cortisol 2 and 24 h post-stressor in trout exposed to Se-Met.

Concentrations of cortisone in blood plasma were not significantly different between trout exposed to dietary Se-Met sampled prior to the handling stressor and controls sampled prior to the handling stressor (Fig. 1B). Concentrations of cortisone in blood plasma from control trout were significantly greater ($p < 0.05$) 2 and 24 h post-stressor compared to pre-stressor concentrations. Concentrations of cortisone at 24 h post-stressor were significantly less ($p < 0.05$) than those 2 h post-stressor. Concentrations of cortisone in blood plasma from trout exposed to Se-Met were significantly greater ($p < 0.05$) 2 and 24 h post-stressor compared to pre-stressor concentrations. There was no significant difference between concentrations at 2 and 24 h post-stressor in trout exposed to Se-Met.

3.4. Concentrations of glucose and lactate in blood plasma

Concentrations of glucose in blood plasma (Fig. 2A) were not significantly different between trout exposed to dietary Se-Met sampled prior to the handling stressor and trout fed the control diet sampled prior to the handling stressor. Concentrations of glucose in blood plasma were significantly less ($p < 0.05$) in trout fed Se-Met sampled at 2 and 24 h post-stressor compared to controls. Concentrations of glucose in blood plasma from control trout were significantly greater ($p < 0.05$) 2 and 24 h post-stressor compared to concentrations in trout sampled prior to the handling stressor. There was no significant difference between concentrations of glucose in blood plasma from control trout 2 and 24 h post-stressor. Concentrations of glucose in blood plasma from trout exposed to Se-Met were significantly greater ($p < 0.05$) 2 h post-stressor than concentrations in trout sampled before the stressor was applied. There was no significant difference between concentrations of glucose in blood plasma from trout sampled prior to the handling stressor and 24 post-stressor.

Overall concentrations of lactate in blood plasma (Fig. 2B) were significantly greater ($p < 0.05$) 2 h post-stressor compared to concentrations in trout sampled prior to the stressor and 24 h post-stressor. There was no significant difference between overall

Table 2
Mean body mass, fork length, condition factor, liver mass, and hepatosomatic index of female rainbow trout exposed to either a control or Se-Met spiked diet.

Exposure group	Body mass (g)	Fork length (cm)	Condition factor (*k*)	Liver mass (g)	Hepatosomatic index (HSI)
Control	224 ± 15.2	25.8 ± 0.61	1.23 ± 0.03	3.29 ± 0.24	1.51 ± 0.07
Se-Met	234 ± 8.86*	25.9 ± 0.34	1.32 ± 0.02*	4.44 ± 0.18*	1.97 ± 0.05*

* Significantly different from the control group (*t*-test, $p < 0.05$).

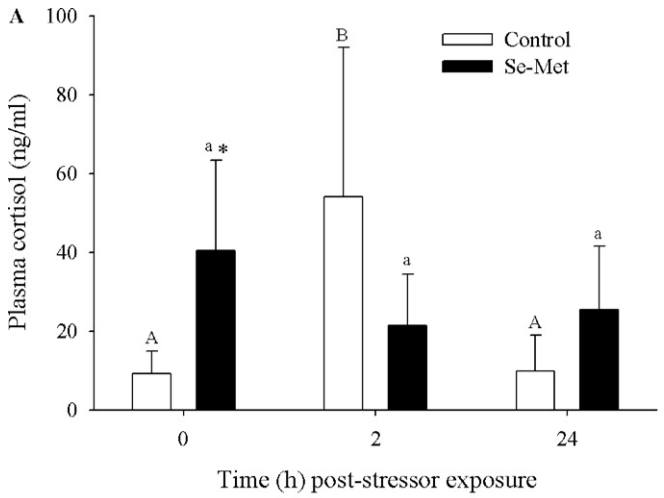

Fig. 1. Effect of a handling stressor on concentrations of (A) cortisol and (B) cortisone in blood plasma from female rainbow trout. Individuals were sampled either immediately prior to stressor exposure (0 h) or 2 or 24 h post-stressor exposure. Bars represent the mean concentration (±S.E.M.) of 8–20 trout. Different uppercase letters denote significant differences with time in the control group and different lowercase letters denote significant differences with time in the Se-Met exposed trout (one-way ANOVA with Tukey's post-hoc test, $p < 0.05$). An asterisk represents a significant difference between control and Se-Met exposed trout at the same sampling time (two-sample *t*-test, $p < 0.05$).

