Consequences of Sublethal Polychlorinated Biphenyl Exposure on the Swimming Performance of Rainbow Trout *Oncorhynchus mykiss*

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Abstract

Freshwater teleost fish often experience natural and anthropogenic conditions that result in fluctuating energy availability, therefore the ability to acquire, transform and use energy is essential for the survival of these fish. Polychlorinated biphenyls (PCB's) are recognized as physiological sources of stress to fish as they incite defense mechanisms that are generally costly in terms of metabolic resources. Over time, such responses may decrease individual performance and possibly fitness by changes in foraging, migration and escape behaviors, and the population in terms of reproductive capacity due to the alterations in energy allocation following an exposure. The main goal of this study was to determine if a sublethal exposure to PCB-126 affects the energy budget of the fish and can therefore be responsible for functional deficiencies associated with their locomotion. Fish were injected low (100 µg/kg) and high (400 µg/kg) concentrations of PCB-126 and swimming performance parameters including critical swimming speed, metabolic rate and recovery ratios were evaluated. EROD activity was also measured in the liver as an indication of PCB-126 intoxication while blood and white muscle tissue metabolites were analyzed to quantify the physiological disturbance levels associated with this exposure. A significant decrease was observed in the swimming performance of rainbow trout for the low and high PCB-126 treatments as well as an impaired recovery with increasing level of PCB exposure following exhaustive exercise. This study also showed the occurrence of physiological disturbance by a reduction in the hepatosomatic and spleen somatic indices and elevation of plasma cortisol and glucose levels, as well as white muscle reductions in glucose and glycogen indicating higher metabolic costs during recovery and muscle restoration for PCB-exposed fish. Overall, this research provides insights into the sublethal effects of toxic organic compounds on fish.

Résumé

Les poissons téléostéens d'eau douce subissent souvent des conditions naturelles et anthropogéniques de disponibilité d'énergie fluctuante, donc la capacité d'acquérir, de transformer et d'utiliser l'énergie est essentielle pour la survie de ces poissons. Les biphényles polychlorés (BPC) sont reconnus comme source de stress physiologique chez les poissons en incitant des mécanismes de défense qui sont généralement très coûteux en termes de ressources métaboliques. Ces mécanismes peuvent diminuer le rendement individuel ou le « fitness » du poisson par des changements comportementaux et aussi en terme de capacité de reproduction pour la population en raison de la modification dans la répartition de l'énergie suite à une intoxication. Le but principal de cette étude était de déterminer si une exposition au BPC 126 affecte le budget énergétique des poissons puis serait responsable des déficiences fonctionnelles. Les poissons ont été injectés avec des concentrations faible (100 µg/kg) et élevée (400 µg/kg) de BPC 126 puis des paramètres associés à la performance de nage tels que la vitesse de nage critique, le taux métabolique et les ratios de récupération ont été évalués. L'activité enzymatique EROD a également été mesurée dans le foie comme indication de l'intoxication par le BPC 126 tandis que des métabolites du sang et du muscle blanc ont été analysés pour quantifier les niveaux de perturbation physiologiques associés à cette exposition. Une diminution significative de la performance de nage des poissons a été observée pour les deux traitements de BPC 126 ainsi qu'une récupération affaiblie suite à un exercice exhaustif. Cette étude a également démontré la présence de perturbations physiologiques par une réduction des indices somatiques du foie et de la rate, par l'élévation des niveaux de cortisol et glucose plasmatique ainsi que des réductions en concentrations de glucose et glycogène dans le muscle blanc. Ceci confirme une hausse des coûts métaboliques au cours de la récupération et la restauration des muscles pour les poissons exposés aux BPCs. Globalement, cette recherche permet de mieux comprendre les effets sublétaux des composés organiques toxiques sur les poissons.

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AA – amino acids
ACTH – adrenocorticotropic hormone
AFA – aerobic scope for activity
AhR – aryl hydrocarbon receptor
AMR – activity metabolic rate
ANS – autonomic nervous system
Arnt – aryl receptor nuclear translocator
ATP – adenosine-5'-triphosphate
BL – body length
CORT – cortisol
CRH – corticotropin releasing hormone
CYP1A – cytochrome P450 1A
DF – degrees of freedom
DNA – deoxyribonucleic acid
EDTA – ethylenediaminetetraacetic acid
EMG – electromyogram
EROD – ethoxyresorufin-O-decthylase
F – statistical F-value
GAS – general adaptation syndrome
GLC – glucose
GLY – glycogen
H – statistical H-value
HIF-1 –hypoxic inducible factor 1
HESI – heart somatic index
HSI – hepatosomatic index
IP – intraperitoneal
K – condition factor
Kow – octanol/water partition coefficient
LAC – lactate
MMR – maximum metabolic rate
MO_2 – metabolic rate (oxygen consumption)
NADPH – nicotinamide adenine dinucleotide phosphate
p – statistical probability
PCB – polychlorinated biphenyl
R – aerobic metabolism
R_s – standard metabolism
R_r – routine metabolism
R_f – heat increment
R_a – active metabolism
RMR – routine metabolic rate
RR – recovery ratio
SEM – standard error mean
SDA – specific dynamic action
SMR – standard metabolic rate
SSI – spleen somatic index
t – statistical t-test value
T_3 – Triiodothyronine
T_4 – Thyroxine
TCDD – 2,3,7,8-tetrachlorodibenxo-p-dioxin
TEF – toxic equivalent factor
TL – total length
TL_i – initial total length
TL_f – final total length
TP – total protein
TRH – thyrotropin releasing hormone
TRI – triglycerides
TSH – thyroid stimulating hormone
Ucrit – critical swimming speed
W_i – initial weight
W_f – final weight

Chapter 1

General Introduction

1.0. Introduction

Aquatic environments worldwide are suffering from accelerated rates of environmental degradation due to emissions of toxic compounds from anthropogenic sources (Van der Oost *et al.*, 2003). Over the past fifteen years, this exposure to toxins has resulted in the loss of more than 400 plant and animal species (Stabeneau *et al.*, 2008). Unfortunately this number is increasing at an alarming rate especially for fish as they represent over half of the known vertebrate species. There are approximately 25,000 documented fish species, of which 15,000 are found in marine ecosystems while the remaining 10,000 occupy freshwater environments (Nelson, 1994). In many of these aquatic ecosystems, fish provide a wide range of services as keystone species, which benefit both wildlife and human populations (Holmlund and Hammer, 1999). Regulating and linking services (Table 1.1) are particularly valuable for wildlife populations as they include top-down effects regulating population dynamics and nutrient availability, bioturbation in or near sediments, active transport of nutrients, carbon and energy exchange between the pelagic and either hard or soft bottoms, or the littoral zone. They also include passive transport of nutrients between ecosystems when fish eggs, fry, juveniles, adults, and carcasses are preyed upon by birds and mammals (Holmlund and Hammer, 1999).

Humans benefit especially from information and cultural services (Table 1.1) generated by fish populations. Information services include using fish as indicators of ecosystem health, recovery and resilience, as well as environmental recorders (Holling, 1973). Also, their movement patterns within temporal and spatial scales of ecological systems

Table 1.1. Ecosystem services generated by marine and freshwater fish populations. All these services rendered by fish species can be divided into fundamental ecosystem services or demand-derived services. Fundamental services ensure the proper functioning and resilience of a particular ecosystem therefore they are essential for human existence. Demand-derived services are formed by human values but are not necessarily fundamental for the survival of human societies.

Fundamental ecosystem services	
Regulating services	Linking services
Regulation of food web dynamics	Linkage within aquatic ecosystems
Recycling of nutrients	Linkage between aquatic and terrestrial ecosystems
Regulation of ecosystem resilience	Transport of nutrients, carbon and minerals
Redristibution of bottom substrates	Transport of energy
Regulation of carbon fluxes from water to the atmosphere	Acting as ecological memory
Maintenance of sediment processes	
Maintenance of genetics, species, ecosystem biodiversity	

Demand-derived ecosystem services	
Cultural services	Information services
Production of food	Assessment of ecosystem stress
Aquaculture production	Assessment of ecosystem resilience
Production of medicine	Revealing evolutionary tracks
Control of hazardous diseases	Provision of historical information
Control of algae and macrophytes	Provision of scientific and educational information
Reduction of waste	
Supply of aesthetic values	
Supply of recreational activities	

Source: Modified from Holmund and Hammer, 1999

enhance the functional importance of fish as ecological memory in the form of energy, nutrients, genetic reserves, and information (Chapin *et al.*, 1997). Cultural services consider fish as goods for purifying water, recreation, mitigating the spread of diseases, and aquaculture as a protein food source (Holmlund and Hammer, 1999). Moreover, fish generate employment and can be used as management tools to stimulate human interest in nature or to provide aesthetic and recreational values (Harris, 1995).

All of these services generated by fish populations worldwide emphasize the importance of studying the effects of pollutants on these valuable vertebrates. Furthermore, fish remain an essential part of a healthy diet as a good source of protein and essential fatty acids, however many consumption advisories are currently in effect across North America as some species contain contaminant levels that are a health concern for humans (WHO, 2003; MOE, 2009). Recognition and prevention of contamination issues would help to ensure sustainability of this important resource for both wildlife and humans (Brandt and Hartman, 1993). In addition, a better knowledge of these problems would help to preserve the integrity of aquatic ecosystems and subsequently prevent unexpected negative consequences from the alterations of fish populations on their role and function that could strongly impact human societies and the environment.

The main focus of this research is the impact and consequence of toxic organic compounds on fish biochemical and physiological processes. The effects of polychlorinated biphenyls (PCBs) on energetic metabolism factors including swimming performance and oxygen consumption rate, will allow us to determine if exposure to sublethal levels of these chemicals could be responsible for functional deficiencies in fish by using a bioenergetics approach. This chapter will introduce the concepts of energetics and stress, provide a short

overview of PCB compounds, and discuss parameters associated with fish swimming performance and respirometry as they relate to endpoints for the study of sublethal impacts of PCBs on fish.

1.1. Bioenergetics and Stress

1.1.1. Energy Metabolism in Fish

Energy is defined as the capacity of a system to do work, and animals generate energy through the catabolism and oxidation of dietary carbohydrates, lipids and proteins within the body (Cho, 1992; Farbridge and Leatherland, 1992). These major food nutrients are required by animals not only as essential provisions for the production of tissues, but also as sources of stored chemical energy in the form of adenosine triphosphate (ATP) that fuels the energy-requiring biochemical processes of life (Warren and Davis, 1967). Energy is therefore essential for the maintenance of vital processes such as cellular metabolism, growth, reproduction, and physical activity (Bar *et al.*, 2007). Thus, the ability to efficiently acquire, transform and use energy is an important factor in the fitness of an animal including the relationship between a fish and its environment (Elliott, 1976). Consequently, it is important to understand how fish partition energy and how exposure to various environmental contaminants can affect their energy budgets.

Bioenergetics describe the relationships between energy supply and expenditures (demands), which relies on the first law of thermodynamics implying that energy and matter are conserved and that energy allocations follow a hierarchical distribution (Fig.1.1). Fish obtain energy through feeding at various trophic levels to support body maintenance processes. Any remaining energy is then allocated to other processes including growth,

reproduction, locomotion and behavioural activities. However, only a portion of the total energy ingested is available to the fish as 45 % of this energy is eliminated at various stages in these processes either as undigested food (feces), metabolic excretion or heat (Elliott and Davison, 1975; Brett and Groves, 1979; Rice, 1990; Beyers *et al.*, 1998; 1999). These energy losses can vary depending on feeding regime, water temperature, fish size and the physiological status of the animal (Cho, 1992).

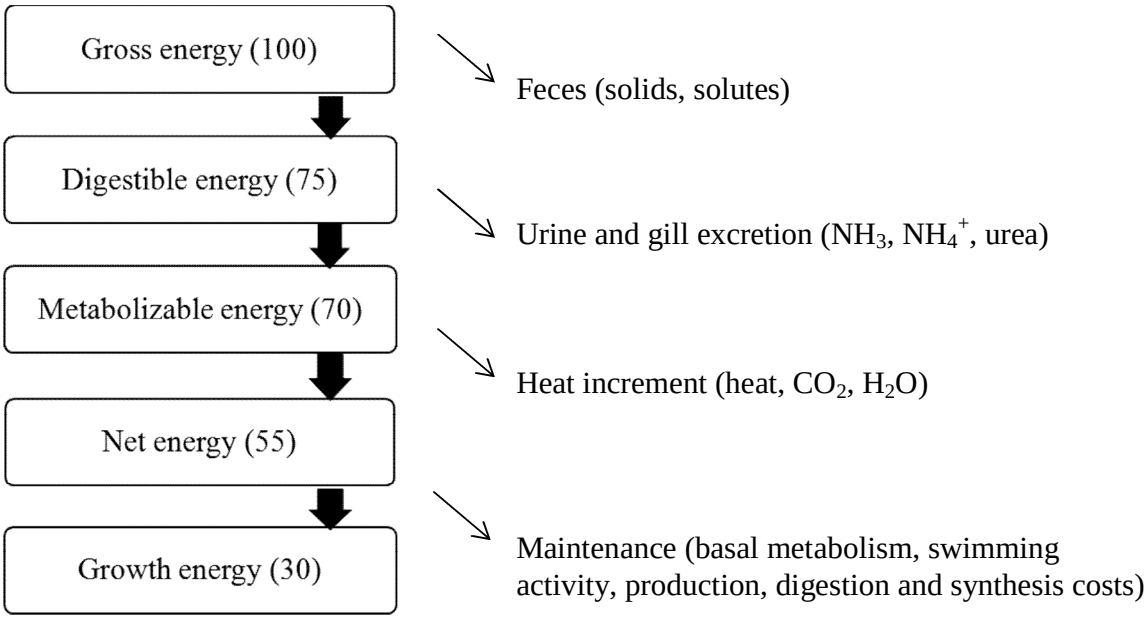

Fig. 1.1. General energy budget of fish. The energy percentage available to the organism at each level of the hierarchy is indicated in parentheses.

The distribution of energy can be translated into a generalized energy budget equation which balances the intake of food consumed against the costs of maintenance and other important activities: $C = R + A + S + F + U + \triangle B$, where C = food consumption, R = metabolism, A = activity, S = specific dynamic action (cost of processing food), F = egestion (feces), U = excretion (urine), and $\triangle B$ = somatic growth (Beyers *et al.*, 1998; 1999). Hence,

energy intake or assimilation represents the net energy acquired from feeding after digestion and absorption, whereas energy expenditures are associated with somatic maintenance, growth, reproduction and locomotion (Campbell *et al.*, 2002).

Energy metabolism in fish is different from that of terrestrial animals in two important aspects. First, in contrast to endothermic animals (birds and mammals), fish are aquatic ectotherms and are not required to expend energy in maintaining a constant body temperature of 37°C (or slightly higher). This implies that they have much lower maintenance energy requirements than terrestrial animals (Cho and Kaushik, 1985). Second, fish are able to obtain 10–20% more energy from the catabolism of proteins than terrestrial animals, since they do not have to convert ammonia (the end product of protein catabolism) into less toxic substances such as urea or uric acid prior to excretion (Brett and Groves, 1979). Thus, the excretion of waste nitrogen requires less energy in fish so they can allocate a higher proportion for other vital processes.

1.1.2. Energetic Costs and Stressors

Many natural and anthropogenic factors are known to influence the energy requirements of fish. They are considered stressful conditions imposed on fish that have the potential to alter energy allocations within their ordinary energy budget (Calow, 1991; Forbes and Calow, 1997). Therefore, it is possible to describe energetic costs imposed on fish resulting from these changes by quantifying stress in terms of energy (Beyers *et al.*, 1999). An energy budget is thus a convenient way of illustrating how environmental conditions influence energy expenditure over time. It provides a means for quantifying the value of available resources as well as associated acquisition costs and can account for physiological effects of

suboptimal environmental conditions that may deplete energy reserves (Calow, 1991; Forbes and Calow, 1998).

