ACUTE SUBLETHAL TOXICITIES OF 6PPD-QUINONE TO JUVENILE SALMONIDS

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Toxicology Graduate Program,

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Summer Jane Selinger

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work or, in their absence, by the Dean of the College in which my thesis work was done. It is understood that any copying, publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Chair of the Toxicology Graduate Program

Toxicology Centre

University of Saskatchewan

44 Campus Drive

Saskatoon, Saskatchewan, Canada, S7N 5B3

Dean

College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan, Canada, S7N 5C9

ABSTRACT

This thesis investigated the effects of N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone), an environmental transformation product of the widely used rubber tire antioxidant 6PPD, on juvenile salmonids. Increasingly recognized as a significant environmental pollutant present in road runoff and surface waters, 6PPD-quinone is notorious for inducing acute lethality in select salmonid species at extremely low concentrations (≤1 μg/L). Conversely, other salmonid species show insensitivity, even when exposed to substantially higher concentrations (3.8-50 μg/L). Sensitive species exhibit distinctive symptoms and atypical swimming behaviour such as gasping, spiraling, increased ventilation, erratic movements, tumbling. and loss of equilibrium, indicating potential impacts on cardiorespiratory physiology. Despite these observations, this thesis is the first to characterize these effects in salmonids comprehensively. Unlike the predominant focus on acute toxicity in existing literature, this study primarily investigated sublethal toxicities. Specifically, it examined the impacts of 6PPD-quinone exposure on salmonids of varying sensitivity: two sensitive species, rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*), and one tolerant species, Arctic char (*Salvelinus alpinus*). Fish were exposed to a solvent control or 0.59, 0.46, or 7.15 μg/L 6PPD-quinone, respectively, for 20-48 hours. In both rainbow trout and Arctic char, increases in standard metabolic rate were observed. Only rainbow trout showed a decrease in end systolic volume and an increase in passive ventricular filling, cardiac output, and PR interval length, indicating cardiac stimulation. The cardiorespiratory symptoms seen in sensitive species might be partly due to a significant increase in methemoglobin, impairing their ability to oxygenate tissues. In lake trout,

6PPD-quinone exposure impaired swimming performance, demonstrated by a decrease in critical swimming speed. Exposure also resulted in significant decreases in active metabolic rate, although no changes were observed in standard metabolic rate. Additionally, decreased concentrations of white muscle triglycerides of swam fish were also observed in lake trout. These findings suggest that environmentally relevant concentrations of 6PPD-quinone can disrupt aerobic metabolism, swimming performance, and cardiovascular function in salmonids, potentially affecting fish survival. The findings of this thesis provide crucial insights into the sublethal toxicities of 6PPD-quinone to juvenile salmonids, offering valuable information for ecological risk assessment.

ACKNOWLEDGEMENTS

I am deeply thankful to my supervisors, Dr. David Janz and Dr. Markus Brinkmann, for their advice, encouragement, and support. Under their guidance, challenges became growing experiences, improving my confidence as a scientist and an individual. My thanks also go to my committee members, Dr. Markus Hecker and Dr. Lynn Weber, for their guidance and the vast knowledge they imparted to me during my degree. I am also profoundly grateful to my lab manager, Luciene Kapronczai, for the continuous motivation and endless assistance she provided during my experiments. Her help, from outdoor breaks and pep talks to weekend calls guiding me through any problem, was invaluable. I am also sincerely thankful to Emily Kennedy for simultaneously being the best role model and friend I could ask for, for the endless laughs, for answering my stupid questions, and for making our days together on conference trips or in the office something I will never forget. I would also like to thank my brother Zane for being a great roommate and listening ear, enduring my endless rants about failed trials and insignificant results. And of course, I am forever thankful to my parents for their unwavering support throughout every aspect of my life, including my academic career. For always answering my late-night calls after I checked on my fish or analyzed sonographs all night long, for listening to me practice my presentations 25 times before I gave them, and for sending a few extra dollars when I overspent at a conference. Finally, I would like to express my gratitude to the other students and staff at the Toxicology Centre for making it the special, welcoming place it is. With the encouragement of these people, no obstacle seemed too big to overcome and no problem too difficult to solve, both in life and at work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

β	Beta
µg	Microgram
µg/L	Micrograms per litre
µm	Micrometre
ºC	Degrees centigrade
%	Percent
AMR	Active metabolic rate
AnGap	Anion gap
ATP	Adenosine triphosphate
ATRF	Aquatic Toxicology Research Centre
AV	Atrioventricular
Aquacalm	Metomidate hydrochloride
AS	Aerobic scope
BE(B)	Base excess
BL/sec	Body lengths per second
B-mode	Brightness mode
BPM	Beats per minute
Ca^{+2}	Calcium
Cl^{-}	Chloride
cm	Centimetre
CO	Cardiac output
CoT	Cost of transport
$ctCO_2$	Concentration of CO_2 in plasma
CTRL	Control
d	Day
DMSO	Dimethyl sulfoxide
ECG	Electrocardiography
EDV	End diastolic volume
EF	Ejection fraction
ESV	End systolic volume
FO_2Hb	Fraction of oxygenated hemoglobin
FCOHb	Fraction of carboxyhemoglobin
FHHb	Fraction of deoxyhemoglobin
FMetHb	Fraction of methemoglobin
f_H	Heart rate
G	Gauge
g	Gram
g/L	Grams per litre
Glu	Glucose
h	Hour
HCO3	Bicarbonate
HPLC	High-performance liquid chromatography
IU	International unit
K^{+}	Potassium

kg	Kilogram
L	Litre
Lac	Lactate
LC_{50}	Lethal concentration 50
LC-HRMS	Liquid chromatography high-resolution mass spectrometry
LOD	Limit of detection
mg	Milligram
mg/g	Milligrams per gram
mg/L	Milligrams per litre
mg O_2/kg/h	Milligrams oxygen per kilogram per hour
MHz	Megahertz
mL	Millilitre
mm	Millimetre
mmHg	Millimetre of mercury
mmol/kg	Millimoles per kilogram
mmol/L	Millimoles per litre
MO_2	Oxygen consumption
mOsm	Osmolarity
MS-222	Tricaine methane sulfonate
n	Number of samples
Na^+	Sodium
ND	Not detected
ng/L	Nanograms per litre
O_2	Oxygen
p	Probability
pCO_2	Partial pressure of CO_2
pO_2	Partial pressure of O_2
PVF	Passive ventricular filling
ROS	Reactive oxygen species
s	Second
SC	Solvent control
SD	Standard deviation
SEM	Standard error of the mean
SMR	Standard metabolic rate
tHb	Total blood hemoglobin
TWP	Tire wear particle
U_{crit}	Critical swimming speed
UHPLC	Ultra-high-performance liquid chromatography
URMS	Urban runoff mortality syndrome
VB	Ventriculobulbar
V_S	Stroke volume
6PPD	*N*-(1,3-Dimethylbutyl)-*N*'-phenyl-*p*-phenylenediamine
6PPD-quinone	*N*-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone

NOTE TO READERS

This thesis was prepared in a manuscript style and will therefore have some redundancies across sections of research chapters. To reduce these redundancies, specific descriptions of methods and statistics are found in their respective chapters. Chapter 1 is a general introduction, and chapters 2-3 are written in the style of publishable manuscripts. Chapter 4 serves as a summary and conclusion to the overall thesis. Chapters 2 and 3 have recently been submitted for publication. To avoid redundancies in citation lists, all citations have been provided in a combined section at the end of this thesis.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 6PPD-Quinone

The health of aquatic systems is increasingly threatened by a growing influx of contaminants originating from urban environments, with stormwater runoff serving a major role in facilitating the flow of these pollutants into waterways. Among these pollutants are tire-derived chemicals, which have recently emerged as critical contaminants of concern due to their widespread presence and capacity to inflict severe ecological damage. Most notably, (*N*-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), has garnered urgent scientific and regulatory attention for its acute toxicity to fish, particularly salmonids. The initial concern with this chemical arose from its identification as the primary toxicant responsible for urban runoff mortality syndrome (URMS), a phenomenon linked to extensive die-offs of coho salmon (*Oncorhynchus kisutch;* 24-h LC_{50} = 95 ng/L) in the Pacific Northwest (Tian et al., 2021, 2022). Continued investigation has demonstrated that 6PPD-quinone also induces acute lethality in a number of additional salmonid species at comparably low concentrations (≤1 μg/L) (Brinkmann et al., 2022; Hiki & Yamamoto, 2022b; Roberts et al., 2025). However, in contrast, other studies have demonstrated that some salmonid species can withstand exposure to 6PPD-quinone, even at considerably elevated levels (3.8-50 μg/L) (Brinkmann et al., 2022; Foldvik et al., 2022, 2024; French et al., 2022; Greer et al., 2023; Hiki & Yamamoto, 2022b; Montgomery et al., 2023). These findings underscore the need for further investigation into the physiological changes that may explain the stark species-specific differences in toxicity, as well as the sublethal impacts and broader ecological consequences of 6PPD-quinone exposure. Moreover, gaining insight into the environmental behavior and

underlying mechanisms of 6PPD-quinone is crucial for developing strategies to reduce its effects and protect vulnerable aquatic populations.

1.1.1 Sources of 6PPD-quinone

6PPD-quinone is a transformation product generated through the environmental ozonation of 6PPD, a commonly used antiozonant and antioxidant that helps prevent the degradation of rubber products such as motor vehicle tires. Among others, antioxidants such as 6PPD are added to elastomers like rubber to prevent or slow damage caused by oxidation, maintaining their structure and thereby prolonging their service life. The additive is designed to diffuse to the surface of the tire and react with environmental oxidants such as ozone, unintentionally forming 6PPD-quinone in the process (Lattimer et al., 1983). This formation occurs when the amine group of 6PPD (*N*-(1,3-Dimethylbutyl)-*N*'-phenyl-*p*-phenylenediamine) reacts with ozone or oxygen, producing 6PPD-quinone (Lattimer et al., 1983). As friction occurs between tires and the road surface, particularly when braking, turning, and accelerating, small fragments of rubber wear off. These tire wear particles (TWPs) collect on roadways and are subsequently washed into aquatic environments through road runoff following rain and storm events, causing notable elevations in the concentration of 6PPD-quinone in surface waters (Johannessen, 2021; Peter et al., 2018; Seiwert et al., 2022; Tian et al., 2021; Werbowski et al., 2021). As tires and other rubber products are used ubiquitously, 6PPD-quinone is predicted to exist at detectable concentrations in aquatic environments around the globe (Huang et al., 2021; Johannessen et al., 2021; Cao et al., 2022; Hiki & Yamamoto, 2022).

Having been in use since the 1960s, 6PPD currently remains the only endorsed additive that meets the rigorous safety and performance standards for vehicle tires (Department of

Toxic Substances Control, 2022). Consequently, virtually all tires in use today and expected in the coming years contain this compound, meaning that its presence in the environment will be a persistent issue. Although a nontoxic substitute may be found, the presence of 6PPD and 6PPD-quinone will continue to be a problem, given the significant number of tires still in use or disposed of in landfills and tire graveyards. Even though efforts to recycle this large amount of scrap tire are well-intentioned, reuse typically involves the further breakdown of rubber for uses such as mulch and playground surfaces, increasing the opportunity for larger quantities of 6PPD-quinone and other rubber-derived chemicals to form and subsequently enter the environment. Overall, the recent discovery and greater awareness of the potential risks that 6PPD-quinone might pose to aquatic environments has emphasized the need for further research to determine its potential ecological effects.

1.1.2 6PPD-quinone in aquatic environments

Stormwater runoff is an important transport pathway for contaminants in urban areas (Masoner et al., 2019). Tire-rubber-related compounds, including 6PPD-quinone, have been identified as significant contaminants in urban runoff, with higher concentrations detected in areas with a greater degree of urbanization (O'Dell, 2001). However, recent findings suggest a complex relationship between population density, urbanization, and 6PPD-quinone concentrations in roadway runoff. Higher concentrations of 6PPD-quinone have been detected in runoff from heavily urbanized and densely populated cities such as Seattle, USA (2024 population: 3.5 million), Los Angeles, USA (2024 population: 12.6 million), and Hong Kong, China (2024 population: 7.5 million). However, smaller cities like Saskatoon, Canada (2024 population: 0.3 million) and Leipzig, Germany (2024 population: 0.6 million) have also shown significant levels of 6PPD-quinone. Specifically, concentrations in roadway runoff ranged

from 0.05-1.3 μg/L in the Seattle area, 0.3-0.4 μg/L in the Los Angeles area, and 0.21-2.4 μg/L in Hong Kong (Cao et al., 2022a; Tian et al., 2021, 2022), while concentrations ranged from 0.086-1.4 μg/L in Saskatoon and 0.11-0.43 μg/L in Leipzig (Challis et al., 2021; Maurer et al., 2023). This indicates that factors beyond population size may contribute to 6PPD-quinnoe levels in different regions. Furthermore, due to the dilution that occurs when roadway runoff discharges into receiving waters, the concentrations of 6PPD-quinone measured in surface waters are reported to be lower than that of roadway runoff, with measured concentrations ranging between 0.06-2.3 μg/L in San Francisco, USA, 0.01-0.2 μg/L in Seattle, USA, and 0.012-0.037 μg/L in Kent County, USA (Nedrich, 2022; Tian et al., 2021, 2022). To date, the highest detected concentration of 6PPD-quinone in North American surface waters,2.3 μg/L, was measured in the Don River, Toronto, Canada (Johannessen et al., 2022a). As rubber tires, roads, and storm events occur globally, these concentrations are likely representative of the global prevalence of 6PPD-quinone.

Recent studies have also shown the presence of 6PPD-quinone in snowmelt samples, suggesting that this compound is able to sequester into snow. This hypothesis was proposed by Johannessen et al. (2021), who detected 6PPD-quinone in surface water samples following a rapid snow melt, and confirmed by Challis et al. (2021), who measured a mean concentration of 0.367 ± 0.206 μg/L in 100% of snowmelt samples collected in Saskatoon, CA. Such events can result in a significant discharge of 6PPD-quinone in short, concentrated pulses during spring snowmelt, posing risks to a variety of aquatic organisms (Meyer & Wania, 2008). Salmonid species, including those assessed in this study, generally spawn in the fall, allowing their embryos to develop over the winter and emerge as fry from the substrate in the spring (Quinn, 2005). Given that some species of salmonid fry are acutely sensitivity to 6PPD-

quinone, pulses of this compound entering aquatic systems during this critical developmental stage may significantly increase the risk of toxicity (Philibert et al., 2024; Roberts et al., 2025). These findings emphasize the importance of assessing seasonal and hydrological factors when evaluating the ecological risks associated with 6PPD-quinone.

Since tire-wear compounds such as 6PPD-quinone are present across urban roadways, it is plausible that the quantity of these chemicals transferred into surface waters is directly related to the size of a storm event. This was observed by Johannessen et al. (2022b), who reported a positive correlation (R^2= 0.777) between the size of a hydrological event and the resulting mass loading of 6PPD-quinone. Similarly, it is expected that areas experiencing a rain event preceded by an extended period of dry conditions would have a higher influx in surface water concentrations than areas experiencing repetitive precipitation events, due to the increased time for TWP accumulation. However, this has not been consistently observed, with some reports showing low concentrations of 6PPD-quinone (0.0862 μg/L) after a rain event (10 mm) preceded by dry conditions (<0.4 mm) for an extended period (~7 d), and some reports showing high concentrations (1.402 μg/L) after a rain event (24 mm) preceded by similar conditions (Challis et al., 2021). Although this difference can partly be attributed to the size of the precipitation event, the fate and transportation of the compound are presumably related.

Laboratory studies analyzing the persistence of 6PPD-quinone in the environment have yielded varying results. One study reported a half-life of 33 h in dechlorinated tap water at 23°; however, another study reported half-lives of 12.8-13.2 d in untreated river water at 25°C, and 15.2-16.3 d in ultrapure water at 25°C (Di et al., 2022; Hiki et al., 2021). In the environment, elevated concentrations of 6PPD-quinone have been reported to persist in surface waters for more than 48 h following a storm event (Rauert et al., 2022). Collectively, these studies

suggest a prolonged presence of 6PPD-quinone in the environment, indicating a need to further study the duration required for elevated concentrations to leave the aquatic system. However, even as these compounds dissipate from the water column, studies have shown that 6PPD-quinone readily associates with particulates and can accumulate in sediments (Hu et al., 2023; Zeng et al., 2023; Zhou et al., 2024). These sediments act as a sink, storing 6PPD-quinone for extended periods and potentially serving as a secondary source of contamination as the compound slowly desorbs back into the water. Together, these observations highlight the complexity of 6PPD-quinone's environmental fate and its potential risk to aquatic organisms. Overall, the frequent detection of 6PPD-quinone in the environment supports the idea that rubber-derived organic chemicals are found globally in rivers and streams within proximity to urban areas. Furthermore, the concentrations of 6PPD-quinone measured in North American surface waters often exceed the median lethal concentration (LC_{50}) reported for coho salmon (Tian et al., 2021, 2022). This highlights the need for improved treatment of roadway runoff prior to its discharge into receiving waters, as well as a better assessment of the occurrence, fate, and persistence of this chemical.

1.1.3 Toxicological effects of 6PPD-quinone on aquatic species

Since 6PPD-quinone was identified as the primary compound responsible for urban runoff mortality syndrome and the mass mortality of coho salmon, multiple studies have aimed to determine its toxicity to other aquatic species. Initially, Tian et al. (2021, 2022) determined the median lethal concentration (24-h LC_{50}) of 6PPD-quinone to juvenile coho salmon as 0.095 µg/L. Following these findings, additional salmonids such as rainbow trout (*Oncorhynchus mykiss*; 72-h LC_{50} = 1.00 µg/L), brook trout (*Salvelinus fontinalis*; 24-h LC_{50} = 0.59 µg/L), lake trout (*Salvelinus namaycush;* 96-h LC_{50} = 0.50 µg/L), and white-spotted char

(*Salvelinus leucomaenis pluvius;* 24-h LC_{50} = 0.51 µg/L) were found to be acutely sensitive to exposure (Brinkmann et al., 2022; Hiki & Yamamoto, 2022b; Roberts et al., 2025). In contrast, a number of other salmonid species such as Arctic char (*Salvelinus alpinus*), brown trout (*Salmo trutta*), westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), Atlantic salmon *(Salmo salar),* masu salmon (*Oncorhynchus masou masou)*, Dolly Varden trout (*Salvelinus curilus*), and pink salmon (*Oncorhynchus gorbuscha*) have shown to be tolerant, even when exposed to significantly greater concentrations (3.8-50 µg/L) (Brinkmann et al., 2022; Foldvik et al., 2022, 2024; French et al., 2022; Greer et al., 2023; Hiki & Yamamoto, 2022b; Montgomery et al., 2023). The underlying cause of this species-specific sensitivity remains unidentified; however, these findings demonstrate that evolutionary proximity does not consistently predict toxicity in salmonid fishes. In addition to salmonids, other fish species such as zebrafish (*Danio rerio*), white sturgeon (*Acipenser transmontanus*), Japanese medaka (*Oryzias latipes*), red drum (*Sciaenops ocellatus*), and Chinese rare minnow (*Gobiocypris rarus*) were also insensitive to 6PPD-quinone exposure (Ackerly et al., 2024; Brinkmann et al., 2022; Di et al., 2022; Hiki et al., 2021; Varshney et al., 2022). Freshwater invertebrates such as larval burrowing mayfly (*Hexagenia* spp.), file ramshorn snail embryos (*Planorbella pilsbryi)*, adult washboard mussel (*Megalonaias nervosa)*, the water flea *Daphnia magna*, and the amphipod *Hyalella azteca* have also shown to be tolerant to 6PPD-quinone exposure (Hiki et al., 2021; Prosser et al., 2023). To summarize, acute lethality following 6PPD-quinone exposure has been observed solely in salmonids, with no other taxa showing sensitivity to date.

1.1.4 Mechanisms of 6PPD-quinone toxicity

At the present time, the biological mechanism(s) by which 6PPD-quinone elicits species-specific acute lethality are unknown. However, behaviours observed prior to the

mortality of sensitive species, such as hovering close to the water surface, increased ventilation, gasping, spiraling, and loss of equilibrium, suggest that the toxicity of 6PPD-quinone involves the dysfunction of cardiorespiratory and metabolic systems (Brinkmann et al., 2022; Tian et al., 2021). These symptoms, which were originally observed during mass mortality die-offs of coho salmon, have also been reported to precede the mortality of other sensitive species such as rainbow trout and brook trout (Brinkmann et al., 2022; Chow et al., 2019; Scholz et al., 2011). Observed behaviours (gasping and increased ventilation) used to increase oxygen consumption may suggest inhibition of cellular respiration (Salin et al., 2015; Souders et al., 2018). This was also reported by Mahoney et al. (2022) who found that exposure to 6PPD-quinone (20 μg/L) resulted in a 2-fold increase in the oxygen consumption rate of RTgill-W1 (gill) cells by uncoupling the mitochondrial electron transport chain. However, this was not observed in RTL-W1 (liver) cells, suggesting that the gill may be the primary target organ. In addition to impairing respiration, 6PPD-quinone is suspected to generate reactive oxygen species (ROS), a mechanism of toxicity that is well-established for quinones in general (Kakizaki et al., 1969; Link et al., 1985; Magos, 1964). This hypothesis is further supported by Varshney et al. (2022), who suggested that 6PPD-quinone induces oxidative stress, potentially leading to inflammatory responses observed in zebrafish larvae. Anderson-Bain et al. (2023) also observed elevated oxidative stress markers in the liver and gills of fathead minnows *(Pimephales promelas),* with gills being the primary site of ROS-related changes. However, it remains unclear whether oxidative stress plays a significant role in the toxicity of 6PPD-quinone. Cardiorespiratory dysfunction has also been documented by other researchers. Varshney et al. (2022) observed reduced heart rates in zebrafish larvae exposed to 6PPD-quinone at concentrations of 10 μg/L or higher, while Zhang et al. (2023)

reported similar effects at concentrations exceeding 25,000 µg/L. This decrease in heart rate is indicative of bradycardia, a common response in fish experiencing hypoxia (Farrell, 2007). In addition, Varshney et al. (2023) measured increased oxygen consumption rates in zebrafish at concentrations above 1 µg/L. However, these findings contrast those from Ricarte et al. (2023), who observed no difference in oxygen consumption rates at concentrations up to 2 µg/L but reported increased heart rate at concentrations above 0.02 µg/L. An increase in the blood glucose levels was also reported by Brinkmann et al. (2022), with effects observed in brook trout at concentrations as low as 0.72 µg/L and in rainbow trout at 2.78 µg/L, suggesting that 6PPD-quinone adversely affects energy homeostasis. Collectively, these findings suggest that impaired cardiovascular or metabolic physiology may be a driver of 6PPD-quinone toxicity. However, further research is needed to validate this hypothesis.

1.2 *Oncorhynchus mykiss* as a Model Organism

Rainbow trout, native to rivers and lakes throughout western North America, are a freshwater fish that derive their name from their vibrant mid-side markings that range from red and pink to purple. This salmonid species ranks among the top five sport fish in the United States, highlighting their commercial and recreational value (Simonds, 2016). Globally recognized as the most widely known trout species, rainbow trout have been introduced to every continent except Antarctica, reflecting their global popularity (Lu & Luo, 2020; US National Park Service, 2015). Beyond their recreational appeal, rainbow trout are also commonly used in aquaculture due to their high growth rates and nutritional value, providing significant economic benefits. Rainbow trout are highly adaptable, thriving in environments ranging from fast-flowing rivers to still lakes. While some populations will remain in the same area for their entire life, the anadromous form, known as steelhead trout, migrates to the ocean

to mature before returning to its natal stream to spawn (Hall, 2022). Ranking second only to zebrafish in research interest worldwide, rainbow trout are a highly studied fish that are extensively used as a bioindicator species in aquatic toxicity studies (Potter et al., 2020). They are favored as a model organism due to their wide distribution, ease of breeding and maintenance in laboratories, and high sensitivity to various aquatic pollutants (Besser et al., 2020; Environment Canada, 1990; Thorgaard et al., 2002; US EPA, 1996). Typically ranging from 14-50 cm in length, these freshwater fish are well-suited for studies requiring tissues and organ samples, as well as blood extraction (Fisheries and Oceans Canada, 2016). For these reasons, as well as their acute sensitivity to 6PPD-quinone (72-h LC_{50} = 1.00 µg/L; (Brinkmann et al., 2022), rainbow trout are an ideal model organism for this study.

1.3 *Salvelinus alpinus* as a Model Organism

Arctic char, another member of the Salmonidae family, are distinguished as the northernmost distributed freshwater fish in the world (Wilsterman et al., 2019) Known for its profound cultural significance, Arctic char have served as a primary food source for Arctic communities for thousands of years (Fisheries and Oceans Canada, 2018). Although their appearance can vary both seasonally and geographically, Arctic char can be identified by their dark dorsal side with light colored spots, as well as their light ventral side (Finstad et al., 2006). In spawning adults, most commonly in males, the ventral side may turn a vibrant shade of red, orange, or yellow (Morton, 1965). Depending on lake productivity, maximum sizes can vary greatly, with some individuals reaching lengths as large as 95 cm, making them ideal candidates for tissue, organ, and blood sampling (Government of Alaska, 2022). Arctic char are well adapted to live in cold, oligotrophic lakes and rivers across the Arctic and subarctic regions, preferring temperature from 4-16°C (Alaska Department of Fish & Game, 2006;

Gilbert, 2020). In contrast to their migratory relatives like the anadromous steelhead trout, Arctic char tend to remain with a certain area, rarely undergoing extensive migrations (Tallman et al., 1996). Their sedentary nature is supported by their ability to thrive in harsh, low-productivity habitats where other species might struggle. Arctic char have demonstrated tolerance to acute 6PPD-quinone exposure, even at concentrations as high as 14.2 µg/L (Brinkmann et al., 2022). Hence, Arctic char serve as an excellent comparative model for studying the sublethal effects of 6PPD-quinone exposure, offering insights into differential responses among tolerant fish species.

1.4 *Salvelinus namaycush* as a Model Organism

The lake trout is a freshwater salmonid predominately found in the cold, deep lakes of North America. Although Canadian and Alaskan waters host the largest populations of lake trout, they have been introduced to many regions beyond their native range (Government of British Columbia, 1997). As apex predators in their ecosystems, adult lake trout play a vital role in regulating prey populations and maintaining ecological balance. In addition to their ecological importance, lake trout are prized in recreational fishing for their large size and remarkable stamina, ranking them among the most popular freshwater game species (Simonds, 2016). Like many other trout species, lake trout are solitary by nature and do not typically form large groups. However, unlike many trout species, lake trout are not known for migratory behaviour (Fisheries and Oceans Canada, 1986). Thriving in temperatures ranging from 10-13°C, they tend to reside in the deep ends of lakes during summer, at depths exceeding 30 feet, where they remain relatively inactive. However, in winter or northern waters, lake trout transition to shallower waters or closer to the lake surface where they become popular targets for anglers (Redick, 1978). Even though lake trout can vary considerably in appearance within

the same lake, they are recognizable by their long, narrow bodies and small spots, which range from light gray to green against a darker background (Redick, 1978). Unlike many other freshwater species, lake trout have a delayed maturation period, taking several years to achieve maturity. Despite this, they are notable for their long lifespans, commonly living between 15 to 20 years under normal conditions, and occasionally surpassing 40 years in some instances (Redick, 1978). Lake trout share many physiological similarities with other salmonid species, making them a great model organism for predicting how different conditions of toxicants might affect other salmonids. Like many other trout, lake trout are also highly sensitivity to changes in their aquatic environment such as water quality, temperature, and the presence of toxicants (Cook et al., 2003; Walker et al., 1991). A recent study highlighted their acute sensitivity to 6PPD-quinone, with acute lethality occurring at just 0.50 μg/L (Roberts et al., 2025). In summary, the lake trout's longevity, sensitivity, ecological significance, and recreational importance make them a valuable model organism for scientific studies.

