

**THE EFFECTS OF SELENIUM ON THE PHYSIOLOGICAL STRESS
RESPONSE IN FISH**

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ABSTRACT

Selenium (Se), an essential element, can bioaccumulate and become toxic. The main toxicity symptom in fish, teratogenicity, is mediated by oxidative stress; however, little is known about the effect of Se on the physiological stress response (PSR). The effects of Se on the PSR and oxidative stress parameters were investigated in rainbow trout, brook trout, and white suckers. The PSR was activated by acute and sub-chronic laboratory exposures to NaSeO_3 , but not exposure to environmental levels of Se. Species-specific sensitivity to Se may be explained by biochemical differences. Rainbow trout (exhibit oxidative damage with Se exposure) have greater GSH reserves (cycles with Se causing oxidative stress) than brook trout. Selenium in agricultural drain water did not accumulate to dangerous levels in white suckers, but additional stressors (e.g. pesticides) were present. Differences in sensitivity to Se may alter competitive interactions between species, changing community compositions and putting additional pressure on threatened species.

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LIST OF ABBREVIATIONS

ACTH – adrenocorticotrophic hormone
AscA – ascorbic acid
ATPase – adenosine triphosphatase
BKTR – brook trout
CAR – carotenoids
°C – degrees Celsius
CAT – catalase
cAMP – cyclic adenosine monophosphate
Cd – cadmium
cP450_{ssc} – cytochrome P450 side chain cleavage
CRH – corticotrophin releasing hormone
Cu – copper
DNA – deoxyribonucleic acid
DD – DNA damage
GPx – glutathione peroxidase
GR – glutathione reductase
GSSG – oxidized glutathione
GSH – reduced glutathione
4-HA – 4 hydroxyalkenal
HPI – hypothalamic-pituitary interrenal
K – condition factor
KB – 2-ketobutyrate
LC₅₀ – lethal concentration that kills 50% of the test population
LPO – lipid peroxidation
LSI – liver somatic index
MDA - malondialdehyde
MEM – minimal essential medium
Met-SeOH – methylselenol
MITHE-RN – Metals In The Human Environment Research Network
Na⁺/K⁺ ATPase – sodium potassium adenosine triphosphatase
NC – no significant change
NSERC – National Science and Engineering Research Council
PSR – physiological stress response
PP – protein peroxidation
RBTR – rainbow trout
RET - retinols
ROS – reactive oxygen species
S – sulfur
Se – selenium
SE – standard error
Se-Met - selenomethionine
SOD – superoxide dismutase

StAR – steroidogenic acute regulatory
T3 - triiodothyronine
T4 - thyroxine
TOC – tocopherols
U - units
USA – United States of America
Zn - Zinc

CHAPTER 1. LINKING SELENIUM TOXICITY, THE PHYSIOLOGICAL STRESS RESPONSE, AND OXIDATIVE STRESS IN FISH.

Selenium (Se), an essential element required for glutathione peroxidase and other selenoenzymes, bioaccumulates and becomes toxic (Lemly 1999). The range of concentrations required for Se homeostasis is narrow compared to other essential elements such as zinc or copper. In aquatic systems, the main effects in birds and fish are teratogenesis and the subsequent population decline (Hamilton 2004). Several different toxicity mechanisms have been proposed including oxidative stress. Oxidative stress refers to damage caused by reactive oxygen species (compounds with unpaired electrons) not removed by a cell's antioxidant enzymatic and scavenging defenses. Oxidative stress has been implicated in many different diseases including arthritis, aging, malaria, and Parkinson's disease in humans (Halliwell 1987). Chemical stressors, such as copper, may increase oxidative stress in fish leading to toxicity symptoms observed (Berntssen et al. 2000).

Animals respond to chemical stressors by activating the physiological stress response (PSR). This enables them to respond appropriately to the stressor (the agent that triggers the response called the stress response) and maintain homeostasis. Hormones released during this process may suppress growth and reproduction (Mommsen et al. 1999). Even though acute exposure to numerous chemical stressors elevate plasma cortisol, the stress response, specifically cortisol secretion, can be impaired by chronic field exposures (Laflamme et al. 2000) or acute *in vitro* exposure (Lacroix and Hontela 2004) to

contaminants such as heavy metals; however, the effect of Se on the PSR in fish is unknown.

The objective of this review is to examine the literature and investigate the link between Se toxicity, the PSR, and oxidative stress in fish. Specifically this chapter will (i) summarize the literature on Se toxicity, the PSR, and oxidative stress for fish, (ii) identify the knowledge gaps, and (iii) identify the implications of linking Se exposure, the PSR and oxidative stress.

1.1. Selenium

1.1.1. Properties and Sources

Selenium is a metalloid that exists in four different oxidation states: -2, +2, +4, and +6 (Banks 1997). It is used in a number of industrial and manufacturing processes including photoelectric cells, steel manufacture, anti-dandruff shampoos, fungicide, and glass manufacturing (Nagpal 2001). Selenides (-2) are usually present as organic compounds (Bowie et al. 1996), such as selenomethionine and selenocysteine (Combs and Combs 1986), and are physically and chemically similar to sulphides (Banks 1997). Selenite (+4) is easily reduced at low pH, and may exist as selenium dioxide (SeO_2), selenite (SeO_2^{-2}), or selenious acid (H_2SeO_3). Selenate (+6), may form selenic acid (H_2SeO_4), or selenate salts (SeO_4^{-2}). Most selenate compounds are soluble in water and are more stable than other Se compounds (Combs and Combs 1986).

The highest Se concentrations (600 ppm) are found in black shales. Other natural sources include phosphate rocks (1-300 ppm), limestone (0.08 ppm), and igneous rock (0.004-1.5 ppm) (Haygarth 1994). Soil Se ranges from 0.01 mg/kg to 38 mg/kg

(Haygarth 1994). In areas with high Se in the soil, Se may accumulate in vegetation and toxicity symptoms may appear in wildlife and livestock (Mayland 1994). Conversely, in areas with low soil Se, Se supplements are given to livestock to avoid deficiency problems (Haygarth 1994).

Major anthropogenic sources of Se include fossil fuel combustion, mining, and agricultural drainwater (Haygarth 1994; Lemly 1999). Belews Lake (North Carolina, USA) was contaminated by wastewater from a coal-fired power plant for 12 years, and a decade later the biological effects of increased Se are still being documented (Lemly 2002). On extensively irrigated land, salts build up and the soil must be flushed to correct the salt balance (Haygarth 1994). In areas with high Se, Se leaches out of the soil and is flushed off the field with the drainwater. At Kesterson Reservoir (California, USA), Se bioaccumulated killing many aquatic birds (Ohlendorf and Santolo 1994). Other anthropogenic sources that may increase Se contamination are open pit phosphate mining, wetlands constructed to treat Se-laden wastewater, and feedlot waste (Lemly 1999).

1.1.2. Toxicokinetics

Upon entering an aquatic ecosystem, Se may be absorbed or ingested by aquatic organisms, bind to particulate matter, or stay free in solution (Lemly and Smith 1987). The majority of Se is taken up by aquatic organisms or bound to particulate matter (Lemly and Smith 1987) leading to Se accumulation in the sediments; however, the aquatic sediments are not permanent storage because biotic and abiotic factors frequently disturb them (Lemly and Smith 1987; Hamilton 2004). Studies have shown that 10 years

after the Se source was removed, a moderate Se hazard from sediments can persist (Lemly 2002).

Selenium accumulation in aquatic systems occurs at relatively low Se water concentrations. For example, Belews Lake fish accumulated 3975 times more Se in their tissue than present in the water (Lemly 2002). Phytoplankton and bacteria actively incorporate selenite and selenate into their cell walls at a rate proportional to its concentration in the water column (Bowie et al. 1996). Zooplankton and fish receive the majority of Se from their diet, but in fish waterborne and dietary Se are additive (Bowie et al. 1996). Commonly, the more selenium in the diet, the more Se accumulated in the tissues (Cleveland et al. 1993; Lorentzen et al. 1994). Selenium accumulates primarily in the liver, followed by the gonad and muscle tissue (Kennedy et al. 2000). Thus, even small inputs of Se into a water body may quickly accumulate up the food chain poisoning higher trophic levels.

Different Se species have different uptake routes and distribution within an organism. For example, fathead minnows accumulate more selenite and selenomethionine (Kleinow and Brooks 1986a), but selenate is absorbed more efficiently by the gastrointestinal tract than selenite (Kleinow and Brooks 1986a; Kleinow and Brooks 1986b). Little is known about the uptake mechanism in fish, but in mice, selenomethionine is taken up through an amino acid transport mechanism in the duodenum (Andersen et al. 1994). Fish may also take up Se through their gills, as Se accumulation in rainbow trout is greater after gill development (Hodson et al. 1986). The primary Se elimination route is urine, although significant amounts of Se are also removed by gills and bile (Kleinow and Brooks

1986b). The elimination rates of selenite and selenate are very similar suggesting a common metabolic pool of Se for these two compounds (Kleinow and Brooks 1986a).

1.1.3. Deficiency

Selenium was first discovered as an essential element in 1957 (Mayland 1994). Since then it has been identified as a part of glutathione peroxidase (an enzyme that defends against oxidative stress), deiodinases (Köhrle et al. 2005), thioredoxin reductases (Arner and Holmgren 2000), selenophosphate synthetase (Köhrle et al. 2005), and many other selenoproteins whose functions are not known (Köhrle et al. 2005). Selenium deficient fish show decreased glutathione peroxidase activity, decreased liver and plasma Se, and increased pyruvate kinase and glutathione transferase activity (Bell et al. 1986).

Increased pyruvate kinase may be due to leakage from damaged cells (Bell et al. 1986) and increased glutathione transferase compensates for the loss of glutathione peroxidase activity (Javanovic et al. 1997). Other deficiency symptoms include growth depression (Watanabe et al. 1997), abnormal swimming patterns (Bell et al. 1986), liver degeneration, and muscle degeneration (Hilton et al. 1980).

1.1.4. Toxicity

In fish, a variety of toxicity symptoms have been identified. The most significant effect of chronic selenosis is teratogenesis and the successive population decline.

Selenium accumulates in the eggs of exposed fish (Kennedy et al. 2000; Lemly 2002) causing teratogenic deformities, a permanent pathological marker of selenosis (Lemly 1997). Teratogenesis symptoms include lordosis (concave spine), kyphosis (convex spine), missing/deformed fins, missing/deformed gills, missing/deformed eyes and a

deformed mouth (Lemly 1997). They occur at exponentially greater rates when $>10 \mu\text{g}$ Se per g tissue is present. These deformities are lethal when they affect feeding or respiration and may increase predation if they impact swimming ability (Lemly 2002). In severe cases, fish populations decrease or disappear due to rapidly declining recruitment (Lemly 1999); however, not all species respond to Se in the same way. Selenium has negatively impacted the reproductive success in rainbow trout (Holm et al. 2005), and razorback suckers (Cooke et al. 2005), but not brook trout (Holm et al. 2005), cutthroat trout (Kennedy et al. 2000) and white suckers (de Rosemond et al. 2005). The effect of Se on fathead minnow's reproduction is more controversial as some studies show an adverse effect of Se on reproduction (Hermanutz 1992; Pyle et al. 2001) and others do not (Ogle and Knight 1989). Other symptoms of selenosis include cataracts, protruding eyeballs, swollen lamellae, hemorrhaging, edema, anemia, increased blood ion levels, pericarditis, myocarditis, liver necrosis, kidney necrosis, and decreased white blood counts (Sorensen et al. 1982; Lemly 1997; Lohner et al. 2001a; Lohner et al. 2001c; Lemly 2002).

The concentrations that kills fifty percent of the test population (LC_{50}) for a variety of fish and exposure routes have been determined (Table 1.1). The toxicity of selenium varies considerably depending on the fish species, the Se form, the life stage of the organism, and many other factors. Generally, selenite (+4) is more toxic than selenate (+6), and juveniles (after gill development) are the most sensitive (Table 1.2; Hodson et al. 1986; Buhl and Hamilton 1991). Acute toxicity tests for selenium compounds use waterborne exposure, not dietary exposures; however, in many environmental exposures diet is the dominant exposure route. Laboratory studies have shown that dietary

concentrations of 9-13 µg Se per g food can cause mortality in rainbow trout (Goelttl and Davies 1976; Hilton et al. 1980) and increased Se decreased bluegill condition factor (Cleveland et al. 1993).

1.1.5. Interactions

Many different compounds interact with Se. Vitamin E alleviates Se deficiency symptoms in a variety of organisms. For example, rainbow trout fed Se deficient diets are healthy as long as they have adequate vitamin E (Bell et al. 1986). Metals can interact with Se and alter how it affects organisms. Se toxicity is reduced by antimony, germanium, tungsten, arsenic, and copper. Additionally, Se reduces the toxicity of arsenic, cadmium, mercury, silver, and thallium (Marier and Jaworski 1983). Se and arsenic can decrease each other's toxicity by increasing biliary excretion of protein bound As or Se compounds (Marier and Jaworski 1983). The interaction that has received the most attention is the ability of Se to alleviate mercury poisoning. Selenium does not aid the excretion of mercury, but increases the accumulation of an inert form, HgSe (Himeno and Imura 2002).

1.1.6. Mechanisms of Action and Resistance

Three major Se toxicity mechanisms have been suggested: generation of superoxide radicals, substitution of Se for sulfur (S) in proteins, and inhibition of methylation (Spallholz and Hoffman 2002). Avian literature suggests selenite and selenocysteine are capable of producing free radicals, but selenate and selenomethionine are not (Spallholz and Hoffman 2002). However, Palace et al. (2004) showed that selenomethionine can be transformed into a superoxide radical by methioninase in rainbow trout (Palace et al.

2004). Additionally, selenides can oxidize thiols and reduce oxygen to produce more superoxide radicals (Bébién et al. 2002; Peakall and Burger 2003). Mammalian cell line studies have shown that Se requires reduced glutathione to inhibit cell growth, supporting this theory (Wu et al. 1995). In bacteria, Se oxides increase enzyme antioxidant levels and cultures without these enzymes were unable to tolerate the selenium oxides (Bébién et al. 2002). This also suggests Se creates a superoxide radical as cultures without antioxidants did not survive and the other cultures increased antioxidant levels in response to Se exposure.

Substitution of Se for S is the mechanism responsible for the damage to hair, feathers, and hooves (Spallholz and Hoffman 2002). Selenium and S have similar properties, but at biological pH Se is reduced while S is oxidized altering protein structure (Combs and Combs 1986). The inhibition of the methylation process (a detoxification method) may result in more Se to substitute for S or to create more superoxide radicals (Spallholz and Hoffman 2002).

Resistance to Se toxicity varies widely between closely related species. For example, rainbow trout are impacted by Se induced teratogenesis, but brook trout are not (Holm et al. 2005). Additionally, bluegill disappeared from Belews Lake after Se contamination, but green sunfish recolonized the lake successfully (Hamilton 2004). Three mechanisms have been suggested for sensitivity differences: enzyme induction, intestinal availability, and differential accumulation rates. Fathead minnows exposed to increases in dietary Se increased Se elimination suggesting Se may induce proteins and enzymes involved in elimination (Kleinow and Brooks 1986a). Additionally, studies of mammalian cell lines suggest that overexpression of α -glutathione transferase may increase Se resistance (Wu

et al. 1995). The availability of Se for uptake may also vary between species. Ogle & Knight (1989) suggested Se availability to fathead minnows may be low because their gut contains fewer and less diverse microflora than many other fish; thus, there is less Se incorporated into amino acids for uptake. Additionally, fathead minnows' gut is less acidic than other fish's, favoring the less toxic selenate form over selenites and selenides (Ogle and Knight 1989). Finally, different accumulation rates may also affect an organism's ability to resist Se toxicity. Both brook trout and rainbow trout accumulate Se in their muscle and eggs; however, brook trout (Se induced teratogenesis rare) accumulate less Se in their eggs for equal muscle concentrations than rainbow trout (Se induced teratogenesis common) (Holm et al. 2005).

1.1.7. Summary

Selenium is a fascinating element as both the lack, and the excess can adversely affect mammals, birds, and fish. Toxicity and deficiency symptoms are similar and both may be due to oxidative stress; however, there are still large knowledge gaps in Se's mechanism of action. More work is needed to fully understand the ability of Se to generate oxidative stress in fish, how it causes various symptoms, why symptoms of deficiency and toxicity are similar, and how Se is metabolized within a variety of organisms, especially fish. Most of the current Se research with fish has focused on the bioaccumulation, food chain transfer, and its reproductive effects. More research is needed to determine how chronic Se exposure influences other indicators of fish health (e.g. the PSR) and the implications of these on the ability of fish to cope with environmental changes such as global warming and introduced species.

1.2. Physiological Stress Response

A “stressor” is a stimulus that acts on a biological system and a “stress response” is the animal’s reaction to the stimulus (Pickering 1981; Barton 2002). According to the general adaptation syndrome, a stress response consists of three stages: alarm, resistance, and exhaustion (Pickering 1981). A lethal stressor exhausts an animal’s ability to resist and adapt (Wedemeyer and McLeay 1981). Sub-lethal stressors do not exhaust, but the energy used for resistance decreases energy availability for growth, immune function, and adaptation to other stressors (Wedemeyer and McLeay 1981). An acute stress response occurs when the stress is removed before the animal has time to compensate and develop a resistance mechanism (Schreck 1990). Stress responses are characterized as primary, secondary and tertiary. The primary stress response is a neuroendocrine response leading to corticosteroid and catecholamine release. The secondary stress response includes changes in plasma and tissue ion and metabolite levels induced by neuroendocrine hormones. The changes in disease resistance, growth, condition factor, and behaviors at a whole organism level are tertiary responses (Wedemeyer and McLeay 1981; Barton 2002).

1.2.1. HPI Axis

The hypothalamic–pituitary interrenal (HPI) axis is responsible for releasing corticosteroids and catecholamines in response to a stressor. Cortisol is the major corticosteroid in teleost fish and most mammals (Hontela 1997). When a fish perceives a stimulus as a stressor, the hypothalamus releases corticotropin releasing hormone (CRH) that stimulates the pituitary gland to release adrenocorticotrophic hormone (ACTH). ACTH enters the bloodstream and stimulates the steroidogenic interrenal cells in the

anterior head kidney to synthesize cortisol (Hontela 1998). ACTH binds to its receptor in the cell membrane, and cyclic adenosine monophosphate (cAMP) activates protein kinase A. Protein kinase A activates cholesterol ester hydrolase releasing free cholesterol from cholesterol esters within the cellular matrix (Stocco 2000). At the outer mitochondrial membrane, steroidogenic acute regulatory (StAR) protein regulates the passage of cholesterol to the inner membrane where cytochrome P450 side chain cleavage (cP450_{scc}) mediates the transformation of cholesterol to pregnenolone (Stocco 2000). This is a rate limiting step in cortisol synthesis and can be disrupted by contaminants (e.g. RoundUp (Walsh et al. 2000)). Pregnenolone moves to the cytoplasm where cytochromes P450_{17 α} and P450_{C21} create 11-deoxycortisol, that is shunted back to the mitochondria to be transformed into cortisol by cytochrome P450_{11 β} (Hontela 1997). Cortisol regulates its own production through a negative feedback loop by altering ATCH secretion at the pituitary and hypothalamus (Hontela 1998).

Cortisol affects a variety of systems that regulate homeostasis. It can induce metallothioneines to sequester metals (Hyliner et al. 1989), stimulate protein degradation (Freeman and Idler 1973), increase mobilization of liver glycogen reserves, increase plasma glucose, increase Na⁺/K⁺ATPase activity (Shrimpton and McCormick 1999), suppress the immune system, suppress maturation, and suppress sex steroid secretion (Carragher and Sumpter 1990; Hontela 1997). High plasma cortisol levels indicate the animal is under acute or sub-chronic stress, while low levels indicate no stress, interrenal exhaustion or impairment of the HPI axis (Wedemeyer and McLeay 1981; Girard et al. 1998; Hontela 1998).

Corticosteroids can also be influenced by factors other than stressors. Genetic differences between species may result in very different cortisol levels (Barton 2002) and developmental stage may also be important. Some fish are more sensitive to stress during smolting and may decrease their cortisol production as they age (Barton 2002). The stress response can also be affected by a number of other factors, including diurnal secretion cycles, temperature, background color, light wavelength, nutritional state, and disease (Gilham and Baker 1985; Barton 2002). Finally, multiple stressors can act in a synergistic fashion and a previous stressor may influence the response to a new stressor (Barton 2002). Due to all the potential confounding factors, care must be taken during experimental design to ensure the stressor under investigation is the only factor altering the stress response in treated groups.

1.2.2. Effect of pollutants on HPI axis

Pollutants can also activate the stress response in fish. At low chronic levels, pollutants may cause a slow increase in plasma cortisol followed by a decrease to basal levels as the fish adapts (Donaldson 1981); however, the decrease may also be due to interrenal exhaustion or impairment of the HPI axis (Hontela 1997). Moderate pollutant levels cause a similar response, but there may be a second cortisol peak if the fish is unable to adapt to the pollutant (Donaldson 1981). At high acute exposures, the common response is a quick cortisol spike, often maintained until death (Donaldson 1981).

Exposure to metals such as cadmium (Cd), zinc (Zn) and copper (Cu) can interfere with the HPI axis. A field study showed that perch from Cd, Zn, and Cu polluted lakes were unable to generate a normal stress response when challenged by physical confinement (Laflamme et al. 2000). Another study showed that chronic exposure to

heavy metals and organic contaminants decreased cortisol secretion in response to capture stressors or ACTH injections (Girard et al. 1998). Earlier studies have shown that salmon exposed to Cd died without a cortisol increase, but Cu created a dose-dependent cortisol response (Schreck and Lorz 1978). These results indicate the HPI axis' response to chronic and acute heavy metal stress varies widely with the dose, exposure duration, species, and metal present; therefore, results must be interpreted carefully.

Pesticides can also influence the stress response of fish. *In vitro*, diazinon decreased cortisol secretion and it could not be restored by ACTH (stimulates steroidogenic cells to synthesize cortisol) or cAMP (activates protein kinase A) stimulation (Bisson and Hontela 2002). This suggests that diazinon acts downstream of both ACTH and cAMP in the cortisol synthesis signaling pathway and may affect other important proteins and enzymes such as protein kinase A (stimulates release of free cholesterol) or StAR protein (mediates transfer of cholesterol across the mitochondrial membrane to cP450_{scc}). The pesticides endosulfan and mancozeb also disrupt cortisol synthesis in a similar fashion (Bisson and Hontela 2002). Conversely after atrazine exposure, cortisol secretion was restored by ACTH, but not cAMP indicating it acts upstream of cAMP, but downstream of ACTH (Bisson and Hontela 2002). Not all pesticides impair the stress response. Aldrin, dieldrin, endrin, dibenzothiophene, and carbazol activated the HPI axis and increased plasma cortisol levels (Hontela 1997). Other pesticides, such as lindane and DDT, are able to be impair the HPI axis without raising cortisol levels (Hontela 1997). This may be due to differences in exposure levels. Low chronic exposures may exert adrenotoxic effects, but not activate the stress response. On the other hand, high acute

exposures activate the stress response and interfere with adrenal function simultaneously. A fungicide, didecyldimethylammonium chloride, elevated cortisol and other primary and secondary indicators of the stress response in rainbow trout after 24 hours, but swimming performance (a tertiary indicator of the stress response) decreased after 12 and 24 hours of exposure (Wood et al. 1996). In this case, swimming performance was the most sensitive stress response indicator. Agricultural chemicals can have diverse effects on the stress response of fish and should be carefully investigated to determine how they affect fish and what impact the effects will have on survival and reproduction.

Over stimulation of the HPI axis by numerous stressors may rapidly deplete an animal's energy reserves leaving little energy for growth or reproduction. For example, the growth rate of Atlantic salmon decreased with increased handling stressor (McCormick et al. 1998). Cortisol can also suppress the immune system and reproduction (Hontela 1997); however, fish may adapt to the stressor or become less sensitive to cortisol. For example, in rainbow trout chronic elevated cortisol caused a downregulation of cortisol receptors decreasing their sensitivity to cortisol (Shrimpton and Randall 1994). On the other hand, if the HPI axis is impaired and cortisol is not produced, then the fish may be unable to respond appropriately to a stressor (Hontela 1997). This can have serious consequences for the fish's survival and reproduction. For example, glycogen stores are not used as efficiently in impaired fish (Levesque et al. 2002); however, more research is needed.

Social status can also influence cortisol levels in fish. Both subordinate rainbow trout and brook trout had higher plasma cortisol levels than dominant individuals (Sloman et al. 2001a). Social status is a chronic stressor and may be a good model to study the

effects of chronically elevated cortisol. Juvenile rainbow trout with artificially raised cortisol showed decreased appetite, growth rate, condition factor, and food conversion efficiency; however, swimming performance was unchanged (Gregory and Wood 1999). The possible cumulative effects of elevated cortisol from contaminant exposure and social status may also be a concern. Social hierarchies in brown trout broke down during exposure to a simulated environmental stressor (drought); however, plasma cortisol levels were the same in the control and drought fish (Sloman et al. 2001b). This suggests that as the hierarchies broke down, the cortisol elevation in the drought tank was due to the environmental stressor, but the cortisol levels in the control tank were due to the social stressor. By extension, low levels of contaminants that chronically elevate plasma cortisol may not add an additional increase in chronic cortisol levels, but replace the cortisol elevation from social status. Regardless, the consequences of chronically elevated cortisol levels should become apparent in fish stressed by chronic contaminant exposure.

There has been very little research on the effect of Se on the HPI axis and available research is conflicting. In gray seals, selenium dioxide decreased the corticosterone produced (Freeman and Sangalang 1977), but in rat cells sodium selenite had no significant effect on adrenal steroid production (Nishiyama et al. 1985). On the other hand, injections of 1.6-3.8 mg/kg of sodium selenite raised the cortisol, ACTH, glucose, and corticosterone levels in male rats (Potmis et al. 1993). In birds, Se reduced corticosteroid production of female, but not male eiders (Wayland et al. 2002). Some of the differences in the HPI axis response may be due to taxonomic variation, Se dose, and exposure route; however, the conflicting results suggest much more research is needed.

More research is also needed because there has been no work on the effect of excess Se on the HPI axis in fish or on hormone synthesis in general.

1.2.3. Summary

The HPI axis has been well defined; however, the effect of various stressors on the HPI axis and their specific target sites will always require further studies, as new chemicals are continuously appearing on the market. Selenium is an element whose beneficial and toxic properties are also well known, but little is known about its effects on the HPI axis. More research is necessary to determine this effect, the mechanism, and the cellular targets of excess Se.

1.3. Oxidative Stress

Oxidative stress, or the cellular damage from reactive oxygen species (ROS), occurs when ROS production exceeds cellular defense mechanisms. This happens if there is a sudden increase in ROS production, impairment of cellular defenses, or a failure to repair ROS damage (Dorval and Hontela 2003; Oakes and McMaster 2004). Oxidative stress has been implicated in many different diseases, including Keshan disease (Se deficiency in humans), arthritis, cancer initiation and promotion, malaria, stroke, aging, multiple sclerosis, cataracts and many more (Halliwell 1987; Packer 1991). Oxidative stress may also be involved in Se toxicity (Palace et al. 2004b). Finally, parameters associated with oxidative stress are being investigated as early pollution biomarkers allowing contaminant effects to be documented before population declines are observed.

1.3.1. Damage from ROS

Reactive oxygen species are generated in several different ways. They are intermediates in the cellular respiration pathway and approximately 5% escape (Kelly et al. 1998). ROS are also byproducts of oxidizing enzymes (Livingstone 2001), and they are involved in phagocytosis (Winston and Di Giulio 1991). Additionally, ROS production may be enhanced by redox cycling of xenobiotics (Kelly et al. 1998). ROS have many useful roles in biological systems. They are cytotoxic agents against pathogens, they act as neurotransmitters, and transcription factors (Kelly et al. 1998); however, when ROS accumulate they cause serious damage to cell components and subsequently cell function.

Reactive oxygen species damage lipids, proteins, and DNA. ROS can react with lipids in a process called lipid peroxidation (LPO) (Livingstone 2001). Lipid peroxidation is a chain reaction that occurs in polyunsaturated fatty acids when a ROS removes a hydrogen atom from a methylene carbon (initiation) resulting in the formation of another ROS that can react with other fatty acids (propagation). This continues until two ROS join together (termination) (Kelly et al. 1998). Lipid peroxidation causes structural damage affecting membrane permeability and fluidity (Kelly et al. 1998). Species or tissues with more polyunsaturated fatty acids may be more vulnerable to LPO (Oakes et al. 2003; Oakes and Van Der Kraak 2003). Damage is commonly assessed by measuring the formation of LPO end products such as malonaldehydes and 4-hydroxyalkenals (Kelly et al. 1998).

ROS may also cause protein peroxidation (Livingstone 2001). Protein carbonyls, an end product, are used as a direct indicator of protein peroxidation (Parvez and

Raisuddin 2005). Protein peroxidation is less commonly measured as an indicator of oxidative stress than LPO. DNA damage occurs when the ROS attack the bases or the sugar-phosphate backbone. They can cause intermolecular complexes and crosslinks, as well as hydroxylation and base fragmentation (Kelly et al. 1998).

1.3.2. Cellular Defenses

There are various cellular mechanisms to remove excess ROS and avoid oxidative damage. These oxidative stress defenses, or antioxidants, can be divided into two categories: enzymes and scavengers. Enzymatic defenses include glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). Glutathione peroxidase, a selenium dependent enzyme, (Orbea et al. 2000), inactivates organic ROS and hydrogen peroxide with reduced glutathione (GSH) (Kelly et al. 1998). Glutathione peroxidase appears at stage 33 of fry development in rainbow trout (Aceto et al. 1994) and is important during the larval stage (Mourente et al. 1999). Its activity decreases in the kidney, but increases in gills and white muscle as rainbow trout age (Otto and Moon 1996). Catalase breaks down hydrogen peroxide forming water and oxygen. It is primarily found in peroxisomes, although it is present in the mitochondria and cytosol as well (Kelly et al. 1998). Catalase appears in stage 19-20 of rainbow trout development, but the activity was greatly increased during fry development (Aceto et al. 1994). Additionally, it decreases in rainbow trout liver as they age, but increases in the gill and red muscle (Otto and Moon 1996). Superoxide dismutase is another enzymatic antioxidant. There are three different forms of SOD, and all remove free oxygen radicals, forming hydrogen peroxide and oxygen (Kelly et al. 1998). Hydrogen peroxide can then be eliminated by CAT or GPx. Manganese SOD is found in the mitochondria, the

copper/zinc form in the cytosol, and the extracellular form in the extracellular matrix (Kelly et al. 1998). In fish, SOD activity appears during stages 19-20 and increases greatly during rainbow trout fry development (Aceto et al. 1994). It decreases in the liver and white muscle of rainbow trout with age, but increases in the gill (Otto and Moon 1996). Glutathione reductase does not directly remove any ROS, but it recycles oxidized glutathione (GSSG) back to the reduced form (GSH) that is required for GPx.

Antioxidant scavengers include GSH, vitamin E (tocopherols), vitamin A (retinols), vitamin C (ascorbic acid), and carotenoids. GSH is the most important intracellular scavenger (Di Mascio et al. 1991) and the most intracellularly abundant thiol (Kelly et al. 1998). It can directly reduce many ROS while being oxidized to GSSG (Kelly et al. 1998). For example, it has been shown to protect adrenal cells against oxidative stress induced by the pesticide endosulfan (Dorval and Hontela 2003). GSH increases in the liver, kidney, red muscle, heart and brain of rainbow trout as they age, but decreases in the gills (Otto and Moon 1996). Tocopherols are lipid soluble and membrane bound scavengers (Kelly et al. 1998). They defend against LPO by removing an electron from the intermediate ROS species that propagates LPO stopping the chain reaction (Sies and Stahl 1995). Fish fed a vitamin E deficient diet have elevated LPO and increase their enzymatic defenses to compensate (Tocher et al. 2002; Puangkaew et al. 2005). Ascorbic acid is the most important antioxidant found in the extracellular fluid (Sies and Stahl 1995). It is water soluble and scavenges a number of different ROS including thiol radicals generated during xenobiotic reduction (Kelly et al. 1998). Ascorbic acid regenerates α -tocopherol as it scavenges ROS (Kelly et al. 1998) further increasing its value as an antioxidant. Retinols (Vitamin A) combine with peroxyl

radicals, quench ROS, or scavenge thiol radicals (Palace et al. 1999). Carotenoids, another lipid soluble antioxidant (Kelly et al. 1998), capture many different ROS including peroxy radicals, thiol radicals, and oxygen radicals (Palozza and Krinsky 1992; Palace et al. 1999). The scavenging and enzymatic antioxidants are linked in many ways. Often if one is deficient, others increase to compensate (Puangkaew et al. 2005). The antioxidant defense system is very elaborate and interconnected; however, it is not indestructible and damage from ROS does occur.

1.3.3. Contaminants and oxidative stress

Recently there has been an explosion of research linking oxidative stress parameters and exposure to contaminants. The primary goal of the research is to find biomarkers that can be used as early indicators of pollution. For example, SOD has been identified as an early warning sign of chromium pollution (Roberts and Oris 2004). Subcellular markers are very useful as they identify target organisms within an ecosystem before potentially irreversible population effects are seen. The other objective of studies investigating causal links between oxidative stress and contaminant exposure is to elucidate toxicity mechanisms of the various pollutants. For example, oxidative stress is a proposed mechanism for blue sac disease in fish; however, a study with retene was unable to conclusively prove this mechanism due to conflicting results (Bauder et al. 2005).

The effect of contaminants on enzyme antioxidants is unclear (Table 1.2) as there are many different variables that influence the outcome. Antioxidant status can change with age (Otto and Moon 1996), nutritional status (Pascual et al. 2003), and amount of toxicant (Bauder et al. 2005). Generally, LPO increases with exposure to contaminants

(Table 1.2); however, there are exceptions. LPO decreased with Se exposure in rainbow trout (Orun et al. 2005) possibly because Se can defend against oxidative stress as a part of GPx. The response of GPx to contaminants is very variable (Table 1.2) changing with both species and contaminant. SOD increased with most contaminants; although, it decreased with cadmium exposure for pearl dace, white sucker, and lake charr (Palace and Klaverkamp 1993). SOD activity may increase as a way to cope with increased ROS, but then decrease when the enzyme itself is damaged by the ROS. This suggests the cadmium exposure of the dace, sucker, and charr was severe in the study reported by Palace and Klaverkamp (1993). The response of CAT to pollutants appears contaminant and species specific (Table 1.2). It decreases with agricultural runoff and pesticides (endosulfan), but increases or shows no change with metals (copper and cadmium). There has been much less work done on GR, but it appears to decrease with increasing copper concentrations (Lui et al. 2005). Results from contaminant studies must be carefully interpreted because there are many variables other than contaminant level that may affect antioxidant enzyme levels.

Scavenging antioxidants levels also vary with contaminant type, exposure level, and animal species exposed (Table 1.3). Reduced glutathione generally decreases with increasing contaminants supporting the hypothesis that GSH removes ROS. Tocopherols and retinols appear to decrease with increasing contaminants; however, there is little information on the effects of contaminants on vitamin levels. All of the antioxidant enzymes and scavengers work together to protect the fish from oxidative stress and overall response patterns may vary with species, contaminant, age, or tissue studied.