The concept of a balanced energy budget has been applied to toxic organic compounds, as they are recognized as pollutants and sources of physiological stress to fish. An exposure to these compounds can be followed by a metabolic disruption at many physiological levels as the fish must meet the cost of stress by either increasing energy intake, reducing energy expenditure, and/or reallocating existing energy (Campbell *et al.*, 2002). Moreover under stressful conditions, the amount of energy ingested is generally lower due to a loss of appetite and changes in foraging activities (Warren and Davis, 1967). Therefore, a large proportion of the ingested energy is allocated primarily to maintenance, resulting in adverse effects on the remaining processes because the fish must invest in detoxification and repair mechanisms. As a result, these modifications in energy allocation caused by toxic exposure may provoke inappropriate behavioural responses including changes in food acquisition, predator response, competitive ability, mating opportunity, environmental tolerance as well as sensitivity to pathogenic agents (Davis and Schreck, 1997, Forbes and Calow, 1997; Parker, 1999; Brown *et al.*, 2002; Seegal and Bowers, 2002). Over time, all of these responses may lead to a decrease in individual performance or fitness of the fish and subsequently alter its role and function within aquatic environments.

1.1.3. Physiological Stress Response

Exposure to a stressor stimulates a physiological response within vertebrates including fish called the General Adaptation Syndrome (G.A.S); a mechanism that describes the body's short and long-term reactions to a stressor (Selye, 1950) and is often quantified by using

energy as a response variable (Beyers *et al.*, 1998; 1999). This syndrome involves the activation of the nervous and endocrine systems, which are sustained by a sequence of biochemical responses (Fig. 1.2) that attempt to counteract the potentially harmful effects of the stressor (e.g. a toxic compound) on the proper functioning of the organism's internal systems (Selye, 1950; 1973). These responses involve all levels of organization from the cell to a whole population or ecosystem (Adams, 1990; Barton and Iwama, 1991; Hightower, 1991) and are classified into three separate phases called alarm, resistance and exhaustion.

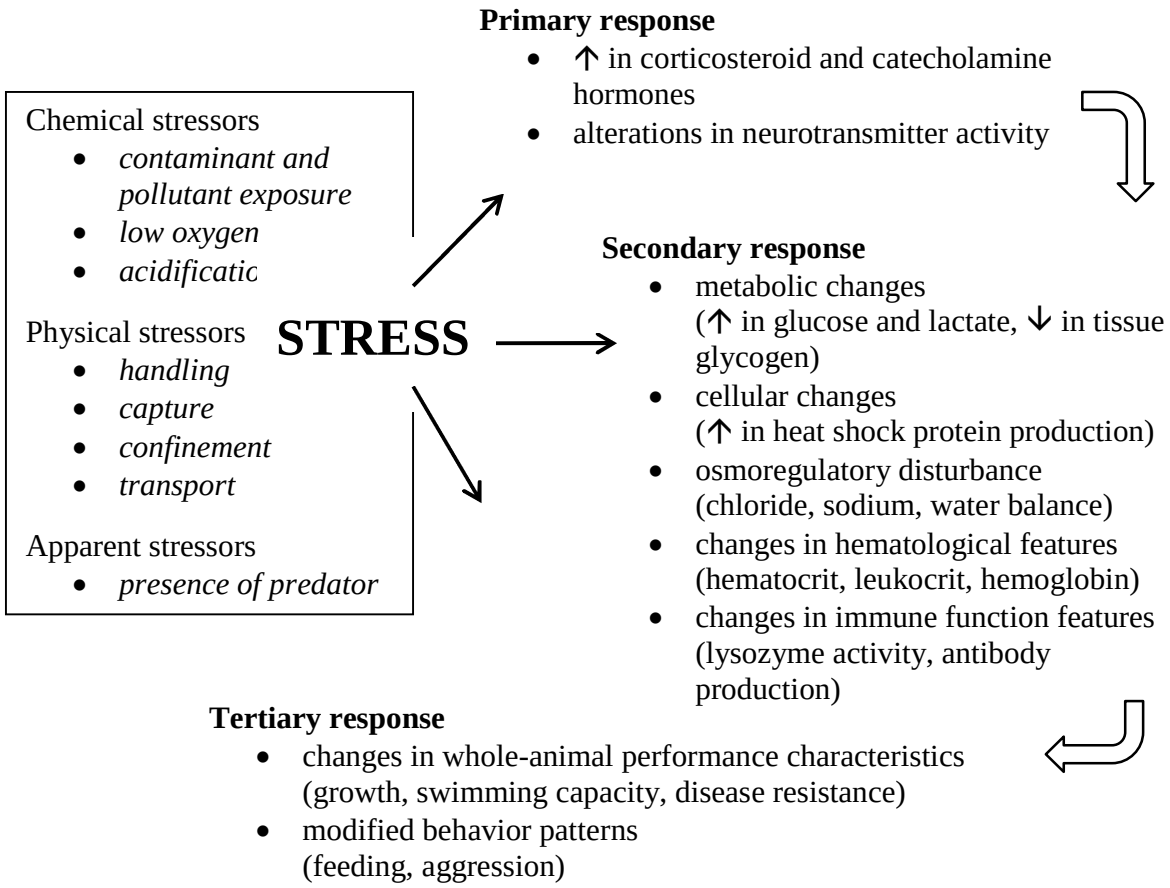

Fig. 1.2. Physiological stress response in fish (*modified from Barton, 2002*)

The alarm (primary response) phase represents the immediate reaction to a stressor. This initial response denotes the perception of an altered state and initiates a neuroendocrine response that forms part of the generalized stress response in fish (Gamperl *et al.*, 1994). It occurs when a stressor upsets organism homeostasis and corresponds to an effort by the organism to readjust its physiological equilibrium (Beyers *et al.*, 1999; Wendelaar Bonga, 1991; Barton, 1997; 2002). In this stage, the hypothalamus preoptic area of the brain detects this disruption and initiates an increase in autonomic nervous system (ANS) activity and the subsequent release of the corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). In turn, CRH stimulates the anterior pituitary gland to secrete adrenocorticotropic hormones (ACTH), which signals the adrenal gland located in the dorsal region of the kidney or the steroidogenic cells of the fish head-kidney to produce and secrete glucocorticoids including cortisone and primarily cortisol (Balm *et al.*, 1994). Increased ANS activity also results in activation of the chromaffin cells of the adrenal gland or interrenal tissue (in fish) to increase the release of the catecholamines adrenaline and noradrenaline (Randall and Perry, 1992). Increased plasma corticosteroid levels are believed to stimulate protein catabolism and hepatic gluconeogenesis, while the catecholamines increase hepatic glycogenolysis (glycogen breakdown) all leading to maintaining blood glycaemic levels appropriate to fuel the increased energy demands associated with the response to the stressor (Mazeaud *et al.*, 1977; Leach and Taylor, 1980; Wendelaar Bonga, 1991, 1997; Barton, 1997, 2002). In addition, the thyroid gland is stimulated by the release of the thyrotropin-secreting hormone (TSH) from the pituitary to secrete thyroxine. The quantity of each hormone released by the pituitary depends on the magnitude of the stressor thus it will be more stimulated if the brain perceives a higher danger or threat (Selye, 1950). The alarm phase may last only a few

seconds to minutes, since adrenaline secretion and manifestation of its effects is a very rapid response. All of these biochemical responses are believed to facilitate a rapid mobilization of energy reserves, allowing the fish to meet the increased energy demands associated with exposure and resistance to stressors (Thomas, 1990; Rice, 1990; Callow, 1991; Wendelaar Bonga, 1991; Forbes and Callow, 1997, 1998).

The stage of resistance (secondary response) occurs when physiological adjustments are accomplished therefore compensating for the effects of the stressor is becoming part of the normal cost of living (Beyers *et al*., 1999). This response comprises the various biochemical and physiological effects associated with stress, and mediated to a large extent by the above stress hormones. The stress hormones activate a number of metabolic pathways that result in alterations in blood chemistry and haematology (Barton and Iwama, 1991; Randall and Perry, 1992; Vijayan *et al.* 1994a; 1994b; Mommsen *et al.*, 1999; Barton, 2002). Over time, the pituitary reduces its secretion of hormones as the effects of the alarm phase lessen (Selye, 1950). Organisms can stay within this resistance phase for an undetermined period while attempting to regain their original energy level and meet the new demands imposed by the environment. In most circumstances the organism is able to recover in the course of time unless additional stressors are encountered.

The final stage, exhaustion (tertiary response), only occurs if the stressor is present in sufficient magnitude and applied over an extended period or if additional stressors are encountered. Essential body resources are eventually depleted due to prolonged, frequent or strong stress responses and the body is unable to maintain normal function (Selye, 1973). The physiological/biochemical systems responsible for compensating for such effects become exhausted and stop functioning as they are forced to function well beyond their capacity

(Selye, 1973). This response represents whole animal and population level changes associated with stress. If the fish is unable to acclimate or adapt to the stressor, whole animal changes may occur, including decreased reproductive capacity and growth (Heath, 1995). This might be associated with stress-mediated energy-repartitioning that diverts energy substrates away from vital life processes, such as reproduction and growth, in order to cope with the enhanced energy demand associated with the stress. Decreased recruitment and productivity may also alter community species abundance and diversity (Barton, 2002). In other words, the ability of an organism to adequately respond to a stressor will determine its ability to adjust to its environment and contribute effectively at the population level.

1.2. Polychlorinated Biphenyls

1.2.1. Physical and Chemical Properties

Polychlorinated biphenyls (PCBs) are industrial contaminants composed of two benzene rings to which 1-10 chlorine or hydrogen atoms are attached to the carbon atoms on the biphenyl molecule (Buikema *et al.*, 1979). Their general chemical formula is $C_{12}H_{10-x}Cl_x$, where x denotes the number of chlorine atoms. PCBs are classified as persistent organic pollutants and represent a class of synthetic halogenated aromatic hydrocarbons or non-polar hydrophobic compounds (Mayer *et al.*, 1977; Safe, 1984). There are 209 PCB congeners, which are similar structural compounds although they differ in their degree of chlorination and the position of the chlorine atoms on the biphenyl molecule (Lang, 1992; Van der Oost *et al.*, 2003). These molecular arrangements determine the physicochemical properties of the congeners, environmental pathways and toxicity (Gdaniec-Pietryka *et al.*, 2007). The two biphenyl rings in a PCB molecule can rotate around the bond connecting them. The shape of

the molecule is further influenced by the repulsion between nearby bulky chlorine atoms thus the rings of a specific congener will either lie approximately in the same plane (coplanar) or in different, more perpendicular (non-planar) planes (Safe, 1984; 1985).

All PCB congeners were anthropogenically produced by a controlled reaction of the biphenyl molecule with elemental chlorine under the catalytic influence of iron or iron chloride (Gdaniec-Pietryka *et al.*, 2007). The commercial production of PCBs started in 1929 based on their resistance to acids, bases and heat. They were used in electrical equipment, primarily as coolants or insulating fluids for transformers and capacitors, but were also utilized for stabilizing additives in flexible PVC coatings or electrical wiring and electric components, pesticide extenders, cutting oils, flame retardants, hydraulic fluids, sealants, adhesives, wood floor finishes, paints, de-dusting agents, carbonless copy paper and many more (Van der Oost *et al.*, 2003). The wide variety of applications was possible because of their different physicochemical properties such as flammability and electric conductivity with varying degrees of chlorination. As the degree of chlorination increases, melting point and lipophilicity also increase while vapour pressure and water solubility decrease (Buckman *et al.*, 2004) resulting in an overall increase in toxicity. Over the years, public, legal and scientific concerns arose on the potential of PCBs to adversely impact humans and wildlife causing the establishment of extensive regulatory actions worldwide (Safe, 1985; 1994). Although production was banned in North America in the late 1970's followed by Japan and a few European countries due to possible risks to human health and the environment, PCBs are still considered amongst the most widespread and hazardous synthetic pollutants remaining in the environment (Buckman *et al.*, 2004) due to their highly unreactive nature and resistance to chemical and biological degradation.

1.2.2. Sources and Pathways in the Environment

The global production of PCBs is estimated at 2 million tons beginning in 1929 (Ivanov and Sandell, 1992; Rantanen, 1992), of which about 10% remains widely distributed in our environment (Buikema *et al.*, 1979). The main sources of PCBs are from commercial production or by-products of combustion processes (Brown *et al.*, 1995) released due to inappropriate disposal practices, accidents, and leakages from industrial facilities. Landfills containing transformers, capacitors, and other old electrical equipment containing PCB waste can release these chemicals into the atmosphere or into adjacent waterways. Also, the incineration of municipal wastes may lead to PCB pollution and produce dangerous by-products including hydrogen chloride and dioxins (Beyer and Vermeulen, 2003; WHO, 2003).

Once in the environment, PCBs in the gaseous phase partition among air, water, soil and sediments as they are transported through several pathways including deposition and evaporation (Billiard *et al.*, 2002; Gdaniec-Pietryka *et al.*, 2007). The octanol-water partition coefficient (Kow) predicts their chemical distribution in the environment as the octanol represents the organic (lipophilic) phase, whereas the water represents the aqueous (hydrophilic) phase. Most PCB congeners have a relatively high Kow (5-8) therefore they bind strongly to organic particles and are mostly found in soils, sediments and animal fatty tissues (Hawker and Connell, 1988).

Small dissolved amounts of PCBs can be found in surface waters because of their low solubility, but the molecules mainly attach to organic matter, clay or micro-particles and remain buried for long periods of time in the sediments before being slowly released to the water column when temperatures warm and concentrations are high. These toxic molecules can then evaporate in the air from the contaminated water bodies. In the atmosphere, PCB

molecules can reach the ground by wet deposition (snow or rain) or dry deposition when the suspended particles that are attached to dust or soot settle by gravity (Iwata *et al.*, 1993; Beyer and Biziuk, 2009). Some PCBs, especially low-chlorinated congeners, have high vapor pressures therefore their volatility is increased permitting them to be transported very long distances and deposited in colder northern regions.

Organic pollutants within the water column can also build up in living organisms either by uptake from the environment over time (bioaccumulation) or along the food chain (biomagnification) since these compounds are not very soluble in water but are readily soluble in fatty tissues (Farrington, 1991; Mackay and Fraser, 2000). This solubility leads to greater PCB concentrations in organisms overtime that are higher in the food chain, such as predatory fish, birds or mammals. The degree of solubility is largely dependent on the number and position of chlorine atoms on the PCB molecule (Farrington, 1991) whereas the degree of bioaccumulation depends on how quickly the compounds are assimilated and then eliminated by the organism. In turn, this depends on body lipid content, habitat, dietary uptake, growth rate and metabolism of each species as well as the specific PCB congener involved (Sijm *et al.*, 1992). Humans can also accumulate PCBs from consumption of animal products such as dairy, fish and meats (Oliver and Niimi, 1988; Borlakoglu and Haegele, 1991; Mackay and Fraser, 2000). These compounds can be measured in biological samples including human blood, milk, fatty tissues, and foods or in environmental samples including air, drinking water, soil, sediment and solid wastes.

All PCBs can be broken down either by the combined effects of sunlight and atmospheric ozone or by microorganisms in the soil or sediments. The extent to which a PCB molecule can be broken down or transformed in the environment depends on the number of

chlorine atoms it contains and their location (Safe, 1994). Highly chlorinated molecules are generally less available to organisms since they are more strongly bound to soil and sediment particles and are usually present in lower quantities within the environment. In these conditions the molecules are usually more persistent and the degradation process is slower. Less chlorinated molecules are more readily metabolized and eliminated, therefore biodegradation is faster and this also prevents them from bioaccumulating in food webs (McFarland and Clarke, 1989).