1.5 Endpoints and Biomarkers

1.5.1 Metabolic responses

All organisms use energy for life-sustaining functions such as maintaining homeostasis, osmoregulation, and protein synthesis (Principiotta et al., 2003; Tseng & Hwang, 2008). Consequently, metabolic rate, which measures energy consumption over time, is an important physiological variable for understanding how stressors like low oxygen, the presence of predators, or toxicant exposure affect organisms (Chabot et al., 2016a, 2016b). Aerobic metabolism, or cellular respiration, is the chemical process in which glucose and oxygen are converted into carbon dioxide, water, and energy (ATP). In this reaction, oxygen is used as the final electron acceptor in the electron transport chain (Molnar & Gair, 2015). For this reason,

changes in the oxygen consumption rate of an organism can be used as a proxy to understand metabolic effects (J. H. Brown et al., 2004).

Metabolic responses to environmental stressors are complex, ranging from alterations in energy allocation, to changes in the efficiency of ATP production (Sokolova, 2013). More specifically, organisms under toxicant-induced stress may prioritize energy towards essential functions like repairing damaged tissues or detoxification mechanisms (Gashkina, 2024). Stress-induced changes in metabolic rate can also impact behavior, as increases in energy demands may reduce the energy available for other vital behaviours like foraging and mating (C. Brown et al., 2005). As a result, metabolic changes can affect the overall health and fitness of organisms, potentially leading to long-term ecological consequences. Previous research has demonstrated that exposure to 6PPD-quinone affects metabolic function by uncoupling the electron transport chain in RT-gill-W1 cells, leading to increased oxygen consumption rates (Mahoney et al., 2022). My thesis research aims to investigate this further by using respirometry to determine the oxygen consumption rates of both resting (standard metabolic rate; SMR) and active (active metabolic rate; AMR) fish following sublethal exposure to 6PPD-quinone and compare these findings with those from unexposed control fish.

1.5.2 Cardiovascular responses

Unlike mammals, fishes have a two-chambered heart comprised of a single ventricle and atrium. This type of heart is part of a single circulatory loop, facilitating efficient blood flow through the heart (Farrell & Jones, 1992). Deoxygenated blood from the body enters the fish heart through a vein called the sinus venosus, where it proceeds through the sinoatrial valve into the atrium. Initially, the atrioventricular (AV) valve opens, allowing the ventricle to fill passively (Farrell & Jones, 1992). The atrium then contracts, completing the movement of

blood into the ventricle. The ventricle contracts next, pushing the deoxygenated blood through the ventriculobulbar (VB) valve into the bulbus arteriosus (Farrell & Jones, 1992). From here, blood travels to the gill capillaries where it becomes oxygenated. Once oxygenated, the blood is distributed through the systemic circulation to nourish body tissues. As oxygen is utilized in systemic capillary beds, the deoxygenated blood returns to the sinus venosus through the venous circulation, restarting the circulatory process (Johansen & Burggren, 1980; Rantin et al., 2020; Yamauchi, 1980).

Cardiac function can be altered in response to fluctuations in oxygen levels or exposure to contaminants (Lind et al., 2021). Traditionally, research has depended on invasive and lethal methods such as the surgical implantation of Doppler probes to monitor changes in blood flow (Keen & Gamperl, 2012). However, recent advancements in non-invasive technologies, such as high-frequency ultrasound, provide an alternative method for examining cardiac structure and function, eliminating the need for euthanization. In this thesis research, cardiac ultrasound will be used to comprehensively assess the effects of acute 6PPD-quinone exposure on various aspects of cardiac performance. This will be done by measuring parameters such as passive ventricular filling velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. By comparing these measurements with those from unexposed controls, this study aims to understand how 6PPD-quinone influences the heart's ability to fill, contract, and pump blood effectively. Furthermore, through the comparison of these parameters across salmonids of differing sensitivity, this research aims to identify factors that may contribute to the species-specific sensitivity in salmonids.

In a resting fish, the majority (~80%) of ventricular filling occurs during the passive phase, prior to atrial contraction and the expulsion of the remaining blood (~20%) into the

ventricle (Farrell & Jones, 1992). Analyzing changes in passive ventricular velocity allows us to assess how exposure may affect ventricular elasticity, which is required for proficient filling and a key determinant of cardiac output (Tota et al., 1983; Usui et al., 2022; Y. Wang et al., 2018). Significant increases may be indicative of ventricular compliance, or the ventricle's ability to expand and accommodate incoming blood during diastole, reflecting the ability for the fish heart to adapt to stressors (Gaasch et al., 1976). Characterization of stroke volume, which is the amount of blood ejected from the ventricle with each heartbeat, serves as an important marker of cardiac performance by allowing researchers to gauge the efficiency of the heart. By examining changes in stroke volume, one can determine if exposure influences the elasticity of the ventricle, of the ventricular compliance. Furthermore, ventricular and atrial contractile rates can be used to determine the AV ratio, which can be used to detect electrical abnormalities (Farrell & Jones, 1992; Johansen & Burggren, 1980). In a healthy fish, this ratio should be close to one, with AV ratios below indicating possible AV block, a condition where the electrical signal that controls the heartbeat is partially or completely blocked. There are three forms of AV block. In first-degree AV block, the mildest type, the electrical pulse can travel from the atrium to the ventricle, but at a slower rate than normal. Second-degree AV block, also referred to as Wenckebach's AV block, occurs when the ventricle occasionally skips a beat, and does not contract following the atrium (P wave occurs, but QRS complex does not). Third-degree AV block happens when the electrical conduction is completely blocked, causing the atrium and ventricle to beat independently of one another. As the most dangerous form of AV block, third-degree block often leads to cardiac arrest (Barold, 2018; Cicini et al., 2022; Lee et al., 2021). This study will also measure cardiac output, a measurement of the blood volume expelled from the heart per minute. Maintaining stable cardiac output is required to

keep adequate blood pressure to supply sufficient oxygenated blood to the body. Significant reductions in cardiac output are a clear indication of cardiac dysfunction (Rantin et al., 2020). In conclusion, a comprehensive assessment of ventricular filling dynamics, stroke volume, contractile rates, and cardiac output will provide a thorough understanding of how 6PPD-quinone exposure impacts the performance of the fish heart.

As previously discussed, the contraction of the heart is controlled by an electrical excitation. This occurs when an action potential is transmitted from the atrium, through the AV node, to the ventricle. The AV node slows this excitation, causing a slight pause between the contraction of each chamber. This delay is critical to proper cardiac function, as it allows time for the atrium to empty into the ventricle prior to its contraction (Farrell & Jones, 1992; Haverinen & Vornanen, 2014). Given that 6PPD-quinone is suspected to generate ROS and the AV node is particularly vulnerable to oxidative stress, examining the effects of exposure on the heart's electrical activity is critical for understanding how 6PPD-quinone causes toxicity (Anderson-Bain et al., 2023; Varshney et al., 2022). This can also be achieved using electrocardiography, which looks at changes in voltage over time to provide a detailed analysis of the passage of electrical current sequentially through the heart muscle (Duong et al., 2021; Zhao et al., 2019). To assess the effects of 6PPD-quinone exposure on the electrical activity of the heart, electrocardiography will be used to analyze changes in the duration of the PR and QT intervals in exposed fishes and compared to that of unexposed controls. Briefly, the duration of the PR interval is the length of time it takes for the excitation to travel from the atrium to the ventricle, whereas the duration of the QT interval is the length of time it takes for the ventricle to recover from excitation (Y. Zhang et al., 2011). Similar to the AV ratio, the PR interval can be used to determine the occurrence of a first-degree AV block (Cheng et al.,

2009). Changes in the length of the QT interval can be indicative of potassium channel function, which is critical to ventricular recovery (repolarization) following contraction (depolarization) (Moss, 2005). Ultimately, these findings will help determine whether 6PPD-quinone impacts cardiac electrical activity, offering insight into the specific factors contributing to the physiological impairments observed in affected fish.

The primary function of blood is to carry oxygen and nutrients to tissues. To assess potential effects of 6PPD-quinone on hematological function, changes in 19 blood gas parameters (see 2.3.9) were analyzed in exposed fishes and compared with those in unexposed controls. Changes in certain parameters, such as total hemoglobin and methemoglobin, can have drastic effects on the oxygen-carrying capacity of the blood. Hemoglobin, a protein in red blood cells, binds oxygen through four iron-containing heme groups that are typically in the ferrous (Fe^{2+}) state where they can easily bind and release oxygen. When one of these groups is oxidized to the ferric state (Fe^{3+}), methemoglobin is formed, which has a decreased ability to bind oxygen (Ludlow et al., 2022; Soldatov, 2021a). Due to their strong oxidative properties, quinones such as 6PPD-quinone may promote hemoglobin oxidation, elevating methemoglobin levels and impairing tissue oxygenation (Devi & Mehendale, 2014). Blood gas analysis can also be used to detect changes in blood electrolytes, which are crucial for generating and conducting electrical impulses that control cardiac activity (Randall, 1970). For example, hypokalemia (low blood potassium levels) can prolong the QT interval, delaying ventricular repolarization (Widimsky, 2008). Therefore, significant shifts in these blood gas parameters could contribute to the observed cardiorespiratory effects in sensitive species following acute 6PPD-quinone exposure.

1.5.3 Swim performance

Behaviors such as migration, predator avoidance, prey capture, reproduction, and habitat selection depend on swim performance in fishes, deeming it a main determinant of survival. Swimming performance can be analyzed by measuring three types of swimming speed: sustained, prolonged, and burst (Plaut, 2001a). Sustained swimming speeds are those that can be maintained for longer than 200 minutes, burst swimming speeds are those that can be maintained for less than 20 seconds, and prolonged swimming speeds cover the range in between (Beamish, 1978; Hoar & Randall, 1978). Energy used for sustained and prolonged swimming is derived from aerobic respiration, while burst swimming relies on anaerobic processes (Hoar & Randall, 1978; Peake, 2004). In the second chapter of my thesis research, the sublethal effects of acute 6PPD-quinone exposure on prolonged swimming speed were determined in exposed rainbow trout and analyzed in comparison to unexposed controls. Prolonged swimming performance was measured by determining critical swimming speed (U_{crit}). This was conducted by placing individuals into a swim tunnel, which challenges fish with incremental changes in water velocity until they reach exhaustion. To normalize for body length's influence on swimming speed, U_{crit} was corrected for total body length and expressed in body lengths per second (BL/sec) (Brett, 1965). In combination with AMR, the energetic cost of transport (CoT; the amount of energy required to move a given distance) was also analyzed (see 3.3.4).

1.5.4 Triglyceride and glycogen stores

Glycogen and triacylglycerols (triglycerides) are the two major forms of energy storage in fishes (Johnson et al., 2004). When glycogen stores have reached capacity, glucose is converted into triglycerides and stored in skeletal muscle, liver, or adipose tissue. Exposure to

toxicants has been shown to alter energy storage, which can impact behaviours such as swimming, reproduction, and growth (Mi et al., 2021; Öner et al., 2008). Triglycerides, a type of lipid, are broken down *via* β-oxidation to produce energy (Schulz, 1991). This process mainly occurs in the liver, which is crucial for lipid homeostasis (Alves-Bezerra & Cohen, 2017). However, in highly active fish, significant β-oxidation also occurs in skeletal muscle (Stubhaug et al., 2005). Slow oxidative red skeletal muscle fibers utilize triglycerides as the primary fuel source through aerobic metabolism for sustained and prolonged swimming speeds. In contrast, fast glycolytic white skeletal muscle fibers rely on glycogen as the main fuel for burst swimming, obtained through anaerobic metabolism (Hammer, 1995). Triglyceride and glycogen concentrations was determined in muscle collected from lake trout following a 20-h exposure to 6PPD-quinone and subsequent critical swimming challenge. These endpoints were measured in exposed fishes and compared to those of unexposed controls to determine if 6PPD-quinone exposure alters the storage or mobilization of these energy stores.

1.6 Purpose of Research

Due to the pervasive use of tires and rubber products, 6PPD-quinone is likely to be present at detectable levels in aquatic environments worldwide. Existing research has mainly concentrated on acute lethality, creating a paucity in research aimed at assessing sublethal responses that may occur at lower concentrations. Acutely sensitive species show characteristic symptoms that imply an impact on cardiorespiratory and metabolic physiology, highlighting the need for further investigation. Therefore, the purpose of my research is to further investigate mechanisms of 6PPD-quinone toxicity in salmonids of differing sensitivities,

specifically the underlying metabolic and cardiovascular responses of acute exposure in juvenile rainbow trout, Arctic char, and lake trout.

1.6.1 Objectives

1. To examine the effects of 6PPD-quinone exposure on cardiovascular and metabolic processes in juvenile rainbow trout and Arctic char.
2. To examine the effects of 6PPD-quinone exposure on the swim performance of juvenile lake trout.

1.6.2 Hypotheses

1. H_0: Exposure to 6PPD-quinone will have no effect on cardiovascular function or energy homeostasis in juvenile rainbow trout and Arctic char.
2. H_0: Exposure to 6PPD-quinone will have no effect on swim performance in juvenile lake trout.

CHAPTER 2

2.0 ACUTE CARDIORESPIRATORY EFFECTS OF 6PPD-QUINONE ON JUVENILE RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* AND ARCTIC CHAR *(SALVELINUS ALPINUS)*

Preface

The purpose of the research in this chapter was to further expand the understanding of the sublethal effects of 6PPD-quinone exposure in juvenile salmonids. This was done by using respirometry, cardiovascular ultrasound, electrocardiography, and blood gas analysis to investigate the underlying cardiovascular and metabolic responses of 6PPD-quinone exposure in juvenile rainbow trout and Arctic char. Oxygen consumption, standard metabolic rate, atrial and ventricular heart rate, AV ratio, passive ventricular filling, end diastolic and systolic volume, stroke volume, ejection fraction, cardiac output PR and QT interval length, and 19 blood gas parameters were determined to investigate mechanisms by which 6PPD-quinone exerts toxicity differs among sensitive and tolerant species. The findings of this study suggest that environmentally relevant exposure to 6PPD-quinone results in altered cardiometabolic physiology, primarily in the sensitive species, rainbow trout.

This chapter has been submitted to the journal Aquatic Toxicology. The anticipated citation is:

Selinger, S. J., Montgomery, D., Wiseman, S., Hecker, M., Weber, L., Brinkmann, M., Janz, D., 2025. Acute Cardiorespiratory Effects of 6PPD-Quinone on Juvenile Rainbow Trout (*Oncorhynchus mykiss*) and Arctic Char (*Salvelinus alpinus*). (In preparation).

The author contributions to chapter 2 of this thesis were as follows:

Summer J. Selinger (University of Saskatchewan) collected, processed, and analyzed all samples, performed all statistical analyses, and drafted the manuscript.

David Montgomery (University of Saskatchewan) performed ultrahigh-performance liquid chromatography (UHPLC) to analyze water samples for 6PPD-quinone concentrations.

Steve Wiseman (University of Lethbridge) reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Markus Hecker (University of Saskatchewan) reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Lynn Weber (University of Saskatchewan) provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

David M. Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Markus Brinkmann (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

2.1 Abstract

N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is an environmental transformation product of the widely used rubber tire antioxidant, 6PPD. Found in stormwater runoff, 6PPD-quinone has been reported to cause acute lethality at ≤1 μg/L in salmonids like coho salmon, rainbow trout, and brook trout. Conversely, other species such as Arctic char and brown trout are insensitive, even when exposed to significantly greater concentrations (3.8-50 μg/L). Sensitive species exhibit symptoms such as gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. This study investigated sublethal 6PPD-quinone toxicities, focusing on cardiovascular and metabolic effects in two salmonids of varying sensitivity: a sensitive species, rainbow trout (*Oncorhynchus mykiss)* and a tolerant species, Arctic char (*Salvelinus alpinus*). Fish were exposed to measured concentrations of 0.59 or 7.15 μg/L 6PPD-quinone, respectively, in respirometry chambers for 48 h to assess temporal changes in resting oxygen consumption compared to unexposed controls. Following exposure, cardiac ultrasound and electrocardiography characterized cardiac function *in vivo,* while blood gas analysis examined blood composition changes. In both species, changes in resting oxygen consumption were observed. In rainbow trout only, a decrease in end systolic volume and an increase in passive ventricular filling, cardiac output, and PR interval length were observed, indicating cardiac stimulation. Cardiorespiratory symptoms observed following rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in an impaired ability to oxygenate tissues. This study is the first to examine the effects of 6PPD-quinone exposure on the cardiorespiratory system of salmonid fishes and provides information invaluable to a better understanding of the mechanism of 6PPD-quinone toxicity.

2.2 Introduction

Recently, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has received considerable attention, particularly for its ability to induce lethality in a variety of salmonid species, such as coho salmon *(Oncorhynchus kisutch)*, at alarmingly low concentrations (24-h LC_{50} = 95 ng/L) (Tian et al., 2022). Since the 1990s, the migration of adult coho salmon from marine to freshwater environments in the Pacific Northwest during spawn was marked by a noteworthy phenomenon: a widespread occurrence of mass mortality (40-90%) during rain events, commonly referred to as urban runoff mortality syndrome (URMS) (Chow et al., 2019; Myers et al., 2011). However, the primary compound responsible for this widespread mortality, 6PPD-quinone, was not identified until recently (Tian et al., 2021). This discovery suggests that 6PPD-quinone is among the most potent chemicals known to cause acute lethality to aquatic life, second only to the organophosphate pesticide parathion (Sanders, 1972; Tian et al., 2022).

6PPD-quinone is an environmental transformation product of 6PPD, a protective additive used in rubber tires to prevent or slow damage caused by ozone. This compound enters the environment through the production of tire wear particles (TWPs), which originate from the friction between tires and the road surface. TWPs are subsequently washed into aquatic environments through road runoff following rain and storm events, causing notable elevations in the concentration of 6PPD-quinone in surface waters (Peter et al., 2018; Werbowski et al., 2021). As more than 2.5 billion tires are produced around the globe annually, each containing around 0.4-2% 6PPD by mass, 6PPD-quinone is predicted to exist at detectable concentrations in aquatic environments globally (Czarna-Juszkiewicz et al., 2023; Krüger et al., 2005; Tian et al., 2021).

The adverse effects of 6PPD-quinone have been studied in a variety of species including aquatic invertebrates, freshwater crustaceans, fish, fish larvae, mice, plants, and humans (Castan et al., 2023; Fang et al., 2023; Hiki et al., 2021; Prosser et al., 2023; Varshney et al., 2022; Z. Zhang et al., 2024). However, the primary focus remains on salmonids, the only taxa of fishes for which acute lethality has been documented thus far. Interestingly, among the salmonid species that have been studied to date, a distinct species-specific sensitivity has been observed. In addition to coho salmon, 6PPD-quinone has been reported to cause acute lethality in salmonids such as rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush)*, brook trout (*Salvelinus fontinalis*), and white-spotted char (*Salvelinus leucomaenis pluvius*), at very low concentrations (72-h LC_{50} = 1.00 μg/L; 96-h LC_{50} = 0.50 μg/L; 24 h LC_{50} = 0.59 and 0.51 μg/L, respectively) (Brinkmann et al., 2022; Hiki & Yamamoto, 2022b; Roberts et al., 2025). Conversely, salmonid species such as Arctic char *(Salvelinus alpinus*), westslope cutthroat trout *(Oncorhynchus clarkii lewisi*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), Dolly Varden trout *(Salvelinus curilus*), masu salmon (*Oncorhynchus masou masou*), sockeye salmon *(Oncorhynchus nerka)*, and pink salmon (*Oncorhynchus gorbuscha*) were tolerant to exposure, meaning that no adverse effects were observed, even when exposed to significantly higher concentrations (3.8-50 μg/L) (Brinkmann et al., 2022; Foldvik et al., 2022, 2024; French et al., 2022; Greer et al., 2023; Hiki & Yamamoto, 2022b; Montgomery et al., 2023). The exact cause for this species-specific sensitivity is currently unknown, yet it is evident that phylogenetic proximity is not a dependable predictor of toxicity. Although this has been previously acknowledged, there has been limited effort to understand the underlying cause of these sensitivity differences (Khan et al., 2024; Montgomery et al., 2023). In addition,

despite considerable research on the acute toxicity of 6PPD-quinone, there is a paucity of studies focused on assessing sublethal responses that may occur at lower concentrations.

Prior to death, sensitive species show distinctive symptoms including increased ventilation, gasping, spiraling, and loss of equilibrium, suggesting a possible impact on cardiometabolic physiology (Brinkmann et al., 2022; Lo et al., 2023). Previous studies have shown that 6PPD-quinone exposure is able to cause metabolic disruption, both *in vivo* and *in vitro*. For example, increased oxygen consumption was observed in both zebrafish (*Danio rerio)* larvae and rainbow trout gill cells (RTgill-W1) (Mahoney et al., 2022; Varshney et al., 2022). In addition, multiple studies have reported chronotropic effects on the hearts of exposed zebrafish larvae (Ricarte et al., 2023; Varshney et al., 2022; Zhang et al., 2023). Cardiovascular variables such as heart rate and cardiac output are closely linked with metabolic rate, enabling the heart function to meet rising oxygen demands (Brodeur et al., 2001). However, despite evidence of both cardiovascular and metabolic toxicity, there have been no studies conducted to date that have characterized these responses in salmonid fishes.

The overall goal of this study was to further investigate the mechanisms of 6PPD-quinone toxicity to salmonids of differing sensitivity, in an effort to understand why related species exhibit varying responses to exposure. This was achieved by analysing cardiovascular and metabolic responses in juvenile individuals of two salmonid species: a tolerant species, Arctic char, and a sensitive species, rainbow trout. Contrary to previous studies, which focused primarily on acute lethality, this study aimed to assess acute sublethal effects that occur at lower concentrations. Intermittent-flow respirometry was used to characterize aerobic metabolism. Doppler and brightness mode (B-mode) ultrasonography and electrocardiography (ECG) were used to determine cardiac and electrical function *in vivo*. Blood gas analysis was

used to evaluate blood parameters associated with gas exchange dynamics and acid-base equilibrium.

2.3 Materials and Methods

2.3.1 Test compound and stock preparation

Native and mass-labeled (d_5) 6PPD-quinone (≥ 97% purities) were purchased from Toronto Research Chemicals (Toronto, CA). Analytical standard solutions of native and mass-labeled 6PPD-quinone were prepared in HPLC-grade methanol. 2.4 and 24 mg dry mass of 6PPD-quinone were weighed and dissolved in 10 mL dimethyl sulfoxide (DMSO) via sonication (15 minutes). This stock was further diluted by addition of 50 mL DMSO to achieve a nominal stock concentration of 40 and 400 mg/L for rainbow trout and Arctic char, respectively. Stock solutions for exposure of fish to 6PPD-quinone were prepared using dimethyl sulfoxide (DMSO) to achieve a final solvent concentration of 0.0025% (v/v) during exposures. Stocks were then stored in amber glass vials at 4°C until exposure.

2.3.2 Test species

Arctic char from Miracle Springs Inc. (North Vancouver, CA) were raised from embryos in the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan. Once they reached the juvenile stage, fish were fed with a commercial fish feed at a daily rate of 1% body weight/day. For each trial, four fish of similar weight and length were randomly selected from the larger population and moved into a separate 500-L tank, where they were fasted for 48 h prior to respirometry trials. Rainbow trout were purchased from Lyndon Hatcheries (New Dundee, CA) as eyed embryos and also raised in the ATRF. Trout were fed size-appropriate commercial fish feed at a daily ration of 3% body weight until they reached the juvenile stage, when they were switched to a maintenance ration of 1% body

weight/day to maintain size between trials. Both species were kept under continuous flow conditions in facility water with controlled temperature (12.0 ± 1.0°C) and photoperiod (14 h light: 10 h dark), in the culture tank and while fasting. Experiments were approved by the University of Saskatchewan Animal Care Committee (Protocol 20070049) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

2.3.3 Aquatic exposure

Due to logistical constraints, exposures were completed in a series of trials. Each trial consisted of two fish per treatment group (DMSO control and 6PPD-quinone). Ten trials were completed for rainbow trout, resulting in a sample size of 20 fish for each treatment group, and nine trials were completed for Arctic char, resulting in a sample size of 18 fish for each treatment group. Following a 48-h fasting period, individual fish were placed into 1-L respirometry chambers (Loligo Systems, Viborg, DK) for 72 h to measure temporal changes in standard metabolic rate (SMR), which is the minimal maintenance metabolic rate of resting, unfed fish, using fiber optic oxygen sensors. Respirometry chambers were held within 50-L tanks, which were visually shielded from external disturbance and supplied with water from a 700-L glass-fiber Min-o-Cool tank containing 400 L of test solution. The first 24 h was an acclimation period that allowed the collection of a baseline oxygen consumption rate for each individual. Following the acclimation period, fish were exposed to their respective 6PPD-quinone concentrations under static renewal conditions (rainbow trout: nominal 1.00 µg/L, measured 0.59 µg/L; Arctic char: nominal 10.0 µg/L, measured 7.15 µg/L) and changes in oxygen consumption rate (MO_2; mg O_2/kg/h) were measured over a 48-h exposure period. Control chambers received the DMSO solvent at the same level as those exposed to 6PPD-quinone [0.0025% (v/v)]. At the 24-h point of each trial, 75% water renewal was performed to

maintain water quality and a consistent exposure concentration. Following the 48-h exposure, all four fish were anesthetized with metomidate hydrochloride (Aquacalm™; Western Chemical Inc., Ferndale, USA) and assessed using cardiac ultrasound, ECG, and blood gas analysis (detailed procedures are outlined below). Blood samples were obtained from the caudal vein using 2 mL 80 IU electrolyte-balanced heparin arterial blood sampler syringes (Radiometer Medical, Bronshoj, DK) with 20 G needles. Fish were then euthanized using an overdose of buffered tricaine methane sulfonate (MS-222; 1 g/L) followed by spinal severance. Following euthanasia, morphometric data were obtained for each individual, including the standard length, fork length, total length, total body weight, and liver weight.

2.3.4 Analytical chemistry

Water samples were collected for analytical confirmation of 6PPD-quinone concentrations at various points throughout the exposure period. This included samples before and after the initial dosing, before and after the 24-h water change, as well as a final sample prior to the removal of fish from respirometry chambers. Samples were taken ~45 minutes after 6PPD-quinone was introduced. Samples were immediately spiked at 50 µg/L with 6PPD-quinone-d_5 and stored at -20°C until they were analyzed. Analysis was completed using a Vanquish UHPLC instrument coupled with a Q-Exactive HF Quadrupole-Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) based on methods previously described by Brinkmann et al. (2022). An isotope dilution strategy using 6PPD-quinone-d_5 was applied for quantification.