Oxidative stress is a proposed Se toxicity mechanism (Palace et al. 2004b) and it is also the mechanism associated with Se deficiency. Se is an important constituent of glutathione peroxidase and fish fed a Se deficient diet have decreased liver GPx activity (Bell et al. 1986). Other studies have shown if Se deficient diets are supplemented with vitamin E (both GPx and tocopherols remove organic peroxides), deficiency symptoms disappear (Gursu et al. 2003). Moreover, rats fed Se deficient diets had significantly lower liver and adrenal glutathione peroxidase activity, and the corresponding increase in oxidative stress interfered with corticosterone response to ACTH (Chanoine et al. 2004). Additionally, mice fed Se deficient diet had lower GPx levels and decreased serum lutenizing hormone, follicle-stimulating hormone, and testosterone levels (Kaur and Bansal 2004). Thus, oxidative stress from Se deficiency can affect both adrenal and sex steroidogenesis. In a mouse tumor cell line, Se deficiency decreased GPx activity and decreased steroid hormone production; however, it did not affect StARs ability to transfer cholesterol across the mitochondrial membrane (Chanoine et al. 2001). The decrease in GPx suggests that oxidative stress is responsible for decreased hormone production in tumor cells. These studies suggest symptoms of Se deficiency are a direct result of increased oxidative stress; however to confirm this, damage indicators, such as LPO levels, should be assessed in Se deficient animals, including fish.

The proposed pathways that link oxidative stress and Se also include glutathione metabolism (Figure 1.1). Selenium species react with GSH creating ROS that can cause oxidative damage if not removed by antioxidants. Selenites, selenium oxides, and selenomethionine may be capable of creating ROS in this manner. Specifically, rainbow trout embryos can metabolize selenomethionine that accumulates in the eggs, generating

a ROS responsible for malformations in the embryo (Palace et al. 2004b). Selenite can also react with GSH to produce ROS and elemental Se (Spallholz 1993).

In adult fish, Se does not increase LPO, and only SOD activity (Orun et al. 2005) and GSH:GSSG (Holm et al. 2005) increased with Se exposure. Adult and larval fish may have different Se toxicity mechanisms, but there are only two studies that have examined the relationship between Se and oxidative stress in adult fish. More work is needed to characterize the relationship between Se and oxidative stress in both larval and adult fish.

Additional support for oxidative stress as a Se toxicity mechanism comes from cancer research. Selenium is being investigated as a cancer treatment because of its ability to generate ROS (Spallholz 1993; Spallholz et al. 2004). Reduced glutathione acts as a pro-oxidant in the presence of Se increasing ROS production (Shen et al. 2000). The ROS induce apoptosis and interfere with the cell cycle in *in vitro* systems (Ip 1998) slowing or preventing tumor growth. At higher concentrations Se may use similar mechanisms to damage non-cancerous cells disrupting vital cellular processes; however, more research is needed.

1.3.4. Summary

Oxidative stress is a topic highly relevant to toxicology. Parameters associated with oxidative stress can be used as non-specific early indicators of pollution in aquatic ecosystems. Eventually, with contaminant specific research, patterns in the oxidative stress response may emerge creating a set of biomarkers specific to a class of contaminants. The development of sub-cellular biomarkers is useful because it allows intervention before population effects are seen; however, much more research is needed

to identify contaminant specific patterns. Oxidative stress may also be an important toxicity mechanism. After a response pattern has been established, carefully designed in vitro and in vivo experiments may identify the mechanisms that caused the observed pathologies. Differences in species sensitivity to toxicants may also be useful in designing these experiments.

1.4. General Summary

Selenium is an essential element that bioaccumulates and becomes toxic at concentrations slightly higher than required to avoid deficiency symptoms. Toxicity and deficiency symptoms are similar and both involve oxidative stress; however much more information is needed to determine the exact toxicity mechanism. Additionally, Se sensitivity varies widely, even between closely related species, and investigation of these differences may provide further insight into toxicity mechanisms. In fish, the majority of research has focused on Se's effect on reproduction (i.e., teratogenesis), but many other systems may be adversely altered by chronic Se exposure.

There is very little research on the effect of Se on the PSR mediated by cortisol. In some organisms, Se appears to increase corticosteroid production (the animal recognizes it as a stressor) and in others it has no effect or decreases steroid production. More research on fish, a group of organisms exposed to increasing Se from anthropogenic sources, is needed to determine the effect of Se on the HPI axis. If Se impairs the HPI axis, a fish's ability to raise cortisol levels in response to an additional stress (e.g. increased temperature or another contaminant) may be impaired and they will be unable to reallocate energy resources to optimize survival. At the other extreme, if Se

sensitizes the HPI axis they may respond maximally to small disturbances (e.g., a rock falling into the water) using up energy needed for other processes such as growth and reproduction. Chronically stressed fish will be more susceptible to disease and have decreased reproductive success because cortisol suppresses the immune system and sex steroid secretion (Pankhurst et al. 1995). Furthermore, if a toxicant interferes with steroidogenesis in the HPI axis, it may also interfere with the production of other steroids. The above examples are extreme cases, but they illustrate the importance of determining the effects of toxicants on the HPI axis.

Oxidative stress is a growing area of research in toxicology because increased LPO, altered antioxidant enzyme levels, and altered antioxidant scavenger levels may be early biomarkers of contamination. Oxidative stress may also be the mechanism of action for many contaminants. In fish, Se has been shown to produce ROS in the presence of GSH (Spallholz et al. 2004) suggesting that oxidative stress is a key toxicity mechanism; however, this has been only shown for teratogenesis and there are many other effects to be investigated. Understanding how Se affects the HPI axis will provide more information about Se toxicity mechanisms and determine if oxidative stress is a factor. It will also determine if oxidative stress parameters can be used as early biomarkers of Se contamination.

There are several important knowledge gaps that need to be addressed to link Se toxicity, the PSR, and oxidative stress in fish. These gaps are: (i) the effect of Se on the PSR in different species, (ii) the response of antioxidant enzymes and scavengers to Se exposure, and (iii) the effect of Se on oxidative stress damage indicators (e.g., LPO). Linking Se toxicity, the PSR, and oxidative stress is important because it will provide

valuable information on the toxicity mechanism of Se, the effect of Se on the HPI axis, and identify biomarkers for early detection of Se toxicity.

Table 1.1. Acute selenium toxicity levels for various fish species.

Organism	Scientific Names	Life Stage	Se Species	Test	Se Concentration (mg/L)	Source
Arctic Grayling	<i>Thumallus arcticus</i>	alevin	selenate ^b	96hr LC ₅₀	100.0	(Buhl and Hamilton 1991)
Arctic Grayling		alevin	selenite ^a	96hr LC ₅₀	76.0	(Buhl and Hamilton 1991)
Arctic Grayling		juvenile	selenate ^b	96hr LC ₅₀	180.0	(Buhl and Hamilton 1991)
Arctic Grayling		juvenile	selenite ^a	96hr LC ₅₀	34.3	(Buhl and Hamilton 1991)
Bluegill	<i>Lepomis macrochirus</i>		selenium dioxide	LC ₅₀	28.5	(USEPA 1980)
Brook trout	<i>Salvelinus fontinalis</i>		selenium dioxide	LC ₅₀	10.2	(USEPA 1980)
Coho Salmon	<i>Oncorhynchus kisutch</i>	alevin	selenate ^b	96hr LC ₅₀	379.0	(Buhl and Hamilton 1991)
Coho Salmon		alevin	selenite ^a	96hr LC ₅₀	80.0	(Buhl and Hamilton 1991)
Coho Salmon		juvenile	selenate ^b	96hr LC ₅₀	74.0	(Buhl and Hamilton 1991)
Coho Salmon		juvenile	selenite ^a	96hr LC ₅₀	7.8	(Buhl and Hamilton 1991)
Fathead Minnow	<i>Pimephales promelas</i>		selenite	LC ₅₀	2.2 – 10.5	(Adams 1976)
Fathead Minnow		fry	selenite ^a	96hr LC ₅₀	1.1	(Halter et al. 1980)
Goldfish	<i>Carassius auratus</i>		selenium dioxide	LC ₅₀	26.1	(USEPA 1980)
Mosquito fish	<i>Cambosia affinis</i>		selenite ^a	LC ₅₀	12.6	(USEPA 1980)
Rainbow Trout	<i>Oncorhynchus mykiss</i>	alevin	selenate ^b	96hr LC ₅₀	47.0	(Buhl and Hamilton 1991)
Rainbow Trout		alevin	selenite ^a	96hr LC ₅₀	118.0	(Buhl and Hamilton 1991)
Rainbow Trout		juvenile	selenate ^b	96hr LC ₅₀	32.3	(Buhl and Hamilton 1991)
Rainbow Trout		juvenile	selenite ^a	96hr LC ₅₀	9.0	(Buhl and Hamilton 1991)
Rainbow Trout			selenite ^a	96hr LC ₅₀	1.8	(Hunn et al. 1987)
Rainbow Trout			selenite ^a	96hr LC ₅₀	7.2	(Hodson et al. 1980)
Rainbow Trout			selenite ^a	96hr LC ₅₀	4.2-4.5	(Adams 1976)
Rainbow Trout		juvenile	selenite ^a	96hr LC ₅₀	12.5	(Goeltl and Davies 1976)

^a NaSeO₃^b NaSeO₄

Table 1.2. The effects of increasing contaminant exposure on the enzymatic defenses (glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and lipid peroxidation (LPO) in fish. Responses are an increase (↑), a decrease (↓), no significant change (NC), an increase followed by a decrease (↑/↓), and a decrease followed by an increase (↓/↑).

Contaminant	Species	Tissue	LPO	GPx	SOD	CAT	GR	Reference
agricultural runoff	Cyprinidae	liver	↑		↑	↓		(Güll et al. 2004)
	white sucker	liver	↑	↓		↓		(Dorval et al. 2005)
	white sucker	adrenal	↑	↓		↓		(Dorval et al. 2005)
β-naphtho-flavone	eel	gill	↑	↑		↑		(Ahmad et al. 2005)
	eel	kidney	↑	NC		NC		(Ahmad et al. 2005)
cadmium	pearl dace	liver		NC	↓	NC		(Palace and Klaverkamp 1993)
	white sucker	liver		NC	↓	NC		(Palace and Klaverkamp 1993)
	lake charr	liver		NC	↓	NC		(Palace and Klaverkamp 1993)
	rainbow trout	liver			↑			(Aït-Aïssa et al. 2003)
	sea bass	Kidney	NC			↓		(Roméo et al. 2000)
chromium	rainbow trout	liver	↑/↓		↑			(Roberts and Oris 2004)
	rainbow trout	gill	↓/↑		NC			(Roberts and Oris 2004)
copper	Stickleback	liver		↑	↑	↑		(Sanchez et al. 2005)
	goldfish	liver					↓	(Lui et al. 2005)
	eel	gill	NC	NC		NC		(Ahmad et al. 2005)
	eel	kidney	NC	↓		↓		(Ahmad et al. 2005)
	sea bass	Kidney	↑			NC		(Roméo et al. 2000)
	Atlantic salmon	intestine	↑	↑/↓				(Berntssen et al. 2000)
	Atlantic salmon	liver	↑	↑				(Berntssen et al. 2000)
	Atlantic salmon	kidney	↑	NC				(Berntssen et al. 2000)
dichlorophenol	goldfish	liver		↓/↑	↑/↓	↓/↑	NC	(Zhang et al. 2005)
endosulfan	rainbow trout	adrenal cells		↓		↑/↓		(Dorval et al. 2003)

Contaminant	Species	Tissue	LPO	GPx	SOD	CAT	GR	Reference
hydrocarbons	dab	liver	↑/↓	NC	↑/↓	↑/↓		(Livingstone et al. 1993)
	channel catfish	liver	↑		↑	↑		(Di Giulio et al. 1993)
mercury	catfish	liver		↑			NC	(Elia et al. 2003)
Paraquat	snake-head catfish							
	(<i>Channa punctata</i>)	liver	NC					(Parvez and Raisuddin 2006)
	snake-head catfish	kidney	NC					(Parvez and Raisuddin 2006)
	snake-head catfish	gill	NC					(Parvez and Raisuddin 2006)
PCDFs	lake sturgeon	liver	↑	NC	↑/↓			(Palace et al. 1996)
	lake sturgeon	kidney		NC				(Palace et al. 1996)
pulp & paper effluent	longnose sucker	liver	↑					(Oakes and McMaster 2004)
	longnose sucker	gonad	↑					(Oakes and McMaster 2004)
	white sucker	liver	↑					(Oakes and Van Der Kraak 2003)
	white sucker	gonad	↑					(Oakes and Van Der Kraak 2003)
retene	rainbow trout	larvae	NC					(Bauder et al. 2005)
selenium	rainbow trout	brain	↓	NC	↑			(Orun et al. 2005)
	rainbow trout	heart	↓	NC	↑			(Orun et al. 2005)
	rainbow trout	spleen	↓	NC	↑			(Orun et al. 2005)
	rainbow trout	liver	↓	↑	↑			(Orun et al. 2005)
sewage effluent	longnose sucker	liver	NC					(Oakes and McMaster 2004)
	longnose sucker	gonad	NC					(Oakes and McMaster 2004)

Table 1.3. The effects of increased contaminants exposure on the scavenging defenses (reduced glutathione (GSH), reduced to oxidized glutathione ratio (GSH:GSSG), tocopherols (TOC), retinols (RET), and ascorbic acid (AscA)) in fish. Responses are an increase (↑), a decrease (↓), no significant change (NC), an increase followed by a decrease (↑/↓), and a decrease followed by an increase (↓/↑).

Contaminant	Organism	Organ	GSH	GSH: GSSG	TOC	RET	AscA	Reference
agricultural runoff	white sucker	liver	↓					(Dorval et al. 2005)
	white sucker	adrenal	↓					(Dorval et al. 2005)
β-naphthoflavone	eel	gill	↓					(Ahmad et al. 2005)
	eel	kidney	NC					(Ahmad et al. 2005)
cadmium	rainbow trout	liver	↑	NC				(Aït-Aïssa et al. 2003)
	Atlantic salmon	intestine	NC		NC			(Berntssen et al. 2000)
	Atlantic salmon	liver	NC		NC			(Berntssen et al. 2000)
	Atlantic salmon	kidney			NC			(Berntssen et al. 2000)
copper	stickleback	liver	↓					(Sanchez et al. 2005)
	goldfish	liver	↑/↓	↑				(Lui et al. 2005)
	eel	gill	↓					(Ahmad et al. 2005)
	eel	kidney	↓					(Ahmad et al. 2005)
	Atlantic salmon	intestine	↓		↓			(Berntssen et al. 2000)
	Atlantic salmon	liver	↓		NC			(Berntssen et al. 2000)
	Atlantic salmon	kidney			NC			(Berntssen et al. 2000)
dichlorophenol	goldfish	liver	↓	↓*				(Zhang et al. 2005)
		adrenal						
endosulfan	rainbow trout	cells	↓					(Dorval et al. 2003)
hydrocarbons	channel catfish	liver	↑					(Di Giulio et al. 1993)
mercury	catfish	liver	↓/↑ ^t					(Elia et al. 2003)
mining effluent	lake trout	liver				↓		(Payne et al. 1998)
paraquat	snake-head catfish	liver	↓				↑	(Parvez and Raisuddin 2006)

Contaminant	Organism	Organ	GSH	GSH: GSSG	TOC	RET	AscA	Reference
paraquat	snake-head catfish	kidney	NC				NC	(Parvez and Raisuddin 2006)
	snake-head catfish	gill	↓				NC	(Parvez and Raisuddin 2006)
PCBs	lake charr	liver			NC	↓		(Palace and Brown 1994)
	lake harr	kidney			↑	NC***		(Palace and Brown 1994)
	lake charr	plasma			NC	↓		(Palace and Brown 1994)
	Arctic grayling	plasma				NC		(Palace et al. 2001)
	Arctic grayling	liver			↓	↑ ^{tt}		(Palace et al. 2001)
PCDFs	lake sturgeon	liver			↓	NC		(Palace et al. 1996)
	lake sturgeon	kidney			↓			(Palace et al. 1996)
	lake sturgeon	plasma			↑			(Palace et al. 1996)
pulp and paper effluent	longnose sucker	liver					↓	(Oakes and McMaster 2004)
	White sucker	liver			↑			(Oakes and Van Der Kraak 2003)
	White sucker	gonad						(Oakes and Van Der Kraak 2003)
retene	rainbow trout	larvae	↓		↓			(Bauder et al. 2005)
selenium	rainbow trout	liver		↑				(Holm 2002)
	rainbow trout	plasma			NC	NC		(Holm 2002)
sewage effluent	longnose sucker	liver					↑**	(Oakes and McMaster 2004)

* decreased, then increased, then decreased to lowest point

** females only

*** retinol and dehyroretinol had no change, but retinyl palmitate increased

^t total glutathione (GSH + GSSG)

^{tt} females only after 90 days exposure

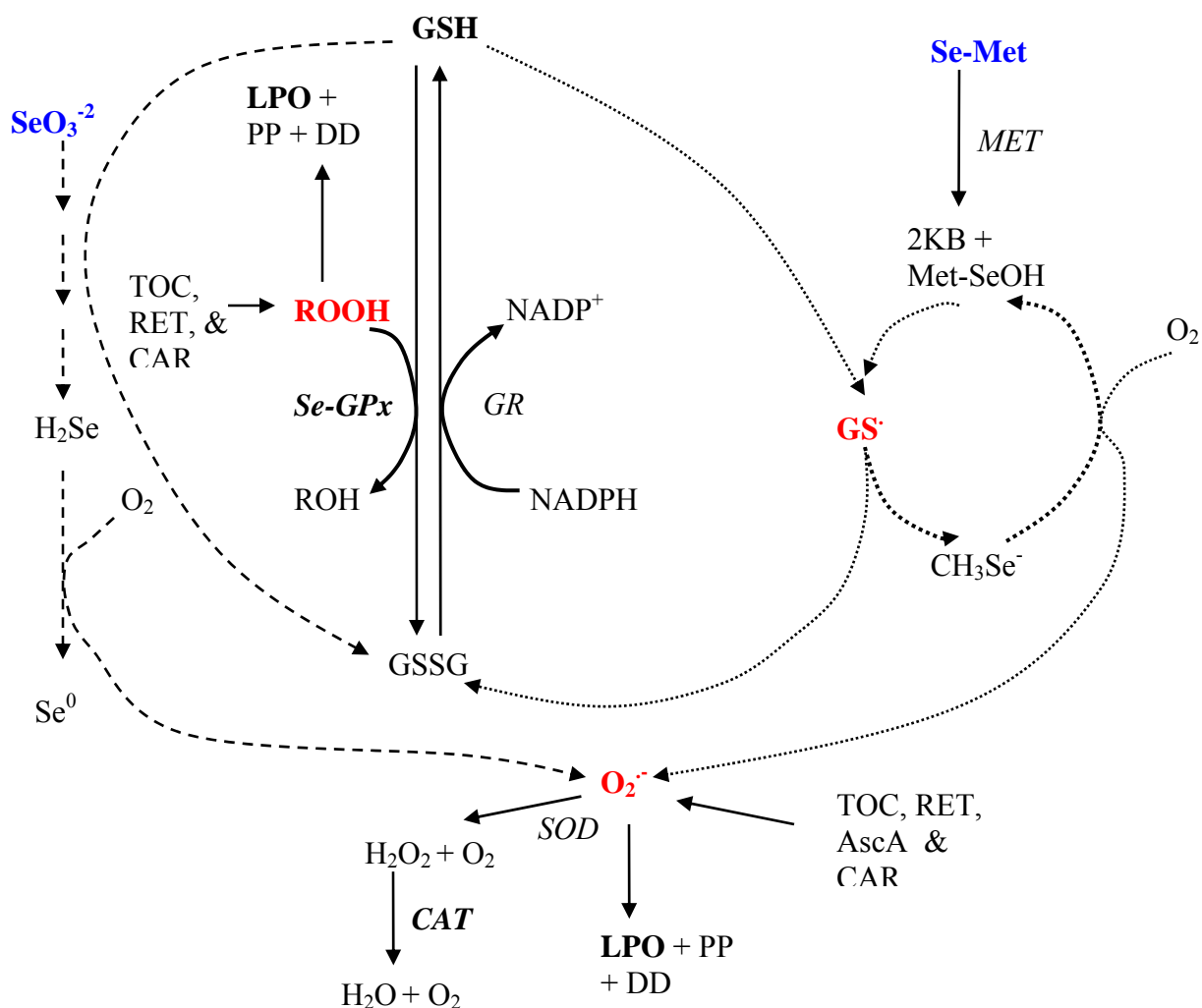


Figure 1.1. Summary of possible ROS formation by Se in the presence of GSH and defense mechanisms. Solid lines are known pathways (Kelly et al. 1998), dashed lines indicate pathways reviewed by Spallholz (1993) and dotted lines indicated pathways proposed by Palace, Spallholz et al. (2004). Italicized terms are enzymes and bold terms are compounds that were measured. Blue indicates initial Se compounds and red indicates reactive species produced.

Abbreviations: Se – selenium; LPO – lipid peroxidation; PP – protein peroxidation; DD – DNA damage; CAT – catalase; SOD – superoxide dismutase; CAR – carotenoids; TOC – tocopherols; AscA – ascorbic acid; RET – retinols; GR – glutathione reductase; GPx – glutathione peroxidase; KB – 2-ketobutyrate; Se-Met – selenomethionine; MET – methionase; Met-SeOH – methylselenol; GSH – reduced glutathione; GSSG – oxidized glutathione

1.5. Literature Cited

- Aceto A, Amicarelli F, Sacchetta P, Dragani B, Bucciarelli T, Masiocco L, Miranda M, Di Ilio C. 1994. Developmental aspects of detoxifying enzymes in fish (*Salmo iridaeus*). Free Radical Research 21(5):285-294.
- Adams W. 1976. The toxicity and residue dynamics of selenium in fish and aquatic invertebrates: Michigan State University.
- Ahmad I, Oliveira M, Pacheco M, Santos M. 2005. *Anguilla anguilla* L. oxidative stress biomarkers responses to copper exposure with or without β -naphthoflavone pre-exposure. Chemosphere 61:267-275.
- Aït-Aïssa S, Ausseil O, Palleul O, Vindimian E, Garnier-LaPlace J, Procher J-M. 2003. Biomarker responses in juvenile rainbow trout (*Oncorhynchus mykiss*) after a single and combined exposure to low doses of cadmium, zinc, PCB77 and 17 β -oestradiol. Biomarkers 8(6):491-508.
- Andersen O, Nielsen J, Sorensen J, Scherrebeck L. 1994. Experimental localization of intestinal uptake sites for metals (Cd, Hg, Zn, Se) *in vivo* in mice. Environmental Health Perspectives 103(Supplement 3):199-206.
- Arner E, Holmgren A. 2000. Physiological functions of thioredoxin and thioredoxin reductase. European Journal of Biochemistry 267:6102-6109.
- Banks A. 1997. Selenium. In: Lagowski J, editor. Macmillan Encyclopedia of Chemistry. New York: Simon and Schuster Macmillan. p 1334.
- Barton B. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.
- Bauder M, Palace V, Hodson P. 2005. Is oxidative stress the mechanism of blue sac disease in retene-exposed trout larvae? Environmental Toxicology and Chemistry 24(3):694-702.
- Bébian M, Lagniel G, Garin J, Touati D, Verméglio A, Labarre J. 2002. Involvement of superoxide dismutases in the response of *Escherichia coli* to selenium oxides. Journal of Bacteriology 184(6):1556-1564.
- Bell J, Pirie B, Adron J, Cowey C. 1986. Some effects of selenium deficiency on glutathione peroxidase (EC 1.11.1.9) activity and tissue pathology in rainbow trout (*Salmo gairdneri*). British Journal of Nutrition 55:305-311.
- Berntssen M, Lundebye A, Hamre K. 2000. Tissue lipid peroxidative responses in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. Fish Physiology and Biochemistry 23:35-48.
- Bisson M, Hontela A. 2002. Cytotoxic and endocrine-disrupting potential of atrazine, diazinon, endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed *in vitro*. Toxicology and Applied Pharmacology 180:110-117.
- Bowie G, Sanders J, Riedel G, Gilmour C, Breitburg D, Cutter G, Porcella D. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. Water, Air and Soil Pollution 90:93-104.

- Buhl K, Hamilton S. 1991. Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. *Ecotoxicology and Environmental Safety* 22:184-197.
- Carragher J, Sumpter J. 1990. The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. *General and Comparative Endocrinology* 77:403-407.
- Chanoine J-P, Compagnone N, Wong A, Mellon S. 2001. Modulation of steroidogenesis by selenium in a novel adrenal cell line developed using targeted tumorigenesis. *BioFactors* 14:229-238.
- Chanoine J-P, Wong A, Lavoie J-C. 2004. Selenium deficiency impairs corticosterone and leptin responses to adrenocorticotropin in the rat. *BioFactors* 20:109-118.
- Cleveland L, Little E, Buckler D, Wiedmeyer R. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquatic Toxicology* 27:265-280.
- Combs G, Jr., Combs S. 1986. The role of selenium in nutrition. Orlando: Academic Press, Inc. 532 p.
- Cooke S, Bunt C, Hamilton S, Jennings CP, MP, Cooperman M, Markle D. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. *Biological Conservation* 121:317-331.
- Di Giulio R, Habig C, Gallagher E. 1993. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. *Aquatic Toxicology* 26:1-22.
- Di Mascio P, Murphy M, Sies H. 1991. Antioxidant defense system: the role of carotenoids, tocopherols, and thiols. *American Journal of Clinical Nutrition* 53:194S-200S.
- Donaldson E. 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering A, editor. *Stress and Fish*. London: Academic Press. p 11-47.
- Dorval J, Hontela A. 2003. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Toxicology and Applied Pharmacology* 192:191-200.
- Dorval J, Leblond V, Deblois C, Hontela A. 2005. Oxidative stress and endocrine endpoints in white sucker (*Catostomus commersoni*) from a river impacted by agricultural chemicals. *Environmental Toxicology and Chemistry* 24(5):1273-1280.
- Dorval J, Leblond V, Hontela A. 2003. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquatic Toxicology* 63:229-241.
- Elia A, Galarini R, Taticchi M, Dörr A, Mantilacci L. 2003. Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicology and Environmental Safety* 55:162-167.
- Freeman H, Idler D. 1973. Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdnerii*) and the brook trout (*Salvelinus fontinalis*). *General and Comparative Endocrinology* 20:69-75.

- Freeman H, Sangalang G. 1977. A study of the effects of methyl mercury, cadmium, arsenic, selenium, and PCB, (Aroclor 1254) on adrenal and testicular steroidogenesis *in vitro*, by the gray seal *Halichoerus grypus*. Archives of Environmental Contamination 5:369-383.
- Gilham I, Baker B. 1985. A black background facilitates the response to stress in teleosts. Journal of Endocrinology 105:99-105.
- Girard C, Brodeur J, Hontela A. 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. Canadian Journal of Fisheries and Aquatic Sciences 55:438-450.
- Goeltl J, Davies P. 1976. Water Pollution Studies. In: Wildlife CDo, editor: Department of Natural Resources.
- Gregory T, Wood C. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Physiological and Biochemical Zoology 72(3):286-295.
- Güll Ş, Belge-Kurutaş E, Yıldız E, Şahan A, Doran F. 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyrinidae) living in Seyhan Dam Lake, Turkey. Environment International 30:605-609.
- Gursu M, Sahin N, Kucuk O. 2003. Effects of vitamin E and selenium on thyroid status, adrenocorticotropin hormone, and blood serum metabolite and mineral concentrations of Japanese Quails reared under heat stress (34°C). Journal of Trace Elements in Experimental Medicine 16:95-104.
- Halliwell B. 1987. Oxidants and human disease: some new concepts. FASEB 1(5):358-364.
- Halter M, Adams W, Johnson H. 1980. Selenium toxicity to *Daphnia magna*, *Hyallela azteca*, and the fathead minnow in hard water. Bulletin of Environmental Contamination and Toxicology 24:102-107.
- Hamilton S. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment 326:1-31.
- Haygarth P. 1994. Global importance and global cycling of selenium. In: Frankenberger W, Jr., Benson S, editors. Selenium In The Environment. New York: Marcel Dekker Inc. p 1-28.
- Hermanutz R. 1992. Malformation of the fathead minnow (*Pimephales promelas*) in an ecosystem with elevated selenium concentrations. Bulletin of Environmental Contamination and Toxicology 49:290-294.
- Hilton J, Hodson P, Slinger S. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). Journal of Nutrition 110:2527-2535.
- Himeno S, Imura N. 2002. Selenium in nutrition and toxicology. In: Sarker B, editor. Heavy Metals in the Environment. New York: Marcel Dekker Inc. p 587-629.
- Hodson P, Hilton J, Slinger S. 1986. Accumulation of waterborne selenium by rainbow trout (*Salmo gairdneri*) eggs, fry, and juveniles. Fish Physiology and Biochemistry 1(4):187-196.
- Hodson P, Spry D, Blunt B. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Canadian Fisheries Research Board of Canada 37(2):233-240.
- Holm J. 2002. Sublethal effects of selenium on rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Winnipeg: University of Manitoba.

- Holm J, Palace V, Siwik P, Sterling G, Evans R, Baron C, Weerner J, Wautier K. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environmental Toxicology and Chemistry* 24(9):2373-2381.
- Hontela A. 1997. Endocrine and physiological responses of fish of xenobiotics: role of glucocorticosteroid hormones. *Reviews in Toxicology* 1:1-46.
- Hontela A. 1998. Interrenal dysfunction in fish from contaminated sites: *in vivo* and *in vitro* assessment. *Environmental Toxicology and Chemistry* 17(1):44-48.
- Hunn J, Hamilton S, Buckler D. 1987. Toxicity of sodium selenite to rainbow trout fry. *Water Research* 21(2):233-238.
- Hyliner H, Andersson T, Haux C, Olsson P-E. 1989. Cortisol induction of metallothionein in primary culture of rainbow trout hepatocytes. *Journal of Cell Physiology* 139:24-28.
- Ip C. 1998. Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition* 128:1845-1854.
- Javanovic A, Grubor-Lajsic G, Djukic N, Gardinovacki G, Matic A, Spasic M. 1997. The effect of selenium on antioxidant system in erythrocytes and liver of the carp (*Cyprinus carpio* L.). *Critical Review in Food Science and Nutrition* 37(5):443-448.
- Kaur P, Bansal M. 2004. Effect of experimental oxidative stress on steroidogenesis and DNA damage in mouse testis. *Journal of Biomedical Science* 11:391-397.
- Kelly S, Havrilla C, Brady TA, KH, Levin E. 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environmental Health Perspectives* 106(7):375-384.
- Kennedy C, McDonald L, Loveridge R, Strosher M. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarki lewisi*). *Archives of Environmental Contamination and Toxicology* 39:46-52.
- Kleinow K, Brooks A. 1986a. Selenium compounds in the fathead minnow (*Pimephales promelas*) - II. quantitative approach to gastrointestinal absorption, routes of elimination and influence of dietary pretreatment. *Comparative Biochemistry and Physiology Part C* 83(1):71-76.
- Kleinow K, Brooks A. 1986b. Selenium compounds in the fathead minnow (*Pimephales promelas*) - I. uptake, distribution, and elimination of orally administered selenate, selenite and *l*-selenomethionine. *Comparative Biochemistry and Physiology Part C* 83(1):61-69.
- Köhrle J, Jakob F, Contempré B, Dumont J. 2005. Selenium, the thyroid, and the endocrine system. *Endocrine Reviews* 26(7):944-984.
- Lacroix A, Hontela A. 2004. A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*. *Aquatic Toxicology* 67:13-21.
- Laflamme J-S, Couillard Y, Campbell P, Hontela A. 2000. Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 57:1692-1700.
- Lemly A. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. *Ecotoxicology and Environmental Safety* 37:259-266.

- Lemly A. 1999. Selenium impacts on fish: an insidious time bomb. *Human and Ecological Risk Assessment* 5(6):1139-1151.
- Lemly A. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. *Aquatic Toxicology* 57:39-49.
- Lemly A, Smith G. 1987. Aquatic cycling of selenium: Implications for fish and wildlife. Washington, DC: US Fish and Wildlife Service. Report nr 12. 10 p.
- Levesque H, Moon T, Campbell P, Hontela A. 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquatic Toxicology* 60:257-267.
- Livingstone D. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin* 42(8):656-666.
- Livingstone D, Lemaire P, Matthews A, Peters L, Bucke D, Law R. 1993. Pro-oxidant, antioxidant and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses in liver of dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Marine Pollution Bulletin* 26(11):602-606.
- Lohner T, Reash R, Willet V, Rose L. 2001a. Assessment of tolerant sunfish populations (*Lipomis* sp.) inhabiting selenium-laden coal ash effluents 1. Hematological and population level assessment. *Ecotoxicology and Environmental Safety* 50:203-216.
- Lohner T, Reash R, Willet V, Rose L. 2001c. Assessment of tolerant sunfish populations (*Lepomis* sp.) inhabiting selenium-laden coal ash effluents 3. serum chemistry and fish health indicators. *Ecotoxicology and Environmental Safety* 50:225-232.
- Lorentzen M, Maage A, Julshamn K. 1994. Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (*Salmo salar*). *Aquaculture* 121:359-367.
- Lui J, Zhang J, Shen H, Wang X, Wang W. 2005. Impact of copper and its EDTA complex on the glutathione dependant system in freshwater fish (*Carassius auratus*). *Bulletin of Environmental Contamination and Toxicology* 74:1111-1117.
- Marier J, Jaworski J. 1983. Interactions of Selenium. Ottawa: National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality. Report nr NRCC 20643.
- Mayland H. 1994. Selenium in plant and animal nutrition. In: Frankenberger W, Jr., Benson S, editors. *Selenium in the Environment*. New York: Marcel Dekker Inc. p 29-45.
- McCormick S, Shrimpton J, Carey J, O'Dea M, Sloan K, Moriyama S, Björnsson B. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture* 168:221-235.
- Mommsen T, Vijayan M, Moon T. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* 9:211-268.
- Mourente G, Tocher D, Diaz E, Grau A, Pastor E. 1999. Relationships between antioxidants, antioxidant enzyme activities and lipid peroxidation products during early development in *Dentex dentex* eggs and larvae. *Aquaculture* 179:309-324.

