INTERACTIVE EFFECTS OF MALATHION EXPOSURE AND FASTING ON THE BIOENERGETIC INDICES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

by

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ABSTRACT

Overwintering is a critical period for salmonids living in British Columbia, when fish face reduced energy intake. While exposure to current-use pesticides (CUP) may exacerbate the problem of a limited energy budget, increasing over-winter mortalities, there exists little information on the interaction between xenobiotics and overwintering in salmonids. To examine this, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to sublethal malathion concentrations (1.5 and 16.2μg/L) in either a fed or fasted treatment group under low water temperatures (10^{o}C). Fed fish displayed enhanced growth (300% mass increase), higher plasma cortisol (4.5±1.1 ng/mL) and elevated energetic indices (liver somatic index (LSI) 0.8±0.01%, muscle lipid 34.4±1.0 mg/g) compared to fasted fish (18% mass decrease, 1.7±1.1 ng/mL plasma cortisol, LSI 0.76±0.02%, muscle lipid 19.6±1.1 mg/g). Altered body mass was observed in exposed fish, with few statistically significant differences for bioenergetic or stress indicators. There appear to be low energy costs associated with malathion defence.

Keywords: Bioenergetics, overwintering, malathion, sublethal, rainbow trout

DEDICATION

I would like to dedicate this work to my sister Lindsay, who convinced me I could do it. Her persistence in difficult times has always inspired me and reminded me of how much we are capable.

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
ANOVA	Analysis of variance
CF	Condition factor
CUPs	Current use pesticides
DAG	Diacylglycerides
GST	Glutathione-S-transferase
K_{ow}	Octanol-water partition coefficient
LC_{50}	Lethal concentration to 50% of test organisms
LSI	Liver somatic index
OPs	Organophosphorus insecticides
PL	Phospholipids
TAG	Triacylglycerides

1: GENERAL INTRODUCTION

1.1 Bioenergetics

Bioenergetics is the study of how energy is ingested, distributed, transformed and excreted within an organism (Lucas, 1996). Organisms take in energy-yielding molecules, distribute those molecules to various systems, and transform them into usable energy, with energy depleted molecules being excreted as waste. Bioenergetics involves the study of the relationship between energy inputs versus outputs, often referred to in the form of an energy budget (Lucas, 1996). Energy budgets illustrate the concept that all biological processes cost energy; budgets account for the materials consumed (or energy inputs), the costs of transforming those materials and the loss of waste materials (outputs). Assimilated energy is used for a variety of functions such as general metabolism, growth and development, reproduction, and activity.

Metabolic rates are used to describe energy expenditure over time. The minimum metabolic or standard metabolic rate is the minimum amount of energy required to maintain basic organism functions such as respiration, ion transport, and cellular maintenance (Jobling, 1993). Those processes that are essential to sustaining life are also referred to as a maintenance requirement. The maximum metabolic or maximum aerobic rate describes the highest level of energy use during aerobic activity that is achievable, above that of the minimum metabolic rate. The metabolic scope, or aerobic scope, is defined as the range of energy use between the minimal metabolic rate and maximum

aerobic output (Jobling, 1993). The scope may also be described as the metabolic cost for an organism to function, and is often measured as the rate of oxygen consumption.

Energy budgets and allocation of energy to priority systems or energy reservoirs

The amount of energy available to an organism is often limiting in aquatic systems, and the allocation of assimilated energy is first directed to those maintenance systems that are vital to immediate survival. It is estimated that the maintenance requirement of rainbow trout is approximately 10-30% of the total assimilated energy (Rodehutscord and Pfeffer, 1999). After this requirement has been met, energy can be directed toward other processes such as growth and/or reproduction, although the regulatory control involved in energy allocation between these processes is not clearly understood.

Aquatic organisms that have not reached sexual maturity will allocate most of their available energy toward somatic growth. Sexually mature organisms will put energy toward both growth and reproduction. When organisms reach sexual maturity, the ability to produce gametes and successfully pass on genetic material becomes a priority. Two energy allocation strategies for fish exist in this regard: to direct energy toward maintaining constant somatic growth and vary the amount of reproductive tissue produced, or to allocate energy towards producing a constant amount of gametes at the expense of somatic growth (Adams, 1999). Most species find a balance between these two strategies for distributing energy. Adult fish will generally distribute energy between both growth and reproduction (Lucas, 1996), although if energy is limiting during reproductively active times, growth rates may decline to allow for successful reproduction. Similarly, energy in fish is described as being distributed in a “hierarchical

fashion" with maintenance costs being met first, followed by growth and reproduction (Beyers *et al*., 1999). It is possible that with enough available energy both somatic and reproduction tissue may undergo increased growth, however if energy is limiting then one or the other may be sacrificed, depending on an organism's life stage. For example, in male Atlantic salmon (*Salmo salar*), sexual maturity was delayed by having low mesenteric lipid reserves, suggesting that there is a lipid or reserve threshold these fish must reach for sexual maturation to occur (Rowe *et al*., 1991). Similarly, energy in migratory and resident brown trout (*Salmo trutta*) that is allocated towards reproductive or somatic purposes is influenced by the abundance of energy reserves, rather than any regulatory control (Jonsson and Jonssan, 1997).

At times when surplus energy is available and can be assimilated, it is stored in energy reserves. Juvenile rainbow trout generally find a balance between maximizing somatic growth during summer months and storing energy as lipid reserves (Post and Parkinson, 2001). Fish that are able to switch from somatic growth to lipid storage have a better chance of overwintering survival (Adams, 1999). As juvenile fish are highly susceptible to predation, it is important to escape the gape limit of predators by increasing in size, while it is also beneficial to maximize lipid stores for the winter months. This appears to be size dependent as larger fish are more likely to store energy as lipids and smaller fish likely to use energy to increase somatic production (Jonsson and Jonssan, 1997). For fish, energy storage can be found in several forms, such as lipids, mainly triacylglycerides (TAGs) (Higgs *et al*., 1995). TAGs are found in fish skeletal muscle, the area surrounding the abdominal mesenteries, and the liver (Adams, 1999). The liver is also an important reservoir in fish and stores energy in the form of glycogen (Hinton *et*

al., 2008). When energy is needed, individual glucose monomers are released from glycogen molecules during glycogenolysis in the liver or from fatty acids that are released from TAGs. Proteins can be an important source of energy, as salmonids are capable of catabolizing the high amounts of protein characteristic of marine and freshwater food sources. Amino acids from proteins provide the precursors for glucose (Higgs *et al*., 1995), which is synthesized in the liver and kidneys via gluconeogenesis (Johnston and Dunn, 1987).

Energy is transported to various tissue and organ systems as glucose or fatty acid. Within cells, these molecules are catabolized to release stored energy in the form of reducing equivalents (NADH and $FADH_2$) and adenosine triphosphate (ATP).

1.2 Modifiers of energetic cost

For an organism to function at optimal capacity there is an energetic requirement or cost, which can be influenced by environmental factors such as alterations in food availability and environmental temperature. Changes in food availability and environmental temperature alter the way energy is distributed within a biological system. These natural variables are common in temperate regions, where the changing seasons can produce cooler temperatures and shorter photoperiods. As a result, food is often limiting as primary productivity decreases and its decline felt throughout the food web during the winter. Aquatic organisms living in northern and southern temperate regions at must be able to survive periods of reduced food availability and cold temperatures.

Effects of environmental stressors on bioenergetics: food availability

Many species regularly encounter periods of decreased food availability. This can be when food sources decline, such as during overwintering conditions; or when an

organism is experiencing physiological changes, for example when adult salmon enter freshwater to spawn. If a deficit is created by lack of assimilated energy (less food), the minimal maintenance requirement may be met but at the expense of somatic or reproductive growth (Beyers *et al*., 1999; Vijayan and Moon, 1992). In a juvenile organism, this may display itself as reduced somatic growth or physical condition. For example, juvenile coho salmon (*Oncorhynchus kisutch*) kept under winter conditions (2.5^{o}C, fasted) experienced a significantly reduced condition factor compared to fish kept under 2.5^{o}C and fed (Larsen *et al*., 2001). In the wild, a reduction in overall condition may have significant negative consequences, as fish may not be able to forage effectively or avoid predation. Likewise, completely exhausting energy reserves have been documented to cause mortalities (Adams, 1999). Lipids constitute a major portion of the energy supply in fish. As the energy content per unit weight (energy density) in fish is a function of lipid concentration in the tissues (Trudel *et al*., 2005), complete depletion of lipid content could be detrimental to fish. Mortality has been attributed to starvation when whole-body lipid reserves of juvenile chum salmon decline to less than 2% (Akiyama and Nose, 1980).

Effects of environmental stressors on bioenergetics: alterations in environmental temperature

Acute temperature changes can affect the metabolic rates of ectothermic organisms and therefore alter energy use. Lower temperatures commonly experienced during seasonal shifts can alter biological processes in teleosts, such as reduced feed conversion (Levesque *et al*., 2005), reduced growth rates (Larsen *et al*., 2001), and reduced maintenance requirements (Brett, 1971). As well, metabolic rate can be reduced

by slower rates of enzymatic reactions. During this time, fish may conserve energy by becoming less active and reducing the time spent feeding and foraging (Johnston and Dunn, 1987); however with reduced energy intake, this may lead to lipid reserves being depleted at a faster rate (Lemly, 1996). Declining temperatures result in reduced maintenance requirements; for example, the maintenance requirement of sockeye salmon (*Oncorhynchus nerka*) was reduced by 50% when the surrounding water temperature was reduced from 17^{o}C to 5^{o}C (Brett, 1971). This is likely due to the reduced energy demand for cellular reactions that are associated with lower temperatures (Clarke and Fraser, 2004).

A rise in temperature will increase the metabolic rates of fish leading to increased energy demands and subsequent increases in the use of glycogen, lipid reserves, and/or oxygen consumption (Adams, 1999; Jobling, 1993). For example, increasing water temperature from 7.5 to 10^{o}C doubled the minimum metabolic rate in rainbow trout (Rodehutscord and Pfeffer, 1999). Growth rates of fish will generally increase with rising water temperatures; however, there must be enough food energy available to satisfy the higher metabolic demand (Lucas, 1996).

1.3 Costs of xenobiotic defence

Natural stressors can alter the energy budgets of teleosts by decreasing the amount of available energy. For example, the shorter photoperiod and colder temperatures of winter months can reduce the energy intake of fish through reductions in available food (Ref?). Reduced energy can therefore limit the amount of energy available for specific physiological processes such as chemical defence or repair.

Environmental contaminants entering a biological system must be removed or modified to a less toxic form in order to maintain chemical homeostasis and prevent toxic thresholds from being reached. Maintaining a working biotransformation system has been an evolutionary benefit providing a marked survival advantage to organisms. For this reason, the energetic demands of the defence system may be prioritized above that of other activities such as growth or reproduction, since it is difficult for an organism to grow or reproduce if its fitness is compromised. The ability to biotransform xenobiotics may pose a significant energy cost to an organism, however, the significance of the costs associated with chemical defence remains poorly understood. Several studies suggest that the cost accrued for defence may be substantial; for example, rainbow trout hepatocytes exposed to dehydroabietic acid (DHAA), a major component of wood-industry effluent, increased oxygen consumption and cellular heat production in a concentration-dependent manner (Rissanen *et al.*, 2003). In another study, concentrations of liver and muscle tissue ATP concentrations were significantly reduced in copper-exposed bluegill (*Lepomis macrochirus*). It was concluded that decreases in ATP in the liver were attributed to the energy requirements for detoxification (Heath, 1984).

There are several processes involved in chemical defence that may constitute a significant energy cost: 1) the generalized stress response, 2) biotransformation enzyme reactions, 3) efflux transporter proteins, and 4) repairing damaged biological systems.

Costs of xenobiotic defence: The physiological stress response

The presence of an environmental stressor such as a xenobiotic can initiate a chain of events that communicate to the body the need to mobilize resources to defend against the stressor. A number of xenobiotics have been demonstrated to induce a

physiological stress response, including crude oil (Alkini *et al*., 1996; Thomas and Rice, 1987), methyl mercury and mercury chloride (Bleau *et al.,* 1996), pulp and paper mill contaminants (Kennedy *et al.,* 1995) amongst others. The physiological stress response in fish has been described as having several stages; the alarm phase, characterized by behavioral or feeding changes, the adjustment phase, observed as increases in metabolism, and the fatigue phase, the exhaustion of resources, which can result in death (Beyers *et al*., 1999). These events are regulated by glucocorticoids, cortisol and catecholamines, which play an active role in the stress response (Kleinow *et al.,* 2008). In fish, stressors stimulate the hypothalamus to secrete hormones that result in the secretion of cortisol from the interrenal cells and catecholamines from chromaffin cells in the head kidney (Thomas, 2008). Cortisol initiates glycogenolysis in the liver, increasing the amount of circulating free glucose. This available energy can be put towards an immediate fight or flight response. Increases in activity also require increases in oxygen consumption to meet the energy demand. In juvenile steelhead (*Oncorhynchus mykiss*), for example, increased oxygen consumption correlates with elevated cortisol levels in stressed fish; it was also suggested by these authors that stress to juveniles may reduce available energy for other activities by approximately 25% (Barton and Schrek, 1987).

Cost of xenobiotic defence: Phase I, Phase II, and Phase III defence systems

Biotransformation enzymes appeared early on in evolutionary history, likely in response to natural toxins produced by plants and animals (Walters, 2011). Proteins that evolved to counter the adverse effects of toxins have been conserved in prokaryotes and eukaryotes (Schlenk *et al*., 2008) and have developed into the more specialized biotransformation enzymes and expulsion pumps used for cellular detoxification.

Biotransformation enzymes convert hydrophobic xenobiotics into more polar molecules that can be more readily removed from cells and excreted from the body. These proteins are classified as either Phase I or Phase II enzymes.

Phase I reactions are commonly mediated by the cytochrome P450 (CYP450) family of enzymes which play a major role in the biotransformation of numerous xenobiotics. These reactions include hydrolysis, reduction and oxidation reactions, whose purpose is to either introduce or expose a functional group (e.g. –OH, $–NH_2$, –SH, –COOH) onto a xenobiotic, resulting in an increase in molecular hydrophilicity. Several studies have attempted to indirectly determine the metabolic energy use associated with Phase I metabolism of xenobiotics. For example, rainbow trout hepatocytes exposed to increasing concentrations of pyrene correlated with significantly increased rates of oxygen consumption (Bains and Kennedy, 2004).

In Phase II reactions, large endogenous polar molecules are conjugated onto xenobiotics or their metabolites, greatly increasing their hydrophilicity; these reactions include sulfation, glucuronidation, acetylation, and methylation reactions (Parkinson and Ogilvie, 2010). A study examining the sublethal effects of chlorothalonil found that induction of the phase II enzyme glutathione-S-transferase (GST), in congolli (*Pseudaphritis urvillii*) resulted in a dose-dependent increase in oxygen consumption, suggesting a significant cost to Phase II conjugation of the compound (Davies *et al*., 1994).

Stress and the production of acidic Phase II conjugated metabolites may alter the acid-base balance of body fluids in organisms (Brown, 1993; Evans, 1987). Stress alters acid-base balance by affecting ion regulation by the epithelial tissue of fish gills

(Erickson *et al*., 2008; Evans *et al*., 2005; Evans, 1987). Marsupial consumption of certain plants containing high amounts of the allelochemicals terpene and phenol requires substantial Phase I and Phase II biotransformation activity (Foley, 1992). The production of a mixture of conjugated metabolites when consuming certain foods resulted in acidic urine (pH 5.7) of these animals. Since a major component was often glucuronic acid, it was concluded that severe acidosis was caused by the accumulation of organic acids during the biotransformation of plant toxins. The pH balance was restored rapidly since buffering internal pH is a priority (Foley, 1992), a process which is likely to have significant energetic costs associated with it.

Multixenobiotic resistance (MXR: sometimes referred to as Phase III of cellular defence) is the process by which organisms exposed to natural toxins or anthropogenic contaminants remove intracellular contaminants. The key proteins in the MXR process are members of the ATP binding cassette (ABC) superfamily. These are large membrane-bound enzyme complexes which function to efflux foreign compounds and reduce intracellular concentration. MXR complexes have the ability to efflux a variety of structurally unrelated hydrophobic compounds (Lawrence *et al*., 2003). One such complex, the permeability glycoprotein (P-gp) is a broad substrate specificity ABC transporter (Kleinow *et al*., 2008). This efflux pump has been localized to fish liver and bile canaliculi in trout (Sturm *et al*., 2001) and catfish (Doi *et al*., 2001), and functions to transport moderately hydrophobic compounds out of organs into excretory products such as urine or bile (Kleinow *et al.*, 2008). Efflux pumps come at a metabolic cost as it takes energy in the form of ATP to run active transport pumps. A recent study examined the fluctuations in adenylate concentration within cells that had been exposed to doxorubicin,

a P-gp substrate. It was observed that the efflux of doxorubicin resulted in decreased intracellular ATP concentrations, which were strongly correlated with increasing ADP, AMP, and inorganic phosphate concentrations. This indicates the efflux of the doxorubicin by P-gp may have a significant energetic cost, although whether this cost will affect an organism's energy budget is unclear (Hildebrand *et al*., 2009).