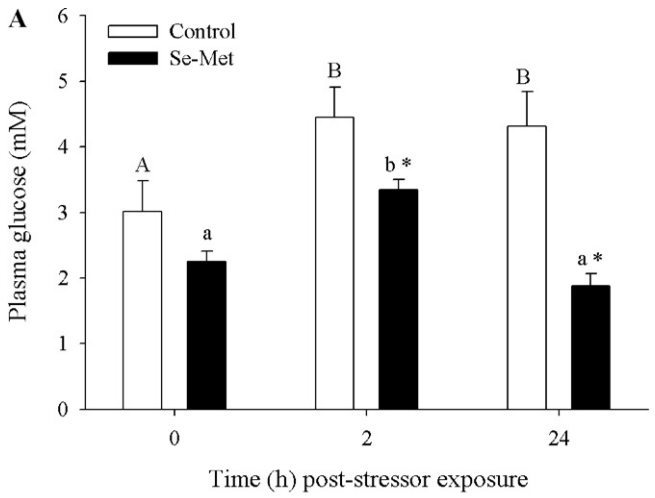

Fig. 2. Effect of a handling stressor on concentrations of (A) glucose and (B) lactate in blood plasma from female rainbow trout. Individuals were sampled either immediately prior to stressor exposure (0 h) or 2 or 24 h post-stressor exposure. Bars represent the mean concentration (±S.E.M.) of 12–13 trout. In the analysis of concentrations of glucose in blood plasma, different uppercase letters denote significant differences with time in the control trout and different lowercase letters denote significant differences with time in the Se-Met exposed trout (one-way ANOVA with Tukey's post-hoc test, $p < 0.05$). An asterisk represents a significant difference between control and Se-Met exposed trout at the same sampling time (two-sample *t*-test, $p < 0.05$). In the analysis of concentrations of lactate in blood plasma, a different uppercase letter represents a significant effect of time, regardless of Se-Met exposure (two-way ANOVA with Tukey's post-hoc test, $p < 0.05$).

concentrations of lactate pre-stressor and 24 h post-stressor. There was no significant effect of exposure to the Se-Met augmented diet on concentrations of lactate in blood plasma. There was no significant interaction between diet and the handling stressor on concentrations of lactate in blood plasma.

3.5. Concentrations of glycogen and triglycerides in liver

There was no significant effect of diet or the handling stressor on concentrations of glycogen or triglycerides in liver (data not shown).

3.6. Concentrations of glycogen and triglycerides in muscle

Overall concentrations of glycogen in muscle tissue (Fig. 3A) were significantly greater ($p < 0.05$) in trout fed the Se-Met compared to the controls. Concentrations of glycogen in muscle were significantly less ($p < 0.05$) 24 h post-stressor compared to concentrations pre-stressor and 2 h post-stressor. There was no significant interaction between diet and time post-handling stressor for concentrations of glycogen in muscle.

Concentrations of triglycerides in muscle were significantly greater ($p < 0.05$) in trout exposed to Se-Met sampled prior to the handling stressor compared to controls sampled prior to the handling stressor. Concentrations of triglycerides in muscle of control trout sampled at 2 h and 24 h post-stressor exposure were not significantly different from concentrations in trout sampled prior to the handling stressor (Fig. 4B). Concentrations of triglycerides in muscle of trout fed Se-Met were significantly less 24 h post-stressor compared to concentrations pre-stressor.

3.7. Abundance of steroidogenic protein transcripts

When abundances of transcripts of corticosteroidogenic proteins/enzymes were quantified in control trout and trout exposed to Se-Met sampled prior to the handling stressor, the abundance