- Nagpal N. 2001. Ambient water quality guidelines for selenium - overview. In: Branch WP, editor: Ministry of Water, Land and Air.
- Nishiyama S, Nakamura K, Ogawa M. 1985. Effects of heavy metals on corticosteroid production in cultured rat adrenocortical cells. *Toxicology and Applied Pharmacology* 81:174-176.
- Oakes K, McMaster M, Pryce AM, KR, Portt C, Hewitt L, MacLean D, Van Der Kraak G. 2003. Oxidative stress and bioindicators of reproductive function in pulp and paper mill effluent exposed white sucker. *Toxicological Sciences* 74:51-65.
- Oakes K, McMaster M, Van Der Kraak, G. 2004. Oxidative stress responses in longnose sucker (*Catostomus catostomus*) exposed to pulp and paper mill and municipal sewage effluents. *Aquatic Toxicology* 67(3):255-271.
- Oakes K, Van Der Kraak G. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology* 63:447-463.
- Ogle R, Knight A. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). *Archives of Environmental Contamination and Toxicology* 18:795-803.
- Ohlendorf H, Santolo G. 1994. Kesterson reservoir - past, present, and future: an ecological risk assessment. In: Frankenberger W, Jr., Benson S, editors. *Selenium in the Environment*. New York: Marcel Dekker Inc. p 69-117.
- Orbea A, Fahimi H, Cajaraville M. 2000. Immunolocalization of four antioxidant enzymes in digestive glands of mollusks and crustaceans and fish liver. *Histochemistry and Cell Biology* 114:393-404.
- Orun I, Ates B, Selamoglu Z, Yazlak H, Ozturk E, Yilmaz I. 2005. Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Fresenius Environmental Bulletin* 14(1):18-22.
- Otto D, Moon T. 1996. Endogenous antioxidant systems of two teleost fish, the rainbow trout and the black bullhead, and the effect of age. *Fish Physiology and Biochemistry* 15(4):349-358.
- Packer L. 1991. Protective role of vitamin E in biological systems. *American Journal of Clinical Nutrition* 53:1050S-1055S.
- Palace V, Allen-Gil S, Brown S, Evans R, Metner D, Landers D, Curtis L, Klaverkamp J, Baron C, Lockhart W. 2001. Vitamin and thyroid status in arctic grayling (*Thymallus arcticus*) exposed to doses of 3,3',4,4'-tetrachlorobiphenyl that induce the phase I enzyme system. *Chemosphere* 45:185-193.
- Palace V, Brown S. 1994. HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (*Salvelinus namaycush*) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. *Environmental Toxicology and Chemistry* 13(3):473-476.
- Palace V, Dick T, Brown S, Baron C, Klaverkamp J. 1996. Oxidative stress in lake sturgeon (*Acipenser fulvescens*) orally exposed to 2,3,7,8-tetrachlorodibenzofuran. *Aquatic Toxicology* 35:79-92.
- Palace V, Khaper N, Quin Q, Singal P. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biology and Medicine* 26(5/6):746-761.

- Palace V, Klaverkamp J. 1993. Variation of hepatic enzymes in three species of freshwater fish from precambrian shield lakes and the effect of cadmium exposure. *Comparative Biochemistry and Physiology Part C* 104(1):147-154.
- Palace V, Spallholz J, Holm J, Wautier K, Evans R, Baron C. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. *Ecotoxicology and Environmental Safety* 58:17-21.
- Palozza P, Krinsky N. 1992. Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview. *Methods in Enzymology* 213:403-421.
- Pankhurst N, VanDerKraak G, Peter R. 1995. Evidence that the inhibitory effects of stress on reproduction in teleost fish are not mediated by the action of cortisol on ovarian steroidogenesis. *General and Comparative Endocrinology* 99:249-257.
- Parvez S, Raisuddin S. 2005. Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). *Environmental Toxicology and Pharmacology* 20:112-117.
- Parvez S, Raisuddin S. 2006. Effects of paraquat on the freshwater fish *Channa punctata* (Bloch): non-enzymatic antioxidants as biomarkers of exposure. *Archives of Environmental Contamination and Toxicology* 50:392-397.
- Pascual P, Pedrajas J, Toribio F, López-Barea J, Peinado J. 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chemico-Biological Interactions* 145:191-199.
- Payne J, Malins D, Gunselman SR, A, Yeats P. 1998. DNA oxidative damage and vitamin A reduction in fish from a large lake system in Labrador, Newfoundland, contaminated with iron-ore mine tailings. *Marine Environmental Research* 46(1-5):289-294.
- Peakall D, Burger J. 2003. Methodologies for assessing exposure to metals: speciation, bioavailability of metals, and ecological host factors. *Ecotoxicology and Environmental Safety* 56:110-121.
- Pickering A. 1981. Introduction: the concept of biological stress. In: Pickering A, editor. *Stress and Fish*. London: Academic Press. p 1-9.
- Potmis R, Nonavinakere V, Rasekh H, Early J. 1993. Effect of selenium (Se) on plasma ACTH, β -endorphin, corticosterone and glucose in rat: influence of adrenal enucleation and metyrapone pretreatment. *Toxicology* 79:1-9.
- Puangkaew J, Kiron V, Satoh S, Watanabe T. 2005. Antioxidant defense of rainbow trout (*Oncorhynchus mykiss*) in relation to dietary n-3 highly unsaturated fatty acids and vitamin E contents. *Comparative Biochemistry and Physiology Part C* 140:1987-1996.
- Pyle G, Swanson S, Lehmkuhl D. 2001. Toxicity of uranium mine-receiving waters to caged fathead minnows, *Pimephales promelas*. *Ecotoxicology and Environmental Safety* 48:202-214.
- Roberts A, Oris J. 2004. Multiple biomarker response in rainbow trout during exposure to hexavalent chromium. *Comparative Biochemistry and Physiology Part C* 138:221-228.
- Roméo M, Gnassia-Barelli M, Lafaurie M, Girard J. 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of sea bass *Dicentrarchus labrax*. *Aquatic Toxicology* 48:185-194.

- Rosemond de S, Liber K, Rosaasen A. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker (*Catostomus commersoni*) from a northern Canadian Lake. *Bulletin of Environmental Contamination and Toxicology* 74:1134-1142.
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher J-M, Aït-Aïssa S. 2005. Copper-induced oxidative stress in three-spine stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology* 19:177-183.
- Schreck C. 1990. Physiological, behavioural, and performance indicators of stress. *American Fisheries Society Symposium* 8:29-37.
- Schreck C, Lorz H. 1978. Stress response of Coho Salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. *Canadian Fisheries Research Board of Canada* 35:1124-1129.
- Shen H-M, Yang C-F, Lui J, Ong C-N. 2000. Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells. *Free Radical Biology and Medicine* 28(7):1115-1124.
- Shrimpton J, McCormick S. 1999. Responsiveness of gill Na^+/K^+ -ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *The Journal of Experimental Biology* 202:987-995.
- Shrimpton J, Randall D. 1994. Downregulation of corticosteroid receptors in fillets of coho salmon due to stress and cortisol treatment. *American Journal of Physiology* 267(2):R432-R438.
- Sies H, Stahl W. 1995. Vitamins E and C, β -carotene, and other carotenoids as antioxidants. *American Journal of Clinical Nutrition* 62:1315S-1321S.
- Sloman K, Metcalfe C, Taylor A, Gilmour K. 2001a. Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. *Physiological and Biochemical Zoology* 74(3):383-389.
- Sloman K, Taylor AM, NB, Gilmour K. 2001b. Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. *Animal Behaviour* 61:325-333.
- Sorensen E, Bauer T, Bell J, Harlan C. 1982. Selenium accumulation and cytotoxicity in teleosts following chronic, environmental exposure. *Bulletin of Environmental Contaminant Toxicology* 29:688-696.
- Spallholz J. 1993. On the nature of selenium toxicity and carcinostatic activity. *Free Radical Biology and Medicine* 17(1):45-64.
- Spallholz J, Hoffman D. 2002. Selenium toxicity: cause and effects in aquatic birds. *Aquatic Toxicology* 57:27-37.
- Spallholz J, Palace V, Reid T. 2004. Methioninase and selenomethionine but not Se-methylselenocysteine generate methylselenol and superoxide in an *in vitro* chemiluminescent assay: Implications for the nutritional carcinostatic activity of selenoamino acids. *Biochemical Pharmacology* 67:547-554.
- Stocco D. 2000. Intramitochondrial cholesterol transfer. *Biochimica et Biophysica Acta* 1486:184-187.
- Tocher D, Mourente G, Van Der Eecken A, Evjemo J, Diaz E, Bell J, Geurden I, Lavens P, Olsen Y. 2002. Effects of dietary vitamin E on antioxidant defense mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus*

- hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquaculture Nutrition 8:195-207.
- USEPA. 1980. Ambient water quality for: selenium. United States Environmental Protection Agency.
- Walsh L, McCormick C, Martin C, Stocco D. 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental and Health Perspectives 108:769-776.
- Watanabe T, Kiron V, Satoh S. 1997. Trace minerals in fish nutrition. Aquaculture 151:185-207.
- Wayland M, Gilchrist H, Marchant T, Keating J, Smits J. 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. Environmental Research Section A 90:47-60.
- Wedemeyer G, McLeay D. 1981. Methods for determining the tolerance of fishes to environmental stressors. In: Pickering A, editor. Stress and Fish. London: Academic Press. p 247-275.
- Winston G, Di Giulio R. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology 19:137-161.
- Wood A, Johnston B, Farrell A, Kennedy C. 1996. Effects of didecyldimethylammonium chloride (DDAC) on the swimming performance, gill morphology, disease resistance, and biochemistry of rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 53:2424-2432.
- Wu L, McGarry L, Lanfear J, Harrison P. 1995. Altered selenium-binding protein levels associated with selenium resistance. Carcinogenesis 16(11):2819-2824.
- Zhang J, Liu J, Sun Y, Wang X, Wu J, Xue Y. 2005. Responses of the antioxidant defenses of the goldfish *Carassius auratus*, exposed to 2,4-dichlorophenol. Environmental Toxicology and Pharmacology 19:185-190.

CHAPTER 2. THE EFFECT OF WATERBORNE SELENIUM ON THE PHYSIOLOGICAL STRESS RESPONSE AND OXIDATIVE STRESS INDICATORS IN JUVENILE RAINBOW TROUT: ACUTE AND SUB-CHRONIC IN VIVO EXPOSURES

2.1. Introduction

Selenium (Se) is an essential element that bioaccumulates and becomes toxic at concentrations above the homeostatic requirement (Bowie et al. 1996). High concentrations can be found in soil derived from black shales and phosphate rocks (Haygarth 1994) and anthropogenic activities that disturb these soils may release significant amounts of previously unavailable Se into aquatic environments. Major anthropogenic sources of Se include coal and uranium mine runoff, coal fired power plant fly ash, and irrigation drainwater (Lemly 1999). Selenium is a constituent of the enzyme antioxidant glutathione peroxidase, of deiodinase required for the synthesis of thyroid hormones (Köhrle et al. 2005), and thioredoxin reductase involved in DNA synthesis, oxidative stress defence, and protein repair (Arnér and Holmgren 2000). In fish, deficiency symptoms may include growth depression (Watanabe et al. 1997), abnormal swimming patterns (Bell et al. 1986), and liver and muscle degeneration (Hilton et al. 1980).

The LC_{50} varies with fish species, Se species, and life stage. The 96 hour LC_{50} for selenite (+4 oxidation state) in rainbow trout, arctic grayling, coho salmon, and mosquito fish ranges from 1.8 mg/L for juvenile rainbow trout (Hunn et al. 1987) to 118 mg/L for

alevin rainbow trout (Buhl and Hamilton 1991). Selenate (+6 oxidation state) is less toxic to the same species, with the 96 hour LC₅₀ ranging from 32 mg/L for juvenile rainbow trout to 379 mg/L for alevin coho salmon (Buhl and Hamilton 1991). The 96 hour sodium selenite (NaSeO₃) LC₅₀ for juvenile rainbow trout ranges from 4.2 to 12.5 mg/L depending on the exposure system (Adams 1976; Goeltl and Davies 1976; Hodson et al. 1980; Buhl and Hamilton 1991). The most significant effect of excess Se in fish and also in aquatic birds is the accumulation of Se in eggs and subsequent larval deformities (Lemly 1997; Lemly 2002; Holm et al. 2005). Other documented effects in fish include skin lesions, cataracts, protruding eyeballs, swollen lamellae, edema, myocarditis, liver necrosis, kidney necrosis, and decreased white blood cell counts (Goeltl and Davies 1976; Sorensen et al. 1982; Lemly 1997; Lohner et al. 2001a; Lemly 2002). Three major mechanisms have been suggested for Se toxicity: (1) substitution of Se for sulfur in proteins, (2) inhibition of detoxification through methylation, and (3) membrane and protein damage from a Se generated reactive oxygen species (ROS) (Spallholz and Hoffman 2002). Most Se toxicity studies have focused on the reproductive effects, but other systems, such as the physiological stress response (PSR), may also be impacted.

The PSR enables fish to maintain the internal homeostasis that is required for survival, growth and reproduction in a changing environment. It consists of responses at three levels: primary endocrine response, secondary metabolite and tissue responses, and tertiary or whole organism responses (Wedemeyer and McLeay 1981; Barton 2002). When a fish perceives an environmental cue as a stressor, along with the release of catecholamines, the hypothalamus stimulates the pituitary gland to secrete

adrenocorticotrophic hormone (ACTH), a primary response. The ACTH stimulates steroidogenic cells in the head kidney to produce cortisol, the major corticosteroid in fish (Hontela 1997; Mommsen et al. 1999). Cortisol promotes protein degradation and glycogen deposition in the liver, increases gill Na^+/K^+ -ATPase activity, and suppresses the immune system, sex steroid secretion, and gonad maturation (Hontela 1997; Carr and Norris 2006). Elevated plasma glucose (a secondary response) is also associated with the classic stress response, as cortisol increases the liver glycogen reserves while catecholamines such as epinephrine mobilize glucose (Hontela 1997).

Many toxicants activate the PSR at acute and chronic exposures (e.g., copper Gagnon et al. 2006); however, chronic exposures may also exhaust or impair the PSR (Hontela 2005; Vijayan et al. 2005). Fish exposed to metals and pesticides in the field have an impaired ability, compared to fish from reference sites, to raise their plasma cortisol when challenged with an ACTH injection (Girard et al. 1998; Laflamme et al. 2000). The effects of excess Se on the PSR have not been firmly established. Acute exposure to Se activates the PSR in rats (Potmis et al. 1993) and decreases the response in gray seals, *Halichoerus grypus*, (Freeman and Sangalang 1977) and eiders, *Somateria mollissima borealis*, (Wayland et al. 2002). It is not known whether Se activates or impairs the PSR in fish. However, Se is a constituent of glutathione peroxidase (GPx), a key enzymatic antioxidant (Köhrle et al. 2005) and it has been reported that some contaminants impair the PSR through oxidative stress (Dorval et al. 2003).

Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the cellular defences and damage proteins, membranes, and DNA (Kelly et al. 1998). Reactive oxygen species are by-products of electron transport chains, enzymes and redox cycling

(Livingstone 2001) and their production may be enhanced by xenobiotics (Winston and Di Giulio 1991). Normally ROS are removed by cellular defenses such as GPx, superoxide dismutase, catalase (CAT), reduced glutathione (GSH), vitamin E, and carotenoids (Kelly et al. 1998). However, when ROS become too numerous, damage to membranes, proteins, and DNA results. Selenium can both cause and defend against oxidative stress. It is required for GPx, an enzyme that removes organic peroxides (Kelly et al. 1998), but it can also generate ROS (Spallholz et al. 2004).

The objectives of this study are: (1) to determine the effect of Se on the PSR (as measured by plasma cortisol, glucose and T3/T4, gill Na^+/K^+ -ATPase activity, and the ability of the head kidney to secrete cortisol), and (2) to determine if Se causes oxidative damage (as measured by LPO) or changes the antioxidant status (as measured by hepatic GPx activity, GSH reserves, and CAT activity). Juvenile rainbow trout were exposed to Se (acute and sub-chronic exposures) to test the following hypotheses: (1) Se alters the PSR, (2) Se alters antioxidant status, and (3) Se increases LPO.

2.2. Materials and Methods

2.2.1. Chemicals

Sodium selenite (NaSeO_3), sucrose, EDTA, imidazole-HCl, NaCl, KCl, ouabain, Na_2ATP , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, H_2SO_4 , potassium phosphate, TRIS-HCl, 2-mercaptoethanol, metaphosphoric acid, BHT, hydrogen peroxide, GSH, NADPH, GSH reductase, sodium azide, porcine ACTH I-39, MEM, bovine serum albumin, NaHCO_3 , and Bradford reagent were purchased from Sigma-Aldrich. MS-222 was purchased from MPBiomedicals. Cortisol (Catalogue # 07-221102), T3 (Catalogue

06B-254215), and T4 (Catalogue # 06B-254011) radioimmunoassay kits were purchased from Mediacorp. Bioxytech GSH 400 (Catalogue # 21011) and LPO 596 (Catalogue # 21012) kits were also purchased from Mediacorp. GOD-PAP reagent was purchased from Roche Diagnostic. Ultra pure nitric acid was purchased from Fischer Scientific.

2.2.2. Fish and Exposures

Juvenile rainbow trout, *Oncorhynchus mykiss*, were obtained from the Allison Creek Provincial Hatchery. Fish were kept in a large tank (1000L) at 14 °C for a minimum of 2 weeks and then moved to the experimental exposure tanks (50L, static system, 25% daily water renewal, 12 °C) and allowed 7 days to acclimate before exposures were begun. Fish were fed extruded floating steelhead food (Nelson's Silver Cup Fish Feed).

Acute Exposure: Fish were exposed to waterborne sodium selenite (NaSeO_3) at 0%, 10%, 25%, 35%, and 50% of the LC_{50} for 96 hours. The 96 hour $\text{LC}_{50} = 7.2 \text{ mg/L}$ for juvenile rainbow trout exposed to sodium selenite was used (Hodson et al. 1980). Eight fish ($18.1 \text{ g} \pm 1.2 \text{ g}$) were randomly assigned to each tank. Temperature, dissolved oxygen, pH, and conductivity were monitored and water samples for total Se analysis were collected daily. After 96 hours the fish were sacrificed and sampled (see section 2c).

Sub-chronic Exposure: Fish were exposed to waterborne sodium selenite for 30 days at 0%, 1%, and 5% of the 96 hour LC_{50} . Twenty-four fish ($28.55 \pm 1.3 \text{ g}$) were randomly assigned to each treatment and each treatment consisted of three tanks with eight fish per tank. Acclimation period, feeding, water replacement, and temperature monitoring

occurred as described for the acute exposure. Oxygen content, conductivity, pH, and water samples were collected twice a week. On days 8 and 22 fish were lightly anesthetised with MS-222 and a blood sample was taken from the caudal vasculature for cortisol and glucose analyses. After 30 days the fish were sacrificed and sampled (see section 2c).

Water Se Analysis: Water samples were collected in acid washed bottles and composites of the daily water samples collected from each tank in the acute exposure were acidified with 0.05% ultra pure nitric acid and analysed for total Se at the Ultra-Clean Trace Elements Laboratory, University of Manitoba. Weekly composites were similarly analysed for each tank in the sub-chronic exposure.

2.2.3. Sampling

Fish were removed from tanks and immediately anesthetised in MS-222. Blood samples were taken from the caudal vasculature and fish were sacrificed by a spinal transection. Plasma was collected and flash frozen in liquid nitrogen. Fork length, weight, and liver weight were recorded. Condition factor (K) and liver somatic index (LSI) were calculated. Plasma and tissues (liver and gill) were stored at -80°C until analysis.

2.2.4. Plasma cortisol, glucose, T3 and T4

Cortisol was measured with a radioimmunoassay kit. Glucose was determined in a spectrophotometric assay (510 nm) using the GOD-PAP reagent. T3 and T4 were measured with radioimmunoassay kits in remaining plasma.

2.2.5. Gill Na^+/K^+ -ATPase Activity

Na^+/K^+ -ATPase activity in a gill homogenate was measured by liberating PO_4 from a hydrolysis reaction with ATPase (Morgan et al. 1997; Levesque et al. 2003). Gills were homogenized in a buffer (pH = 7.2) containing 250mM sucrose, 6mM EDTA, and 20mM imidazole-HCl. The homogenate was incubated (10 minutes, 30°C) in a medium without ouabain, an ATPase inhibitor, (167mM NaCl, 50mM KCl, 33mM imidazole-HCl, pH = 7.2) and with ouabain (237mM NaCl, 1.67mM ouabain, 33mM imidazole-HCl, pH = 7.2). Phosphate was liberated during an incubation period (30 minutes at 30°C) with ATP (25mM Na_2ATP , 50mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) and measured at 700nm with a spectrophotometer. The phosphate forms a blue complex with Bonting solution (8.1mM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 176mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 560mM H_2SO_4) during a 20 minute incubation at room temperature. Na^+/K^+ -ATPase activity is expressed as $\mu\text{mol PO}_4$ liberated per mg of protein. Protein was measured using a spectrophotometric assay (595 nm) and the Bradford reagent.

2.2.6. Oxidative Stress Parameters

A portion of the liver (0.1g) was homogenized in a 50 mM potassium phosphate buffer with 1mM EDTA, pH 7.4 for GSH, LPO, and CAT determination. A second portion of liver (0.05g) was homogenized in 50mM TRIS-HCl buffer with 5mM EDTA and 1mM 2-mercaptoethanol for GPx determination. Metaphosphoric acid was added to a portion of the first homogenate for GSH analysis (final concentration 0.209M) and BHT was added to a portion of the first homogenate for LPO analysis (final concentration 5 mM). Homogenates were centrifuged at 4°C at $3270 \times g$ for 20 minutes and

supernatants removed. GSH was assayed within an hour, and supernatants for CAT, GPx, and LPO were kept at -80°C for a maximum of 2 days before analysis.

GSH: Reduced glutathione reserves were determined using the Bioxytech GSH 400 kit. GSH forms a thioether that reacts with a reagent forming a thiol (400nm). GSH is expressed as μM GSH per mg protein.

GPx: Total cellular GPx activity was determined by measuring the decrease in absorbance (340nm) due to the decline in NADPH at 23-25 °C (Lorentzen et al. 1994). The sample (70 μl) was added to 350 μl phosphate buffer, 350 μl 0.25mM hydrogen peroxide and 350 μl of reagent. The reagent contained 1mM GSH, 0.2mM NADPH, 1 unit GSH reductase and 2mM sodium azide in a phosphate buffer (described previously). Absorbance was monitored for 3 minutes at 340nm. The activity of GPx was expressed as mU/mg protein and one mU is defined as 1 nmol of NADPH consumed/minute/mL of sample.

LPO: Lipid peroxidation was determined using the Bioxytech LPO-596 kit. 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 586nm. LPO is expressed as U/mg protein, where one unit is one μM MDA and 4-HA.

CAT: Catalase activity was determined by measuring the rate of hydrogen peroxide consumption at 240nm (Beers and Sizer 1952). One unit of catalase activity is 1 μ mol hydrogen peroxide consumed/min/mg of protein.

2.2.7. Adrenocortical challenge

The ability of fish to secrete cortisol was tested by stimulating cortisol production in a coarse head kidney homogenate with ACTH *in vitro*. The head kidney was removed and rinsed on ice in a solution containing MEM, bovine serum albumin, and NaHCO₃. A coarse head kidney homogenate in MEM was incubated at 15°C for two hours to remove any cortisol secretion due to capture stress. After the incubation medium was removed, MEM was added to one well (basal), and 2 units of ACTH in MEM were added to a second well (stimulated) for each fish. The plate was incubated at 15°C for one hour, then the supernatant was removed and frozen for later cortisol analysis. The ability of the head kidney to secrete cortisol is expressed as the change in cortisol secretion (ng/mL) after stimulation with 2 units of ACTH (stimulated cortisol – basal cortisol).

2.2.8. Statistical Analysis

Data was analyzed using JMP IN 5.1.2 (1989-2004 SAS Institute Inc.). Data that were not normally distributed were transformed to respect normality. Treatment groups were compared using a one-way ANOVA and a Tukey-Kramer HSD. If data could not be transformed to normality a Kruskal – Wallis test was used to compare treatment groups, followed by multiple *post hoc* Wilcoxon tests with the Bonferroni correction to compare each exposure group to the control. The change in plasma cortisol and glucose levels in the sub-chronic exposure were calculated (level at day 30 – level at day 8) and

the treatment groups were compared as discussed above. Samples below the detection limit (0.01 ng/mL) of the radioimmunoassays were entered as 0.01 ng/ml. All the statistical tests use $\alpha = 0.05$ unless otherwise stated.

2.3. Results

2.3.1. Exposure Characteristics

Temperature, dissolved oxygen, pH, conductivity measurements, and Se water concentrations during exposures are presented in Table 2.1. The observed water Se concentrations during the acute 96 hour exposure were similar to expected values, although the concentration for the 50% LC₅₀ group was higher than expected (Table 2.1). Selenium exposures ranged from 0.00 mg/L (control) to 2.97mg/L (50% LC₅₀).

Water quality parameters in the 30 day sub-chronic sodium selenite exposures were similar to those measured in the acute exposures (Table 2.1). Water Se concentrations ranged from 0.00 mg/L (control) to 0.16 mg/L (5% LC₅₀), and the observed and nominal values were very similar.

2.3.2. Physiological Stress Response

Plasma cortisol levels were significantly higher ($p < 0.05$) in groups exposed to 35% and 50% of the LC₅₀ for 96 hours than in the control (Figure 2.1A). Plasma glucose levels (Figure 2.1B) were greater ($p < 0.05$) than the control group in the 25% and 50% 96 hour exposures. Gill Na⁺/K⁺ ATPase (Table 2.2), plasma T3 (mean = 2.23 ± 0.36 ng/ml plasma, data not shown), T4 (mean = 23.04 ± 2.40 ng/ml plasma, data not shown),

condition factor (Table 2.2), and LSI (Table 2.2) were not significantly affected by sodium selenite exposure for 96 hours.

After the 30 day (sub-chronic) sodium selenite exposure, plasma cortisol levels were higher ($p < 0.05$) in the 5% LC_{50} group than the 1% LC_{50} group (Figure 2.2A). The change in plasma cortisol between day 30 and day 8 was significantly greater ($p < 0.05$) in the 5% LC_{50} than the other two treatment groups (Figure 2.2A inset). Plasma cortisol in the control and 1% LC_{50} groups decreased over the 22 days, but increased in the 5% LC_{50} group (inset). There were no significant differences between treatment groups in plasma glucose levels after 30 days (mean = 0.78 ± 0.01 ng/ml plasma, data not shown) or in the change of plasma glucose levels (mean = 0.13 ± 0.04 ng/ml plasma, data not shown). Sodium selenite did not significantly change gill Na^+/K^+ -ATPase activity after 30 days (Table 2.2). Finally, the 5% LC_{50} group had significantly higher plasma T3 and T4 levels ($p < 0.05$) than the control on day 30 of the sub-chronic sodium selenite exposure (Figure 2.3).

The ability of the head kidney to respond to an ACTH challenge did not change significantly with exposure to sodium selenite for 30 days, even though a trend for a lower cortisol response in the 5% LC_{50} group than the control and 1% groups was noted (Figure 2.2B). Condition factor and LSI were not different among treatment groups (Table 2.2).

2.3.3. Oxidative Stress Parameters

Hepatic oxidative stress parameters were modified by acute (96 hours) sodium selenite exposure. Lipid peroxidation levels were lower in the 35% LC_{50} and 50% LC_{50} groups; although, only the 35% LC_{50} exposure was significantly ($p < 0.05$) lower than the

control (Figure 2.4A). Rainbow trout had significantly less hepatic GSH reserves ($p < 0.05$) in the 35% LC_{50} and 50% LC_{50} exposures compared to the 25% LC_{50} group (Figure 2.4B). Hepatic GPx activity ($p < 0.05$) varied between the treatment groups (Figure 2.5A). It decreased in the 35% LC_{50} treatment then increased to control levels in the 50% LC_{50} treatment. Catalase activity did not significantly change, but the highest exposure (50% LC_{50}) had the highest CAT activity (Figure 2.5B).

Hepatic oxidative stress parameters did not significantly change (Table 2.3) with 30 days of sodium selenite exposure. Liver GSH concentrations and lipid peroxidation were similar across all treatments, while GPx and catalase activities exhibited a non-significant trend to decrease in the highest (5% LC_{50}) exposure.

2.4. Discussion

The objectives of these experiments were to (1) determine the effect of Se on the PSR, and (2) determine if Se causes oxidative damage, and alters antioxidant levels in the liver. Juvenile rainbow trout were exposed for 96 hours to high sodium selenite concentrations (acute exposure) or 30 days to low sodium selenite concentrations (sub-chronic exposure). Sodium selenite exposure activated the PSR in juvenile rainbow trout in both the high and low exposures. Selenium did not cause oxidative damage at the sodium selenite concentrations tested; however, exposure to high concentrations of sodium selenite (acute exposure) reduced hepatic GSH levels while the enzymatic antioxidants (GPx, CAT) were unchanged. At concentrations approaching environmental Se concentrations (sub-chronic exposure), sodium selenite exposure for 30 days did not alter hepatic antioxidant levels or activities.

An important feature of the present study was the use, during the sub-chronic (30 day) exposures, of Se water concentrations that approach environmental exposures. Streams receiving runoff from coal mines in northern Alberta contain 0.032 mg/L Se and developmental deformities have been observed in the resident rainbow trout population (Holm et al. 2005). In lentic systems, reproductive effects have been documented in fish exposed to water Se concentrations as low as 0.005 mg/L (Lemly 1999). The lowest Se exposure used in this study was 0.05 mg/L in the 1% LC₅₀ group, a concentration that approaches the Se concentrations found in the coal mine- impacted streams of northern Alberta where reproductive effects have been documented. Therefore the exposure concentrations used in the present laboratory study are environmentally relevant.

2.4.1. Physiological Stress Response

Acute (96 hour) exposure to sodium selenite activated the PSR in juvenile rainbow trout. It raised plasma cortisol (a primary response) and plasma glucose (a secondary response), however other stress related responses (gill Na⁺/K⁺-ATPase activity and plasma T3 and T4 levels), including some of the tertiary responses (condition factor and LSI) were not affected by the acute 96 hours exposure to Se. The sub-chronic (30 day) sodium selenite exposure also activated the PSR. Plasma cortisol levels increased significantly in the 5% LC₅₀ group, although the acute exposure raised the cortisol much higher. This suggests exposures to waterborne Se between 1.15 and 2.67 mg Se/L for 96 hours are more stressful to the fish than 0.16 mg Se/L for 30 days. Plasma T4 and T3 levels (stress related responses) were significantly increased in the highest (5% LC₅₀) exposure group, but the other stress indicators (plasma glucose levels and gill Na⁺/K⁺-ATPase activity), and the tertiary responses (condition and LSI) were unaffected. Plasma

T4 and T3 levels may have increased because the PSR was activated and cortisol influences thyroid hormone metabolism (Brown et al. 1991). Moreover, Se is an integral part of the deiodinase enzymes involved in thyroid hormone synthesis (Köhrle et al. 2005). Thus, the synthesis of thyroid hormones may have increased simply because more Se, and subsequently more deiodinase, was available. Up to 0.16 mg/L (5%LC50) of sodium selenite exposure may not harm juvenile rainbow trout, but more research is needed to determine why the thyroid hormones increased with low levels of Se over 30 days but not during the acute 96 hour exposure.

The common primary response pattern to a chemical stressor during an acute exposure is a quick cortisol spike maintained until death or until the stressful stimulus is removed (Donaldson 1981; Schreck 1990). As expected, a cortisol spike was observed in the two highest exposures to sodium selenite for 96 hours. Presumably, the sodium selenite exposure was too short for elevated cortisol to significantly affect secondary stress indicators, other than plasma glucose, or the tertiary responses. Numerous other studies have shown that pollutants such as crude oil (Kennedy and Farrel 2005), copper (Pelgron et al. 1995; Dethloff et al. 1999), or cadmium (Pelgron et al. 1995) increase plasma cortisol activating the PSR in fish. However, the present study provides the first evidence that elevated Se activates the PSR in fish and increases plasma cortisol. A similar response to Se was reported in male rats (Potmis et al. 1993); however, Se decreased plasma corticosterone levels in gray seals (Freeman and Sangalang 1977) and female eiders (Wayland et al. 2002).

In chronic contaminant exposures, a slow increase in plasma cortisol is generally followed by a decrease as the fish acclimates (Donaldson 1981), the response is impaired,

or interrenal cells become exhausted (Hontela 1997). In the present study, cortisol increased between day 8 and day 30 in the highest exposure (5% LC₅₀) group, but it decreased in the control and 1% LC₅₀ groups. This pattern suggests Se is still causing a slow cortisol increase after 30 days. Thus, the fish have not completely acclimated to Se and their interrenal cells are not impaired or exhausted. Chronic exposure to some contaminants, such as heavy metals, can decrease the cortisol response to capture stress and ACTH stimulation (Girard et al. 1998). For example, Girard et al.'s (1998) study supports the impairment or exhaustion hypothesis as the fish from metal contaminated lakes responded less to ACTH stimulation than the controls. The ability of the head kidney to secrete cortisol was tested in the sub-chronic (30 day) Se exposure in the present study to determine if the cortisol response was impaired or exhausted. There was no significant difference in cortisol secretion after ACTH stimulation *in vitro*; thus, the head kidney of juvenile rainbow trout was not adversely affected by *in vivo* exposure to 0.16 mg/L Se for 30 days. However, a trend for lower cortisol secretion *in vitro* was noted in the highest group suggesting Se may impair or exhaust the interrenal system but the exposure was not long enough to detect the difference. Further studies are needed to determine if rainbow trout acclimate to Se or if Se impairs or exhausts the PSR.

2.4.2. Oxidative Stress Biomarkers

In the acute exposure, sodium selenite decreased LPO in the two highest exposure groups, although it was only significantly lower than the control in the 35% LC₅₀ group. These results were unexpected as most contaminants including cadmium (Roberts and Oris 2004), pulp and paper effluent (Oakes et al. 2003; Oakes and McMaster 2004), agricultural runoff (Güll et al. 2004; Dorval et al. 2005), copper (Berntssen et al. 2000),

and hydrocarbons (Di Giulio et al. 1993; Livingstone et al. 1993) increased LPO in fish tissues. Conversely, 2 – 6 mg/L sodium selenite decreased hepatic LPO in rainbow trout exposed for 72 hours in a dose-dependent manner, although the differences were not statistically significant (Orun et al. 2005). Lipid peroxidation may decrease with increased Se exposure because Se is a constituent of the antioxidants GPx and thioredoxin reductase (Steinbrenner et al. 2006) and at lower levels protects fish from oxidative damage. However, this was not apparent in the present 96 hour exposure as GPx activity did not increase with decreasing LPO. Instead, sodium selenite may have induced other antioxidants that were not measured in the present study (e.g. superoxide dismutase).

Selenium had an effect on hepatic antioxidant levels in rainbow trout exposed for 96 hours. It significantly decreased liver GSH concentrations in the two highest exposure groups. There is extensive evidence that reduced glutathione is a powerful antioxidant and anti-toxicant as it binds to many different toxicants, inactivating them (Parkinson 2003). In fish, reduced glutathione reserves decrease with exposure to endosulfan (Dorval et al. 2003), agricultural runoff (Dorval et al. 2005), copper (Ahmad et al. 2005), cadmium (Berntssen et al. 2000), and mercury (Payne et al. 1998). The decrease in GSH observed with increased Se in the present study supports the proposed mechanism for superoxide radical generation in rainbow trout embryos in which GSH reacts with methylselenol to produce a Se-ROS (Palace et al. 2004). Damage indicators (LPO, protein peroxidation, DNA damage) should increase if the combination of GSH and Se is producing ROS in the liver; however, LPO decreased in the 35% group in the present study suggesting at this exposure level GSH is acting as an antioxidant inactivating Se

and not creating ROS. However, in the 50% group LPO levels are beginning to increase indicating increasing oxidative damage. At higher sodium selenite exposures GSH may act as a prooxidant generating ROS in conjunction with Se.