Cost of Xenobiotic defence: Repair

Many xenobiotics have a direct mode of action at target sites, however contaminants may also cause damage indirectly. If xenobiotics reach a threshold level of toxicity in cells, tissue and organ damage may occur. Tissue damage may create an added metabolic demand in order to restore cellular homeostasis. For example, a study using rainbow trout (*Oncorhynchus mykiss*) exposed to the insecticide permethrin found increased standard metabolic rates associated with tissue repair (Kumaraguru and Beamish, 1983).

Overall, fish exposed to xenobiotics that lead to higher metabolic costs whether it be through stress, Phase I, II or III defence, or damage repair (or all simultaneously) will reduce the amount of surplus energy available for other activities, such as somatic growth and reproduction (Beyers *et al.,* 1999; Chellappa *et al*., 1989; Heath, 1995; Lawrence *et al*., 2003). Energy in fish is described as following a distribution hierarchy, with maintenance costs being met first, followed by growth and reproduction (Beyers *et al*., 1999). When energy is limiting there must be a trade-off between detoxification and growth/reproduction, although the prioritization of energy to systems following maintenance requirements is unknown.

1.4 Pesticides

Synthetic pesticides are commonly used to control a variety of insects, plants, rodents and parasites, amongst other organisms. Pesticides are used globally in agricultural, domestic, and industrial applications, and may be applied to soil, water, weeds, or food. Depending on their method of application, pesticides may be transported far from their original site of application, by spray drift, soil erosion, run-off, volatilization or deposition as precipitation (McEwan and Stephenson, 1979). The organochlorines (OCs) are a group of pesticides that were heavily used during the 20th century; however due to their toxicity, persistence in the environment, and potential to bioaccumulate in organisms, many were banned in the 1970s. The organophosphorous (OPs) compounds, a group of pesticides developed shortly after the OCs, quickly replaced the OCs because they were less persistent and less bioaccumulative (Gupta, 2006). These are known as current-use pesticides (CUPs), to distinguish them from the more harmful "legacy" chemicals that have been banned from use. OPs are susceptible to hydrolysis, photodegradation and microbial degradation (Druzina and Stegu, 2007; Zhao and Hwang, 2009; Zheng and Hwang, 2006), allowing them to break down over time and reducing the potential to bioaccumulate (Gupta, 2006). Though CUPs are generally less persistent than OCs, many are still detected in the environment long after their initial application (Verrin *et al*., 2004). As well, many of these chemicals display lethal (Brewer *et al*., 2001; Laetz *et al*., 2009; Levine and Oris, 1999) and sublethal effects (Awasthi *et al*., 1984; Eaton, 1970; Ferrari *et al.,* 2007; Jarvinen *et al*., 1983; Huculeci *et al*., 1999; Little *et al*., 1990; Sandahl *et al*., 2005) at relatively low concentrations.

Organophosphorus (OP) compounds

OPs are estimated to be the most widely used class of CUPs in industrialized countries (Druzina & Stegu, 2007). The insecticides diazinon, and chlorpyrifos, for example, have been detected frequently in North American urban streams (USGS, 2001), and are both among the top 10 pesticide ingredients sold in British Columbia (BC) and Alberta, Canada (Enkon, 2005). There was a reported 4,666,709 kg of pesticide ingredients sold or used in BC in 2003, with 20 ingredients making up 93% of total sales; of those top 20, the OP diazinon accounted for 27,074 kg sold or 0.6% or the total (Enkon, 2005). In a sampling survey done by Environment Canada (2011) to determine the presence and level of priority pesticides, the OPs diazinon and chlorpyrifos were detected at 83% and 58% of sites sampled in the Lower Fraser Valley, BC, respectively. The BC Agricultural Land Reserve (ALR) accounts for 4.7 million hectares, 2.5 million hectares of which are used for agricultural purposes (Verrin *et al*., 2004). Much of this land is found within valley systems close to major waterways, increasing the risk of pesticide exposure to aquatic organisms, including Pacific salmonids.

OPs are organic phosphoric esters that act at muscarinic and nicotinic cholinergic receptors in central and peripheral neuromuscular junctions. The mode of action of OPs is to bind acetylcholinesterase (AChE) in the synaptic cleft and inhibit the enzyme from degrading and recycling its natural substrate acetylcholine (ACh) (Bradbury *et al*., 2008). The neurotransmitter's function is to bind to post-synaptic receptors on muscle fibers creating an action potential that stimulates muscle contraction. The loss of this regulatory control results in continuous polarization of the axon and overstimulation of the cholinergic system that in fish can lead to muscular spasms, convulsions, paralysis, and

eventually death (Carr and Chambers, 2001). Some parent OPs such as malathion do not have an especially high affinity for AChE, but metabolic activation within an organism alters chemical structure and toxicity is enhanced (Timchalk, 2006). Some OPs become a thousand fold (Tang *et al*., 2006) more toxic than the parent compound. Malathion is a phosphorodithioate compound (Gupta, 2006) and when oxidized by CYP450 enzymes, the more toxic malaoxon metabolite with a phosphate-oxygen (P=O) moiety is formed. The oxon metabolite phosphorylates the hydroxyl group on the serine residue within AChE, preventing interaction with its substrate ACh. Once phosphorylated, the P=O moiety will lose its substituent alkyl groups in a process known as "aging" (Sultatos, 2006). This binding of malaoxon to AChE is considered irreversible, as the reverse reaction is extremely slow. Therefore, new enzyme must be synthesized in order to regain regulatory control (Sultatos, 2006).

An estimated 15 million kg of malathion is used annually in the U.S., most being applied to agricultural crops, the remainder used in ditches, roads, buildings, particularly for medfly and mosquito control (U.S. EPA, 2006). In Canada, OPs such as malathion are used in domestic (lawns, gardens), industrial (landscaping, golf courses, forestry right of ways), and agricultural settings (crops, mosquito control). Although the reported sales from malathion in BC have decreased from 6691 kg to 4658 kg between 1999-2003, malathion was still detected at 22% of sites sampled in the Lower Fraser Valley during 2003-2005 (EC, 2011). Despite its marketed low persistence, malathion has been detected in waters of the Fraser River at concentrations considered to cause effects to aquatic biota (Verrin *et al*., 2004). A watershed study done in Puget Sound detected several of the OP insecticides such as chlorpyrifos, malathion, and diazinon at higher

concentrations in urban streams compared to agricultural streams (Stout, 1986; U.S. Geological Survey, 2001).

The persistence of malathion is largely dependent on pH, water temperature, sunlight, and the presence of a healthy microbial community. Malathion has been shown to be readily hydrolyzable under alkaline conditions (pH 8.0, 25^{o}C $t_{1/2}$=20 d, Druzina and Stegu, 2007), as well as higher temperatures (pH 8.0, 27^{o}C $t_{1/2}$=36 hrs, Wolfe *et al*., 1975) (Bondarenko *et al*., 2004; Zheng *et al*., 2006), and in the presence of sunlight (pH 8.0, $t_{1/2}$= 15 d, Druzina and Stegu, 2007). Several studies have shown that malathion is quickly degraded in the presence of bacteria (Wolfe *et al*., 1975; Zheng *et al*., 2006) but it can remain unchanged for months under aseptic conditions. Malathion was formulated to be slightly lipophilic in order to pass through the dermal layer or digestive tract of an insect and because of this can accumulate to a small degree in the lipid tissue of non-target organisms (Jokanovic, 2001). However, with an octanol-water partition coefficient (K_{ow}) ranging from 2.36 to 3.25 and water solubility of 1.45 mg/L, malathion is also able to associate with aqueous matrices such as the blood (ATDSR, 2003).

Malathion is relatively non-toxic to mammals (Jokanovic, 2001); the acute oral LD_{50} for rats was reported as 2000 mg/kg, while the dermal LD_{50} was measured at 4000 mg/kg (McEwan and Stephenson, 1979). Mammals contain high levels of carboxylesterases, that can hydrolyze ester-containing xenobiotics such as malathion to more easily excreted metabolites (Hosokawa and Satoh, 2006). Insects and fish do not have these enzymes in the same abundance (Jokanovic, 2001; Schlenk *et al*., 2006), consequently, malathion is bioactivated to its toxic conjugate malaoxon more quickly than endogenous carboxylesterases can break it down.

A review of acute toxicity data compiled from the ECOTOX database (USEPA, 2007), found a range of malathion LC_{50} values for rainbow trout (*Oncorhynchus mykiss*) to be 111-234 μg/L (Douglas *et al*., 1986; Macek and McAllister, 1970; Marking *et al*., 1984; Mayer, 1974; Post and Schroeder, 1971). Acute effects may include decreased heart rate and decreased gill oxygen uptake efficiency (McKim *et al*., 1987). Observations of fish exposed to sublethal aqueous concentrations of malathion have found inhibitory behavior such as loss of equilibrium, reduced feeding and swimming activity (Brewer *et al*., 2001; Little *et al*., 1990; Sandahl *et al*., 2005; Tierney *et al*., 2007a), and stimulatory behavior, such as muscle spasms, tremors, or hyperactivity (Heath, 1995; Bradbury *et al*., 2008). Sublethal aqueous concentrations of malathion were also found to alter red blood cell, hematocrit, hemoglobin, and leukocytes levels in catfish (*Ictalurus punctatus* and *Heteropneustes fossilis)* (Areechon and Plumb, 1990; Dutta *et al*., 1992), reduce resistance to *Yersinia ruckeri* infection in Japanese medaka (*Oryzias latipes*) (Beaman *et al*., 1999), and decrease gonadosomatic index, plasma testosterone and estradiol in *Monopterus albus* (Singh, 1989).

1.5 Measuring bioenergetic cost

Currently, concern exists for salmonids in the Pacific Northwest that are exposed to sublethal levels of CUPs combined with other environmental stressors, such as declining food resources and fluctuating water temperatures. Considering the widespread use of CUPs in Canada and the U.S., and the proximity of urban sites and agricultural land to major river systems, particularly in BC, it is likely that salmonids are exposed to OPs at some point in their life cycle. Chronic, sublethal concentrations of xenobiotics may not always produce direct or obvious effects in organisms: for example, if there are

significant energetic costs associated with chemical defence, low level exposures may affect energy budgets and the bioenergetics of these fish. This can be particularly significant for temperate fish species, if exposure occurs during winter under conditions of reduced food intake and lower environmental temperatures.

This may be particularly important for certain life stages of Pacific salmonids. The maintenance energy required for small fish is greater than that required for larger fish (Cho, 1992), therefore, if energy is limiting, xenobiotics that impact energy use may be much more detrimental to juvenile fish. This has been shown indirectly, as studies have found that sublethal levels of various contaminants can reduce the growth rate of juvenile fish (Arunachalam *et al*., 1980; Borgmann and Ralph, 1986; Heath, 1995; Oikari *et al*., 1988). A chronic exposure (40 d) to crude oil depressed food conversion ability and the growth of Atlantic salmon parr, presumably by increasing metabolic costs of maintenance (Vignier *et al*., 1992). Similarly, Coho salmon (*Oncorhynchus kisutch*) fry exposed to sublethal concentrations of toluene and naphthalene for 40 days experienced concentration-dependent decreases in growth (Moles *et al*., 1981), although they displayed the same feed intake as unexposed fish. This may be due to less energy being available for somatic growth, and more energy being allocated toward repairing damage and/or biotransforming the contaminants. The consequence of reduced growth rates may be prolonged time required to reach maturity, potentially increasing the risk of predation, increased overwintering mortality, and later in life, delaying reproduction.

Energy use in teleosts has been determined through several basic condition measures such as mass, growth rates, and condition factor (CF) (OECD, 1993). Growth is a reasonable indicator of energy status in fish when reproduction is not a priority

(Beyers *et al*., 1999; Jobling, 1993). Combining these measurements with measures of energy storage (e.g. liver glycogen, organo-somatic indices, lipid content and lipid classes) can provide further information on energy assimilation, energy use, and potentially allocation. If energy costs are typically higher in stressed fish, several additional parameters could be used as indicators of energetic use and include plasma cortisol (which is a stress-induced steroid hormone) and hematocrit (a measure of the aerobic or oxygen-carrying capacity in fish that increases during stress).

The objectives of this research were to determine whether sublethal exposure to the CUP malathion affects energy status as measured by several indicators (e.g. growth rate, glycogen and lipid content) under chronic over-wintering conditions of reduced feed and colder water temperatures. Research is needed to determine the possible adverse effects of increased energy use for xenobiotic defence and how this may impact organisms under energy-limiting conditions such as those found during overwintering periods. To accomplish this, juvenile rainbow trout were fed commercial diets or fasted and exposed to several sublethal concentrations of malathion.

2: Interactive effects of malathion exposure and fasting on bioenergetic indices of rainbow trout (*Oncorhynchus mykiss*)

2.1 Introduction

Pacific salmon play an important role in aquatic and riparian ecosystems of British Columbia (B.C.) and adjacent areas, both ecologically and commercially (Cooke *et al*., 2008; Naiman *et al*., 2002; Scholz *et al*., 2006). Concern over declining wild salmon stocks in this region have prompted investigations into possible causes (Laetz *et al*., 2009) including exposure to current-use pesticides (CUP) such as organophosphorus compounds (OPs) (Harris *et al*., 2008). OPs are generally perceived to be less of an environmental concern than the more toxic legacy chemicals (e.g. DDT), as they have shorter half-lives and are less likely to bioaccumulate in non-target organisms (Gupta, 2006). Depending on their method of application, pesticides may be transported far from their original site of application, either by spray drift, soil erosion, run-off, volatilization or deposition as precipitation (McEwan and Stephenson, 1979).

The widespread use of OPs in North America, and the proximity of urban and agricultural sites to major river systems have contributed to the detection of these chemicals in aquatic habitats (EC, 2011; Verrin *et al*., 2004). Despite their marked low persistence, OPs have been detected in many important salmon-bearing stream and river habitats along the west coast of Canada and the U.S. (USGS, 2001) at concentrations considered to cause effects to aquatic biota (Verrin *et al*., 2004). Sublethal levels of OPs have been noted to cause a range of adverse behavioural effects in salmonids, including

reduced feeding and swimming performance (Sandahl *et al*., 2005), and disruption of predator avoidance and homing behaviours in salmon (Scholz *et al*., 2000).

The amount of available ingestible energy for salmonids is often limiting in aquatic systems, and the allocation of assimilated energy directed first to those maintenance systems that are vital to maintaining homeostasis (Jobling, 1993). Environmental contaminants have the potential to be metabolically costly and must be removed or modified to a less toxic form in order to reduce intracellular concentrations and toxicity. Increasing energetic demands of the defence system may result in a trade-off of energy allocated to other systems, such as growth or reproduction, (Heath, 1995; Lawrence *et al*., 2003). There are several potential energy costs associated with xenobiotic exposure including the initiation of a physiological stress response (Barton and Schrek, 1987; Davis and Schrek, 1997), biotransformation reactions (Bains & Kennedy, 2004; Davies *et al*., 1994), xenobiotic and metabolite efflux pumps (use a Kurulec or Bard reference here), and the repair of damaged biomolecules, cells, or tissues (Kumaraguru and Beamish, 1983).