PCBs in the atmosphere tend to react with ozone and water under the effect of sunlight which results in the removal of chlorine atoms (Safe, 1984; 1985). The time required for half the initial PCBs to be broken down ranges from 3.5 to 83 days for molecules with 1-5 chlorine atoms (Brown *et al.*, 1995). In water, PCBs are essentially broken down by the effects of sunlight (photolysis). During summer in shallow water, it takes 17-210 days for half the amount initially present to be broken down for molecules with 1 to 4 chlorine atoms (Lang, 1992). This breakdown by sunlight is slower during winter. PCB congeners with a greater number of chlorine atoms (>7) absorb more sunlight and are thus broken down more easily. PCBs in soils and sediments are degraded primarily by microorganisms. The speed of degradation depends on several factors including the number and location of chlorine atoms, PCB concentration, the types of microorganism present, available nutrients and temperature (Lang, 1992). Although this breakdown is generally slow, it can occur with or without the presence of oxygen and also to some extent in water.

1.2.3. Toxic Mechanisms of Action

Coplanar congeners are of primary concern because they are very stable compounds containing 4-7 chlorine atoms out of 10 possible substitutions at the *para, meta* and *ortho*

positions on the molecule which makes them nearly chemically inert and not readily degradable in the environment (Veith and Lee, 1970; McFarland and Clarke, 1989). Therefore they remain available to organisms within the environment for uptake and tend to bioaccumulate in the food chain thus increasing their overall potential toxicity. They may be destroyed by chemical, thermal and biochemical processes; however full destruction is difficult to achieve and poses a risk of creating extremely toxic dibenzodioxin and dibenzofuran compounds through partial oxidation (Van der Oost *et al.*, 2003).

These PCB congeners are considered the most toxicologically active compounds (Giesy and Kannan, 1998) and are divided into non-*ortho* (PCB 77, 126, 169), mono-*ortho* (PCB 105, 114, 118, 123, 156, 157, 167, 189) or di-*ortho* (PCB 170, 180) substituted PCBs (Alcock *et al.*, 1998). Coplanar congeners share the same toxic mechanism for producing biological and toxicological effects as dioxins due to their planar configuration and similar structure to TCDD or 2,3,7,8-tetrachlorodibenxo-p-dioxin molecule (Veith and Lee, 1970; Safe, 1990, 1994, 1995; Bhavsar *et al.*, 2007a). This implies that they can incur toxic effects at lower concentrations compared to the non-planar congeners.

PCBs are toxic to aquatic, avian and mammalian species through their binding to the aryl-hydrocarbon receptor (AhR), a cytosolic receptor protein found in vertebrate tissues; however fish (especially at early life stages) are amongst the most sensitive vertebrates (Billiard *et al.*, 2002). The PCB-AhR complex moves to the nucleus and heterodimerizes with the Ah-receptor nuclear translocator (Arnt) protein. Arnt has been found to be the β-subunit of the hypoxic-inducible factor 1 (HIF-1). In turn, this AhR-Arnt complex interacts with specific DNA sequences called xenobiotic response elements in the promoter regions of xenobiotic-sensitive genes to alter their expression (Brown *et al.*, 1998; Hahn, 1998).

However, for fish to efficiently biotransform or metabolize organochlorine contaminants these contaminants must be either planar or have adjacent *ortho* and *meta* positions that are not chlorine-substituted. Coplanar congeners have no reactive functional groups so they must be hydroxylated to increase their polarity and be subjected to excretion. This occurs mainly by inducing the hepatic P-450-dependent monooxygenase enzymes including CYP1A activities (Siroka and Drastichova, 2004). The CYP1 family are amongst the most sensitive indicators of exposure to xenobiotics in aquatic environments. They are important oxidation enzymes of phase 1 xenobiotic metabolism (hydroxylation) and are present at high activities in the liver, but are also found in the intestine, kidney, lungs, brain, skin, prostate gland, and placenta. These enzymes are classified as b-type hemeproteins associated with membranes of the endoplasmic reticulum but also bound to mitochondrial membranes (Siroka and Drastichova, 2004). The toxicity of pollutants is related to their degree of affinity for the AhR; therefore an increase binding affinity indicates an increase capacity to induce CYP1A and lead to adverse consequences (Billiard *et al.*, 2002). The induction of the CYP enzymes therefore results in toxic effects on the cell (Safe, 1990; 1994; Ahlborg *et al.*, 1992; 1994) reflected by a plethora of downstream events including hormonal systems, cellular growth and development which in turn can alter certain enzyme types involved in energy production (Billiard *et al.*, 2002). In fish, differences in CYP catalytic activities among individuals of one population as well as among populations are related to factors including species, sex, diet and seasonality.

The toxicity of “dioxin-like” compounds can also be expressed as the toxic equivalent factor (TEF) which indicates the toxicity of a compound compared to the equivalent amount of 2,3,7,8-TCDD with a reference value of 1 (Bhavsar *et al.*, 2007b; 2008). Thus the toxicity

potential of PCBs in fish vary such that non-*ortho* substituted congeners (PCB 77, 126, 169) have higher TEFs ranging from 0.005-0.1 compared to the much lower values (0.00001-0.001) of the mono-*ortho* and di-*ortho* substituted molecules mentioned above (Alcock *et al.*, 1998).

Non-planar PCBs do not bind to the AhR as they do not have dioxin-like properties so they are usually assessed separately as they express different toxicological hazards (Bhavsar *et al.*, 2007a). However, at high doses they can have effects on the development of the nervous system and on dopamine levels which can contribute to the formation of tumors. In addition to the actual PCB compounds, some of their breakdown products can act as endocrine disruptors having negative effects on thyroid hormone levels, inhibiting enzyme activity in the adrenal glands, interacting with estrogen receptors, altering cellular processes in the lung and affecting the reproductive system (WHO, 2003).

1.2.4. Sublethal Toxicity in Vertebrates

The concentrations of PCBs found in the environment are generally in the low parts per trillion (ppt) range which is considered to be below the necessary levels to result in acute toxicity or death (MOE, 2009). However, these concentrations are still toxicologically relevant levels (Bhavsar *et al.*, 2008) for sublethal effects to occur and alter important processes including growth, reproduction, behavior and development. Sublethal effects of toxic organic compounds on vertebrates should be studied at different levels of biological organization (e.g., cell, organs, tissues, individuals) as each level offers unique insights on a particular response (Heath, 1995). Furthermore, the toxicity mechanism of chemicals suspected of causing adverse ecological effects should be focused on potential interference in biochemical and endocrinological control mechanisms that are important for the survival of individuals within populations including growth, reproduction, energy utilization and osmoregulation (Alcaraz,

2000). They are confident indices of physical fitness because the overall capability and efficiency of the organism to cope with changing environmental conditions can be determined (Seegal and Bowers, 2002). The changes observed at the individual level, and their consequences, can then be extrapolated to whole populations, communities and ecosystems.

1.2.4.1 Anatomical and Behavioural Effects

Previously observed effects of persistent organic pollutants on vertebrates include liver damage, anemia, chloracne, skin legions, stomach or thyroid gland injuries, immune system changes, behavioral alterations, impaired reproduction as well as teratogenic effects (Brown *et al.*, 1998; Forbes and Calow, 1998; Clotfelter *et al.*, 2004; Zala and Penn, 2004; Breuner *et al.*, 2008). Disrupting behavior could include changes to foraging, predator avoidance, reproduction and social hierarchies. Sensory, hormonal, neurological and metabolic systems are physiological effects that contribute to alter the expression of these behaviours (Scott and Sloman, 2004). Predator avoidance can be diminished when an organism is exposed to contaminants increasing the prey's susceptibility to predation by the disruption of sensory systems and lack of motivation to respond appropriately (Weis *et al.*, 2001). This results in a disruption in the ability of the animal to escape a predation event, which in turn reduces the survival of the individual. Toxicants can also disrupt schooling behavior in fish by forcing a decrease in group cohesion, or by increasing occurrences of schooling, which decreases the time available for other crucial activities such as reproduction and feeding (Scott and Sloman, 2004). Organic pollutants also have the ability to alter the timing of reproductive behaviours, the criteria for mate selection (Parsons, 1997), and ultimately disrupt reproductive performance (Donaldson, 1990). They can also alter spawning site selection and natal homing,

which for some species is crucial for successful reproduction (De Coen and Janssen, 1997). Furthermore PCBs are shown to alter social hierarchies by increasing agonistic acts such as threats, nips and chases with higher toxicant concentrations by dominant males (Scott and Sloman, 2004).

1.2.4.2. Cellular, Biochemical and Physiological Effects

Organic compounds can also be associated with cellular, biochemical and physiological toxic responses including thymic involution, carcinogenicity, teratogenicity, changes in thyroid function and altered vitamin A metabolism. Sensory disruption is an important effect since olfaction is believed to be a predominant sense in fish (Scott and Sloman, 2004). Chemoreception allows these organisms to respond to their environment and by accumulating toxicants in cells of the olfactory system this could subsequently cause cell damage or death (Scott and Sloman, 2004). Toxicants can also disrupt electrical transmission of sensory information from the olfactory epithelium to the brain (Rehnberg and Schreck, 1986). Endocrine disruption is also an important consideration since toxicants may agonize or antagonize endogenous hormones, disrupting their synthesis or metabolism as well as their receptors. In turn this may disrupt growth and thyroid hormone function (Breuner *et al.*, 2008), as well as hormones associated with the physiological stress response. Thyroid function is associated directly and indirectly with nearly all major physiological and biochemical functions in fish, thus alteration of its normal function could affect the ability of fish to adapt and survive changes in its natural environment (Mayer *et al.*, 1977). Thyroid hormones (T_3 and T_4) regulate many metabolic processes such as early development, growth and reproduction; therefore exposure of fish to environmental contaminants during critical periods

could disrupt the thyroid endocrine system and compromise overall fitness and survival (Buckman *et al.,* 2007). These hormones are essential to the proper development and differentiation of all cells in addition to regulating protein, fat and carbohydrate metabolism which means they affect how cells use energy compounds and protein synthesis. Thyroid hormones, in conjunction with other hormone systems and vitamins, promote growth, development and early reproductive events (Clotfelter *et al.,* 2004). They are generally associated with protein anabolism in fish. The endocrine system might be affected by PCBs through neurological dysfunctions, which may include alterations in brain cholinesterase activity or neurotransmitter levels (Porter *et al.,* 1984). Other brain enzymes involved in general cellular functions can also be altered, thus directly affecting the electrophysiological properties of the fish brain (Anderson, 1990). As previously mentioned, toxicants may also disrupt various aspects of metabolism in fish. The major downstream effect of this metabolic disruption is an alteration of energy allocations that could have potential implications for numerous aspects of fish behavior. Also, an animal exposed to a toxicant may experience changes in food and energy requirements and, most probably, have a decreased ability to assimilate nutrients from food (Zala and Penn, 2004). Finally, some contaminants have the ability to adversely impact the development of fetuses or embryos. This can result in decreased growth rates, functional deficiencies, anatomical malformations and, in the worst case, fetal or embryonic death (Bodammer, 1993).

1.3. Fish Locomotion and Swimming Performance

Axial undulation is the primitive swimming mode of fish, usually employed to travel long distances while maintaining a constant speed (Sfakiotalis *et al.,* 1999). This type of

locomotion involves the lateral bending of the body and oscillatory movement of the tail. These movements are powered by the myotomal musculature and involve several key steps. In order to swim, the axial muscles must generate force and then transmit this force along the myotome to the skin and tail of the fish. Once transmitted, these forces are converted into thrust by the propulsive structures of the fish, which in undulatory swimmers are the body and the unpaired fins (Coughlin *et al.*, 2002; Müller and van Leeuwen, 2006). In undulatory swimming, a backward-travelling wave of bending is generated by the sequential activation of the segmental myotomes from head to tail. As the body bends, and the oscillations travel down the fish, the body and caudal fin push against the water, generating forward thrust (Altringham and Ellerby, 1999). Most fish possess a narrow strip of red aerobic or slow-twitch muscle fibres that power this continuous swimming while the bulk of the axial musculature is made up of white anaerobic or fast-twitch muscle fibres that power fast starts and sprints. Hence, slow or steady axial swimming is the product of alternating contractions of red muscle that propagate from anterior to posterior (Rome *et al.*, 1993; Wardle *et al.*, 1995; Coughlin and Rome, 1996; Hammond *et al.*, 1998; Coughlin, 2000). Power production at higher, but still steady swimming speeds is improved by the pink or intermediate muscle in some fish species (Coughlin and Rome, 1996). At higher swimming speeds, swimming becomes unsteady and unsustainable as white or fast muscles are recruited to power swimming (Rome *et al.*, 1988; Jayne and Lauder, 1994, 1995; Coughlin and Rome, 1999).

The swimming performance of fish is characterized by the relation of swimming speed and endurance time, and is classified into three categories: sustained, prolonged, and burst swimming (Brett, 1964; Beamish, 1978; Hammer, 1995). Sustained swimming is observed when a certain low speed is maintained for more than 200 min aerobically without fatigue, as

the metabolic demands (primarily fatty acids) are balanced by waste production (primarily CO_2) and immediate disposal. Prolonged swimming speed can be maintained between 20 s to 200 min and always ends in “fatigue”, considered as the inability of the fish to continue swimming. It contains uneven activity periods of cruising and bursts. Burst speed is described as the highest swimming speed performed anaerobically and maintained for less than 20 s. It is usually terminated by exhaustion as intracellular energy supplies decrease (primarily creatine phosphate and glycogen) and/or waste products accumulate (primarily lactic acid). It can also be classified into either steady (sprints) or unsteady (accelerations) periods (Kieffer, 2010).

Swimming performance depends on a variety of biological and physical factors that can be species-specific. These include body shape, fin form, muscle mass and function, fish size and swimming mode. Moreover, swimming performance can also be influenced by several environmental factors including water pH, oxygen tension, photoperiod, salinity, water temperature, and the presence of pollutants (Brett, 1967; Jain and Farrell, 1997, 2003; Janz *et al.*, 1991; Lee *et al.*, 2003a). Therefore, swimming performance is a key factor in determining the survival of a fish species within their aquatic environment (Wood, 1991; Kieffer, 2000). Fish spend a large portion of their daily energy budget on swimming activities (Boisclair and Sirois, 1993) used essentially for survival behaviours including foraging and escaping predators (Campbell *et al.*, 2002). Predator-prey interactions, reproductive behaviours including spawning migrations, habitat shifts and dispersal are also of profound ecological importance and depend on the capacity of an individual for locomotion (Kolok, 1999, 2001; Reidy *et al.*, 2000). Swimming speed and endurance are directly related to food capture, escape from predators and reproduction, and therefore subjected to strong selection pressures that enhance evolutionary fitness (Beamish, 1978; Videler and Wardle, 1991; Videler, 1993).

Consequently, swimming performance is a good sublethal indicator of stress and an index of fitness as it indicates chances of survival or long-term opportunity to reproduce and directly reflects the functional integrity of the fish and is of immediate relevance to their ecology.