Hepatic GSH may be a useful indicator of Se toxicity as it began to decrease in rainbow trout exposed for 96 hours to 35% and 50% of the LC₅₀ (water concentrations above 1.15 mg/L). Most field exposures are much lower (e.g., water concentrations of 0.006 mg/L to 0.032 mg/L; Holm et al. 2005), but fish are exposed for longer than 96 hours or 30 days. Thus, the decrease in liver GSH resulting from the 96 hour acute exposure suggests that chronic environmental exposures might also decrease GSH levels. This will be determined in future field studies.

The activity of GPx decreased in the 35% LC₅₀ group, but did not change in the other treatment groups. Additionally, there was no significant change in CAT levels with Se exposure. This suggests Se did not induce enzymatic defenses and it may have inactivated or depleted GPx in the 35% LC₅₀ group. Alternatively, the Se exposures used may have been too low to generate enough ROS to alter CAT or GPx. Many other enzymatic and non-enzymatic defenses (e.g., superoxide dismutase, vitamin A, vitamin E, etc) could have changed with Se exposure. The response of CAT and GPx to toxicants varies with the toxicant. For example, CAT and GPx are both induced by *B*-naphthoflavone (Ahmad et al. 2005), and copper (Sanchez et al. 2005), but decreased by dichlorophenol (Zhang et al. 2005) and agricultural runoff (Dorval et al. 2005). Additionally, hepatic GPx has been shown to increase significantly at exposures over 4 mg/L sodium selenite (Orun et al. 2005). The present study's highest exposure was only 2.67 mg/L; thus, the exposures may have been too low to induce GPx. Hepatic GPx and

CAT may not be useful early indicators of Se toxicity in juvenile rainbow trout as they were not consistently affected by Se exposure.

In contrast to the acute (96 hour) Se exposure, 30 days of sodium selenite (sub-chronic) exposure, did not affect oxidative damage (LPO) or alter any antioxidant levels and activities (CAT, GPx, GSH). The exposures may not have been high or long enough to cause oxidative stress and alter antioxidant levels and activities. Measuring oxidative damage and antioxidant activity during the 30 day period, will give a time series data set to determine acclimation patterns. Acclimation can help protect against oxidative stress. For example, acclimation to low levels of copper protected fish against oxidative stress (Pandey et al. 2001); however, at higher levels copper causes oxidative stress (Berntssen et al. 2000; Roméo et al. 2000). Selenium may act in a similar manner. At higher levels, similar to the 96 hour acute exposure, it may begin to alter antioxidant status, but at lower levels it may protect the liver from damage. More research is needed to determine the threshold level at which Se stops acting as an antioxidant and begins to act as a prooxidant.

2.4.3. Conclusions

This present study provides evidence, for the first time, that sodium selenite activates the PSR in fish subjected to acute and sub-chronic exposures. After 30 days of 0.16 mg/L sodium selenite exposure, plasma cortisol levels were elevated and the ability of juvenile rainbow trout to secrete cortisol was not altered; however, more research is needed to ensure that sodium selenite does not exhaust or impair the PSR. Hepatic GSH may be an indicator of Se toxicity, as it decreased in juvenile rainbow trout exposed to Se levels above 1.15 mg/L; however most field exposures are much lower and this effect

may not be observed. Reduced glutathione may combine with Se and act as a prooxidant and/or an antioxidant, but more research is needed to define the interaction between Se and GSH. No clear response patterns in GPx activity, CAT activity, or LPO levels of fish exposed to sodium selenite were observed; thus, these parameters are not indicators of Se toxicity. The absence of changes in antioxidant levels and oxidative damage after 30 days of sodium selenite exposure suggests rainbow trout are able to acclimate to up to 0.16 mg/L Se.

2.5. Acknowledgments

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Table 2.1. Water quality characteristics (mean \pm SE) and water Se concentrations for the 96 hour and 30 day sodium selenite (NaSeO_3) exposures.

Experiment	Treatment	Temperature ^a (°C)	Oxygen ^a (mg/L)	pH ^a	Conductivity ^a (μs)	Nominal NaSeO_3 (mg/L)	Nominal Se (mg/L)	Measured Se ^{bc} (mg/L)
96 hours (acute)	Control	13.0 \pm 0.1	6.8 \pm 0.3	7.9 \pm 0.16	318 \pm 42	0.00	0.00	0.00
	10% LC_{50}	13.4 \pm 0.2	6.5 \pm 0.5	8.0 \pm 0.06	293 \pm 1.9	0.72	0.38	0.39
	25% LC_{50}	13.3 \pm 0.3	6.5 \pm 0.2	7.9 \pm 0.10	310 \pm 41	1.80	0.95	0.99
	35% LC_{50}	12.2 \pm 0.3	7.6 \pm 0.5	7.9 \pm 0.27	323 \pm 60	2.52	1.33	1.15
	50% LC_{50}	12.4 \pm 0.5	7.5 \pm 0.8	7.5 \pm 0.40	284 \pm 1.2	3.60	1.90	2.67
30 days (sub- chronic)	Control	12.6 \pm 0.1	9.3 \pm 0.4	7.6 \pm 0.04	276 \pm 6.1	0.00	0.00	0.00
	1% LC_{50}	12.2 \pm 0.1	8.7 \pm 0.3	7.7 \pm 0.03	287 \pm 7.5	0.07	0.04	0.05
	5% LC_{50}	11.9 \pm 0.1	8.5 \pm 0.2	7.7 \pm 0.03	290 \pm 2.0	0.36	0.19	0.16

^a n = 4

^b Composite of daily water samples for acute exposure (n = 3)

^c Average (n = 4) of weekly composite samples (2 samples/week) for sub-chronic exposure

Table 2.2. Morphological and physiological parameters (mean \pm SE) of juvenile rainbow trout exposed for 96 hours or 30 days to waterborne sodium selenite (NaSeO₃).

Experiment	Treatment	Sample Size	Condition Factor ^a	Liver Somatic Index ^b	Gill Na ⁺ /K ⁺ ATPase ^c
96 hours (acute)	Control	8	1.11 \pm 0.03	1.21 \pm 0.12	1.15 \pm 0.33
	10% LC ₅₀	8	1.14 \pm 0.08	1.19 \pm 0.16	1.66 \pm 0.32
	25% LC ₅₀	8	1.16 \pm 0.02	1.78 \pm 0.13	1.83 \pm 0.24
	35% LC ₅₀	8	1.09 \pm 0.04	1.20 \pm 0.16	1.98 \pm 0.42
	50% LC ₅₀	8	1.09 \pm 0.04	1.21 \pm 0.17	2.27 \pm 0.14
30 days (sub-chronic)	Control	21	1.10 \pm 0.02	1.14 \pm 0.04	0.76 \pm 0.19
	1% LC ₅₀	19	1.10 \pm 0.02	1.17 \pm 0.05	0.92 \pm 0.13
	5% LC ₅₀	18	1.10 \pm 0.02	1.11 \pm 0.05	0.72 \pm 0.16

^a $K = \frac{w_f \times 100}{l^3}$, where K is condition factor, w_f is the final weight and l is the fork length.

^b $LSI = \frac{lv}{w_f} \times 100$, where LSI is the liver somatic index, lv is the liver weight and w_f is the final weight

^c activity expressed in units (U/mg protein), where one unit is one μ mol of liberated PO₄

Table 2.3. Oxidative stress biomarkers (mean \pm SE) in the liver of juvenile rainbow trout exposed for 30 days to waterborne sodium selenite (NaSeO₃).

	GSH	GPx ^a	CAT ^b	LPO ^c
	$\mu\text{mol/mg protein}$	mU/mg protein	U/mg protein	U/mg protein
Control ^d	25.75 ± 2.09	4.07 ± 0.62	599 ± 243	0.05 ± 0.006
1% LC ₅₀	23.59 ± 2.03	4.16 ± 0.88	452 ± 169	0.05 ± 0.005
5% LC ₅₀	27.81 ± 2.86	3.32 ± 0.62	113 ± 18.2	0.06 ± 0.005

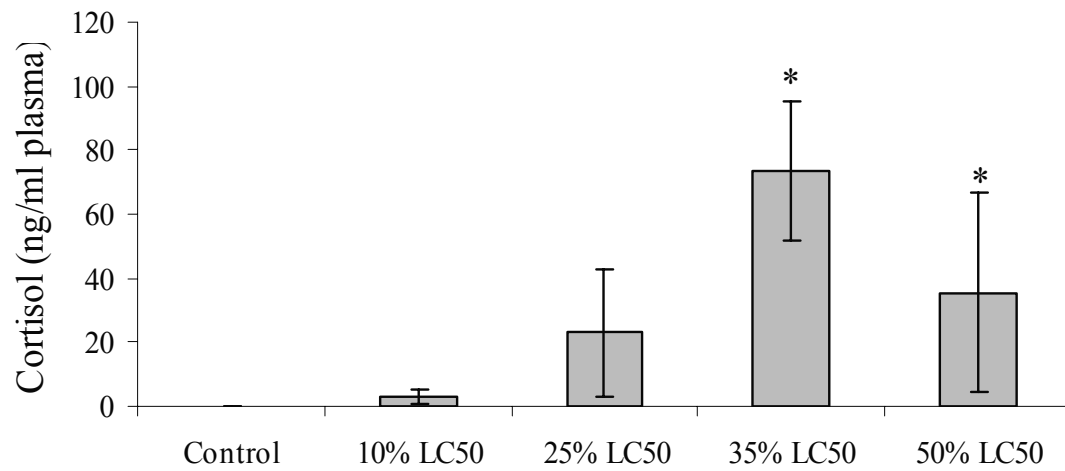
^a one mU is one nmol NADPH consumed per minute

^b one U is one $\mu\text{mol H}_2\text{O}_2$ consumed per minute

^c one U is one $\mu\text{mol MDA}$ and 4-HA

^d number of fish per group, n = 9-12

(A)



(B)

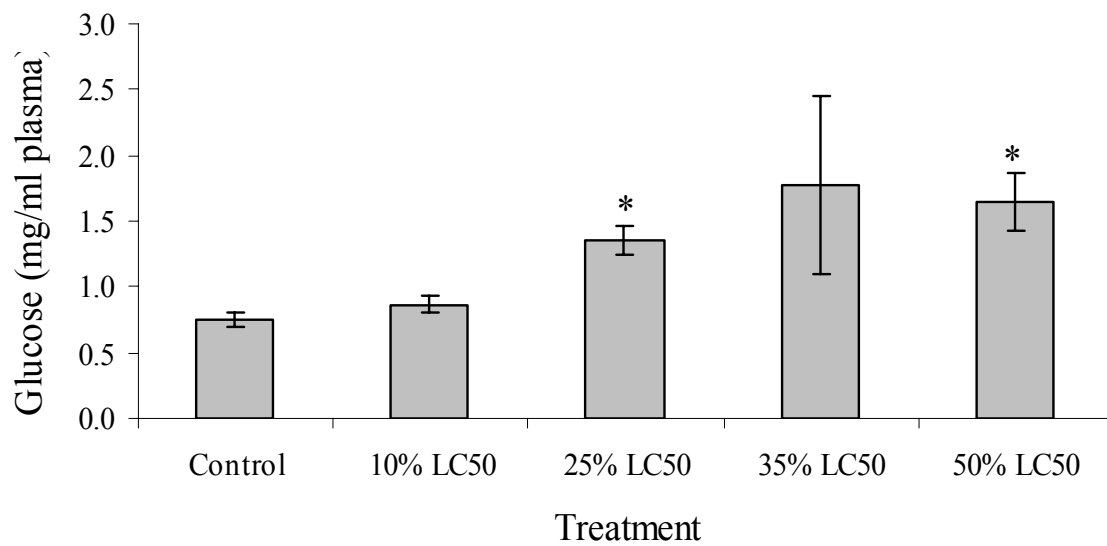
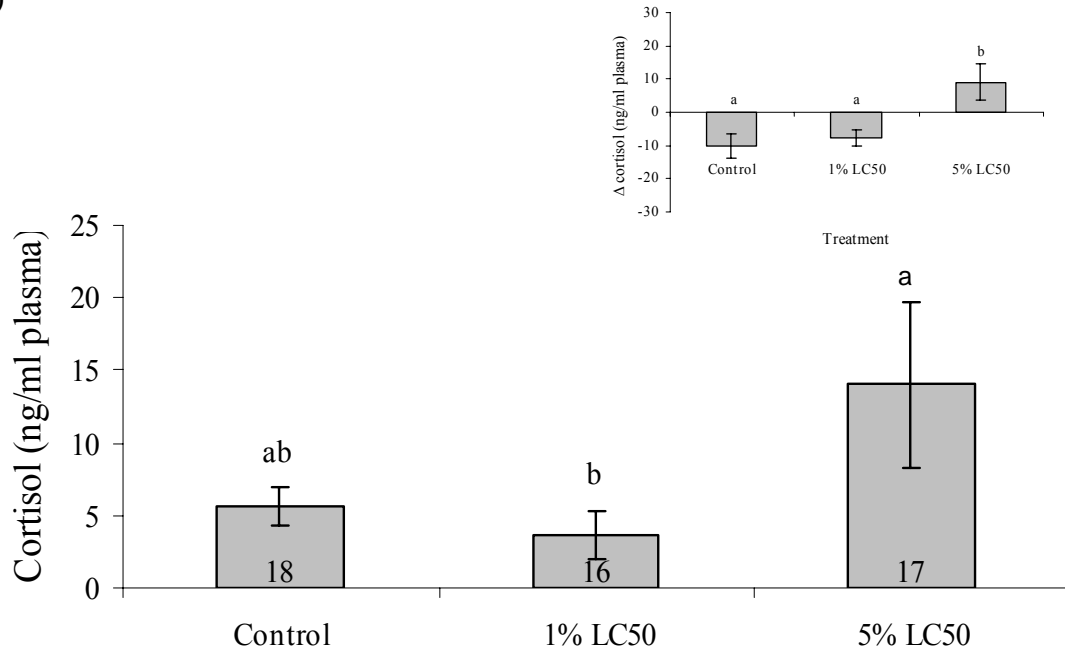


Figure 2.1. Plasma cortisol (A) and plasma glucose (B) of juvenile rainbow trout (mean \pm SE) exposed for 96 hours to waterborne sodium selenite (NaSeO_3), $n = 8$. * indicates a significant difference from the control group (Kruskal-Wallis).

(A)



(B)

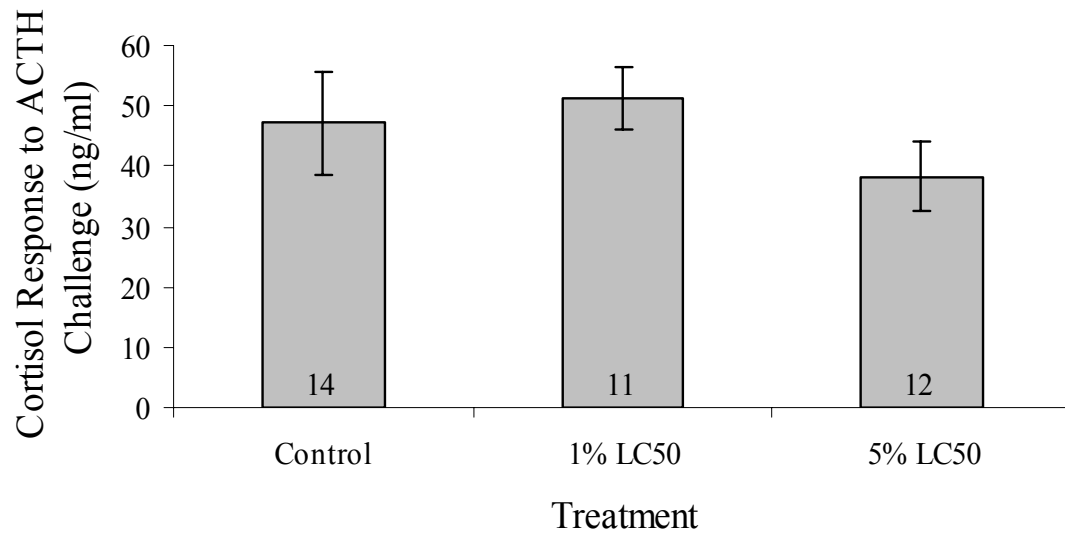


Figure 2.2. (A) Plasma cortisol levels and Δ cortisol (in the inset graph) expressed as change in plasma cortisol levels between day 30 and day 8 and (B) the ability of the head kidney to secrete cortisol in juvenile rainbow trout (mean \pm SE) exposed for 30 days to waterborne sodium selenite (NaSeO_3). Groups with different letters are significantly different (Kruskal-Wallis) in A. No significant differences were observed in B (ANOVA). Sample size indicated in the bars.

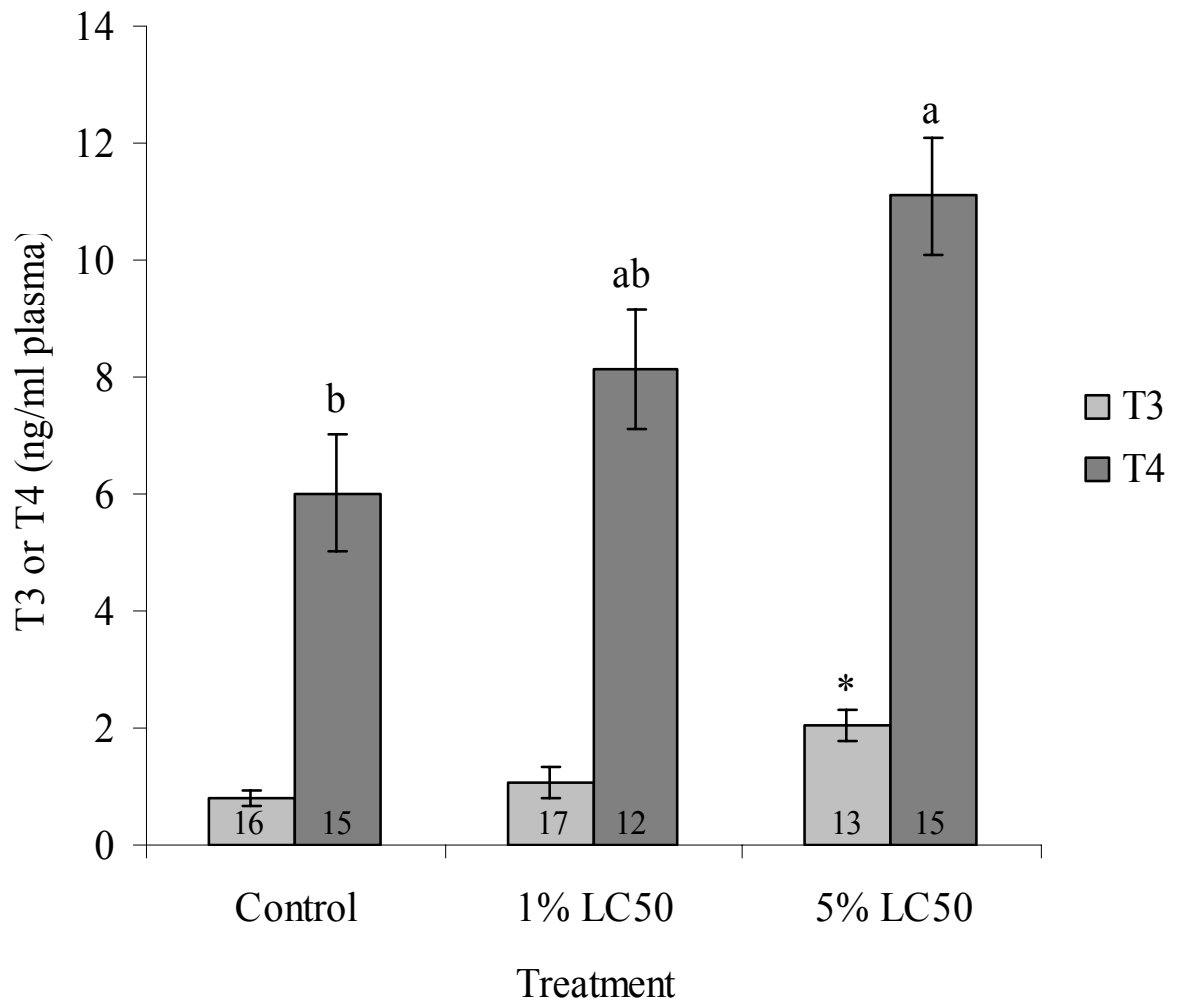
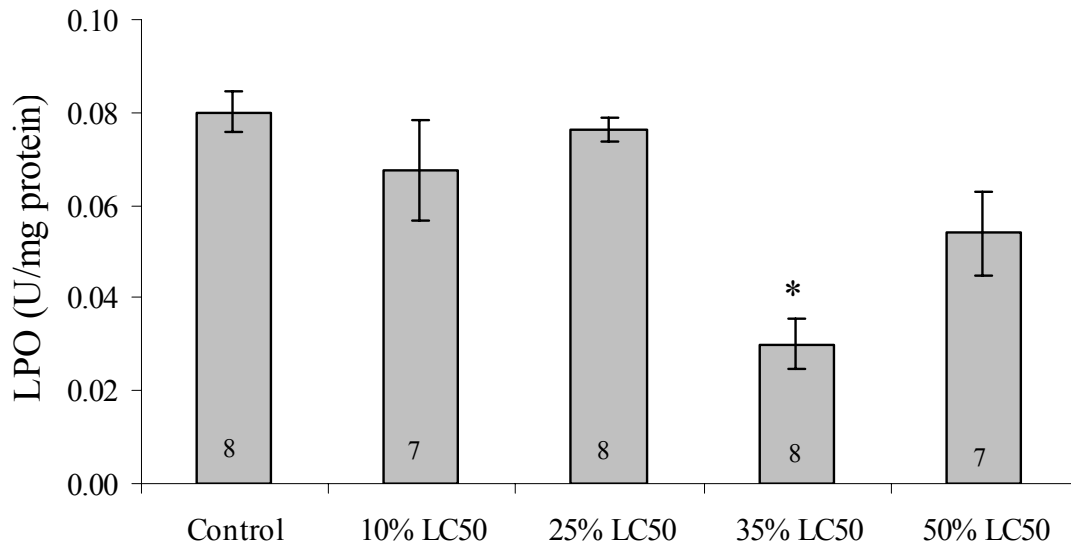


Figure 2.3. Plasma T3 and T4 levels (mean \pm SE) in juvenile rainbow trout exposed to 30 days of waterborne sodium selenite (NaSeO_3). * indicates a significant difference from the control (Kruskal-Wallis), groups with different letters are significantly different (ANOVA). Sample size indicated in bars.

(A)



(B)

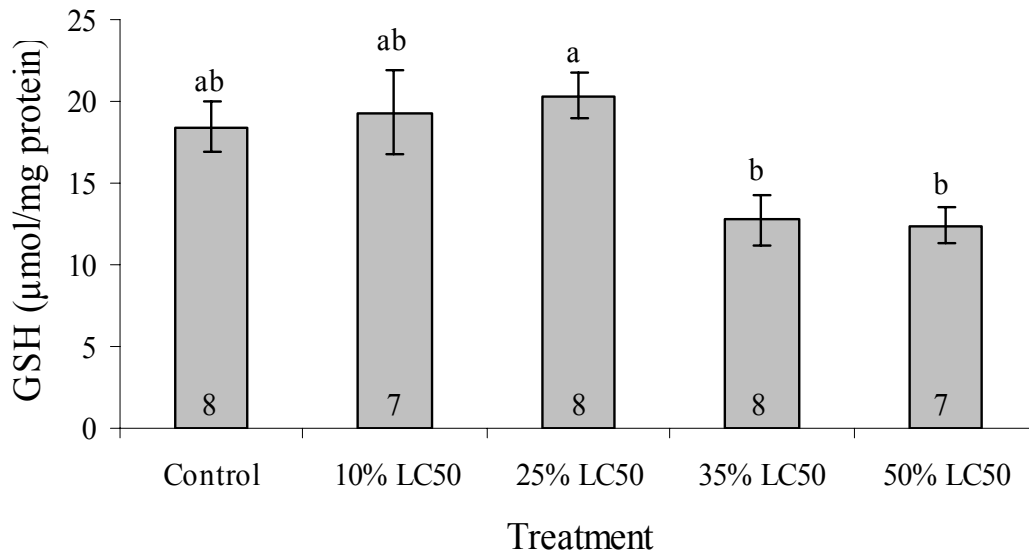
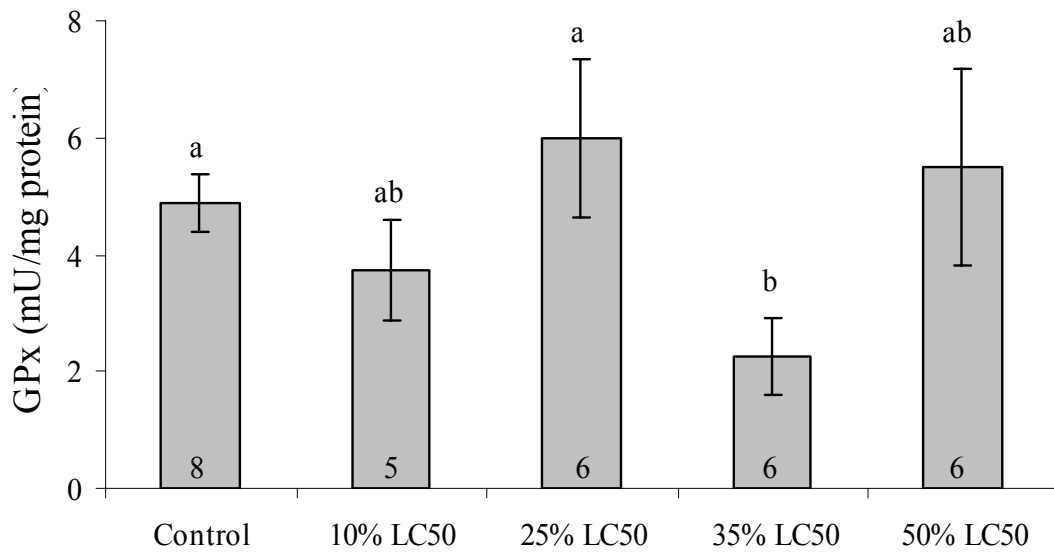


Figure 2.4. Lipid peroxidation (A) and GSH concentrations (B) in the liver of juvenile rainbow trout (mean \pm SE) exposed for 96 hours to waterborne sodium selenite (NaSeO_3). Groups with different letters are significantly different (ANOVA), * indicates a significant difference from the control group (Kruskal-Wallis). Sample size indicated in the bars.

(A)



(B)

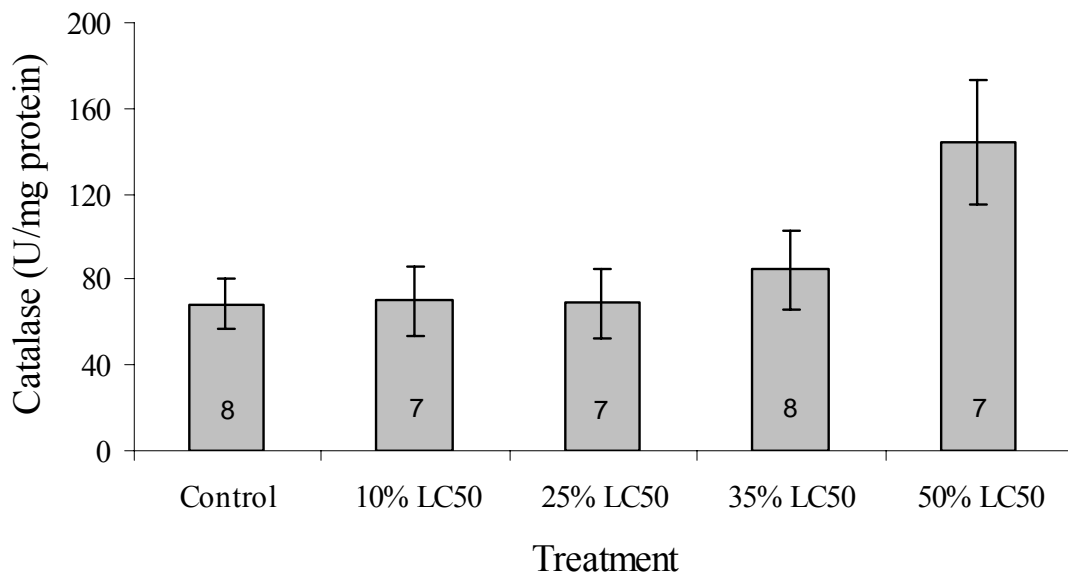


Figure 2.5. Enzyme antioxidants (mean \pm SE) in the liver of juvenile rainbow trout exposed for 96 hours to waterborne sodium selenite (NaSeO_3). (A) GPx, and (B) CAT. Groups with different letters are significantly different (ANOVA. Sample size indicated in the bars.

2.4 Literature Cited

- Adams W. 1976. The toxicity and residue dynamics of selenium in fish and aquatic invertebrates: Michigan State University.
- Ahmad I, Oliveira M, Pacheco M, Santos M. 2005. *Anguilla anguilla* L. oxidative stress biomarkers responses to copper exposure with or without β -naphthoflavone pre-exposure. *Chemosphere* 61:267-275.
- Arnér E, Holmgren A. 2000. Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry* 267:6102-6109.
- Barton B. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42:517-525.
- Beers R, Sizer I. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry* 195:133-140.
- Bell J, Pirie B, Adron J, Cowey C. 1986. Some effects of selenium deficiency on glutathione peroxidase (*EC 1.11.1.9*) activity and tissue pathology in rainbow trout (*Salmo gairdneri*). *British Journal of Nutrition* 55:305-311.
- Berntssen M, Lundebye A, Hamre K. 2000. Tissue lipid peroxidative responses in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. *Fish Physiology and Biochemistry* 23:35-48.
- Bowie G, Sanders J, Riedel G, Gilmour C, Breitburg D, Cutter G, Porcella D. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. *Water, Air and Soil Pollution* 90:93-104.
- Brown S, MacLatchy D, Hara T, Eales J. 1991. Effects of cortisol on aspects of 3,5,3'-triiodo-L-thyronine metabolism in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* 8:207-216.
- Buhl K, Hamilton S. 1991. Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. *Ecotoxicology and Environmental Safety* 22:184-197.
- Carr J, Norris D. 2006a. The adrenal gland. In: Norris D, Carr J, editors. *Endocrine disruption: biological bases for health effects in wildlife and humans*. New York: Oxford University Press. p 111-134.
- Dethloff G, Schlenk D, Khan S, Bailey H. 1999. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Contamination and Toxicology* 36(4):415-423.
- Di Giulio R, Habig C, Gallagher E. 1993. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. *Aquatic Toxicology* 26:1-22.
- Donaldson E. 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering A, editor. *Stress and Fish*. London: Academic Press. p 11-47.
- Dorval J, Leblond V, Deblois C, Hontela A. 2005. Oxidative stress and endocrine endpoints in white sucker (*Catostomus commersoni*) from a river impacted by agricultural chemicals. *Environmental Toxicology and Chemistry* 24(5):1273-1280.

- Dorval J, Leblond V, Hontela A. 2003. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquatic Toxicology* 63:229-241.
- Freeman H, Sangalang G. 1977. A study of the effects of methyl mercury, cadmium, arsenic, selenium, and PCB, (Aroclor 1254) on adrenal and testicular steroidogeneses *in vitro*, by the gray seal *Halichoerus grypus*. *Archives of Environmental Contamination* 5:369-383.
- Gagnon A, Jumarie C, Hontela A. 2006. Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 78:59-65.
- Girard C, Brodeur J, Hontela A. 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. *Canadian Journal of Fisheries and Aquatic Sciences* 55:438-450.
- Goeltl J, Davies P. 1976. Water Pollution Studies. In: Wildlife CDo, editor: Department of Natural Resources.
- Güll Ş, Belge-Kurutaş E, Yıldız E, Şahan A, Doran F. 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyrinidae) living in Seyhan Dam Lake, Turkey. *Environment International* 30:605-609.
- Haygarth P. 1994. Global importance and global cycling of selenium. In: Frankenberger W, Jr., Benson S, editors. *Selenium In The Environment*. New York: Marcel Dekker Inc. p 1-28.
- Hilton J, Hodson P, Slinger S. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* 110:2527-2535.
- Hodson P, Spry D, Blunt B. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. *Canadian Fisheries Research Board of Canada* 37(2):233-240.
- Holm J, Palace V, Siwik P, Sterling G, Evans R, Baron C, Weerner J, Wautier K. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environmental Toxicology and Chemistry* 24(9):2373-2381.
- Hontela A. 1997. Endocrine and physiological responses of fish of xenobiotics: role of glucocorticosteroid hormones. *Reviews in Toxicology* 1:1-46.
- Hontela A. 2005. Adrenal toxicology: environmental pollutants and the HPI axis. In: Mommsen T, Moon T, editors. *Biochemistry and Molecular Biology of Fishes*. Amsterdam: Elsevier B.V. p 331-363.
- Hunn J, Hamilton S, Buckler D. 1987. Toxicity of sodium selenite to rainbow trout fry. *Water Research* 21(2):233-238.
- Kelly S, Havrilla C, Brady TA, KH, Levin E. 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environmental Health Perspectives* 106(7):375-384.
- Kennedy C, Farrel A. 2005. Ion homeostasis and interrenal stress responses in juvenile Pacific herring, *Clupea pallasii*, exposed to the water-soluble fraction of crude oil. *Journal of Experimental Marine Biology* 323:43-56.
- Köhrle J, Jakob F, Contempré B, Dumont J. 2005. Selenium, the thyroid, and the endocrine system. *Endocrine Reviews* 26(7):944-984.
- Laflamme J-S, Couillard Y, Campbell P, Hontela A. 2000. Interrenal metallothioneine and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca*

- flavescens*, from Abitibi lakes. Canadian Journal of Fisheries and Aquatic Sciences 57:1692-1700.
- Lemly A. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety 37:259-266.
- Lemly A. 1999. Selenium impacts on fish: an insidious time bomb. Human and Ecological Risk Assessment 5(6):1139-1151.
- Lemly A. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57:39-49.
- Levesque H, Dorval J, Hontela A, Van Der Kraak G, Campbell P. 2003. Hormonal, morphological, and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. Journal of Toxicology and Environmental Health, Part A 66:657-676.
- Livingstone D. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin 42(8):656-666.
- Livingstone D, Lemaire P, Matthews A, Peters L, Bucke D, Law R. 1993. Pro-oxidant, antioxidant and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses in liver of dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. Marine Pollution Bulletin 26(11):602-606.
- Lohner T, Reash R, Willet V, Rose L. 2001a. Assessment of tolerant sunfish populations (*Lipomis* sp.) inhabiting selenium-laden coal ash effluents 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety 50:203-216.
- Lorentzen M, Maage A, Julshamn K. 1994. Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (*Salmo salar*). Aquaculture 121:359-367.
- Mommsen T, Vijayan M, Moon T. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9:211-268.
- Morgan I, Henry R, Wood C. 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na⁺ and Cl⁻ transport. Aquatic Toxicology 38:145-163.
- Oakes K, McMaster M, Pryce AM, KR, Portt C, Hewitt L, MacLean D, Van Der Kraak G. 2003. Oxidative stress and bioindicators of reproductive function in pulp and paper mill effluent exposed white sucker. Toxicological Sciences 74:51-65.
- Oakes K, McMaster MVDK, GJ. 2004. Oxidative stress responses in longnose sucker (*Catostomus catostomus*) exposed to pulp and paper mill and municipal sewage effluents. Aquatic Toxicology 67(3):255-271.
- Orun I, Ates B, Selamoglu Z, Yazlak H, Ozturk E, Yilmaz I. 2005. Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). Fresenius Environmental Bulletin 14(1):18-22.
- Palace V, Spallholz J, Holm J, Wautier K, Evans R, Baron C. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety 58:17-21.
- Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S. 2001. Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Block: 1.