Any extra incurred energy costs due to sublethal contaminant exposure would likely have greater impacts on fish under conditions of energy limitation. Salmonids living in B.C. regularly encounter periods of decreased food availability and declining environmental temperature in winter. During overwintering periods, they may conserve energy by becoming less active and reducing the time spent feeding and foraging (Johnston and Dunn, 1987), however, with limited energy intake, lipid reserves often become depleted (Lemly, 1996a). An energy deficit created by a lack of assimilated energy may meet minimal maintenance requirements, but this is often at the expense of

somatic or reproductive growth (Beyers *et al*., 1999; Vijayan and Moon, 1992). The regulatory control of energy allocation between physiological systems and activities is not clearly understood. During winter, declining water temperatures can result in reduced maintenance requirements (Brett, 1971) through reductions in metabolic reaction rates (Clarke and Fraser, 2004). For example, in sockeye salmon (*Oncorhynchus nerka*), maintenance requirements are reduced by up to 50% when water temperatures are reduced from 17^{o}C to 5^{o}C (Brett, 1971). The "salmon overwintering strategy" put forward by Nagasawa (2000) suggests that salmon may overwinter in colder waters as a survival strategy; that during times of reduced food availability, Pacific salmon will seek out colder waters in order to decrease their metabolic rate and retain their lipid stores, an obvious energetic advantage in times of fasting (Clarke and Johnston, 1999). The "winter stress syndrome" described by Lemly (1996b) suggests that lower temperatures and reduced food combined with xenobiotic exposure can result in an enhanced reduction of energy reserves in fish, and possibly overall fitness (Lemly, 1993). The combined expenditure of maintaining a standard metabolic rate overwinter as well as chemical defence may result in an energy deficit that could negatively impact energy reserves. For example, sublethal levels of various contaminants have been found to slow the rate of growth of juvenile fish (Arunachalam *et al*., 1980; Borgmann and Ralph, 1986; Heath, 1995), and reduced early growth such as this has been associated with lower overwintering survival rates (Beamish *et al*., 2004), therefore xenobiotic exposure during winter may impact the overwintering success of salmonids.

Little information exists regarding the potential indirect adverse effects of xenobiotic defence through increases in energy use. The objectives of this study were to

determine whether sublethal concentrations of the CUP malathion affects bioenergetic parameters by increasing the energy use in juvenile salmonids, particularly under chronic over-wintering conditions of reduced feed and cold temperatures. To accomplish this, juvenile rainbow trout were fed commercial diets or fasted while being exposed to several sublethal concentrations of malathion and examined for changes in fish bioenergetics through several indirect measures.

2.2 Materials and Methods

2.2.1 Fish

Juvenile rainbow trout (*Oncorhynchus mykiss*) with an average body weight (BW) of 12.5±2.5 g (mean±SD), length 9.0±0.6 cm were obtained from Miracle Springs Trout Farm (Mission, BC). Fish were sorted by size to reduce mass variation (OECD, 1993) between individuals and to avoid extensive hierarchy formation that could lead to unbalanced feeding (Jobling, 1993). Fish were acclimated to laboratory conditions for 4 weeks prior to experiments. Fish were housed in 450 L fiberglass tanks supplied with a continuous flow of dechlorinated municipal water (pH 6.8, hardness 6.3 mg/L $CaCO_3$, O_2 saturation 91%, temperature 10^{o}C) under a 10 h light:14 h dark photoperiod. Pacific feed® for salmonids (2 to 4 mm) was used and consisted of 50% protein, 18% fat, 2% fiber, remaining 30% vitamins and nutrients (EWOS Canada Ltd). All work conducted with the animals was in accordance with Canadian Council of Animal Care (CCAC) guidelines, under an approved SFU Animal Care protocol.

2.2.2 Chemicals

Malathion was obtained from Gardex Chemicals Ltd., (commercial grade 96.5% pure chemical, the remainder reported as inert compounds). Reagent grade ethyl alcohol (Commercial Alcohols, Brampton, ON, Canada), acetone (Anachemia Canada Inc., Montreal, QC, Canada), and HPLC grade hexane (EMD Chemicals Group, Gibbstown, N.J, USA) were used in lipid extraction analysis.

2.2.3 Exposure

Exposure to malathion was achieved in a flow-through system with fish receiving a continuous water flow of at least 1 L/min/kg fish. Trout (n = 30) were randomly assigned to tanks following acclimation, and divided into two groups, those that were fed daily (2% BW/day) (Gourley and Kennedy, 2009) and those that were fasted. Feeding treatments were exposed to either a solvent control, low, or high malathion concentrations, resulting in 6 treatment groups. All treatment groups were performed in duplicate. Sublethal concentrations of malathion were calculated as 1% and 10% of the average LC_{50} values for rainbow trout (USEPA, 2007). Malathion delivered to tanks was *via* a peristaltic pump mixed with dechlorinated water (final concentrations in treatment tanks was 1.5 and 16.2 μg/L). In stock solutions (made fresh every 2 d) malathion was dissolved in analytical grade ethanol (0.002% v/v) to ensure adequate solubility in the tank water. Water samples were taken from all treatment tanks and stored in solvent rinsed amber glass jars and analyzed for malathion concentration using OP/carbamate assay kits (Abraxis, 2010).

2.2.4 Morphometric and biochemical parameters

Fish (n=10) in each tank were randomly sampled and weighed following light anaesthetization with 0.3 g/L $NaHCO_3$-buffered tricaine methanesulfonate (MS 222 [Argent Chemical Laboratories, Redmond, WA, USA]) every 2 weeks during the exposure period. At the end of the exposure period (85 d), fish were sacrificed by anesthetization in 0.5 g/L buffered MS222. Blood was then collected from the severed caudal vein in heparinized capillary tubes and centrifuged for 3 min at 700 x *g*. Hematocrit was measured and the remaining plasma stored in microtubes at -80^{o}C. The liver was removed and weighed, and stored frozen at -80^{o}C for glycogen analysis. Frozen liver samples were thawed and homogenized in distilled water using a Mixer Mill 300 with 2 mm stainless steel beads (Retsch GmbH & Co., Hann, Germany). Glycogen was determined by measuring glucose concentration before and after the addition of glucoamylase (Biovision Glycogen Assay Kit, 2010). Frozen plasma was thawed and cortisol was measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits (Neogen, 2010).

The gastrointestinal (GI) tract along with the visceral mesenteries were dissected out and stored in chloroform containing butylated hydroxytoluene (BHT) at -20^{o}C until further analysis. Duplicate samples of muscle fillets were also removed from the dorsal side of a fish between the dorsal and adipose fins, and stored in the dark in chloroform containing BHT at -20^{o}C (Kang et al., 1998).

2.2.5 Muscle lipid content

Chloroform-methanol lipid extraction was conducted on all muscle tissue from fed and fasted fish and GI tracts from only fasted fish (Folch *et al.*, 1957). Muscle tissues (0.5 g) were homogenized over ice using a Polytron Series PT homogenizer (Kinematica Inc., Bohemia, NY), and stored overnight in a chloroform and methanol solution (2:1 v/v). Homogenized samples were filtered using Whatmann® size 4 filter paper into solvent rinsed tubes and 4.5 mL of a 0.88% NaCl solution was added to separate the aqueous and organic layers. The aqueous layer was removed and 2 g $NaSO_4$ added to remove any remaining water. The lipid-containing organic layer was transferred to a pre-weighed solvent rinsed tube and the solvent removed under a gentle stream of nitrogen for approximately 40 min. The remaining lipids and tube were weighed and the percentage of total muscle lipid calculated using the original muscle sample weight. Muscle samples were also prepared for lipid class analysis: extracted lipids were dissolved in chloroform to a concentration of 15 mg/mL, nitrogen purged and stored in the dark at -20^{o}C until further analysis (Kang et al., 1998).

2.2.5.1 Lipid class analysis

The procedure for lipid class separation was adapted from Kang *et al*. (1998) and lipid classes determined using normal phase thin-layer chromatography and flame ionization detection (TLC-FID), using an Iatroscan MK-5 TLC/FID Chromatographic Analyzer (Iatron Laboratories Inc., Tokyo, Japan). The thin layers consist of silica coated onto a quartz rod (chromarods) with diameter 0.9 mm and length 15 cm. The Iatroscan was standardized using lipid concentrations ranging from 2 to 10 mg/mL (Doosan Serdiary Research Laboratories, Toronto, ON). Concentrated muscle lipid

extracts (1 µL) were spotted onto triplicate chromarods and the samples focused at the point of origin by placing in a focusing chamber containing acetone, and repeated once. Chromarods were deactivated in a humidor for 5 min (76% solution NaCl), then developed for 20 min in a saturated elution chamber containing hexane, ethyl ether and formic acid (65:35:0.04 v/v/v). When development was complete, rods were removed and dried in an oven at 100^{o}C for 3 min, then processed through the flame ionization detector at a speed 0.36 cm/sec. Flow rates were maintained at 160 mL/min hydrogen and 2000 mL/min for air, as recommended for optimal detector response and reproducibility (Iatron Laboratories Inc., Tokyo, Japan).

2.2.6 Calculations and statistical analysis

Specific growth rates (MGR) were calculated using the following formula (Jobling, 1983):

$$MGR = (\log W_1 - \log W_0) / (t) \times 100 \quad [1]$$

Where W_1 is the final weight, W_0 is the initial weight, and t is the time in days. Condition factor (CF) was measured as the ratio of body mass to cubic fork length using:

$$CF = (BW/L^3) \times 100 \quad [2]$$

The LSI was calculated as:

$$LSI = (\text{Liver weight}/BW) \times 100 \quad [3]$$

All statistical analyses were performed using JMP 8.0.2 (SAS Institute Inc., Cary, NC, USA), except for slopes of graphs which were calculated using Prism® (Graphpad Software Inc., San Diego, CA, USA). Results are reported as mean ± standard error of the mean (SEM). Data was tested for normality of residuals using a Shapiro-Wilk test. Outliers were determined using Grubb's test (GraphPad Software Inc., San Diego, CA,

USA). Duplicate tanks for all treatment groups were t-tested to ensure there were no tank effects. Where no differences were detected ($p<0.05$) data from each fish in the replicate tanks was combined and subsequent statistical analysis performed on pooled data. A Student's t-test was used to determine if there were significant differences between fed and fasted groups for all condition, energetic, and stress indicators. Effects from malathion exposure within either fed or fasted treatments were analyzed using one-way analysis of variance (ANOVA, $p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). When data was not normally distributed, it was log transformed and analyzed as above. Where log transformed data was not normally distributed, a non-parametric Kruskal-Wallis test was used.

2.3 Results

2.3.1 Water chemistry:

Water temperature ranged from 9.5 to 11.7^{o}C during the 12-week exposure period. Malathion was detected in the high malathion concentration tanks for both fed and fasted treatment groups, with an average concentration of 14.0±2.3 μg/L (mean±SD), which is within 15% of the target concentration of 16.2 μg/L. Control and low tanks had non-detectable levels. Water quality parameters were maintained within suggested guidelines as per the OECD (1993) (Table 1).

Table 1: Summary of water quality parameters measured during a 12-week exposure of juvenile rainbow trout to control, 1.5, and 16.2 μg/L concentrations of malathion. Water temperature was monitored every 2 days; pH and dissolved oxygen were measured weekly. Values from all tanks were averaged for each month.

	March	April	May	June
Temperature (^{o}C)	9.5	10.0	10.8	11.7
Dissolved oxygen (% saturation)	90	79	96	101
pH	6.6	6.9	6.8	6.7

2.3.2 Mass, growth rate, and condition factor

Figure 1 shows the changes in mass over time and associated regression lines for fed and fasted fish exposed to either a solvent control, low or high malathion concentrations. Fed fish had significantly higher body mass than fasted fish observed 2 weeks after exposure began and continuing for the entire 12-week exposure period. Fed fish in all treatment groups had increased their mass by at least 300% by the end of the 12-week exposure period, whereas fasted fish lost mass during the 12-week exposure period. There were no significant differences in the growth rates or slopes for fed or fasted groups exposed to different malathion concentrations. However, body mass for fed fish in the high malathion exposure group was significantly higher than the body mass in the control group at weeks 8 and 12 of the exposure (Figure 1).

Mean growth rates (MGR) for fed and fasted fish during the exposure period are shown in Figure 2. Fed fish displayed positive MGRs for the entire exposure period, whereas fasted fish exhibited mainly negative MGRs, with the exception of week 12 where the fed control displayed a negative MGR and the fasted control a positive MGR. For fed fish, only the control group at week 12 showed a significantly decreased MGR, compared to the low and high malathion exposure groups. Similarly, fasted control fish at week 12 had significantly increased MGR compared to the low and high malathion exposure groups (Figure 2).

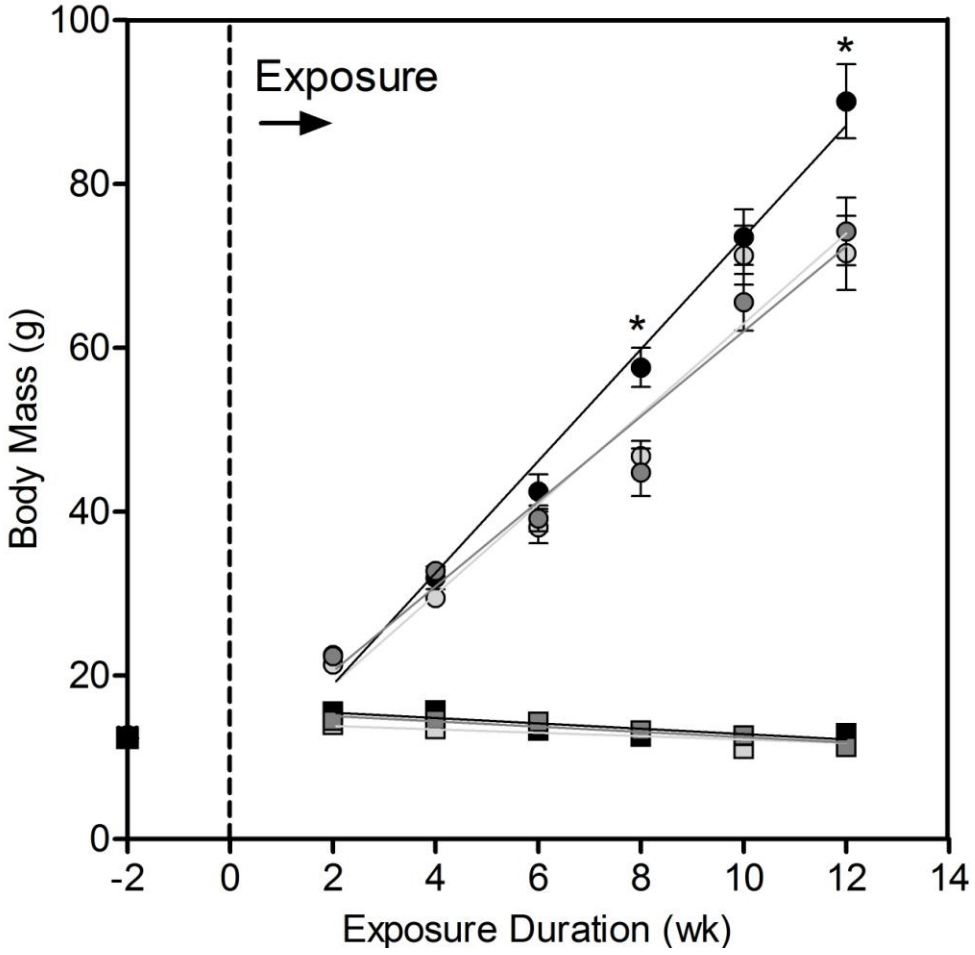

Figure 1: Fed fish had significantly higher body mass than fasted fish during the 12-week exposure period. Fed fish exposed to high malathion concentration showed greater body mass than the controls at weeks 8 and 12. Data points indicate the mean±SEM (n=20). Effects from malathion exposure were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). *****Indicates body mass in high treatment group was significantly higher than the control for individual time point. (□) Fasted control; (■) fasted low; (■) fasted high; (○) fed control; (●) fed low; (●) fed high.