2.3.5 Intermittent-flow respirometry

Intermittent-flow respirometry, the most commonly used method for measuring rates of oxygen consumption in gill-breathing organisms, was performed in order to assess the

effects of 6PPD-quinone exposure on aerobic metabolism (Svendsen et al., 2016). This approach involves extended measurement intervals within a sealed respirometer, followed by brief flushing periods to ensure thorough exchange of water within the chamber. Respirometry was conducted utilizing a 1-L horizontal acrylic respirometry chamber, NETIO and Witrox 4 acquisition devices, in combination with AutoResp 2.3.0 software (Loligo Systems). For the duration of the experiment, fish were kept at the same temperature and photoperiod as during culture and fasting. Oxygen consumption was recorded over a 10.5-minute cycle that included a 420-s closed measuring period, followed by a wait period of 30-s, and a flush period of 180-s. These cycle times ensured that dissolved oxygen levels in the chamber remained above 80% for the duration of the experiment. Air stones were placed into the ambient tanks to maintain dissolved oxygen concentration (≥ 97%), and circulating pumps were placed within the reservoir tank to ensure adequate flow. The total respirometric volume was calculated by combining the chamber volume (1 L), the volume of the recirculating loop (78.9 mL) and subtracting fish volume (estimated from wet weight). Ratios (unitless) between the respirometric volume and the wet weight volume, which should be between 10 and 20 for measurements in resting animals, ranged between 10.1 and 14.2 for rainbow trout and 10.1 and 18.2 for Arctic char.

2.3.6 Cardiac ultrasound

Ultrahigh resolution B-mode and pulse-wave Doppler ultrasonography were used to assess changes in cardiac structure and function of rainbow trout and Arctic char. This non-invasive method was completed using a VEVO 3100 high frequency ultrasound machine (VisualSonics, Markham, CA) according to methods previously published by our laboratory (Gerger et al., 2015; Pettem et al., 2017). The average duration of anesthesia, from initiation to

the conclusion of ultrasonography, was approximately 15 minutes. Following the induction of anesthesia, fish were placed ventral side up into a foam insert, submerged in a 5.5-L bucket supplied with continuous flow of clean aerated water under light anesthesia (10 mg/L) of Aquacalm™. Temperature was maintained at 12.5 ± 0.1°C and aerated using a chilled recirculating water bath (Isotemp 250, Thermo Fisher Scientific Inc., Waltham, USA). A stabilization period of approximately 10 minutes was provided to allow any mild cardiodepressive effects of Aquacalm™, which stabilize within 5-10 minutes, to subside and for cardiac function to reach steady-state conditions. A handheld MX400 transducer was then used to emit 20-46 MHz pulses, producing real-time imaging within the body cavity at a resolution of 30 μm. B-mode ultrasonography was used to quantify the outer volume of the ventricle at its largest (diastole) and smallest (systole) states using Simpson's rule of discs (Equation 1) (Mercier et al., 1982). Using VEVO LAB analysis software 5.7.1 (VisualSonics, Markham, Canada), the short axis view was divided into three ventricular discs, and the area of each was determined (A_1, A_2 and A_3). These values were then multiplied by the height of each disc (⅓ of the ventricular length) to estimate the end systolic volume (ESV) and end diastolic volume (EDV). A minimum of 3 images for each view at each stage were averaged per individual.

$$(1)\ V = (A_1 + A_2)h + \left(\frac{A_3 h}{2}\right) + \left(\frac{\pi}{6(h^3)}\right)$$

The difference between ESV and EDV was calculated to give the ventricular stroke volume (V_S), which is a measurement of the volume of blood ejected from the heart during each beat (Equation 2). To facilitate comparison between individuals of different sizes, these volume measurements (V_S, EDV, ESV) were corrected for individual fish body weight.

$$(2)\ V_S = EDV - ESV$$

The stroke volume and end diastolic volume were used to determine the ejection fraction (EF), a measure of the percent (%) volume of blood ejected from the heart with each beat (Equation 3).

$$(3)\ EF = 100 \times (V_S / EDV)$$

Pulse-wave Doppler ultrasonography was used to measure the passive ventricular velocity (PVF), a measure of the speed of blood traveling through the atrioventricular (AV) valve, as well as in the determination of atrial (atrial f_H) and ventricular (ventricular f_H) contractile rates. Contraction rates were calculated by counting individual beats in 15 s video loops, and then converting to beats per minute (BPM).

Atrial contractile rates were divided by ventricular contractile rates to determine the AV ratio for each individual, allowing us to assess how effectively the atrial excitation travels through the AV node to stimulate ventricular contraction, and diagnose cardiac issues such as AV block (Equation 4). In optimal physiological conditions, this ratio should be equal to 1.0.

$$(4)\ \text{AV Ratio} = \text{Atrial } f_H / \text{Ventricular } f_H$$

Coupling the information determined using B-mode and pulse-wave Doppler ultrasonography allowed us to determine the cardiac output (CO), a measure of the volume of blood pumped from the heart per minute (Equation 5). Cardiac output data were also corrected for individual fish body weight.

$$(5)\ CO = \text{Ventricular } f_H \times V_S$$

2.3.7 Electrocardiography

ECG was used to assess changes in the electrical activity of rainbow trout and Arctic char hearts *in vivo*. Immediately following cardiac ultrasound, an ECG measurement was taken in anaesthetized individuals to assess cardiac function using the PowerLab 26T system and

LabChart7Pro software (ADInstruments Inc., Colorado Springs, USA). This technique was adapted from a rodent model to fish, using methods previously published by our laboratory (Wildemann et al., 2014). For ECG analyses, ten stable readings were used in analyses from the ECG at 30-s intervals. The parameters of interest (PR interval duration and QT interval duration) were measured in at least ten cardiac cycles and averaged to obtain a value for each fish.

2.3.8 Blood gas and biochemical analysis

Whole blood samples were analyzed to characterize changes in 19 blood gas parameters. This involved acid-base measurements including pH, partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), bicarbonate (HCO_3), base excess (BE(B)), and the concentration of CO_2 in plasma ($ctCO_2$), and pulse CO-oximetry measurements including total blood hemoglobin (tHb), the fraction of oxygenated hemoglobin (FO_2Hb), and the fraction of deoxyhemoglobin (FHHb), as well as the fraction of dyshemoglobins such as carboxyhemoglobin (FCOHb) and methemoglobin (FMetHb). Electrolytes such as sodium (Na^+), potassium (K^+), calcium (Ca^{+2}) and chloride (Cl^-) were also determined, as well as the anion gap (AnGap) and osmolality (mOsm). Metabolites such as glucose (Glu) and lactate (Lac) were also measured. Blood samples were obtained from the caudal vein using 2-mL 80 IU electrolyte-balanced heparin arterial blood sampler syringes (Radiometer Medical, Bronshoj, DK) with 20 G needles. Analysis of blood was completed within five minutes of sampling, using a RAPIDPoint 500 Blood Gas Analyzer (Siemens Healthcare Limited, Oakville, CA).

2.3.9 Statistical analysis

Oxygen consumption (MO_2) data with a corresponding R^2 less than 0.95 were trimmed from both the acclimation and exposure period. Outliers were identified and excluded using the robust regression and outlier removal (ROUT) method available in GraphPad Prism 9.5.1 (San Diego, USA). This method fits a robust regression model to the data, minimizing the influence of outliers, and then applies the false discovery rate to identify outliers based on their distance from the model's predictions, with the maximum false discovery rate set to 1%. Oxygen consumption data obtained during the acclimation period were analyzed to determine a baseline consumption rate for each individual. Exposure data are presented as a percentage (mean ± standard deviation) of the SMR determined for each individual during acclimation. Exposure data were analyzed to determine periods of activity (higher oxygen consumption) and periods of inactivity (lower oxygen consumption) and averaged among these periods to determine oxygen consumption rates for each individual. Data were analyzed using a paired two-tailed t-test to look for differences between overall consumption, consumption during the active period, and consumption during the inactive period.

Data obtained using cardiac ultrasound, electrocardiography, and blood gas analysis were tested for normality using the Shapiro-Wilk test and homogeneity of variance with the F test using GraphPad Prism 9.5.1 (San Diego, CA, USA). A two-tailed t-test was used to determine significant differences between control and treated groups. An alpha value of 0.05 was used in all statistical tests to determine statistical significance. Data are presented as mean ± standard error of the mean (SEM).

2.4 Results

2.4.1 Analytical chemistry

Mean concentrations of 6PPD-quinone measured over the exposure periods deviated <58% from nominal values in Arctic char, and <63% in rainbow trout (Table 2.1). There was a mean loss of 56% and 52% of the test chemical over the 24 h window between water changes in Arctic char and rainbow trout, respectively. 6PPD-quinone was not detected in control groups at any point throughout the exposure, in either species. Mean concentrations in treatment groups (nominal concentrations 10 and 1 μg/L) were 7.15 ± 3.41 and 0.59 ± 0.28 μg/L in Arctic char and rainbow trout, respectively.

Table 2.1. Nominal versus measured concentrations of 6PPD-quinone during exposure experiments with Arctic char and rainbow trout as determined using LC-HRMS. Concentrations were measured ~45 minutes after dosing, before and after renewal at 24 h, and at 48 h prior to removal of fish from respirometry chambers.

Measured concentration (μg/L)					
Nominal concentration	0 h	24 h		48 h	Average during exposure
		Before	After		
Arctic char					
CTRL	ND	ND	ND	ND	ND
10 μg/L	10.88 ± 2.13	4.40 ± 1.54	9.12 ± 2.21	4.20 ± 0.90	7.15 ± 3.41
Rainbow trout					
CTRL	ND	ND	ND	ND	ND
1 μg/L	0.93 ± 0.17	0.37 ± 0.07	0.70 ± 0.20	0.38 ± 0.09	0.59 ± 0.28

Data are presented as mean ± SD. *ND: < LOD.*

2.4.2 Fish morphometrics

In Arctic char, total fish length across control and 6PPD-quinone treated groups was 20.6 ± 0.69 cm and body weight of fish was 74.8 ± 4.37 g. In rainbow trout, total fish length in control and 6PPD-quinone treated groups was 19.8 ± 0.28 cm and body weight of fish was 81.1 ± 3.16 g. Liver weight for control and 6PPD-quinone exposed Arctic char was 0.733 ± 0.043 g and 0.843 ± 0.03 g in rainbow trout. There were no statistically significant differences in any of the morphometric measures between exposed and control fish in either species (Table 2.2).

Table 2.2. Total length, body weight, and liver weight of juvenile Arctic char and rainbow trout following exposure to 6PPD-quinone or solvent control for 48 h.

Treatment	Total length (cm)	Body weight (g)	Liver weight (g)
Arctic char			
CTRL	21.3 ± 1.28	75.2 ± 6.65	0.740 ± 0.065
7.15 µg/L	20.0 ± 0.392	74.5 ± 5.83	0.726 ± 0.055
Rainbow trout			
CTRL	19.9 ± 0.394	81.0 ± 4.49	0.828 ± 0.050
0.59 µg/L	19.7 ± 0.408	81.2 ± 4.56	0.857 ± 0.048

Data are presented as mean ± SEM.

2.4.3 Intermittent-flow respirometry

6PPD-quinone had a measurable effect on SMR in both Arctic char and rainbow trout (Figure 2.1; Table 2.3). To minimize effects related to metabolic variation across individual fishes, data are presented as a percentage of the baseline SMR determined for each fish during the 24 h acclimation period using a method described by Chabot (2016a). The baseline SMR was 62.2 ± 14.2 and 87.2 ± 24.1 mg O_2/kg/h in Arctic char and rainbow trout, respectively. In Arctic char, oxygen consumption was significantly greater ($p \leq 0.0001$) in 6PPD-quinone

exposed fish (105.4 ± 7.5%) than control fish (101.8 ± 7.6%). Similarly, treated rainbow trout (100.7 ± 5.87%) exhibited a significant increase ($p \leq 0.01$) from the baseline SMR measured during the acclimation period, when compared to that of control fish (99.17 ± 9.33%). Oxygen consumption rates were highly variable among fish when first placed into respirometry chambers, but stabilized overnight, suggesting that the ~24 h acclimation phase was sufficient to achieve resting state. In both species, higher MO_2 values were observed during periods of activity that typically correlated with the beginning of the dark period (Figure 2.1). Thus, data were also analyzed for differences during the active period (5-14 and 27-36 h) and the inactive period (1-5, 14-27, and 36-48 h). In short, resting oxygen consumption was significantly greater in 6PPD-quinone treated Arctic char overall ($p \leq 0.0001$), during the active period ($p \leq 0.001$), and during the inactive period ($p \leq 0.0001$; Table 2.3). In contrast, no activity-dependent effects were observed in rainbow trout, but an overall increase in resting oxygen consumption was also observed ($p \leq 0.01$; Table 2.3).

Table 2.3. Standard metabolic rate in juvenile Arctic char and rainbow trout during 48 h exposure to 6PPD-quinone or solvent control.

Treatment	Active Period	Inactive Period	Overall
Arctic char			
CTRL	104.9 ± 13.370	99.36 ± 5.666	101.8 ± 7.580
7.15 µg/L	110.4 ± 8.676***	102.4 ± 4.589****	105.4 ± 7.504****
Rainbow trout			
CTRL	100.4 ± 8.878	98.41 ± 9.538	99.17 ± 9.330
0.59 µg/L	101.8 ± 5.659	100.1 ± 5.916	100.7 ± 5.867**

**$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$ compared to control using t-test. Data are presented as a percentage (mean ± SD of n = 17-20 fish) of the baseline SMR determined for each individual during a 24 h acclimation period.

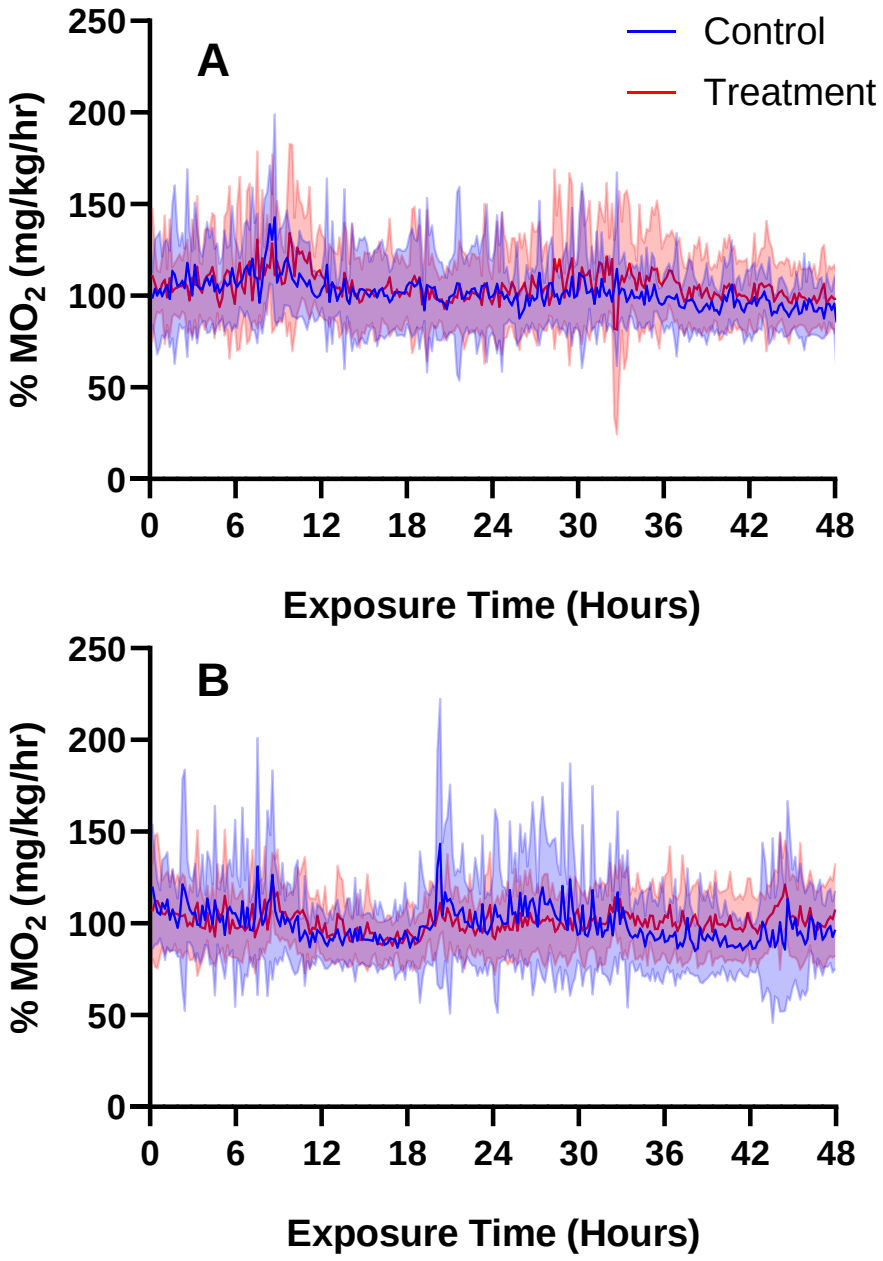

Figure 2.1. Changes in standard metabolic rate (SMR; the minimal maintenance MO_2 of unfed fish) in juvenile salmonids exposed to 6PPD-quinone (0.59 or 7.15 µg/L) or a solvent control (SC) for 48 h. A, Arctic char and B, rainbow trout. Data are presented as a percentage (mean ± SD of *n* = 17-20 fish) of the baseline SMR determined for each individual during a 24 h acclimation period.

2.4.4 Cardiovascular ultrasound

6PPD-quinone exposure resulted in significantly altered cardiac function in juvenile rainbow trout (Figure 2.2E-H; Table 2.5). In contrast, no significant effects were observed in Arctic char (Figure 2.2A-D; Table 2.4). Although no statistically significant effect was observed on V_S or EF (Table 2.5), CO was significantly increased ($p \leq 0.05$) in rainbow trout exposed to 6PPD-quinone (48.7 ± 3.87 μL/g/min; mean ± SEM) when compared to control (35.3 ± 2.63 μL/g/min; Figure 2.2H). Exposure did not result in a change in atrial f_H, ventricular f_H, or AV ratio in either species (Table 2.4, 2.5). In addition, ESV was significantly reduced ($p \leq 0.05$) in rainbow trout following exposure (0.812 ± 0.019 μL/g) compared to the control (0.916 ± 0.038 μL/g; Figure 2.2F), while EDV (Figure 2.2E) remained unchanged. Quantitative analyses of the pulse-wave Doppler sonograms showed that exposure had a marked influence on PVF. This is characterized as the velocity of blood flowing through the AV valve following active atrial contraction, or the passive flow from the atrium to ventricle during atrial diastole, which was significantly greater ($p \leq 0.0001$) in 6PPD-quinone exposed trout (173 ± 9.04 mm/sec) compared to the control (113 ± 5.34 mm/sec).

Table 2.4. Biometrics of cardiac function in juvenile Arctic char exposed to 6PPD-quinone or solvent control for 48 h.

	Control	**7.15 µg/L 6PPD-quinone**
Atrial heart rate (BPM)	51.5 ± 5.43	55.0 ± 6.77
Ventricular heart rate (BPM)	50.6 ± 5.29	52.5 ± 6.79
AV ratio	1.00 ± 0.0115	1.05 ± 0.0254
Stroke volume (µL/g)	1.38 ± 0.0769	1.21 ± 0.0603
Ejection fraction (%)	50.7 ± 1.83	50.5 ± 2.02

Data are expressed as mean ± SEM of n = 17-18 fish.

Table 2.5. Biometrics of cardiac function in juvenile rainbow trout exposed to 6PPD-quinone or solvent control for 48 h.

	Control	**0.59 µg/L 6PPD-quinone**
Atrial heart rate (BPM)	46.5 ± 3.67	55.5 ± 4.36
Ventricular heart rate (BPM)	47.3 ± 3.73	56.8 ± 4.24
AV ratio	0.983 ± 0.00933	1.00 ± 0.00509
Stroke volume (µL/g)	0.906 ± 0.0704	0.928 ± 0.0683
Ejection fraction (%)	49.2 ± 1.75	50.7 ± 1.67

*$p \leq 0.05$ compared to control using t-test. Data are expressed as mean ± SEM of n = 16-20 fish.

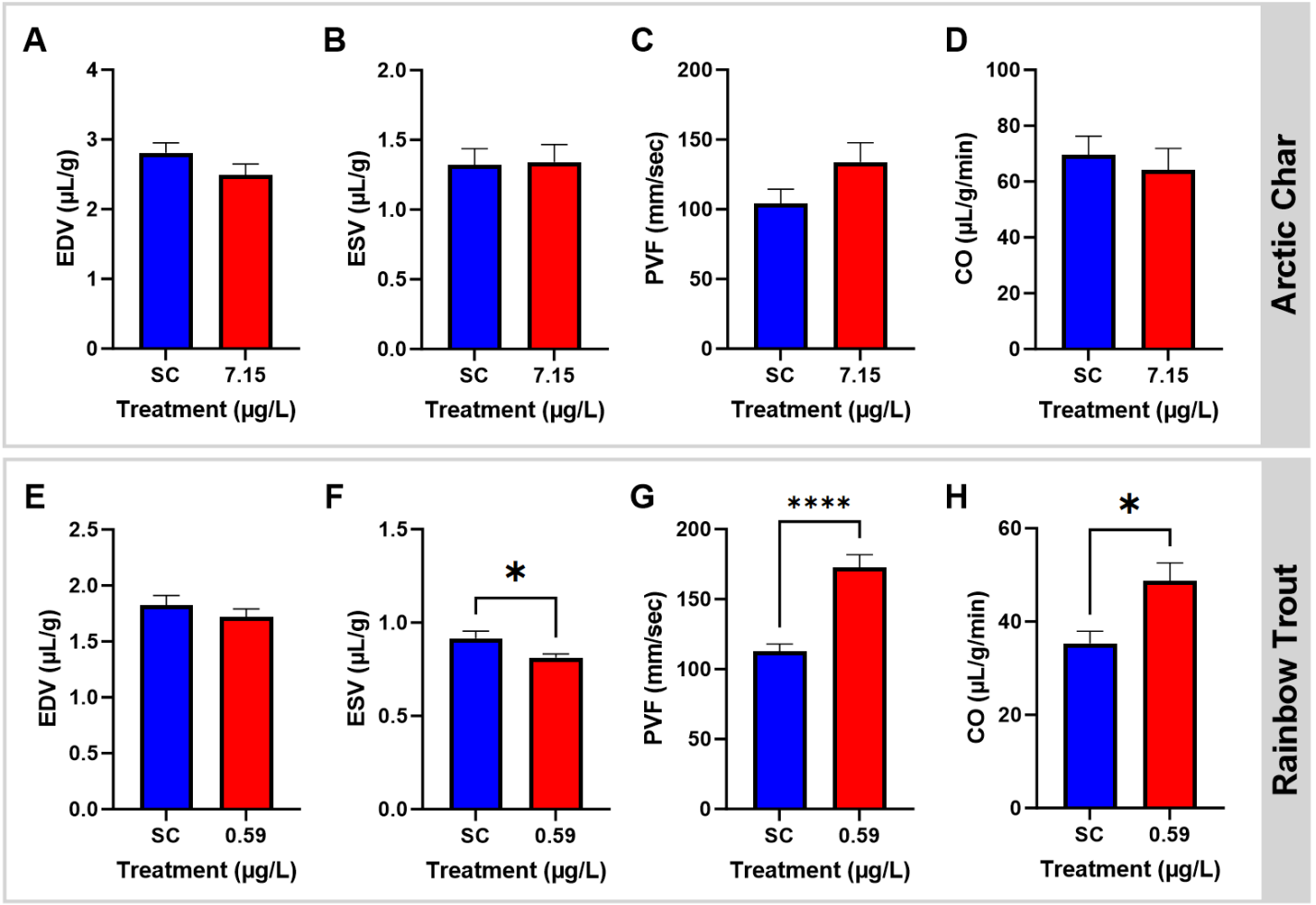

Figure 2.2. Cardiac function determined using cardiac ultrasound in Arctic char (A-D) and rainbow trout (E-H) exposed to 6PPD-quinone (0.59 or 7.15 µg/L) or a solvent control (SC) for 48 h. End diastolic volume (EDV; A, E), end systolic volume (ESV; B, F), passive ventricular filling (PVF) velocity measured as velocity of passive blood flow movement through the atrioventricular (AV) valve (C, G), and cardiac output (CO; D, H). Data are expressed as mean ± SEM of n = 17-20 fish. Significantly different from control group using t-test (*$p \leq 0.05$, ****$p \leq 0.0001$).

2.4.5 Electrocardiography

Exposure to 6PPD-quinone substantially altered the electrical function of the juvenile rainbow trout heart yet showed no statistically significant impact on Arctic char (Figure 2.3). After the 48-h exposure, a significantly prolonged PR interval ($p \leq 0.05$) was observed in exposed trout (0.155 ± 0.0042 seconds) compared to control (0.140 ± 0.0048 seconds; Figure 2.3G). However, the length of the QT interval was unchanged in either species (Figure 2.3D, H).

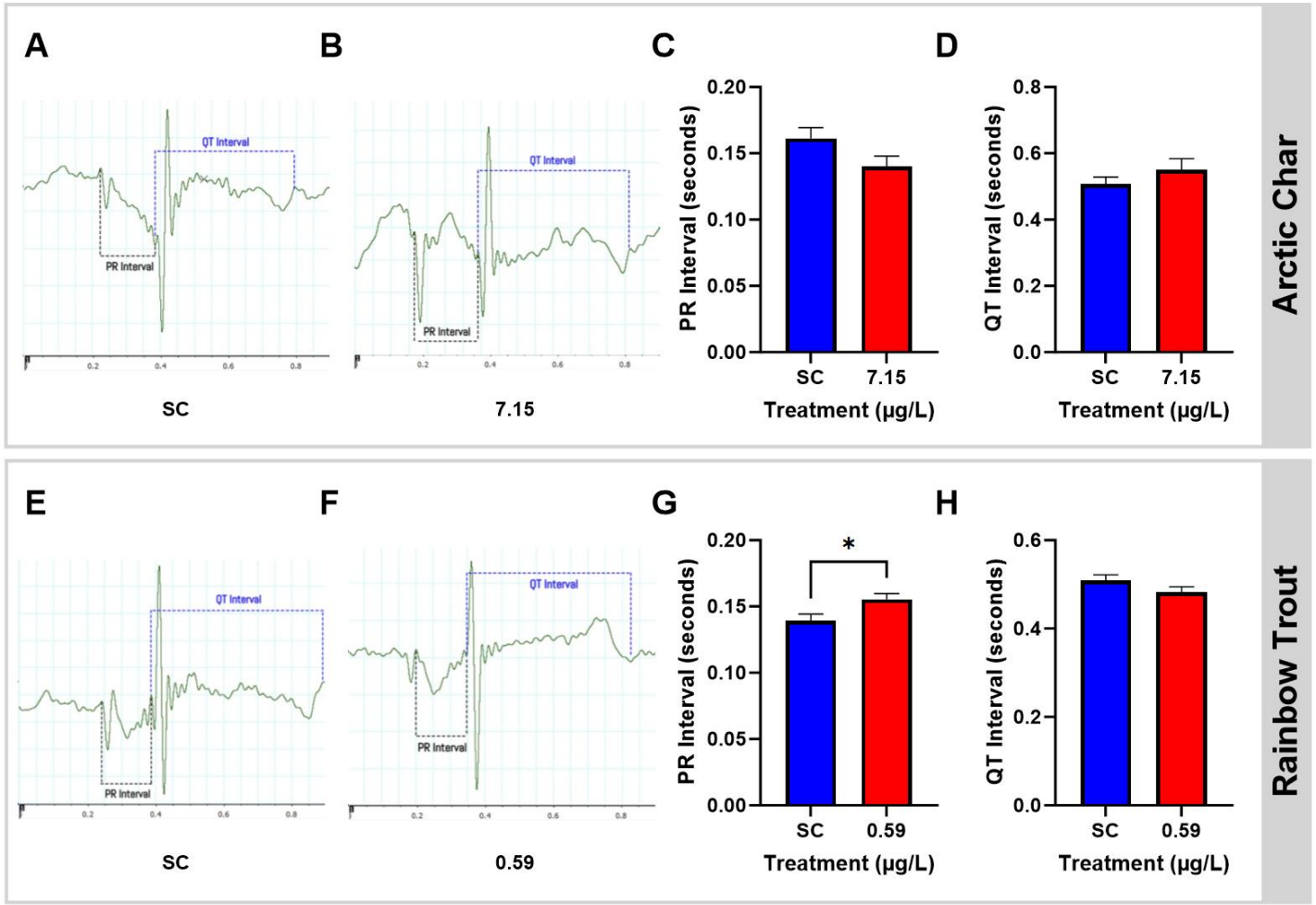

Figure 2.3. Cardiac function determined using electrocardiography in Arctic char (A-D) and rainbow trout (E-H) exposed to 6PPD-quinone (0.59 or 7.15 µg/L) or a solvent control (SC) for 48 h. PR interval length, the amount of time it takes for the excitation to travel from the atrium to the ventricle (C, G) and QT interval length, the amount of time it takes for the ventricle to recover from excitation (D, H). Data are expressed as mean ± SEM of n = 17-20 fish. Significantly different from control group using t-test (*$p \leq$ 0.05).