- protection against peroxidation in liver by copper preexposure. *Archives of Environmental Contamination and Toxicology* 41:345-352.
- Parkinson A. 2003. Biotransformations of xenobiotics. In: Klaasen C, Watkins J, editors. *Casarett and Doull's essentials of toxicology*. New York: The McGraw-Hill Companies, Inc. p 71-97.
- Payne J, Malins D, Gunselman SR, A, Yeats P. 1998. DNA oxidative damage and vitamin A reduction in fish from a large lake system in Labrador, Newfoundland, contaminated with iron-ore mine tailings. *Marine Environmental Research* 46(1-5):289-294.
- Pelgron S, Lock R, Balm P, Wendelaar Bonga S. 1995. Effects of combined waterborne Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, *Oreochromis mossombicus*. *Comparative Biochemistry and Physiology Part C* 111(2):227-235.
- Potmis R, Nonavinakere V, Rasekh H, Early J. 1993. Effect of selenium (Se) on plasma ACTH, β -endorphin, corticosterone and glucose in rat: influence of adrenal enucleation and metyrapone pretreatment. *Toxicology* 79:1-9.
- Roberts A, Oris J. 2004. Multiple biomarker response in rainbow trout during exposure to hexavalent chromium. *Comparative Biochemistry and Physiology Part C* 138:221-228.
- Roméo M, Gnassia-Barelli M, Lafaurie M, Girard J. 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of sea bass *Dicentrarchus labrax*. *Aquatic Toxicology* 48:185-194.
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher J-M, Aït-Aïssa S. 2005. Copper-induced oxidative stress in three-spine stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology* 19:177-183.
- Schreck C. 1990. Physiological, behavioural, and performance indicators of stress. *American Fisheries Society Symposium* 8:29-37.
- Sorensen E, Bauer T, Bell J, Harlan C. 1982. Selenium accumulation and cytotoxicity in teleosts following chronic, environmental exposure. *Bulletin of Environmental Contaminant Toxicology* 29:688-696.
- Spallholz J, Hoffman D. 2002. Selenium toxicity: cause and effects in aquatic birds. *Aquatic Toxicology* 57:27-37.
- Spallholz J, Palace V, Reid T. 2004. Methioninase and selenomethionine but not Se-methylselenocystiene generate methylselenol and superoxide in an *in vitro* chemiluminescent assay: Implications for the nutritional carcinostatic activity of selenoamino acids. *Biochemical Pharmacology* 67:547-554.
- Steinbrenner H, Alili L, Bilgic E, Sies H, Brenneisen P. 2006. Involvement of selenoprotein P in protection of human astrocytes from oxidative damage. *Free Radical Biology and Medicine* 40:1513-1523.
- Vijayan M, Prunet P, Boone A. 2005. Xenobiotic impact on corticosteroid signaling. In: Mommsen T, Moon T, editors. *Biochemistry and Molecular Biology of Fishes*. Amsterdam: Elsevier B.V. p 365-394.
- Watanabe T, Kiron V, Satoh S. 1997. Trace minerals in fish nutrition. *Aquaculture* 151:185-207.
- Wayland M, Gilchrist H, Marchant T, Keating J, Smits J. 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to

- cadmium, mercury, and selenium concentrations. Environmental Research Section A 90:47-60.
- Wedemeyer G, McLeay D. 1981. Methods for determining the tolerance of fishes to environmental stressors. In: Pickering A, editor. Stress and Fish. London: Academic Press. p 247-275.
- Winston G, Di Giulio R. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology 19:137-161.
- Zhang J, Liu J, Sun Y, Wang X, Wu J, Xue Y. 2005. Responses of the antioxidant defenses of the goldfish *Carassius auratus*, exposed to 2,4-dichlorophenol. Environmental Toxicology and Pharmacology 19:185-190.

CHAPTER 3. THE EFFECT OF CHRONIC SELENIUM EXPOSURE ON THE PHYSIOLOGICAL STRESS RESPONSE AND OXIDATIVE STRESS BIOMARKERS IN RAINBOW TROUT AND BROOK TROUT FROM A COAL MINING AREA

3.1. Introduction

Selenium (Se) is an essential element as a constituent of several key enzymes including glutathione peroxidase, an antioxidant, deiodinase, important for thyroid hormone synthesis, and thioredoxin reductases, another antioxidant (Köhrle et al. 2005). At concentrations in excess of the homeostatic requirements, Se can bioaccumulate 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) and anthropogenic activities, such as coal mining, can release previously unavailable Se into aquatic environments (Lemly 1999). Elevated Se has been documented on the north-eastern 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) in streams impacted by coal mining.

The major toxic effect of Se in fish and aquatic birds is its accumulation in eggs and the resulting teratogenic deformities occurring when Se accumulates above 10 µg/g in the egg (Lemly 1997). Different fish species have very different Se sensitivities. Selenium - linked teratogenesis has been documented in rainbow trout, *Oncorhynchus mykiss*, (Holm et al. 2005), 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 cutthroat trout, *Oncorhynchus clarki* (Kennedy et al. 2000), brook trout, *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 and Cairns 1996) and rainbow trout (Hilton et al. 1980), but not bluegill (Lohner et al. 2001a). There is little research on how excess Se affects systems other than reproduction in fish. Other systems, such as the physiological stress response, might be influenced by Se and modify its toxicity and physiological impacts. Moreover, species-specific responses to Se may mediate the effect of Se on reproduction.

The physiological stress response (PSR) enables a fish to respond appropriately to stressors and maintain homeostasis (Carr and Norris 2006). The primary responses (release of stress hormones, such as cortisol, into plasma) trigger secondary responses including metabolic effects (such as the elevation of plasma glucose) that eventually lead to tertiary or whole organism responses (e.g. lower condition factor) (Barton 2002). Little is known about the effect of Se on the PSR in fish. It activated the PSR in rats (Potmis et al. 1993) and eiders (Wayland et al. 2002) by increasing stress hormone levels, but decreased cortisol levels in grey seals (Freeman and Sangalang 1977). Contaminants can also impair the PSR (Girard et al. 1998; Bisson and Hontela 2002) causing damage to cellular components within the steroidogenic tissue. For example, cortisol secretion impairment in head kidney cells exposed, *in vitro*, to the pesticide endosulfan is mediated by cellular damage through oxidative stress (Dorval and Hontela 2003; Dorval et al. 2003).

Oxidative stress occurs when reactive oxygen species (ROS) overwhelm cellular antioxidants and damage lipids, proteins, and DNA. ROS are by-products of cellular process, such as cellular respiration, but contaminant exposure can increase their production as well (Kelly et al. 1998). For example, Se creates a ROS when it combines with GSH in rainbow trout larvae (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 catalase (CAT) and glutathione peroxidase (GPx) provide a measure of the capacity to remove dangerous peroxides (Kelly et al. 1998). Non-enzymatic antioxidants such as reduced glutathione (GSH) and vitamins A and E also remove ROS (Kelly et al. 1998). Generally, LPO increases with contaminant exposure (Berntssen et al. 2000), but it decreased with Se exposure in rainbow trout (Orun et al. 2005). An increase in antioxidants suggests they are being up regulated to potentially deal with increased ROS (Sanchez et al. 2005), while a decrease suggests they are depleted or inhibited by the contaminant (Zhang et al. 2005). Oxidative stress biomarkers levels may be useful indicators of contaminant exposure and even effects, in the case of LPO; however, careful research is needed to establish contaminant-induced species specific patterns. There are few reports on species-specific differences in contaminant-induced oxidative stress and vulnerability to contaminants (Hasspieler et al. 1994; Di Giulio et al. 1995).

The objectives of this study were: (1) to determine if and how chronic exposure to environmental levels of Se influences the PSR in rainbow trout and brook trout, (2) to determine if chronic exposure to environmental levels of Se alters biomarkers of

oxidative stress in rainbow trout and brook trout, and (3) to compare the PSR and oxidative stress biomarkers in rainbow trout and brook trout from a coal mining area in the north-eastern slopes of the Canadian Rocky Mountains. The following hypotheses will be tested: (1) exposure to chronic environmental Se alters the PSR, (2) exposure to chronic environmental Se alters biomarkers of oxidative stress, and (3) there are species-specific differences in the PSR and oxidative stress biomarkers of rainbow trout and brook trout.

3.2. Materials and Methods

3.2.1. Chemicals

Porcine ACTH 1-39 and Bradford reagent were purchased from Sigma-Aldrich and MS-222 was purchased from MPBiomedicals. Cortisol (catalogue #07-221102), T3 (catalogue #06B-254215), T4 (catalogue #06B-254011) radioimmunoassay kits, Bioxytech GSH 400 (catalogue #21011), and LPO 596 (catalogue #21012) kits were purchased from Mediacorp. GOD-PAP reagent was purchased from Roche Diagnostic. Ultra pure nitric acid was purchased from Fischer Scientific.

3.2.2. Fish

Post-spawning adult and immature rainbow trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, were sampled from the north-eastern slopes of the Rocky Mountains near Hinton, Alberta, Canada, a region with active open pit coal mines. They were captured with a Smith-Root LR-24 electroshocker from reference and Se contaminated streams over three days. Rainbow trout were captured in early June

2005 from Wampus Creek (reference), Deerlick Creek (reference), the Gregg River (low Se) and Luscar Creek (high Se). Brook trout were captured in late October 2005 from Cold Creek (reference), the Gregg River (low Se), and Luscar Creek (high Se). Water samples were collected in acid washed bottles and acidified with 0.05% ultra pure nitric acid. Samples were analyzed for total Se (ICP-MS) at the Ultra-Clean Trace Elements Laboratory at the University of Manitoba.

Sampling: After electroshocking, fish were kept in secure enclosures overnight *in situ*. The next morning, the fish were transported (up to 0.5 hours) to the sampling station and kept in oxygenated large bins until sampling the same morning. Fish were anesthetised with MS-222, and blood samples were taken from the caudal blood vessels. Fish were killed by spinal transection and the plasma was recovered and flash frozen in liquid nitrogen. Carcasses were kept on ice (maximum 4 hours) until dissection. Fork length, weight, sex, maturity, and liver weight were recorded. Livers and gills were removed and flash frozen in liquid nitrogen. Muscle samples for Se analysis were taken from the left side of the fish under the dorsal fin and sent to the Department of Fisheries and Oceans' Freshwater Institute, Winnipeg, Manitoba for total Se analysis. Condition factor and liver somatic index were calculated.

3.2.3. Plasma

Cortisol (ng/ml plasma) was measured with a radioimmunoassay kit.

Glucose (mg/ml plasma) was determined in a spectrophotometric assay (510 nm) using the GOD-PAP reagent.

T3 and T4 (ng/ml plasma) were measured with radioimmunoassay kits in remaining plasma.

3.2.4. Gills

Na⁺/K⁺-ATPase activity in a gill homogenate was measured by liberating PO₄ from a hydrolysis reaction with ATPase in a gill homogenate (Morgan et al. 1997) as in Chapter 2. Na⁺/K⁺ ATPase activity is expressed as U per mg protein and one unit is one μmol liberated PO₄.

Protein was measured using a spectrophotometric assay (595 nm) and the Bradford reagent.

3.2.5. Liver

Liver homogenates were prepared in a 50 mM potassium phosphate buffer as described in Chapter 2. GSH was assayed within an hour, and supernatants for CAT, GPx, and LPO analysis were kept at -80°C for a maximum of 2 days before analysis.

Total Liver Cellular GPx was determined by measuring the decrease in absorbance (340nm) due to the decline in NADPH at 23-25°C (Lorentzen et al. 1994) as described in Chapter 2. GPx activity was expressed as mU/mg protein and one mU is defined as 1 nmol of NADPH consumed/minute.

Live CAT was determined by measuring the rate of hydrogen peroxide consumption at 240nm (Beers and Sizer 1952) and is expressed as U/mg protein. One unit of catalase activity is 1 μmol hydrogen peroxide consumed/min.

Liver GSH was determined using the Bioxytech GSH 400 kit. GSH forms a thioether that reacts with a reagent forming a thiol (400nm). GSH is expressed as $\mu\text{mol GSH}$ per mg protein.

Liver LPO was determined using the Bioxytech LPO-596 kit. 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 586nm. LPO is expressed as U/mg protein, where one unit is one μM MDA and 4-HA.

3.2.6. 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 in Chapter 2. The ability of the head kidney to secrete cortisol is expressed as the change in cortisol secretion ($\Delta \text{ ng/mL}$) after stimulation with 2 units of ACTH (stimulated cortisol – control cortisol).

3.2.7. Statistical Analysis

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 brook trout and rainbow trout. Species, muscle Se levels (as an indicator of Se exposure), site type (an indicator of other stressors), length, and sex 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.

3.3. Results

3.3.1. Selenium Exposure

Total water Se concentrations were highest at Luscar Creek, followed by the Gregg River, and then the reference sites (Table 3.1). Muscle Se concentrations followed a similar pattern in both brook trout and rainbow trout with the highest Se concentrations occurring in fish from Luscar Creek (Table 3.1). At each site, muscle Se levels were similar between the two species. Muscle moisture levels are also given in Table 3.1 to allow conversion between wet and dry weight Se measurements.

3.3.2. Physiological Stress Response

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 3.2. The sample sizes, means, transformed means, and transformed SE for each species at reference, low Se and high Se sites are given in Table 3.3. Plasma cortisol levels were significantly influenced by species, sex, and site type, but not muscle Se levels (Table 3.2). Female brook trout had higher plasma cortisol levels than the male fish (Figure 3.1A); however, female rainbow trout had similar plasma cortisol levels to the males (Figure 3.1B).

Plasma glucose levels were significantly ($p < 0.05$) influenced by species and site type (Table 3.2). Plasma glucose levels were lower in fish from reference sites compared to fish from high Se sites and this increase was greater in the rainbow trout than the brook trout (Figure 3.2A). Gill Na^+/K^+ -ATPase activities were significantly ($p < 0.05$) influenced by species and sex (Table 3.2). Brook trout had greater gill Na^+/K^+ ATPase activities than rainbow trout and female rainbow trout had the lowest activity (Figure 3.2B). Plasma thyroid hormone (T3 and T4) levels were significantly ($p < 0.05$) influenced by species and fish length and T4 was also influenced by sex (Table 3.2). Larger rainbow trout had higher plasma T3 and T4 levels, but length did not influence plasma thyroid hormone levels in brook trout (Figure 3.3).

Condition factor and the liver somatic index were both significantly ($p < 0.05$) influenced by muscle Se levels (Table 3.2). Condition factor was also influenced by species, and the liver somatic index was additionally influenced by sex. Fish with greater muscle Se levels had a greater condition factor in both species, but for similar muscle Se levels, rainbow trout had a greater condition factor than brook trout (Figure 3.4A). Similarly, fish with greater muscle Se levels had a greater liver somatic index and, for similar muscle Se levels, female fish had greater liver somatic indices than males or immature fish (Figure 3.4B).

3.3.3. Oxidative Stress Response

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 3.2. The sample sizes, means, transformed means, and transformed SE for each

species at reference, low Se and high Se sites are given in Table 3.4. The antioxidant defences were not significantly influenced by muscle Se levels; however, they were influenced ($p < 0.05$) by species and/or sex (Table 3.2). Hepatic GPx activities were greater in rainbow trout than brook trout (Figure 3.5A) and hepatic CAT activities were slightly greater in female fish than male and immature fish (Figure 3.5B). Rainbow trout had greater hepatic GSH levels than brook trout (Figure 3.5C). Male and immature rainbow trout had greater GSH levels than female rainbow trout, but GSH levels are similar in the male and female brook trout (Figure 3.5C). Hepatic LPO levels were significantly ($p < 0.05$) influenced by both species and muscle Se levels (Table 3.2). In rainbow trout LPO levels are beginning to increase with increasing muscle Se levels; however in the brook trout, LPO levels decrease with increasing muscle Se levels (Figure 3.6).

3.4. Discussion

The objectives of this study were: (1) to determine if chronic exposures to environmental Se levels influence the PSR, (2) to determine if chronic exposures to environmental Se levels alter biomarkers of oxidative stress, and (3) to compare the PSR and oxidative stress biomarkers in rainbow trout and brook trout exposed to environmental Se levels. Brook trout and rainbow trout were exposed to similar increasing amounts of Se, as indicated by muscle Se levels at sites ranging from reference to high Se exposure, but species-specific responses of the PSR and oxidative stress parameters were evident. Components of the PSR were influenced by muscle Se levels, species, length, sex, and/or site type. The hepatic antioxidant defences were not

influenced by muscle Se levels, but by species and/or sex. Lipid peroxidation, an indicator of oxidative damage, was increased by increasing muscle Se levels in rainbow trout, but it decreased in brook trout.

3.4.1. Selenium Exposure

Brook trout and rainbow trout were caught from the same Se-exposed sites, but from different reference sites; however, the Se muscle levels were low at all of the reference sites. These sites have also been used in other projects that investigated the effect of Se on teratogenic deformities in fish (Holm et al. 2005) and on invertebrate populations (Wayland and Crosley 2006). Water Se levels were similar between all of the studies at these sites (Holm et al. 2005; Wayland and Crosley 2006).

In this study, muscle Se levels were used as an indicator of Se exposure, instead of site type, because all the streams are interconnected and the fish may move in and out of the Se-impacted streams. Muscle Se levels indicate the true exposure of the fish, as they accumulate Se from both water and food, and may depurate a significant amount when they move to cleaner environments (Hamilton et al. 2005b). The muscle Se levels found in the rainbow trout and brook trout in the present study were similar to those identified in Holm's (2005) study, where a positive correlation between Se egg concentrations and deformities in rainbow trout, but not brook trout, was identified. Other effects that have been documented in rainbow trout are growth reduction (Hilton and Hodson 1983), mortality (Hunn et al. 1987), and decreased red blood cell volumes (Hodson et al. 1980). The effects of Se on brook trout, other than teratogenesis, have not been investigated.

3.4.2. Physiological Stress Response

Plasma cortisol levels (a primary response) were influenced by species, sex, and site type, but not muscle Se levels. Selenium did activate the PSR and raised plasma cortisol levels in rainbow trout subjected to acute and sub-chronic exposures in the laboratory (Chapter 2). In the field, fish may have acclimated to the Se present and no longer respond to it as a stressor. Alternatively, Se levels may have been too low to activate the PSR as the environmental exposures (0.11 – 32.2 µg/L) were lower than the concentrations used in the laboratory exposures (50 – 2670 µg/L; Chapter 2). Selenium is an essential element, so water concentrations near the Se concentrations required for growth should not activate the PSR.

Plasma cortisol levels were influenced by species, sex, and site type. Both species elevated their plasma cortisol levels to similar levels in response to a capture and confinement stress; however, female brook trout had consistently higher levels than male brook trout, while female rainbow trout had similar cortisol levels than the male rainbow trout. This suggests that female brook trout were more stressed by the capture and confinement stress than the males, but that the female and male rainbow trout were similarly stressed. Benfey and Biron (2000) reported that rainbow trout were more sensitive to confinement stress and raised their plasma cortisol levels higher than brook trout (Benfey and Biron 2000). Moreover, female fish are more sensitive to stressors than male fish, although it has been reported that during the breeding season females may not respond to a stressor (Kubokawa et al. 1999). In the present study a difference between males and females was observed, but it was species-specific as the female rainbow trout had similar plasma cortisol levels to the males, but female brook trout

always had higher plasma cortisol levels than the males. Thus, female brook trout were more sensitive to the confinement stressor than the males. Site type encompasses unquantified site-specific stressors (other than Se) that may be present in the system. There are no obvious patterns in plasma cortisol levels to suggest that one site has an additional stressor when site type is compared within sex and species.

Plasma glucose levels and gill Na^+/K^+ -ATPase activities are both secondary stress responses. Cortisol has been shown to increase plasma glucose levels by triggering glucose synthesis from amino acids and lipids as well as decreasing muscle glucose uptake (Carr and Norris 2006a). Cortisol may also increase gill Na^+/K^+ -ATPase activity (Shrimpton and McCormick 1999) and this is one of the major mechanisms enabling smolts to tolerate salt water (Carr and Norris 2006).

Plasma glucose levels of brook trout and rainbow trout were not influenced by Se exposure, but they were influenced by species and site type. In both species, plasma glucose levels increased from reference to high Se site types, but this change was more pronounced in the rainbow trout. This suggests that rainbow trout are more sensitive to a stressor that is present at the low and high Se sites than the brook trout. Our models suggest that this stressor is not Se as muscle Se levels did not significantly influence glucose levels, but it may be an additional parameter which occurs with Se. For example, strontium, an element that was also elevated in eggs collected from rainbow trout and brook trout (Holm et al. 2005) could be a chemical stressor influencing glucose levels.

Gill Na^+/K^+ -ATPase activities were influenced by species and sex, but not muscle Se levels. This is expected as Se did not significantly influence plasma cortisol levels and cortisol increases gill Na^+/K^+ -ATPase activity (Shrimpton and McCormick 1999). Gill

Na^+/K^+ -ATPase activity in brook trout was greater than in rainbow trout, suggesting a fundamental species-specific difference in the activities of this enzyme. Higher gill Na^+/K^+ -ATPase activity may give brook trout an advantage in areas with higher salt contents. Luscar Creek (high Se) and the Gregg River (low Se) did have higher conductivity than the reference sites (Holm et al. 2005). Higher gill Na^+/K^+ ATPase activity may give brook trout a physiological advantage over rainbow trout in streams with higher conductivity such as the Se-impacted streams near Hinton, Alberta, Canada.

Plasma thyroid hormones (T3 and T4) levels are also influenced by cortisol and are part of the secondary stress response (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). Often exposure to chemical stressors increases both plasma T3 and T4 levels; juvenile rainbow trout increased plasma T3 and T4 levels in response to a laboratory sub-chronic Se exposure (Chapter 2). Moreover, deiodinase, the enzyme that converts T4 to T3, requires Se (Köhrle et al. 2005). However, in this field study the thyroid hormones levels were not influenced by muscle Se levels, confirming that these fish are not selenium deficient and most likely have sufficient deiodinase levels. Our models suggest that plasma thyroid hormones were influenced by fork length and fish species. In rainbow trout larger fish had greater T3 and T4 levels, but this relationship was not evident in the brook trout. Thyroid hormone levels in fish may be linked to growth (Nankervis and Southgate 2006), but the species-specific relationships between growth and thyroid function have not yet been investigated.

Condition factor and liver somatic index are well established tertiary stress responses at the whole organism level resulting from altered energy allocation. In this study, the

cortisol secretory capacity was also considered a tertiary response as it specifically tests the functionality of a system that integrates processes in the entire organism. Exposure to chronic stressors may also impact other tertiary responses such as sex steroid secretion and reproduction (Carr and Norris 2006a).

The cortisol secretory capacity tested, in an *in vitro* system, the functional integrity of the head kidney of fish exposed to environmental levels of Se. If the PSR is impaired, the fish will be unable to respond to stressors (e.g., predators) and allocate their energy reserves. The secretory capacity of the head kidneys was not influenced by any parameter tested (muscle Se levels, species, sex, length or site type); therefore, Se or any other contaminant at the sites did not impair the PSR in brook trout or rainbow trout. These fish are expected to normally respond to environmental stressors encountered in their habitat.

Condition factor and LSI were significantly influenced by muscle Se levels. Unexpectedly, they increased with increasing Se exposure. Generally, with exposure to a contaminant, condition factor and sometimes liver somatic index decrease as the fish use their energy to deal with the contaminant, rather than for growth and energy reserves. Yellow perch from lakes contaminated with heavy metals had lower LSI than those from clean lakes (Girard et al. 1998; Laflamme et al. 2000) and bluegill exposed to waterborne Se had a decreased condition factor (Cleveland et al. 1993). Increased condition factor and LSI may result from stimulated growth in the contaminated systems when the contaminant alters the foodweb by eliminating competitors or changing prey abundance (Campbell et al. 2003). Selenium, an essential element, may stimulate growth at the same exposure concentrations it induces teratogenesis in some species, possibly by

reducing recruitment and therefore increasing food availability for the survivors. This complex relationship between Se and species-specific processes may ultimately result in smaller unstable populations of larger fish, but this must be investigated further.

3.4.3. Oxidative Stress Response

The hepatic antioxidant defenses were not influenced by Se exposure in brook trout or rainbow trout. Glutathione peroxidase activities were significantly influenced by species. Rainbow trout GPx activities were greater than brook trout activities, but activities in both were elevated above levels previously measured in laboratory studies (Hilton et al. 1980; Orun et al. 2005). Catalase activities were only influenced by sex, although the differences were only slight. Reduced glutathione levels were affected by species and sex, but not Se. In a previous laboratory studies, acute Se exposure decreased liver GSH levels in rainbow trout (Chapter 2), but in the field, Se exposures may have been too low to deplete hepatic GSH.

Lipid peroxidation levels, an indicator of oxidative damage, were influenced by muscle Se levels and species. They increased with increasing muscle Se levels in rainbow trout, but they decreased with increasing Se levels in brook trout. Thus, Se exposure appears to cause oxidative stress in rainbow trout, but protects against it in brook trout. These results are in agreement with previous studies investigating species-specific toxicity of Se, as Se caused a greater rate of teratogenic deformities in rainbow trout than in brook trout (Holm et al. 2005). Our results indicate that rainbow trout are more sensitive to Se toxicity than brook trout.

Biochemical differences may explain the different Se sensitivities observed.

Rainbow trout had more hepatic GSH than brook trout and GSH can react with Se to create ROS (Spallholz et al. 2004). Thus, rainbow trout may have more GSH available than brook trout to bind to Se and create more ROS. Rainbow trout in our field study also had greater GPx activities than brook trout. These additional defences may not have been sufficient to counteract the ROS production and, therefore, more oxidative damage (LPO) occurred in rainbow trout. More research is needed to determine if the difference in GSH levels is the underlying cause of the species-specific LPO response or if other mechanisms contribute to species specific vulnerability to Se.

3.4.4. Conclusions

Chronic exposure to environmental Se did not activate or impair the PSR in rainbow trout or brook trout and Se did not influence cortisol secretion, glycemia, or thyroid status; however, there were species differences in some of the PSR parameters as brook trout had greater gill Na^+/K^+ -ATPase activities than rainbow trout. Such a physiological difference may enable brook trout to tolerate a greater range of osmotic perturbations and underlie differences in species sensitivities to toxicants and competitive interactions. Species-specific sensitivity to Se should be further investigated to elucidate Se's toxicity mechanism and to predict population responses.

Selenium exposure did not alter antioxidant levels (GPx, CAT, GSH), but it did increase oxidative damage (LPO) in rainbow trout. This finding supports the hypothesis that oxidative stress is a mechanism of Se toxicity. Species differences were also evident

in the oxidative stress parameters and the difference in GSH reserves may have led to a greater susceptibility to Se-induced oxidative stress (measured by LPO) in rainbow trout.

In the Hinton area, the rainbow trout are Athabaskan rainbow trout, a genetically distinct population of native rainbow trout (Carl et al. 1994) and the only native rainbow trout on the east side of the Rocky Mountains (Nelson and Paetz 1992). In contrast, the brook trout are introduced to the area from eastern Canada (Nelson and Paetz 1992) and have been moving into the native rainbow trout habitat. The physiological and biochemical differences between these two species may account for different Se sensitivities and competitive abilities. More specifically, the higher gill Na⁺/K⁺ ATPase activities and lower hepatic GSH levels may allow the brook trout to osmoregulate more efficiently and protect them from oxidative stress in the impacted streams. However, more research is needed to link cellular (biochemical) effects to population (competition) effects in this system.

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Table 3.1 Selenium water levels, muscle Se content and muscle water content of brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated sites near Hinton, Alberta, Canada.

Site	Site Type	Water Se ($\mu\text{g/L}$)	Sample Size		Muscle Se ($\mu\text{g/g}$) ^a		Muscle Moisture (%)	
			RBTR	BKTR	RBTR	BKTR	RBTR	BKTR
Deerlick Creek	Reference	-	10	-	0.71 ± 0.05	-	78.47 ± 0.20	-
Wampus Creek	Reference	-	12	-	0.74 ± 0.06	-	79.18 ± 0.22	-
Cold Creek	Reference	0.11	-	14	-	0.35 ± 0.02	-	78.26 ± 0.31
Gregg River	Low Se	1.43	10	14	1.51 ± 0.08	1.23 ± 0.10	78.61 ± 0.23	78.43 ± 0.17
Luscar Creek	High Se	32.22	6	15	3.30 ± 0.74	3.90 ± 0.33	78.29 ± 0.20	76.55 ± 0.61

^a wet weight

Table 3.2 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 near Hinton, Alberta, Canada.

Parameter	Data Transformation ^a	Adjusted r ²	Model
<i>Physiological Stress Response</i>			
Cortisol	0.4	0.2552	Sex + Species + Site Type
Glucose	-0.8	0.2611	Species + Site Type + Species*Site Type
T3	0.4	0.1550	Length + Species + Length*Species
T4	-	0.4217	Length + Species + Sex + Length*Species
Gill Na ⁺ /K ⁺ -ATPase	-	0.3815	Species + Sex
Cortisol Secretory Capacity	0.4	-	No significant model ^b
Condition Factor	log	0.3885	Selenium + Species
Liver Somatic Index	0.2	0.1712	Selenium + Sex
<i>Hepatic Oxidative Stress Parameters</i>			
Glutathione peroxidase	log	0.2044	Species
Catalase	-0.4	0.0548	Sex
Reduced Glutathione	-	0.5217	Species + Sex
Lipid Peroxidation	0.2	0.1676	Selenium + Species + Selenium*Species

^a the number indicates the λ value from a Box-Cox transformation.

^b none of the parameters investigated explained a significant amount of the variation in the brook trout or rainbow trout's ability to secrete cortisol.

Table 3.3 Physiological stress response parameters in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada.

Parameter	Reference		Low Se		High Se	
	BKTR	RBTR	BKTR	RBTR	BKTR	RBTR
<i>Cortisol (ng/ml plasma)</i>						
n	14	22	14	10	15	6
Mean	75	165	182	179	125	119
Transformed Mean	175	270	270	283	230	228
Transformed SE	22	16	21	21	21	31
<i>Glucose (mg/ml plasma)</i>						
n	14	20	14	10	14	6
Mean	0.90	0.90	0.96	1.45	1.02	1.40
Transformed Mean	-0.15	-0.19	-0.06	0.04	-0.02	0.17
Transformed SE	0.06	0.07	0.04	0.06	0.06	0.15
<i>T3 (ng/ml plasma)</i>						
n	13	16	11	7	13	3
Mean	4.58	6.14	3.64	5.26	4.90	2.10
Transformed Mean	4.69	5.78	3.72	5.03	4.90	1.50
Transformed SE	0.49	0.69	0.56	1.08	0.55	1.61
<i>T4 (ng/ml plasma)</i>						
n	13	13	11	5	13	3
Mean	10.98	18.83	13.18	18.56	14.09	8.34
Transformed Mean	-	-	-	-	-	-
Transformed SE	-	-	-	-	-	-
<i>Gill Na⁺/K⁺-ATPase (U/mg protein)^a</i>						
N	14	13	14	6	15	5
Mean	0.55	0.28	0.62	0.30	0.54	0.49
Transformed Mean	-	-	-	-	-	-
Transformed SE	-	-	-	-	-	-
<i>Cortisol Secretory Capacity (Δ ng/ml)^b</i>						
N	9	8	12	7	9	6
Mean	25.36	24.97	29.41	29.50	33.86	12.52
Transformed Mean	27.05	31.06	36.59	35.01	37.49	22.45
Transformed SE	8.25	6.39	3.79	6.85	6.43	3.64
<i>Condition Factor (K)^c</i>						
N	14	22	14	10	15	6
Mean	0.94	1.08	-0.93	1.13	1.05	1.18
Transformed Mean	-0.08	0.07	-0.10	0.11	0.02	0.15
Transformed SE	0.03	0.02	0.03	0.03	0.03	0.03

Table 3.3 Continued

Parameter	Reference		Low Se		High Se	
	BKTR	RBTR	BKTR	RBTR	BKTR	RBTR
<i>Liver Somatic Index (LSI)^d</i>						
N	14	22	14	10	15	6
Mean	0.81	1.13	0.97	0.90	1.37	1.02
Transformed Mean	-0.22	0.11	-0.07	-0.16	0.28	0.00
Transformed SE	0.05	0.06	0.08	0.13	0.09	0.10

^a one unit is defined as one $\mu\text{mol PO}_4$ liberated

^b Δ = stimulated cortisol (ng/ml) – basal cortisol (ng/ml)

^c $K = \frac{\text{weight} \times 100}{\text{length}^3}$

^d $LSI = \frac{\text{weight}_{\text{liver}}}{\text{weight}_{\text{body}}} \times 100$

Table 3.4 Oxidative stress parameters in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada.