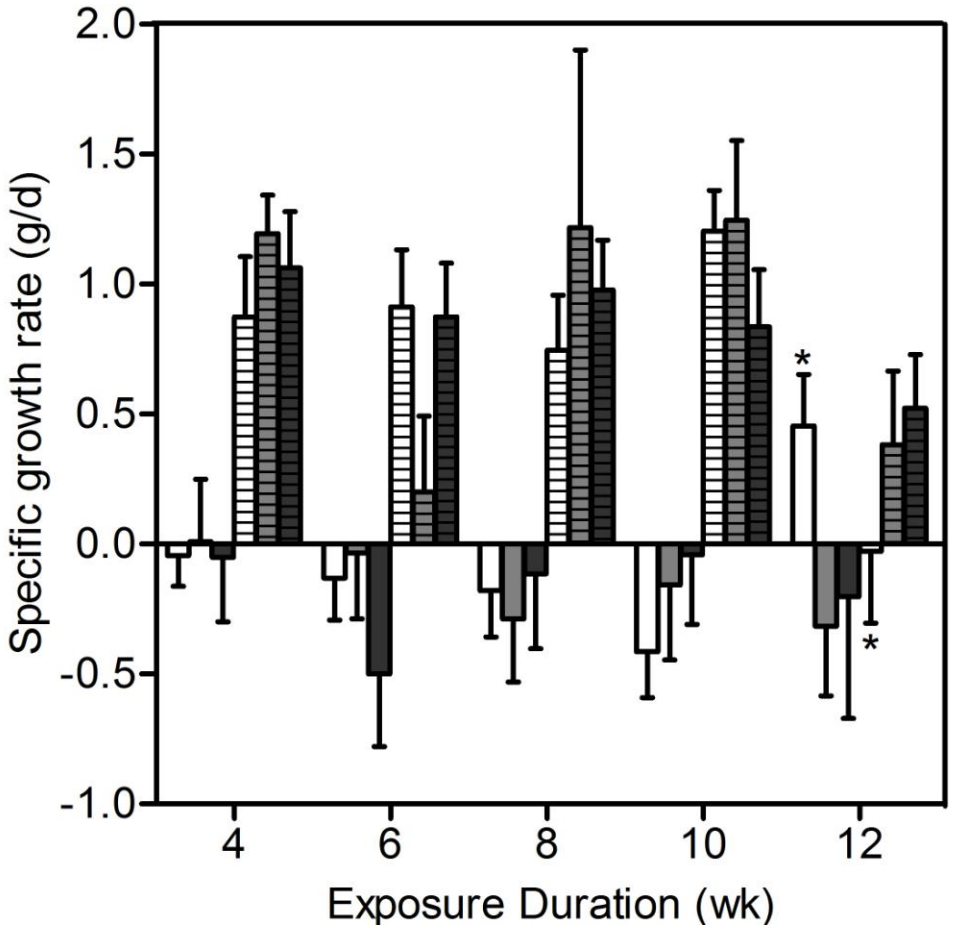

Figure 2: Mean weekly growth rates (MGR) for fed and fasted juvenile rainbow trout exposed to control, low, and high malathion concentrations for 12 weeks. Data point indicate the mean±SEM (n=20). Effects of malathion exposure to body mass and slopes were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). *Indicates week value is different for week 12. (□) Fasted control; (▒) fasted low; (■) fasted high; (▤) fed control; (▒) fed low; (■) fed high.

Figure 3 shows changes in fish condition factors (CFs) over the exposure period in both fed and fasted fish exposed to varying concentrations of malathion. The CFs at the end of the 12-week exposure period for fed fish (1.71±0.02 g/cm^3) was significantly higher than the value for fasted fish (1.33±0.02 g/cm^3). There was a trend for CFs to decrease with time for fed fish, however no significant difference was found between malathion treatment groups at any given time points or in the rate of change of CF values over time. In a similar manner, there was a trend for CFs to decrease over time for fasted fish, with no significant differences seen between malathion treatment groups at any given time point with the exception of week 2 where the fasted control CF value was significantly higher than the low malathion exposure group.

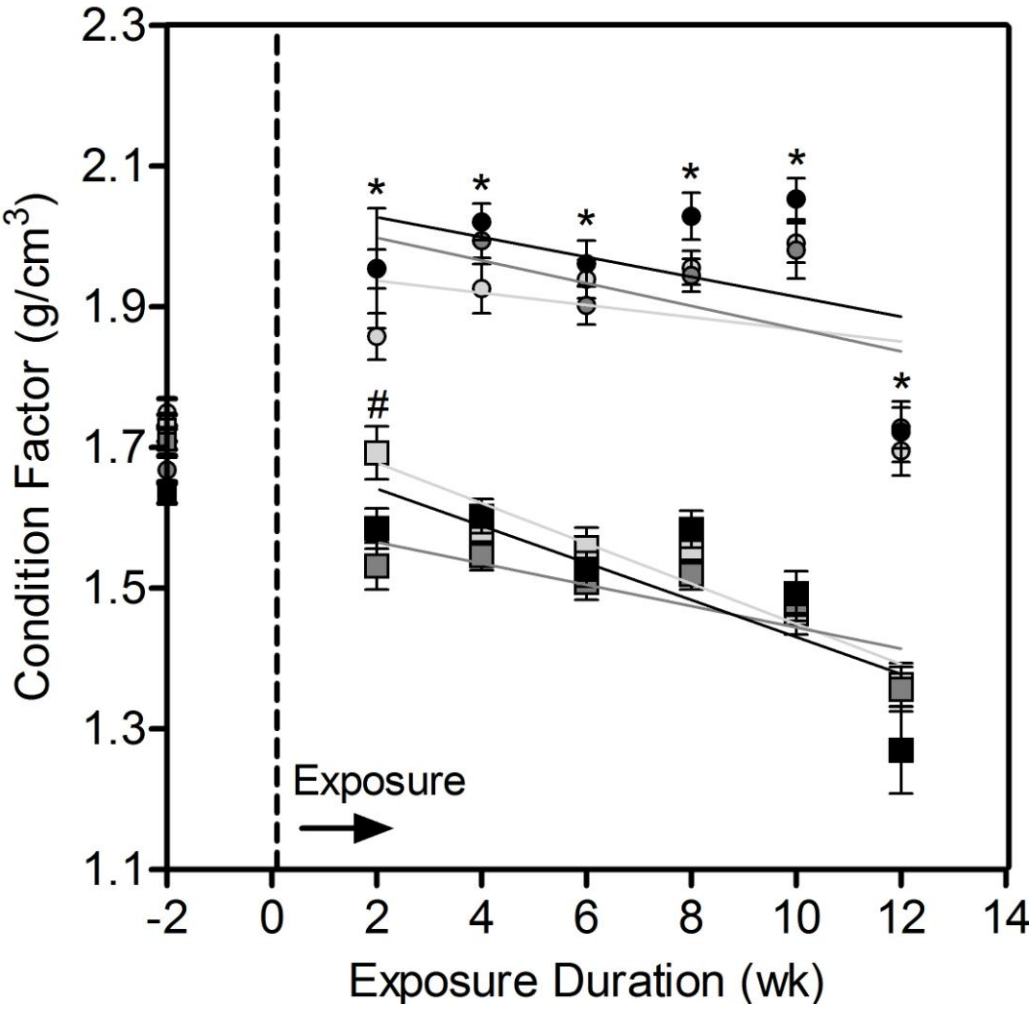

Figure 3: Condition factor for fed and fasted juvenile rainbow trout during a 12-week exposure to control, low, and high malathion concentrations. Data points indicate the mean±SEM. Effects from malathion exposure were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). *Indicates the mean for fed fish are significantly greater than fasted fish. # Indicates the mean between malathion exposure groups are significantly different within either the fed or fasted groups. (□) Fasted control; (▒) fasted low; (■) fasted high; (○) fed control; (●) fed low; (●) fed high.

2.3.3 Stress Indices: Plasma cortisol and hematocrit

Both hematocrit and plasma cortisol concentrations in fed and fasted fish are shown in Figure 4. Fed fish had significantly higher hematocrit values compared to fasted fish in the control group (38.4±0.7% and 31.0±0.7% for fed and fasted fish, respectively) and in the high malathion treatment group (35.4±1.1% and 30.1±0.9%, respectively) but not the low malathion treatment group. Within fed fish, there were no significant differences in hematocrit values between malathion treatment groups, however, within the fasted treatment group, fish exposed to low malathion concentrations had elevated hematocrit levels (38.2±1.0%) compared to the high malathion treatment group and controls (Figure 4A).

Significantly higher plasma cortisol concentrations were found in fed fish compared to fasted fish for the control (4.4±1.2 and 1.2±1.3 ng/mL for fed and fasted fish, respectively) and high (6.2±1.2 and 2.1±1.2 ng/mL, respectively) malathion treatment groups but not the low malathion treatment group. There were no differences found between malathion treatment groups for either fed or fasted fish (Figure 4B).

2.3.4 Energetic indices: liver somatic index (LSI), glycogen, lipid content and lipid classes

Fed fish had significantly higher LSI values compared to fasted fish for the control (0.86±0.02% and 0.77±0.03% for fed and fasted fish, respectively) and high (0.82±0.02% and 0.72±0.02%, respectively) malathion treatment groups but not the low exposure group (Figure 5A). Fed fish also had significantly higher liver glycogen levels compared to fasted fish for the low malathion exposure (3.9±0.3 mg/g and 1.2±1.1 mg/g

liver, for fed and fasted fish, respectively) (Figure 5B). There were no differences found between malathion treatment groups for either fed or fasted fish

Total muscle lipid concentrations were significantly higher in fed fish compared to fasted fish, for the control (32.5±1.1 mg/g and 17.7±1.1 mg/g, for fed and fasted, respectively), low (33.0±1.1 mg/g and 23.0±.1.1 mg/g, respectively) and high exposure groups (36.2±1.1 and 18.8±1.1 mg/g, respectively). There were no significant differences found between exposure treatment groups for fed or fasted fish in total muscle lipid values (Figure 5C).

Quality control testing done to ensure samples were not compromised during storage and transport found no significant differences between samples that had been stored at different temperatures or frozen repeatedly were found. In Figure 6 the concentrations of individual lipid classes in muscle tissues from fed and fasted fish exposed to different concentrations of malathion are shown. Fed fish had significantly higher amounts of triacylglycerides (TAGs) compared to muscle from fasted fish for all malathion exposure groups (average 26.66±2.49 mg/g muscle and 10.72±1.29 mg/g muscle, for fed and fasted, respectively) (Figure 5). Diacylglycerides (DAG) were similar between fed and fasted fish (average 1.29±0.15 mg/g muscle and 1.04±0.11 mg/g muscle, respectively), whereas phospholipids (PLs) were lower in fed fish compared to fasted (average 5.44±0.18 mg/g muscle and 9.47±0.66 mg/g muscle, respectively). Malathion exposure had no effect on the concentrations of individual lipid classes in muscle in either fed or fasted fish (Figure 6).

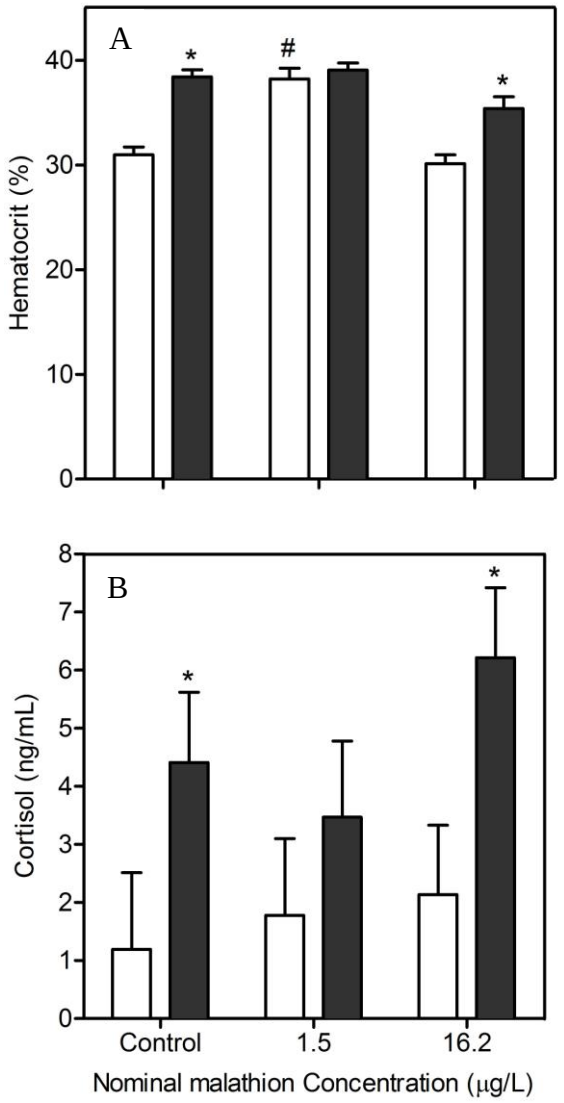

Figure 4: Stress indices for fed and fasted juvenile rainbow trout after 12-week exposure to control, low, and high malathion concentrations. A: Hematocrit B: Cortisol, clear bars fasted, shaded bars fed. Values are given as the mean±SEM. Effects from malathion exposure were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). *Indicates the mean for fed fish are significantly greater than fasted fish. #Indicates the mean between malathion exposure groups are significantly different within the fed or fasted group.

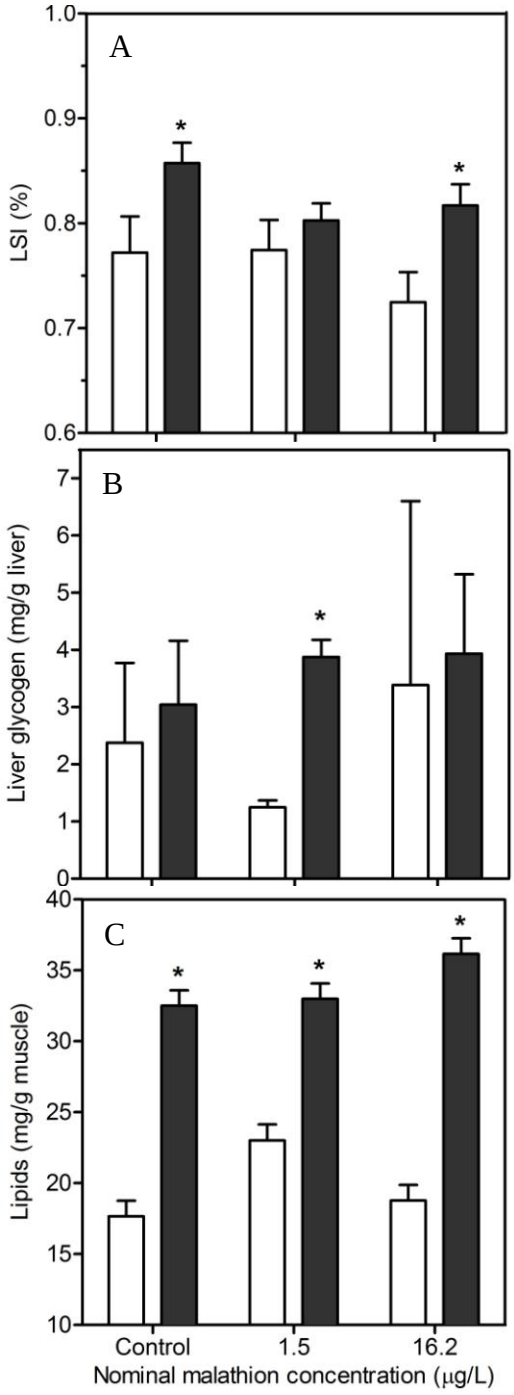

Figure 5: Energetic indicators of fed and fasted fish after a 12-week exposure to control, low, and high malathion concentrations. A: Liver somatic index (LSI) B: Liver glycogen C: Total lipids in muscle tissue, clear bars fasted, shaded bars fed. Values are given as the mean±SEM. Effects from malathion exposure were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). *Indicates the mean of exposed fed fish was significantly higher than fasted fish.

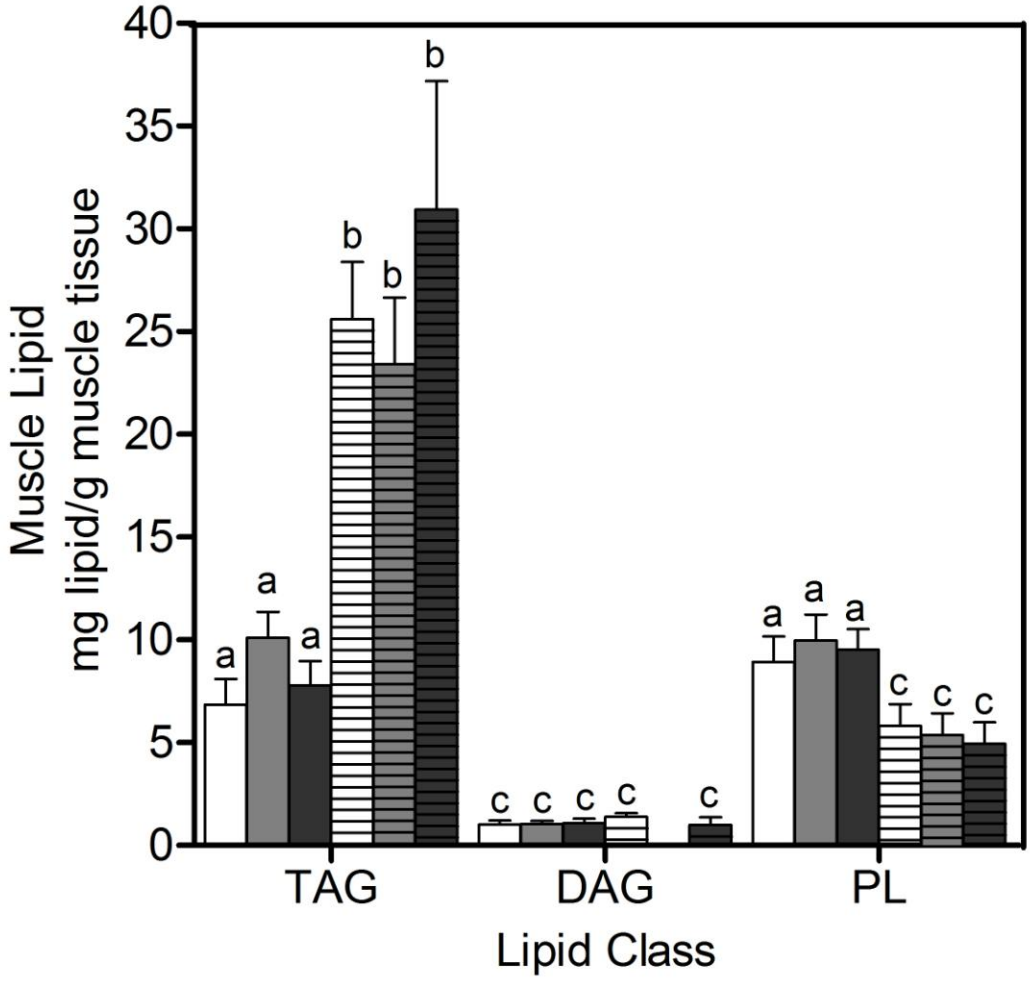

Figure 6: Lipid classes measured in muscle tissue of juvenile rainbow trout during a 12-week exposure to control, low, and high malathion for fed and fasted fish. Values are given as the mean±SEM. Effects from malathion exposure were detected using ANOVA ($p<0.05$), followed by Tukey-Kramer's HSD multiple comparison test ($\alpha=0.05$). Bars not connected by the same letter are significantly different. Lipids are classified as triacylglycerides (TAG), diacylglycerols (DAG), and phospholipids (PL). (□) Fasted control; (▒) fasted low; (■) fasted high; (▤) fed control; (▒) fed low; (■) fed high.