1.3.1. Critical Swimming Speed

Prolonged swimming is the best studied form of fish locomotion, as the water velocities necessary to exercise the fish are reproducible in a laboratory with a swim chamber/tunnel and because fatigue, or the inability of the fish to continue swimming, is a discrete and identifiable endpoint (Kolok, 1999). The parameter used to evaluate prolonged swim performance is the critical swimming speed (Ucrit). This parameter estimates the maximum velocity a fish can swim aerobically and is measured by subjecting an individual, for a prolonged period of time, to an increasing flow of water in a swim tube until the onset of fatigue (Blazka, 1960; Brett, 1964; Hammer, 1995). Two experimental procedures were suggested by Brett (1964, 1967) to assess swim performance: fixed or fatigue velocity tests, and incremental or increased velocity tests. The incremental velocity test is widely used in swimming performance tests because it takes a shorter time to conduct and uses fewer fish without decreasing statistical power (Farrell, 2008). Also, results of this test are specific to each individual as Ucrit performance is repeatable in control or healthy fish after a relatively short recovery period (Jain *et al.*, 1998; Kolok *et al.*, 1998, Kolok, 1999; Farrell, 1998; Farrell *et al.*, 2003; McKenzie *et al.*, 2007), and the rank order for swimming performance remains unchanged among individuals within a group of fishes when environmental conditions are changed (Kolok, 1992; Kolok and Farrell, 1994; Kolok *et al.*, 1998; Nelson *et al.*, 2002; Claireaux *et al.*, 2007).

Assessment of swimming performance is considered a good means of evaluating sublethal effects and an important criterion for the quantification of the sublethal effects of toxicants on fish (Hammer, 1995). Significant reductions in Ucrit are documented after exposure to a number of stressors, including physiological challenges such as the effects of burst swimming on aerobic swimming (Randall *et al*., 1987), transfer to seawater (Brauner *et al.*, 1994), increased water acidity (Beaumont *et al.*, 1995a), and bacterial, fungal or other parasitic infections (Swanson, 2002; Tierney and Farrell, 2004; Wagner *et al.*, 2005). In addition, exposure to sublethal concentrations of organic and inorganic toxicants resulted in impaired swimming performance in several studies (Beamish, 1978; Little and Finger, 1990; Heath, 1995; Nikl and Farrell, 1993; Beaumont *et al.*, 1995b; Wood *et al.*, 1996; Jain *et al.*, 1998; Baltz *et al.*, 2005; McKenzie *et al.*, 2007). These studies reported a significant decrease in Ucrit ranging from 6.5% to 50% depending on the compound as well as the exposure dose and duration; it is important however to note that not all studies in the primary literature demonstrated reductions in swimming performance.

1.3.2. Recovery Ratio

Repeat swimming performance measurements can be compared by using the recovery ratio. This ratio is an indication of the individual's capacity to return to its full swimming ability after an initial bout of exhaustive exercise. This recovery period is separated by two Ucrit performance tests, therefore the recovery ratio can be calculated by dividing $Ucrit_2$ by $Ucrit_1$ (Jain *et al.*, 1998). A ratio near 1 indicates that swim performance remains unchanged and the fish effectively recovered from both the exhaustive exercise and imposed chemical stress. These ratios are ecologically important since changes in water quality or health status,

induced by contaminant exposure, can alter fish metabolism, which would impair their recovery times (Kieffer, 2000). This is a useful tool for assessing fish health and can be utilized to calculate the maximum duration of exposure to a contaminant that a given fish population can endure before they deplete their energy resources. In normal conditions, Ucrit values are repeatable, however in stressful conditions, the second Ucrit performance is usually reduced and therefore the recovery ratio is decreased. This implies that intoxicated fish require a longer period of time to adjust and regain their original energy levels compared to completely healthy fish (Jain *et al.*, 1998) which might become problematic as a rapid rate of recovery may be beneficial if the activity is important to survival and reproduction.

1.3.3. Respirometry and Oxygen Consumption

Respirometry is an indirect method of determining metabolic rate by measuring the rate of oxygen consumption which is useful in modeling physiological requirements of fish under various conditions (Beamish and Mookherjii, 1964; Fry, 1991). Respiratory rates are used in numerous papers to monitor pollutant stress in fishes as they are rapid and useful for identifying short-term pollution events (Morgan and Küln, 1974; 1984; Sellers *et al.*, 1975; Travis and Heath, 1981). Oxygen consumption following strenuous activity is considered to be another good stress index to evaluate contaminant exposure (Brett, 1964, Milligan *et al.*, 2000). Oxygen uptake is higher at the end of an exercise bout since higher levels are needed to restore the body to a resting state and recover from the exercise just performed including any incurred oxygen debt. Thus, this effect is greatest soon after the exercise is completed and decays to a lower value over time (Milligan, 1996; Lee *et al.*, 2003b). Oxygen consumption rates show a clear linear dose-response in many organisms and for many chemicals, and are

often used as a surrogate for metabolic rate (Erickson and McKim, 1991; Randall *et al.*, 1996). Estimates of oxygen consumption rates in fish are thus associated with swimming performance (i.e. active metabolism) and involve the use of one of a variety of respirometry methods (Steffensen, 1989). Closed or constant-volume respirometry measurements are made in a sealed chamber of known volume. The oxygen content of the water is measured initially, then the respirometer is closed and at the end of the experiment the oxygen content is measured again. An advantage of this method is simplicity while a disadvantage is that the measurements are never made at a constant oxygen level due to the continuous use of oxygen by the fish inside the chamber. Furthermore, metabolites from the fish (e.g. CO_2, ammonia) accumulate in the water, thus limiting the duration of measurements. This prevents the fish from fully recovering from the initial handling stress which will often increase fish respiration significantly for several hours, thus overestimating oxygen consumption rates (Steffensen, 1984; 1989).

Open or flow-through respirometry is a more sophisticated method for oxygen consumption estimates where the fish is placed in a flow-through chamber with a known flow rate. Oxygen is measured in the water inflow and outflow with the difference being the rate of uptake by the fish. The advantages of this method are that the duration of the experiment can be unlimited, there is no accumulation of CO_2 and other metabolites, and respiration is estimated as a constant level of oxygen. However, this method has one significant disadvantage; to determine oxygen consumption, it is crucial that the system is in steady state which means that the oxygen content of the inflowing and outflowing water, as well as the oxygen consumption of the fish must be constant at all times. If the oxygen consumption of the fish changes for some reason during the experiment, oxygen consumption rates cannot be

assessed until the system returns to steady state to avoid over- or under-estimating values and represent with better accuracy the linear decline in oxygen consumption. Thus, open respirometry measurements are time consuming and are not suitable to determine the oxygen consumption for organisms with a highly variable respiration rate, such as fish (Steffensen, 1984; 1989).

Intermittent flow respirometry combines the best of both described methods. The fish is placed in a respirometer immersed in an ambient tank and a recirculating pump ensures proper mixing of the water inside the chamber and an adequate flow past the oxygen probe. During this period, a flush pump exchanges the water inside the respirometer with water from the ambient tank. During measurements of oxygen consumption, this flush pump is turned off and the system operates like a closed respirometry system. During swimming trials, the flush pump is turned back on and pumps ambient water into the respirometer to bring the oxygen content back to pre-measurement levels. This avoids problems with accumulating metabolites and severe changes in oxygen levels due to animal respiration. The most important advantages of this method are the unlimited duration of the experiment and the greater time resolution of this method. Oxygen consumption rates can be determined frequently over periods of hours and even days which make intermittent flow respirometry particularly suited for uncovering short term variations in respiration rates of fish (Steffensen, 1984; 1989).

Aerobic metabolism (R) is calculated by using the following equation: $R = R_s + R_r + R_f + R_a$, where R represents the total metabolism, R_s = standard metabolism, R_r = routine metabolism, R_f = heat increment of feeding also called the specific dynamic action (SDA), and R_a= active metabolism associated with locomotion (Jobling, 1981; Hunt van Herbing and White, 2002). Standard metabolic rate (SMR) represents the minimum amount of energy

required for maintenance functions when the animal is at rest or in a post-absorptive state. Routine metabolic rate (RMR) describes a fish that is feeding, growing, producing sexual products or any other activities that elevate the routine metabolic rate (Fry, 1971; Brett, 1972; Brett and Groves, 1979). The lowest level of metabolism can be either the oxygen consumption associated with SMR of a fish at rest or the lowest activity level of the RMR (Fry, 1947). Swimming (activity) metabolism or active metabolic rate (AMR) represents the highest sustainable swim speed and maximum oxygen consumption of the fish (Forstner, 1983; Cech, 1990; Fry, 1991). On some occasions, metabolic rates higher than the AMR can be achieved (Bushnell *et al.*, 1994) which are termed the maximum metabolic rate (MMR). The highest level of metabolism is thus the oxygen consumption of a fish swimming at the maximum sustainable speed in a swim tunnel. The difference between the highest achievable metabolic rates and the lowest represents the scope for activity which is a good indicator of the survival capacity and energy allocations available for vital processes.

1.3. Question and Rationale

The main objective of my research is to determine if an exposure to a PCB congener at a sublethal concentration affects the energy budget of fish and can therefore be responsible for functional deficiencies associated with the locomotion of a fish. Behavioural and physiological responses that give relevant information on the environmental impacts of chemicals describe performance, account for the health of individual organisms and indicate the chances for survival or long-term opportunities to reproduce. Because swimming involves the coordinated efforts of a host of physiological mechanisms, measurements of swimming performance can be viewed as a reliable index of general health and stress in fish (Bennett, 1991; Wood, 1991;

Heath, 1995). The first part of my research is devoted to swimming performance experiments by evaluating parameters including critical swimming speed, metabolic rate and recovery ratios. I hypothesize that exposure to a PCB will disrupt the energy budget of the fish because PCBs stored in lipids will be released to the bloodstream during high periods of activity when energy stored in these tissues must be mobilized as a result of increased metabolic demands. This leads to two predictions: 1) that critical swimming speed will decrease substantially with an increasing concentration level of PCBs; and, 2) that recovery ratios of intoxicated fish will be reduced following an exhaustive exercise bout compared with non-exposed fish. The effects of exposure to sublethal pollutant concentrations can also be measured in terms of biochemical or physiological responses by the fish; therefore the second objective of my research involves assessing blood and white muscle tissue metabolites to quantify physiological disturbances and fuel mobilization associated with this exposure. The PCB intoxication will also be validated by measuring EROD activity in the liver. Overall, this research will provide insights into the effects of sublethal toxicant exposure on fish and contribute to a rather small body of literature on this topic.

Chapter 2

Swimming Performance and Energetic Metabolism of Rainbow Trout in Response to a Sublethal Polychlorinated Biphenyl Exposure

2.0. Introduction

Freshwater teleost fish often experience natural and anthropogenic conditions that result in fluctuating energy availability (Bureau *et al.*, 2002) therefore the ability to acquire, transform and use energy is essential for the survival of these fish. Energy partition models in rainbow trout (*Oncorhynchus mykiss*) demonstrate that at 15 °C approximately 69% of ingested energy is available for body maintenance, activity, growth and development, or reproductive investments (Caddy and Sharp, 1986; Cho, 1992). Swimming performance is believed to be one of the crucial factors determining the survival or fitness of a fish species within its environment (Nelson *et al.*, 2002; Claireaux *et al.*, 2007) as locomotion is required for vital activities including foraging and escaping predators, as well as reproductive behaviors including spawning migrations, habitat shifts and dispersal (Wood, 1991; Kolok, 1999, 2001; Kieffer, 2000; Reidy *et al.*, 2000). Because swimming involves the coordinated efforts of numerous physiological processes across multiple levels of biological organization (i.e., molecule, cell, organ, organ system), performance can be viewed as a reliable index of general health and stress in fish (Kolok, 1999; 2001).

Polychlorinated biphenyls (PCBs) are industrial contaminants that are considered amongst the most widespread and hazardous synthetic pollutants remaining in the environment (Buckman *et al.*, 2004) due to their highly unreactive nature and resistance to chemical and biological degradation. These toxic organic compounds are recognized as sources of stress to fish by disrupting various aspects of metabolism from whole-organism

responses (e.g. metabolic rate and swim performance) to tissue responses (e.g. metabolite availability and enzyme activity) which are generally energy-demanding and costly in terms or metabolic resources (Calow, 1989; Wendelaar Bonga, 1991; 1997; Iwata *et al.*, 2004; McKenzie *et al.*, 2007; Beyers *et al.*, 1998; Gourley and Kennedy, 2009). Over time, such responses may decrease individual performance and possibly fitness (Alcaraz, 2000) by changes in foraging, migration and escape behaviours, and the population in terms of reproductive capacity (De Coen and Janssen, 2003; DuRant *et al.*, 2007) due to the alterations in energy allocation.

The first part of my research is devoted to swimming performance experiments in order to evaluate parameters including critical swimming speed, metabolic rate and recovery ratios to test the hypothesis that a sublethal exposure to PCB 126 will affect the energy budget of the fish and can therefore be responsible for functional deficiencies associated with their locomotion. PCB 126 is a lipophilic compound that accumulates in lipids and can be mobilized from fat then released to the bloodstream of the fish during high periods of activity when energy stored in these tissues must be mobilized due to the increasing metabolic demands. This leads to the predictions that critical swimming speed will decrease substantially with an increasing concentration level of PCB 126 and, that recovery ratios of following an exhaustive exercise compared to non-exposed fish.

The sublethal effects of exposure to pollutants can also be measured in terms of biochemical and physiological intoxicated fish will be reduced responses by the fish in order to understand the mechanisms underlying the behaviour of swimming (Farrell *et al.*, 2001; Peake and Farrell, 2006; Veiseth *et al.*, 2006; Lee-Jeekins *et al.*, 2007). As such, the second part of my research involves an analysis of blood and white muscle tissue metabolites as well

as somatic indices to quantify physiological disturbance levels and nutritional conditions associated with this exposure. The PCB 126 intoxication will also be validated by measuring EROD activity in the liver as an indication of exposure. Overall, this research will provide insights into the sublethal effects of toxic organic compounds on fish.

2.1. Materials and Methods

2.1.1. Study Animals and Fish Maintenance

Adult female rainbow trout (*Oncorhynchus mykiss*) were obtained from Linwood Acres Trout Farm (Peterborough, ON) and placed in 115-L re-circulating freshwater holding tanks located in the University of Ottawa Aquatic Care Facility. Salmonids are the target species of numerous swim performance studies for practical reasons including the availability of various fish sizes and their easy maintenance in a laboratory setting, in addition to the physiological reason that they are considered a high performance species (Moyes and West, 1995). All fish were prophylactically treated with a 1 h bath of 125 ppm formalin 3-times every 2nd day for a week at arrival to reduce the potential for disease development associated with transport and handling. The tank systems were operated at a recycle rate that allowed 12% of the circulating flow to be continually replaced. The recycled water was denitrified through biofiltration and re-oxygenated or degassed by ambient air gravity pass-through columns. Holding tank physical and chemical parameters reflected natural trout habitats which are well-oxygenated and low temperature (13^{o}C) environments. Chemical parameters including pH, salinity, water hardness and ammonia levels were also monitored. Photoperiod was set at a 12 h light:dark cycle. Rainbow trout prefer low light levels therefore the holding rooms were lit with dimmed fluorescent green lights and the fish were further shaded by

garbage bags placed on top of the tanks to reduce stress levels. Experiments were conducted between August 1 and November 15, 2009.