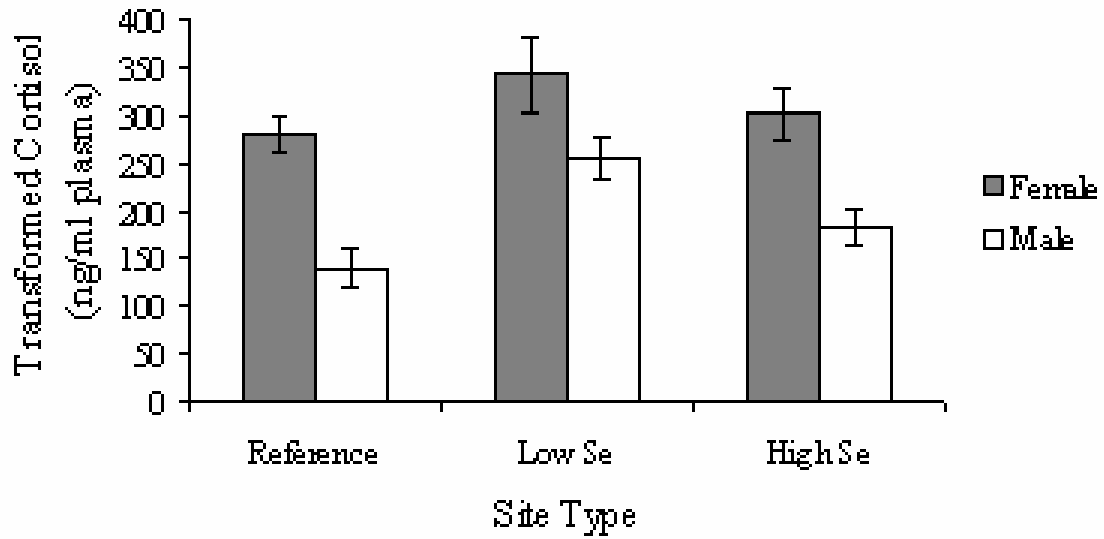
Parameter	Reference		Low Se		High Se	
	BKTR	RBTR	BKTR	RBTR	BKTR	RBTR
<i>Glutathione Peroxidase (mU/mg protein)^a</i>						
N	14	20	12	9	15	3
Mean	3.61	11.98	5.58	7.43	5.00	6.73
Transformed Mean	5.50	11.57	7.09	8.44	7.37	9.59
Transformed SE	1.00	0.77	1.23	1.40	0.80	0.91
<i>Catalase (U/mg protein)^b</i>						
N	14	21	13	7	15	6
Mean	145	168	143	328	202	93
Transformed Mean	1291	1266	1269	1357	1216	1244
Transformed SE	20	22	22	30	20	49
<i>Reduced Glutathione (μmol/mg protein)</i>						
N	14	21	13	8	15	6
Mean	11.07	16.56	10.22	17.25	9.59	14.84
Transformed Mean	-	-	-	-	-	-
Transformed SE	-	-	-	-	-	-
<i>Lipid Peroxidation (U/mg protein)^c</i>						
N	14	21	13	8	14	5
Mean	0.16	0.10	0.09	0.08	0.08	0.12
Transformed Mean	-0.24	-0.29	-0.30	-0.30	-0.31	-0.28
Transformed SE	0.01	0.01	0.01	0.01	0.01	0.02

^a one mU is defined as one nmol of NADPH consumption/min

^b one U is defined as one μmol hydrogen peroxide consumption/min

^c one U is defined as one μmol MDA and 4-HAs

(A) Brook Trout



(B) Rainbow Trout

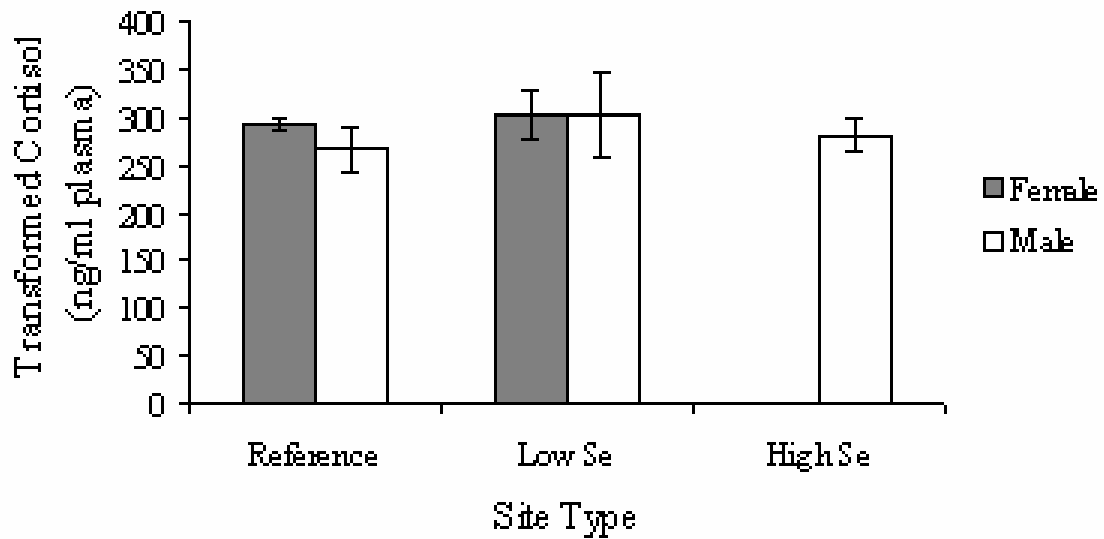
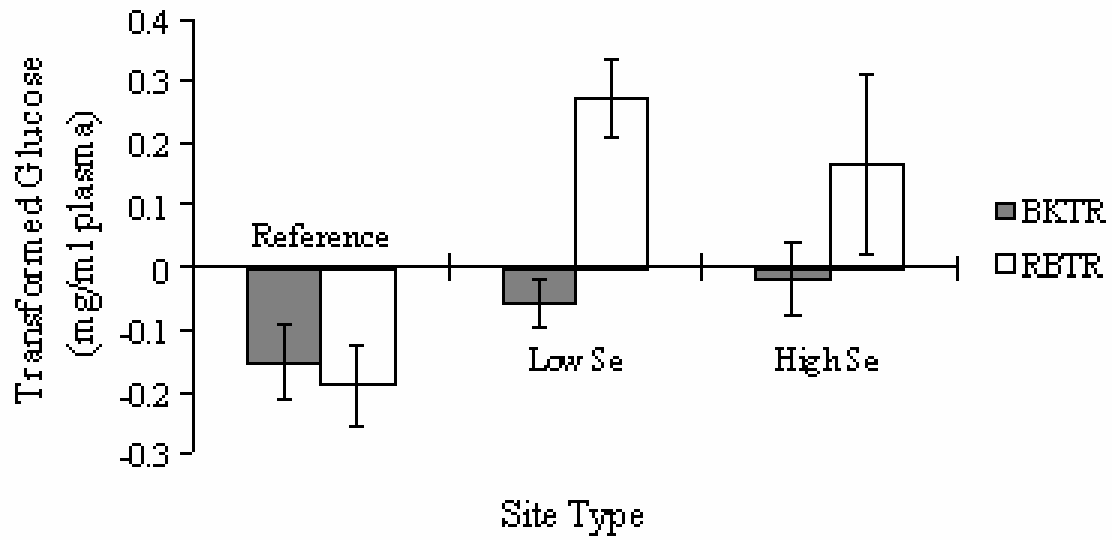


Figure 3.1. Plasma cortisol levels in male and female (A) brook trout, and (B) rainbow trout from reference and Se-contaminated streams near Hinton, Alberta, Canada. Data (mean \pm SE) is transformed. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured.

(A)



(B)

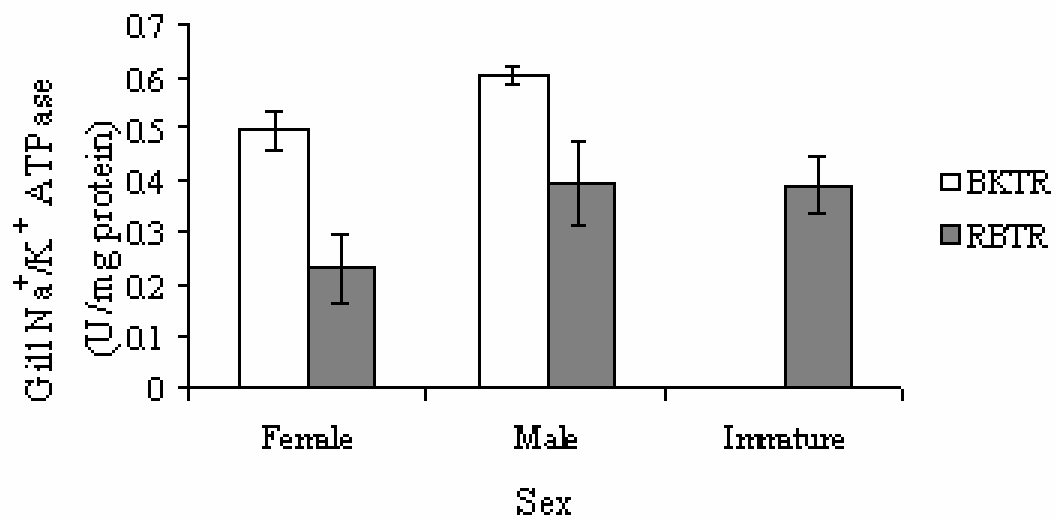


Figure 3.2 (A) Plasma glucose levels (mean \pm SE) and (B) gill Na^+/K^+ -ATPase activities (mean \pm SE) in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured.

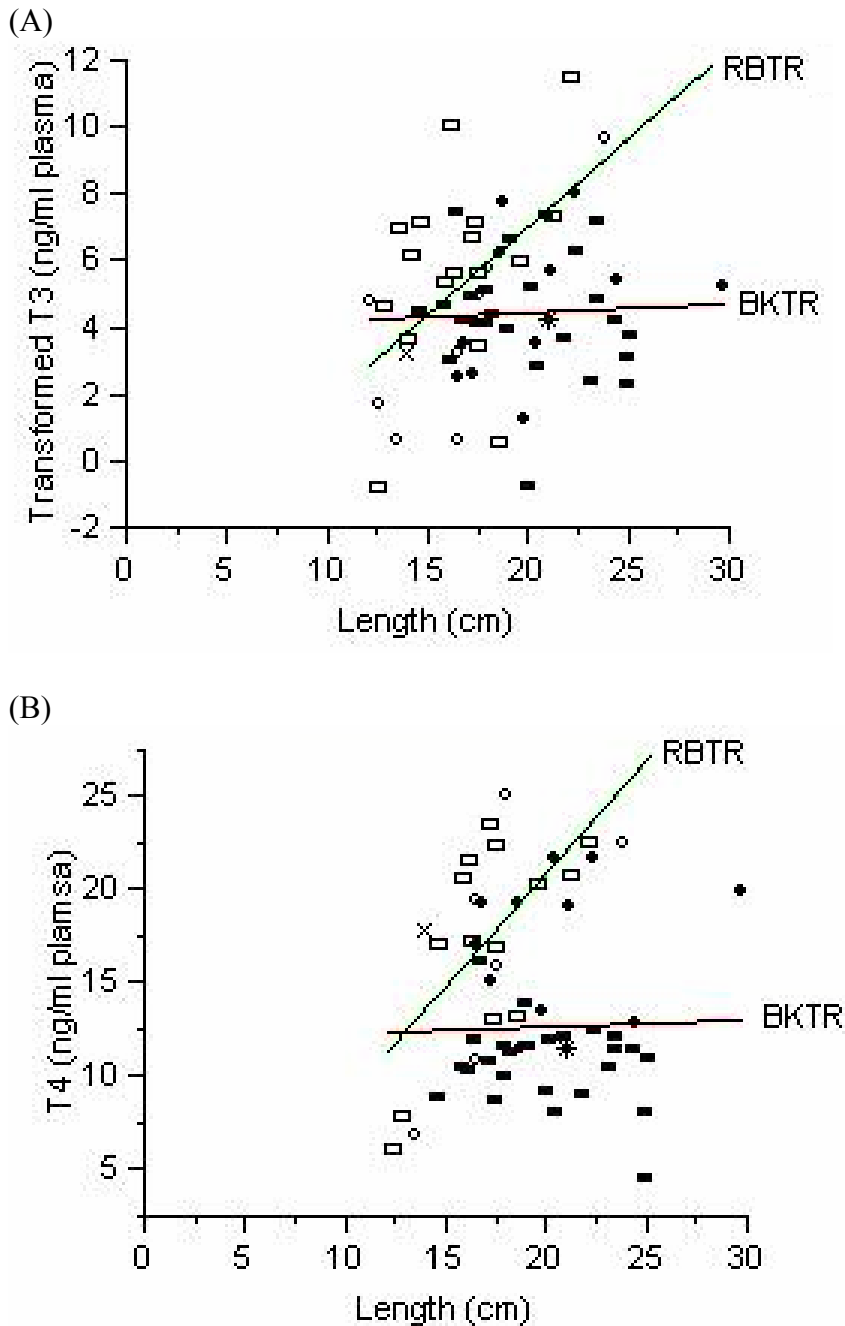


Figure 3.3 The effect of length and sex on plasma T3 (A) and T4 (B) levels in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured. Closed symbols represent BKTR female (●), male (■) and immature (*) fish and open symbols represent RBTR female (○), male (□) and immature (×) fish.

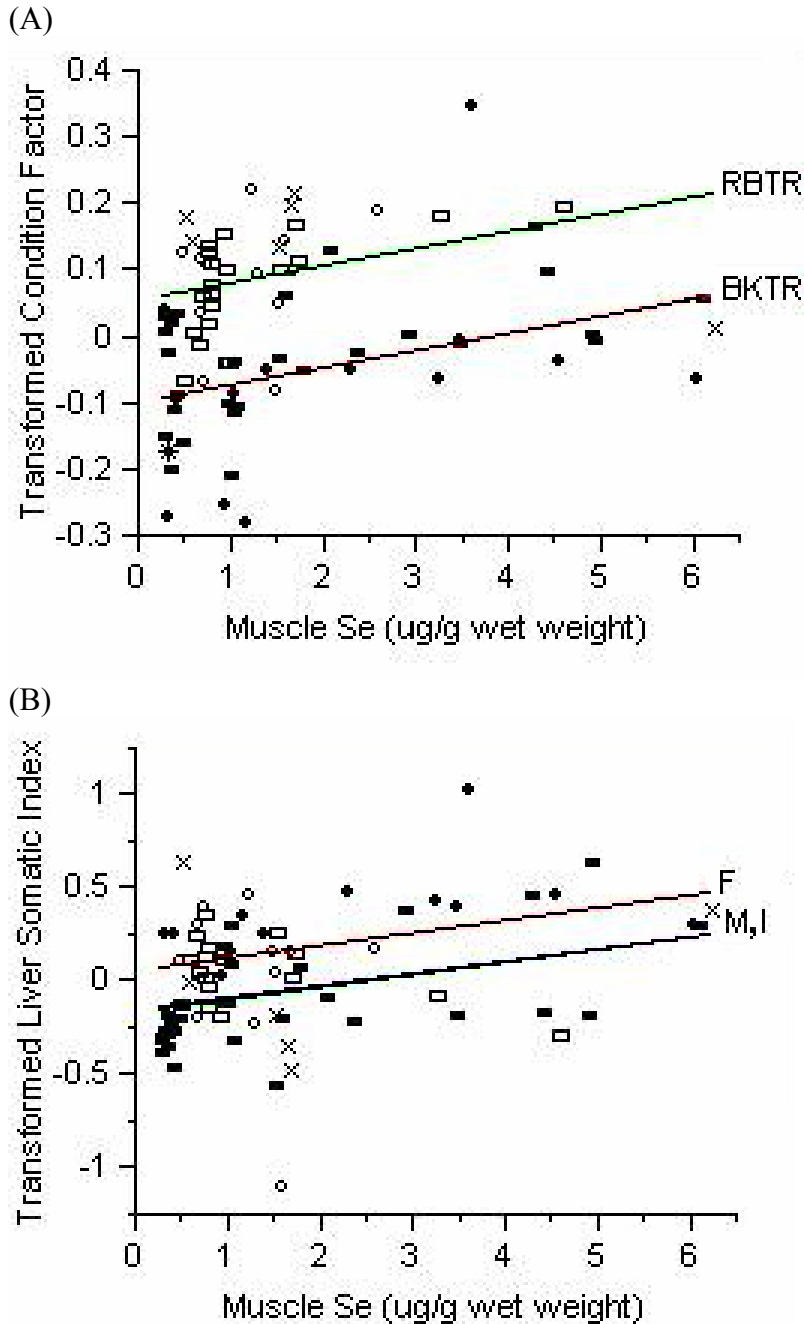
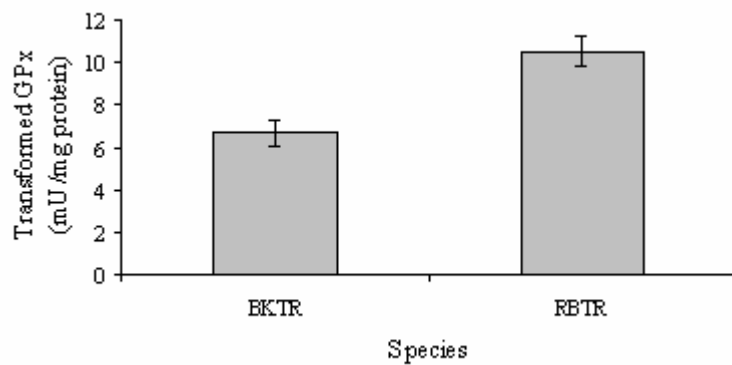
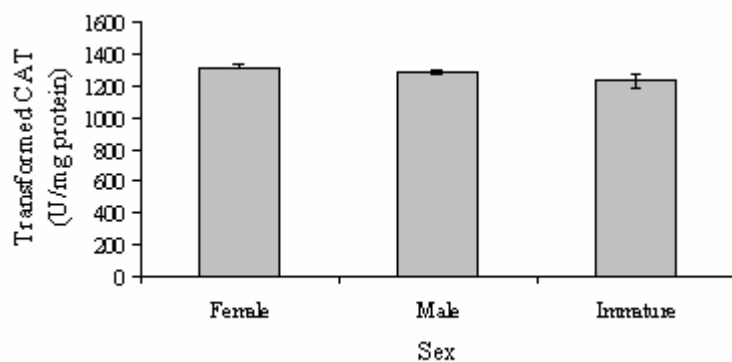


Figure 3.4 The effect of muscle Se levels on condition factor (A) and liver somatic index (B) in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured. Closed symbols represent BKTR female (●), male (■) and immature (*) fish and open symbols represent RBTR female (○), male (□) and immature (×) fish.

(A)



(B)



(C)

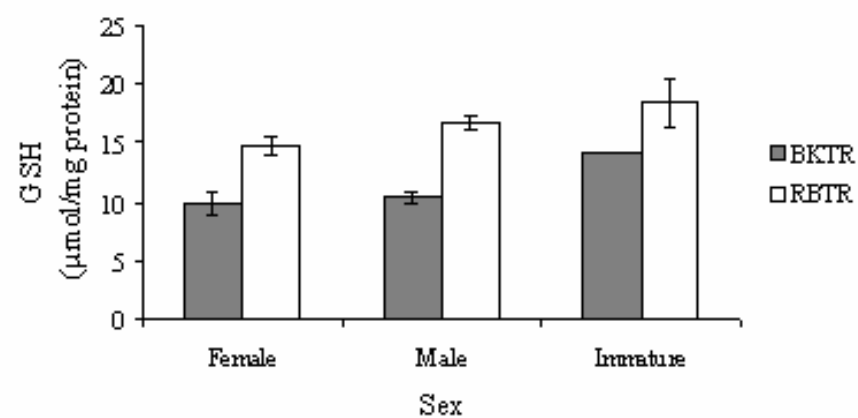


Figure 3.5 Hepatic glutathione peroxidase (GPx) activities (A), catalase activities (B), and reduced glutathione (GSH) levels (C) in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada. Data is mean \pm SE. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured.

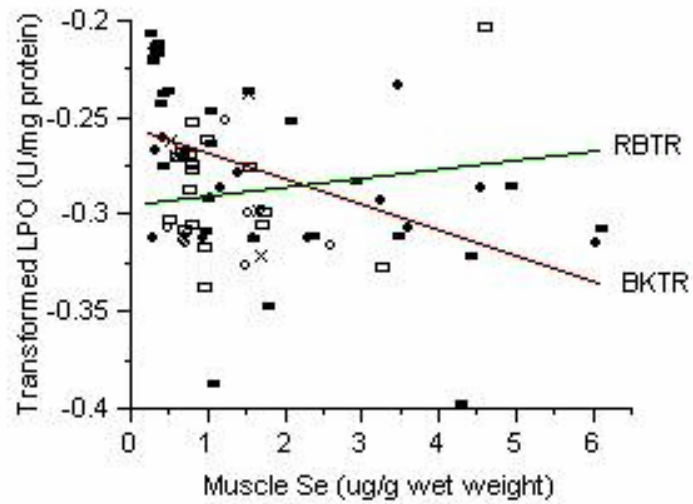


Figure 3.6. The effect of muscle Se levels on lipid peroxidation (LPO) levels in brook trout (BKTR) and rainbow trout (RBTR) from reference and Se-contaminated streams near Hinton, Alberta, Canada. Refer to Table 3.2 for transformations and models describing the significant influences on the parameters measured. Closed symbols represent BKTR female (●), male (■) and immature (*) fish and open symbols represent RBTR female (○), male (□) and immature (×) fish.

3.6. Literature Cited

- Barton B. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42:517-525.
- Beers R, Sizer I. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry* 195:133-140.
- Benfey TJ, Biron M. 2000. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Aquaculture* 184:167-176.
- Berntssen M, Lundebye A, Hamre K. 2000. Tissue lipid peroxidative responses in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. *Fish Physiology and Biochemistry* 23:35-48.
- Bisson M, Hontela A. 2002. Cytotoxic and endocrine-disrupting potential of atrazine, diazinon, endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed *in vitro*. *Toxicology and Applied Pharmacology* 180:110-117.
- Brown S, MacLachy D, Hara T, Eales J. 1991. Effects of cortisol on aspects of 3,5,3'-triiodo-L-thyronine metabolism in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* 8:207-216.
- Campbell PGC, Hontela A, Rasmussen JB, Giguere A, Gravel A, Kraemer L, Kovescs J, Lacroix A, Levesque H, Sherwood G. 2003. Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. *Human And Ecological Risk Assessment* 9(4):847-866.
- Carl LM, Hunt C, Ihssen PE. 1994. Rainbow trout of the Athabasca River, Alberta: a unique population. *Transactions of the American Fisheries Society* 123(2):129-140.
- Carr J, Norris D. 2006. The adrenal gland. In: Norris D, Carr J, editors. *Endocrine disruption: biological bases for health effects in wildlife and humans*. New York: Oxford University Press. p 111-134.
- Cleveland L, Little E, Buckler D, Wiedmeyer R. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquatic Toxicology* 27:265-280.
- de Rosemond S, Liber K, Rosaasen A. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker (*Catostomus commersoni*) from a northern Canadian Lake. *Bulletin of Environmental Contamination and Toxicology* 74:1134-1142.
- Di Giulio R, Behar J, Carlson D, Hasspieler B, Watson D. 1995. Determinants of species susceptibility to oxidative stress: a comparison of channel catfish and brown bullhead. *Marine Environmental Research* 39:175-179.
- Dobbs MC, DS, Cairns J. 1996. Toxicity and bioaccumulation of selenium to a three-trophic food chain. *Environmental Toxicology and Chemistry* 15(3):340-347.
- Dorval J, Hontela A. 2003. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow

- trout (*Oncorhynchus mykiss*). Toxicology and Applied Pharmacology 192:191-200.
- Dorval J, Leblond V, Hontela A. 2003. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. Aquatic Toxicology 63:229-241.
- Freeman H, Sangalang G. 1977. A study of the effects of methyl mercury, cadmium, arsenic, selenium, and PCB, (Aroclor 1254) on adrenal and testicular steroidogeneses *in vitro*, by the gray seal *Halichoerus grypus*. Archives of Environmental Contamination 5:369-383.
- Girard C, Brodeur J, Hontela A. 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. Canadian Journal of Fisheries and Aquatic Sciences 55:438-450.
- Halter M, Adams W, Johnson H. 1980. Selenium toxicity to *Daphnia magna*, *Hyallela azteca*, and the fathead minnow in hard water. Bulletin of Environmental Contamination and Toxicology 24:102-107.
- Hamilton S. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment 326:1-31.
- Hamilton S, Holley K, Buhl K, Bullard F. 2005a. Selenium impacts on razorback sucker, Colorado River, Colorado II. Eggs. Ecotoxicology and Environmental Safety 61:32-43.
- Hamilton S, Holley K, Buhl KB, FA, Weston L, McDonald S. 2005b. Selenium impacts on razorback sucker, Colorado River, Colorado I. Adults. Ecotoxicology and Environmental Safety 61:7-31.
- Hasspieler B, Behar J, Di Giulio R. 1994. Glutathione-dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameriurus nebulosus*). Ecotoxicology and Environmental Safety 28:82-90.
- Haygarth P. 1994. Global importance and global cycling of selenium. In: Frankenberger W, Jr., Benson S, editors. Selenium In The Environment. New York: Marcel Dekker Inc. p 1-28.
- Hilton J, Hodson P. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdner*). Journal of Nutrition 113:1241-1248.
- Hilton J, Hodson P, Slinger S. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). Journal of Nutrition 110:2527-2535.
- Hodson P, Spry D, Blunt B. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Canadian Fisheries Research Board of Canada 37(2):233-240.
- Holm J, Palace V, Siwik P, Sterling G, Evans R, Baron C, Weerner J, Wautier K. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. Environmental Toxicology and Chemistry 24(9):2373-2381.
- Hunn J, Hamilton S, Buckler D. 1987. Toxicity of sodium selenite to rainbow trout fry. Water Research 21(2):233-238.
- Kelly S, Havrilla C, Brady TA, KH, Levin E. 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environmental Health Perspectives 106(7):375-384.

- Kennedy C, McDonald L, Loveridge R, Strosher M. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarki lewisi*). Archives of Environmental Contamination and Toxicology 39:46-52.
- Köhrle J, Jakob F, Contempré B, Dumont J. 2005. Selenium, the thyroid, and the endocrine system. Endocrine Reviews 26(7):944-984.
- Kubokawa K, Watanabe T, Yoshioka Y, Iwata M. 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172:335-349.
- Laflamme J-S, Couillard Y, Campbell P, Hontela A. 2000. Interrenal metallothioneine and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. Canadian Journal of Fisheries and Aquatic Sciences 57:1692-1700.
- Lemly A. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety 37:259-266.
- Lemly A. 1999. Selenium impacts on fish: an insidious time bomb. Human and Ecological Risk Assessment 5(6):1139-1151.
- Lemly A. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57:39-49.
- Lohner T, Reash R, Willet V, Rose L. 2001a. Assessment of tolerant sunfish populations (*Lipomis* sp.) inhabiting selenium-laden coal ash effluents 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety 50:203-216.
- Lorentzen M, Maage A, Julshamn K. 1994. Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (*Salmo salar*). Aquaculture 121:359-367.
- Morgan I, Henry R, Wood C. 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na⁺ and Cl⁻ transport. Aquatic Toxicology 38:145-163.
- Nankervis L, Southgate PC. 2006. The influence of dietary protein and energy levels on growth, survival and thyroid hormone (T3 and T4) composition of Lates calcarifer larvae. Aquaculture Nutrition 12(3):219-226.
- Nelson J, Paetz M. 1992. The fishes of Alberta. Edmonton: The University of Alberta Press.
- Orun I, Ates B, Selamoglu Z, Yazlak H, Ozturk E, Yilmaz I. 2005. Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). Fresenius Environmental Bulletin 14(1):18-22.
- Palace V, Baron C, Evans R, Holm J, Kollar S, Wautier K, Werner J, Siwik P, Sterling G, Johnson C. 2004a. An assessment of the potential for selenium to impair reproduction in bull trout, *Salvelinus confluentus*, from an area of active coal mining. Environmental Biology of Fishes 70(2):169-174.
- Palace V, Spallholz J, Holm J, Wautier K, Evans R, Baron C. 2004b. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety 58:17-21.

- Potmis R, Nonavinakere V, Rasekh H, Early J. 1993. Effect of selenium (Se) on plasma ACTH, β -endorphin, corticosterone and glucose in rat: influence of adrenal enucleation and metyrapone pretreatment. *Toxicology* 79:1-9.
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher J-M, Aït-Aïssa S. 2005. Copper-induced oxidative stress in three-spine stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology* 19:177-183.
- Shrimpton J, McCormick S. 1999. Responsiveness of gill Na^+/K^+ -ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *The Journal of Experimental Biology* 202:987-995.
- Spallholz J, Palace V, Reid T. 2004. Methioninase and selenomethionine but not Se-methylselenocystiene generate methylselenol and superoxide in an *in vitro* chemiluminescent assay: Implications for the nutritional carcinostatic activity of selenoamino acids. *Biochemical Pharmacology* 67:547-554.
- Teh S, Deng X, Deng D-F, Teh F-C, Hung S, Fan T-M, Liu J, Higashi R. 2004. Chronic effects of dietary selenium on juvenile sacramento splittail (*Pogonichthys macrolepidotus*). *Environmental Science Technology* 38:6085-6093.
- Wayland M, Crosley R. 2006. Selenium and other trace elements in aquatic insects in coal mine-affected streams in the Rocky Mountains of Alberta, Canada. *Archives of Environmental Contamination and Toxicology* 50:511-522.
- Wayland M, Gilchrist H, Marchant T, Keating J, Smits J. 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environmental Research Section A* 90:47-60.
- Zhang J, Liu J, Sun Y, Wang X, Wu J, Xue Y. 2005. Responses of the antioxidant defenses of the goldfish *Carassius auratus*, exposed to 2,4-dichlorophenol. *Environmental Toxicology and Pharmacology* 19:185-190.

CHAPTER 4. SEASONAL VARIATION IN THE PHYSIOLOGICAL STRESS RESPONSE OF FISH EXPOSED TO AGRICULTURAL DRAIN WATER IN SOUTHERN ALBERTA: THE INFLUENCE OF PESTICIDES AND SELENIUM.

4.1. Introduction

Agriculture (grain and animal production) is one of the primary industries in southern Alberta, a semi-arid region where irrigation is common. Irrigation return flows and other agricultural drain water may contain multiple contaminants including pesticides (Anderson 2005), selenium (Ohlendorf and Santolo 1994), veterinary pharmaceuticals (Forrest et al. 2006), salts (Schlenk et al. 2003), particulates (Velasco et al. 2006), and nutrients (Velasco et al. 2006). These chemicals have the potential to adversely impact fish species in the irrigation canals and also in the rivers receiving return flows. The goal of this field study is to assess the physiological status of fish in irrigation canals and to quantify the specific effects of selenium and pesticide exposure.

Exposure to pesticides, specifically carbamate and organophosphate insecticides, inhibits the activity of acetylcholinesterase (AChE), the enzyme that metabolizes the neurotransmitter acetylcholine. Thus, decreased AChE activity can be used as a biomarker of pesticide exposure in fish and other non-target species (Sturm et al. 1999). Acetylcholinesterase inhibition results in excessive stimulation of the nervous system and this can cause many toxicity symptoms, including mortality (Sibley et al. 2000). Pesticide exposures have also been associated with malformations, liver disease, and ulcerations (Austin 1999).

Selenium, an essential element, bioaccumulates and can be toxic at concentrations only slightly greater than needed to maintain homeostasis (Hilton et al. 1980). The major toxicity symptoms in fish and aquatic birds are teratogenic deformities (Lemly 2002; Spallholz and Hoffman 2002). At the Kesterson reservoir, a wetland that receives agricultural drain water from the San Joaquin Valley, CA, USA, many aquatic birds and native fish populations species were negatively impacted by Se-induced teratogenesis (Ohlendorf and Santolo 1994). The effect of Se on the reproductive ability of fish has been well studied; however, the effects of Se on other systems such as the physiological stress response (PSR) have not been investigated.

The PSR mobilizes energy reserves to maintain homeostasis during exposure to a stressor. When a fish perceives a stimulus (such as a predator, contaminant, or temperature change) as a stressor, cortisol and catecholamines are released (a primary stress response) and their concentrations in plasma increase. Cortisol is synthesised by the interrenal cells of the head kidney and is the major stress hormone in fish (Hontela 1998). Elevated cortisol levels lead to secondary responses such as metallothioneine induction (Hyliner et al. 1989), protein degradation (Freeman and Idler 1973), increased plasma glucose, and increased Na^+/K^+ ATPase activity (Shrimpton and McCormick 1999). Cortisol can also suppress the immune system, gonadal maturation, and sex steroid secretion (Carragher and Sumpter 1990; Hontela 1997).

Contaminants can activate, exhaust, or impair the PSR (Hontela 2005), depending on the length of exposure and concentration of the contaminant. Recently it was reported that acute and sub-chronic laboratory exposures to Se activate the PSR in rainbow trout, but Se does not exhaust or impair it (Chapter 2). Chronic exposure to pesticides can

impair the PSR (Dorval et al. 2003), potentially interfering with the ability of the fish to appropriately allocate energy reserves to handle additional stressors. Chronically elevated cortisol, due to continuous exposure to single or multiple stressors, may also have important physiological consequences. Chronically stressed fish can have decreased growth, decreased reproductive success, and increased susceptibility to disease (Hontela 1997). They may also have decreased appetite and food conversion efficiency (Gregory and Wood 1999). It has been proposed that in chronically stressed fish, energy has been reallocated from somatic and gonadal growth to mechanisms enabling the fish to handle the stressors, such as the ability to escape (Gregory and Wood 1999).

There is evidence that fish from contaminated and reference sites may have different seasonal energy reserve patterns. Yellow perch from metal contaminated lakes exhibited little or no seasonal cycling in glycogen reserves, while perch from reference lakes seasonally increased their glycogen (Levesque et al. 2002). Winter stress syndrome is an extension of this phenomenon where chemical stressors, such as selenium (Lemly 1993a) and the natural reduction in feeding during the winter result in mortality due to depleted energy reserves (Lemly 1996). Together these fluctuations in biotic and chemical stressors may significantly alter energy metabolism of fish exposed to agricultural drain water.

Most ecotoxicological studies focused on large fish species, even though forage fish (e.g., dace (*Rhinichthys*), shiners (*Notropis*), and fathead minnows (*Pimephales*)) may also be useful bioindicators of contaminant exposure and effects. They are common throughout North America (Nelson and Paetz 1992) and are found in irrigation return flows. Fathead minnows are often used in laboratory toxicology studies (Dobbs and

Cairns 1996) and reference data are available, facilitating laboratory and field comparisons. Using forage fish as bioindicators or sentinel species is an emerging research area, but more data are needed on the basic physiology and ecology of such fish. More specifically, values for normal and exposed fish are needed for bioindicators such as AchE activity (marker of pesticide exposure), metallothionein (metal exposure), lipid peroxidation (oxidative damage), and plasma cortisol (PSR).

The objectives of this study were to determine (1) if Se accumulates in fish exposed to agricultural runoff in southern Alberta, (2) if agricultural drain water alters the PSR, (3) what components of agricultural drain water alter the PSR, and (4) if the responses of forage fish can be used as indicators of pesticide contamination and PSR status. The following hypotheses were tested (1) fish exposed to agricultural drain water accumulate Se, and (2) the contaminants in agricultural drain water, specifically Se and pesticides, activate the PSR.

4.2. Methods

4.2.1. Chemicals

Acetylthiocholine iodide, MS-222, KH_2PO_4 , Na_2HPO_4 , OMPA, DNTB, Tris-buffer, Bradford reagent, KOH, glacial acetic acid, sodium acetate trihydrate, and amyloglucosidase were purchased from Sigma-Aldrich. The GOD-PAP reagent was purchased from Roche Diagnostic and the cortisol radioimmuno assay (RIA) kit (catalogue #07-221102) was purchased from Mediacorp. Ultra pure nitric acid was purchased from Fisher Scientific.

4.2.2. Study Sites

Fish were sampled from four different drains (canals receiving agricultural runoff from an irrigation canal network and returning that water to the river) in the Lethbridge Northern Irrigation District (LNID) and the Little Bow River in Alberta, Canada (Figure 4.1). The LNID drainage canals each receive drain water from a large irrigated agricultural area with intensive grain and animal production, and they are only filled between April and October. The Little Bow River receives drain water from the dry land agricultural areas and flows all year. Fish were sampled in the summer (late June to early August) and the fall (mid September to mid October) at all sites. Water samples were collected from each site in the summer and the fall. They were acidified with 0.05% ultra pure nitric acid and analysed for total Se at the Ultra-Clean Trace Elements Laboratory, University of Manitoba. Water and air temperatures were recorded at each site (Table 4.1).