2.4 Discussion

Overwintering is a critical time for juvenile salmonids when fasting conditions and declining water temperatures present suboptimal conditions for obtaining energy, constraining fish to lipid reserves as the sole source of energy. Fish that are able to adjust more easily from the process of somatic growth to that entailing lipid storage have a better chance of overwintering survival (Adams, 1999), since completely exhausting energy reserves has been documented to cause mortalities (Adams, 1999). For this reason juvenile salmonids generally find a balance between maximizing somatic growth and storing energy as lipid reserves during the summer months (Post and Parkinson, 2001).

The presence of CUPs in North American freshwater environments has been confirmed by a number of water quality monitoring programs (EC, 2011; Verrin *et al*., 2004), highlighting the likelihood of exposure in juvenile salmonids during their freshwater life stage. The combined effects of sublethal CUP exposure and reduced energy availability, on the energetic status of salmonids during overwintering are largely unknown. Reduced energy assimilation in conjunction with a potential increase in metabolic costs brought on by xenobiotic exposure and resulting chemical defence could lead to reduced survival of young fish, particularly juveniles in their first year (Lemly, 1996).

Growth measurements in fish can have a substantial degree of uncertainty as the endpoints can be affected by many factors, such as environmental temperature, energy content of food, and swimming activity (Jobling, 1993), however, growth is still considered a useful endpoint when examining effects from natural or man-made stressors,

particularly in studies involving bioenergetics. In this study, fed fish significantly increased body mass (300% increase) and had positive MGRs over the 12-week exposure period, although MGR values declined over time. Abundant food sources allow fish to meet the minimum metabolic requirement and experience increased rates of growth, evidenced by positive growth rates. Reductions in mass due to fasting were expected, and were observed in feed deprivation (Vijayan and Moon, 1992) and fasting (Damsgard and Gill, 1998) studies. The response to fasting conditions in the present study was a significant reduction in body mass (17-20%) over the 12-week exposure period with negative MGRs over time. A similar mass reduction was observed in juvenile trout fasted for 9 weeks (~30% mass reduction) (Gourley and Kennedy, 2009). Further evidence of fasting conditions was lower CF in fasted fish compared to fed fish. Fulton's method of determining the condition of fish describes the relationship between body mass and length, and will increase if mass increases or length decreases relative to the other. Reduced CF under fasting conditions has been observed in several past studies comparing fed salmonids versus those that are fasted (Barton *et al*., 1988; Larsen *et al*., 2001; Vijayan and Moon, 1992).

To maintain energy levels during times of fasting or stress, salmonids will preferentially utilize lipid stores, however liver glycogen can also provide significant energy in the form of carbohydrates. A higher amount of glycogen has been positively correlated with increased LSI (Simpkins *et al*., 2003) and can be a reasonable indicator of energetic status. Fed fish in this study had higher liver glycogen concentrations and LSI values compared to fasted fish, observations that are similar to previous studies (Barton *et al*., 1988; Gourley and Kennedy, 2009; Vijayan and Moon, 1992). This can be attributed

to fasted fish depleting liver glycogen stores, as glycogen is used to maintain blood glucose levels, consequently reducing liver mass.

Lipids may provide up to 2 fold more digestible energy per weight than protein (Higgs *et al*., 1995) and are an essential biological component for growth, membrane structure, and function. Fish mobilize energy reserves during critical periods related to survival when food is not abundant or energy costs increase (Naesje *et al*., 2006), and therefore lipid content (or stores) can be considered ecologically relevant indicators of energetic status in salmonids (Toneys and Coble, 1980). In the present study, fasted fish were found to have significantly lower concentrations of muscle lipid compared to fed fish, although not at levels that would be considered to be detrimental to fish health (Akiyama and Nose, 1980). This is not surprising, as juvenile rainbow trout have been shown to be able to survive fasting conditions for up to 147 days (Simpkins *et al*., 2003). Reduced lipid levels are normally seen during winter months (Cunjak, 1988; Naesje *et al*., 2006), and more specifically, it has been observed that the highest rate of lipid depletion occurs in early winter, when food supply and water temperatures drop rapidly, rather than in late winter when conditions are least favourable but are stable (Simpkins *et al*., 2003).

In salmonids, lipid classification is often based on polarity, with nonpolar lipids such as TAGs and DAGs serving as the main energy storage forms, and polar lipids such as PLs serving as essential structural components of biological membranes (Higgs *et al*., 1995). When energy is needed, storage lipids (TAGs) and intermediary lipids (DAGs) are used preferentially (Simpkins *et al*., 2003), with structural lipids (PLs) and proteins being used as a last resort (Adams, 1999; Naesje *et al*., 2006). Fasted and fed fish in this

study had similar amounts of DAGs and PL, although fed fish showed greater amounts of storage lipids (TAGs). This was not surprising as fasted fish were expected to mobilize their TAG stores to meet the requirements of minimum metabolic rate and movement.

Cortisol is considered one of the primary biochemical indicators of acute stress in fish, and elevated levels have been observed in response to a variety of environment stressors (Barton and Schrek, 1987; Thomas, 2008). The higher plasma cortisol concentrations measured in fed fish compared to fasted fish coincides with reports from previous studies (Thomas *et al*., 1986; Vijayan and Moon, 1992), although the concentrations for both groups were not above those measured in resting, non-stressed juvenile Pacific salmon (0-10 ng/mL) (Schreck, 1981). The packed red blood cell volume, or hematocrit is a secondary indicator of stress, and is often increased in the presence of cortisol, in response to acute stress or environmental perturbation (Heath, 1995; Schlenk *et al*., 2008). In this study, higher hematocrit values were consistent with higher cortisol concentrations in fed fish, although neither parameter was considered to be out of range of normal values. Hematocrit in Pacific salmon is normally in the range of 20-40% (Randall and Wright, 1995).

Effects from malathion exposure was observed in the greater body mass of exposed fed fish compared to unexposed controls during weeks 8 and 12. Greater body mass in these groups did not relate to significantly elevated energy storage (LSI, liver glycogen or muscle lipid concentrations. It is possible that malathion stimulated the appetite of exposed fish. Although this is in opposition to a study that found reduced strike feeding activity in rainbow trout exposed to sublethal concentrations of parathion (Little *et al*., 1990), as well as a study that correlated inhibition of brain AChE with

reduced feeding activity, upon exposure of Coho to sublethal levels of chlorpyrifos (Sandahl *et al*., 2005). No differences were observed in CFs between chemical treatment groups for fed or fasted fish. There can be a substantial degree of variability when using growth (Woltering, 1984) and CF as biological endpoints (although very valuable as ecologically relevant endpoints), as these parameters are affected by a number of variables including water temperature variation, energy intake, and competition between individuals. Extraneous variables can act synergistically to either increase, decrease, or produce no effect on the final growth endpoint (Schlenk *et al*., 2008). For example, previous studies have found reduced CFs in juvenile bluegill *(Lepomis macrochirus)* associated with selenium exposure (Lemly, 1993). In contrast, CF was not affected when Gudgeon (*Gobio gobio*) were exposed to sewage effluent (Faller *et al*., 2003). Effects from malathion may not have been observed for several reasons. There may be low energy costs associated with the defence strategies resulting from malathion exposure such as its biotransformation, or the sublethal concentrations of malathion used in this study may not have been high enough to cause significant or measurable changes in the selected endpoints.

Teleosts exposed to xenobiotics have displayed depleted glycogen reserves often coinciding with decreasing LSI values (Awasthi *et al*., 1984; Simpkins *et al*., 2003). Depleting liver reserves are thought to compensate for the additional energy needed to cope with environmental stressors (Heath, 1995). There were no significant differences observed in glycogen concentrations or LSI values when fed and fasted fish were exposed to malathion. However, large variation in some of these endpoints, particularly glycogen, may potentially mask any detectable effects of malathion. This is in contrast to

a study, which observed that sublethal malathion exposure caused a gradual reduction in liver glycogen content of a freshwater teleost (Awasthu *et al.,* 1984). Trout are capable of maintaining their liver weight relatively long periods before liver mass is reduced (Simpkins *et al*., 2003). Salmonids have an innate ability to produce glucose during gluconeogenesis, using non-carbohydrate sources, such as lactate, glycerol, and amino acids (Higgs *et al*., 1995). Fish in this study may have compensated for the increased energy demand of malathion exposure by using non-carbohydrate precursors during gluconeogenesis to maintain blood glucose levels and therefore reduce the use of glycogen stores.

Measuring the level of lipid stores in fish has been used as an indicator of xenobiotic impact on the energetic status of fish. For example, the total lipid content in eel (*Anguilla Anguilla)* was found to decrease when exposed to sublethal concentrations of the OP fenitrothion (Sancho *et al*., 1998). In contrast, some studies have found increased lipid stores when exposed to xenobiotics. Overwintering in mining effluent-exposed waters resulted in greater levels of body lipids and TAGs for Northern pike (*Esox lucius*), burbot (*Lota lota*) (Bennett and Janz, 2007) and juvenile fathead minnows (*Pimephales promelas)* (Driedger *et al*., 2009). The increased lipid stores were attributed to several direct and indirect factors, such as contaminated effluent causing an increase in primary productivity of the lakes and therefore increased food supply. Size-dependent mortality was also suggested, as smaller fish with less energy stores would be more likely to experience a higher rate of mortality overwinter, leaving the larger survivors with more energy stores to be sampled in spring. No significant differences were detected in muscle lipid content between malathion exposure groups in fed or fasted fish. As there were no

additional nutritional inputs or significant size-dependent mortality in our study, it is possible fish in our study experienced a reduction in metabolic rates due to lower temperatures, which allowed them to retain their lipid stores (Nagasawa, 2000). Malathion exposure had no effect on the amounts of TAGs or PLs within the fed or fasted group, however juvenile Northern pike in effluent contaminated lakes also showed no difference for muscle TAG content between controls and low or high exposure lakes (Kelly and Janz, 2008).

Generally, exposure to xenobiotics has resulted in increases in plasma cortisol, for example, crude oil (Alkindi *et al*., 1996; Thomas and Rice, 1987) and pulp and paper mill contaminants (Kennedy *et al.*, 1995) have all been associated with a rise in plasma cortisol concentrations. Exposure, however, may also cause a decrease or have no effect of cortisol concentrations. This may be for several reasons; fish may acclimate to chronic xenobiotic exposure and the stressor no longer elicits an appropriate stress response. This has been observed in perch (*Perca flavescens)* and northern pike (*Esox lucius)* chronically exposed to PCB, and PAHs, which did not result in the expected rise in plasma cortisol when exposed to handling (Hontela *et al*., 1992). A xenobiotic may also directly affect the secretion of cortisol, as cortisol exerts negative feedback control that can inhibit secretion of additional hormone (Basu *et al*., 2002; Thomas, 2008). Chronically elevated plasma cortisol may eventually eliminate the ability of fish to respond to an acute stress, possibly from impaired interrenal cell function (Thomas, 2008). Xenobiotics may directly damage interrenal cells, reducing the cortisol response (Bisson and Hontela, 2002; Dorval *et al*., 2003), such as with acute exposure to methyl-parathion in jundiá *Rhamdia quelen* (*Heptapteridae Teleostei*) (Cericato *et al*., 2008).

At the conclusion of this study, exposure to malathion did not have a significant effect on cortisol levels in fed or fasted fish. Malathion may not induce elevations in cortisol at sublethal concentrations, as it may be rapidly biotransformed and never build up to concentrations capable of eliciting a characteristic stress response. However, because cortisol was measured at the end of the 12-week exposure period, it is possible that a significant stress response occurred earlier during the exposure period and was missed.

As a secondary response to physiological stress, increases in hematocrit can be used as an indicator of xenobiotic exposure, for example, catfish were observed to have increased hematocrit levels in response to acute malathion exposure (Areechon and Plumb, 1992). Although as a stress indicator, it is subject to the same limitations discussed above for cortisol. In the present study, the only changes observed in response to malathion exposure were within the fasted treatment group. Fasted fish exposed to low malathion exposure had the highest hematocrit levels compared to the controls and high malathion exposure. It is unclear why hematocrit levels increased in the low malathion exposure group and not in the high malathion exposure, as higher concentrations of malathion would theoretically represent a greater stressor and require greater levels of oxygen to maintain energy levels. However hematocrit has been suggested to be a less reliable indicator of oxygen-carrying capacity in response to xenobiotics, as blood parameters may change depending on an organism's life cycle stage, nutritional status, or stress-induced responses unrelated to xenobiotic exposure (Schlenk *et al*., 2008).

A variety of endpoints can be used when determining the potential impacts of xenobiotics on fish bioenergetics. All measured biological responses will have a degree of variability due to the complex and integrative nature of physiological testing. The most practical approach when assessing xenobiotic exposure is to choose endpoints that provide information from several levels of biological organizations. In the present study, an integrated approach was used by using biochemical (e.g. glycogen, cortisol), organ (e.g. LSI), and whole organism (e.g. growth) measurements. Even with this approach, which is likely to be sensitive due to the integrative nature of the measures, the sublethal concentrations of malathion used in this study did not elicit a significant bioenergetic response of rainbow trout. It is possible malathion has a low metabolic expense associated with biotransformation and excretion. It is also possible the pesticide concentration in the exposure tanks was not high enough to cause costly cellular damage or elicit a stress response that would cause a change in the measured endpoints. Fasting alone seemed to be the only factor that impacted the condition of fish, rather than xenobiotic exposure.

2.5 Conclusion

The growth, stress, and energetic indices of rainbow trout in this study were modified by 12-weeks of fasting. Fish provided with an abundant food source experienced increased growth and CF compared to fasted fish, while after 12 weeks of fasting led to unfed fish having significantly reduced energetic indices such as liver glycogen, total lipids and muscle TAGs. Effects from sublethal exposure to malathion were observed in the greater body mass of exposed fed fish compared to unexposed

controls during weeks 8 and 12. However malathion did not appear to have an effect on the growth rates, CF, plasma cortisol or energetic indices of fish, although large variation in some of these endpoints, particularly glycogen, may potentially mask any detectable effects of malathion. This could be amended by increasing the number of values (n) used when performing statistical analysis. It is possible malathion may not elicit a strong stress response, it may also incur low energy costs associated with its biotransformation and excretion, or the sublethal concentrations of malathion used in this study may not have been high enough to cause effects.

2.6 References

Adams, S.M. 1999. Ecological role of lipids in the health and success of fish populations, in: *Lipids in Freshwater Ecosystems*. M.T. Arts and B.C. Wainman (Eds.), Springer-Verlag, New York, NY., pp. 132-160.

Ackerman, P.A., Forsyth, R.B., Mazur, C.F., and G.K. Iwama. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry*, 23: 327-336.

Akiyama, T. and T. Nose. 1980. Changes in body weight, condition factor and body composition of fingerling chum salmon with various sizes during starvation. *Bulletin of the Natural Resources Institute of Aquaculture* (Japan), 1: 71-78.