2.4.6 Blood gas analysis

Quantitative blood gas analyses revealed substantial changes in the composition of both rainbow trout and Arctic char blood following 6PPD-quinone exposure (Table 2.6, 2.7). Notable effects measured using CO-oximetry were observed only in rainbow trout (Figure 2.4E-H), such as a significant increase ($p \leq 0.05$) in *F*MetHb (2.02 ± 0.312%) when compared to the control (1.09 ± 0.329%; Figure 2.4G). Furthermore, trout exposed to 6PPD-quinone showed a significant decrease ($p \leq 0.05$) in FO_2Hb (9.63 ± 1.33%) and significant increase ($p \leq 0.05$) in *F*HHb (86.5 ± 1.57%) when compared to the control (16.4 ± 2.76 and 78.9 ± 2.93%, respectively; Figure 2.4E, F). In addition, no changes in *F*COHb were observed (Figure 2.4H).

Exposure to 6PPD-quinone led to alterations in electrolyte and metabolite concentrations. In both rainbow trout and Arctic char, a significant decrease ($p \leq 0.05$) in Lac (2.74 ± 0.269 and 3.38 ± 0.603 mmol/L, respectively) was observed when compared to the controls (3.53 ± 0.273 and 4.24 ± 0.260 mmol/L, respectively). In just Arctic char, a significant increase ($p \leq 0.05$) in Na+ was observed in treated fish (148 ± 0.914 mmol/L) when compared to the control (125 ± 8.48 mmol/L). Contrarily, in just rainbow trout, a significant increase ($p \leq 0.05$) in Cl- was observed (130 ± 0.770 mmol/L) in comparison to control fish (117 ± 6.99 mmol/L). Exposure to 6PPD-quinone also caused changes to acid-base balance in both species. A significant decrease ($p \leq 0.05$) in pCO_2 was observed in exposed Arctic char (18.8 ± 0.994 mmHg) when compared to that of control fish (21.4 ± 0.660 mmHg). Similarly, a significant decrease ($p \leq 0.05$) in HCO_3 and $ctCO_2$ was observed in exposed rainbow trout (7.16 ± 0.216 and 7.81 ± 0.220 mmol/L, respectively), when compared to the controls (7.97 ± 0.252 and 8.67 ± 0.251 mmol/L, respectively).

Table 2.6. Quantitative analyses using a blood gas analyzer in juvenile Arctic char exposed to 6PPD-quinone or solvent control for 48 h.

	Control	**7.15 µg/L 6PPD-quinone**
pH	7.10 ± 0.0120	7.19 ± 0.0419
Partial pressure of CO2 (mmHg)	21.4 ± 0.660	18.8 ± 0.994*
Partial pressure of O2 (mmHg)	44.7 ± 9.82	61.7 ± 11.6
Bicarbonate (mmol/L)	6.45 ± 0.195	6.98 ± 0.364
Base excess (mmol/L)	-18.2 ± 3.11	-19.2 ± 1.12
Total CO2 in plasma (mmol/L)	7.13 ± 0.190	7.55 ± 0.354
Total hemoglobin (g/L)	80.8 ± 7.24	87.8 ± 8.84
Sodium (mmol/L)	125 ± 8.48	148 ± 0.914*
Potassium (mmol/L)	2.58 ± 0.195	2.68 ± 0.206
Calcium (mmol/L)	1.39 ± 0.0176	1.34 ± 0.0228
Chloride (mmol/L)	130 ± 0.750	130 ± 0.783
Anion gap (mmol/L)	15.0 ± 0.798	13.9 ± 0.991
Osmotic concentration (mmol/kg)	302 ± 2.25	299 ± 1.88
Glucose (mmol/L)	3.54 ± 0.188	3.34 ± 0.201
Lactate (mmol/L)	4.24 ± 0.260	3.38 ± 0.603*

*$p \leq 0.05$ compared to control using t-test. Data are expressed as mean ± SEM of n = 11-16 fish.

Table 2.7. Quantitative analyses using a blood gas analyzer in juvenile rainbow trout exposed to 6PPD-quinone or solvent control for 48 h.

	Control	**0.59 µg/L 6PPD-quinone**
pH	6.80 ± 0.397	7.16 ± 0.0220
Partial pressure of CO2 (mmHg)	21.4 ± 0.799	20.8 ± 0.824
Partial pressure of O2 (mmHg)	52.8 ± 9.36	37.7 ± 5.57
Bicarbonate (mmol/L)	7.97 ± 0.252	7.16 ± 0.216*
Base excess (mmol/L)	-18.2 ± 0.765	-19.6 ± 0.624
Total CO2 in plasma (mmol/L)	8.67 ± 0.251	7.81 ± 0.220*
Total hemoglobin (g/L)	107 ± 6.00	97.3 ± 5.16
Sodium (mmol/L)	144 ± 3.08	149 ± 0.893
Potassium (mmol/L)	2.72 ± 0.346	2.26 ± 0.124
Calcium (mmol/L)	1.43 ± 0.0379	1.47 ± 0.0219
Chloride (mmol/L)	117 ± 6.99	130 ± 0.770*
Anion gap (mmol/L)	25.6 ± 10.7	13.2 ± 0.952
Osmotic concentration (mmol/kg)	292 ± 6.94	302 ± 1.95
Glucose (mmol/L)	4.91 ± 0.280	4.93 ± 0.256
Lactate (mmol/L)	3.53 ± 0.273	2.74 ± 0.269*

*$p \leq 0.05$ compared to control using t-test. Data are expressed as mean ± SEM of n = 9-19 fish.

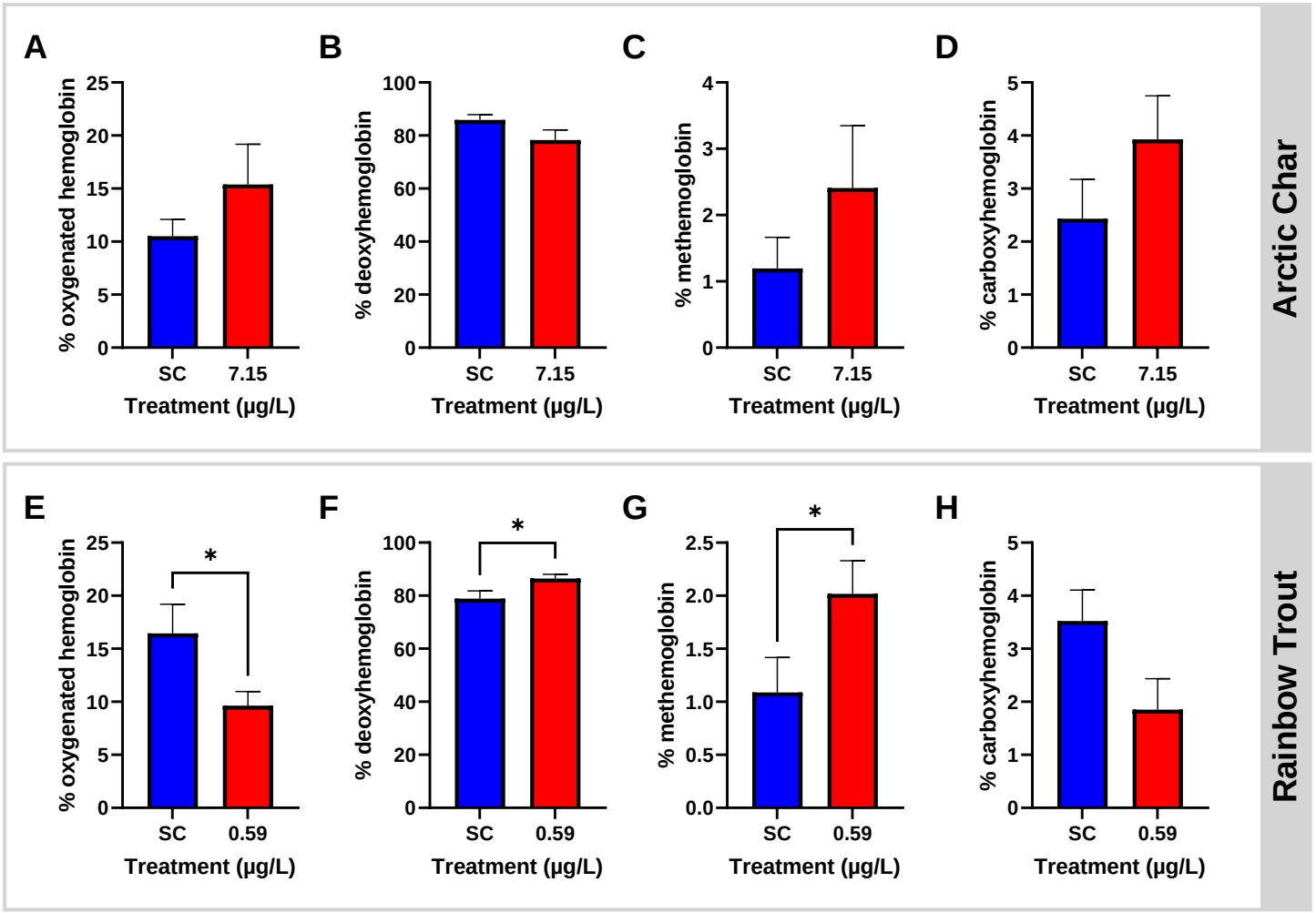

Figure 2.4. Changes in the oxygen carrying state of hemoglobin determined using CO-oximetry in Arctic char (A-D) and rainbow trout (E-H) exposed to 6PPD-quinone (0.59 or 7.15 µg/L) or a solvent control (SC) for 48 h. Oxygenated hemoglobin (A, E), deoxyhemoglobin (B, F), methemoglobin (C, G), and carboxyhemoglobin (D, H). Data are expressed as a percentage (mean ± SEM of n = 17-20 fish) of the total hemoglobin. Significantly different from control group using t-test (*$p \leq 0.05$).

2.5 Discussion

To our knowledge, this is the first study to investigate cardiovascular and metabolic effects of 6PPD-quinone exposure in salmonids. Overall, it was demonstrated that exposure to an environmentally relevant concentration of 6PPD-quinone exposure can disrupt normal cardiovascular function, the oxygen carrying state of hemoglobin, and oxygen consumption in juvenile rainbow trout. Similar alterations in oxygen consumption were also observed in juvenile Arctic char. However, despite being exposed to a concentration over 10 times higher than that of rainbow trout, Arctic char did not exhibit changes in cardiovascular performance. In contrast, rainbow trout showed a variety of adaptive cardiovascular responses, including decreased ESV, increased PVF, increased cardiac output, and prolonged PR interval length. Moreover, blood gas analysis in rainbow trout, but not Arctic char, showed changes such as decreases in FO_2Hb, increases in *F*HHb, and increases in *F*MetHb.

2.5.1 6PPD-quinone exposures

The selection of 6PPD-quinone concentrations was informed by a previous study in our laboratory, which determined acute lethality in juvenile rainbow trout and Arctic char (Brinkmann et al., 2022). In the present study, fish were subjected to a 48 h exposure, utilizing nominal concentrations (rainbow trout, 1.00 µg/L; Arctic char, 10.0 µg/L) that closely approached previously determined LC_{50} values (rainbow trout, 72-h LC_{50} = 1.00 µg/L) or maximum exposure levels (Arctic char, 96 h 14.2 µg/L). The aim of this approach was to observe and analyze the sublethal effects of 6PPD-quinone exposure immediately preceding mortality. While previous studies have reported mortality in rainbow trout exposed to 6PPD-quinone, we did not observe any mortality in our study at the lower concentration of 0.59 µg/L over the 48-hour exposure period, consistent with our focus on sublethal effects. Rainbow

trout, chosen for their sensitivity, were exposed to an environmentally relevant concentration (0.59 μg/L) reflective of surface water levels measured across North America (0.086-2.3 μg/L) (Challis et al., 2021; Johannessen et al., 2022; Tian et al., 2021, 2022). Arctic char, chosen for their tolerance, were exposed to a concentration (7.15 μg/L) which exceeds levels measured in the environment to date (Challis et al., 2021; Johannessen et al., 2022; Tian et al., 2021, 2022). The intentional use of differing concentrations allowed for an assessment of each species within its specific tolerance level, helping to provide a more meaningful comparison of their physiological responses. In the present study, concentration decreases over the exposure duration are within the range of those observed in previous studies (Brinkmann et al., 2022; Foldvik et al., 2022; Hiki et al., 2021; Hiki & Yamamoto, 2022).

2.5.2 Metabolic and blood gas changes

6PPD-quinone exposure significantly dysregulated aerobic metabolism in juvenile rainbow trout and Arctic char. Aerobic metabolism is a fundamental biochemical process that utilizes glucose and oxygen to produce carbon dioxide, water, and energy (ATP), using oxygen as the final electron acceptor in the electron transport chain (Berg et al., 2002). For this reason, oxygen consumption rate is often used as proxy for metabolic rate (Nelson, 2016). Our findings revealed increased SMR in both rainbow trout and Arctic char following exposure to 6PPD-quinone. Although overall mean oxygen consumption values show modest differences, the paired analysis accounts for individual variability over time, revealing subtle but significant effects that might be missed when comparing overall means. Despite small differences in oxygen consumption between treated and control fish, the statistical significance reflects a consistent metabolic response across both species, emphasizing the effects of 6PPD-quinone on metabolic function. This increase contrasts with a study by (Ricarte et al., 2023),

who observed no differences in oxygen consumption rate between exposed and control zebrafish larvae at concentrations up to 2 μg/L 6PPD-quinone. Conversely, these observations align with results reported by Varshney et al. (2022), who observed dose- and time-dependent increases in oxygen consumption rates of zebrafish larvae treated at concentrations higher than 10 μg/L. These findings also corroborate a previous study from our group, which found that oxygen consumption rates increased 2-fold in rainbow trout gill cells exposed to 20 μg/L of 6PPD-quinone. The absence of comparable effects in liver cells suggested that gill cells may be the primary target of toxicity of 6PPD-quinone toxicity (Mahoney et al., 2022). This can potentially lead to respiratory stress and increased respiration as fish attempt to compensate for reduced oxygen availability or impaired gas exchange at the gills.

The observed increase in oxygen consumption rates may also reflect an increased demand for ATP production to meet cellular needs or support detoxification processes in response to exposure (Gashkina, 2024). It was previously hypothesized that 6PPD-quinone affects metabolic function by uncoupling the electron transport chain, prompting an increase in oxygen consumption as cells attempt to maintain energy homeostasis despite reduced ATP production efficiency (Mahoney et al., 2022). While the exact mechanism of toxicity remains unclear, changes in hemoglobin species, such as oxygenated hemoglobin and non-oxygen carrying but normal hemoglobin (deoxyhemoglobin), measured in rainbow trout following exposure confirm impaired oxygen utilization. These changes in oxygenation drive cardiac responses aimed at increasing oxygen delivery. However, as hypoxia progresses, these compensatory mechanisms may become overwhelmed, causing oxygenated hemoglobin levels to decrease and deoxyhemoglobin levels to increase as the tissues become increasingly deprived of oxygen.

In addition to impaired oxygen utilization, cardiorespiratory symptoms observed in sensitive species following 6PPD-quinone exposure might partly be driven by a significant increase in methemoglobin, leading to impaired tissue oxygenation. Significant increases in methemoglobin concentrations in teleost fish have previously been observed under hypoxic conditions, although at levels greater than those observed in this study (Affonso et al., 2002; Soldatov, 2021). Methemoglobin is produced when the ferrous iron in the heme groups of hemoglobin is oxidized to the ferric state. This oxidized form has a decreased ability to bind oxygen and can cause tissue hypoxia when elevated substantially (Tönz, 1968). As quinones are strong oxidants, 6PPD-quinone could potentially be facilitating this oxidation, leading to increased methemoglobin production — a mechanism that has been previously observed following exposure to quinones (Kakizaki et al., 1969; Link et al., 1985; Magos, 1964). Increased methemoglobin production might also be attributed to oxidative stress, where reactive oxygen species or other oxidizing agents oxidize hemoglobin, driving this conversion. In addition, methemoglobin elevation may result from impaired activity or reduced expression of enzymes such as cytochrome b5 reductase, which is primarily responsible for converting methemoglobin back to hemoglobin under normal physiological conditions (Kitao et al., 1974). When its function is compromised, the enzyme's ability to restore methemoglobin to its functional state is reduced, leading to its accumulation. Moreover, oxidative stress, such as that induced by quinones, can potentially overwhelm or inhibit cytochrome b5 reductase, further decreasing its capacity to manage methemoglobin levels (Hyun & Lee, 2015). However, further research is needed to elucidate the specific mechanisms involved in this increase.

Exposure to 6PPD-quinone significantly altered electrolyte and metabolite concentrations, suggesting potential disruptions in energy production and ion homeostasis in

juvenile salmonids. When oxygen delivery is insufficient, anaerobic glycolysis usually increases, resulting in a buildup of metabolites such as lactate (Gerard et al., 2014). However, this study found a significant decrease in lactate levels in both species following 6PPD-quinone exposure. This suggests that while aerobic metabolism may be disrupted, as indicated by increased oxygen consumption rates and elevated cardiac output, anaerobic metabolism may also be impaired, potentially due to mitochondrial dysfunction. When the electron transport chain is impaired or uncoupled, NADH cannot be efficiently oxidized back to NAD+, a necessary cofactor for glycolysis (Gerard et al., 2014). Without sufficient NAD+, glycolysis becomes inefficient, impairing energy production and reducing lactate levels despite heightened metabolic demand. Additionally, elevated levels of sodium in Arctic char and chloride in rainbow trout may reflect osmoregulatory stress induced by 6PPD-quinone exposure. Given that the gill has been proposed as the primary target organ, exposure could impair normal ion transport mechanisms, leading to abnormal blood ion concentrations. When fish experience osmoregulatory imbalance, they exhibit behaviors such as lethargy, erratic swimming, increased ventilation, and difficulty maintaining buoyancy, all of which have been observed in sensitive species following exposure (Brinkmann et al., 2022; Jin et al., 2022; Lo et al., 2023). Although the observed changes in sodium, chloride, and lactate levels are statistically significant, they remain within normal concentration ranges (Jones & Moffitt, 2004; Milligan & Girard, 1993). However, even moderate fluctuations can trigger stress responses, especially if they persist or occur rapidly. The biological significance of these changes and their role in the cardiorespiratory symptoms is still unknown, requiring further investigation to clarify their effects on fish.

In contrast to rainbow trout, Arctic char showed an increase in oxygen consumption without corresponding changes in cardiovascular function or hemoglobin oxygenation status. This discrepancy may stem from differences in metabolic efficiency between the two species. A previous study from our laboratory found that tolerant fish species appear to possess more efficient biotransformation pathways, enabling them to process 6PPD-quinone more effectively (Montgomery et al., 2023). Specifically, species such as chinook salmon and westslope cutthroat trout exhibited higher levels of 6PPD-quinone metabolites in their bile compared to sensitive species like coho salmon and rainbow trout. This suggests that Arctic char may metabolize 6PPD-quinone more effectively, protecting them from advancing to toxicity stages that require cardiovascular compensation. In contrast, the cardiovascular and hemoglobin changes observed in rainbow trout indicate that their reduced metabolic capacity triggers physiological disruptions that lead to the onset of cardiac symptoms. Taken together, these findings demonstrate that metabolic function plays a key role in determining the toxicological responses of fish species to 6PPD-quinone, providing new insights into species-specific sensitivity differences.

2.5.3 Cardiovascular changes

6PPD-quinone significantly impacted cardiac function in juvenile rainbow trout but had no effect in Arctic char, emphasizing the acute sensitivity of rainbow trout to this compound. When oxygen uptake across the gills is impaired, or aerobic metabolism is disrupted due to mitochondrial toxicity, energy production is disrupted. Consequently, the body compensates by enhancing cardiac function as it attempts to increase oxygen delivery to deprived tissues and meet increased metabolic demands caused by 6PPD-quinone exposure. However, despite symptoms indicating cardiorespiratory issues in sensitive species following

exposure, research has not investigated these effects in salmonids, instead focusing solely on heart rate changes in zebrafish embryos (Ricarte et al., 2023; Varshney et al., 2022; Zhang et al., 2023). Moreover, these studies present conflicting results regarding heart rate modulation, which contrast the findings in salmonids observed in this study. Specifically, Varshney et al. (2022) and Zhang et al. (2023) reported decreases in heart rate, while Ricarte et al. (2023) documented increases. However, neither juvenile rainbow trout nor Arctic char exhibited changes in atrial or ventricular heart rate. While efforts to enhance cardiac function and oxygen delivery typically lead to increases in heart rate and shortened ECG interval lengths, our study revealed a contrary result. Specifically, we observed a significant prolongation of the PR interval, which represents the time needed for the electrical impulse to travel from the atrium to the ventricle (Chan, 2005). Normally, a prolonged PR interval would lead to a slower heart rate, yet we did not observe this, suggesting the presence of electrical impairments such as first-degree atrioventricular (AV) block. When combined with cardiac stimulation aiming to increase heart rate, these conditions would likely offset each other, causing heart rate to remain unchanged. As electrical excitation travels through the heart, it passes through the AV node, briefly delaying the impulse before it reaches the ventricle. This delay allows the atrium to contract and ventricular filling to occur prior to ventricular contraction, facilitating smooth blood flow (Hoar & Randall, 1969). A prolonged PR interval indicates delayed conduction between the atrium and ventricle, indicating dysfunction in the atrioventricular node (Chan, 2005). The susceptibility of the AV node to oxidative stress-induced damage suggests that this mechanism may contribute to the observed increase in PR interval duration in exposed rainbow trout (Langenbacher et al., 2020). Varshney et al. (2022) suggested that 6PPD-quinone may be inducing oxidative stress, potentially leading to inflammatory responses

observed in exposed zebrafish larvae. This hypothesis was further explored by Anderson-Bain et al. (2023), who observed elevated levels of oxidative stress-related metabolites such gluconolactone and methionine sulfoxide in the liver and gills of fathead minnow (*Pimephales promelas*). While most of these changes were observed in gills, further research is needed to confirm if similar changes are occurring in the heart. This will provide a clearer understanding of whether oxidative stress is indeed the cause of the prolonged PR interval observed in this study. The reduced end systolic volume observed in exposed trout indicates a positive inotropic response, a common mechanism in fish to enhance cardiac output and meet metabolic demands (Shiels et al., 2006). This response refers to an increase in the force of cardiac muscle contraction, which causes the ventricle to empty more fully. To enhance oxygen delivery, cardiac stimulation increases cardiac output by increasing heart rate, contractility, or both (Froelicher & Myers, 2007). With heart rate unchanged, the decreased ESV (indicating increased contractility) appears to be the primary driver for the significant increase in cardiac output observed in rainbow trout in this study. The increase aligns with speculation by Varshney et al. (2022) that fish exposed to 6PPD-quinone would need to elevate cardiac output to meet the increased demand for oxygen. In addition to increased myocardial contractility, decreased ESV can result from factors such as reduced afterload (the pressure the heart must overcome to eject blood) and improved passive ventricular filling (LaCombe et al., 2023). In the present study, a significant increase in passive ventricular filling was also observed in rainbow trout, which refers to the process by which blood returns to the heart and fills the ventricle during diastole, the relaxation phase of the heart. This response may signify changes in preload (the blood volume stretching the ventricle prior to contraction), afterload, or increased ventricular elasticity (Klabunde, 2011). Ventricular elasticity, or compliance,

represents the ventricle's ability to expand and accommodate incoming blood during diastole (Gaasch et al., 1976). However, further analysis of the ventricle's structural properties will be needed to confirm this hypothesis.

Overall, the collective changes observed in contractility, cardiac output, and passive filling, combined with the increased oxygen consumption rates in treated rainbow trout, reflect alterations in cardiovascular and respiratory function as fish strive to meet the elevated metabolic demands induced by 6PPD-quinone exposure. Responses like this may be indicative of sympathetic nervous system stimulation, potentially mediated by increased levels of catecholamines acting through β-adrenergic mechanisms (Taylor et al., 2009; Wendelaar Bonga, 1997). Previous research has shown that 6PPD-quinone exposure increases the levels of norepinephrine and epinephrine in zebrafish larvae, suggesting that exposure to 6PPD-quinone could induce similar effects in rainbow trout (Ricarte et al., 2023). Given these findings, it is plausible that 6PPD-quinone may similarly influence the catecholaminergic system in rainbow trout, though evidence is still lacking. Whatever the mechanism of cardiac stimulation in 6PPD-quinone exposed trout, the physiological responses appear to be directed toward increasing blood output to maintain tissue perfusion and oxygen delivery in the presence of physiological stress. Although this study primarily focused on metabolic and cardiovascular responses, previous research suggests that neurotoxic effects, indicated by behavioural changes in fish, may also play a role in the overall physiological response to 6PPD-quinone exposure (Brinkmann et al., 2022; Lo et al., 2023; Ricarte et al., 2023). Therefore, further research is needed to confirm the underlying mechanisms driving these responses, including the potential role of sympathetic stimulation, and to assess their implications for fish health and survival in contaminated environments.

2.6 Conclusion

The results of this study revealed significant cardiovascular and metabolic effects caused by 6PPD-quinone exposure in juvenile salmonids. Environmentally relevant concentrations of 6PPD-quinone induced a variety of effects, including alterations in cardiovascular function, hemoglobin dynamics, and increased metabolism in the more sensitive species, rainbow trout. These responses likely reflect cardiovascular changes as a secondary consequence of metabolic disruptions, rather than direct cardiac toxicity. This may include compensatory adjustments arising from impaired oxygen utilization or decreased oxygen transport to tissues, potentially influenced by oxidative stress or sympathetic nervous system activation. In contrast, Arctic char exhibited substantially fewer and less pronounced effects at much greater concentrations of 6PPD-quinone, with significant impacts only observed on oxygen consumption rates. By investigating the cardiorespiratory responses to 6PPD-quinone exposure in these species of differing sensitivity, this study provides valuable insights into the species-specific toxicity observed in salmonids. However, as many cardiovascular changes were interpreted based on mammalian studies, further research is needed to definitively determine the underlying mechanisms driving these responses. This study not only highlights the complex responses of salmonids to 6PPD-quinone exposure but also stresses the importance of studying sublethal effects. As rainbow trout and Arctic char are ecologically, commercially, and culturally significant species that migrate long distances and have a narrow range of both oxygen and temperature tolerance, even sublethal effects on their cardiovascular or metabolic fitness could potentially have effects on the health of their populations.