4.2.3. Fish Sampling

Confinement-Stressed Fish. White suckers, *Catostomus commersoni*, (8.4 to 28.0 cm) were captured with a Smith Root LR-24 electroshocker in the morning (0900 to 1200 hours) and kept in stream enclosures until sampling (1300 – 1400 hours). Suckers were anaesthetised with MS-222 and blood samples were taken from the caudal blood vessels. Fish were then sacrificed by spinal transection and plasma for AchE, cortisol, and glucose analyses was flash frozen in liquid nitrogen. Fork length and weight were recorded and condition factor (K) and liver somatic index (LSI) were calculated. Livers for glycogen analysis and gills for Na^+/K^+ -ATPase activity were removed and flash

frozen in liquid nitrogen. Muscle samples for Se analysis were taken from the left side of the fish under the dorsal fin. Muscle samples were sent to the Department of Fisheries and Oceans' Freshwater Institute, Winnipeg, Manitoba for total Se analysis (ICP-MS). Water and air temperatures were taken during electrofishing.

Basal plasma cortisol and glucose. To determine basal plasma parameters in white suckers sampled from agricultural drain water, fish (7.5 to 38.7 cm) were immediately anaesthetized in MS-222 while they were still stunned from the electroshocker and blood was taken from the caudal blood vessels. Fish were released after they recovered from the anaesthetic. Plasma was flash frozen in liquid nitrogen for cortisol and glucose analysis.

Forage Fish. Emerald shiners (*Notropis atherinoides*), longnose dace (*Rhinichthys cataractae*), fathead minnows (*Pimephales promelas*), and juvenile white suckers (1.6 to 6.4 cm), captured and handled as the confinement-stressed white suckers, were anaesthetized with MS-222 and immediately frozen in liquid nitrogen. The head and trunk (posterior to operculum including the caudal peduncle) were homogenized separately in a phosphate buffer (1.2 mM KH_2PO_4 and 8.9 mM Na_2HPO_4) at a 1:3 ratio of tissue:buffer. Samples were centrifuged three times at 9000 x g for 10 minutes at 5°C. Acetylcholinesterase (AChE) activity and protein were measured in the head supernatant. Cortisol, glucose, and protein were measured in the trunk supernatant.

4.2.4. Biochemical Parameters

Acetylcholinesterase was measured with a kinetic spectrophotometric assay (Ellman et al. 1961) in a 96-well microplate. The final reaction solution (142 μ L) contained 5 mM OMPA, 0.07mM DNTB, and 4.2 MM acetylthiocholine iodide in Tris Buffer and 2 μ L of sample. The change in absorbance was measured at 405 nm for 10 minutes.

Activity expressed as μ mol DNTB degradation per minute per mL plasma or mg protein.

Cortisol was measured with a RIA kit. Glucose was determined in a spectrophotometric assay (510 nm) using the GOD-PAP reagent.

Na^+/K^+ ATPase activity in a gill homogenate was measured by liberating PO_4 from a hydrolysis reaction with ATPase in a gill homogenate (Morgan et al. 1997) as previously described (Chapter 2). Protein was measured using a spectrophotometric assay (595 nm) and the Bradford reagent.

Liver glycogen was measured by digesting glycogen with amyloglucosidase and determining the resulting glucose concentration (Bleau et al. 1996). Livers were initially digested with KOH (1N) and acetic acid (1.5 N). Samples were centrifuged at 16,000 x g for 10 minutes and the glycogen in the supernatant was digested in a 1 N acetate buffer with amyloglucosidase (30 min at 37 °C). Glycogen levels were measured with an endpoint spectrophotometric assay using the GOD-PAP reagent (510 nm). Results were expressed as mg glycogen per g wet liver weight.

4.2.5. Statistical Analysis

Data was analyzed using JMP IN 5.1.2. (1989-2002 SAS Institute Inc.) and all tests used $\alpha = 0.05$. To determine different exposure characteristics, site and season were

compared with a 2-way ANOVA and *post hoc* Tukey Kramer HSD test (normal data) or a Kruskal-Wallis test followed by multiple Wilcoxon tests with the Bonferroni correction (non parametric data).

To determine the effects of multiple stressors on the PSR of white suckers (stressed and basal) an analysis of covariance was used. Muscle Se levels (indicator of Se exposure), plasma AchE activities (indicator of pesticide exposure), site, season, and length were considered as covariates. The PSR parameters (plasma cortisol, plasma glucose, gill Na^+/K^+ ATPase activity, liver glycogen, condition factor, and liver somatic index) were transformed with the Box-Cox transformation to meet the assumption of normally distributed residuals.

Data from the forage fish (head AchE activity, trunk cortisol, and trunk glucose levels) were analyzed with a Kruskal-Wallis test.

4.3. Results

4.3.1. Site and exposure characteristics

Fish were sampled from 5 different sites in the irrigation canals and the Little Bow River of southern Alberta (Figure 4.1) during the summer and the fall. During electrofishing, water temperatures in the summer (Table 4.1) ranged from 17.0 to 24.5 °C and air temperatures from 21.0 to 28.5 °C. In the fall, water temperatures ranged from 8.5 to 10.5 °C and air temperatures ranged from 12.5 to 17.5 °C.

Water Se levels were the highest at the Monarch site in both the summer and the fall, although muscle Se levels in stressed white suckers were significantly ($p < 0.05$) elevated at both the Monarch and BASF sites during both seasons (Figure 4.2). Muscle

Se levels were significantly ($p < 0.05$) lower in the fall than the summer at Battersea and BASF (Figure 4.2). Muscle water content of the stressed suckers was $78.4 \pm 0.23\%$. Selenium concentrations were not measured in the basal suckers (sampled without confinement stress) or the forage fish.

Pesticide exposure was estimated with AchE activity (Sturm et al. 1999). Plasma AchE activity of stressed white suckers was significantly lower in the fall than the summer ($p < 0.05$) at all sites (Figure 4.3A). In the summer, fish from the Little Bow River had lower ($p < 0.05$) plasma AchE activity than fish from Monarch, BASF, and Battersea (Figure 4.3A). There was a significant ($p < 0.05$) negative relationship between the fork length of stressed white sucker and plasma AchE activity (Figure 4.4). This relationship was not evident in the basal suckers (sampled without confinement stress).

Basal white suckers in the fall had lower ($p < 0.05$) plasma AchE activity than in the summer at all sites except BASF (Figure 4.3B); however, plasma AchE levels were similar between sites both in the summer and the fall (Figure 4.3B). Monarch and Pyami are not included in the comparison of basal white suckers due to low sample size ($n = 1-3$) in the fall.

4.3.2. Physiological Stress Response (PSR)

Models including the covariates that accounted for a significant ($p < 0.05$) amount of variation in each PSR parameter for stressed and basal white suckers are given in Table 4.2. The sample sizes, means, and transformed values are given in Tables 4.3 (stressed white suckers) and 4.4 (basal white suckers). Plasma cortisol levels in confinement-stressed white suckers were not influenced by muscle Se levels or plasma

AchE activity, but they were significantly influenced ($p < 0.05$) by site and season (Table 4.2). Plasma cortisol levels were higher in fall than in summer (Figure 4.5A). Gill Na^+/K^+ ATPase activities of stressed white suckers were significantly influenced only by site and season (Table 4.2) and lower levels were observed in fall (Figure 4.5B). Condition factor was only influenced by season, not site, muscle Se levels or plasma AchE activities (Table 4.2). The condition of stressed white suckers was lower in the fall than in summer (Figure 4.5C).

Plasma glucose levels were significantly influenced by muscle Se levels, site and season, but not plasma AchE activity (Table 4.2). Plasma glucose levels increased with increasing muscle Se levels at all sites, both in the summer and the fall (Figure 4.6A). Liver glycogen levels were significantly influenced by plasma AchE activities, site and season, but not muscle Se levels (Table 4.2). In the summer, fish with higher plasma AchE activities had lower liver glycogen levels, but in the fall the glycogen levels were higher and no such relationship was evident (Figure 4.6B). The LSI was significantly affected by length, season and site, but not muscle Se levels or plasma AchE activities (Table 4.2). Larger stressed white suckers had a smaller LSI (Figure 4.6C). Site and season were the only factors that influenced plasma cortisol and plasma glucose levels of basal suckers (Table 4.2), but no seasonal patterns were evident (Figure 4.7).

4.3.3. Forage Fish

Emerald shiners (*Notropis atherinoides*), fathead minnows (*Pimephales promelas*), longnose dace (*Rhinichthys cataractae*), and small white suckers were caught in the LNID drainage canals and the Little Bow River. However, there were not enough

fish of each species caught to allow site and season comparisons. Head AchE activities, trunk cortisol levels, and plasma glucose levels were measured in all forage fish species and significant ($p < 0.05$) species differences were evident (Table 4.5). Head AchE levels were ranked as follows: white suckers > emerald shiners > fathead minnows = longnose dace. Trunk cortisol levels were ranked as: fathead minnow = white sucker > longnose dace = emerald shiners. There were no significant differences in trunk glucose levels between species (Table 4.5).

4.4. Discussion

The objectives of this study were to determine: (1) if Se accumulates in fish exposed to agricultural runoff in southern Alberta, (2) if agricultural drain water alters the PSR, (3) what components of agricultural drain water alter the PSR, and (4) if forage fish can be used as indicators of pesticide contamination and PSR status. Multiple stressors were present in the drain water from the LNID drainage canals and the Little Bow River and they could impact fish. Water Se concentrations and white sucker muscle Se levels were elevated at two sites (Monarch and BASF), but plasma AchE activity, an indicator of pesticide exposure, did not vary between sites. Plasma AchE activities were lower in the fall than the summer in white suckers sampled with or without confinement stress.

Exposure to agricultural drain water that may contain many different stressors including pesticides, Se, veterinary pharmaceuticals, nutrients, and particulates, affected PSR parameters (plasma cortisol, plasma glucose, gill Na^+/K^+ ATPase, liver glycogen, condition factor and liver somatic index), but site and season were the main factors that influenced them. Our models suggested that muscle selenium levels influenced plasma

glucose levels, while plasma AchE levels were correlated with liver glycogen levels. There was also a relationship between length and liver somatic index in stressed white suckers. Forage fish were investigated as indicators of pesticide contamination and PSR status in this study. Acetylcholinesterase activities, cortisol and glucose levels were detected; however, significant species differences exist in head AchE activities and trunk cortisol levels, thus fish from different sites could not be compared or pooled.

4.4.1. Site Exposure and Characteristics

The highest Se muscle concentrations (1.52 ± 0.12 µg/g wet weight) were detected in white suckers from the BASF drain and the Monarch drain (1.23 ± 0.08 µg/g wet weight). Increased deformities have been documented in adult white suckers with 42 µg/g dry weight whole body Se (Lemly 1993b) and in embryos with 1.8 µg/g wet weight Se levels (de Rosemond et al. 2005). Assuming that the embryos have similar Se levels as the adult fish from the BASF drain, deformities may be occurring at these two sites. However, deformities were not observed, possibly because white suckers spawn in the spring (Nelson and Paetz 1992) and they were only exposed to elevated Se as they moved into the affected drains from the Oldman River when the canals filled in the spring. These fish may not be exposed to Se long enough for Se to accumulate in the eggs and cause teratogenic deformities. The main documented effect of Se levels between 1.3 and 1.5 µg/g wet weight is increased Se accumulation (Hamilton et al. 2005; Allert et al. 2006). Thus, Se is probably not an important chemical stressor in agricultural drain water in the LNID drainage canals and the Little Bow River, although it is beginning to accumulate in white suckers from some sites.

Plasma AchE activity is a common indicator of exposure to carbamate and organophosphate pesticides; lower activities corresponding to greater pesticide exposures (Whitehead et al. 2005). In both the stressed and basal white suckers, plasma AchE activities did not vary between sites, but the activities were significantly lower in suckers sampled in the fall than the summer. This suggests fish are exposed to greater concentrations of pesticides in the fall or the cumulative exposure to low levels of pesticides throughout the summer progressively lowers AchE activities. However, other factors, such as water temperature and body size, may influence AchE activities.

Cholinesterase activities in goldfish decrease with decreasing temperature (Hazel 1969), but other studies did not observe the same effect of temperature (Beauvais et al. 2002; Phillips et al. 2002). Generally, temperature differences within 10°C do not appear to significantly influence cholinesterase activities in fish (Beauvais et al. 2002). The difference between summer and fall water temperatures was 9 to 15°C; thus the decrease in AchE activity in the fall may be partially due to lower water temperature. Future studies need to determine how temperature influences AchE activities in fish generally, and more specifically how temperature affects white sucker plasma AchE activities.

Size may also influence AchE levels. Significant negative relationships between length or weight and AchE activities in stickleback (Sturm et al. 1999) and bluegill (Beauvais et al. 2002) have been documented. A similar relationship was observed in the stressed white suckers from the LNID drainage canals and the Little Bow River.

To determine if the AchE activities measured in the white suckers are normal or inhibited is difficult as white suckers are not commonly monitored for AchE activity in the field or used in laboratory exposures. Additionally, plasma AchE levels (a non-lethal

sample) are not commonly used in fish, instead brain and muscle activities are used. There is only one study that investigated the effect of agricultural chemicals on plasma AchE activities in white suckers (Dorval et al. 2005) and this study used a different method to measure AchE, making comparisons difficult. Nonetheless, sites may be compared to determine relative exposures; however, variation in water temperature and fish size needs to be considered. Other variables that may have contributed to the lower AchE activities in the fall are photoperiod and reproductive cycles; however, the effects of temperature, photoperiod and reproductive status on AchE activities in white suckers and fish in general have not been characterized. Such data are needed to validate the use of AchE activities in monitoring pesticide contamination.

The effects of multiple stressors on aquatic organisms are becoming more common as streams are impacted by many diverse processes (Adams 2001). At the site level, white suckers were exposed to both Se and pesticides. Other relevant stressors include veterinary pharmaceuticals (Forrest et al. 2006), pesticides other than carbamates and organophosphates, flow changes (Flodmark et al. 2002), turbidity (Humborstad et al. 2006) and temperature (Roche and Bogé 1996). To determine the effect of multiple stressors on fish, it is important to study a physiological system that is influenced by all of the stressors affecting the fish. The PSR is such a system as it integrates the effect of all the stressors into one response.

4.4.2. Physiological Stress Response

The PSR is a series of responses, including mobilization of energy reserves, that allow the fish to adjust to the new environmental or internal conditions. The primary

stress response was assessed by measuring plasma cortisol levels. In stressed white suckers cortisol levels were influenced only by site and season. Generally, confinement-stressed white suckers had higher plasma cortisol levels in the fall suggesting they are more stressed in the fall than the summer. Plasma cortisol levels from white suckers without confinement stress were much lower than the levels from confinement-stressed fish, suggesting they represent true basal levels. They were only influenced by site and season, although no obvious patterns were observed.

Season effects may be mediated by water temperatures as they were lower in the fall (8-11°C) than the summer (17-25°C). The white suckers thermal preference is approximately 27°C, although lower temperature preferences (14.1 – 21.2°C) have also been reported (Cincotta and Stauffer 1984). The PSR of white suckers may be altered by season because they are experiencing temperatures outside of their thermal preference in the fall. Alternatively, the season effect may come from differences in photoperiod and annual cortisol cycles, flow changes from irrigation activities (Flodmark et al. 2002) and contaminant exposure duration. Site differences may be due to additional chemical stressors, such as veterinary pharmaceuticals, that were not quantified. Site differences in turbidity and flow may have also contributed.

Plasma glucose levels, a secondary response, were significantly influenced by muscle Se levels, site, and season in confinement-stressed white suckers. Plasma glucose levels increased with increasing muscle Se levels in the summer and the fall. This response was slightly greater in the fall than the summer. This suggests white suckers exposed to increased Se may mobilize more glucose to meet the higher energy demands associated with a chemical stressor. White suckers exposed to bleached kraft mill

effluent have a similar response. Fish from exposed sites had elevated plasma glucose levels (McMaster et al. 1994; Jardine et al. 1996) suggesting they mobilize the extra energy to cope with the chemical stressor. Basal levels of plasma glucose were lower than the levels in confinement-stressed white suckers; however, they were influenced only by site and season, not muscle Se levels. This suggests that muscle Se levels may affect how the fish respond to confinement stress (mobilize more energy reserves), but do not act as a stressor.

Gill Na^+/K^+ ATPase activity, a secondary response, was influenced by site and season. At some sites the activity was lower in the fall than the summer (Pyami, Little Bow, and BASF), but at Monarch it was higher in the fall than the summer. Fish may express different variants and levels of gill Na^+/K^+ ATPase in the summer and the winter (Packer and Garvin 1998). However, in the LNID drainage canals and the Little Bow River, season effects appear to be influenced by site differences as a consistent seasonal pattern is not observed across all sites. Chemical stressors can also influence gill Na^+/K^+ ATPase activity. For example, exposure to silver (Morgan et al. 1997) and copper (Gagnon et al. 2006) decreased gill Na^+/K^+ ATPase activity in rainbow trout. There may be an additional contaminants present at Little Bow, Pyami and BASF (the sites with lower gill Na^+/K^+ ATPase activity in the fall) that cumulatively decreases ATPase activity over the summer. More research is needed to determine what component of the agricultural drain water is influencing gill Na^+/K^+ ATPase activity in stressed white suckers as muscle Se levels and plasma AchE activity did not significantly explain the variation in gill Na^+/K^+ ATPase activity observed.

Liver glycogen levels, another secondary response, were influenced by AchE activity as well as site and season. In the summer, stressed white suckers with higher AchE levels (lower pesticide exposures) had lower liver glycogen levels, but in the fall this relationship disappears. This is unexpected because contaminant exposure generally decreases glycogen reserves (Bleau et al. 1996), as the fish metabolize the glycogen to meet increased energy demands during exposure to a chemical stressor. However, if the pesticides interrupt the pathway leading to glycogen degradation, exposed fish may have greater glycogen reserves than unexposed fish, as has been previously reported for yellow perch (Hontela et al. 1995). This difference may be exaggerated by additional stressors that stimulate glycogen degradation in the functional fish. In the fall, fish normally increase their glycogen reserves in preparation for winter (Lemly 1996), masking the effect (inhibited glycogen degradation) of pesticide exposure on glycogen reserves. It has not been established if carbamate and organophosphate pesticides can interrupt glycogen degradation in white suckers and how this influences their response to additional environmental stressors.

Condition factor, a tertiary response, was only significantly influenced by season. Condition factor of stressed white suckers was lower in the fall, suggesting that there is an energetic cost associated with living in agricultural drain water for an extended period of time. Fish exposed to organic contaminants and heavy metals experienced a similar energetic cost and had lower condition factors than the reference fish (Hontela et al. 1995). Condition factor integrates the energetic cost of all the different stressors in the agricultural drain water over the exposure period and is not attributed specifically to one contaminant or site. Alternatively, the lower condition in the fall may reflect a natural

seasonal cycle in white suckers. Cod also spawn in the spring and have maximum condition indices in the fall (Mello and Rose 2005), thus the lowered condition factor observed in white suckers probably reflects exposure duration not seasonal cycling; however, the effect of season on condition factor in white suckers is unknown. The energetic costs of the agricultural drain water to white suckers needs to be quantified to determine if exposure duration drives the seasonal change observed or if it is a natural cycle.

The LSI, another tertiary response, was influenced by season, site and length. In the summer, stressed white suckers had similar LSI independent of length; however, in the fall, larger fish had a lower LSI than the smaller fish. A similar result was observed in mummichogs. Large mummichogs had heavier livers than the smaller fish suggesting the larger fish may have allocated more energy to somatic growth than energy storage (Leamon et al. 2000). This difference in resource partitioning may be occurring in stressed white suckers as they prepare for the winter season.

Generally, site and season appear to have the largest influence on the PSR of confinement-stressed white suckers; however, Se and pesticide exposure do influence some aspects of the PSR. If the additional stressors (e.g. veterinary pharmaceuticals) were quantified and included in the model, they may have influenced specific aspects of the PSR as well. The PSR is a useful model system for evaluating the effects of multiple stressors because adverse consequences can be identified without explicitly quantifying all of the stressors within a system. More research is needed to identify normal levels of PSR parameters in healthy white suckers and other sentinel species.

4.4.3. Forage Fish

Four different species of forage fish (emerald shiners, fathead minnows, longnose dace and juvenile white suckers) were sampled in the LNID drainage canals and the Little Bow River, but there were not enough fish of each species to allow site and season comparisons. Head AchE activities were detectable in all four species, thus forage fish may be used as biomarkers of pesticide exposure in field studies. However, species may not be pooled due to species differences in head AchE activities. Fathead minnows are commonly used in laboratory toxicology studies and normal brain AchE levels have been characterized. Brain AchE activities of unexposed fish were 75 $\mu\text{mol}/\text{min}/\text{mg}$ protein (Stansley 1992) which is greater than the 33 $\mu\text{mol}/\text{min}/\text{mg}$ protein measured in the fish captured in the LNID drainage canals and the Little Bow River in the present study. This suggests the fathead minnows were exposed to pesticides and they may be used to monitor pesticide levels. The normal and exposed AchE activities in the other forage fish species remain to be characterized.

Trunk cortisol and glucose levels were also measurable in all species, suggesting the PSR of forage fish may be used to monitor the effect of multiple stressors. There were significant species specific differences in cortisol levels, but not in glucose levels; thus different species of forage fish cannot be pooled when evaluating the PSR. The basal and stressed levels of PSR responses for the different species need to be determined and validated before they can be used as reliable field biomarkers.

4.4.4. Conclusions

Selenium accumulates in the muscle of white suckers at some sites within the LNID drainage canals. The muscle levels are approaching thresholds for teratogenic

deformities, but the main effect at these levels is accumulation. Plasma AchE levels were similar at all sites, although levels were lower in the fall than the spring. This may be due to the duration of exposure, declining temperatures or another variable. More research is needed to determine what variables influence AchE activity in white suckers to improve its use as a biomarker of pesticide exposure.

Agricultural drain water influences the PSR of white suckers, but the primary influences were site and season. Season effects may be mediated by decreasing temperature, decreasing photoperiod, reproductive cycles, or different exposure durations. Site effects may have been driven by unidentified contaminant exposures (e.g. veterinary pharmaceuticals), and differences in flow or turbidity. Selenium exposure increased plasma glucose levels, and pesticide exposure increased liver glycogen levels in the summer, but not in the fall.

White suckers appear to be a good sentinel species as they are found in many North American systems (Nelson and Paetz 1992). Forage fish such as emerald shiners, longnose dace, fathead minnow, and juvenile white sucker may also be used to monitor the multiple stressors with the PSR as they are also well distributed and common (Nelson and Paetz 1992). The PSR is a good model system for monitoring multiple stressors as it integrates the responses to all the stimuli the fish perceive as stressors, even though different chemical stressors influence different components of the system.

4.5. Acknowledgments

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Table 4.1. Water and air temperatures from LNID drainage canals and the Little Bow River, Southern Alberta, Canada in the summer and the fall. Measurements collected during electrofishing.

Site	Water (°C)		Air (°C)	
	Summer	Fall	Summer	Fall
Little Bow	18.0	8.5	16.0	15.8
Monarch	17.0	10.3	21.0	12.5
BASF	17.3	8.7	24.3	13.3
Pyami	21.3	10.5	25.6	16.5
Battersea	24.5	9.5	28.5	17.5

Table 4.2. Analysis of covariance models that describe a significant ($p < 0.05$) portion of the variation in the PSR parameters of confinement-stressed and basal white suckers.

Parameter	Transformation	Adjusted r^2	Model
<i>Stressed White Suckers*</i>			
Plasma Cortisol	0.6	0.1980	Site + Season
Plasma Glucose	0.8	0.2692	Selenium + Site + Season + Site*Season
Gill Na^+/K^+ ATPase	-0.2	0.3954	Site + Season + Site*Season
Liver Glycogen	0.4	0.7185	AchE + Site + Season + Site*Season
Condition Factor	2.0	0.1185	Season
Liver Somatic Index	0.4	0.2617	Site + Season + Length + Site*Season
<i>Basal White Suckers</i>			
Plasma Cortisol	Log	0.0758	Site + Season + Site*Season
Plasma Glucose	-	0.5973	Site + Season + Site*Season

* a Box-Cox transformation (λ indicated) was used for the stressed white suckers

Table 4.3. Physiological stress response parameters in confinement-stressed white suckers from the LNID drainage canals and the Little Bow River in the summer (S) and the fall (F).

Parameter	Little Bow		Monarch		BASF		Pyami		Battersea	
	S	F	S	F	S	F	S	F	S	F
<i>Cortisol (ng/ml plasma)</i>										
N	16	14	13	16	12	10	17	14	12	10
Mean	98	150	115	139	100	105	168	229	136	236
Transformed mean	157	198	173	194	151	162	218	262	191	276
Transformed SE	9	23	14	15	21	18	17	28	19	18
<i>Glucose (mg/ml plasma)</i>										
N	17	14	13	14	12	10	15	13	11	10
Mean	1.62	1.57	1.47	1.57	1.34	1.85	2.26	1.89	1.87	1.37
Transformed mean	0.64	0.58	0.48	0.62	0.34	0.86	1.25	0.91	0.88	0.39
Transformed SE	0.11	0.13	0.12	0.10	0.18	0.15	0.16	0.09	0.14	0.05
<i>Gill Na⁺/K⁺ ATPase (μmol/mg protein)</i>										
N	16	12	16	17	13	10	17	14	16	10
Mean	0.72	0.30	0.31	0.49	0.50	0.36	0.59	0.38	0.41	0.36
Transformed mean	-0.16	-0.51	-0.50	-0.29	-0.27	-0.43	-0.22	-0.39	-0.35	-0.41
Transformed SE	0.04	0.05	0.03	0.03	0.03	0.05	0.03	0.04	0.03	0.04
<i>Glycogen (mg/g liver)</i>										
N	17	14	15	17	12	10	17	14	16	10
Mean	19	80	20	48	36	100	33	126	34	83
Transformed mean	50	107	50	81	69	120	65	134	65	110
Transformed SE	3.1	4.6	5.1	5.1	6.6	4.5	5.7	2.6	6.6	4.5
<i>Condition Factor (K)^a</i>										
N	17	14	16	17	13	10	17	14	16	10
Mean	1.34	1.22	1.24	1.19	1.31	1.19	1.20	1.19	1.30	1.19
Transformed mean	0.05	-0.04	0.00	-0.07	0.04	-0.05	-0.02	-0.05	0.04	-0.05
Transformed SE	0.02	0.02	0.02	0.02	0.04	0.02	0.03	0.03	0.03	0.04
<i>Liver Somatic Index (LSI)^b</i>										
N	17	14	16	17	13	10	17	14	16	10
Mean	1.67	2.86	1.75	1.63	2.58	2.22	2.01	2.76	1.82	1.62
Transformed mean	0.80	1.89	0.78	0.77	1.57	1.36	1.05	1.79	0.95	0.75
Transformed SE	0.11	0.14	0.24	0.10	0.27	0.15	0.24	0.17	0.14	0.15

$$^a K = \frac{\text{weight} \times 100}{\text{length}^{3.09}}$$

$$^b LSI = \frac{\text{weight}_{\text{liver}}}{\text{weight}_{\text{body}}} \times 100$$

Table 4.4. Physiological stress response parameters in basal white suckers from the LNID drainage canals and the Little Bow River in the summer (S) and the fall (F).

Parameter	Little Bow		BASF		Battersea	
	S	F	S	F	S	F
<i>Cortisol (ng/ml plasma)</i>						
N	24	12	6	11	20	9
Mean	16.07	0.81	0.88	2.19	21.81	0.28
Transformed mean	0.09	-0.04	0.27	-0.49	-0.45	0.21
Transformed SE	0.18	0.19	0.21	0.28	0.14	0.23
<i>Glucose (mg/ml plasma)</i>						
N	25	12	8	11	21	9
Mean	0.46	0.38	0.64	0.32	0.94	0.39
Transformed mean	0.10	-0.05	-0.03	0.06	-0.10	0.05
Transformed SE	0.04	0.07	0.04	0.03	0.05	0.03

Table 4.5. Head AchE activities, and trunk cortisol and glucose levels (mean \pm SE) of forage fish caught in the Little Bow River and the LNID drainage canals in the summer and the fall.

Species [#]	Head AchE [*] (μ mol/min/mg protein)	Trunk Cortisol [*] (ng/ml protein)	Trunk Glucose (mg/mg protein)
EMSH (n = 28)	56.18 \pm 10.11 ^b	0.08 \pm 0.01 ^a	0.78 \pm 0.09
FHMN (n = 41)	33.15 \pm 5.40 ^a	0.11 \pm 0.02 ^b	0.50 \pm 0.07
LNDC (n = 68)	33.08 \pm 2.70 ^a	0.07 \pm 0.00 ^a	1.01 \pm 0.09
WHSC (n = 33)	78.98 \pm 4.96 ^c	0.10 \pm 0.02 ^b	0.40 \pm 0.08

* Different letters indicate a significant difference between species.

[#] EMSH – emerald shiner; FHMN – fathead minnow; LNDC – longnose dace; WHSC – white sucker.

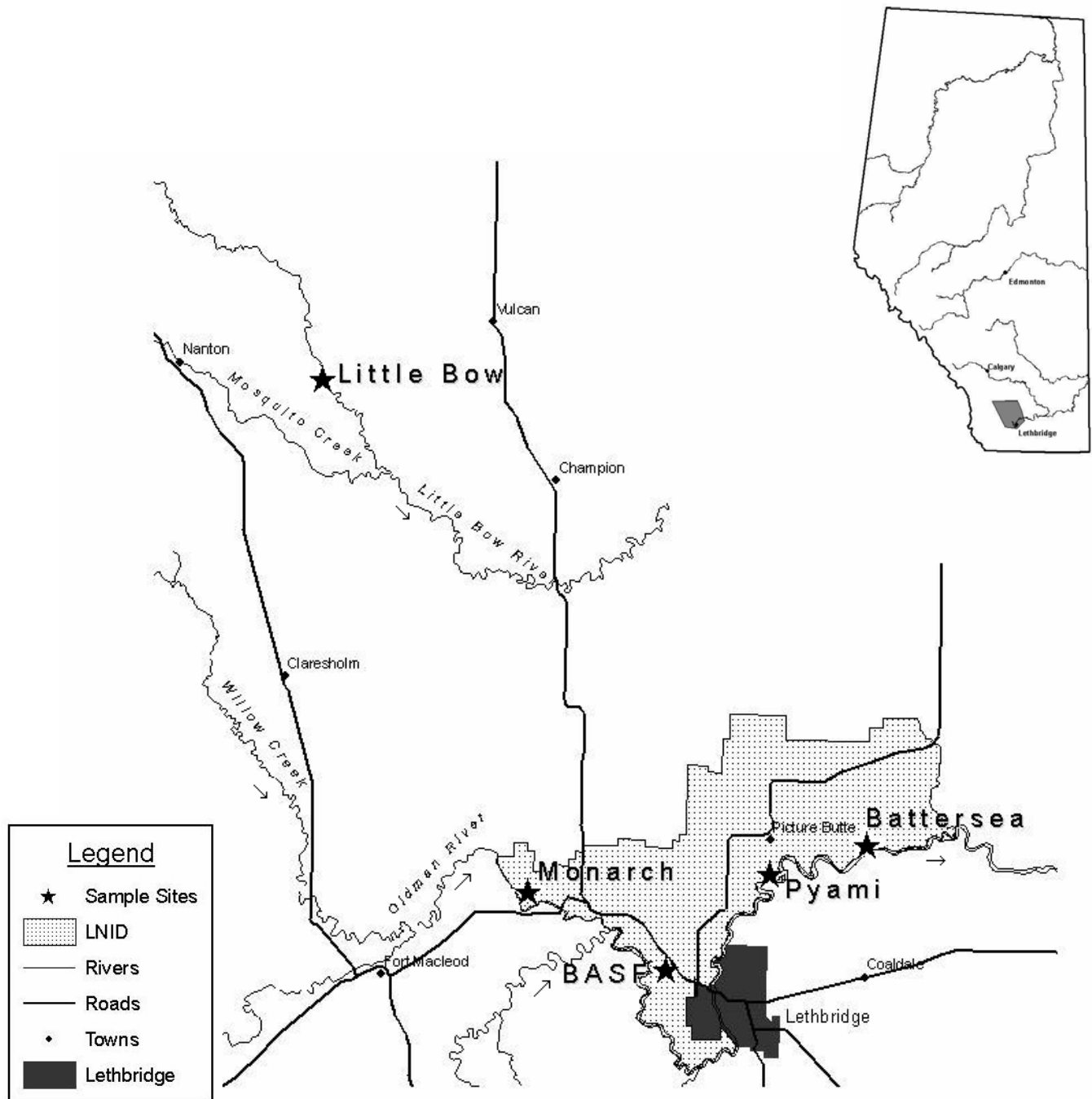


Figure 4.1. The sample study sites in the Lethbridge Northern Irrigation District (LNID) drainage canals and the Little Bow River, Southern Alberta, Canada. → indicates direction of water flow.