Alkindi, A.Y.A., Brown, J.A., Waring, C.P. and J.E. Collins. 1996. Endocrine, osmoregulatory, respiratory and haematological parameters in flounder exposed to the water soluble fraction of crude oil. *Journal of Fish Biology*, 49: 1291-1305.

Andersen, D.E., Reid, S.D., Moon, T.W., and S.F. Perry. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 48 (9): 1811-1817.

Areechon, N. and J. A. Plumb. 1990. Sublethal Effects of Malathion on Channel Catfish, *Ictalurus punctatus*. *Bulletin of Environmental Contamination and Toxicology*, 44: 435-442.

Arunachalam, S., Jeyalakshmi, K. and S. Aboobucker. 1980. Toxic and sublethal effects of carbaryl on a freshwater catfish, *Mystus vittatus* (Bloch). *Archives of Environmental Contamination and Toxicology*, 9: 307-316.

Awasthi, M., Shah, P., Dubale, M.S., and P. Gadhia. 1984. Metabolic changes induced by organophosphates in the piscine organs. *Environmental Research*, 35: 320-325.

Bains, O.S. and C.J. Kennedy. 2004. Energetic costs of pyrene metabolism in isolated hepatocytes of rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology*, 67: 217-226.

Barton, B.A. and C.B. Schrek. 1987. Metabolic costs of acute physical stress in juvenile steelhead. *Transactions of the American Fisheries Society*, 116: 257-263.

Barton, B.A., Schreck, C.B., and L.D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress esponses in juvenile rainbow trout. *Diseases of Aquatic Organisms*, 2: 173-185.

Barton, B.A., Schreck, C.B., and L.G. Fowler. 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. *The Progressive Fish Culturist*, 50: 16-22.

Basu, N., Kennedy, C.J., Hodson, P.V., and G.K. Iwama. 2002. Altered stress responses in rainbow trout following a dietary administration of cortisol and β-napthoflavone. *Fish Physiology and Biochemistry*, 25: 131-140.

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

Bisson, M. and A. Hontela. 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.

Bleau, H., Chevalier, D.G., van Tra, H. and A. Hontela. 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: 221-235.

Borgmann, U. and K.M. Ralph. 1986. Effects of cadmium, 2,4-dichlorophenol, and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. *Archives of Environmental Contamination and Toxicology*, 15: 473-480.

Brett, J.R.1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *American Zoologist*, 11: 99-113.

Brown S. B., Fisk, A.T., Brown, M., Villella, M., Muir, D.C.G., Evans, R.E., Lockhart, W.L., Metner, D.A., and H.M. Cooley. 2002. Dietary accumulation and biochemical responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to 3,3,4,4,5-pentachlorobiphenyl (PCB 126). *Aquatic Toxicology* 59: 139–152.

Centers for Disease Control and Prevention, Department of Health and Human Services. 2009. *Fourth national Report on Human Exposure to Environmental Chemicals*. Atlanta, GA.

Cericato, L. Machado Neto, J.G. Fagundes, M.. Kreutz, L.C., Quevedo, R.M., Finco, J., Santos da Rosa, J.G., Koakoski, G., Centenaro, L. Pottker, E. Anziliero, D., Gil Barcellos, L.J. 2008. Cortisol response to acute stress in jundiá *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. *Comparative Biochemistry and Physiology, Part C*, 148: 281-286.

Clarke, A. and K.P.P. Fraser. 2004. Why does metabolism scale with temperature? *Functional Ecology,* 18:243-251.

Clarke, A. and N.M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68: 893-905.

Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Healey, M.C., Macdonald, J.S., Shrimpton, J.M., Young, J.L., Lister, A., Van Der Kraak, G. and A.P. Farrell. 2008. Physiological correlates of coastal arrival and river entry

timing in late summer Fraser River sockeye salmon (*Oncorhynchus nerka*). Behavioral Ecology, 19 (4): 747-758.

Cunjak, R.A. 1988. Physiological consequences of overwintering in streams: the cost of acclimatization? *Canadian Journal of Fisheries and Aquatic Science*, 45: 443-452.

Damsgard, B. and L.M. Gill. 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch. Behavioral Ecology*, 9(1): 26-32.

Davies, P.E., Cook, L.S.J. and D. Goenarso. 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. *Environmental Toxicology and Chemistry*, 13(8): 1341-1354.

Davis, L.E. and C.B. Schrek. 1997. The energetic response to handling stress in juvenile coho salmon. *Transactions of the American Fisheries Society*, 126: 248-258.

Dorval, J., Leblond, V.S. and A. Hontela. 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 (3): 229-241.

Driedger, K., Weber, L.P., Rickwood, C.J., Dube, M.G., and D.M. Janz. 2009. Overwintering alterations in energy stores and growth in juvenile fishes inhabiting areas receiving metal mining and municipal wastewater effluents. Environmental Toxicology and Chemistry, 28 (2): 296-304.

Environment Canada. 2011. Presence and levels of priority pesticides in selected Canadian aquatic ecosystems, Water Science and Technology Directorate, Environment Canada Cat.No.: En14-40/2011E-PDF

Faller, P., Kobler, B., Peter, A., Sumpter, J.P., and Burkhardt-Holm, P. 2003. Stress status of gudgeon (*Gobio gobio*) from rivers in Switzerland with and without input of sewage treatment plant effluent. *Environmental Toxicology and Chemistry*, 22: 2063-2072.

Gourley, M.E. and C.J. Kennedy. 2009. Energy allocations to xenobiotic transport and biotransformation reactions in rainbow trout (*Oncorhynchus mykiss*) during energy intake restriction. *Comparative Biochemistry and Physiology Part C*, 150: 270-278.

Guderley, H., Pierre, J.St., Couture, P., and A.J. Hulbert. 1997. Plasticity of the properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. *Fish Physiology and Biochemistry*, 16: 531-541.

Gupta, R.C. 2006. Classification and uses of organophosphates and carbamates, in: *Toxicology of organophosphate & carbamate compounds*, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.5-24.

Handy, R.D., Sims, D.W., Giles, A., Campbell, H.A. and M.M. Musonda. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. *Aquatic toxicology*, 47: 23-47.

Harris, K.A., Dangerfield, N., Woudneh, M., Brown, T., Verrin, S. and P.S. Ross. 2008. Partitioning of current-use and legacy pesticides in salmon habitat in British Columbia, Canada. *Environmental Toxicology and Chemistry*, 27(11): 2253-2262.

Heath, A.G. 1995. *Water Pollution and Fish Physiology 2nd Ed.*, CRC Press, Boca Raton, FL, pp. 125-139, 171-216, 325-342.

Higgs, D.A., Macdonald, J.S., Levings, C.D., and B.S. Dosanjh. 1995. Nutrition and feeding habits in relation to life history stage, in: *Physiological Ecology of Pacific Salmon*, UBC Press, Canada, pp.159-316.

Hildebrand, J.L., Bains, O.S., Lee, D.S.H., and C.J. Kennedy. 2009. Functional and energetic characterization of P-gp-mediated doxorubicin transport in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Comparative Biochemistry and Physiology*, Part C, 149: 65-72.

Hontela, A., Rasmussen, J.B., Audet, C. and G. Chevalier. 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Achives of Environmental and Contamination Toxicology*, 22: 278-283.

Jacobs, J.M., Rhodes, M.R., Martin, B., McIntosh, D., Van Heukelem, W.F. and R.M. Harrell. 2008. Tissue-specific sampling for the estimation of striped bass whole-body proximate composition. *Transactions of the American Fisheries Society*, 137: 386-392.

Jobling, M. 1983. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpines* (L.). *Journal of Fish Biology*, 22: 471-475.

Jobling, M. 1993. Bioenergetics: Feed intake and energy partitioning, in: *Fish Ecophysiology*, Ranking, J.C. and F.B. Jensen (Eds.), Chapman and Hall, London, pp. 1-40.

Jobling, M. 2002. Environmental factors and rates of development and growth, in: *Handbook of Fish Biology and Fisheries*, Vol.1. Blackwell Publishing Ltd., Malden, MA, pp. 97-122.

Kang, S-J., Timmins, M.C.A. and R.G. Ackman. 1998. Similarities in the lipid class profiles of oils from Atlantic and Pacific dogfish livers *Journal of the American Oil Chemists' Society*, 75 (11): 1667-1672.

Kelly, J.M., and D.M. Janz. 2008. Altered energetic and parasitism in juvenile northern pike (*Esox lucius*) inhabiting metal-mining contaminated lakes. *Ecotoxicology and Environmental Safety*, 70: 357-369.

Kennedy, C.J., Sweeting, R.M., Johansen, J.A., Farrell, A.P. and B.A. McKeown. 1995. Acute effects of chlorinated resin acid exposure on juvenile rainbow trout,*Oncorhynchus mykiss*. *Environmental Toxicology and Chemistry*, 14 (6): 977-982.

Kleinow, K.M., Nichols, J.W., Hayton, W.L., McKim, J.M., and M.G. Barron. 2008. Toxicokinetics in Fishes, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 55-134.

Kumaraguru, A.K. and F.W.H. Beamish. 1983. Bioenergetics of acclimation to permethrin (NRDS-143) by rainbow trout. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 75(2): 247-252.

Laetz, C.A., Baldwin, D.H., Collier, T.K., Hebert, V., Stark, J.D. and N.L. Scholz. 2009. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. *Environmental Health Perspectives:* 117 (3): 348-353.

Larsen, D.A., Beckman, B.R., and W.W. Dickhoff. 2001. The effect of low temperature and fasting during the winter on growth and smoltification of coho salmon. *North American Journal of Agriculture*, 63: 1-10.

Lawrence, A.J., Arukwe, A., Moore, M., Sayer, M., and J. Thain. 2003. Molecular/cellular processes and the physiological response to pollution, in: *Effects of Pollution on Fish*. Blackwell Science Ltd. Oxford, UK, pp. 83-133.

Lemly, A.D. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquatic Toxicology*, 27: 133-158.

Lemly, A.D. 1996a. Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. *Ecotoxicology and Environmental Safety*, 34: 223-227.

Lemly, A.D. 1996a. Wastewater discharges may be most hazardous to fish during winter. *Environmental Pollution*, 93 (2): 169-174.

Levesque, H.M., Short, C., Moon, T.W., Ballantyne, J.S., and W.R. Driedzic. 2005. Effects of seasonal temperature and photoperiod on Atlantic cod (*Gadus morhua*), morphometric parameters and metabolites. *Canadian Journal of Aquatic Science*, 62: 2854-2863.

Little, E.E., Archeski, R.D., Flerov, B.A., and V.I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. *Achives of Environment and Contaminant Toxicology*, 19: 380-385.

McEwan, F.L. and G.R. Stephenson. 1979. *The Use and Significance of Pesticides in the Environment*. Wiley Interscience, New York, pp. 155-215.

Næsje T.F., Thorstad, E.B., Forseth, T., Aursand, M., Saksga˚rd, R. and A.G. Finstad. 2006. Lipid class content as an indicator of critical periods for survival in juvenile Atlantic salmon (*Salmo salar*). *Ecology of Freshwater Fish*, 15: 572–577.

Naiman,,R.J., Bilby, R.E., Schindler, D.E. and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems*, 5: 399 – 417.

Organization for Economic Cooperation and Development (OECD). 1993. Fish, early-life stage toxicity test. OECD Guideline 210. Fish, juvenile growth test OECD Guideline 204 (January, 2000). Paris,France.

Parkinson, A. and B.W. Ogilvie. 2010. Biotransformation of Xenobiotics, in: *Casarett and Doull's Essentials of Toxicology*, *2nd Ed.*, Klaasen, C.D. and J.B. Watkins III (Eds.). The McGraw-Hill Companies, NY., pp.72-75.

Ptashynski, M.D., Pedlar, R.M., Evans, R.E., Baron, C.L., and Klaverkamp, J.F. 2002. Toxicology of dietary nickel in lake whitefish (*Coregonus clupeaformis*). *Aquatic Toxicology*, 58: 229-247.

Sancho, E., Ferrando, M.D. and E. Andreu. 1998. Effects of sublethal exposure to a pesticide on levels of energetic compounds in *Anguilla anguilla*. *Journal of Environmental Science and Health*, B33(4): 411-424.

Sandahl, J.F., Baldwin, D.H., Jenkins, J.J. and N.L. Scholz. 2005. Comparative thresholds for Acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry*, 24(1): 136-145.

Scholz, N., Truelove, N.K., French, B.L. Berejikian, B.A., Quinn, T.P., Casillas, E., and T.K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in Chinook salmon. *Canadian Journal of Fisheries and Aquatic Science*, 57: 1911-1918.

Scholz, N.L., Truelove, N.K., Labenia, J.S., Baldwin, D.H. and T.K. Collier. 2006. Dose- additive inhibition of Chinook salmon acetylcholinesterase activity by mixtures of organophosphate and carbamate insecticides. *Environmental Toxicology and Chemistry*, 25 (5): 1200-1207.

Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., Van der Hurk, P., and K. Willett. 2008. Biotransformation in fishes, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 153-234.

Schrek, C.B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors, in: *Stress and Fish*, A.D. Pickering (Ed.), Academica Press, London, pp. 295-321.

Simpkins, D.G., Hubert, W.A, Martinez Del Rio, C., and D.C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. *Transactions of the American Fisheries Society*, 132: 576-589.

Smolders, R., De Boeck, G. and R. Blust. 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environmental Toxicology and Chemistry*, 22 (4): 890-899.

Stout, V. F. 1986. What is happening to PCBs? Elements of environmental monitoring as illustrated by an analysis of PCB trends in terrestrial and aquatic organisms, in: *PCBs and the Environment*, Vol. 1., Waid, J. S. (*eds.*), CRC Press, Boca Raton, Florida, pp.163-206.

Strange, R.J. and C.B. Schreck. 1978. Anaesthetic and handling stress on survival and cortisol concentrations in yearling Chinook salmon (Oncorhynchus tshawytscha). *Journal of the Fisheries Resource Board of Canada*, 35: 345-349.

Sweeting, R.M. 1989. Aspects of growth hormone in the physiology of smoltification and seawater adaptation of Coho salmon. Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, Simon Fraser University.

Thibault, M., Blier, P.U., and H. Guderley. 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, 16(2): 139-155.

Thomas, P. 2008. The endocrine system, in: *The Toxicology of Fishes*, R.T. Di Giulio & D.E. Hinton, (Eds.), CRC Press, Boca Raton, FL, pp. 457-488.

Thomas, R.E., Gharrett, J.A., Carls, M.G., Rice, S.D., Moles, A., and S. Korn. 1986. Effects of fluctuating temperature on mortality, stress, and energy reserves of juvenile coho salmon. *Transactions of the American Society*, 115: 52-59.

Thomas, R.E. and S.D. Rice. 1987. Effect of Effect of water-soluble fraction ofCook Inlet crude oil on swimming performance and plasma cortisolin juvenile coho salmon (*Oncorhynchus kisutch*). *Comparative Biochemistry and Physiology Part C*, 87:177–180.

Toneys, M.L. and D.W. Coble. 1980. Mortality, hematocrit, osmolality, electrolyte regulation, and fat depletion of young-of-the-year freshwater fishes under simulated winter conditions. *Canadian Journal of Fisheries and Aquatic sciences*, 37: 225-232.

United States Environmental Protection Agency (EPA). 2007. ECOTOX User Guide: ECOTOXicology Database System. Version4.0. Available: http:/www.epa.gov/ecotox/, retrieved February, 2010.

United States Geological Survey. 2001. Selected findings and current perspectives on urban and agricultural water quality by the national water-quality assessment program (NAWQA). FS-047-01. http://water.usgs.gov/pubs/FS/fs-047-01/.

Verrin, S.M., Begg, S.J., and P.S. Ross. 2004. Pesticide use in British Columbia and the Yukon: An assessment of types, applications and risks to aquatic biota. *Canadian Technical Report of Fisheries and Aquatic Sciences 2517*. Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, BC.

Vijayan M.M. and T.W. Moon. 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Science*, 49: 2260-2266.

Woltering, D.M. 1984. The growth response in fish chronic and early life stage toxicity tests: A critical review. *Aquatic Toxicology*, 5: 1-21.

Wooton, R.J. 1998. *Ecology of Teleost Fishes 2nd Ed*. Kluwer Academic Publishers, The Netherlands, pp.1-86.