2.1.2. Contaminant, Injections and Exposures

PCB 126 (3,3',4 ,4',5-pentachlorobiphenyl) is a coplanar non-*ortho* substituted PCB congener that is commonly found at high concentrations in the environment (Bhavsar *et al., 2007*) due to its long half-life, high assimilation efficiency, as well as high bioaccumulation capacity (Giesy and Kannan, 1998). Hence, this congener was used in this study because of its high potential for toxicity and environmental relevance. PCB 126 (99% purity) was obtained from Ultra Scientific (North Kingstown, RI) and the entire contents of the vial (5 mg) was diluted in 1 mL iso-octane and sonicated for 30 min in an ultrasonic bath to agitate particles and speed dissolution. In the meantime, a small amount of coconut oil was heated in the oven until liquid. Three solutions were prepared to represent a control (800 µL coconut oil in 200 µL iso-octane), low (100 µg/kg or 960 µL coconut oil in 40 µL iso-octane/PCB 126) and high (400 µg/kg or 840 µL coconut oil in 160 µL iso-octane/PCB 126) treatments. These concentrations were based on values previously found in fish or used in other similar studies for the same congener (Brown *et al.*, 2002; Luk, 2002; WHO, 2003; MOE, 2009).

A total of 45 fish were used for this experiment. Nine fish (3 per treatment) were selected and placed at random in 5 small re-circulating experimental tanks according to their post-injection swim period: 24 h, 3 days, 5 days, 7 days or 9 days. Each individual fish was lightly anesthetized with a solution of benzocaine (0.01 g/mL) in order to measure their initial weight (g) and total length (cm) from the tip of snout to the longest part of caudal fin. Each fish was then intraperitoneally (IP) injected once using a 1 mL B-D syringe (26 G 3/8 needle)

with the appropriate treatment solution. The volume injected was proportional to fish mass (100 µL/0.2 kg). Each fish was identified by inserting a distinctive vinyl tag into the muscle just below the dorsal fin with a fine fabric pistol grip tagging gun and needle. The fish were then returned to their respective tanks until their post-injection swim periods. There was no feeding throughout the experiment in order to ensure a post-absorptive state during the swimming challenges (Farbridge and Leatherland, 1992) and to examine how body energy stores provide energy under dietary restriction that may be used towards maintaining defense activities. All experiments were approved by the University of Ottawa Animal Care Protocol Review Committee and adhered to the published guidelines of the Canadian Council on Animal Care for the use of animals in teaching and research.

2.1.3. Swim Performance and Respirometry

The following tests consisted of evaluating fish swimming performance at various post-injection times (24 h, 3 days, 5 days, 7 days or 9 days) to the chosen levels of the PCB 126. Fish were removed from their experimental holding tanks and placed in a 56-L intermittent flow modified Blazka-type respirometer. They were given a standard overnight acclimation period at a low speed of 10 cm/s (Plaut, 2001; Kieffer, 2010) before the start of the swim trials. The water flow was generated by a propeller and could be regulated via the power supplied to the motor driving the propeller (Appendix I). All tests were conducted between 9 am and 6 pm in order to account for diurnal effects or variations in the swimming performance and respiration of the fish.

2.1.3.1. Critical Swimming Speed (Ucrit)

This protocol followed the standard test for critical swimming speed (Brett, 1964; Beamish, 1978) though fish were subjected to two step tests ($Ucrit_1$ and $Ucrit_2$), where the motor speed was increased by 10 cm/s every 5 min until the fish fatigued. These parameters were chosen based on preliminary trials for the same species and size. The fatigue endpoint was characterized by the fish either hitting the rear grid of the tunnel or by not removing its tail for more than 5 s from the grid. After both swim trials, the critical swimming speed was calculated using the formula: $Ucrit = u_i + (t_i/t_{ii} \times u_{ii})$, where u_i = highest velocity maintained for the entire swimming interval (cm/s), t_i = time fish swam at fatigue velocity (min), t_{ii} = prescribed interval time (min) and u_{ii} = velocity increment (cm/s). The Ucrit values were converted from absolute (cm/s) to relative (BL/s) values in order to account for variation in fish length.

2.1.3.2. Recovery Ratio (RR)

The two performance tests were separated by a 1 h recovery period in which the fish was kept in the respirometer at a speed of 10 cm/s. The recovery ratio was calculated by dividing $Ucrit_2$ by $Ucrit_1$ (Jain *et al.*, 1998), which is an indication of the time required by an individual fish to return to its full swimming ability after an exhaustive exercise where a ratio near 1 indicates an unchanged swim performance.

2.1.3.3. Oxygen Consumption (MO_2)

Metabolic rates were calculated by recording oxygen values after closure of water inlets/outlets, with a flow rate across the electrode of 350 mL/min and a low swim tunnel

motor setting of 10 cm/s. A standard metabolic rate measurement was taken in the morning following the overnight acclimation period, followed by the first maximum metabolic rate after the first step test ($Ucrit_1$). The routine metabolic rate followed the recovery period while the second maximum metabolic rate was calculated at the end of the second swim trial ($Ucrit_2$). Oxygen values were recorded every 5 min for a period of 30 min, and metabolic rates were calculated using the formula: MO_2 = [$\triangle O_2$ concentration (mg/L) x respirometer H_2O volume (L)]/ [fish mass (kg) x time (h)]. An oxygenation column was connected to the swim tunnel to ensure that the water within the respirometer was maintained at or above 80% air saturation to maintain the proper dissolved oxygen concentrations for a temperature of 13^{o}C. All dissolved oxygen values recorded over the duration of the experiment were in the range of 8.0-9.9 mg/L.

2.1.4. Physiological and Biochemical Disturbance

2.1.4.1. Somatic Indices and Visual Examination

All fish were sacrificed within 5 min following the measurement of the second maximum metabolic rate with a lethal dose of benzocaine (0.1 g/mL). Fish final mass (g) and total length (cm) were recorded and used to calculate the condition factor (K) as an indication of fish health using the formula K = 100 * (fish mass/length^3). The liver, heart and spleen were weighed in order to calculate somatic indices by dividing the organ weight (g) by the body weight (g) of the fish times 100. An external and internal visual examination was also conducted to assess general fish health according to the methods outlined in Tierney and Farrell (2004; Appendix II).

2.1.4.2. Blood Analysis

Blood samples were collected by caudal vessel puncture using a heparinized syringe coated with ethylenediaminetetraacetic acid **(**EDTA) to reduce coagulation. The samples were placed in small 1 mL tubes and kept on ice. These tubes were then centrifuged for 5 min at 14,000 xg and the plasma obtained divided in smaller aliquots and kept at -80^{o}C until further biochemical analysis. Glucose concentrations were measured using an enzymatic assay method described by Darrow and Colowick (1962). Amino acids were analyzed using the methods as outlined by Troll and Cannan (1953) while levels of lactate were determined using the protocol defined in Gutmann and Wahlefeld (1974). Total protein levels were determined with a bicinchoninic acid (BCA) protein assay as described by Smith *et al.* (1985). Cortisol levels were determined using a radioimmunoassay RIA kit (MP Biomedicals, CA, US) while triglyceride levels were analyzed using TECO Diagnostics Triglyceride GPO reagents (Appendix III).

2.1.4.3. EROD Activity

Livers were analyzed for EROD (ethoxyresorufin-O-deethylase) activity, an enzyme used as an indicator of cytochrome P450 (or CYP1A) enzymes that are sensitive indicators of PCB exposure (Whyte *et al*., 2000). To isolate the microsomal fraction containing the endoplasmic reticulum and CYP1A enzymes, an ice cold homogenization buffer (10 mM HEPES, 1 mM EDTA, 10% (v/v) glycerol, pH 7.4) was added to liver tissues. Tissues were then minced with scissors, homogenized and centrifuged at 9,000 xg for 20 min at 4°C. The supernatant was removed and centrifuged at 100,000 xg for 60 min in a Beckman L7-65 ultracentrifuge at 4°C. The supernatant was discarded and the microsomal pellet was re-

suspended in a suspension buffer (50mM Tris, 1mM EDTA 10% (v/v) glycerol, pH 7.4), then aliquoted and stored at -80°C. The assay was conducted using the method described by Pohl and Fouts (1980) in which the substrate 7-ethoxyresorufin (1 mM) was added to 10 µL microsomal fractions in a 96-well microplate. After a 10 min incubation period in the dark, 20 mg/mL of nicotinamide adenine dinucleotide phosphate (NADPH) was added to each well in order to start the reaction. CYP1A-mediated resorufin production was measured kinetically for 20-30 minutes with 530 nm and 590 nm excitation and emission filters. Afterwards, 0.3 mg/mL of fluorescamine was added to each well to stop the reaction and CYP1A protein concentration was measured at 400 and 460 nm excitation and emission filters.

2.1.4.4. White Muscle Tissue Analysis

White muscle tissue was used for extractions of glucose, glycogen, proteins and amino acids. Tissues were frozen in liquid nitrogen then ground to a powder using a mortar and pestle. They were sonicated in 4 vol/wt of 70% PCA for 1-2 min, centrifuged at 14,000 xg for 5 min then the supernatant was collected for subsequent assays. Glucose, protein and amino acid levels were measured using the microplate assay methods as described above in the above section for blood plasma. Glycogen levels were determined using the method of Keppler and Decker (1975) by analyzing glucose after digestions with amyloglucosidase.

2.1.5. Data Analysis and Statistics

The statistical analysis of the data was performed using SigmaPlot 11.0 (2008). Initial and final fish measurements were compared with a t-test for standard means while comparisons between treatments across post-injection time periods were classified as either

short (24 h, 3 days) or long (7 days, 9 days) term to increase the sample size and statistical power. These comparisons were also carried out with a t-test for standard means or a non-parametric Mann-Whitney rank sum test if the Shapiro-Wilk normality test failed. Multiple comparisons across treatments were carried out with a one-way ANOVA, or Kruskal-Wallis on ranks if the normality test failed, to determine if a significant statistical difference existed between the mean values of the control group relative to low and high exposure treatments. If a significant difference across treatments was detected, a Dunnett's post-hoc test was used to determine which treatment in particular was different from the control. A two-way ANOVA was conducted to test for differences in metabolic rates using all time periods and metabolism types as factors. A Holm-Sidak post-hoc pairwise test was conducted if significant differences were observed. In all cases, $p < 0.05$ was used to establish significant differences for statistical comparisons.

2.2. Results

2.2.1. Body Parameters and Condition Factor

Comparisons between the initial and final size measurements (i.e. mass, length) of rainbow trout did not differ significantly among the control, low-dose and high-dose treatments when post-injection time periods were excluded from the analysis. However, significant differences in initial and final measurements as well as the condition factor (Table 2.1) were observed across all treatment groups.

Table 2.1. Length, mass and condition factor of rainbow trout prior to experimentation (W_i, TL_i) and at termination (W_f, TL_f) for the three treatments: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. Condition factor = 100 m/l^3, where *m* is body mass (g) and *l* is total body length (cm) measured from the tip of the snout to the longest part of the caudal fin. Letters indicate a significant difference between the initial and final measurements or the condition factor across treatments.

	Measurements				**Condition Factor**
Treatment	W_i (g)	W_f (g)	TL_i (cm)	TL_f(cm)	K
Control	291.4 ± 7.2	290.1 ± 7.2	31 ± 0.26	30.9 ± 0.25	1.13 ± 0.03
Low-dose	292.9 ± 5.0	290.2 ± 4.5	30.7 ± 0.21	30.6 ± 0.22	1.13 ± 0.03
High-dose	304.7 ± 4.4 [a]	307.9 ± 5.5 [b]	31.5 ± 0.19	31.4 ± 0.22	1.24 ± 0.02 [c]

[a]: n = 45, df = 44, p = 0.001, [b]: n = 45, df = 44, p = 0.002, [c]: n = 45, df = 44, p = 0.005

2.2.2. Critical Swimming Speed

The critical swimming speed of all three treatment groups was generally higher for the first swim challenge ($Ucrit_1$) than the second ($Ucrit_2$) trial (Fig. 2.1). Also, the critical swimming speed values increased at the higher level of exposure. Variation in $Ucrit_1$ and $Ucrit_2$ within treatments between the short and long-term periods was only significant for the $Ucrit_1$ of the low-dose treatment (*n = 6, t = -2.29, df = 10, p = 0.04*). No significant difference in $Ucrit_2$ across treatments was observed. However, variation in $Ucrit_1$ across treatments was detected (Fig. 2.1) when time periods were excluded from the model. Since the fish in the high-dose treatment were significantly bigger in size than the other treatments (Table 2.1), linear regressions between the absolute critical swimming speed value (cm/s) and the total length (cm) were conducted to establish if size would skew the data; however none of the treatments demonstrated a statistically significant relationship indicating that the results could be interpreted without adjusting this variable.

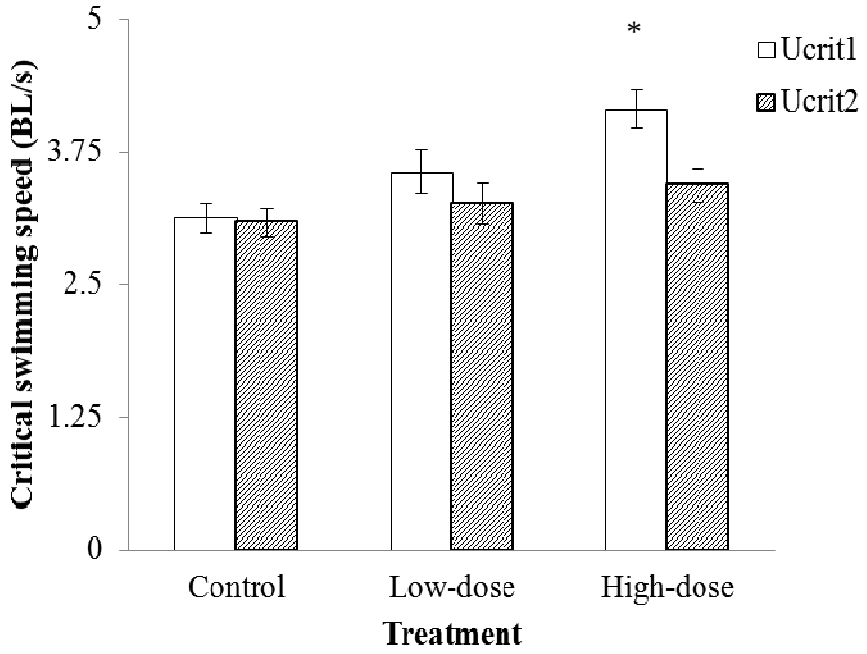

Fig. 2.1. Critical swimming speed values of rainbow trout during the first ($Ucrit_1$) and second ($Ucrit_2$) swim performance challenges compared among treatment groups: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. An asterisk represents a significant difference in critical swimming speed compared to the control group (*n = 45, df = 44, p = 0.001, F = 8.16*).

2.2.3. Recovery Ratios

There was a decrease in recovery ratios ($Ucrit_2/Ucrit_1$) with increasing levels of PCB exposure. No variation was detected within treatments between short and long-term periods for all treatments. However, significant differences in recovery ratios were detected across treatments (Fig. 2.2) when the time period factor was excluded from the model.

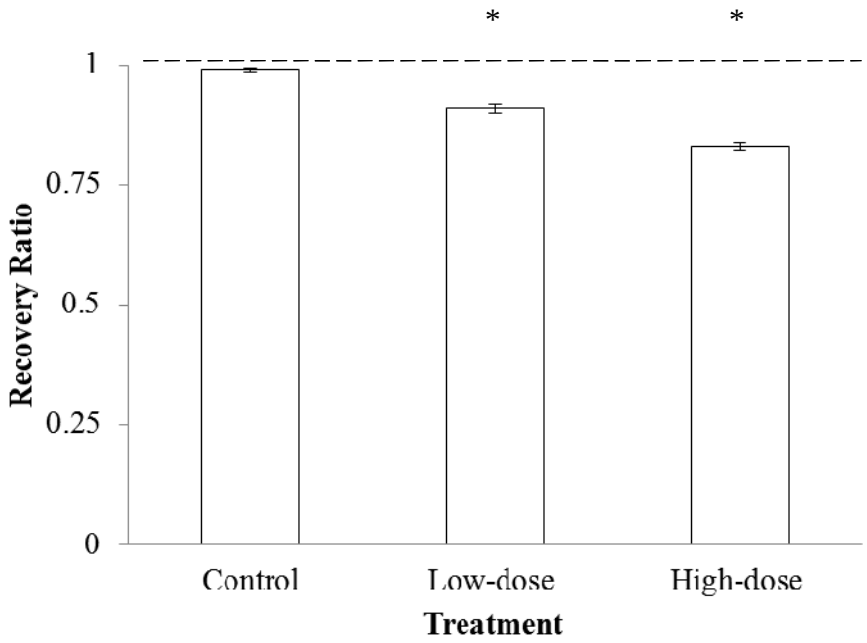

Fig.2.2. Recovery ratios for rainbow trout in relation to treatment: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. A single asterisk represents a significant difference in recovery ratio from the low or high-dose treatments compared to the control group (*n = 45, df = 2, p = 0.001, H = 37.8*). The dashed line (---) represents a recovery ratio of unity.