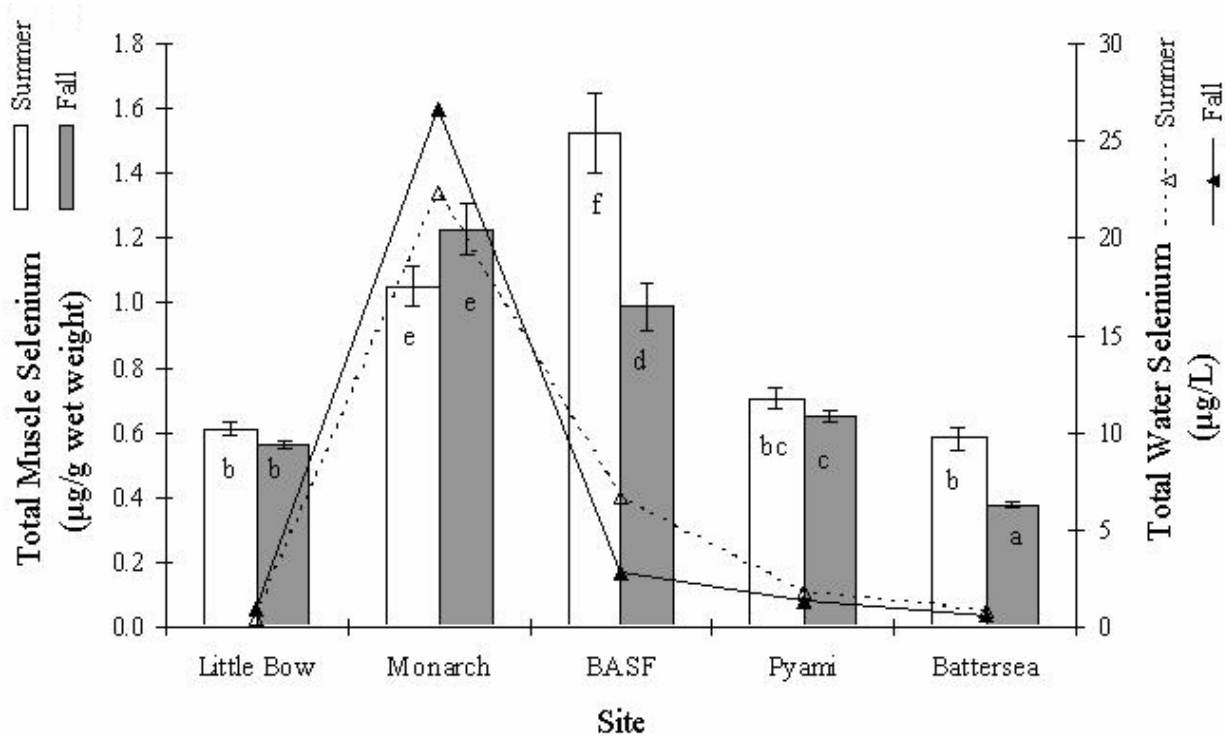
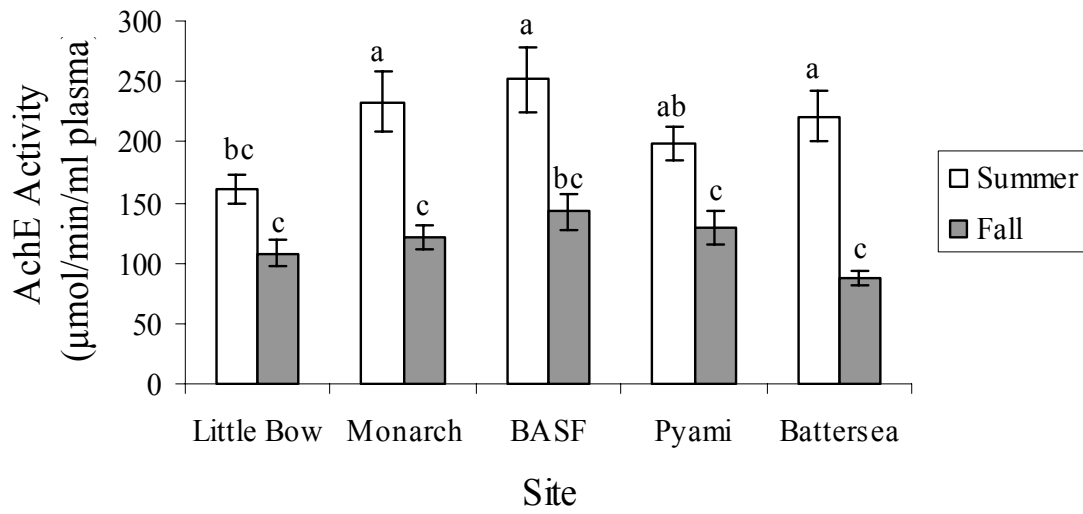


Figure 4.2. Selenium concentrations in the muscle of confinement-stressed white suckers (n = 10-17) and water (n = 1) from LNID drainage canals and the Little Bow River in the summer and the fall. Different letters indicate a significant difference.

(A)



(B)

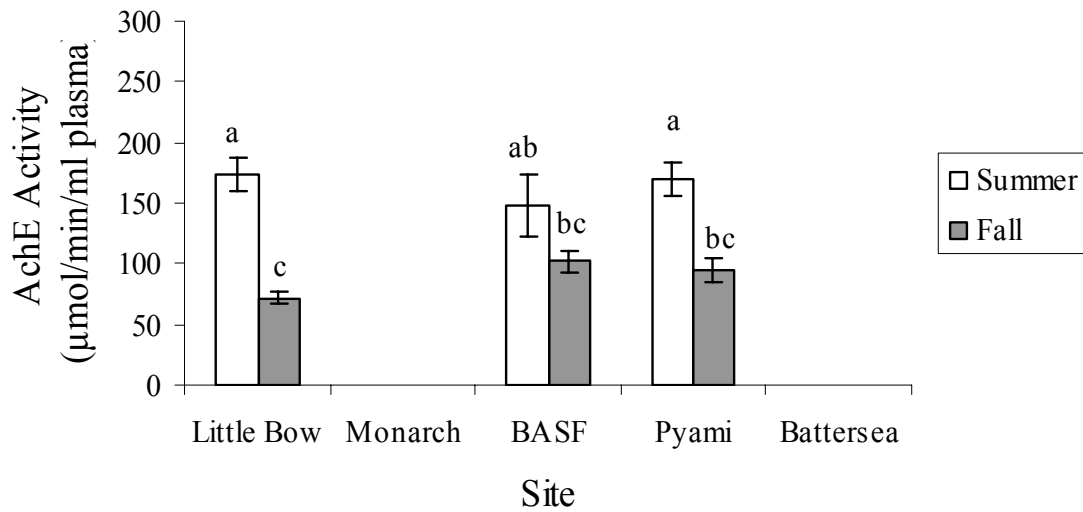


Figure 4.3. Plasma AchE activities of (A) confinement-stressed white suckers (n = 10-17) and (B) basal white suckers (n= 8-25) from the LNID drainage canals and the Little Bow River. Different letters indicate a significant difference.

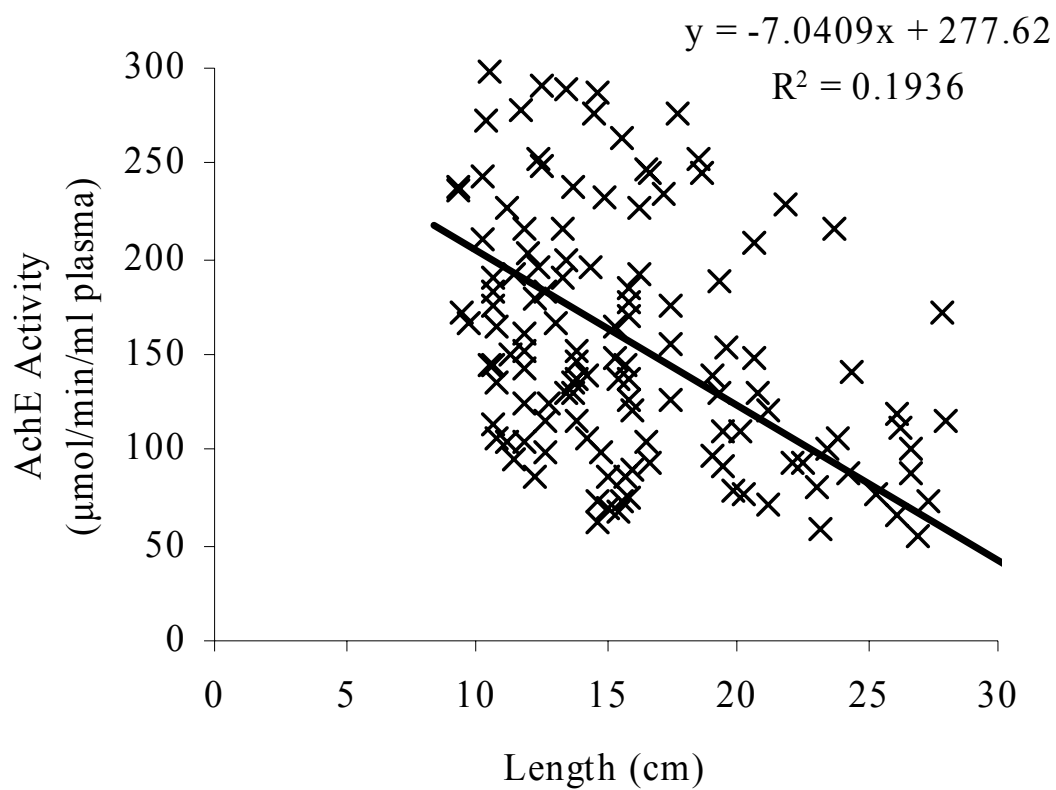
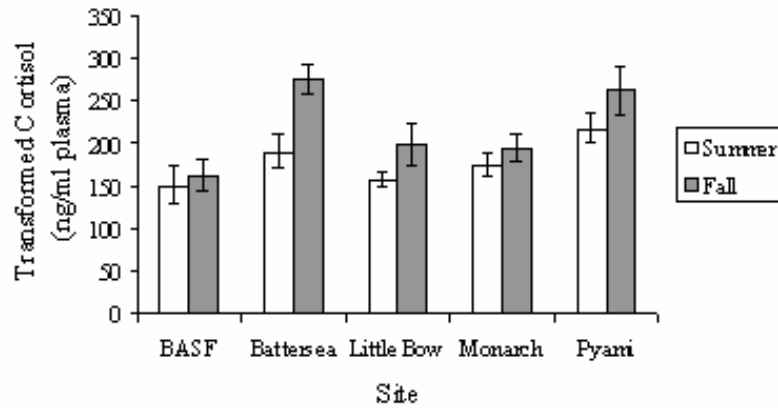
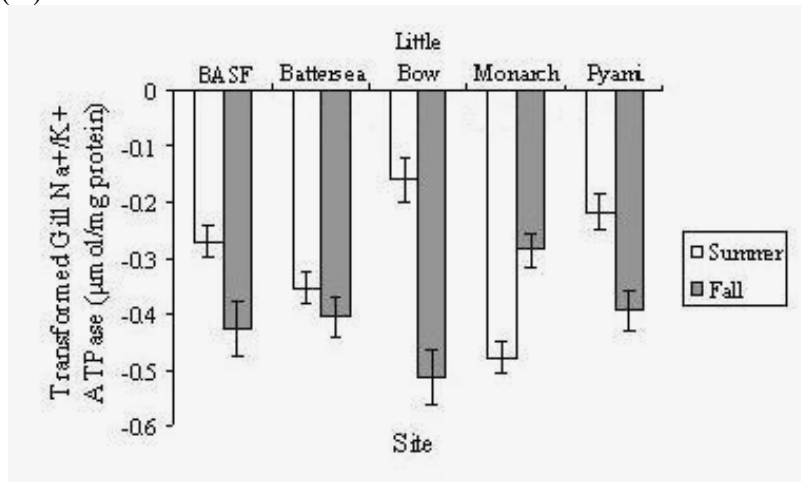


Figure 4.4. The correlation between length and plasma AchE activity in confinement-stressed white suckers (n = 136) from the LNID drainage canals and the Little Bow River in the summer and the fall.

(A)



(B)



(C)

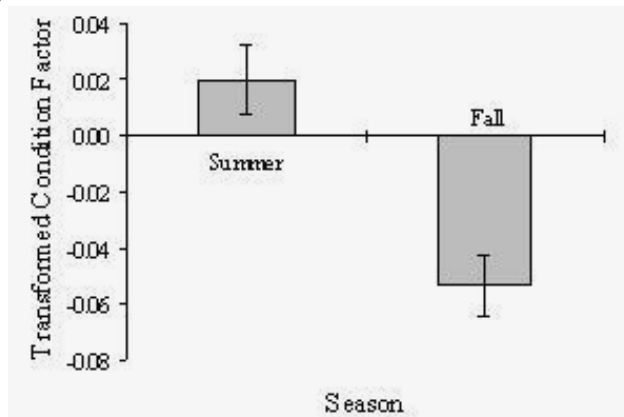


Figure 4.5. Plasma cortisol levels (A), gill Na⁺/K⁺ ATPase activity (B), and condition factor (C) in confinement-stressed white suckers from the LNID drainage canals and the Little Bow River. Data (mean \pm SE) are transformed (refer to Table 4.2 for transformations and models describing the significant influences on the PSR parameters).

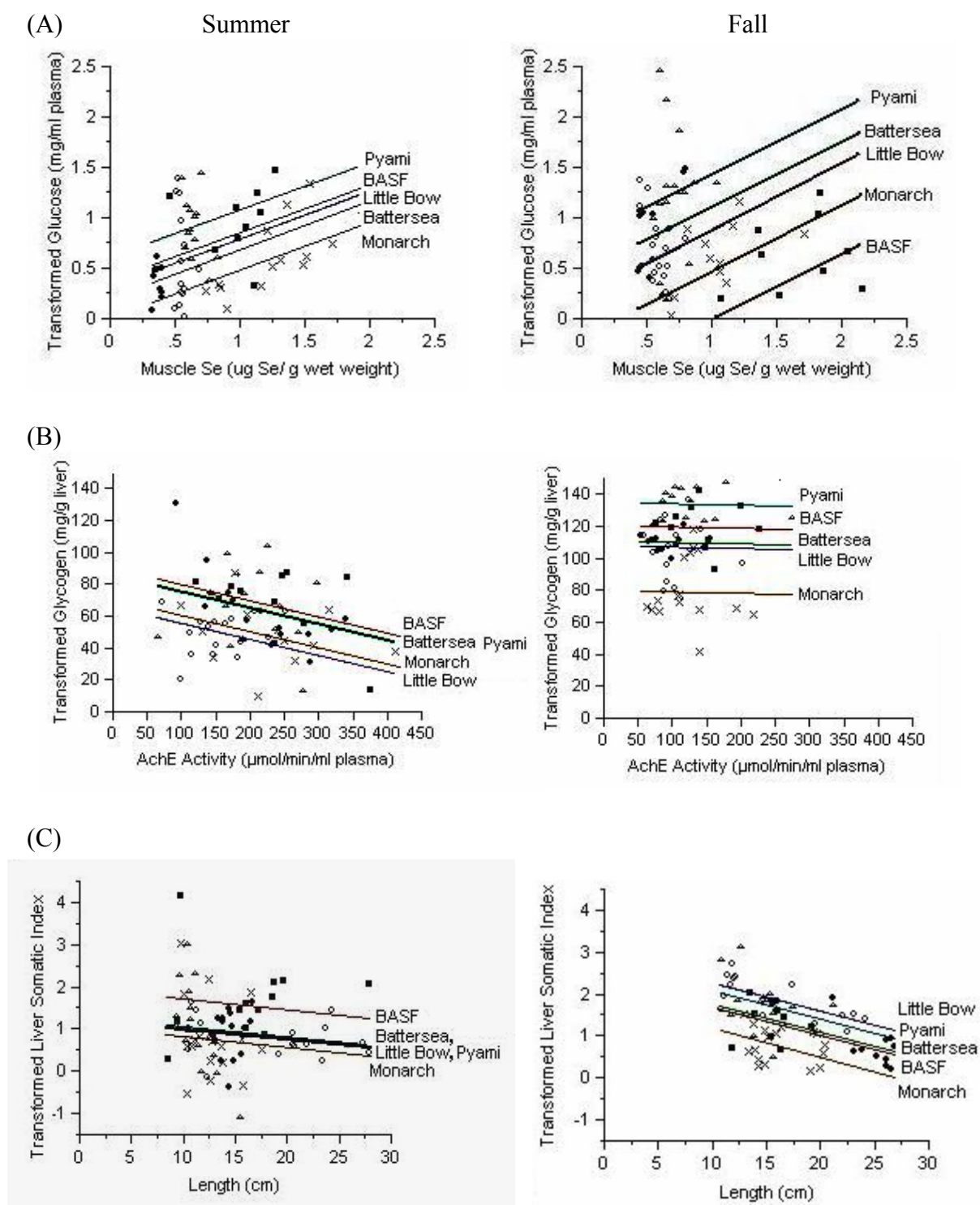


Figure 4.6. The effect of (A) muscle Se on plasma glucose levels in the summer and the fall, (B) plasma AchE activity on liver glycogen levels in the summer and the fall, and (C) length on the liver somatic index in the summer and the fall in confinement-stressed white suckers from LNID drainage canals (Monarch (×), BASF (■), Pyami (Δ), and Battersea (●) and the Little Bow River (○). Lines represent the ANCOVA model that describes r^2 (refer to Table 4.2) of the variation in the parameter.

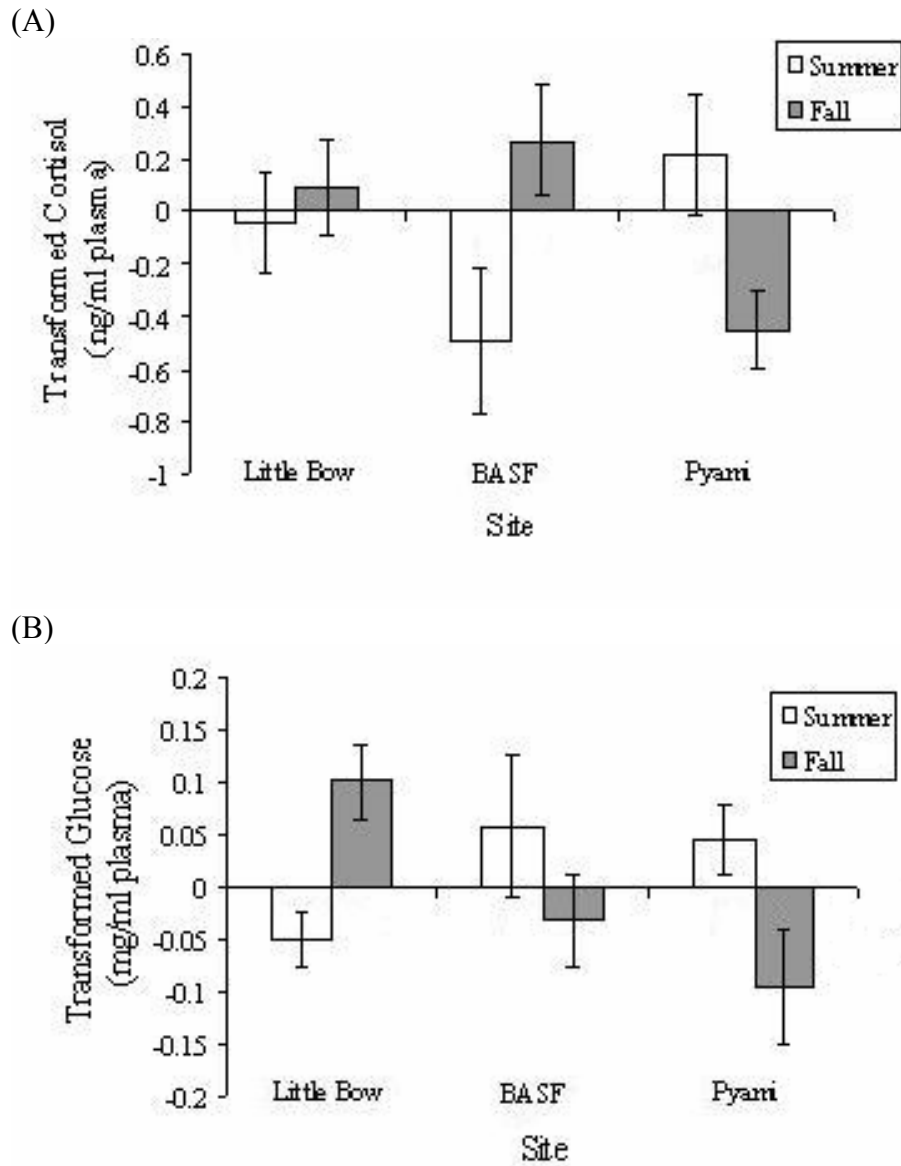


Figure 4.7. The effect of site and season on (A) plasma cortisol levels and plasma glucose levels in basal white suckers (mean \pm SE) from LNID drainage canals and the Little Bow River.

4.6. Literature Cited

- Adams SM. 2001. Biomarker/bioindicator response profiles of organisms can help differentiate between sources of anthropogenic stressors in aquatic systems. *Biomarkers* 6(1):33-44.
- Allert A, Fairchild J, May T, Sappington L, Darnall N, Wilson M. 2006. Using on-site bioassays to determine selenium risk to propagated endangered fishes. *North American Journal of Fisheries Management* 26:308-316.
- Anderson A. 2005. Overview of pesticide data in Alberta surface waters since 1995. Edmonton: Alberta Environment.
- Austin B. 1999. The effects of pollution on fish health. *Journal of Applied Microbiology Symposium Supplement* 85:234S-242S.
- Beauvais SC, KJ, Atchison G, Coffey M. 2002. Factors affecting brain cholinesterase activity in bluegill (*Lepomis macrochirus*). *Water, Air, and Soil Pollution* 135:249-264.
- Bleau H, Daniel C, Chevalier G, van Tra H, Hontela A. 1996. Effects of acute exposure to mercury chloride and methylmercury on plasma cortisol, T3, T4, glucose and liver glycogen in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 34(3):221.
- Carragher J, Sumpter J. 1990. The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. *General and Comparative Endocrinology* 77:403-407.
- Cincotta DA, Stauffer JR. 1984. Temperature preference and avoidance studies of six North American freshwater fish species. *Hydrobiologia* 109:173-177.
- de Rosemond S, Liber K, Rosaasen A. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker (*Catostomus commersoni*) from a northern Canadian Lake. *Bulletin of Environmental Contamination and Toxicology* 74:1134-1142.
- Dobbs MC, DS, Cairns J. 1996. Toxicity and bioaccumulation of selenium to a three-trophic food chain. *Environmental Toxicology and Chemistry* 15(3):340-347.
- Dorval J, Leblond V, Deblois C, Hontela A. 2005. Oxidative stress and endocrine endpoints in white sucker (*Catostomus commersoni*) from a river impacted by agricultural chemicals. *Environmental Toxicology and Chemistry* 24(5):1273-1280.
- Dorval J, Leblond V, Hontela A. 2003. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquatic Toxicology* 63:229-241.
- Ellman GC, KD, Andres V, Featherstone R. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7:88-95.
- Flodmark LEW, Urke HA, Halleraker JH, Arnekleiv JV, Vøllestad LA, Peléo ABS. 2002. Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. *Journal of Fish Biology* 60:238-248.

- Forrest F, Keenlside J, Kendall J, Thompson T, Noot D, Wuite J. 2006. Livestock pharmaceuticals in agricultural streams: a scoping study for Alberta. Edmonton: Alberta Agriculture, Food, and Rural Development.
- Freeman H, Idler D. 1973. Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdnerii*) and the brook trout (*Salvelinus fontinalis*). *General and Comparative Endocrinology* 20:69-75.
- Gagnon A, Jumarie C, Hontela A. 2006. Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 78:59-65.
- Gregory T, Wood C. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* 72(3):286-295.
- Hamilton S, Holley K, Buhl KB, FA, Weston L, McDonald S. 2005. Selenium impacts on razorback sucker, Colorado River, Colorado I. Adults. *Ecotoxicology and Environmental Safety* 61:7-31.
- Hazel J. 1969. The effect of thermal acclimation upon brain acetylcholinesterase activity of *Carassius auratus* and *Fundulus heterolitus*. *Life Sciences* 8(2):775-784.
- Hilton J, Hodson P, Slinger S. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* 110:2527-2535.
- Hontela A. 1997. Endocrine and physiological responses of fish of xenobiotics: role of glucocorticosteroid hormones. *Reviews in Toxicology* 1:1-46.
- Hontela A. 1998. Interrenal dysfunction in fish from contaminated sites: *in vivo* and *in vitro* assessment. *Environmental Toxicology and Chemistry* 17(1):44-48.
- Hontela A. 2005. Adrenal toxicology: environmental pollutants and the HPI axis. In: Mommsen T, Moon T, editors. *Biochemistry and Molecular Biology of Fishes*. Amsterdam: Elsevier B.V. p 331-363.
- Hontela A, Dumont J, Duclos D, Fortin R. 1995. Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. *Environmental Toxicology and Chemistry* 14(4):725-731.
- Humborstad O, Jørgensen T, Grotmol S. 2006. Exposure of cod *Gadus morhua* to resuspended sediment: an experimental study of the impact of bottom trawling. *Marine Ecology Progress Series* 306:247-254.
- Hyliner H, Andersson T, Haux C, Olsson P-E. 1989. Cortisol induction of metallothionein in primary culture of rainbow trout hepatocytes. *Journal of Cell Physiology* 139:24-28.
- Jardine J, VanDerKraak G, Munkittrick K. 1996. Capture and confinement stress in white sucker exposed to bleached kraft pulp mill effluent. *Ecotoxicology and Environmental Safety* 33:287-298.
- Leamon JH, Schultz ET, Crivello JF. 2000. Variation among four health indices in natural populations of the estuarine fish, *Fundulus heteroclitus* (Pisces, Cyprinodontidae) from five different geographically proximate estuaries. *Environmental Biology of Fishes* 57:451-458.
- Lemly A. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquatic Toxicology* 27:133-158.

- Lemly A. 1993b. Teratogenic effects of selenium in natural populations of freshwater fish. *Ecotoxicology and Environmental Safety* 26:181-204.
- Lemly A. 1996. Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. *Ecotoxicology and Environmental Safety* 34:223-227.
- Lemly A. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. *Aquatic Toxicology* 57:39-49.
- Levesque H, Moon T, Campbell P, Hontela A. 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquatic Toxicology* 60:257-267.
- McMaster M, Munkittrick K, Luxon P, VanDerKraak G. 1994. Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. *Ecotoxicology and Environmental Safety* 27:251-264.
- McMaster M, Munkittrick K, Luxon P, VanDerKraak G. 1994. Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. *Ecotoxicology and Environmental Safety* 27:251-264.
- Mello LGS, Rose GA. 2005. Seasonal cycles in weight and condition in Atlantic cod (*Gadus morhua* L.) in relation to fisheries. *ICES Journal of Marine Science* 62(5):1006.
- Morgan I, Henry R, Wood C. 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na⁺ and Cl⁻ transport. *Aquatic Toxicology* 38:145-163.
- Nelson J, Paetz M. 1992. The fishes of Alberta. Edmonton: The University of Alberta Press.
- Ohlendorf H, Santolo G. 1994. Kesterson reservoir - past, present, and future: an ecological risk assessment. In: Frankenberger W, Jr., Benson S, editors. *Selenium in the Environment*. New York: Marcel Dekker Inc. p 69-117.
- Packer R, Garvin J. 1998. Seasonal differences in activity of perch (*Perca flavescens*) gill Na⁺/K⁺ ATPase. *Comparative Biochemistry and Physiology Part B* 120:777-783.
- Phillips T, Summerfelt R, Atchison G. 2002. Environmental, biological, and methodological factors affecting cholinesterase activity in walleye (*Stizostedion vitreum*). *Archives of Environmental Contamination* 43:75-80.
- Roche H, Bogé G. 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Marine Environmental Research* 41(1):27-43.
- Schlenk D, Zubcov N, Zubcov E. 2003. Effects of salinity on the uptake, biotransformation, and toxicity of dietary seleno-L-methionine to rainbow trout. *Toxicological Sciences* 75:309-313.
- Shrimpton J, McCormick S. 1999. Responsiveness of gill Na⁺/K⁺ -ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *The Journal of Experimental Biology* 202:987-995.
- Sibley PK, Chappel MJ, George TK, Solomon KR, Liber K. 2000. Integrating effects of stressors across levels of biological organization: examples using

- organophosphorus insecticide mixtures in field-level exposures. *Journal of Aquatic Ecosystem Stress and Recovery*:117-130.
- Spallholz J, Hoffman D. 2002. Selenium toxicity: cause and effects in aquatic birds. *Aquatic Toxicology* 57:27-37.
- Stansley W. 1992. Postmortem changes in uninhibited brain cholinesterase activity in the fathead minnow. *Transactions of the American Fisheries Society* 121:137-139.
- Sturm A, Wogram J, Hansen P-D, Liess M. 1999. Potential use of cholinesterase in monitoring low levels of organophosphates in small streams: natural variability in three-spined stickleback (*Gasterosteus aculeatus*) and relation to pollution. *Environmental Toxicology and Chemistry* 18(2):194-200.
- Velasco J, Lloret J, Millan L, Marin L, Barahona J, Abellan P, Sanchez-Fernandez D. 2006. Nutrient and particulate inputs into the Mar Menor Lagoon (SE Spain) from an intensive agricultural watershed. *Water, Air, and Soil Pollution* 176:37-56.
- Whitehead A, Anerson S, Ramirez A, Wilson B. 2005. Cholinesterases in aquatic biomonitoring: assay optimization and species-specific characterization for a California native fish. *Ecotoxicology* 14:597-606.

CHAPTER 5. A SUMMARY OF THE EFFECTS OF SELENIUM ON THE PHYSIOLOGICAL STRESS RESPONSE AND OXIDATIVE STRESS BIOMARKERS IN FISH FROM ALBERTA

5.1. Research Objectives

The unifying objectives of this project were to determine:

- if Se contamination is a problem in areas affected by coal mining and irrigation in Alberta
- if Se alters the physiological stress response of fish
- if Se alters biomarkers of oxidative stress in fish

These objectives were met by investigating the following hypotheses :

- Se, at concentrations above the homeostatic requirement, activates the physiological stress response
- Se mediates its toxic effects through an alteration of oxidative status at a cellular level

5.2. Summary of Findings

5.2.1. Chapter 1: Linking selenium toxicity, the physiological stress response, and oxidative stress in fish

A literature review dealing with selenium toxicity, the physiological stress response and oxidative stress in fish was presented in this chapter. The current knowledge can be summarized as follows:

- Selenium, an essential element, bioaccumulates and becomes toxic at concentrations slightly higher than those required for homeostasis; although species sensitivity to Se toxicity varies.
- The physiological stress response (PSR) enables fish to reallocate energy reserves to handle external and internal stimuli they perceive as stressors.
- Contaminants may activate, impair, or exhaust the PSR; however, little is known about the effect of Se on the PSR in fish.
- Selenium toxicity and deficiency symptoms are similar and both involve oxidative stress.
- Oxidative stress, damage to cellular components that occurs when reactive oxygen species overwhelm cellular defences, is a mechanism of Se toxicity.

5.2.2. Chapter 2: The effect of waterborne selenium on the physiological stress response and oxidative stress indicators in juvenile rainbow trout: acute and sub-chronic *in vivo* exposures

- Acute and sub-chronic exposures to waterborne sodium selenite in the laboratory activated the PSR in juvenile rainbow trout as plasma cortisol levels were elevated by selenium in both exposures.
- Sub-chronic sodium selenite exposure did not change the ability to secrete cortisol, thus selenium does not impair or exhaust the PSR of juvenile rainbow at the concentrations tested.
- Hepatic GSH levels were decreased by acute exposure to sodium selenite. This suggests GSH binds to Se and may act as an antioxidant and/or prooxidant.

5.2.3. Chapter 3: The effect of chronic selenium exposure on the physiological stress response and oxidative stress biomarkers in rainbow trout and brook trout from a coal mining area

- The PSR of rainbow trout and brook trout was not activated, impaired or exhausted by exposure to chronic environmental Se as plasma cortisol levels and the ability to secrete cortisol were not influenced by muscle Se levels.
- Brook trout had greater gill Na^+/K^+ -ATPase activity than rainbow trout. This physiological difference may allow the brook trout to osmoregulate more efficiently in Se impacted streams.
- In rainbow trout, oxidative damage (LPO levels) increased with increasing Se exposure (muscle Se levels), but in brook trout oxidative damage decreased with increasing Se exposure. Thus, Se exposure may cause oxidative damage in rainbow trout but protect against it in brook trout.
- Rainbow trout also had greater hepatic GSH levels than brook trout. This biochemical species difference may explain the species difference in oxidative damage as the rainbow trout have more glutathione available to create reactive oxygen species than brook trout.

5.2.4. Chapter 4: Seasonal variation in the physiological stress response of fish exposed to agricultural drain water in southern Alberta: the influence of selenium and pesticides

- Agricultural drain water may contain multiple stressors (selenium, pesticides, veterinary pharmaceuticals, nutrients, and particulates)

- Selenium accumulated in white suckers from the Monarch and BASF drain in the Lethbridge Northern Irrigation District, although teratogenic deformities are unlikely.
- Plasma AchE activities (indicator of pesticide exposure) were lower in the fall than the spring suggesting fish were exposed to more pesticides in the fall than the spring. However, AchE activities may also be influenced by size, temperature and exposure duration.
- Agricultural drain water influenced the PSR, but selenium and pesticide exposure only influenced plasma glucose and liver glycogen levels respectively. The main influences on PSR parameters were site (other stressors such as veterinary pharmaceuticals) and season (temperature, photoperiod, exposure duration or reproductive cycles).
- Forage fish (emerald shiners, fathead minnows, longnose dace, and juvenile white suckers) may be used as indicators of pesticide exposure and PSR status as head AchE activities, trunk cortisol and trunk glucose levels can be measured; however, species cannot be pooled as species-specific differences in AchE activities and cortisol levels are evident.

5.3. Conclusions

- Selenium contamination is occurring in Alberta as muscle Se levels were elevated in rainbow trout caught in streams impacted by coal mines in north-eastern Alberta, and in white suckers caught in irrigation drainage canals in southern Alberta.

- Selenium activates the PSR of juvenile rainbow trout exposed to acute and sub-chronic sodium selenite in the laboratory; however Se did not activate the PSR of trout or white suckers exposed to chronic environmental levels. Additionally, Se did not impair or exhaust the PSR.
- Selenium increased oxidative damage (LPO) in rainbow trout, but not brook trout exposed to chronic environmental levels of Se. It also depleted GSH in juvenile rainbow trout exposed to acute levels of sodium selenite in the laboratory, but not in fish exposed to sub-chronic or chronic environmental levels of Se.
- Selenium and oxidative stress are linked as Se increases oxidative damage in rainbow trout, but protects against it in brook trout. Selenium did activate the PSR in the laboratory exposures; however, this was not linked to oxidative stress as Se did not impair the PSR (cortisol secretory capacity was unaltered).

5.4. Significance and Management Implications

- Juvenile rainbow trout respond to acute (96 hours) and sub-chronic (30 days) Se exposures as stressors, and to my knowledge this is the first time this has been demonstrated in fish. However, at environmental levels Se does not activate, impair or exhaust the PSR in fish. This is important because it means the fish are still able to appropriately respond to new stressors (such as an increase in temperature) and reallocate their energy reserves.
- Selenium caused oxidative damage in rainbow trout from Se impacted streams and depleted GSH reserves in laboratory exposed fish; thus, oxidative stress is a mechanism of Se toxicity in rainbow trout.

- Physiological and biochemical differences (e.g. gill Na^+/K^+ -ATPase activities and hepatic GSH levels) between rainbow trout and brook trout may drive the different species sensitivities to Se. These differences may allow introduced brook trout to gain a foothold in Se impacted streams previously primarily occupied by native Athabasca rainbow trout and slowly replace them.
- The PSR is a good model system to assess the effects of multiple stressors as it integrates the response to many different stressors into one system. Exposure to agricultural drain water (contains multiple stressors) altered components of the white suckers' PSR, but site (contaminants other than Se and pesticides) and season (temperature or exposure duration) had the largest influence on the PSR.
- Muscle Se levels were elevated in white suckers from two of the Lethbridge Northern Irrigation District drainage canals, but these levels are below those causing teratogenic deformities.
- Emerald shiners, fathead minnows, longnose dace and juvenile white suckers may be used as sentinel species as pesticide exposure and PSR parameters may be evaluated. This is important as they are widespread, occur in many impacted systems, and are not valued as game fish.

5.5. Future Research

- Examine the interactions between GSH and Se to determine when GSH acts as an antioxidant and when it acts as a prooxidant using the head kidney *in vitro* model.
- Further investigate the physiological and biochemical differences between rainbow trout and brook trout that mediate different Se sensitivities.

- Investigate the implications of different Se sensitivities on rainbow trout and brook trout populations and their competitive interactions in Se-impacted systems.
- Investigate the influence of temperature and photoperiod on AchE activities in white sucker, emerald shiner, fathead minnow, and longnose dace to validate it as a biomarker of pesticide exposure.
- Validate trunk cortisol and glucose levels as indicators of exposure to stressors in emerald shiners, fathead minnows, longnose dace and juvenile rainbow trout.