3: CONCLUSIONS

Salmonids living in the Pacific Northwest regularly encounter conditions of energy limitation during overwintering periods. Due to the widespread use of the CUPs in North America, the overall aim of this study was to determine whether CUP exposure during the critical period of overwintering would affect the bioenergetics of juvenile salmonids, thus potentially affecting salmonid populations in the wild in contaminated freshwater habitats. Fish were separated into two groups, one fed and one fasted, and each group exposed to several concentrations of the organophosphorous insecticide malathion at low water temperatures. The major findings of this project were:

1) Fish that were fed over the 12-week exposure period experienced increased body mass, positive growth rates and increased condition factors. In comparison, fish exposed to 12 weeks of fasting had significantly reduced body mass, negative growth rates, and reduced condition factors.

2) The physiological stress response indicators in fed fish were higher than those in fasted fish, although the values of these indicators in both groups of fish suggested that neither were subject to a significantly elevated stress response at the end of the 12-week exposure.

3) Fasted fish had significantly lower energetic indices including liver somatic index, muscle lipid content and TAGs in muscle compared to fed fish.

4) Effects from sublethal exposure to malathion were observed in the greater body mass of exposed fed fish compared to unexposed controls during weeks 8 and 12. However malathion did not appear to have an effect on the growth rates, CF, plasma cortisol or energetic indices of fish Malathion exposure at the sublethal concentrations used did not elicit a physiological stress response in fish in this study. As well, there appear to be low energy costs associated with malathion defence (biotransformation, excretion, or toxic repair).

This study is one of the first to examine the combined effects of sublethal xenobiotic exposure and overwintering conditions in salmonids, and may be of use in aiding regulatory bodies when reviewing Canadian water quality guidelines for sublethal effects of CUPs in freshwater systems. Chronic in-vivo studies such as these using juvenile salmonids provide insight into changes in individual growth and condition parameters and therefore population dynamics and are valuable due to their ecological relevance. Gathering information from several levels of biological organization such as biochemical (e.g. cortisol), organ (e.g. LSI), and whole organism (e.g. growth) measurements such as in the present study can provide a more comprehensive assessment of contaminant effects on teleosts.

Reference List

Adams, S.M. 1999. Ecological role of lipids in the health and success of fish populations, in: *Lipids in Freshwater Ecosystems*. M.T. Arts and B.C. Wainman (Eds.), Springer-Verlag, New York, NY., pp. 132-160.

Ackerman, P.A., Forsyth, R.B., Mazur, C.F., and G.K. Iwama. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry*, 23: 327-336.

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological profile for Malathion. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Akiyama, T. and T. Nose. 1980. Changes in body weight, condition factor and body composition of fingerling chum salmon with various sizes during starvation. *Bulletin of the Natural Resources Institute of Aquaculture* (Japan), 1: 71-78.

Alkini, A.Y.A., Brown, J.A., Waring, C.P. and J.E. Collins. 1996. Endocrine, osmoregulatory, respiratory and haematological parameters in flounder exposed to the water soluble fraction of crude oil. *Journal of Fish Biology*, 49: 1291-1305.

Andersen, D.E., Reid, S.D., Moon, T.W., and S.F. Perry. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 48 (9): 1811-1817.

Andersson, T. 1986. Cytochrome P-450-dependent metabolism in isolated liver cells from fed and starved rainbow trout, *Salmo gairdneri*. *Fish Physiology and Biochemistry*, 1: 105-111.

Areechon, N. and J. A. Plumb. 1990. Sublethal Effects of Malathion on Channel Catfish, *Ictalurus punctatus*. *Bulletin of Environmental Contamination and Toxicology*, 44: 435-442.

Arunachalam, S., Jeyalakshmi, K. and S. Aboobucker. 1980. Toxic and sublethal effects of carbaryl on a freshwater catfish, *Mystus vittatus* (Bloch). *Archives of Environmental Contamination and Toxicology*, 9: 307-316.

Awasthi, M., Shah, P., Dubale, M.S., and P. Gadhia. 1984. Metabolic changes induced by organophosphates in the piscine organs. *Environmental Research*, 35: 320-325.

Bains, O.S. and C.J. Kennedy. 2004. Energetic costs of pyrene metabolism in isolated hepatocytes of rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology*, 67: 217-226.

Barton, B.A. and C.B. Schrek. 1987. Metabolic costs of acute physical stress in juvenile steelhead. *Transactions of the American Fisheries Society*, 116: 257-263.

Barton, B.A., Schreck, C.B., and L.D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress esponses in juvenile rainbow trout. *Diseases of Aquatic Organisms*, 2: 173-185.

Barton, B.A., Schreck, C.B., and L.G. Fowler. 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. *The Progressive Fish Culturist*, 50: 16-22.

Basu, N., Kennedy, C.J., Hodson, P.V., and G.K. Iwama. 2002. Altered stress responses in rainbow trout following a dietary administration of cortisol and β-napthoflavone. *Fish Physiology and Biochemistry*, 25: 131-140.

Beamann, J.R., Finch, R., Gardner, H., Hoffman, F., and A. Rosencrance. 1999. Mammalian immunoassays for predicting the toxicity of malathion in a laboratory fish model. *Journal of Toxicology and Environmental Health*, 56: 101-120.

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

Beyers, D.W., Rice, J.A., Clements, W.H. and C.J. Henry. 1999. Estimating physiological costs of chemical exposure: integrating energetic and stress to quantify toxic effects in fish. *Canadian Journal of Fisheries and Aquatic Science*, 56: 814-822.

Bisson, M. and A. Hontela. 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.

Bleau, H., Chevalier, D.G., van Tra, H. and A. Hontela. 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: 221-235.

Bondarenko, S., Gan, J., Haver, D.L., and J.N. Kabashima. 2004. Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. *Environmental Toxicology and Chemistry*, 23 (11): 2649-2654.

Borgmann, U. and K.M. Ralph. 1986. Effects of cadmium, 2,4-dichlorophenol, and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. *Archives of Environmental and Contaminant Toxicology*, 15: 473-480.

Bradbury, S.P., Carlson, R.W., Henry, T.R., Padilla, S. and J. Cowden. 2008. Toxic responses of the fish nervous system, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 417-456.

Brett, J.R.1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *American Zoologist*, 11: 99-113.

Brewer, S.K., Little, E.E., DeLoney, A.J., Beauvais, S.L., Jones, S.B. and M.R. Ellersieck. 2001. Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykiss*) exposed to cholinesterase-inhibiting chemicals. *Archives of Environmental and Contamination Toxicology*, 40: 70-76.

Brown, J.A. 1993. Endocrine responses to environmental pollutants, in: *Fish Ecophysiology*, Ranking, J.C. and F.B. Jensen (Eds.), Chapman and Hall, London, pp. 276-296.

Brown S. B., Fisk, A.T., Brown, M., Villella, M., Muir, D.C.G., Evans, R.E., Lockhart, W.L., Metner, D.A., and H.M. Cooley. 2002. Dietary accumulation and

biochemical responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to 3,3,4,4,5-pentachlorobiphenyl (PCB 126). *Aquatic Toxicology* 59: 139–152.

Carr, R.L. and J.E. Chambers. 2001. Toxic responses of the nervous system, in: *Target organ toxicity in marine and freshwater teleosts*, Vol.2, Schlenk, D. and W.H. Benson (Eds.), Taylor & Francis, London, pp.26-95.

Centers for Disease Control and Prevention, Department of Health and Human Services. 2009. *Fourth national Report on Human Exposure to Environmental Chemicals*. Atlanta, GA.

Cericato, L. Machado Neto, J.G. Fagundes, M.. Kreutz, L.C., Quevedo, R.M., Finco, J., Santos da Rosa, J.G., Koakoski, G., Centenaro, L. Pottker, E. Anziliero, D., Gil Barcellos, L.J. 2008. Cortisol response to acute stress in jundiá *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. *Comparative Biochemistry and Physiology, Part C*, 148: 281-286.

Chellappa, S., Huntingford, F.A., Strang, R.H.C. and R.Y. Thomson. 1989. Annual variation in energy reserves in male three-spined stickleback, *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae). *Journal of Fish Biology*, 35: 275-286.

Cho, C.Y. 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, 100: 107-123.

Clarke, A. and K.P.P. Fraser. 2004. Why does metabolism scale with temperature? *Functional Ecology*, 18:243-251.

Collvin, L. 1985. The effect of copper on growth, food consumption and food conversion of perch (*perca fluviatilis)* offered maximal food rations. *Aquatic Toxicology*, 6: 105-113.

Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Healey, M.C., Macdonald, J.S., Shrimpton, J.M., Young, J.L., Lister, A., Van Der Kraak, G. and A.P. Farrell. 2008. Physiological correlates of coastal arrival and river entry timing in late summer Fraser River sockeye salmon (*Oncorhynchus nerka*). Behavioral Ecology, 19 (4): 747-758.

Crockett, E.L. and J.R. Hazel. 1995. Cholesterol levels explain inverse compensation of membrane order in brush border but not homeoviscous adaptation in basolateral membranes from the intestinal epithelia of rainbow trout. *The Journal of Experimental Biology*, 198: 1105-1113.

Cunjak, R.A. 1988. Physiological consequences of overwintering in streams: the cost of acclimatization? *Canadian Journal of Fisheries and Aquatic Science*, 45: 443-452.

Damsgard, B. and L.M. Gill. 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch. Behavioral Ecology*, 9(1): 26-32.

Davies, P.E., Cook, L.S.J. and D. Goenarso. 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. *Environmental Toxicology and Chemistry*, 13(8): 1341-1354.

Davis, L.E. and C.B. Schrek. 1997. The energetic response to handling stress in juvenile coho salmon. *Transactions of the American Fisheries Society*, 126: 248-258.

Doi, A.M., Holmes, E. and K.M. Kleinow. 2001. P-glycoprotein in the catfish intestine: inducibility by xenobiotics and functional properties. *Aquatic Toxicology*, 55: 157-170.

Dorval, J., Leblond, V.S. and A. Hontela. 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 (3): 229-241.

Douglas, M.T., D.O. Chanter, I.B. Pell, and G.M. Burney. 1986. A proposal for the reduction of animal numbers required for the acute toxicity to fish test (LC_{50} Determination). *Aquatic Toxicology*, 8(4): 243-249.

Driedger, K., Weber, L.P., Rickwood, C.J., Dube, M.G., and D.M. Janz. 2009. Overwintering alterations in energy stores and growth in juvenile fishes inhabiting areas receiving metal mining and municipal wastewater effluents. Environmental Toxicology and Chemistry, 28 (2): 296-304.

Druzina, B. and M. Stegu. 2007. Degradation study of selected organophosphorus insecticides in natural waters. *International Journal of Environmental and Analytical Chemistry*, 87 (15): 1079-1093.

Dutta, H.M., Dogra, J.V., Singh, N.K., Roy, P.K., Nasar, S.S., Adhikari, S., Munshi, J.S., and C. Richmonds. 1992. Malathion induced changes in the serum proteins and hematological parameters of an Indian catfish *Heteropneustes fossilis* (Bloch). *Bulletin of environmental contamination and toxicology*, 49: 91-97.

Eaton, J.G. 1970. Chronic malathion toxicity to the bluegill (*Lepomis macrochirus rafinesque*).*Water Research*, 4: 673-684.

ENKON Environmental Limited. 2005. Survey of pesticide use in British Columbia, 2003. Project#1004-011. Environment Canada and British Columbia Ministry of Environment, Lands and Parks. Surrey, British Columbia.

Environmental Canada. 2011. Presence and levels of priority pesticides in selected Canadian aquatic ecosystems, Water Science and Technology Directorate, Environment Canada Cat.No.: En14-40/2011E-PDF.

Erickson, R.J., Nichols, J.W., Cook, P.M., and G.T. Ankley. 2008. Bioavailability of chemical contaminants in aquatic systems, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 9-54.

Evans, D.H. 1987. The Fish Gill: Site of action and model for toxic effects of environmental pollutants. *Environmental Health Perspective*, 71: 47-58.

Evans, D.H., Piermarini, P.M. and K.P. Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews*, 85: 97–177.

Faller, P., Kobler, B., Peter, A., Sumpter, J.P., and Burkhardt-Holm, P. 2003. Stress status of gudgeon (*Gobio gobio*) from rivers in Switzerland with and without input of sewage treatment plant effluent. *Environmental Toxicology and Chemistry*, 22: 2063-2072.

Ferrari, A., Venturino, A., and A.M. Pechen de D'Angelo. 2007. Effects of carbaryl and azinphos methyl on juvenile rainbow trout detoxifying enzymes. *Pesticide Biochemistry and Physiology*, 88: 134-142.

Foley, W.J. 1992. Nitrogen and energy retention and acid-base status in the common ringtail possum (*Pseudocheirusper egrinus*): Evidenceof the Effects of Absorbed Allelochemicals. *Physiological Zoology*, 65 (2): 403-421.

Gourley, M.E. and C.J. Kennedy. 2009. Energy allocations to xenobiotics transport and biotransformation reactions in rainbow trout (*Oncorhynchus mykiss*) during

energy intake restriction. *Comparative Biochemistry and Physiology Part C*, 150: 270-278.

Guderley, H., Pierre, J.St., Couture, P., and A.J. Hulbert. 1997. Plasticity of the properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. *Fish Physiology and Biochemistry*, 16: 531-541.

Gupta, R.C. 2006. Classification and uses of organophosphates and carbamates, in: *Toxicologyof organophosphate & carbamate compounds*, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.5-24.

Handy, R.D., Sims, D.W., Giles, A., Campbell, H.A. and M.M. Musonda. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. *Aquatic toxicology*, 47: 23-47.

Harris, K.A., Dangerfield, N., Woudneh, M., Brown, T., Verrin, S. and P.S. Ross. 2008. Partitioning of current-use and legacy pesticides in salmon habitat in British Columbia, Canada. *Environmental Toxicology and Chemistry*, 27(11): 2253-2262.

Heath, A.G. 1984. Changes in tissue adenylates and water content of bluegill, *Lepomis macrochirus*, exposed to copper. *Journal of Fish Biology*, 24: 299-309.

Heath, A.G. 1995. *Water Pollution and Fish Physiology 2nd Ed.*, CRC Press, Boca Raton, FL, pp.125-139, 171-216.

Higgs, D.A., Macdonald, J.S., Levings, C.D., and B.S. Dosanjh. 1995. Nutrition and feeding habits in relation to life history stage, in: *Physiological Ecology of Pacific Salmon*, UBC Press, Canada, pp.159-316.

Hildebrand, J.L., Bains, O.S., Lee, D.S.H., and C.J. Kennedy. 2009. Functional and energetic characterization of P-gp-mediated doxorubicin transport in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Comparative Biochemistry and Physiology*, Part C, 149: 65-72.

Hinton, D.E., Segner, H., Au, D.W.T., Kullman, S.W., and R.C. Hardman. 2008. Liver Toxicity, in: *The Toxicology of Fishes,* Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 327-400.

Hontela, A., Rasmussen, J.B., Audet, C. and G. Chevalier. 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Achives of Environmental and Contamination Toxicology*, 22: 278-283.

Hosokawa, M. and T. Satoh. 2006. Structure, function, and regulation of carboxylesterases, in: *Toxicology of organophosphate & carbamate compounds*, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.219-232.

Houlihan, D.F., Mathers, E.M. and A. Foster. 1993. Biochemical correlates of growth rate in fish, in: *Fish Ecophysiology*, Ranking, J.C. and F.B. Jensen (Eds.), Chapman and Hall, London, pp. 45-71.

Huculeci, R., Dinu, D., Staicu, A.C., Munteanu, M.C., Costache, M. and A. Dinischiotu. 1999. Malathion-induced alteration of the antioxidant defence system in kidney, gill, and intestine of *Carassius auratus gibelio*. *Environmental Toxicology*, 24(6): 523–530.

Hylland, K., Feist, S., Thain, J. and L. Forlin. 2003. Molecular/cellular processes and the health of the individual, in: *Effects of Pollution on Fish*. Blackwell Science Ltd. Oxford, UK, pp. 134-178.

Jacobs, J.M., Rhodes, M.R., Martin, B., McIntosh, D., Van Heukelem, W.F. and R.M. Harrell. 2008. Tissue-specific sampling for the estimation of striped bass whole-body proximate composition. *Transactions of the American Fisheries Society*, 137: 386-392.

Jarvinen, A.W., Nordling, B.R. and M.E. Henry. 1983. Chronic toxicity of Dursban (Chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetylcholinesterase inhibition. *Ecotoxicolocy and Environmental Safety,* 7: 423-434.

Jobling, M. 1983. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpines* (L.). *Journal of Fish Biology,* 22: 471-475.

Jobling, M. 1993. Bioenergetics: Feed intake and energy partitioning, in: *Fish Ecophysiology*, Ranking, J.C. and F.B. Jensen (Eds.), Chapman and Hall, London, pp. 1-40.