CHAPTER 3

3.0 SUBLETHAL 6PPD-QUINONE EXPOSURE IMPAIRS SWIMMING PERFORMANCE AND AEROBIC METABOLISM IN JUVENILE LAKE TROUT (*SALVELINUS NAMAYCUSH)*

Preface

The purpose of this study was to explore the physiological and behavioural effects of environmentally relevant 6PPD-quinone exposure in a sensitive salmonid species, lake trout. Swimming performance, oxygen consumption, and concentrations of stored energy (triglycerides and glycogen) were determined in juvenile lake trout exposed to sublethal concentrations of 6PPD-quinone. Exposure resulted in impaired swimming performance, as evident by a decrease in critical swimming speed, and dysregulation of aerobic metabolic capacity, indicated by decreases in active metabolic rate and aerobic scope. Our findings suggest that environmentally relevant exposure to 6PPD-quinone can cause sublethal toxicities in juvenile salmonids.

This chapter has been submitted to the journal Comparative Biochemistry and Physiology Part C. The anticipated citation is:

Selinger, S. J., Hunnie, B., Roberts, C., Amekor, M., Hogan, N., Wiseman, S., Hecker, M., Weber, L., Brinkmann, M., Janz, D., 2025. Sublethal 6PPD-Quinone Exposure Impairs Swimming Performance and Aerobic Metabolism in Juvenile Lake Trout (*Salvelinus namaycush).* (In preparation).

The author contributions to chapter 2 of this thesis were as follows:

Summer J. Selinger (University of Saskatchewan) collected, processed, and analyzed all samples, performed all statistical analyses, and drafted the manuscript.

Blake Hunnie (University of Saskatchewan) performed ultrahigh-performance liquid chromatography (UHPLC) to analyze water samples for 6PPD-quinone concentrations.

Catherine Roberts (University of Saskatchewan) assisted in collection and fertilization of lake trout eggs; raised lake trout embryos.

Mawuli Amekor (University of Saskatchewan) assisted in collection and fertilization of lake trout eggs; raised lake trout embryos.

Natacha Hogan (University of Saskatchewan) reviews and revised the manuscript, providing comments and corrections.

Steve Wiseman (University of Lethbridge) reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Markus Hecker (University of Saskatchewan) reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Lynn Weber (University of Saskatchewan) provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

David M. Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Markus Brinkmann (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

3.1 Abstract

6PPD-quinone, an environmental oxidation product of the rubber tire antioxidant 6PPD, has recently gained recognition as a chemical of concern. Frequently detected in road runoff and surface waters, studies have reported this compound to cause acute lethality in several salmonid species at extremely low concentrations, including lake trout (*Salvelinus namaycush;* 96-h LC_{50} = 0.50 μg/L). Following exposure, species experiencing acute lethality show characteristic symptoms such as gasping, spiraling, increased ventilation, loss of equilibrium, erratic movements, and tumbling. However, there is a deficit of research targeted at understanding sublethal toxicities of 6PPD-quinone exposure, particularly concerning swimming capability and metabolic function. To evaluate these effects, juvenile lake trout were exposed for 20 hours to a measured concentration of 0.46 μg/L 6PPD-quinone in a swim tunnel respirometer to assess temporal changes in standard metabolic rate (SMR) compared to controls. Following exposure, fish underwent a swim trial to determine critical swimming speed (U_{crit}), oxygen consumption rate (MO_2), active metabolic rate (AMR), aerobic scope (AS) and energetic cost of transport (CoT), followed by analysis of muscle triglyceride and glycogen concentrations. Results showed that 6PPD-quinone exposure impaired swimming performance, evident by a decrease in U_{crit}. Additionally, exposure resulted in decreased AMR, although alterations in SMR were not observed. Decreased concentrations of muscle triglycerides of swam fish were also observed. These findings suggest that environmentally relevant concentrations of 6PPD-quinone disrupt aerobic metabolic capacity in juvenile lake trout, producing adverse effects that diminish endurance and maximum swim speeds, which may affect survival of fish populations.

3.2 Introduction

In recent years, 6PPQ-quinone has become a chemical of interest, sparking concern in both scientific and public communities. This is due to its ability cause acute lethality in multiple salmonid species, including coho salmon *(Oncorhynchus kisutch)*, brook trout (*Salvelinus fontinalis*), white-spotted char (*Salvelinus leucomaenis pluvius*), rainbow trout (*Oncorhynchus mykiss*), and lake trout (*Salvelinus namaycush*) at low concentrations (24-h LC_{50} = 0.095, 0.59, and 0.51 μg/L; 72-h LC_{50} = 1.00 μg/L; 96-h LC_{50} = 0.50 μg/L, respectively) (Brinkmann et al., 2022; Hiki & Yamamoto, 2022; Roberts et al., 2025; Tian et al., 2022). As concentrations of 6PPD-quinone measured in aquatic systems worldwide range between 0.086 and 2.43 μg/L (Cao et al., 2022b; Challis et al., 2021; Tian et al., 2021, 2022), these levels may exceed the lethal threshold for several salmonid species, underscoring the potential for both lethal and sublethal effects in affected ecosystems. Although a large number of recent studies assessed the acute lethality of 6PPD-quinone to salmonids, there is currently a paucity of research targeted at understanding its sublethal toxicities. Prior to lethality, sensitive species display a progression of atypical swimming behaviours and characteristic symptoms, beginning with sustained surface swimming, followed by spiraling, loss of equilibrium, erratic movements, tumbling, increased ventilation, and ultimately ending in unresponsiveness (Chow et al., 2019). Proposed mechanisms of toxicity suggest that 6PPD-quinone may inhibit cellular respiration, as indicated by increased oxygen consumption and other cardiometabolic alterations observed in previous studies and the second chapter of this thesis (Mahoney et al., 2022; Selinger et al., 2024). Despite these

irregular behaviours, the effects of 6PPD-quinone exposure on swimming capability and metabolic function have not yet been fully investigated in salmonids.

6PPD-quinone is an environmental transformation product of 6PPD, a widely used antioxidant and antiozonant that helps prevent the degradation of rubber products such as tires. During the operation of motor vehicles, particularly when braking, turning, and accelerating, friction occurs between the tire and road surface, resulting in the production of tire wear particles (TWPs). TWPs build up on road surfaces and are subsequently washed into aquatic environments through roadway runoff following rain events, causing notable elevations in the concentration of 6PPD-quinone in surface waters (Peter et al., 2018; Seiwert et al., 2022; Werbowski et al., 2021). In 2021, it was estimated that 1.7 million tons of TWPs were produced in the US alone (Mayer et al., 2024). As there are currently no endorsed substitutes for 6PPD that achieve the same safety and performance standards, its introduction into the environment is expected to be a persistent issue (Department of Toxic Substances Control, 2022). Even if a nontoxic replacement is developed, the widespread use of tires today ensures that the presence of 6PPD and 6PPD-quinone will remain an environmental concern. Overall, the increased awareness of the potential risk that 6PPD-quinone may pose in aquatic environments has emphasized the need for further research to determine its potential ecological impacts, especially at sublethal concentrations.

In the previous chapter of this thesis, we detected significant effects on cardiorespiratory physiology in rainbow trout exposed to 0.59 μg/L, disrupting normal cardiovascular function, the oxygen carrying state of hemoglobin, and oxygen consumption rate. Furthermore, significant increases in standard (resting) metabolic rate

(SMR), cardiac output, and contractility were observed, suggesting that these changes were compensatory mechanisms aimed at enhancing oxygen delivery to tissues during periods of increased metabolic demand. These findings emphasized the need to assess the effects of 6PPD-quinone exposure on active metabolic functions, particularly during periods of increased activity. Along with the observations of atypical swimming behaviours by Brinkmann et al. (2022) and Lo et al. (2023), other studies have also reported adverse effects on locomotion following exposure. More specifically, Varshney et al. (2022) found that the total distance travelled by zebrafish larvae was significantly reduced following exposure to 10 and 25 μg/L 6PPD-quinone. Conversely, Ricarte et al. (2023) observed an increase in basal locomotor activity in zebrafish larvae exposed to 0.02 and 0.2 μg/L. Adverse effects on swimming performance can severely impair a fish's ability to carry out essential activities necessary for survival, growth, and overall fitness. When swimming performance is compromised, fish may face challenges in meeting the demands of their environment, leading to potential long-term ecological consequences. Thus, it is crucial to assess the effects of environmental contaminants on swimming performance and behavior in order to understand the full ecological consequences of exposure.

Swimming performance can be determined by measuring three types of swimming speed: sustained, prolonged, and burst (Plaut, 2001). Sustained swimming speeds are those that can be maintained for a period longer than 200 minutes, burst swimming speeds are those that can be maintained for less than 20 seconds, and prolonged swimming speeds cover times in between (Beamish, 1978; Hoar & Randall, 1978). Energy used for sustained and prolonged swimming is derived from aerobic

respiration, whereas energy used to reach burst swimming speeds is acquired through anaerobic processes (Hoar & Randall, 1978; Peake, 2004). During aerobic respiration in salmonids, triglycerides are preferentially mobilized and metabolised to use for energy, whereas glycogen is primarily used during anaerobic swimming (Hammer, 1995). Exposure to toxicants has been shown to alter storage and mobilization of energy, which can impact behaviours such as swimming, reproduction, and growth (Mi et al., 2021; Öner et al., 2008).

The overall goal of this study was to further investigate the mechanisms of 6PPD-quinone toxicity to juvenile salmonids, particularly toxicities that arise during exposure to sublethal concentrations. This was achieved by determining swimming performance and metabolism in lake trout, a species sensitive to acute lethality and an apex predator in cold-water systems. Intermittent-flow respirometry was used to determine standard metabolic rate and active metabolic rate (AMR). The latter was determined during a critical swimming challenge that was conducted to characterize effects on critical swimming speed. Rates of oxygen consumption were used to determine aerobic scope and energetic cost of transport. Following exposure, concentrations of muscle glycogen and triglycerides were determined.

3.3 Materials and Methods

3.3.1 Test compound and stock preparation

Native and mass-labeled (d_5) 6PPD-quinone, each with purities 97% or higher, were obtained from Toronto Research Chemicals (Toronto, CA). For analytical purposes, standard solutions were prepared using HPLC-grade methanol. For exposure experiments, 6PPD-quinone was dissolved in dimethyl sulfoxide (DMSO), resulting in a

final solvent concentration of 0.00125% (v/v) in the tanks. Stock solutions were stored in amber glass vials at 4°C until use.

3.3.2 Test species

Eggs were obtained from lake trout collected from Whiteswan Lake (Saskatchewan, CA) in collaboration with the Fisheries Department of the Government of Saskatchewan. Embryos were transported to the University of Saskatchewan under the Government of Saskatchewan fish transport/stocking permit FTP022022. To ensure genetic diversity and maintain a representative population, eggs from ten females were fertilized with milt from two males, creating 20 distinct genetic lines. Embryos were reared in the dark under continuous flow conditions in a heath tray system at the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan, using facility water maintained at a controlled temperature of 10.0 ± 1.0°C. Prior to hatching, eyed embryos were transferred to tanks with a 14-h light and 10-h dark photoperiod. Food was introduced a few days prior to swim-up. Fish were fed commercial fish feed at a daily rate of 3% body weight until they reached the juvenile stage, when they were switched to a maintenance diet of 1% body weight/day to maintain size between trials. Experiments were approved by the University of Saskatchewan Animal Care Committee (Protocols 20230010 and 20220002) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

3.3.3 Aqueous exposure, oxygen consumption, and swim performance

6PPD-quinone exposure, oxygen consumption measurements and swim performance challenge were conducted in a modified Blazka-type, variable speed, miniature swim tunnel respirometer with a DAQ-M control device and AutoResp 2.2.2

software (Loligo Systems, Viborg, DK). Due to logistical constraints, exposures were completed in a series of trials. Each trial consisted of one fish per treatment group (DMSO control and 6PPD-quinone). A total number of 18 trials were completed, resulting in a sample size of 18 fish for each treatment group. Prior to exposure, morphometric data were obtained for each individual, including the total length (cm), wet weight (g), and the width (cm) and depth (cm) measured at the midpoint of the dorsal fin. Individual fish were then placed into a 170 mL swim tunnel submerged in a 10-L buffer tank, which was supplied with 12.0 ± 1.0°C aerated water from a 400-L glass-fiber Min-o-Cool tank containing 200 L of test solution. Dissolved oxygen was measured using a fiber optic oxygen dipping probe that was connected to a Fibox 3 minisensor oxygen meter (Precision Sensing GmbH, Regensburg, DE). AutoResp 2.2.2 software was used to calculate the rate of oxygen consumption (MO_2). The detailed MO_2 measuring principle is explained elsewhere (Steffensen et al., 1984). MO_2 was measured using automated intermittent-flow respirometry in 5-minute loops. Each loop consisted of a 2.5-minute measuring period, followed by a 2-minute flushing period and a 30-second waiting period. Two MO_2 measurements were taken during each water velocity increment, and the average value was used for statistical analysis. To determine the effects of 6PPD-quinone exposure on SMR, fish were introduced to swim tunnels and exposed for 20 h at a minimal water velocity of 0.8 body lengths/second (BL/sec). Fish were exposed to 6PPD-quinone under static non-renewal conditions (nominal, 0.5 μg/L; measured, 0.46 μg/L), and control chambers received the DMSO solvent at the same level as those exposed to 6PPD-quinone [0.00125% (v/v)]. Fish were fasted for 24 h prior to exposure. After 20 h of exposure, fish were subjected to a swimming challenge to assess the effects

of 6PPD-quinone on aerobic swimming capacity. During the swimming challenge, fish were subjected to incremental increases in water velocity (0.5 BL/sec every 10 min) under the same exposure conditions, until they reached exhaustion. If a fish was unable to maintain its position in the water flow during the first velocity increment, the water velocity was temporarily reduced once for 10 seconds to reposition the fish in the middle of the swim tunnel and encourage swimming. Fish that did not resume swimming after this intervention were removed from the swim tunnel and excluded from further analysis. U_{crit} was calculated using an equation previously described by Brett (1964)**.** Fish cross-sectional area was more than 5% of swim tunnel cross-sectional area, so U_{crit} values were corrected for solid blocking effect. Swim performance data (cm/s) were also corrected for total body length of each individual, and consequently, U_{crit} data were expressed as body length per second (BL/sec).

3.3.4 Determination of standard metabolic rate, active metabolic rate, aerobic scope, and energetic cost of transport

Standard metabolic rate, or the minimal maintenance metabolic rate of unfed fish, was calculated by extrapolating MO_2 back to a water velocity of zero. This was done from a plot of swimming speed (m/s) versus MO_2 (mg O_2/kg/h) by use of nonlinear, curve fitting regression analysis (Brett, 1964; Shingles et al., 2001). Active metabolic rate was calculated as the metabolic rate of fish at the maximum sustainable velocity in the U_{crit} test (Fry, 1971). Aerobic scope (AS) was calculated as the difference between SMR and AMR (AMR-SMR) (Fry, 1971). Fish were included in the sample for a specific water velocity increment only if they successfully maintained swimming performance for the entire duration of the increment (10 min). Energetic cost of transport (CoT; J/kg/m) was

calculated by multiplying MO_2 (mg O_2/kg/s) by an oxycaloric value of 14.1 J/mg O_2 and then dividing it by the corresponding swim speed (m/s) (Cai et al., 2014).

3.3.5 Analytical chemistry

Throughout the exposure period, water samples were collected to confirm 6PPD-quinone concentrations. Sampling occurred at several key points: before and after the initial dosing, prior to the critical swimming challenge, and after the critical swimming challenge, prior to the removal of fish from swim tunnels. Approximately 45 minutes after dosing, samples were taken, immediately spiked at 50 µg/L with 6PPD-quinone-d_5, and stored at -20°C until analysis. The samples were analyzed using a Vanquish UHPLC instrument coupled with a Q-Exactive HF Quadrupole-Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) based on methods previously described by Brinkmann et al. (2022). An isotope dilution strategy using 6PPD-quinone-d_5 was applied for quantification. Concentrations were expressed as the time-weighted average (TWA) calculated across all treatment replicates.

3.3.6 Triglyceride and glycogen assays

Following the swim performance challenge, fish were euthanized using an overdose of buffered tricaine methane sulfonate (MS-222; 1 g/L) followed by spinal severance. Mixed muscle tissue was obtained from each individual and stored at -80°C until analysis. Concentrations of muscle triglycerides were determined using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA) based on a lipoprotein lipase and glycerol kinase colorimetric assay. Concentrations of muscle glycogen were determined using an amylase and glucose-based colorimetric assay, which has been previously validated in our laboratory (Weber et al., 2008). Assays were performed with

technical replicates to ensure reliable results. Reagents were purchased from Sigma-Aldrich. Standard curves were prepared using type IX glycogen from bovine liver.

3.3.7 Statistical analysis

Data were tested for normality using the Shapiro-Wilk test and homoscedasticity using the F test (GraphPad Prism 9.5.1, San Diego, CA, USA). A two-tailed t-test was used to determine significant differences in total length, total width, total depth, wet weight, SMR, AMR, AS, U_{crit}, muscle triglycerides, and muscle glycogen between control and treated groups. To account for the substantial number of fish that performed poorly during the critical swimming challenge, separate analyses were conducted to determine significant differences in triglycerides and glycogen in all exposed fish, and in only those capable of swimming during the critical swimming challenge, specifically those that completed at least one full water velocity increment. At each water velocity tested in the U_{crit} analysis, a repeated measures t-test was used to test for differences in MO_2 and CoT of control and 6PPD-quinone exposed lake trout. All data were presented as mean ± SEM. An alpha value of 0.05 was used in all statistical tests to determine statistical significance.

3.4 Results

3.4.1 Analytical chemistry

Mean concentrations of 6PPD-quinone measured at each of the timepoints during the exposure deviated <30% from nominal values. The mean 6PPD-quinone concentration during exposure (nominal concentration 0.5 μg/L) was 0.456 ± 0.082 μg/L (Table 3.1). 6PPD-quinone was not detected in the control group at any point throughout the exposure.

Table 3.1. Nominal versus measured concentrations of 6PPD-quinone during exposure experiments with lake trout as determined using LC-HRMS. Concentrations were measured ~45 minutes after dosing, before the start of the swimming challenge (U_{crit}; 20 h) and after the swimming challenge prior to removal of fish from swim tunnel respirometers (21 h).

Treatment/nominal concentration	**0 h**	**Before U_{crit} test**	**21 h**	**Average during exposure**
SC	ND	ND	ND	ND
0.5 μg/L	0.564 ± 0.0977	0.350 ± 0.0665	0.348 ± 0.0653	0.456 ± 0.0815

Data are presented as mean ± SD. *ND: < LOD.*

3.4.2 Fish morphometrics and mortalities

Total fish length in control and 6PPD-quinone treated groups was 8.07 ± 0.17 cm and wet whole-body weight of fish was 4.15 ± 0.27 g. Total fish width in control and 6PPD-quinone treated groups was 0.59 ± 0.024 cm and total depth 1.07 ± 0.039 cm. No mortalities were observed in the control group. In the treatment group, mortality was 14% (3/21 individuals), with all mortalities occurring between 16 and 20 hours post-exposure.

3.4.3 Oxygen consumption and swim performance

Lake trout exposed to 6PPD-quinone experienced significant dysregulation of aerobic capacity and swimming performance. AMR was significantly lower ($p \leq 0.01$) in treated fish (337 ± 39.3 mg O_2/kg/h) than control fish (478 ± 34.3 mg O_2/kg/h; Figure 3.1B). Conversely, SMR did not significantly differ between control (166 ± 7.47 mg O_2/kg/h) and treated (153 ± 9.14 mg O_2/kg/h; Figure 3.1A) groups. AS was significantly lower ($p \leq 0.05$) in exposed trout (197 ± 31.4 mg O_2/kg/h) compared to control trout (307 ± 37.1 mg O_2/kg/h; Figure 3.1C). During the critical swimming speed challenge, significant differences in MO_2 or energetic CoT of control and 6PPD-quinone exposed

lake trout were not observed at any incremental water velocity (Figure 3.2A, B). Conversely, U_{crit} was significantly lower ($p \leq 0.05$) in treated fish (2.87 ± 0.278 BL/sec) compared to control fish (3.77 ± 0.180 BL/sec, Figure 3.2C).

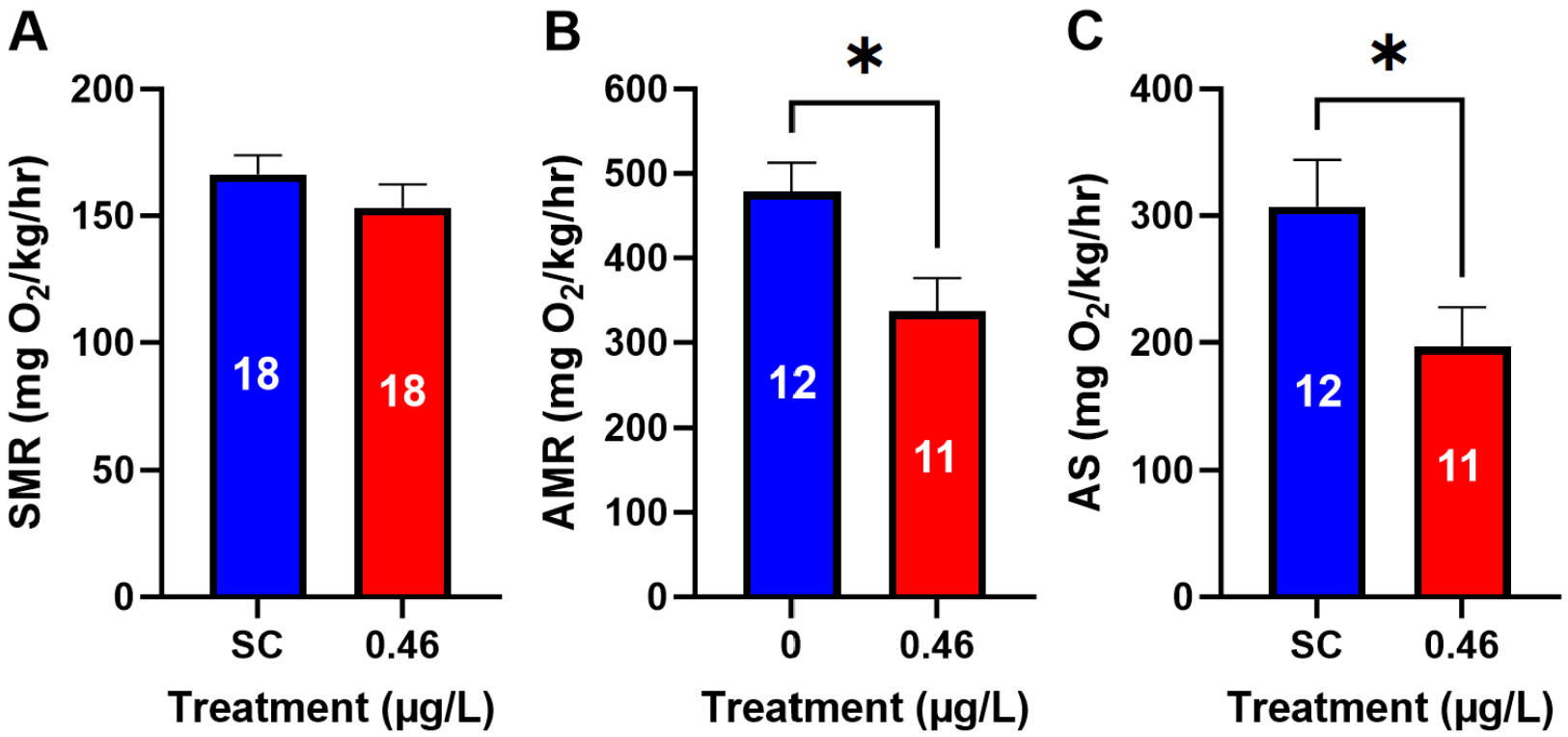

Figure 3.1. Metabolic responses of juvenile lake trout exposed to 0.46 µg/L 6PPD-quinone for 20 h. A, standard metabolic rate (SMR); B, active metabolic rate (AMR); and C, aerobic scope (AS). Data are presented as mean ± SEM of n = 11-18 fish. Actual number of individuals indicated is on the bar for each treatment. Significantly different from control group using t-test (*$p \leq 0.05$).

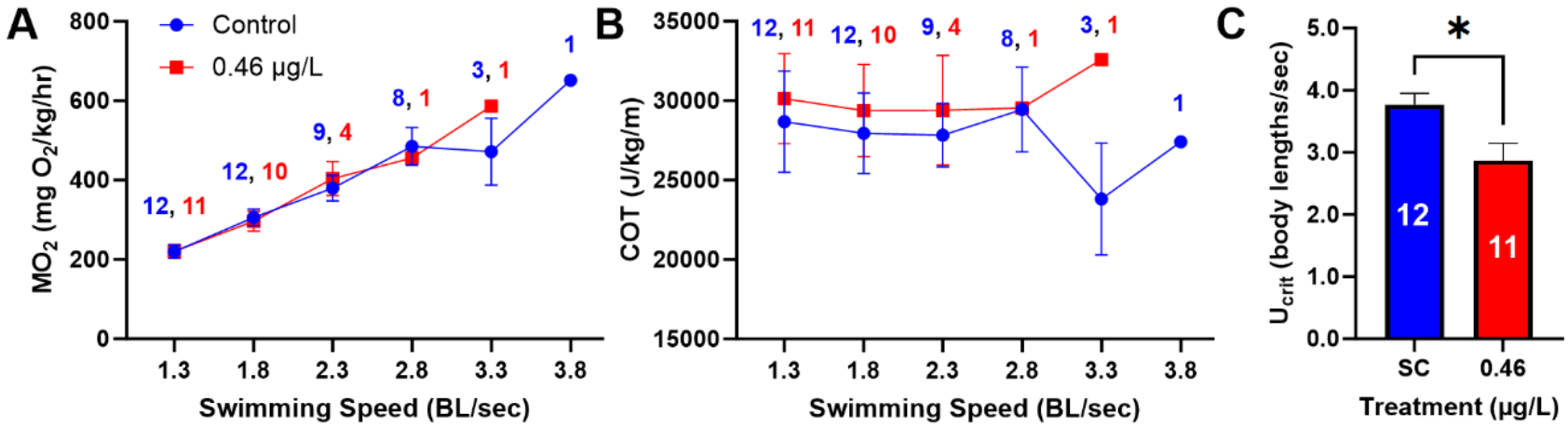

Figure 3.2. Metabolic responses of juvenile lake trout exposed to 0.46 µg/L 6PPD-quinone for 20 h. A, oxygen consumption (MO_2); B, energetic cost of transport (CoT); and C, critical swimming speed (U_{crit}). Fish were included in the sample for a specific water velocity increment only if they successfully maintained swimming performance for the entire duration of the increment. Data are presented as mean ± SEM of n = 1-12 fish. Actual number of individuals for each treatment is indicated on the bar or directly above the data point for each water velocity increment. Significantly different from control group using t-test (*$p \leq 0.05$).