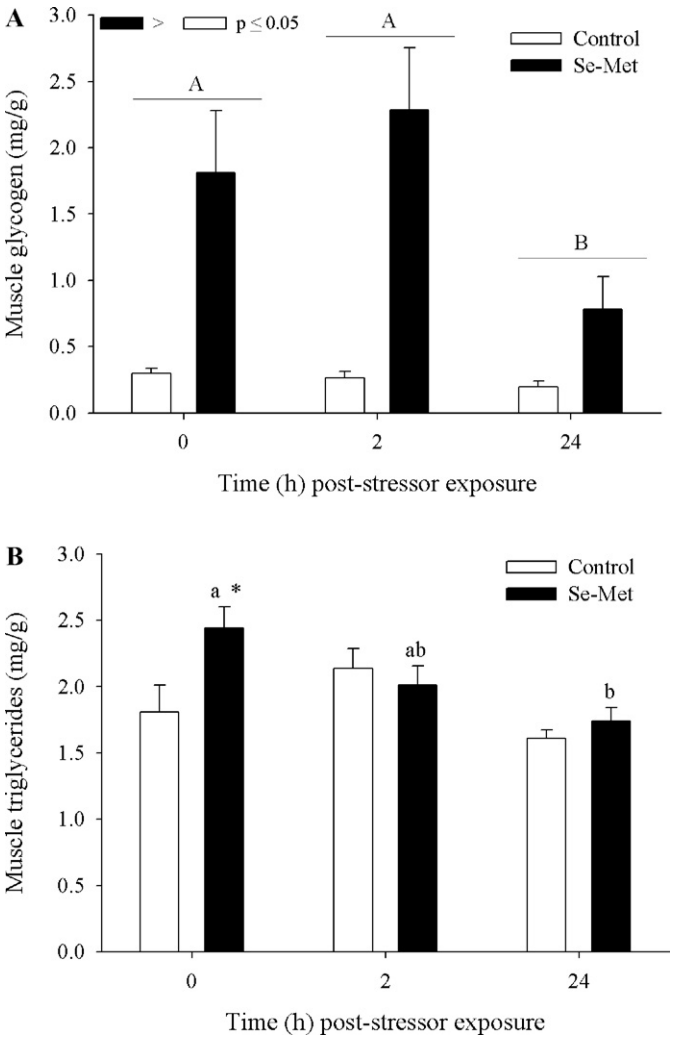

Fig. 3. Effect of a handling stressor on concentrations of (A) glycogen and (B) triglycerides in muscle from female rainbow trout. Individuals were sampled either immediately prior to stressor exposure (0 h) or 2 or 24 h post-stressor exposure. Bars represent the mean concentration (±S.E.M.) of 12–13 trout. Different uppercase letters denote significant differences with time regardless of Se-Met exposure (two-way ANOVA with Tukey's post-hoc test, $p < 0.05$). The inset denotes a significant effect of the Se-Met exposure on concentrations of glycogen in muscle (two-way ANOVA, $p < 0.05$). In the analysis of concentrations of triglycerides in muscle different lowercase letters represents a significant effect of time in the Se-Met exposed trout (one-way ANOVA with Tukey's post-hoc test, $p < 0.05$) and an asterisk represents a significant difference between control and Se-Met exposed trout at the same sampling time-point (two-sample *t*-test, $p < 0.05$).