2.2.4. Oxygen Consumption

Maximum metabolic rates for all treatments were nearly twice as high as the standard and routine values, a tendency more pronounced at an increasing level in exposure (Fig. 2.3). A significant difference in metabolism was only detected between the 24 h and 3 days time periods for the control group (*n = 60, df = 59, t = 3.20, p = 0.03*) while no variation was observed between any time period for the low and high-dose treatments. Excluding time periods, all interactions between standard, first and second maximum, as well as routine metabolisms were significant for the control and low-dose treatments (*n = 45, df = 44, p < 0.001-0.01*) while the high-dose treatment demonstrated no variation between both maximum metabolic rates, or between the routine and standard metabolic rates.

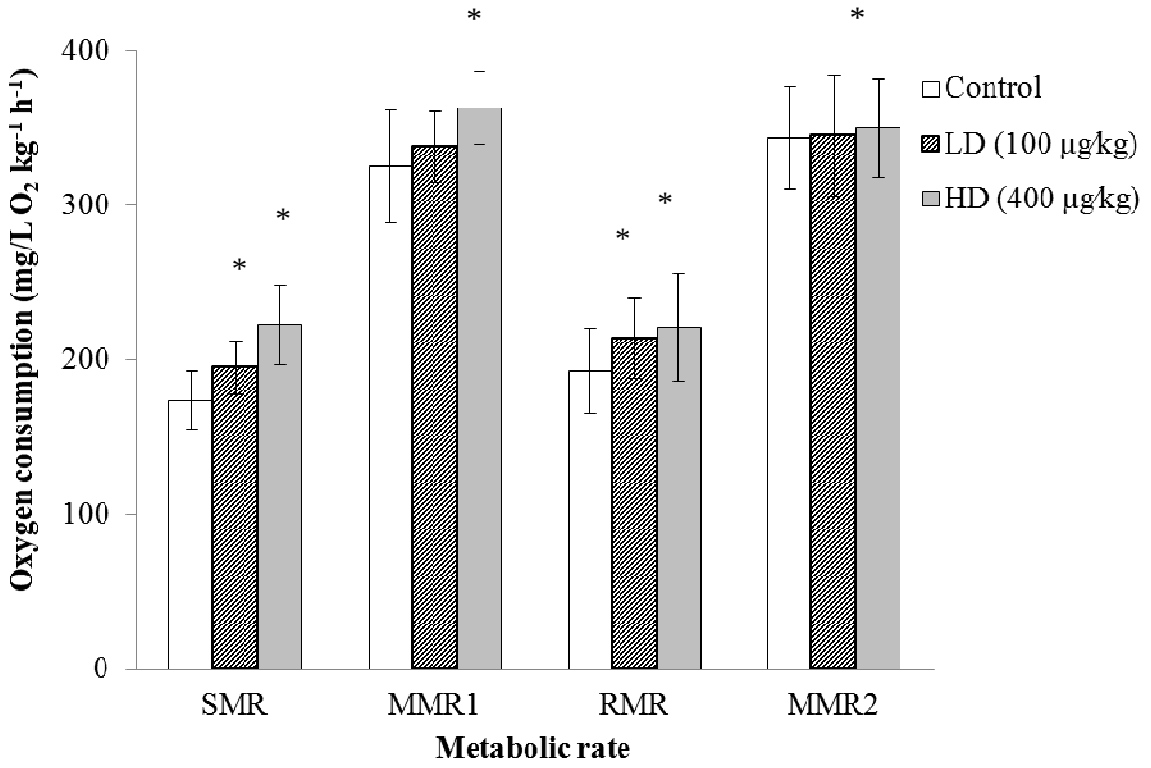

Fig.2.3. Oxygen consumption values of rainbow trout for standard metabolic rate (SMR), maximum metabolic rates (MMR_1, MMR_2) and routine metabolic rate (RMR) for three treatment groups: control, low-dose and high-dose. Values presented are means ± S.E.M; n=15. Temperature was maintained between 13-15^{o}C during the oxygen readings. An asterisk represents a significant difference within each metabolic rate compared to the control treatment (*n = 45, df = 2, p < 0.001 (H = 22.48, 16.604), df = 44, p < 0.001 (F = 30.69), p = 0.012 (F = 4.470*).

For all treatments across time periods, second maximum metabolic rate and standard metabolic rate or routine metabolic rate, first maximum metabolic rate and routine or standard metabolic rates were significantly different (*n = 45, df = 44, p < 0.001*) from each other while interactions between the second and first maximum metabolic rates, as well as the routine and standard metabolic rates were not significant. No significant differences within each

metabolism type (i.e. standard, first and second maximum, routine) were detected between time periods for the control, low and high-dose treatments. However, significant differences were observed across treatments (Fig.2.3) within each of the metabolism types.

Aerobic scope for activity (AFA) was calculated as the difference between the maximum and routine metabolic rates. The metabolic scope for the control group averaged at 150.7 ± 2.1 mg O_2 kg^{-1} h^{-1}, while those for the low and high-dose treatments were 142.7 ± 0.91 mg O_2 kg^{-1} h^{-1} and 140 ± 3.22 mg O_2 kg^{-1} h^{-1}, respectively. A significant difference in AFA was observed across treatments ($n = 45$, $df = 44$, $p = 0.005$), and more specifically AFA was significantly lower in the low and high-dose treatment groups compared to the control ($n = 30$, $df = 14$, $p < 0.05$).

2.2.5. Somatic Indices

A small but significant decrease in liver and spleen somatic indices with increasing exposure level was observed whereas the heart somatic index remained unchanged across all treatment groups (Table 2.2). The hepatosomatic index only varied within the control treatment between short and long-term periods ($n = 6$, $t = -2.50$, $df = 10$, $p = 0.03$). No significant differences were detected within any of the treatments for the heart somatic and spleen somatic indices. Also, significant differences across treatments were observed in the hepatosomatic and spleen somatic indices when time periods were ignored (Table 2.2) in contrast to the heart somatic index.

Table 2.2. Liver (HSI), heart (HESI) and spleen (SSI) somatic index values of rainbow trout in each treatment group: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. Letters represents a significant difference in an index from the low or high treatment groups compared to the control group.

	Somatic index		
Treatment	HSI	HESI	SSI
Control	0.97 ± 0.03	0.13 ± 0.01	0.18 ± 0.01
Low dose	0.96 ± 0.03	0.13 ± 0.01	0.17 ± 0.02
High dose	0.83 ± 0.04 [a]	0.13 ± 0.01	0.11 ± 0.01 [b]

[a]: *n = 45, df = 44, p = 0.005 (F=6.075),* [b]: *n = 45, df = 44, p = 0.002 (F=7.431)*

2.2.6. Physiological and Biochemical Disturbance

2.2.6.1. Visual Examination

Only a few fish demonstrated internal or external abnormalities. Such abnormalities included mild erosion of fins, wounds, some shortening of the opercula, slight hindgut inflammation or a granular spleen. However, no trend could be detected with exposure level as most of these were control fish and these deformities were most likely a result of poor health status, transport stress or diseases rather than PCB exposure.

2.2.6.2. Blood Analysis

Blood metabolites appear to remain constant throughout time periods except for glucose and cortisol levels. Significant variation within treatments between short and long-term periods was only detected for amino acid levels in the high-dose treatment (*n = 6, t = -2.93, df = 10, p = 0.01*) and for triglycerides levels in the control (*n = 6, t = 2.43, df = 10, p = 0.04*), low (*n = 6, t = 4.76, df = 10, p < 0.001*) and high (*n = 6, t = 3.91, df =10, p = 0.003*)

treatments. Significant variations across treatment groups were also observed for the glucose and cortisol levels (Table 2.3) when time periods were excluded.

Table 2.3. Plasma concentrations in rainbow trout of glucose (GLC), amino acids (AA), total protein (TP), cortisol (CORT), lactate (LAC) and triglycerides (TRI) at termination for the three treatments: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. Letters indicate a significant difference in metabolite between the treatment groups compared to the control.

	Treatment		
Metabolites	Control	Low dose	High dose
GLC (mmol/L)	5.33 ± 0.13	6.09 ± 0.14 [a]	6.95 ± 0.17 [a]
AA (mg/mL)	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
TP (mg/mL)	37.4 ± 1.22	36.3 ± 1.38	36.8 ± 1.08
CORT (ng/mL)	6.28 ± 0.14	12.1 ± 0.42 [b]	20.7 ± 0.68 [b]
LAC (mmol/L)	2.51 ± 0.20	2.52 ± 0.21	2.56 ± 0.21
TRI (mmol/L)	5.21 ± 0.17	4.87 ± 0.16	4.84 ± 0.13

[a]: n = 45, df = 44, p < 0.001(F=30.78), [b]: n = 45, df = 2, p < 0.001 (H=39.13)

2.2.6.3. EROD Activity

EROD activities in the liver of fish increased significantly (*n = 45, df = 2, H = 39.1, p < 0.001*) in both the low (208.1 ± 8.44 pmol/min/mg protein) and high-dose (475.8 ± 11.6 pmol/min/mg protein) treatments compared to the control group (2.2 ± 0.17 pmol/min/mg protein) supporting CYP1A activation. Furthermore, a significant difference was observed between short and long-term periods in the low-dose (*n =6, df = 10, t = 4.26, p < 0.001*) and high-dose treatments (*n = 6, df = 10, t = 6.57, p < 0.001*) while no variation was detected for the control group.

2.2.6.4. White Muscle Tissue Analysis

White muscle metabolites were not significantly different between short and long-term periods within all treatment groups. However, significant variations across treatment groups were observed for all metabolites (Table 2.4) when time periods were excluded as factors.

Table 2.4. White muscle tissue concentrations in rainbow trout of glucose (GLC), amino acids (AA), total protein (TP) and glycogen (GLY) at termination for the three treatments: control, low-dose (100 µg/kg) and high-dose (400 µg/kg). Values presented are means ± S.E.M; n=15. Letters indicate a significant difference in metabolite between the treatment groups compared to the control.

	Treatment		
Metabolite	Control	Low dose	High dose
GLC (mg/g)	0.37 ± 0.02	0.31 ± 0.01	0.19 ± 0.01 [a]
AA (ug/g)	40.6 ± 0.94	43.9 ± 0.80 [b]	45.6 ± 0.94 [b]
TP (mg/g)	7.75 ± 0.1	5.61 ± 0.25 [c]	4.64 ± 0.25 [c]
GLY (mg/g)	0.66 ± 0.02	0.59 ± 0.01	0.50 ± 0.01 [d]

[a]: n = 45, df = 2, p < 0.001 (H=26.3), [b]: n = 45, df = 2, p < 0.001 (H=32.0), [c]: n = 45, df = 44, p < 0.001 (F=8.26), [d]: n = 45, df = 2, p < 0.001 (H=31.5)

2.3. Discussion

This research provides evidence that 100 µg/kg and 400 µg/kg PCB-126 concentrations have the ability to impair the swim performance and recovery of rainbow trout and that intoxicated fish allocate a large portion of their energy budget towards the restoration of depleted resources. Body parameters and the health index of fish across all treatment groups only showed a significant increase in fish weight and condition factor in the high-dose

treatment (Table 2.1.**)** possibly due to this group being on average larger than the other groups as a result of random sampling. However, linear regressions between the absolute critical swimming speed value (cm/s) and the total length (cm) of these fish demonstrated very weak relationships suggesting that any changes in metabolic rates or swimming performance was not necessarily due to fish size or condition factor. Furthermore, the significant increase in EROD activity in the low and high PCB-exposed fish confirms that these treatments were responding to the PCB-126 exposure by activating metabolizing enzymes in the liver. This validates the intoxication of PCB-126 to fish and allows us to conclude that further changes in swimming, metabolic or physiological parameters can be considered as a result of PCB exposure rather than fish health or size. The significant difference in EROD activity between short and long-term periods in only the exposed fish indicates that detoxification of PCB-126 was, in fact, higher for the long-term time periods (7 or 9 days post-injection).

This study demonstrated a decrease in the swimming performance of rainbow trout for the low and high PCB-126 dose treatments from the first swimming challenge ($Ucrit_1$) compared to the second ($Ucrit_2$), suggesting an impaired recovery with increasing level of PCB exposure following exhaustive exercise (Fig.2.1). The critical swimming speed values of the low PCB treatment were significantly decreased by 9 % while the high dose treatment were significantly decreased by 17 % when compared between swimming challenges. These values are known to be extremely variable as they depend on fish species and size (Berry and Pimentel, 1985; Watenpaugh and Beitinger, 1985), but the results shown above are consistent with many studies on the sublethal exposures to organic and inorganic toxicants which also demonstrated impaired swimming performance on several occasions (Little and Finger, 1990; Heath, 1995, Beamish, 1978; Nikl and Farrell, 1993; Beaumont *et al.*, 1995b; Wood *et al.*,

1996; Jain *et al.*, 1998; Baltz *et al.*, 2005; McKenzie *et al.*, 2007). However, the significant difference in absolute $Ucrit_1$ between control and high PCB-exposed fish is a bit unusual, as the above studies indicate a lower swimming ability for intoxicated fish, not just in the context of recovery ratios. This is perhaps due to larger fish used in this treatment, or a survival mechanism near the end of the swim challenge when resources become limited. For this group, swimming performance near exhaustion became more of the burst than prolonged type as opposed to control fish that seemed to maintain their swimming speed throughout the swimming challenge.

Recovery ratios are considered sensitive indicators for metabolic disturbance and were measured as the ratio ($Ucrit_2/Ucrit_1$) between both swimming challenges. Fish representing the low-dose treatment exhibited a significant 9 % loss of their recovery ability, a tendency more pronounced in the high-dose treatment where the recovery of fish significantly decreased by 17 % compared to the control group (Fig. 2.2). These reductions in recovery ratios were also documented in sockeye salmon (*Onchorynchus nerka*) exposed to sublethal concentrations of 0.12 – 0.77 mg/L dehydroabietic acid (DHA) and subjected to two swim challenge tests (Jain *et al.*, 1998), as well as in many other studies on various stress factors (Farrell *et al.*, 1998, MacNutt *et al.*, 2004; Peake and Farrell, 2004, Tierney and Farrell, 2004). The decrease in recovery ratios indicates reduced recovery ability with increasing level of exposure which implies that intoxicated fish require longer periods to adapt following an exhaustive exercise or during stressful conditions compared to non-exposed fish. This metabolic disturbance was present in fish exposed to PCB-126, as they required more energy to maintain their swimming activity, which led to a faster exhaustion.