3.4.4 Triglyceride and glycogen assays

Concentrations of muscle glycogen and triglycerides in all individuals, and in just the swam group were determined following exposure. The “swam” group refers to fish that were able to successfully maintain swimming performance for the entire duration of the first water velocity increment, while the "non-swam" group includes those that were unable to complete the first increment, displaying avoidance behaviors such as tail wedging or burst swimming (more detailed observations are outlined below). Concentrations of muscle triglycerides were significantly lower ($p \leq 0.05$) in fish exposed to 6PPD-quinone (3.95 ± 0.67 mg/g) compared to control fish (6.15 ± 0.62 mg/g), within the group that swam during the swim performance challenge (Figure 3.3B). Conversely, when the assessment was based on all individuals (swam and non-swam), concentrations of triglycerides were unchanged in exposed lake trout (5.84 ± 0.91 mg/g) when compared to controls (5.93 ± 0.61 mg/g, Figure 3.3A). Similarly, the concentration of muscle glycogen was also unaffected when the assessment was based on all fish (swam and non-swam) exposed to 6PPD-quinone (5.00 ± 0.90 mg/g) compared to control trout (5.04 ± 0.56 mg/g, Figure 3.3C). Even when limited to the swam fish only, glycogen concentrations did not significantly differ between control (5.44 ± 0.68 mg/g) and treated groups (5.29 ± 1.20 mg/g, Figure 3.3D).

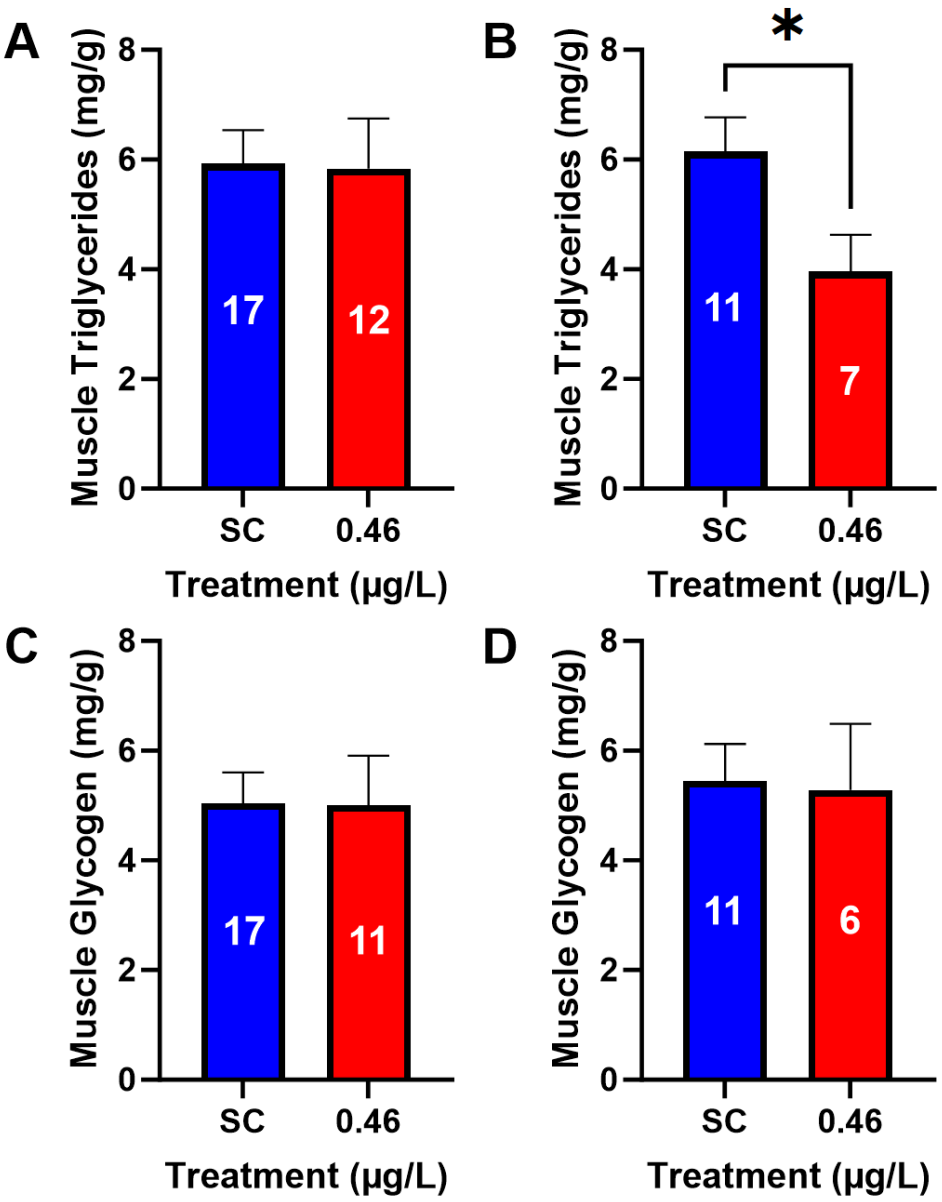

Figure 3.3. Skeletal muscle triglyceride (A, B) and glycogen (C, D) concentrations in juvenile lake trout exposed to 0.46 µg/L 6PPD-quinone or a solvent control for 20 h. All individuals (A, C) and individuals who swam during the U_{crit} test (B, D). Data are presented as mean ± SEM of n = 5-17 fish. Actual number of individuals is indicated on the bar for each treatment. Significantly different from control group using t-test (*$p \leq 0.05$).

3.5 Discussion

To our knowledge, this study is the first investigation into the sublethal impacts of 6PPD-quinone exposure on swimming capability and active metabolism in salmonids. The findings indicated that exposure to environmentally relevant concentrations of 6PPD-quinone had the capacity to disrupt both behavior and physiology in juvenile lake trout, resulting in impaired swim performance and dysregulation of aerobic metabolic capacity. The selection of 6PPD-quinone concentrations was guided by a prior investigation conducted in our laboratory, which assessed the acute toxicity of 6PPD-quinone to lake trout fry (Roberts et al., 2025). In the present study, fish underwent a 20-h exposure to a concentration of 6PPD-quinone that closely approached the previously determined LC_{50} value for lake trout fry (96-h LC_{50} = 0.50 μg/L). This approach was designed to observe and assess the sublethal effects of 6PPD-quinone exposure in the moments that may precede mortality.

Significant alterations in MO_2 were observed in juvenile lake trout following exposure to 6PPD-quinone. Although SMR remained unchanged, exposure resulted in a significant increase in AMR. This observation differed from those made in the second chapter of this thesis, where exposure to 6PPD-quinone resulted in a significant increase in SMR in two salmonid species, rainbow trout and Arctic char (*Salvelinus alpinus*). The lack of change in resting metabolism in lake trout compared to the significant increase observed in rainbow trout and Arctic char may suggest that responses to 6PPD-quinone exposure vary not only between sensitive and tolerant species, but also among species that are sensitive to exposure. However, it is worth highlighting that the concentrations used in the second chapter are relatively lower than the LC_{50} for the species examined,

which may explain the differences observed in resting metabolism. Furthermore, differences in exposure duration and developmental stage between studies could also contribute to the observed variations. In the present study, fish were only exposed to 6PPD-quinone for 20 h, whereas significantly raised metabolic rates were observed in Arctic char and rainbow trout over a 48-h exposure. Thus, it is possible that a longer exposure duration would have been required to observe similar effects in lake trout. Additionally, the exposure concentrations in this study were derived from research conducted on lake trout fry, whereas our study focused on juvenile lake trout (Roberts et al., 2025). Given that developing organisms are generally more susceptible to the adverse effects of pollutants, the concentration selected may not have been high enough to elicit a change in SMR (McKim, 1977). These observations highlight the complexity of metabolic responses to 6PPD-quinone and suggest that additional research is needed to explore the factors driving species-specific metabolic responses.

While several studies have examined the impacts of 6PPD-quinone exposure on resting oxygen consumption rates in fish (Ricarte et al., 2023; Selinger et al., 2024; Varshney et al., 2022), none have examined the impacts on swimming or active oxygen consumption rates at different levels of sustained activity. AMR, a measure of the energy expenditure during periods of increased activity, was determined as the metabolic rate of fish at the maximum sustainable velocity during the swimming challenge. This decrease in AMR could be attributed to internal hypoxia, potentially mediated by physiological limitations in oxygen uptake or utilization (Mahoney et al., 2022; Selinger et al., 2024). Evidence from a previous study in our group demonstrated that oxygen consumption rates increased 2-fold in rainbow trout gill cells exposed to 20 μg/L of 6PPD-quinone,

while no comparable effects were observed in liver cells (Mahoney et al., 2022). While this finding suggests that gill cells could be a primary target of 6PPD-quinone toxicity, resulting in impaired gas exchange or reduced oxygen availability at the gills, it is important to acknowledge that respiratory stress can arise from a variety of mechanisms, including generalized stress responses to toxic insult (Wendelaar Bonga, 1997). Therefore, the specific contribution of the gills remains speculative and warrants further investigation. Additionally, it was hypothesized that 6PPD-quinone exposure may impair oxygen utilization due to electron transport chain uncoupling, which could compromise mitochondrial function and limit the energy available for sustained swimming performance (Mahoney et al., 2022). As a result, fish may reduce their activity levels to conserve energy and adapt to the reduced oxygen supply, leading to a decrease in AMR. These findings may indicate a diminished capacity to sustain energetically demanding activities, which could impair overall physiological performance and reduce survival in competitive environments. The decline in AMR also prompted a decrease in AS, which is defined as the difference between an organism's maximum aerobic metabolic rate and its SMR. This measure represents the range of metabolic rates in which a fish can sustain aerobic activities without experiencing fatigue (Halsey et al., 2018). Reduced aerobic scope has been linked to impaired swimming performance in several studies on fish exposed to environmental stressors or contaminants, leading to decreased endurance and a diminished capacity for sustained swimming (Eliason et al., 2013; Soofiani & Priede, 1985). Specifically, fish with a reduced aerobic scope often experience fatigue more rapidly and are less capable of maintaining critical swimming speeds, which are essential for behaviors such as prey capture, predator avoidance, migration, and habitat selection

(Cano-Barbacil et al., 2020; Fu et al., 2022). Over time, energy expended to compensate for impaired swimming performance could divert resources from growth and development, ultimately affecting the long-term health of fish populations.

Sublethal exposure to 6PPD-quinone resulted in reduced swimming performance in lake trout. This decrease was evident from the significantly lower U_{crit} observed in exposed fish compared to control fish. U_{crit}, known as the critical swimming speed, represents the maximum swimming velocity fish could sustain during the critical swim challenge. This measure is the highest speed fish can swim for an extended period without becoming exhausted, which is crucial for understanding changes in locomotor capabilities (Brett, 1964). During the swim challenges, the decrease in U_{crit} was visually apparent, as a sharp decline occurred in the number of fish capable of swimming after the third incremental increase in water velocity. Specifically, out of the 12 control and 10 treated fish that completed the second velocity (1.8 BL/sec), only 9 control and 4 treated fish managed to complete the third velocity (2.3 BL/sec). However, despite utilizing velocities comparable to those tested in the literature (Massé et al., 2013; Thomas & Janz, 2014), a significant number of fish failed to complete even the first increment (1.3 BL/sec). Specifically, 33% of control and 39% of treated fish were not capable of maintaining swimming performance at this initial velocity. The inability to sustain swimming was largely due to avoidance behaviors, such as wedging their tails into the rear grid of the swim tunnel or burst swimming and biting onto the front of the chamber to avoid swimming against the current. Although similar avoidance behaviors have been observed in other studies (Gilbert et al., 2014; Magnhagen et al., 2018), we visually noted that these behaviours seemed to occur more frequently in treated fish, particularly at the

higher velocities. However, future studies with more precise tracking methods and quantitative assessments will be needed to provide a clearer understanding of these avoidance strategies and their effects on swim performance. Significant dysregulation in the mobilization of energy stores was observed in juvenile lake trout exposed to 6PPD-quinone. While concentrations of muscle glycogen remained unchanged, exposed fish that actively swam during the critical swimming challenge had significantly lower triglyceride reserves than control fish. Glycogen and triglycerides are the two major forms of energy storage in fishes (Johnson et al., 2004). During low-intensity aerobic activities such as sustained and prolonged swimming, slow oxidative red skeletal muscle fibers primarily use triglycerides as a fuel source to produce energy through aerobic metabolism. Conversely, in high-intensity aerobic activities such as burst swimming, fast glycolytic white skeletal muscle fibers primarily use glycogen as a fuel source for energy production through anaerobic metabolism (Hammer, 1995). Thus, the absence of glycogen depletion is likely attributable to fish not reaching water velocities sufficient to induce burst swimming and shift to anaerobic metabolism. Alternatively, the increased depletion of triglycerides may indicate that lake trout exposed to 6PPD-quinone require additional fuel to sustain low-intensity activities, despite a considerable reduction in swim performance. Collectively, these findings provide valuable insights into how 6PPD-quinone exposure alters energy utilization in fish, potentially compromising their ability to perform essential swimming behaviors.

3.6 Conclusion

The findings of this study demonstrate that exposure to 6PPD-quinone resulted in decreased swimming performance and dysregulation of aerobic metabolic capacity in

juvenile lake trout. Specifically, exposure to an environmentally relevant concentration of 6PPD-quinone caused decreases in AMR, AS, U_{crit}, and muscle triglycerides in swimming fish. These observations suggest an adaptive response, where fish reduce activity levels to conserve energy and cope with reduced oxygen supply, possibly due to impaired oxygen utilization. In the future, research should further analyze the sublethal effects, which are often more subtle and may not be immediately apparent. However, these effects can still impart significant long-term implications for the health, growth, reproduction, and survival of fish populations. Studies should aim to incorporate a broader range of concentrations to deepen understanding of dose-response dynamics and their influence on swimming performance and metabolism across different exposure scenarios. Additionally, studies should include repeated critical and burst swimming challenges to assess recovery rates and further characterize impacts on swimming performance. By characterizing these sublethal effects, we can better predict and manage the ecological impacts of 6PPD-quinone, ensuring the long-term health of aquatic ecosystems.

CHAPTER 4

4.0 GENERAL DISCUSSION

4.1 Project Rationale and Summary

Investigating the sublethal effects of 6PPD-quinone on salmonids addresses critical gaps in our understanding of the contaminant's impacts on aquatic species. With increasing reports of 6PPD-quinone in road runoff and surface waters, its strong potential to disrupt fish physiology highlights the need for comprehensive research into both lethal and sublethal effects. While it has been previously established that acute exposure to 6PPD-quinone is lethal to many salmonid species at environmentally relevant concentrations, my thesis research has, for the first time, confirmed that acute exposure also results in significant sublethal toxicities in juvenile salmonids. Particularly, this study demonstrated that 6PPD-quinone exposure adversely affects cardiovascular function, metabolic capacity, and swimming performance in sensitive species such as rainbow trout and lake trout. Additionally, this research revealed that even tolerant species, such as Arctic char, experience metabolic changes when exposed to 6PPD-quinone. This discovery contributes to the growing body of evidence that species previously considered resilient to this contaminant are impacted by exposure.

Chapter two aimed to compare the cardiovascular and metabolic effects of 6PPD-quinone exposure in two salmonid species of differing sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. This study was designed to shed light on the underlying mechanisms of species-specific toxicity observed in related salmonids. The findings revealed distinct cardiovascular and metabolic responses between the species, with nearly all significant effects observed in the sensitive rainbow trout.

Exposure to 6PPD-quinone led to increased oxygen consumption rates and altered hemoglobin dynamics in rainbow trout, suggesting impaired oxygen utilization. This impairment triggered a range of compensatory cardiac responses aimed at improving oxygen delivery. The most notable changes included prolonged PR interval length, decreased end-systolic volume, and increased passive ventricular filling, contributing to a significant increase in cardiac output. In contrast, the primary effect observed in Arctic char was an increase in oxygen consumption, with no significant changes in cardiovascular physiology. This reflects the acute tolerance of Arctic char to 6PPD-quinone, which did not display compensatory cardiovascular adjustments like those seen in rainbow trout.

Chapter three aimed to assess changes in aerobic metabolism and swimming performance in a sensitive species, lake trout. This study was designed to analyze the specific components of metabolism that are altered by 6PPD-quinone exposure, such as resting metabolism, active metabolism, and energy homeostasis. The results of this study demonstrate that environmentally relevant 6PPD-quinone exposure adversely affected swimming performance and dysregulates aerobic metabolic capacity in juvenile lake trout. Specifically, exposure resulted in significant decreases in active metabolic rate, aerobic scope, critical swimming speed, and muscle triglycerides in swam fish. These findings indicate that 6PPD-quinone impaired the ability to efficiently utilize stored energy or oxygen, metabolic functions that are essential for sustained swimming.

Overall, the findings in my thesis highlight the need for further research efforts to assess the sublethal effects of 6PPD-quinone exposure on aquatic life. While urban fish exposed to higher levels of 6PPD-quinone are known to be at risk, this study revealed

that even fish in areas with lower concentrations are also vulnerable to adverse impacts. Moreover, these data are invaluable for understanding the underlying causes of the characteristic cardiorespiratory symptoms and atypical swimming behaviour observed in sensitive species before lethality occurs. By uncovering these sublethal impacts, this research contributes to a more comprehensive understanding of the risk 6PPD-quinone poses to aquatic life.

4.2 Overall Findings

4.2.1 6PPD-quinone exposures

Like most aquatic contaminants, fish are primarily exposed to 6PPD-quinone through the gills, which have also been hypothesized as the primary target organ (Mahoney et al., 2022). Therefore, to conduct acute exposures with lake trout, rainbow trout, and Arctic char, 6PPD-quinone was added into facility water. The concentrations and durations of exposure were carefully selected based on previously reported LC_{50} values (sensitive species) or maximum exposure concentration (tolerant species). For instance, in the cardiorespiratory study, rainbow trout were exposed to a nominal concentration of 1.00 μg/L for 48 h, which was based on a previously measured 72-h LC_{50} of 1.00 μg/L (Brinkmann et al., 2022). This resulted in a measured concentration of 0.59 μg/L, which ensured that sublethal effects were captured without inducing mortality. Likewise, Arctic char were exposed to a nominal concentration of 10 μg/L for 48 h, slightly below the maximum exposure concentration reported, which was 96 h at 14.2 μg/L (Brinkmann et al., 2022). A similar approach was applied in determining exposure concentrations for the swimming performance study. In this case, lake trout were exposed to a nominal concentration of 0.5 μg/L for 20 h, resulting in a measured concentration of

0.46 µg/L. This was just below the reported 96-h LC_{50} of 0.50 µg/L for lake trout (Roberts et al., 2025). The rationale of choosing these concentrations and durations was to allow the observation of sublethal effects under non-lethal concentrations where significant physiological impacts could be detected. For the sensitive species, lake trout and rainbow trout, the chosen exposure concentrations fell well within the range of surface water concentrations reported in North America (0.086-2.3 µg/L) (Challis et al., 2021; Johannessen et al., 2022a; Tian et al., 2021, 2022). In addition, 6PPD-quinone was not detected in the control tanks at any point during the exposures, ensuring that the observed effects could be attributed solely to 6PPD-quinone exposure. Moreover, concentrations remained consistent and statistically comparable among repeated trials, suggesting good reproducibility of the experimental design.

Exposure durations were relatively short due to the transient and pulsed nature of 6PPD-quinone occurrence in the environment. Analytical results indicated a mean loss of 56% and 52% of 6PPD-quinone over a 24-h period in Arctic char and rainbow trout, respectively. Thus, daily renewals were performed to maintain stable exposure levels throughout the experiments. In the lake trout exposures, a mean loss of 38% was recorded over the 20-h exposure period. In this study, static renewal was not performed due to the shorter exposure duration. Observed losses across all three species were notably higher than those reported by other researchers, such as Brinkmann et al. (2022), who reported an average loss of 14% over a similar 24-h period. This discrepancy may be attributed to additional equipment or plastic tubing used in our setup, which was required to perform respirometry over the exposure period. It is hypothesized that the extra material

potentially provided a sorption site for the compound, thereby reducing concentrations in the exposure water.

4.2.2 Metabolic changes

Exposure to 6PPD-quinone altered the metabolic physiology of all three salmonid species analyzed in this thesis research. Blood gas changes were assessed only in rainbow trout and Arctic char and significant effects of 6PPD-quinone exposure were observed solely in rainbow trout. These findings provide valuable insights into how 6PPD-quinone disrupts physiological processes in fish. In chapter two, it was shown that exposure to 6PPD-quinone dysregulated aerobic metabolism in juvenile rainbow trout and Arctic char. Specifically, increased resting oxygen consumption rates (SMR) were observed in both species, indicating a heightened metabolic demand following exposure. This increase aligns with observations from a previous study in our laboratory, which reported a two-fold increase in oxygen consumption rates in rainbow trout gill cells exposed to 6PPD-quinone (Mahoney et al., 2022). However, these findings did not align with results from chapter three, which did not observe significant changes to SMR in lake trout. The absence of a change in SMR in lake trout agrees with findings by Ricarte et al. (2023), who reported no change in oxygen consumption rates in a non-salmonid species, zebrafish. These findings indicate that responses to 6PPD-quinone exposure vary not only between sensitive and tolerant species, but also among sensitive species. Although it is not definitively known why SMR increased in Arctic char and rainbow trout but not lake trout, this difference may be attributable to differences in exposure duration. Arctic char and rainbow trout were exposed for more than twice as long as lake trout (48 h vs. 20 h), which may have been insufficient to induce a detectable change in SMR in lake trout.

Moreover, the exposure concentrations in chapter two were based on research conducted using fry, while this study focused on juvenile individuals. Developing organisms are generally more sensitive to pollutants, which suggests that the chosen concentration may not have been adequate to trigger a change in SMR in older lake trout (McKim, 1977). This heightened sensitivity in younger individuals has already been observed with 6PPD-quinone, where juvenile coho salmon displayed an LC_{50} that was 2.3 times lower than that of 1+-year-old coho salmon (Lo et al., 2023; Tian et al., 2021, 2022).

Although SMR remained unchanged, 6PPD-quinone significantly decreased both AS and AMR in lake trout. AMR, defined as the maximum metabolic rate of fish at their maximum sustainable velocity during the critical swimming test, can be used to determine AS — a measure of a fish's capacity to sustain physical exertion. If data from one sensitive species (rainbow trout) can be extrapolated to another sensitive species (lake trout), it may be hypothesized that the decreased active metabolic rate and aerobic scope are linked to increased methemoglobin, which impairs oxygen delivery to the tissues. Regardless of the underlying mechanism — whether mitochondrial toxicity, disruption of gill function, or impaired oxygen transport — when fish experience internal hypoxia, they may compensate by reducing activity levels to conserve energy (Z. Wang et al., 2023). This behavioural adaption, while beneficial in the short term, could have long-term implications for growth, reproductive success, and overall population health.

4.2.3 Cardiovascular changes

Cardiac structure and function were evaluated in both sensitive and tolerant species, yet cardiovascular alterations were observed only in the sensitive species. This thesis represents the first comprehensive examination of the effects of 6PPD-quinone on

the cardiovascular system of fish beyond heart rate, and specifically on the cardiovascular system of salmonid fishes. Cardiac performance is typically regulated in response to oxygen availability and metabolic demand, suggesting increased oxygen consumption would lead to greater cardiac output in both species. However, the findings revealed that only the sensitive species, rainbow trout, exhibited cardiovascular changes following exposure. This aligns with blood gas changes observed exclusively in rainbow trout, such as decreases in oxygenated hemoglobin and increases in deoxyhemoglobin, indicating a state of internal hypoxia. The absence of cardiovascular changes in Arctic char, despite their increased oxygen consumption, suggests several possible explanations. First, Arctic char may possess physiological mechanisms that provide greater resilience to hypoxic conditions, allowing them to maintain cardiovascular stability even under stress. These mechanisms could include more efficient oxygen transport or utilization systems, or an enhanced capacity to buffer against oxidative stress (Ducros et al., 2023). Secondly, toxicokinetic differences may be responsible. Previous research indicated that levels of an intermediary metabolite of 6PPD-quinone were higher in tolerant species than in sensitive ones, suggesting that tolerant species might detoxify 6PPD-quinone more effectively (Montgomery et al., 2023). Additionally, differences in the expression of stress-responsive genes between the two species could play a role. Arctic char might exhibit a higher expression of protective genes that mitigate the adverse effects of 6PPD-quinone exposure, preserving their cardiovascular function (Inderberg et al., 2021). Understanding these mechanisms would require targeted studies to compare the genetic, biochemical and physiological responses of sensitive and tolerant species, enhancing our understanding of the species-specific toxicity of 6PPD-quinone.

The cardiovascular changes observed in rainbow trout suggest a response to increased energy demands induced by exposure to 6PPD-quinone, potentially involving sympathetic stimulation. Under such conditions, the body adjusts cardiac function to increase oxygen delivery to tissues and meet increased metabolic requirements (Reid et al., 1998). Cardiac function is typically upregulated by sympathetic stimulation, which increases heart rate through the release of catecholamines like norepinephrine and epinephrine (Reid et al., 1998). These neurotransmitters bind to β-adrenergic receptors on the pacemaker cells of the heart, increasing the rate of depolarization and resulting in a faster heart rate (Ju & Allen, 1999). This also shortens the PR interval and QT interval due to faster conduction through the AV node and accelerated repolarization (Taylor et al., 2009; Wendelaar Bonga, 1997). Moreover, sympathetic stimulation increases myocardial contractility, leading to a reduction in ESV and an increase in V_S. This facilitates greater blood ejection per heartbeat, thereby enhancing CO, which is the product of heart rate and V_S (Gordan et al., 2015). Additionally, increased calcium influx into cardiac cells enhances contractile strength, further contributing to the decrease in ESV (Taylor et al., 2009; Wendelaar Bonga, 1997). These changes ensure that tissues receive an adequate blood supply under physiological stress and increased metabolic demand. While chapter two indicates possible sympathetic stimulation through decreased end-systolic volume and increased CO, discrepancies arise with findings such as a prolonged PR interval and the absence of expected changes in heart rate, V_S, and QT interval length. This implies that the level of sympathetic stimulation, if present, is occurring at a moderate to low level. Despite reductions in end-systolic volume, these changes may not be sufficient to significantly increase V_S. Moreover, the concurrent

presence of sympathetic stimulation and an electrical abnormality like first-degree AV block could potentially counteract each other, obscuring the effects of sympathetic activity on heart rate. The exact role of sympathetic stimulation in these observed changes remains unclear; future studies should consider introducing an adrenergic blocker to reassess cardiovascular responses and determine if the indicators of sympathetic stimulation persist.

4.2.4 Swim performance and bioenergetics

Lake trout exposed to 6PPD-quinone experience significant disruptions in aerobic capacity, swimming performance, and energy utilization. The impairments in swimming performance are particularly evident through a notably reduced U_{crit}, which indicates a decreased ability to sustain prolonged physical activity. Consequently, a compromised swimming ability increases the risk of predation for exposed fish and makes it more challenging for them to obtain food, directly impacting their survival and growth (Plaut, 2001b). However, the diminished swimming ability in exposed fish is evident not only from their significantly reduced U_{crit}, but also from their reluctance to swim. This was visually apparent, as substantially fewer exposed fish managed to surpass the early swimming velocities during the challenge. For instance, only 25% fewer control fish were able to complete the third velocity (2.3 BL/sec) compared to the second velocity (1.8 BL/sec). In contrast, 60% fewer exposed fish managed to reach the third velocity. This reduced swimming performance was accompanied by abnormal behaviours such as tail wedging and burst swimming to avoid water flow, further illustrating the impact of 6PPD-quinone on the locomotor abilities of juvenile salmonids.