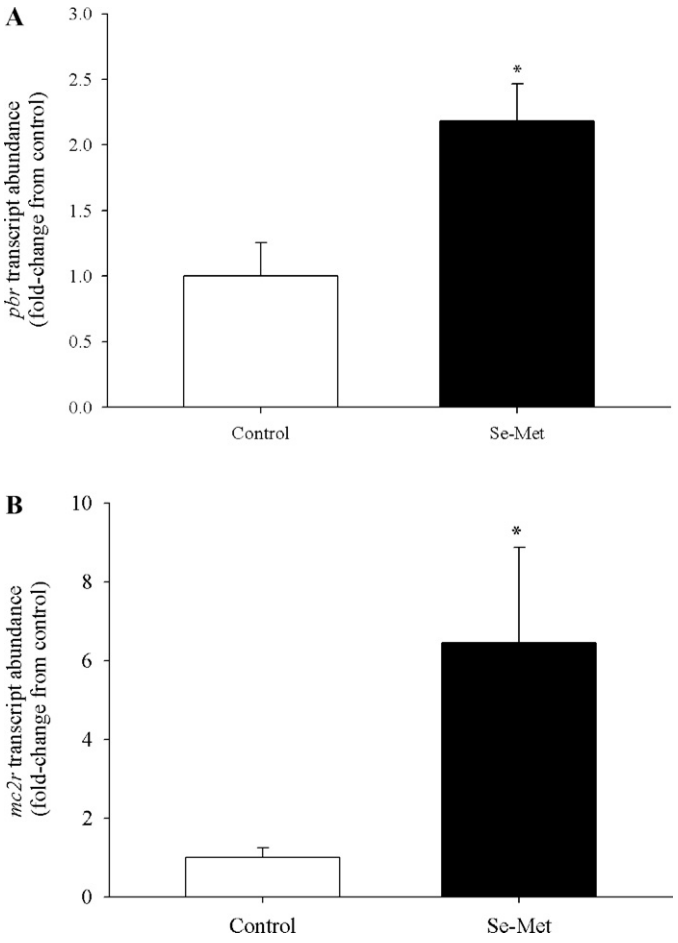

Fig. 4. Transcript abundance of (A) peripheral benzodiazepine receptor (*pbr*) and (B) melanocortin 2 receptor (*mc2r*) in head kidney from female rainbow trout determined by quantitative real-time PCR. Bars represent the mean fold transcript abundance (±S.E.M.) of 8 trout relative to the controls. An asterisk denotes a statistically significant difference from the controls (two sample *t*-test, $p < 0.05$).

of *mc2r* was significantly (6.5 ± 2.1 fold) greater ($p < 0.05$) in trout exposed to Se-Met (Fig. 4A). Abundance of *pbr* was significantly (2.2 ± 0.3 fold) greater ($p < 0.05$) in trout exposed to Se-Met (Fig. 4B). There were no statistically significant differences in abundances of transcripts of *star*, *p450scc* or *cyp11b1* between control trout and trout exposed to Se-Met (data not shown).

4. Discussion

Dietary exposure to an environmentally relevant concentration of Se-Met elicited a stress response in immature female rainbow trout. The greater concentrations of cortisol in blood plasma of trout exposed to Se-Met but not subjected to the handling stressor is consistent with the greater concentrations of cortisol in blood plasma of rainbow trout exposed to waterborne selenite (Miller et al., 2007). Despite the greater concentrations of cortisol in blood plasma, the trout exposed to Se-Met had significantly greater body mass, condition factor, liver mass and HSI relative to the controls. This contrasts the lesser body mass and HSI reported in fish chronically exposed to exogenous cortisol or repeated stressors (Barton et al., 1987). However, several studies have demonstrated that fish exposed to Se have a greater body mass relative to controls (Bennett and Janz, 2007; Driedger et al., 2009; Thomas and Janz, 2011). The greater concentrations of glycogen and triglycerides in muscle of trout fed Se-Met might be partially responsible for their greater body mass. The greater HSI of trout exposed to Se-Met is not consistent with the findings by Miller et al. (2007) whom did not observe changes in HSI of juvenile rainbow trout exposed to waterborne selenite for either 4 or 30 days. Although liver is the primary site of glycogen synthesis and storage, and triglyceride synthesis, the greater HSI of trout exposed to dietary Se-Met does not appear to be due to their glycogen and triglyceride content, since no significant accumulation of these energy substrates was evident in Se-Met exposed trout. In a companion study, trout exposed to dietary Se-Met had greater abundances of transcripts of vitellogenin, which suggested that the greater HSI is likely due to greater concentrations of this protein (Wiseman et al., 2011).

The mechanism that resulted in the greater concentrations of cortisol in blood plasma of trout exposed to Se-Met is unknown. The primary signaling pathway that stimulates corticosteroidogenesis appears to be activation of the MC2R by ACTH. Activation of the MC2R stimulates greater abundances of transcripts of the *mc2r*, *star*