Jobling, M. 2002. Environmental factors and rates of development and growth, in: *Handbook of Fish Biology and Fisheries, Vol.1*, Hart, P.J.B. and J.D. Reynolds (Eds.), Blackwell Publishing, Malden, MA., pp. 97-122.

Johnston, I.A. and J. Dunn. 1987. Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Society for Experimental Biology*, 67-93.

Jokanovic, M. 2001. Biotransformation of organophosphorus compounds. *Toxicology*, 166: 139-160.

Jonsson, N. and B. Jonsson. 1997. Energy allocation in polymorphic brown trout. *Functional Ecology*, 11: 310-317.

Kang, S-J., Timmins, M.C.A. and R.G. Ackman. 1998. Similarities in the lipid class profiles of oils from Atlantic and Pacific dogfish livers *Journal of the American Oil Chemists' Society*, 75 (11): 1667-1672.

Kelly, J.M., and D.M. Janz. 2008. Altered energetic and parasitism in juvenile northern pike (*Esox lucius*) inhabiting metal-mining contaminated lakes. *Ecotoxicology and Environmental Safety*, 70: 357-369.

Kennedy, C.J., Sweeting, R.M., Johansen, J.A., Farrell, A.P. and B.A. McKeown. 1995. Acute effects of chlorinated resin acid exposure on juvenile rainbow trout, *Oncorhynchus mykiss*. *Environmental Toxicology and Chemistry*, 14 (6): 977-982.

Kennedy, C.J., Higgs, D., and K.B. Tierney. 2004. Influence of diet and ration level on benzo(a)pyrene metabolism and excretion in rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Contamination and Toxicology*, 47: 379-386.

Kennedy, C.J. and K.B. Tierney. 2008. Energy intake affects the biotransformation rate, scope for induction, and metabolite profile of benzo(a)pyrene in rainbow trout. *Aquatic Toxicology*, 90: 172-181.

Kleinow, K.M., Nichols, J.W., Hayton, W.L., McKim, J.M., and M.G. Barron. 2008. Toxicokinetics in Fishes, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 55-134.

Kokalis-Burelle, N. and R. Rodriguez-Kabana. 2006. Allelochemicals as biopesticides for management of plant-parasitic nematodes, in: *Disease management of fruits and vegetables, Vol. 2*, Inderjit and K.G. Mukerji (Eds.), Springer, The Netherlands.

Kumaraguru, A.K. and F.W.H. Beamish. 1983. Bioenergetics of acclimation to permethrin (NRDS-143) by rainbow trout. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 75(2): 247-252.

Lackner, R. 1998. "Oxidative stress" in fish by environmental pollutants, in: *Fish Ecotoxicology*, Braunbeck, T., Hinton, D.E. and B. Streit (Eds.), Birkhauser Verlag Basel/Switzerland, pp. 203-224.

Laetz, C.A., Baldwin, D.H., Collier, T.K., Hebert, V., Stark, J.D. and N.L. Scholz. 2009. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. *Environmental Health Perspectives:* 117 (3): 348-353.

Larsen, D.A., Beckman, B.R., and W.W. Dickhoff. 2001. The effect of low temperature and fasting during the winter on growth and smoltification of Coho salmon. *North American Journal of Agriculture*, 63: 1-10.

Lawrence, A.J., Arukwe, A., Moore, M., Sayer, M., and J. Thain. 2003. Molecular/cellular processes and the physiological response to pollution, in: *Effects of Pollution on Fish*. Blackwell Science Ltd. Oxford, UK, pp. 83-133.

Lemly, A.D. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquatic Toxicology*, 27: 133-158.

Lemly, A.D. 1996a. Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. *Ecotoxicology and Environmental Safety*, 34: 223-227.

Lemly, A.D. 1996b. Wastewater discharges may be most hazardous to fish during winter. *Environmental Pollution*, 93 (2): 169-174.

Levesque, H.M., Short, C., Moon, T.W., Ballantyne, J.S., and W.R. Driedzic. 2005. Effects of seasonal temperature and photoperiod on Atlantic cod (*Gadus morhua*), morphometric parameters and metabolites. *Canadian Journal of Aquatic Science*, 62: 2854-2863.

Levine, S.L. and J.T. Oris. 1999. Enhancement of acute parathion toxicity to fathead minnows following pre-exposure to propiconazole. *Pesticide Biochemistry and Physiology*, 65: 102-109.

Little, E.E., Archeski, R.D., Flerov, B.A., and V.I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. *Achives of Environment and Contaminant Toxicology*, 19: 380-385.

Livingstone, D.R. 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. *Revue de Médecine Vétérinaire*, 154 (6): 427-430.

Lucas, A. 1996. *Bioenergetics of Aquatic Animals*, Taylor & Francis, London, pp. 40-64, 82-109.

Macek, K.J., and W.A. McAllister. 1970. Insecticide susceptibility of some common fish family representatives. *Transactions of the American Fisheries Society*, 99(1): 20-27.

Marking, L.L., T.D. Bills, and J.R. Crowther. 1984. Effects of five diets on sensitivity of rainbow trout to eleven chemicals. *Progressive Fish-Culturist*, 46(1): 1-5.

Mayer, F.L. 1974. Pesticides as pollutants, in: *Environmental Engineer's Handbook*, B.G.Liptak (Ed.), Chilton Book Co., Radnor, PA., pp. 405-418.

McEwan, F.L. and G.R. Stephenson. 1979. *The Use and Significance of Pesticides in the Environment*. Wiley Interscience, New York, pp. 155-215.

McKim, J.M., Schmieder, P.K., Niemi, G.J., Carlson, R.W., and T.R. Henry. 1987. Use of respiratory-cardiovascular responses of rainbow trout (*Salmo gairdneri*) in identifying acute toxicity syndromes in fish. Part 2. Malathion, carbaryl, acrolein, and benzaldehyde. *Environmental Toxicology and Chemistry*, 6: 313-328.

Moles, A., Bates, S., Rice, S.D. and S. Korn. 1981. Reduced growth of coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water. *Transaction of the American Fisheries Society*, 110: 430-436.

Næsje T.F., Thorstad, E.B., Forseth, T., Aursand, M., Saksga˚rd, R. and A.G. Finstad. 2006. Lipid class content as an indicator of critical periods for survival in juvenile Atlantic salmon (*Salmo salar*). *Ecology of Freshwater Fish*, 15: 572–577.

Naiman,,R.J., Bilby, R.E., Schindler, D.E. and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems*, 5: 399 – 417.

National Center for Environmental Health (NCEH). 2009. F*ourth National Report on Human Exposure to Environmental Chemicals*. Department of Health and Human Services Centers for Disease Control and Prevention , Atlanta, GA.

Oikari, A., Lindstrom-Seppa, P. and J. Kukkonen. 1988. Subchronic metabolic effects and toxicity of a simulated pulp mill effluent on juvenile lake trout, *Salmo trutta* m. *lacustris*. *Ecotoxicolgy and Environmental Safety*, 16: 202-218.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: *Lipids in Freshwater Ecosystems*. M.T. Arts and B.C. Wainman (Eds.), Springer-Verlag, New York, NY., pp. 161-202.

Organization for Economic Co-operation and Development (OECD). 1993. OECD guidelines for testing of chemicals, Vol.1: Fish, juvenile growth test (January, 2000). Paris, France.

Parkinson, A. and B.W. Ogilvie. 2010. Biotransformation of Xenobiotics, in: *Casarett and Doull's Essentials of Toxicology*, *2nd Ed.*, Klaasen, C.D. and J.B. Watkins III (Eds.). The McGraw-Hill Companies, NY., pp.72-75.

Post, J.R. and E.A. Parkinson. 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology*, 82 (4): 1040-1051.

Post, G., and T.R. Schroeder. 1971. The toxicity of four insecticides to four salmonid species. *Bulletin of Environmental Contamination and Toxicology*, 6(2): 144-155.

Ptashynski, M.D., Pedlar, R.M., Evans, R.E., Baron, C.L., and Klaverkamp, J.F. 2002. Toxicology of dietary nickel in lake whitefish (*Coregonus clupeaformis*). *Aquatic Toxicology*, 58: 229-247.

Rissanen, E., Krumschnabel, G., and M. Nikinmaa. 2003. Dehydroabietic acid, a major component of wood industry effluents, interferes with cellular energetics in rainbow trout hepatocytes, *Aquatic Toxicology* 62:.45-53.

Rodehutscord, M. and E. Pfeffer. 1999. Maintenance requirement for digestible energy and efficiency of utilization of digestible energy for retention in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 179: 95-107.

Rowe, D.K., Thorpe, J.E., and A.M. Shanks. 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Science*, 48: 405-413.

Sancho, E., Ferrando, M.D. and E. Andreu. 1998. Effects of sublethal exposure to a pesticide on levels of energetic compounds in *Anguilla anguilla*. *Journal of Environmental Science and Health*, B33(4): 411-424.

Sandahl, J.F., Baldwin, D.H., Jenkins, J.J. and N.L. Scholz. 2005. Comparative thresholds for Acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry*, 24(1): 136-145.

Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., Van der Hurk, P., and K. Willett. 2008. Biotransformation in fishes, in: *The Toxicology of Fishes*, Di Giulio, R.T. and D.E. Hinton (Eds.), CRC Press, Boca Raton, FL, pp. 153-234.

Scholz, N., Truelove, N.K., French, B.L. Berejikian, B.A., Quinn, T.P., Casillas, E., and T.K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in Chinook salmon. *Canadian Journal of Fisheries and Aquatic Science*, 57: 1911-1918.

Scholz, N.L., Truelove, N.K., Labenia, J.S., Baldwin, D.H. and T.K. Collier. 2006. Dose-additive inhibition of Chinook salmon acetylcholinesterase activity by mixtures of organophosphate and carbamate insecticides. *Environmental Toxicology and Chemistry*, 25 (5): 1200-1207.

Schrek, C.B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors, in: *Stress and Fish*, A.D. Pickering (Ed.), Academica Press, London, pp. 295-321.

Simpkins, D.G. and W.A. Hubert. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Transactions *of the American Fisheries Society*, 132: 576-589.

Singh, H. 1989. Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus albus. Marine Environmental Research,* 28: 285 289.

Smolders, R., De Boeck, G. and R. Blust. 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environmental Toxicology and Chemistry*, 22 (4): 890-899.

Stout, V. F. 1986. What is happening to PCBs? Elements of environmental monitoring as illustrated by an analysis of PCB trends in terrestrial and aquatic organisms, in: *PCBs and the Environment*, Vol. 1., Waid, J. S. (*eds.*), CRC Press, Boca Raton, Florida, pp.163-206.

Strange, R.J. and C.B. Schreck. 1978. Anaesthetic and handling stress on survival and cortisol concentrations in yearling Chinook salmon (Oncorhynchus tshawytscha). *Journal of the Fisheries Resource Board of Canada,* 35: 345-349.

Sturm, A., Cravedi, J.P. and H. Segner. 2001. Prochloraz and nonylphenol diethoxylate inhibit an MDR1-like activity *in-vitro*, but do not alter hepatic levels of P-glycoprotein in trout exposed *in vivo*. *Aquatic Toxicology*, 53: 215-228.

Sultatos, L.G. 2006. Interactions of organophosphorus and Carbamate compounds with cholinesterases, in: *Toxicology of organophosphate & carbamate compounds*, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.209-218.

Sweeting, R.M. 1989. Aspects of growth hormone in the physiology of smoltification and seawater adaptation of Coho salmon. Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, Simon Fraser University.

Tang, J., Rose, R.L. and J.E. Chambers. 2006. Metabolism of organophosphorus and Carbamate pesticides, in: *Toxicology of organophosphate & carbamate compounds*, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.127-144.

Thibault, M., Blier, P.U., and H. Guderley. 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, 16(2): 139-155.

Thomas, P. 2008. The endocrine system, in: *The Toxicology of Fishes* (R.T. Di Giulio & D.E. Hinton, Eds.). CRC Press, Boca Raton, FL, pp. 457-488.

Thomas, R.E., Gharrett, J.A., Carls, M.G., Rice, S.D., Moles, A., and S. Korn. 1986. Effects of fluctuating temperature on mortality, stress and energy reserves of juvenile coho salmon. *Transactions of the American Fisheries Society*, 115: 52-59.

Thomas, R.E. and S.D. Rice. 1987. Effect of water-soluble fraction of Cook Inlet crude oil on swimming performance and plasma cortisolin juvenile coho salmon *Oncorhynchus kisutch*). *Comparative Biochemistry and Physiology Part C*, 87:177–180.

Timchalk, C. 2006. Phsyiologically based pharmacokinetic modelling of organophosphorus and carbamate pesticides, in: *Toxicology of organophosphate*

& carbamate compounds, R.C. Gupta, (Eds.), Elsevier Academic Press, Burlington, MA., pp.103-126.

Tierney, K.B., Casselman, M., Takeda, S., Farrell, T., and C.J. Kennedy. 2007a. The relationship between cholinesterase inhibition and two types of swimming performance in chlorpyrifos-exposed coho salmon (*Oncorhynchus kisutch*). *Environmental Toxicology and Chemistry,* 26 (5): 998-1004.

Tierney, K.B., Singh, C.R., Ross, P.S., and C.J. Kennedy. 2007b. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three current-use pesticides. *Aquatic Toxicology*, 81: 55-64.

Tierney, K.B., Sampson, J.L., Ross, P.S., Sekela, M.A., and C.J. Kennedy. 2008. Salmon olfaction is impaired by an environmentally realistic pesticide mixture. *EnvironmentalScience and Technology*, 42: 4996-5001.

Toneys, M.L. and D.W. Coble. 1980. Mortality, hematocrit, osmolality, electrolyte regulation, and fat depletion of young-of-the-year freshwater fishes under simulated winter conditions. *Canadian Journal of Fisheries and Aquatic sciences*, 37: 225-232.

Trudel, M., Tucker, S., Morris, J.F.T., Higgs, D.A. and D.W. Welch. 2005. Indicators or energetic status in juvenile coho salmon and Chinook salmon. *North American Journal of Fisheries Management*, 25: 374-390.

United States Environmental Protection Agency (U.S. EPA). 2006. Reregistration eligibility decision (RED) Malathion. EPA 738-R- 06-030. Available at URL: http://www.epa.gov/oppsrrd1/REDs/ malathion_red.pdf. 6/1/09.

U.S. EPA. 2007. ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Available: http:/www.epa.gov/ecotox/, retrieved February 2010.

U.S. Geological Survey. 2001. Selected findings and current perspectives on urban and agricultural water quality by the national water-quality assessment program (NAWQA). FS-047-01. http://water.usgs.gov/pubs/FS/fs-047-01/.

Verrin, S.M., Begg, S.J., and P.S. Ross. 2004. Pesticide use in British Columbia and the Yukon: An assessment of types, applications and risks to aquatic biota. *Canadian Technical Report of Fisheries and Aquatic Sciences 2517*. Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, BC.

Vijayan, M.M. and T.W. Moon. 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Science*, 49: 2260-2266.

Vignier, V., Vandermeulen, J.H., and A.J. Fraser. 1992. Growth and food conversion by Atlantic salmon parr during 40 days exposure to crude oil. *Transactions of the American Fisheries Society*, 121: 322-332.

Walters, D.R. 2011. *Plant Defense: Warding off attack by pathogens, herbivores, and parasitic plants.* Wiley-Blackwell, West Sussex, United Kingdom, pp. 15-67.

Wheeler, C.E., Eder, K.J., Werner, I., Huang, H., Jones, P.D., Brammell, B.F., Elskus, A.A., and B.D. Hammock. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology*, 74: 172-192.

Wolfe, N.L., Zepp, R.G., Baughman, G.L., and J.A. Gordon. 1975. Kinetic investigation of malathion degradation in water. *Bulletin of Environmental Contamination and Toxicology*, 13 (6): 707-713.

Woltering, D.M. 1984. The growth response in fish chronic and early life stage toxicity tests: A critical review. *Aquatic Toxicology*, 5: 1-21.

Wooton, R.J. 1998. *Ecology of Teleost Fishes 2nd Ed*. Kluwer Academic Publishers, the Netherlands, pp. 1-86.

Zhao, X. and H. Hwang. 2009. A study of the degradation of organophosphorus pesticides in river waters and the identification of their degradation products by chromatography coupled with mass spectrometry. *Archives of Environmental and Contaminant Toxicology*, 56: 646-653.

Zheng, Y. and H.M. Hwang. 2006. Effects of temperature and microorganisms on malathion transformation in river water. *Bulletin of Environmental Contamination and Toxicology*, 76: 712-719.

Appendix A

Lipid content of GI tracts for fasted fish exposed to control, low, and high malathion concentrations. Clear bars fasted, shaded bars fed. Values are given as the mean±SEM.