Standard and routine metabolic rates were assessed by measuring oxygen consumption at low activity levels while maximum metabolic rates were assessed directly after prolonged exhaustive exercise. Standard MO_2 represents the minimum amount of energy required for maintenance functions when the fish is in a post-absorptive state whereas routine MO_2, in this case, describes a fish that is undergoing activities that elevate the routine metabolic rate, such as recovery (Fry, 1971; Brett, 1972; Brett and Groves, 1979). Maximum metabolic rate (MMR) represents the highest sustainable swim speed and maximum oxygen consumption of the fish (Forstner, 1983; Cech, 1990; Fry, 1991; Bushnell *et al.*, 1994). The difference between the highest achievable metabolic rates (MMR) and the lowest (SMR) represents the aerobic scope for activity (AFA) which is a good indicator of the survival capacity and energy allocations available for vital processes. In this study, AFA was significantly lower in the low and high-dose treatment groups compared to the control indicating higher metabolic disturbance in exposed fish than in control groups. It has generally been established that metabolism is higher following exhaustive exercise, but it has also been observed that oxygen consumption can increase further when exposed to different stressors including the presence of predators, air exposure, light intensity, and pollutants (Brown *et al.* 2005, Sager *et al.* 2000, Thompson *et al.* 2008). The metabolic rate estimates (Fig. 2.3) confirmed that the fish were indeed exhausted and stressed as the maximum metabolic rates were significantly higher than the standard and routine values for all treatments after both swimming challenges. This trend, however, was further enhanced with increasing exposure level which may be associated with the depuration or detoxification of the PCB-126 contaminant (Brown *et al.*, 2002; Billiard *et al.*, 2002) or merely due to bigger fish size within this treatment.

This study also showed a reduction in the hepatosomatic (HSI) and spleen somatic indices (SS1) suggesting that physiological disturbance occurred within these organs (Table 2.2). The liver performs many essential roles including regulation of metabolism, synthesis of plasma proteins, energy or vitamin storage, and transformation or excretion of steroids and xenobiotics (Hylland *et al.*, 2001). The 15% HSI decrease demonstrated at the higher PCB-exposure in this study remains unusual, as the liver is the site of detoxification thus the increased CYP1A enzyme activity with higher PCB-126 exposure established above should have resulted in larger livers (Denton *et al.*, 1976; Gingerich, 1982; Lech *et al.*, 1982). Also, toxicants generally increase lipid energy stores (Hacking *et al.*, 1978) but in this case it seemed as though exposed-fish were suffering from poor nutritional conditions compared to the control group (Heath, 1995) and that a mobilization of fuels as indicated by a reduction in liver glycogen occurred to support swimming. Furthermore the high-dose treatment in general held larger fish, so it is expected that they would have larger livers as related to their body mass. The spleen is involved in immune responses and is considered a storage organ for mainly red but also white blood cells (Hylland *et al.*, 2001). The SSI decreased by 39 % with higher level of exposure, which was expected as the loss of red blood cells in the spleen is a common stress response where the organ becomes empty and thus smaller in size (Stevens, 1968). This has been documented on numerous occasions for fish exposed to pulp mill effluents (Barker *et al.*, 1994; Couillard and Hodson, 1996), polycyclic aromatic hydrocarbons (Hart *et al.*, 1998) and pesticides (Hart *et al.*, 1997). This is usually an immediate response, however in this case we did not observe any significant differences in SSI between short and long-term exposure periods, possibly because swimming required more red blood cells in circulation. Estimates of hematocrit would have supported these changes in SSI but values

were unfortunately not taken in this particular study. The HESI remained the same across treatments, which is understandable given the short duration of the experiment of only 9 days though it would be interesting to see what would happen to this index after a chronic PCB exposure.

The magnitude of the physiological response to exhaustive exercise can be assessed by monitoring the levels of white muscle energy reserves and metabolites as well as blood biochemistry following the exercise challenges. Swimming requires the maintenance of energy in the form of ATP through the oxidation of carbohydrates, lipids and triglycerides (Milligan, 1996) and involves the use of three endogenous fuels stored within the white muscle: glycogen, ATP and phosphocreatine (PCr). In this study, PCB-exposed fish demonstrated a significant decrease in total protein and increase in amino acid levels in the white muscle tissue for the low-dose treatment, while the high-dose treatment also demonstrated these same changes in metabolites in addition to reduced glucose and glycogen levels. These changes could be a result of amino acid breakdown and protein synthesis as well as higher water content in the muscle. The metabolite values obtained for the control group were in accordance with other resting levels of white muscle metabolites (Dobson and Hochachka, 1987; Ferguson *et al.*, 1993; Milligan and Girard, 1993; Kieffer *et al.*, 1994; Wang *et al.*, 1994b; Wang *et al.*, 1994a). Glycogenolysis provides the majority of ATP required to sustain muscular exertion (Dobson and Hochachka, 1987; Wood, 1991; Milligan, 1996) and their recovery patterns often differ substantially among studies and species (Milligan and Wood, 1986; Dobson and Hochachka, 1987; Schulte *et al.*, 1992; Boutilier *et al.*, 1993; Kieffer *et al.*, 1994; Wang *et al.*, 1994b). Fish exposed to the high PCB-exposed treatment demonstrated a 26 % and 49 % decrease in white muscle glycogen and glucose

levels (Table 2.4), respectively, indicating greater depletion than in controls which suggests that more energy was used for prolonged swimming and a larger effort was put towards muscle restoration by fish exposed to PCB-126.

Biochemicals in the plasma may be useful tools to evaluate the health and/or stress conditions of fish (Sadler *et al.* 2000; Campbell, 2004; Wagner and Congleton; 2004) as stress has been reported to elevate cortisol (Pottiner and Mosuwe; 1994, Wendelaar-Bonga; 1997, Pottinger *et al,*. 2003; Haukenes *et al.,* 2008**)** and glucose levels (Silbergeld, 1974; Hattingh, 1977; Wedemeyer and Yasutake, 1977; Balm *et al.,* 1989; Barcellos *et al,*. 1999). Cortisol is the principal glucocorticoid secreted by the interrenal tissue located in the head-kidney of teleost fish (Iwama *et al.,* 1999) released by the activation of the hypothalamus-pituitary-interrenal (HPI) axis (Mommsen *et al.,* 1999) under stressful conditions. Cortisol values in the plasma of salmonids are extremely variable but are often found in the range of < 10 and 40-200 ng/mL for resting and stressed fish (Iwama *et al.*, 2004). The values obtained in this study were in a lower range of 6.28 to 20.7 ng/mL from controls to PCB-exposed fish (Table 2.3); nonetheless demonstrating a significant increase. In most fish, cortisol reaches its highest concentration 1 h after being stressed, and returns to basal levels after 6 h (Iwama *et al.,* 2006) to avoid tissue degeneration or damage of homeostatic mechanisms (Dickhoff, 1989; Stein-Behrens and Sapolsky, 1992; Wendelaar-Bonga, 1997). The low levels of cortisol found in this study may be explained by either the exhaustion of the endocrine system as a result of prolonged hyperactivity (Hontela *et al.,* 1992) or the habituation of fish to PCB-126 exposure. This habituation caused the interrenal tissue of stressed fish to become less sensitive to the action of ACTH or other pituitary hormones (Vijayan and Leatherland, 1990; Mommsen *et al.,* 1999), which decreased the levels or cortisol production.

This elevation of plasma cortisol levels due to stress is frequently associated with subsequent elevation of glucose and lactate levels (Gesto *et al.*, 2008; Barton *et al.*, 2002; Gourley and Kennedy, 2009). Glucose is a carbohydrate that has a major role in the bioenergetics of fish, as it transforms to chemical energy (ATP) that can be expressed as mechanical energy (Cho, 1992; Milligan, 1996). Blood glucose levels in fish and mammals are normally 5 mmol/L or less (Handy and Depledge, 1999). The elevation of glucose from 5.33 mmol/L in the control group to 6.95 mmol/L in high PCB-exposed fish demonstrated in this study indicate a typical secondary response to stressors in fish (Table 2.3). In stressful conditions the chromaffin cells release catecholamine hormones toward blood circulation (Reid *et al.*, 1998). Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Iwama *et al.*, 1999) to cope with the energy demand produced by the stressor. This production can assist the fish by providing energy substrates to tissues such as brain, gills and muscles, in order to cope with the increased energy demand (Barton *et al.*, 2002). The levels indicated above could thus be an indication of enhanced gluconeogenesis in the liver by the release of higher cortisol levels, which occurs mostly during periods of fasting or intense exercise and may be used to restore glycogen levels in the muscle. The increase in glucose production from control to intoxicated fish could also imply that they must release additional metabolites to fully restore their body to normal condition in order to counterbalance the effect of the stressor.

The remaining blood constituents revealed only minimal increases in amino acids (0.07-0.09 mg/mL) and lactate (2.51-2.56 mmol/L) levels and a decrease in triglycerides (5.21-4.84 mmol/L) levels with increasing exposure treatment. No significant variation was noted for total proteins levels which remained around 37 mg/mL across treatments (Table 2.3).

Lactate plays an important role in anaerobic metabolism and is considered a stress indicator in fish because its levels may be enhanced under adverse situations (Thomas *et al.*, 1999; Grutter and Pankhurst, 2000). In rainbow trout, post-exercise lactate values often vary substantially between studies (Wieser *et al.*, 1985; Dobson and Hochachka, 1987; Schulte *et al.*, 1992; Ferguson *et al.*, 1993; Krumschnabel and Lackner, 1993; Milligan and Girard, 1993; Kieffer *et al.*, 1994; Wang *et al.*, 1994b). It was expected that lactate concentrations would have significantly increased in this study as greater plasma lactate levels indicate greater anaerobic effort in exercise; however, PCB-exposed fish lactate levels were comparable to those of the control fish (Table 2.3). An explanation for this could be that when blood was taken from euthanized fish, lactate was already involved in glycogen res-synthesis and had begun to travel to the liver or still remained in the muscle.

Recent evidence suggests that amino acids and fatty acids are also used to support the post-exercise recovery process (Milligan and Girard, 1993; Wang *et al.*, 1994b; Milligan, 1997). In some fish, a small increase in plasma cortisol such as the one demonstrated in this study can lead to an increase in amino acid metabolism (Hopkins *et al.*, 1995), but in this case, no significant changes in plasma levels were observed (Table 2.3) Nonetheless, the slight increase in amino acids and lactate in blood plasma indicate that PCB-exposed fish may have begun to mobilize alternate fuel sources after depletion of glycogen. Fatty acid levels are usually in the range of 5.5 mmol/L in fish (Morris and Davis, 1995; Handy and Depledge, 1999), though animals that are suffering from food deprivation or poor nutrition, such as the fasting in this study, may have lower plasma triglyceride levels. This is what we observed as the triglyceride levels decreased with increasing level of PCB dose, indicating that energy

stores or nutritional conditions of fish in the high-dose treatment were lower than those of the controls prior to the fasting period.

The time needed for these physiological variables to recover after exhaustive exercise is important and often varies between studies. For instance, recovery of muscle lactate may take about 6–8 h in rainbow trout (Milligan and Wood, 1986; Scarabello *et al.*, 1991; Milligan and Girard, 1993; Kieffer *et al.*, 1994) whereas the time required for muscle glycogen resynthesis following exhaustive exercise can also vary from about 4–6 h (Scarabello *et al.*, 1991; Schulte *et al.*, 1992; Milligan and Girard, 1993) to as much as 12 h (Milligan and Wood, 1986). Finally, the recovery patterns of certain blood variables showed in this study may also differ substantially between studies using rainbow trout (Milligan and Wood, 1986; Milligan and Girard, 1993; Kieffer *et al.*, 1994). The changes in metabolites observed in this study indicate alterations in fuel utilization of PCB-exposed fish as endogenous stores were catabolized to meet increasing energy demands (Andersson *et al.*, 1985; Peragón *et al.*, 1999; Boujard *et al.*, 2000; Ruane *et al.*, 2002; Salem *et al.*, 2007) due to the intoxication and exercise.

2.4. Conclusions

This research has important implications in an ecological context, especially for salmonids as they rely heavily on their swimming activities to perform important reproductive duties with high energetic costs associated with migration and spawning, competition, and gonadal development (Thorpe, 1994; Vijayan *et al.*, 2003; Bains and Kennedy, 2005). In terms of ecological relevance, critical swimming speed and recovery ratio values are good indicators of the ability of a salmon to swim through stretches of strong currents during its upstream

migration (Jain *et al.*, 1998). This study demonstrated that PCB-126 can affect the energy budget of fish and cause functional deficiencies. As predicted, critical swimming speed decreased substantially with an increasing concentration level of PCB-126 and recovery ratios of intoxicated fish were reduced following an exhaustive exercise compared to non-exposed fish demonstrating a metabolic disturbance. This study contributes to the literature with additional insights on the sublethal effects of PCBs on fish, and also by integrating physiological and behavioural processes which provides ecological relevance to the results as an impaired swim performance and slower recovery could impact typical salmonid behaviours mentioned above and have serious repercussions at the population or ecosystem levels.

Chapter 3

Summary and Conclusions

3.0. General Conclusions

Fish utilize various behavioral, molecular and physiological strategies to survive challenging environments (Kieffer, 2010). Because exercise involves the interactions of many body systems, it is considered an integrated measure of an animal's fitness for a particular environment (Nelson, 1989). Given that the capacity for exercise is highly variable (Kieffer, 1995; Milligan, 1996), exercise to exhaustion, therefore, can be a useful model system to study regulatory processes and allow the determination of rate limiting factors in exercise performance and recovery in fish (Kieffer, 1995; Milligan, 1996). Exposure to chemical stressors activates detoxification and repair mechanisms in order to maintain homeostasis and increase resistance to the toxicant. These responses are energy-demanding and costly in terms of metabolic resources (Beyers *et al.*, 1998; McKenzie *et al.*, 2007) which may lead to alterations in normal fish function and behaviour including growth, reproduction and locomotion. This may translate to a decrease in individual performance or fitness of the fish (Alcaraz, 2000), and subsequently alter its role and function within aquatic environments.

This research demonstrated a significant decrease in the swimming performance of rainbow trout for the low and high PCB-126 treatments as well as an impaired recovery with increasing level of PCB exposure following exhaustive exercise as energy is allocated to additional functions for detoxification and repair. The decrease in recovery ratios indicates reduced recovery ability with increasing level of exposure implying that intoxicated fish require longer periods to adapt following an exhaustive exercise or during stressful conditions compared to non-exposed fish. As predicted, increased exhaustion metabolic rates and oxygen

consumption were observed in both PCB-exposed groups. This confirmed that the fish were in fact exhausted and stressed as the maximum metabolic rates were significantly higher than the standard and routine values. This is congruent with the reduction in Ucrit values usually associated with interference of O_2 uptake in the gills or reduction of blood O_2 transport (Hammer, 1995). This study also showed the occurrence of physiological disturbance by a reduction in the hepatosomatic and spleen somatic indices and elevation of plasma cortisol and glucose levels, as well as white muscle reductions in glucose and glycogen indicating higher metabolic costs during recovery and muscle restoration for PCB-exposed fish.