and *p450scc* (Aluru and Vijayan, 2008; Sandu and Vijayan, 2011). Similarly, the abundance of transcripts of *cyp11b1* was greater in interrenal cells exposed to ACTH (Hagen et al., 2006). In the current study, the greater abundance of transcripts of *mc2r* in trout exposed to Se-Met suggests that concentrations of ACTH might be greater in blood plasma of these fish. Similarly, the greater abundance of transcripts of the cholesterol transport protein, *pbr*, supports greater availability of cholesterol as a substrate for synthesis of cortisol in trout exposed to Se-Met. However, abundances of transcripts of *star*, *p450scc*, and *cyp11b1* were not significantly greater in trout exposed to Se-Met. Whether the transcript abundance of the steroidogenic enzymes accurately reflects their protein abundance, and therefore the cortisol-producing capacity of the steroidogenic tissue, is not known. In addition, the regulation of steroidogenic enzyme transcript abundance by MC2R under conditions of prolonged stress has not been determined. Alternatively, the greater concentrations of cortisol in blood plasma of trout exposed to Se-Met might have been due to direct activation of the MC2R by Se-Met. Although there is no evidence of interactions of Se-Met with MC2R, a recent study demonstrated that cadmium disrupts cortisol synthesis by directly disrupting MC2R signaling, which suggests that ACTH-independent activation of MC2R signaling is possible (Sandu and Vijayan, 2011). These results suggest that chronic exposure to Se-Met stimulates the primary stress response in exposed female rainbow trout, but the mechanism of this effect requires further investigation.

Dietary exposure to Se-Met had a pronounced effect on the cortisol response to the secondary handling stressor. The significantly greater concentrations of cortisol in blood plasma of control trout at 2 h post-stressor exposure, and the return to pre-stressor concentrations at 24 h post-stressor exposure are typical of the cortisol response to a handling stressor in rainbow trout (Mommsen et al., 1999; Wiseman et al., 2007; Ings et al., 2011). In contrast, there was a clear attenuation of the cortisol response to the handling stressor in trout that had been exposed to dietary Se-Met. The greater abundance of transcripts of *mc2r* in trout exposed to dietary Se-Met and sampled prior to the handling stressor suggests that these individuals should be more sensitive to the handling stressor than unexposed trout. Consequently, the handling stressor should have stimulated a cortisol response. Although the mechanism of the attenuated cortisol response to the handling stressor is unknown, it has been suggested that the inability of fish to mount a stress response to a secondary stressor might be due to depletion of corticotrophs thereby leading to interrenal inactivation (Hontela et al., 1992, 1997; Hontela and Vijayan, 2008; Ings et al., 2011). Alternatively, trout exposed to Se-Met might not have been able to mount a cortisol response due to direct inhibition of the steroidogenic machinery by Se-Met. However, the greater concentrations of cortisol in blood plasma of trout exposed to Se-Met compared to control trout sampled prior to the handling stressor suggests that the inability to mount a stress response to the handling stressor is likely not due to depletion of ACTH or impairment of steroidogenic enzymes. Although the responsiveness to ACTH of steroidogenic cells from rainbow trout chronically exposed to Se-Met is unknown, adrenocorticol cells isolated from rainbow trout exposed to waterborne selenite for 30 d maintained ACTH responsiveness (Miller et al., 2007) and a recent study demonstrated that Se-Met did not impair cortisol biosynthesis in adrenocorticol cells isolated from rainbow trout (Miller and Hontela, 2011). The results of the current study suggest that the lesser concentrations of cortisol in blood plasma from trout exposed to Se-Met and subsequently subjected to the handling stressor likely resulted from greater metabolism of cortisol. The greater concentrations of cortisone in blood plasma from trout exposed to Se-Met sampled at 2 or 24 h post-stressor compared to concentrations in blood plasma of trout that were not exposed to the handling stressor suggests that chronic exposure to Se-Met disrupts the primary stress response by stimulating greater metabolic inactivation of cortisol.

Significant effects on energy substrate partitioning were observed in trout exposed to Se-Met. Overall, the exposure to dietary Se-Met stimulated greater concentrations of glycogen in muscle but not in liver. Similarly, concentrations of triglycerides were significantly greater in muscle of Se-Met exposed trout sampled prior to the handling stressor. These results are in agreement with the greater concentrations of glycogen and triglycerides in whole body of zebrafish exposed to Se-Met in the diet (Thomas and Janz, 2011) and fish sampled from Se-impacted sites (Bennett and Janz, 2007; Kelly and Janz, 2008; Driedger et al., 2009). The greater concentrations of glycogen in muscle could be due to greater glucose production since cortisol stimulates gluconeogenesis by induction of phosphoenolpyruvate carboxykinase (PEPCK) activity (Hanson and Reshef, 1997). The greater concentrations of triglycerides in muscle might have been due to greater activity of fatty acid synthase (FAS) which has been demonstrated in rats exposed to Se (Mueller et al., 2008). It is unknown why concentrations of glycogen and triglycerides were not significantly greater in liver of trout exposed to Se-Met that were sampled prior to the handling stressor. One explanation is that glycogen and triglycerides in the liver are preferentially utilized to provide energy for the metabolic demands associated with the stress caused by exposure to the Se-Met, thus preventing accumulation in the liver.