Bioenergetics in fish, particularly in salmonids, is a crucial area of study as it uncovers how energy is allocated and utilized during various activities, including swimming. In this research, fish exhibited a higher rate of triglyceride depletion despite a reduced swimming performance. This was interesting because it seemed the fish needed more energy to face the challenge. Essentially, the exposed fish use more energy to swim less effectively, resulting in doubly negative outcomes. Although the ability to mobilize energy stores remains unaffected, the amount exposed fish need to mobilize is altered, implying an increased energetic cost or impaired metabolic efficiency, which aligns with other findings of my thesis research. The increased utilization leaves less available for other essential behaviours, resulting in a series of consequences. For example, energy reserves are important for reproductive activities such as mating displays, spawning migrations, or nurturing offspring (Liu et al., 2022). Lower energy reserves can also make fish less able to compete for territory, mates, and food, when compared to healthier individuals. Furthermore, constantly operating at higher energy levels can lead to chronic stress, affecting overall health and immune function (Dai et al., 2023). In addition, energy diverted to meet increasing metabolic demands might have otherwise been used for growth, leading to stunted growth and reduced size over time (Liu et al., 2022). Finally, for species that undergo long migrations, reduced energy reserves can impair their ability to reach spawning or feeding grounds, causing effects at the population level. In conclusion, the findings clearly demonstrate the impact of 6PPD-quinone on the bioenergetics and swimming performance of salmonids, highlighting significant implications for both individual fitness and population dynamics.

4.3 Conclusions

My thesis research offers a thorough evaluation of the sublethal effects of 6PPD-quinone, focusing on the underlying metabolic and cardiovascular responses to acute exposure in juvenile salmonids. By examining physiological disruptions in rainbow trout, Arctic char, and lake trout, this research revealed critical differences in species-specific responses, highlighting the complex nature of toxic effects on different salmonid species. The study employed a variety of methods, such as cardiovascular ultrasound, critical swimming challenges, and biochemical analyses, to assess the impact of 6PPD-quinone. The combination of these techniques allowed for a detailed analysis of both behavioural and physiological responses, providing a comprehensive understanding of the contaminant's effects.

One of the key revelations of this research is the species-specific variation in cardiorespiratory response to 6PPD-quinone exposure. While all three species showed signs of cardiorespiratory disruption, the severity and nature of these effects varied, suggesting differential sensitivity and adaptive mechanisms. Rainbow trout exhibited significant physiological stress responses to 6PPD-quinone, including increased oxygen consumption and compensatory cardiovascular changes such as increased contractility and enhanced cardiac output. These findings highlight the rainbow trout's sensitivity to 6PPD-quinone, and the physiological adjustments made to maintain oxygen homeostasis under toxic stress. In contrast, Arctic char demonstrated a higher tolerance, with increased oxygen consumption as the only observed effect, indicating their relative resilience to exposure. Lake trout showed significant impairments in swimming performance and aerobic metabolism, with decreased active metabolic rates, aerobic

scope, and critical swim speed observed in 6PPD-quinone-exposed fish. The reduced aerobic capacity and increased energy demand observed in exposed fish indicate significant metabolic disruptions, which were further corroborated by the increased utilization of triglycerides in muscle tissue. These changes suggest that 6PPD-quinone exposure disrupts energy balance and increases metabolic demands, resulting in reduced swimming efficiency and overall fitness.

In conclusion, my thesis research has significantly advanced our understanding of the sublethal effects of 6PPD-quinone on juvenile salmonids, providing valuable insights into the metabolic and cardiovascular disruptions it causes. The implications of my findings are significant, particularly for environmental risk assessments and the conservation of salmonid populations. These results emphasize that fish exposed to lethal levels of 6PPD-quinone are not the only ones at risk; even fish in areas with sublethal concentrations are susceptible to adverse impacts. Ultimately, my research not only improved our understanding of the sublethal effects of 6PPD-quinone exposure on juvenile salmonids but also highlighted the importance of species-specific assessments to fully comprehend the complex impacts of such contaminants. Moreover, it highlighted the urgent need for targeted approaches to mitigate the widespread threat posed by 6PPD-quinone in aquatic environments and protect the health of salmonid populations.

4.4 Future Considerations

Despite the novel findings in my research, there are still several areas that need further investigation to provide a complete picture of sublethal 6PPD-quinone toxicities in salmonids.

- In my thesis research, I could demonstrate that environmentally relevant 6PPD-quinone exposure can alter cardiovascular physiology, aerobic metabolism, and swimming performance at sublethal concentrations. Future studies should investigate similar endpoints at lower concentrations to determine the threshold (e.g., EC_{50}) needed to induce these physiological changes.
- In my thesis research, I could demonstrate that sublethal 6PPD-quinone exposure could alter cardiovascular and metabolic physiology in juvenile salmonids. Future studies should assess these endpoints across different life stages to understand how life stage affects the severity and number of sublethal effects.
- In my thesis research, I investigated acute 6PPD-quinone exposure, with all exposure durations under 48 h. Future studies should assess the chronic effects of exposure on growth, reproduction, and survival rates to fully understand the ecological impact of 6PPD-quinone.
- In my thesis research, I investigated the effects of 6PPD-quinone at a fixed temperature and dissolved oxygen concentration. Future studies should explore the interactions between 6PPD-quinone and other environmental stressors, such as temperature fluctuations and hypoxia, to provide a more comprehensive understanding of its impact under real-world conditions.
- In my thesis research, I exposed fish to 6PPD-quinone only. Future studies should investigate the synergistic effects of 6PPD-quinone with other

pollutants commonly found in aquatic environments to gain a more accurate understanding of the challenges aquatic organisms face in the environment.

- In my thesis research, I exposed fish continuously to 6PPD-quinone before cardiovascular function and swimming ability were characterized. Future studies should assess whether movement into freshwater after exposure causes sublethal effects to subside or persist.
- In chapter two, several observed effects, such as prolonged PR interval and increased methemoglobin, may result from oxidative stress. Future studies should look for markers of oxidative stress in the heart, as they have been previously identified in the liver and gill.
- In chapter two, I demonstrated that sublethal 6PPD-quinone exposure caused cardiovascular changes in sensitive species, rainbow trout, but not a tolerant species, Arctic char. Future studies should investigate these endpoints in fish exposed to lethal concentrations at time points closer to mortality to better understand the species-specific lethality observed in salmonids. Additionally, these endpoints should be analyzed in other sensitive species, such as coho salmon, to confirm similar responses and in other tolerant species, such as brown trout, to ensure a lack of cardiovascular changes.
- In chapter two, I demonstrated that 6PPD-quinone exposure can cause cardiovascular alterations suggestive of sympathetic stimulation. Future studies should determine if this mechanism is responsible for observed increases in contractility, passive ventricular filling, and cardiac output by introducing an adrenergic blocking agent to see if responses are reversed.

- In chapter two, I demonstrated that 6PPD-quinone exposure caused increased blood methemoglobin in a sensitive species, rainbow trout. Future studies should investigate the mechanisms involved in its increased production.
- In chapter two, I demonstrated that 6PPD-quinone exposure increased oxygen consumption rates *in vivo* in both Arctic char and rainbow trout. It was previously observed that *in vitro* exposure of rainbow trout gill cells resulted in increased oxygen consumption rates. Future studies should investigate if oxygen consumption rates are also increased in the gill cells of a tolerant species, such as Arctic char.
- In chapter three, I demonstrated that sublethal 6PPD-quinone exposure significantly reduced critical swim speed during a critical swimming challenge. Future studies should better characterize the effects of exposure on swimming performance by conducting repeated critical swimming challenges and burst swimming challenges to gain insights into the recovery rates of fish following exposure.
- In chapter three, I demonstrated that sublethal 6PPD-quinone exposure impaired swimming performance and aerobic metabolism in a sensitive species, lake trout. Future studies should investigate whether sublethal exposure elicits similar effects in a tolerant species, such as Arctic char.
- In chapter three, I demonstrated that sublethal 6PPD-quinone exposure impaired swimming performance in a sensitive species, lake trout. Future research should look for these effects in stronger-swimming salmonid species, such as migratory rainbow trout or coho salmon.

- In chapter three, I demonstrated that sublethal 6PPD-quinone exposure resulted in increased utilization of triglyceride energy stores in lake trout during a critical swimming challenge. Future research should investigate changes in enzyme activities and gene expression related to energy mobilization and lipid metabolism to provide deeper insights into the pathways affected by 6PPD-quinone.

LIST OF REFERENCES

Ackerly, K. L., Roark, K. J., Lu, K., Esbaugh, A. J., Liu, Z., & Nielsen, K. M. (2024). Acute Toxicity Testing of 6PPD-Quinone on the Estuarine-Dependent Sport Fish, *Sciaenops ocellatus*. *Ecotoxicology*, *33*(6), 582–589. https://doi.org/10.1007/s10646-024-02755-x

Affonso, E. G., Polez, V. L. P., Corrêa, C. F., Mazon, A. F., Araújo, M. R. R., Moraes, G., & Rantin, F. T. (2002). Blood Parameters and Metabolites in the Teleost Fish *Colossoma macropomum* Exposed to Sulfide or Hypoxia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(3), 375–382. https://doi.org/10.1016/S1532-0456(02)00127-8

Alaska Department of Fish & Game. (2006). *Arctic Char* (Salvelinus alpinus) *Species Status Review*. https://westernnativetrout.org/wp-content/uploads/2019/07/wnti-species_assessment_arctic-char_adfg_20130318.pdf

Alves-Bezerra, M., & Cohen, D. E. (2017). Triglyceride Metabolism in the Liver. *Comprehensive Physiology*, *8*(1), 1–22. https://doi.org/10.1002/CPHY.C170012

Anderson-Bain, K., Roberts, C., Kohlman, E., Ji, X., Alcaraz, A. J., Miller, J., Gangur-Powell, T., Weber, L., Janz, D., Hecker, M., Montina, T., Brinkmann, M., & Wiseman, S. (2023). Apical and Mechanistic Effects of 6PPD-Quinone on Different Life-Stages of the Fathead Minnow *(Pimephales promelas)*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *271*, 109697. https://doi.org/10.1016/j.cbpc.2023.109697

Barold, S. S. (2018). Type I Wenckebach Second-Degree AV Block: A Matter of Definition. *Clinical Cardiology*, *41*(3), 282–284. https://doi.org/10.1002/clc.22874

Beamish, F. W. H. (1978). Swimming Capacity. In W. S. Hoar & D. J. B. J. R. Randall (Eds.), *Fish Physiology* (Vol. 7, Issue C, pp. 101–187). https://doi.org/10.1016/S1546-5098(08)60164-8

Besser, J. M., Dorman, R., Ivey, C. D., Cleveland, D., & Steevens, J. A. (2020). Sensitivity of Warm-Water Fishes and Rainbow Trout to Selected Contaminants. *Bulletin of Environmental Contamination and Toxicology*, *104*(3), 321–326. https://doi.org/10.1007/s00128-020-02788-y

Brett, J. R. (1964). The Respiratory Metabolism and Swimming Performance of Young Sockeye Salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *21*(5), 1183–1226.

Brett, J. R. (1965). The Relation of Size to Rate of Oxygen Consumption and Sustained Swimming Speed of Sockeye Salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada*, *22*(6), 1491–1501. https://doi.org/10.1139/f65-128

Brinkmann, M., Montgomery, D., Selinger, S., Miller, J. G. P., Stock, E., Alcaraz, A. J., Challis, J. K., Weber, L., Janz, D., Hecker, M., & Wiseman, S. (2022). Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance. *Environmental Science & Technology Letters*, *9*(4), 333–338. https://doi.org/10.1021/acs.estlett.2c00050

Brodeur, J. C., Dixon, D. G., & McKinly, R. S. (2001). Assessment of Cardiac Output as a Predictor of Metabolic Rate in Rainbow Trout. *Journal of Fish Biology*, *58*(2), 439–452. https://doi.org/10.1111/j.1095-8649.2001.tb02263.x

Brown, C., Gardner, C., & Braithwaite, V. A. (2005). Differential Stress Responses in Fish from Areas of High-and Low-Predation Pressure. *Journal of Comparative Physiology B*, *175*(5), 305–312. https://doi.org/10.1007/s00360-005-0486-0

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a Metabolic Theory of Ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Cai, L., Fang, M., Johnson, D., Lin, S., Tu, Z., Liu, G., & Huang, Y. (2014). Interrelationships between feeding, food deprivation and swimming performance in juvenile grass carp. *Aquatic Biology*, *20*(1), 69–76. https://doi.org/10.3354/AB00546

California Department of Toxic Substances Control. (2021). *Product – Chemical Profile for Motor Vehicle Tires Containing N-(1,3-Dimethylbutyl)-N'-phenyl-pphenylenediamine (6PPD)*. *June*, 89. https://dtsc.ca.gov/wp-content/uploads/sites/31/2021/06/2021-Product-Chemical-Profile-for-Motor-Vehicle-Tires-Containing-6PPD-Discussion-Draft_ADA.pdf

Campbell, N. A. , 1946-2004. (1987). *Biology*. Benjamin/Cummings Pub. Co.

Cano-Barbacil, C., Radinger, J., Argudo, M., Rubio-Gracia, F., Vila-Gispert, A., & García-Berthou, E. (2020). Key Factors Explaining Critical Swimming Speed in Freshwater Fish: A Review and Statistical Analysis for Iberian Species. *Scientific Reports*, *10*(1), 18947. https://doi.org/10.1038/s41598-020-75974-x

Cao, G., Wang, W., Zhang, J., Wu, P., Zhao, X., Yang, Z., Hu, D., & Cai, Z. (2022a). New Evidence of Rubber-Derived Quinones in Water, Air, and Soil. *Environmental Science & Technology*, *56*(7), 4142–4150. https://doi.org/10.1021/acs.est.1c07376

Cao, G., Wang, W., Zhang, J., Wu, P., Zhao, X., Yang, Z., Hu, D., & Cai, Z. (2022b). New Evidence of Rubber-Derived Quinones in Water, Air, and Soil. *Environmental Science & Technology*, *56*(7), 4142–4150. https://doi.org/10.1021/acs.est.1c07376

Castan, S., Sherman, A., Peng, R., Zumstein, M. T., Wanek, W., Hüffer, T., & Hofmann, T. (2023). Uptake, Metabolism and Accumulation of Tire Wear Particle-Derived Compounds in Lettuce. *Environmental Science & Technology*, *57*(1), 168–178. https://doi.org/10.1021/acs.est.2c05660

Chabot, D., McKenzie, D. J., & Craig, J. F. (2016). Metabolic Rate in Fishes: Definitions, Methods and Significance for Conservation Physiology. *Journal of Fish Biology*, *88*(1), 1–9. https://doi.org/10.1111/jfb.12873

Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The Determination of Standard Metabolic Rate in Fishes. *Journal of Fish Biology*, *88*(1), 81–121. https://doi.org/10.1111/jfb.12845

Challis, J. K., Popick, H., Prajapati, S., Harder, P., Giesy, J. P., McPhedran, K., & Brinkmann, M. (2021). Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff. *Environmental Science & Technology Letters*, *8*(11), 961–967. https://doi.org/10.1021/acs.estlett.1c00682

Chan, T. C. (2005). P Wave. In *ECG in Emergency Medicine and Acute Care* (pp. 48–52). Elsevier. https://doi.org/10.1016/B978-0-323-01811-1.50020-1

Cheng, S., Keyes, M. J., Larson, M. G., McCabe, E. L., Newton-Cheh, C., Levy, D., Benjamin, E. J., Vasan, R. S., & Wang, T. J. (2009). Long-term Outcomes in Individuals With Prolonged PR Interval or First-Degree Atrioventricular Block. *JAMA*, *301*(24), 2571–2577. https://doi.org/10.1001/JAMA.2009.888

Chow, M. I., Lundin, J. I., Mitchell, C. J., Davis, J. W., Young, G., Scholz, N. L., & McIntyre, J. K. (2019). An Urban Stormwater Runoff Mortality Syndrome in Juvenile Coho Salmon. *Aquatic Toxicology*, *214*, 105231. https://doi.org/10.1016/j.aquatox.2019.105231

Cicini, M. P., Ferretti, G., Morace, N., Nisticò, C., Cognetti, F., & Rulli, F. (2022). Novel Insights from Clinical Practice Second-Degree Type 2 Atrioventricular Block Requiring Permanent Cardiac Pacing in Patients on CDK4/6 Inhibitors: Report of Two Cases Keywords Metastatic breast cancer · 4/6 inhibitors · Case report · Atrioventricular block. *Breast Care*, *17*, 330–334. https://doi.org/10.1159/000519728

Cook, P. M., Robbins, J. A., Endicott, D. D., Lodge, K. B., Guiney, P. D., Walker, M. K., Zabel, E. W., & Peterson, R. E. (2003). Effects of Aryl Hydrocarbon Receptor-Mediated Early Life Stage Toxicity on Lake Trout Populations in Lake Ontario during the 20th Century. *Environmental Science & Technology*, *37*(17), 3864–3877. https://doi.org/10.1021/ES034045M

Czarna-Juszkiewicz, D., Kunecki, P., Cader, J., & Wdowin, M. (2023). Review in Waste Tire Management—Potential Applications in Mitigating Environmental Pollution. *Materials*, *16*(17), 5771. https://doi.org/10.3390/ma16175771

Dai, C., Zheng, J., Qi, L., Deng, P., Wu, M., Li, L., & Yuan, J. (2023). Chronic Stress Boosts Systemic Inflammation and Compromises Antiviral Innate Immunity in *Carassius gibel*. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1105156

Department of Toxic Substances Control. (2022). *Chemical Profile for Motor Vehicle Tires Containing 6PPD*. https://dtsc.ca.gov/wp-content/uploads/sites/31/2022/05/6PPD-in-Tires-Priority-Product-Profile_FINAL-VERSION_accessible.pdf

Devi, S. S., & Mehendale, H. M. (2014). Quinone. *Encyclopedia of Toxicology: Third Edition*, 26–28. https://doi.org/10.1016/B978-0-12-386454-3.00350-X

Di, S., Liu, Z., Zhao, H., Li, Y., Qi, P., Wang, Z., Xu, H., Jin, Y., & Wang, X. (2022). Chiral Perspective Evaluations: Enantioselective Hydrolysis of 6PPD and 6PPD-Quinone in Water and Enantioselective toxicity to *Gobiocypris rarus* and *Oncorhynchus mykiss*. *Environment International*, *166*, 107374. https://doi.org/10.1016/j.envint.2022.107374

Ducros, L., Touaibia, M., Pichaud, N., & Lamarre, S. G. (2023). Resilience and Phenotypic Plasticity of Arctic char (*Salvelinus alpinus*) Facing Cyclic Hypoxia: Insights Into Growth, Energy Stores and Hepatic Metabolism. *Conservation Physiology*, *11*(1). https://doi.org/10.1093/conphys/coad099

Duong, T., Rose, R., Blazeski, A., Fine, N., Woods, C. E., Thole, J. F., Sotoodehnia, N., Soliman, E. Z., Tung, L., McCallion, A. S., & Arking, D. E. (2021). Development and Optimization of an *in vivo* Electrocardiogram Recording Method and Analysis Program for Adult Zebrafish. *Disease Models & Mechanisms*, *14*(8). https://doi.org/10.1242/dmm.048827

Eliason, E. J., Clark, T. D., Hinch, S. G., & Farrell, A. P. (2013). Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conservation Physiology*, *1*(1), cot008. https://doi.org/10.1093/CONPHYS/COT008

Environment Canada. (1990). *Biological Test Method: Acute Lethality Test Using Rainbow Trout*. https://www.canada.ca/content/dam/eccc/migration/main/faunescience-wildlifescience/334bfc88-6f93-4175-a185-bd49fcd5604f/en49-24-1-9-eng.pdf

Fang, L., Fang, C., Di, S., Yu, Y., Wang, C., Wang, X., & Jin, Y. (2023). Oral Exposure to Tire Rubber-derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice. *Science of The Total Environment*, *869*, 161836. https://doi.org/10.1016/j.scitotenv.2023.161836

Farrell, A. P. (2007). Tribute to P. L. Lutz: A Message from the Heart – Why Hypoxic Bradycardia in Fishes? *Journal of Experimental Biology*, *210*(10), 1715–1725. https://doi.org/10.1242/jeb.02781

Farrell, A. P., & Jones, D. R. (1992). The Heart. In W. S. Hoar, D. J. Randall, & A. P. Farrell (Eds.), *Fish Physiology* (Vol. 12a, pp. 1–88).

Finstad, A. G., Ugedal, O., & Berg, O. K. (2006). Growing Large in a Low Grade Environment: Size Dependent Foraging Gain and Niche Shifts to Cannibalism in Arctic Char. *Oikos*, *112*(1), 73–82. https://doi.org/10.1111/j.0030-1299.2006.13990.x

Fisheries and Oceans Canada. (1986). *Lake Trout*. Underwater World. https://www.dfo-mpo.gc.ca/species-especes/profiles-profils/lake-trout-touladi-eng.html

Fisheries and Oceans Canada. (2016). *Rainbow Trout*. https://www.dfo-mpo.gc.ca/species-especes/profiles-profils/rainbow-trout-truite-arc-en-ciel-eng.html

Fisheries and Oceans Canada. (2018). *Arctic Char*. https://www.dfo-mpo.gc.ca/species-especes/profiles-profils/arctic-char-omble-chevalier-eng.html

Foldvik, A., Kryuchkov, F., Sandodden, R., & Uhlig, S. (2022). Acute Toxicity Testing of the Tire Rubber–Derived Chemical 6PPD-quinone on Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Environmental Toxicology and Chemistry*, *41*(12), 3041–3045. https://doi.org/10.1002/etc.5487

Foldvik, A., Kryuchkov, F., Ulvan, E. M., Sandodden, R., & Kvingedal, E. (2024). Acute Toxicity Testing of Pink Salmon (*Oncorhynchus gorbuscha*) with the Tire Rubber–Derived Chemical 6PPD-Quinone. *Environmental Toxicology and Chemistry*, *43*(6), 1332–1338. https://doi.org/10.1002/etc.5875

French, B. F., Baldwin, D. H., Cameron, J., Prat, J., King, K., Davis, J. W., McIntyre, J. K., & Scholz, N. L. (2022). Urban Roadway Runoff Is Lethal to Juvenile Coho, Steelhead, and Chinook Salmonids, But Not Congeneric Sockeye. *Environmental Science & Technology Letters*, *9*(9), 733–738. https://doi.org/10.1021/acs.estlett.2c00467

Froelicher, V. F., & Myers, J. (2007). Manual of Exercise Testing. In *Manual of Exercise Testing*. Mosby. https://doi.org/10.1016/B978-0-323-03302-2.X5001-5

Fry, F. E. J. (1971). The Effect of Environmental Factors on the Physiology of Fish. *Fish Physiology*, *6*(C), 1–98. https://doi.org/10.1016/S1546-5098(08)60146-6

Fu, S.-J., Dong, Y.-W., & Killen, S. S. (2022). Aerobic Scope in Fishes with Different Lifestyles and Across Habitats: Trade-offs Among Hypoxia Tolerance, Swimming Performance and Digestion. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *272*, 111277. https://doi.org/10.1016/j.cbpa.2022.111277

Gaasch, W. H., Levine, H. J., Quinones, M. A., & Alexander, J. K. (1976). Left Ventricular Compliance: Mechanisms and Clinical Implications. *The American Journal of Cardiology*, *38*(5), 645–653. https://doi.org/10.1016/S0002-9149(76)80015-X

Gashkina, N. A. (2024). Metal Toxicity: Effects on Energy Metabolism in Fish. *International Journal of Molecular Sciences*, *25*(9), 5015. https://doi.org/10.3390/ijms25095015

Gerard, M. P., Graaf-Roelfsema, E., Hodgson, D. R., & Van Der Kolk, J. H. (2014). Energetic Considerations of Exercise. In *The Athletic Horse: Principles and Practice of Equine Sports Medicine* (Second, pp. 19–33). Elsevier.