3.1. Swimming Performance and Respirometry

Critical swimming speed measurements has been frequently used by researchers to evaluate the effects of different environmental conditions on fish swimming ability and aerobic metabolism, usually in order to predict their ecological effects on fish in their habitat (Plaut, 2001; Kieffer, 2010, Beamish, 1978; Randall and Brauner, 199; Kolok, 1999; Kieffer and Cooke, 2009). These factors include temperature (Jones *et al.*, 1974; Keen and Farrell, 1994; Taylor *et al.*, 1996; Adams and Parsons, 1998; Kieffer *et al.*, 1998), salinity (Nelson *et al.*, 1996; Kolok and Sharkey, 1997; Swanson *et al.*, 1998; Plaut, 2000a), feeding rate (Gregory and Wood, 1998), body form (Nicoletto, 1991; Plaut, 2002b), the effects of tagging (Davison *et al.*, 1999) and transmitters (Counihan and Frost, 1999), and toxicological effects of pollutants (Beaumont *et al.*, 1995a, 1995b; Hammer, 1995; Heath, 1995; Kennedy *et al.*, 1995). It has generally been agreed that swimming speeds are individually repeatable (Jain *et al.*, 1998; Kolok, 1998, 1999; Farrell *et al.*, 1998; 2003; McKenzie *et al.*, 2007), reflect the maximum aerobic capacity (Brauner *et al.*, 1994), are generally assumed to reflect maximum

oxygen consumption capabilities (Farrell and Steffensen, 1987) and can produce comparable data on general swimming ability of the fish (Plaut, 2001). Although it was suggested that swimming capacity is a major trait affecting fitness (Reidy *et al*., 2000), it has not yet been proven that Ucrit values can be directly correlated to fitness and survival in an ecological system. This presents a debate concerning how environmentally relevant are the results presented in this research; therefore the limitations of these critical swim speed tests will be discussed.

Critical swimming speed values are the easiest available method of measuring swim performance, however using the typical Brett (1964) or Blazka (1960 *et al.*) tunnels (or any modifications) generates limitations. The environment that a fish experiences in a water tunnel under the common experimental protocol of measuring critical swimming speed is rarely, if ever, experienced by fish in their natural habitat. The water flow/swimming conditions far from resemble any existing natural habitat experienced by most fish as they experience mostly calm or turbulent water in nature, whereas swim tunnels produced mostly laminar or uniformly microturbulent water flow (Gordon *et al*., 1989; Plaut and Gordon, 1994). Also, a boundary layer may be created near tunnel walls, ceiling and floor so the fish may find places with slower water velocities and consequently swim at different speeds than prescribed (Webb, 1993b). This boundary layer width is usually correlated with water velocity. In addition, the fish itself may create a blocking effect if it obstructs part of the tunnel cross-section area and creates a faster velocity or different pressure regime using its own body (Bell and Terhune, 1970).

Despite these considerations, it is suggested that critical swimming speeds still provide a relative index by which the physical status of fish can be quantified and compared (Brauner

et al., 1994). Also, its physiological value has been validated by several studies on intra-inter-individual variation in locomotion performance of fish (Kolok, 1992; Gregory and Wood, 1998; Kolok *et al*., 1998) and the effect of locomotion capacity on survival (Taylor and McPhail, 1986; Jayne and Bennett, 1990 a,b,; Watkins, 1996) which suggests environmental relevance. Furthermore, close correlations were found between Ucrit values and ecologically relevant traits such as routine activity level, coloration, male territoriality, growth rate on several accounts (Kolok, 1999). Critical swimming speed thus remains the most convenient way to measure swim performance without harming fish, and is probably the best eco-physiological measurement to estimate swim performance capability and to predict ecological consequences. Also, using more than one Ucrit value and separating them by a recovery period can be used to calculate recovery ratios which are better indicators for metabolic disturbance and of greater ecological relevance (Jain *et al.,* 1998) as the swimming performance of each individual is compared with themselves rather than as a group or treatment.

3.2. Physiological and Biochemical Disturbance

Swimming exercise activates many physiological and biochemical systems, which may influence performance as they approach their limits and metabolic fuels are depleted. These responses to exhaustive exercise in fish are well documented (Milligan and Wood, 1987 b,c; Dalla Via *et al.*, 1989; Nelson, 1990; Milligan *et al.*, 1991; Pagnotta and Milligan, 1991; Wood, 1991; Boutilier *et al.*, 1993; Kieffer *et al.*, 1996; Ferguson *et al.*, 1993; Kieffer *et al.*, 1994; Nelson *et al.*, 1994; Milligan, 1996). In order to explain why the PCB-126 compound limited the exercise performance of fish in this study, it is important to first describe which

processes set the limits for performance. Since white muscle energy stores are used to support swimming, the concentrations of white muscle energy reserves (glycogen, ATP, PCr) are critical (Moyes and West, 1995). Thus, a potential limitation to swimming activity could be the lack of energy reserves within the muscle prior to experimentation. In this study, this could have resulted from malnutrition of the PCB-exposed fish prior to their fasting period. All fish were fed the same ration prior to experimentation; however, intoxicated fish have a general tendency to ingest less food and therefore energy.

Limitations could also be the result of the accumulation of metabolic end-products and/or the ability of the animal to recover from exhaustive exercise. There are some selective advantages associated with rapid recovery from exhaustive exercise. For example, fast recovery of white muscle energy fuels (PCr, ATP and glycogen) following activity would permit fish to perform subsequent bouts of exercise. In nature, this could be important for salmonid species along their upstream migration route when they are exposed to high water velocities and for extended time periods. PCB-exposed fish in this study were allocating most of their energy for muscle restoration and metabolite mobilization therefore this was compensated by a reduced recovery ratio, which in an environmental setting could pose a serious problem to their survival.

Lastly, limitations to exercise performance could be the result of a combination between the levels of anaerobic fuels and the time for recovery following exhaustive exercise. As some literature suggests (Milligan, 1996; Handy *et al.*, 1999; Kieffer, 2000), a metabolic trade-off may exist between balancing the levels of anaerobic fuels with reduced post-exercise recovery times. Thus, it may be more advantageous to store enough fuels to support anaerobic metabolism, but recover them quickly so subsequent and rapid bouts of exercise are possible.

This would probably have a strong ecological component, and would depend on the foraging and reproductive strategies, the life-history stage and the habitat requirements of the species.

3.3. Research Implications

This research has important implications in an ecological context, especially for salmonids as they rely heavily on their swimming activities to perform important reproductive duties such as their migration patterns and spawning. Critical swimming speed values and recovery can measure sublethal effects and provide accurate and relevant information on ecosystems in polluted habitat (Hammer, 1995). In terms of ecological relevance, Ucrit values are a good indicator of the ability of a salmon to swim through stretches of strong current during its migration, or swimming upstream in high velocity rivers or against fast tidal currents (Jain *et al.*, 1998). As demonstrated by our study, an impaired swim performance and slower recovery could alter energy allocations needed to perform these behaviours and have serious repercussions on ecosystem functioning. Life stage is also an important factor to consider as the altered energy allocations can vary and impair different functions within fish. For example, juvenile fish have high metabolic costs due to rapid growth and development, whereas mature fish have high costs associated with migration and spawning, competition, and gonadal development (Vijayan *et al.*, 1993; Thorpe, 1994; Bains and Kennedy, 2005). In turn, these deficiencies could have serious repercussions on fish role and function within an aquatic ecosystem. This research can also be considered as a contribution to a relatively small body of literature published on this topic with respect to the specific PCB congener and concentrations used but most importantly because of the recovery ratio approach rather than only focusing on critical swimming speed values.

3.4. Future Research Considerations

Future research considerations should include repeating this study with different post-injection time periods and PCB concentrations/congeners, as few statistically significant differences existed between short and long-term time periods thus perhaps a chronic study would be a more appropriate approach. Also, sublethal effects of PCB-126 were found for both the low and high-dose treatments, however they weren't always present at the low-dose level. This means that perhaps a threshold exists between the 100 and 400 µg/kg concentrations that should be tested before making assumptions and environmental implications. Swim trials should also be replicated and physiological analysis compared between fish suffering from both an exposure stress and exhaustive exercise stress to fish suffering only from exposure stress. This would isolate the effects demonstrated by either the exercise stress, the PCB exposure, or both.

Some other possible options or follow-up experiments are to evaluate other types of swimming performance such as burst (< 20 s) or sustained (> 200 s) swimming. Sprint and acceleration tests may better reflect the diversity of fish swimming styles and how other types of swimming performance relate to Ucrit. These tests emphasize the ecological and evolutionary implications of variance in aquatic locomotory performance (Nelson *et al.*, 2002) as they are the types found more frequently under natural conditions. Also, the behavioral component of using a swim tunnel should be studied by addressing the relationship between fish behaviour (gait transition) and swimming performance (McFarland and McDonald, 2002; Peake and Farrell, 2006; Peake, 2008) possibly by using PIT tags, video, or EMG telemetry to observe the behaviours of fish in a laboratory setting among each treatment, as the

interpretation of Ucrit results alone are limited because they should take into account this crucial variable for survival.

An additional possibility is to incorporate the results obtained from the swim performance tests into a bioenergetics models first validated in the laboratory and then extrapolated under controlled field conditions to confirm the assumptions made and create a general framework permitting a proper evaluation of contamination within natural ecosystems. Future research should also compare swimming capacity under conditions that are more natural and across populations (Hasler *et al.*, 2009). An experiment should be established to measure the ecological relevance of critical swim speed values. This could be conducted by marking fish with known pre-measured swimming abilities (Ucrit) in the laboratory and release them into their natural habitat and follow their reproductive success by recapturing them and correlating their survival rate with initial Ucrit measurements.

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Appendix I –Velocity Settings and Swimming Speeds

	Time (min)																			
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Motor setting	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10
Swim speed (cm/s)	10	20	30	40	50	60	70	80	100	110	120	130	140	150	160	170	180	190	200	210
Swim speed (BL/s)	0.3	0.6	1.0	1.3	1.6	1.9	2.3	2.6	3.2	3.5	3.9	4.2	4.5	4.8	5.1	5.5	5.8	6.1	6.4	6.8

Appendix II - Visual Inspection Parameters

External examination		
	Condition	Notation
Eyes	Normal	N1, N2
(1=L, 2=R)	Exophthalmia	E1, E2
	Haemorrhagic	H1, H2
	Blind	B1, B2
	Missing	M1, M2
	Other	OT
Pseudobranchs	Normal	N
	Swollen	S
	Lithic	L
	Inflamed	I
	Other	OT
Gills	Normal	N
	Pale	P
	Other	OT
Fins	No active erosion	0
	Mild active erosion	1
	Severe active erosion, haemorrhaging	2
Opercula	No shortening	0
	Shortening, some gill exposed	1
	Severe shortening, gill exposed	2
Wound	None	0
	Mild, < 1 cm^2	1
	Severe, > 1 cm^2	2

Internal examination		
	Condition	Notation
Mesenteric fat	None	0
	Slight, less than 50%	1
	50% coverage	2
	More than 50% coverage	3
	100% fat coverage	4
Spleen	Very dark red	B
	Red	R
	Granular, rough	G
	Nodular	NO
	Enlarged	E
	Other	OT
Hindgut inflammation	None	0
	Mild or slight	1
	Severe	2
Kidney	Normal	N
	Swollen	S
	Mottled	M
	Granular	G
	Urolithiasis	U
	Other	OT
Liver	Red	A
	Lighter, or less red	B
	Fatty, light tan coloured	C
	Nodules in liver	D
	Focal discolouration, marbling	E
	Other	OT
Bile	Yellow, not full	0
	Yellow, full	1
	Light green, green	2
	Dark green, blue green	3

Appendix III – Biochemical Assay Protocols

Glucose (blood and white muscle)

Glucose concentrations for blood plasma and white muscle tissue were determined by using the glucose assay according to Darrow and Colowick (1962). A standard glucose solution was prepared by dissolving 2.5 mg D-glucose in 5 mL distilled water. Standards (0.125, 0.25, 0.375, and 0.5 mg/ml) were set up in triplicates in a 96 well-plate. A 20 mL "soup" was prepared containing 60 nM trizma base (145 mg), 40 mM tris-HCl (126 mg), 1 mM $MgSO_4$ (4.9 mg), 2 mM NAD^+ (29.5 mg), 1 mM ATP (12.1 mg) and 0.1 units/mL G6PH. A plasma sample of 10 µL was added to the plate in duplicates followed by 200 µL of the "soup" mentioned above. The plate was then incubated for 5 minutes at room temperature on the spectrophotometer shaker and pre-read at 340 nm. A solution of 1 mg of hexokinase in 5.18 ml ddH_2O was prepared and 10 µL was added to the plate which was incubated for 30 minutes on the shaker and then read again at 340 nm by spectrophotometric analysis. The glucose concentrations of each sample were determined with the values set by the standard curve.

Amino acids (blood and white muscle)

The amino acid assay was prepared using the method of Troll and Cannan (1953). Leucine dissolved in ddH_2O was used to prepare standards in concentrations of: 100, 75, 50, 25 and 0 µg/mL. A 0.5 mL solution of 400 mg phenol in 100 mL absolute ethanol as well as a 2 ml solution of 0.01 M KCN diluted to 100 mL with NH_3-free pyridine (shaked with 1 g Permutit) were prepared and added to a tube with a 0.25 mL sample containing 0.05-0.5 µmoles of amino acids. The tube was placed in a boiling water bath in the fume hood until all samples reach water temperature and a 0.1 mL solution of 500 mg ninhydrin in 10 mL absolute ethanol was added. Marbles were placed on tubes and boiled between 3-5 minutes. Solution was cooled and brought to 5 mL by adding 60 % ethanol. Samples were plated in triplicates in a 96-well plate and absorbance was read at 570 mm. Proline was not detected by this method and amino acid concentrations were determined with the standard curve.

Protein (blood and white muscle)

Total protein concentrations were determined using the assay methods as described by Smith *et al.* (1985). In a 1.5 ml microfuge tube, a 1 ml of 1 x PBS solution was prepared by diluting 100 µl of 10 x PBS buffer with 900 µl of molecular grade water. Standards were prepared in concentrations of 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0 mg/mL (BSA in 1X PBS). The samples (20 µL) and standards (20 µL) were plated on a 96-well plate with a BCA reagent (150 µL), incubated for 20 min at 37°C in a water bath and read at 562 nm by spectrophotometric analysis.

Lactate (blood and white muscle)

Lactate concentrations were determined using methods as described by Gutmann and Wahlefeld (1974). A solution of perchloric acid (6 %) was prepared by diluting 7 ml of PCA (70%, sp. gr. 1.67) in 93 ml of ddH_2O. A glycine buffer/NAD^+ solution was prepared by dissolving 50 mg NAD^+ (N-7004, MW 663.4) in 20 ml ddH_2O and adding 10 ml of glycine buffer (G-5418). A lactate dehydrogenase (LDH) solution of 1500 (U/ml) in ddH_2O was also prepared. After, 10 mg of L-(+)-lactic acid was dissolved in 10 mL of 1N PCA (1mg/ml or 11.1 mM). The following standards were prepared in 1.5 ml microfuge tubes (0.450, 0.225, 0.09, 0.045, and 0 mg/mL). Standards were set up in a 96 well plate while 5 µL of the plasma sample, along with 242.5 µl of the glycine buffer / NAD^+ and 2.5 µl of the lactate dehydrogenase solutions. The plate was incubated for 45 min and read at 340 nm at room temperature. The standard curve was plotted and sample concentrations were determined according to the standard curve.

Glycogen (white muscle)

Glycogen concentrations were determined using the glycogen assay according to Keppler and Decker (1975). Type II oyster glycogen was used to prepare the following standard concentrations: 0, 2.5, 5, 7.5, 10, 15, and 20 mg/ml in triplicates. Each sample (100 µL) was transferred into a 12x75 mm glass test to which 50 µL of 1M $NaHCO_3$ and 1 ml of amyloglucosidase solution (0.144 ml glacial acetic acid, 0.2925 g sodium acetate, and 30 mg amyloglucosidase in 30 ml of ddH_2O) were added. The samples and standards were incubated at 37°C for 2 h. The incubation was terminated by adding 25 µL of 70% PCA to the tubes. Each sample was transferred into 1.5 ml conical test tubes and centrifuged at 7,000 xg for 2 min. The glucose assay was performed as described above.