Energy, primarily in the form of glucose, is required to meet the metabolic needs associated with recovery from stress (Wendelaar Bonga, 1997; Mommsen et al., 1999). The immediate stressor stimulated increase in concentrations of glucose in blood plasma results from catecholamine stimulated breakdown of stored glycogen (Randall and Perry, 1992; Vijayan and Moon, 1994). The handling stressor had different effects on concentrations of glucose in blood plasma of trout exposed to the control and Se-Met diets. In unexposed trout concentrations of glucose in blood plasma were sustained at greater concentrations following exposure to the handling stressor. The source of this glucose is unclear since concentrations of glycogen in neither muscle nor liver were significantly less following exposure to the handling stressor. In contrast, concentrations of glucose in blood plasma of trout exposed to Se-Met were significantly greater 2 h post-stressor and returned to pre-stressor concentrations by 24 h post-handling stressor. Concentrations of glycogen in liver were not significantly less in either the control trout or trout exposed to Se-Met. The lesser concentration of glycogen in muscle of trout exposed to Se-Met sampled 24 h post-stressor does suggest that glycogen was an important energy source in these trout, but does not explain the greater plasma glucose observed 2 h post-stressor exposure. Moreover, it would be expected that the lesser concentration of glycogen in muscle of trout exposed to Se-Met and sampled 24 h post-stressor exposure would be matched by a greater concentration of glucose in blood plasma from these fish. The absence of such an effect suggests that glucose liberated from the breakdown of glycogen stored in muscle is utilized by the muscle and other tissues to provide energy for recovery from the handling stressor, and that any peak in glucose concentration in blood plasma might have occurred between 2 and 24 h post-stressor exposure.

Triglycerides are an important source of energy during the recovery from a stressor. Cortisol plays a role in the regulation of lipid metabolism by stimulating lipolysis that provides fatty acids as substrates for oxidation and glycerol for gluconeogenesis (Mommsen et al., 1999). Concentrations of triglycerides in liver were not significantly affected by the handling stressor in either the unexposed trout or trout exposed to Se-Met. Similarly, the handling stressor had no significant effects on concentrations of triglycerides in muscle from unexposed trout. However, triglycerides stored in muscle of trout exposed to Se-Met appear to be

an important source of energy during recovery from the handling stressor because concentrations were significantly less 24 h post-stressor exposure compared to the concentrations in trout not exposed to the handling stressor.

In summary, the results of this study suggest that long-term dietary exposure of immature female rainbow trout to an environmentally realistic dietary concentration of Se-Met stimulates a stress response. The mechanism of the greater concentrations of cortisol is unknown although greater concentrations of ACTH or direct effects of Se-Met on the MC2R receptor might be involved. This exposure also attenuates the cortisol response to a secondary handling stressor, possibly by increasing inactivation of cortisol. Exposure to Se-Met also had a pronounced affect on the partitioning of energy substrates required to fuel recovery from the handling stressor. Glycogen and triglycerides were preferentially stored in muscle but not liver, and were utilized to provide energy for recovery from the secondary handling stressor.

Acknowledgements

This work was supported by separate Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to J.P.G. [grant number 326415-07], M.P. [grant number 371538-09] and D.M.J. [grant number 288163-10]. J.P.G. was also supported by a grant from Western Economic Diversification Canada (grant numbers 6971, 6807). The authors acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructures. Prof. Giesy was supported by the Canada Research Chair program, an at large Chair Professorship at the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, The Einstein Professor Program of the Chinese Academy of Sciences and the Visiting Professor Program of King Saud University. The authors wish to thank the following people for their assistance with tissue collection: Amber Tompsett, Fengyan Liu, Jonathan Doering, Brett Tendler, and Shawn Beitel. The authors wish to thank Eric Higley for LC-MS/MS analysis of corticosteroids. The authors acknowledge the support of the Aquatic Toxicology Research Facility (ARTF) at the Toxicology Centre, University of Saskatchewan, in providing space and equipment for the culturing of rainbow trout and the exposure portion of the study.

References

Aluru, N., Vijayan, M.M., 2008. Molecular characterization, tissue-specific expression and regulation of melanocortin 2 receptor in rainbow trout. Endocrinology 149, 4577–4588.

Barton, B.A., Schreck, C.B., Barton, L.D., 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2, 173–185.

Bennett, P.M., Janz, D.M., 2007. Bioenergetics and growth of young-of the-year northern pike (*Esox lucius*) and burbot (*Lota lota*) exposed to metal mining effluent. Ecotoxicol. Environ. Saf. 68, 1–12.

Chang, H., Wan, Y., Hu, J., 2009. Determination and source apportionment of five classes of steroid hormones in urban rivers. Environ. Sci. Technol. 43, 7691–7698.

Chang, H., Wan, Y., Naile, J., Zhang, X.W., Wiseman, S., Hecker, M., Lam, M.H.W., Giesy, J.P., Jones, P.D., 2010. Simultaneous quantification of multiple classes of phenolic compounds in blood plasma by liquid chromatography–electrospray tandem mass spectrometry. J. Chromatogr. A 1217, 506–513.

Driedger, K., Weber, L.P., Rickwood, C.J., Dubé, M.G., Janz, D.M., 2009. Overwinter alterations in energy stores and growth in juvenile fishes inhabiting areas receiving metal mining and municipal wastewater effluents. Environ. Toxicol. Chem. 28, 296–304.

Fan, T.W.M., The, S.J., Hinton, D.E., Higashi, R.M., 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat. Toxicol. 57, 65–84.

Goertzen, M., 2011. Swimming performance and energy homeostatic effects of uranium mill effluent exposure in small-bodied fish. MSc Dissertation. University of Saskatchewan, Saskatoon, SK, Canada.

Gómez-Lechón, M.J., Ponsoda, X., Castell, J.V., 1996. A microassay for measuring glycogen in 96-well-cultured cells. Anal. Biochem. 236, 296–301.

Hagen, I.J., Kusakabe, M., Young, G., 2006. Effects of ACTH and cAMP on steroidogenic acute regulatory protein and P450 11B-hydroxylase messenger RNAs in rainbow trout interrenal cells: Relationships with in vitro cortisol production. Gen. Comp. Endocrinol. 254, 254–262.

Hamilton, S., 2004. Review of selenium toxicity in the aquatic food chain. Sci. Total Environ. 326, 1–31.

Hanson, R.W., Reshef, L., 1997. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. Annu. Rev. Biochem. 66, 581–611.

Hesketh, J., 2008. Nutrigenomics and selenium: gene expression patterns, physiological targets, and genetics. Annu. Rev. Nutr. 28, 157–177.

Hontela, A., Daniel, C., Rasmussen, J.B., 1997. Structural and functional impairment of the hypothalamo–pituitary–interrenal axis in fish exposed to bleached kraft mill effluent in the St Maurice River, Quebec. Ecotoxicology 6, 1–12.

Hontela, A., Rasmussen, J.B., Audet, C., Chevalier, G., 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Arch. Environ. Contam. Toxicol. 22 (3), 278–283.

Hontela, A., Vijayan, M.M., 2008. Adrenocortical toxicology in fishes. In: Harvey, P.W., Everett, D., Springall, C. (Eds.), Adrenal Toxicology. Taylor and Francis, Boca Raton, FL, pp. 233–256.

Ings, J.S., Servos, M.R., Vijayan, M.M., 2011. Exposure to municipal wastewater effluent impacts stress performance in rainbow trout. Aquat. Toxicol. 103, 85–91.

Janz, D.M., 2011. Selenium. In: Wood, C.M., Brauner, C.J., Farrell, A.P. (Eds.), Fish Physiology, vol. 31A: Homeostasis and Toxicology of Essential Metals. Academic Press, San Diego, CA, pp. 327–374.

Janz, D.M., DeForest, D.K., Brooks, M.L., Chapman, P.M., Gilron, G., Hoff, D., Hopkins, W.D., McIntyre, D.O., Mebane, C.A., Palace, V.P., Skorupa, J.P., Wayland, M., 2010. Selenium toxicity to aquatic organisms. In: Chapman, P.M., Adams, W.J., Brooks, M.L., Delos, C.G., Luoma, S.N., Maher, W.A., Ohlendorf, H.M., Presser, T.S., Shaw, D.P. (Eds.), Ecological Assessment of Selenium in the Aquatic Environment. CRC Press, Boca Raton, FL, pp. 141–231.

Kelly, J.M., Janz, D.M., 2008. Altered energetics and parasitism in juvenile northern pike (*Esox lucius*) inhabiting metal-mining contaminated lakes. Ecotoxicol. Environ. Saf. 70, 357–369.

Lemly, D., 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicol. Environ. Saf. 37, 259–266.

McGowan, M.W., Artiss, J.D., Strandbergh, D.R., Zak, B., 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29, 538–542.

Miller, L.L., Rasmussen, J.B., Palace, V.P., Hontela, A., 2009. The physiological stress response and oxidative stress biomarkers in rainbow trout and brook trout from selenium-impacted streams in a coal mining region. J. Appl. Toxicol. 29, 681–688.

Miller, L.L., Wang, F., Palace, V.P., Hontela, A., 2007. Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. Aquat. Toxicol. 83, 263–271.

Miller, L.L., Hontela, A., 2011. Species-specific sensitivity to selenium-induced impairment of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Toxicol. Appl. Pharmacol. 253, 137–144.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268.

Mueller, A.S., Klomann, S.D., Wolf, N.M., Schneider, S., Schmidt, R., Spielmann, J., Stangl, G., Eder, K., Pallauf, J., 2008. Redox regulation of protein tyrosine phosphatase 1B by manipulation of dietary selenium affects the triglyceride concentration in rat liver. J. Nutr. 138, 2328–2336.

Muscatello, J.R., Bennett, P.M., Himbeault, K.T., Belknap, A.M., Janz, D.M., 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environ. Sci. Technol. 40, 6506–6512.

Papadopoulos, V., 2004. In search of the function of the peripheral-type benzodiazepine receptor. Endocr. Res. 30, 677–684.

Payne, A.H., Hales, D.B., 2004. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr. Rev. 25, 947–970.

Randall, D.J., Perry, S.F., 1992. Catecholamines. In: Randall, D.J., Hoar, W.S. (Eds.), Fish Physiology. vol. 12B. The Cardiovascular System. Academic Press, New York, pp. 255–300.

Sandu, N., Vijayan, M.M., 2011. Cadmium-mediated disruption of cortisol biosynthesis involved suppression of corticosteroidogenic genes in rainbow trout. Aquat. Toxicol. 103, 92–100.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Simon, P., 2003. Q-Gene: processing quantitative real-time RT-PCR data. Bioinformatics 19, 1439–1440.

Stocco, D.M., Wang, X.J., Jo, Y., Manna, P.R., 2005. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: more complicated than we thought. Mol. Endocrinol. 19, 2647–2659.

Thomas, J.K., Janz, D.M., 2011. Dietary selenomethionine exposure in adult zebrafish alters swimming performance, energetics and the physiological stress response. Aquat. Toxicol. 102, 79–86.

Vijayan, M.M., Moon, T.W., 1994. The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. Can. J. Zool. 72, 379–386.

Weber, L.P., Higgins, P.S., Carlson, R.I., Janz, D.M., 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. J. Fish Biol. 63, 637–658.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Wiseman, S., Oschaff, H., Bassett, E., Malhotra, J., Bruno, J., VanAggelen, G., Mommsen, T.P., Vijayan, M.M., 2007. Gene expression patterns in the liver during recovery from an acute stressor in rainbow trout. Comp. Biochem. Physiol. Part D: Genomics Proteomics 2, 234–244.

Wiseman, S., Thomas, J.K., Higley, E., Hursky, O., Pietrock, M., Raine, J.C., Giesy, J.P., Janz, D.M., Hecker, M., 2011. Chronic exposure to dietary selenomethionine increases gonadal steroidogenesis in female rainbow trout. Aquat. Toxicol. 105, 218–226.

Woock, S.E., Garrett, W.R., Partin, W.E., Bryan, W.T., 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, *Lepomis macrochirus*. Bull. Environ. Contam. Toxicol. 39, 998–1005.