Gerger, C. J., Thomas, J. K., Janz, D. M., & Weber, L. P. (2015). Acute Effects of β-Naphthoflavone on Cardiorespiratory Function and Metabolism in Adult Zebrafish (*Danio rerio*). *Fish Physiology and Biochemistry*, *41*(1), 289–298. https://doi.org/10.1007/s10695-014-9982-z

Gilbert, M. J. H. (2020). *Thermal Limits to the Cardiorespiratory Performance of Arctic Char (*Salvelinus alpinus*) in a Rapidly Warming North*. https://doi.org/10.14288/1.0394066

Gilbert, M. J. H., Zerulla, T. C., & Tierney, K. B. (2014). Zebrafish *(Danio rerio)* as a Model for the Study of Aging and Exercise: Physical Ability and Trainability Decrease with Age. *Experimental Gerontology*, *50*(1), 106–113. https://doi.org/10.1016/j.exger.2013.11.013

Gordan, R., Gwathmey, J. K., & Xie, L.-H. (2015). Autonomic and Endocrine Control of Cardiovascular Function. *World Journal of Cardiology*, *7*(4), 204. https://doi.org/10.4330/wjc.v7.i4.204

Government of Alaska. (2022). *Arctic Char Species Profile, Alaska Department of Fish and Game*. https://www.adfg.alaska.gov/index.cfm?adfg=arcticchar.main

Government of British Columbia. (1997). *Lake Trout*. B.C. Fish Facts. https://a100.gov.bc.ca/pub/eirs/finishDownloadDocument.do?subdocumentId=942

Greer, J. B., Dalsky, E. M., Lane, R. F., & Hansen, J. D. (2023). Establishing an *in vitro* Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products. *Environmental Science & Technology Letters*, *10*(6), 533–537. https://doi.org/10.1021/acs.estlett.3c00196

Hall, D. (2022). *The Complicated Tale of Salmon and Trout*. https://ocean.si.edu/ocean-life/fish/complicated-tale-salmon-and-trout

Halsey, L. G., Killen, S. S., Clark, T. D., & Norin, T. (2018). Exploring Key Issues of Aerobic Scope Interpretation in Ectotherms: Absolute versus Factorial. *Reviews in Fish Biology and Fisheries*, *28*(2), 405–415. https://doi.org/10.1007/s11160-018-9516-3

Hammer, C. (1995). Fatigue and Exercise Tests with Fish. *Comparative Biochemistry and Physiology Part A: Physiology*, *112*(1), 1–20. https://doi.org/10.1016/0300-9629(95)00060-K

Haverinen, J., & Vornanen, M. (2014). Effects of Deltamethrin on Excitability and Contractility of the Rainbow Trout (*Oncorhynchus mykiss*) Heart. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *159*(1), 1–9. https://doi.org/10.1016/j.cbpc.2013.09.004

Hiki, K., Asahina, K., Kato, K., Yamagishi, T., Omagari, R., Iwasaki, Y., Watanabe, H., & Yamamoto, H. (2021). Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species. *Environmental Science & Technology Letters*, *8*(9), 779–784. https://doi.org/10.1021/acs.estlett.1c00453

Hiki, K., & Yamamoto, H. (2022a). Concentration and Leachability of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) and its Quinone Transformation Product (6PPD-Q) in Road Dust Collected in Tokyo, Japan. *Environmental Pollution*, *302*, 119082. https://doi.org/10.1016/j.envpol.2022.119082

Hiki, K., & Yamamoto, H. (2022b). The Tire-Derived Chemical 6PPD-quinone Is Lethally Toxic to the White-Spotted Char *Salvelinus leucomaenis pluvius* but Not to Two Other Salmonid Species. *Environmental Science & Technology Letters*, *9*(12), 1050–1055. https://doi.org/10.1021/acs.estlett.2c00683

Hoar, W. S., & Randall, D. J. (1978). Preface. In *Fish Physiology* (Vol. 7, Issue C, pp. xi–xii). Elsevier Inc. https://doi.org/10.1016/S1546-5098(08)60161-2

Hu, X., Zhao, H. (Nina), Tian, Z., Peter, K. T., Dodd, M. C., & Kolodziej, E. P. (2023). Chemical Characteristics, Leaching, and Stability of the Ubiquitous Tire Rubber-Derived Toxicant 6PPD-Quinone. *Environmental Science: Processes & Impacts*, *25*(5), 901–911. https://doi.org/10.1039/D3EM00047H

Huang, W., Shi, Y., Huang, J., Deng, C., Tang, S., Liu, X., & Chen, D. (2021). Occurrence of Substituted *p* -Phenylenediamine Antioxidants in Dusts. *Environmental Science & Technology Letters*, *8*(5), 381–385. https://doi.org/10.1021/acs.estlett.1c00148

Hyun, D. H., & Lee, G. H. (2015). Cytochrome b5 reductase, a plasma membrane redox enzyme, protects neuronal cells against metabolic and oxidative stress through maintaining redox state and bioenergetics. *Age*, *37*(6), 122. https://doi.org/10.1007/S11357-015-9859-9

Inderberg, H., Neerland, E. D., McPartland, M., Sparstad, T., Bytingsvik, J., Nikiforov, V. A., Evenset, A., & Krøkje, Å. (2021). Expression of DNA Repair Genes in Arctic Char (*Salvelinus alpinus*) from Bjørnøya in the Norwegian Arctic. *Ecotoxicology and Environmental Safety*, *210*, 111846. https://doi.org/10.1016/j.ecoenv.2020.111846

Jin, X., Wang, X., Tse, W. K. F., & Shi, Y. (2022). Editorial: Homeostasis and physiological regulation in the aquatic animal during osmotic stress. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.977185

Johannessen, C. (2021). *Monitoring and Fate of Selected Tire-Derived Organic Contaminants* (Issue May). https://www.proquest.com/openview/f66bfbf1501609876fed06a5f31e77a5/1?pq-origsite=gscholar&cbl=18750&diss=y

Johannessen, C., Helm, P., Lashuk, B., Yargeau, V., & Metcalfe, C. D. (2022a). The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. *Archives of Environmental Contamination and Toxicology*, *82*(2), 171–179. https://doi.org/10.1007/s00244-021-00878-4

Johannessen, C., Helm, P., Lashuk, B., Yargeau, V., & Metcalfe, C. D. (2022b). The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. *Archives of Environmental Contamination and Toxicology*, *82*(2), 171–179. https://doi.org/10.1007/s00244-021-00878-4

Johannessen, C., Helm, P., & Metcalfe, C. D. (2021). Detection of Selected Tire Wear Compounds in Urban Receiving Waters. *Environmental Pollution*, *287*(March), 117659. https://doi.org/10.1016/j.envpol.2021.117659

Johansen, K., & Burggren, W. (1980). Cardiovascular Function in the Lower Vertebrates. In *Comparative Anatomy and Development* (pp. 61–117). Elsevier. https://doi.org/10.1016/B978-0-12-119401-7.50009-8

Johnson, N. A., Stannard, S. R., & Thompson, M. W. (2004). Muscle Triglyceride and Glycogen in Endurance Exercise. *Sports Medicine*, *34*(3), 151–164. https://doi.org/10.2165/00007256-200434030-00002

Jones, D. T., & Moffitt, C. M. (2004). Swimming Endurance of Bull Trout, Lake Trout, Arctic Char, and Rainbow Trout following Challenge with *Renibacterium salmoninarum*. *Journal of Aquatic Animal Health*, *16*(1), 10–22. https://doi.org/10.1577/H03-028.1

Ju, Y., & Allen, D. G. (1999). How Does β-Adrenergic Stimulation Increase the Heart Rate? The Role of Intracellular Ca^{2+} Release in Amphibian Pacemaker Cells. *The Journal of Physiology*, *516*(3), 793–804. https://doi.org/10.1111/j.1469-7793.1999.0793u.x

Kakizaki, T., Sato, M., Tsuruta, H., & Hasegawa, H. (1969). Oxidation of Haemoglobin by P-Quinone. *Industrial Health*, *7*, 13.

Keen, A. N., & Gamperl, A. K. (2012). Blood Oxygenation and Cardiorespiratory Function in Steelhead Trout (*Oncorhynchus Mykiss*) Challenged with an Acute Temperature Increase and Zatebradine-Induced Bradycardia. *Journal of Thermal Biology*, *37*(3), 201–210. https://doi.org/10.1016/j.jtherbio.2012.01.002

Khan, F. R., Rødland, E. S., Kole, P. J., Van Belleghem, F. G. A. J., Jaén-Gil, A., Hansen, S. F., & Gomiero, A. (2024). An Overview of the Key Topics Related to the Study of

Tire Particles and their Chemical Leachates: From Problems to Solutions. *Trends in Analytical Chemistry*, *172*, 117563. https://doi.org/10.1016/j.trac.2024.117563

Kitao, T., Sugita, Y., Yoneyama, Y., & Hattori, K. (1974). Methemoglobin Reductase (Cytochrome b5 Reductase) Deficiency in Congenital Methemoglobinemia. *Blood*, *44*(6), 879–884. https://doi.org/10.1182/BLOOD.V44.6.879.879

Klabunde, R. E. (2011). Cardiovascular Physiology Concepts. In *Cardiovascular Physiology Concepts 2nd Edition*. Lippincott Williams & Wilkins.

Kruger, R. H., Boissiere, C., Klein-Hartwig, K., & Kretzschmar, H.-J. (2005). New Phenylenediamine Antiozonants for Commodities Based on Natural and Synthetic Rubber. *Food Additives and Contaminants*, *22*(10), 968–974. https://doi.org/10.1080/02652030500098177

LaCombe, P., Tariq, M. A., & Lappin, S. L. (2024). Physiology, Afterload Reduction. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/30757937

Langenbacher, A. D., Shimizu, H., Hsu, W., Zhao, Y., Borges, A., Koehler, C., & Chen, J.-N. (2020). Mitochondrial Calcium Uniporter Deficiency in Zebrafish Causes Cardiomyopathy with Arrhythmia. *Frontiers in Physiology*, *11*, 617492. https://doi.org/10.3389/fphys.2020.617492

Lattimer, R. P., Hooser, E. R., Layer, R. W., & Rhee, C. K. (1983). Mechanisms of Ozonation of N-(1,3-Dimethylbutyl)-N′-Phenyl-p-Phenylenediamine. *Rubber Chemistry and Technology*, *56*(2), 431–439. https://doi.org/10.5254/1.3538136

Lee, G., Badertscher, P., Sticherling, C., & Osswald, S. (2021). A Rare and Reversible Cause of Third-Degree Atrioventricular Block: A Case Report. European Society of Cardiology. *European Heart Journal - Case Reports*, *5*(10). https://doi.org/10.1093/ehjcr/ytab372

Lind, L., Araujo, J. A., Barchowsky, A., Belcher, S., Berridge, B. R., Chiamvimonvat, N., Chiu, W. A., Cogliano, V. J., Elmore, S., Farraj, A. K., Gomes, A. V., McHale, C. M., Meyer-Tamaki, K. B., Posnack, N. G., Vargas, H. M., Yang, X., Zeise, L., Zhou, C., & Smith, M. T. (2021). Key Characteristics of Cardiovascular Toxicants. *Environmental Health Perspectives*, *129*(9). https://doi.org/10.1289/EHP9321

Link, C. M., Theoharides, A. D., Anders, J. C., Chung, H., Canfield, C. J., & Chung, J. C. (1985). Structure-Activity Relationships of Putative Primaquine Metabolites Causing Methemoglobin Formation in Canine Hemolysates. *Toxicology and Applied Pharmacology*, *81*, 192–202.

Liu, Y., Li, X., Li, J., & Li, Y. (2022). Insights Into Energy Accumulation and Allocation Strategy of Reproductive Migration of Black Amur Bream (*Megalobrama terminalis*) in the Pearl River Basin, China. *Frontiers in Ecology and Evolution*, *10*, 848228. https://doi.org/10.3389/fevo.2022.848228

Lo, B. P., Marlatt, V. L., Liao, X., Reger, S., Gallilee, C., Ross, A. R. S., & Brown, T. M. (2023). Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (*Oncorhynchus tshawytscha*) and Coho (*Oncorhynchus kisutch*) Salmon. *Environmental Toxicology and Chemistry*, *42*(4), 815–822. https://doi.org/10.1002/etc.5568

Lu, G., & Luo, M. (2020). Genomes of Major Fishes in World Fisheries and Aquaculture: Status, Application and Perspective. *Aquaculture and Fisheries*, *5*(4), 163–173. https://doi.org/10.1016/j.aaf.2020.05.004

Ludlow, J. T., Wilkerson, R. G., & Nappe, T. M. (2022). Methemoglobinemia. *StatPearls*. https://www-ncbi-nlm-nih-gov.cyber.usask.ca/books/NBK537317/

Magnhagen, C., Backström, T., Nilsson, J., & Brännäs, E. (2018). Oxygen Consumption and Swimming Performance in Arctic Charr with Different Pigmentation Patterns. *Applied Animal Behaviour Science*, *202*, 119–124. https://doi.org/10.1016/j.applanim.2018.01.006

Magos, L. (1964). Globin-Haemochromogen Formation caused by Quinones. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *90*(1), 55–61. https://doi.org/10.1016/0304-4165(64)90118-7

Mahoney, H., da Silva Junior, F. C., Roberts, C., Schultz, M., Ji, X., Alcaraz, A. J., Montgomery, D., Selinger, S., Challis, J. K., Giesy, J. P., Weber, L., Janz, D., Wiseman, S., Hecker, M., & Brinkmann, M. (2022). Exposure to the Tire Rubber-Derived Contaminant 6PPD-Quinone Causes Mitochondrial Dysfunction *in vitro*. *Environmental Science & Technology Letters*, *9*(9), 765–771. https://doi.org/10.1021/acs.estlett.2c00431

Masoner, J. R., Kolpin, D. W., Cozzarelli, I. M., Barber, L. B., Burden, D. S., Foreman, W. T., Forshay, K. J., Furlong, E. T., Groves, J. F., Hladik, M. L., Hopton, M. E., Jaeschke, J. B., Keefe, S. H., Krabbenhoft, D. P., Lowrance, R., Romanok, K. M., Rus, D. L., Selbig, W. R., Williams, B. H., & Bradley, P. M. (2019). Urban Stormwater: An Overlooked Pathway of Extensive Mixed Contaminants to Surface and Groundwaters in the United States. *Environmental Science & Technology*, *53*(17), 10070–10081. https://doi.org/10.1021/acs.est.9b02867

Massé, A. J., Thomas, J. K., & Janz, D. M. (2013). Reduced Swim Performance and Aerobic Capacity in Adult Zebrafish Exposed to Waterborne Selenite. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *157*(3), 266–271. https://doi.org/10.1016/j.cbpc.2012.12.004

Maurer, L., Carmona, E., Machate, O., Schulze, T., Krauss, M., & Brack, W. (2023). Contamination Pattern and Risk Assessment of Polar Compounds in Snow Melt: An Integrative Proxy of Road Runoffs. *Environmental Science & Technology*, *57*(10), 4143–4152. https://doi.org/10.1021/acs.est.2c05784

Mayer, P. M., Moran, K. D., Miller, E. L., Brander, S. M., Harper, S., Garcia-Jaramillo, M., Carrasco-Navarro, V., Ho, K. T., Burgess, R. M., Thornton Hampton, L. M., Granek, E. F., McCauley, M., McIntyre, J. K., Kolodziej, E. P., Hu, X., Williams, A. J., Beckingham, B. A., Jackson, M. E., Sanders-Smith, R. D., … Mendez, M. (2024). Where the Rubber Meets the Road: Emerging Environmental Impacts of Tire Wear Particles and their Chemical Cocktails. *Science of The Total Environment*, *927*, 171153. https://doi.org/10.1016/j.scitotenv.2024.171153

McKim, J. M. (1977). Evaluation of Tests with Early Life Stages of Fish for Predicting Long-Term Toxicity. *Journal of the Fisheries Research Board of Canada*, *34*(8), 1148–1154. https://doi.org/10.1139/f77-172

Mercier, J. C., DiSessa, T. G., Jarmakani, J. M., Nakanishi, T., Hiraishi, S., Isabel-Jones, J., & Friedman, W. F. (1982). Two-Dimensional Echocardiographic Assessment of Left Ventricular Volumes and Ejection Fraction in Children. *Circulation*, *65*(5), 962–969. https://doi.org/10.1161/01.CIR.65.5.962

Meyer, T., & Wania, F. (2008). Organic Contaminant Amplification During Snowmelt. *Water Research*, *42*(8–9), 1847–1865. https://doi.org/10.1016/j.watres.2007.12.016

Mi, K., Chen, X., Lu, K., Zhu, Y., Zhang, M., Yang, H., Wei, W., & Zhang, Y. (2021). Bisphenol A Induces Hepatic Triglyceride Level in Adult Male Rare Minnow *Gobiocypris rarus*. *Ecotoxicology and Environmental Safety*, *213*, 112050. https://doi.org/10.1016/j.ecoenv.2021.112050

Milligan, C. L., & Girard, S. S. (1993). Lactate Metabolism In Rainbow Trout. *Journal of Experimental Biology*, *180*(1), 175–193. https://doi.org/10.1242/jeb.180.1.175

Molnar, C., & Gair, J. (2015). *Citric Acid Cycle and Oxidative Phosphorylation*. Press Books. https://pressbooks.pub/icgbiology/chapter/4-3-citric-acid-cycle-and-oxidative-phosphorylation/

Montgomery, D., Ji, X., Cantin, J., Philibert, D., Foster, G., Selinger, S., Jain, N., Miller, J., McIntyre, J., de Jourdan, B., Wiseman, S., Hecker, M., & Brinkmann, M. (2023). Interspecies Differences in 6PPD-Quinone Toxicity Across Seven Fish Species: Metabolite Identification and Semiquantification. *Environmental Science & Technology*, *57*(50), 21071–21079. https://doi.org/10.1021/acs.est.3c06891

Morton, W. M. (1965). The Taxonomic Significance of the Kype in American Salmonids. *Copeia*, *1965*(1), 14. https://doi.org/10.2307/1441233

Moss, A. J. (2005). Long QT Syndrome: From Channels to Cardiac Arrhythmias. *Journal of Clinical Investigation*, *115*(8), 2018–2024. https://doi.org/10.1172/JCI25537

Nedrich, S. (2022). *Preliminary Investigation of the Occurrence of 6PPD-Quinone in Michigan's Surface Water*. https://doi.org/10.13140/RG.2.2.34478.59204

Nelson, J. A. (2016). Oxygen Consumption Rate *v.* Rate of Energy Utilization of Fishes: A Comparison and Brief History of the Two Measurements. *Journal of Fish Biology*, *88*(1), 10–25. https://doi.org/10.1111/jfb.12824

O'Dell, G. (2001). AHA Environmental Assessment. *Hospitals & Health Networks*, *75*(11), 59–61, 63–69, 2. http://www.ncbi.nlm.nih.gov/pubmed/11769592

Öner, M., Atli, G., & Canli, M. (2008). Changes in Serum Biochemical Parameters of Freshwater Fish *Oreochromis niloticus* Following Prolonged Metal (Ag, Cd, Cr, Cu, Zn) Exposures. *Environmental Toxicology and Chemistry*, *27*(2), 360–366. https://doi.org/10.1897/07-281R.1

Peake, S. (2004). An Evaluation of the Use of Critical Swimming Speed for Determination of Culvert Water Velocity Criteria for Smallmouth Bass. *Transactions of the American Fisheries Society*, *133*(6), 1472–1479. https://doi.org/10.1577/T03-202.1

Peter, K. T., Tian, Z., Wu, C., Lin, P., White, S., Du, B., McIntyre, J. K., Scholz, N. L., & Kolodziej, E. P. (2018). Using High-Resolution Mass Spectrometry to Identify Organic Contaminants Linked to Urban Stormwater Mortality Syndrome in Coho Salmon. *Environmental Science & Technology*, *52*(18), 10317–10327. https://doi.org/10.1021/acs.est.8b03287

Pettem, C. M., Weber, L. P., & Janz, D. M. (2017). Cardiac and Metabolic Effects of Dietary Selenomethionine Exposure in Adult Zebrafish. *Toxicological Sciences*, *159*(2), 449–460. https://doi.org/10.1093/toxsci/kfx149

Philibert, D., Stanton, R. S., Tang, C., Stock, N. L., Benfey, T., Pirrung, M., & de Jourdan, B. (2024). The Lethal and Sublethal Impacts of Two Tire Rubber-Derived Chemicals on Brook Trout (*Salvelinus fontinalis*) Fry and Fingerlings. *Chemosphere*, *360*, 142319. https://doi.org/10.1016/j.chemosphere.2024.142319

Plaut, I. (2001a). Critical Swimming Speed: Its Ecological Relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *131*(1), 41–50. https://doi.org/10.1016/S1095-6433(01)00462-7

Plaut, I. (2001b). Critical Swimming Speed: Its Ecological Relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *131*(1), 41–50. https://doi.org/10.1016/S1095-6433(01)00462-7

Potter, G., Smith, A. S. T., Vo, N. T. K., Muster, J., Weston, W., Bertero, A., Maves, L., Mack, D. L., & Rostain, A. (2020). A More Open Approach Is Needed to Develop Cell-Based Fish Technology: It Starts with Zebrafish. *One Earth*, *3*(1), 54–64. https://doi.org/10.1016/j.oneear.2020.06.005

Princiotta, M. F., Finzi, D., Qian, S.-B., Gibbs, J., Schuchmann, S., Buttgereit, F., Bennink, J. R., & Yewdell, J. W. (2003). Quantitating Protein Synthesis,

Degradation, and Endogenous Antigen Processing. *Immunity*, *18*(3), 343–354. https://doi.org/10.1016/S1074-7613(03)00051-7

Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to Four Freshwater Invertebrate Species. *Environmental Pollution*, *337*, 122512. https://doi.org/10.1016/j.envpol.2023.122512

Quinn, T. P. (2005). The Behavior and Ecology of Pacific Salmon and Trout. In *American Fisheries Society* (2nd ed.). American Fisheries Society.

Randall, D. J. (1970). The Circulatory System. In *Fish Physiology* (Vol. 4, Issue C, pp. 133–172). Academic Press. https://doi.org/10.1016/S1546-5098(08)60129-6

Rantin, F. T., Kalinin, A. L., & Monteiro, D. A. (2020). The Cardiovascular System. In *Biology and Physiology of Freshwater Neotropical Fish* (pp. 185–216). Elsevier. https://doi.org/10.1016/B978-0-12-815872-2.00009-9

Rauert, C., Charlton, N., Okoffo, E. D., Stanton, R. S., Agua, A. R., Pirrung, M. C., & Thomas, K. V. (2022). Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary. *Environmental Science & Technology*, *56*(4), 2421–2431. https://doi.org/10.1021/acs.est.1c07451

Redick, R. (1978). The Lake Trout in Alaska. In *Alaska Department of Fish and Game, Wildlife Notebook Series*. https://myfwp.mt.gov/getRepositoryFile?objectID=39196

Reid, S. G., Bernier, N. J., & Perry, S. F. (1998). The adrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology Part C*, *120*, 1–27.

Ricarte, M., Prats, E., Montemurro, N., Bedrossiantz, J., Bellot, M., Gómez-Canela, C., & Raldúa, D. (2023). Environmental Concentrations of Tire Rubber-Derived 6PPD-Quinone Alter CNS Function in Zebrafish Larvae. *Science of The Total Environment*, *896*, 165240. https://doi.org/10.1016/j.scitotenv.2023.165240

Roberts, C., Lin, J., Kohlman, E., Jain, N., Amekor, M., Alcaraz, A. J., Hogan, N., Hecker, M., & Brinkmann, M. (2024). *Acute and Subchronic Toxicity of 6PPD-Quinone to Early Life Stage Lake Trout (*Salvelinus namaycush*)*. https://doi.org/10.1101/2024.03.26.586843

Roberts, C., Lin, J., Kohlman, E., Jain, N., Amekor, M., Alcaraz, A. J., Hogan, N., Hecker, M., & Brinkmann, M. (2025). Acute and Subchronic Toxicity of 6PPD-Quinone to Early Life Stage Lake Trout (*Salvelinus namaycush*). *Environmental Science & Technology*, *59*(1), 791–797. https://doi.org/10.1021/acs.est.4c09090

Salin, K., Auer, S. K., Rey, B., Selman, C., & Metcalfe, N. B. (2015). Variation in the Link Between Oxygen Consumption and ATP Production, and its Relevance for Animal Performance. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1812), 20151028. https://doi.org/10.1098/rspb.2015.1028

Scholz, N. L., Myers, M. S., McCarthy, S. G., Labenia, J. S., McIntyre, J. K., Ylitalo, G. M., Rhodes, L. D., Laetz, C. A., Stehr, C. M., French, B. L., McMillan, B., Wilson, D., Reed, L., Lynch, K. D., Damm, S., Davis, J. W., & Collier, T. K. (2011). Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams. *PLOS ONE*, *6*(12), e28013. https://doi.org/10.1371/journal.pone.0028013

Schulz, H. (1991). Beta Oxidation of Fatty Acids. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1081*(2), 109–120. https://doi.org/10.1016/0005-2760(91)90015-A

Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Reemtsma, T. (2022). Abiotic Oxidative Transformation of 6-PPD and 6PPD-Quinone from Tires and Occurrence of their Products in Snow from Urban Roads and in Municipal Wastewater. *Water Research*, *212*, 118122. https://doi.org/10.1016/j.watres.2022.118122

Selinger, S. J., Montgomery, D., Wiseman, S., Hecker, M., Weber, L. P., Brinkmann, M., & Janz, D. M. (2024). *Acute Cardiorespiratory Effects of 6ppd-Quinone on Juvenile Rainbow Trout (Oncorhynchus mykiss) and Arctic Char (Salvelinus alpinus)*. https://doi.org/10.2139/ssrn.4956593

Shiels, H. A., Calaghan, S. C., & White, E. (2006). The Cellular Basis for Enhanced Volume-Modulated Cardiac Output in Fish Hearts. *The Journal of General Physiology*, *128*(1), 37–44. https://doi.org/10.1085/jgp.200609543

Shingles, A., McKenzie, D. J., Taylor, E. W., Moretti, A., Butler, P. J., & Ceradini, S. (2001). Effects of Sublethal Ammonia Exposure on Swimming Performance in Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, *204*(15), 2691–2698. https://doi.org/10.1242/jeb.204.15.2691

Simonds, J. (2016). *Top 5 Most Popular Game Fish In America [Freshwater & Saltwater]*. https://www.saltstrong.com/articles/most-popular-game-fish-in-america/

Sokolova, I. M. (2013). Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate the Effects of Multiple Stressors. *Integrative and Comparative Biology*, *53*(4), 597–608. https://doi.org/10.1093/icb/ict028

Soldatov, A. A. (2021a). Content of Methemoglobin in the Blood of Teleost Fish: Effect of Environmental Factors and Natural States of the Organism (Review). *Inland Water Biology*, *14*(6), 747–757. https://doi.org/10.1134/S1995082921060122

Soldatov, A. A. (2021b). Content of Methemoglobin in the Blood of Teleost Fish: Effect of Environmental Factors and Natural States of the Organism (Review). *Inland Water Biology*, *14*(6), 747–757. https://doi.org/10.1134/S1995082921060122

Soofiani, N. M., & Priede, I. G. (1985). Aerobic Metabolic Scope and Swimming Performance in Juvenile Cod, *Gadus morhua L. Journal of Fish Biology*, *26*(2), 127–138. https://doi.org/10.1111/J.1095-8649.1985.TB04249.X

Souders, C. L., Liang, X., Wang, X., Ector, N., Zhao, Y. H., & Martyniuk, C. J. (2018). High-Throughput Assessment of Oxidative Respiration in Fish Embryos: Advancing Adverse Outcome Pathways for Mitochondrial Dysfunction. *Aquatic Toxicology*, *199*, 162–173. https://doi.org/10.1016/j.aquatox.2018.03.031

Steffensen, J. F., Johansen, K., & Bushnell, P. G. (1984). An Automated Swimming Respirometer. *Comparative Biochemistry and Physiology Part A: Physiology*, *79*(3), 437–440. https://doi.org/10.1016/0300-9629(84)90541-3

Stubhaug, I., Frøyland, L., & Torstensen, B. E. (2005). β-Oxidation Capacity of Red and White Muscle and Liver in Atlantic Salmon (*Salmo salar* L.)—Effects of Increasing Dietary Rapeseed Oil and Olive Oil to Replace Capelin Oil. *Lipids*, *40*(1), 39–47. https://doi.org/10.1007/s11745-005-1358-4

Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and Setup of Intermittent-Flow Respirometry System for Aquatic Organisms. *Journal of Fish Biology*, *88*(1), 26–50. https://doi.org/10.1111/jfb.12797

Tallman, R. F., Saurette, F., & Thera, T. (1996). Migration and Life History Variation in Arctic charr, *Salvelinus alpinus*. *Écoscience*, *3*(1), 33–41. https://doi.org/10.1080/11956860.1996.11682312

Taylor, E. W., Leite, C. A. C., & Levings, J. J. (2009). Central Control of Cardiorespiratory Interactions in Fish. *Acta Histochemica*, *111*(3), 257–267. https://doi.org/10.1016/j.acthis.2008.11.006

Thomas, J. K., & Janz, D. M. (2014). *In ovo* Exposure to Selenomethionine via Maternal Transfer Increases Developmental Toxicities and Impairs Swim Performance in F1 Generation Zebrafish *(Danio rerio)*. *Aquatic Toxicology*, *152*, 20–29. https://doi.org/10.1016/j.aquatox.2014.03.022

Thorgaard, G. H., Bailey, G. S., Williams, D., Buhler, D. R., Kaattari, S. L., Ristow, S. S., Hansen, J. D., Winton, J. R., Bartholomew, J. L., Nagler, J. J., Walsh, P. J., Vijayan, M. M., Devlin, R. H., Hardy, R. W., Overturf, K. E., Young, W. P., Robison, B. D., Rexroad, C., & Palti, Y. (2002). Status and Opportunities for Genomics Research with Rainbow Trout. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *133*(4), 609–646. https://doi.org/10.1016/S1096-4959(02)00167-7

Tian, Z., Gonzalez, M., Rideout, C. A., Zhao, H. N., Hu, X., Wetzel, J., Mudrock, E., James, C. A., McIntyre, J. K., & Kolodziej, E. P. (2022). 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. *Environmental Science & Technology Letters*, *9*(2), 140–146. https://doi.org/10.1021/acs.estlett.1c00910

Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A. E., Biswas, R. G., Kock, F. V. C., Soong, R.,

Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., … Kolodziej, E. P. (2021). A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon. *Science*, *371*(6525), 185–189. https://doi.org/10.1126/science.abd6951

Tönz, O. (1969). The Congenital Methemoglobinemias: Physiology and Pathophysiology of the Hemoglobin Metabolism. *Archives of Disease in Childhood*, *44*(233), 141–141. https://doi.org/10.1136/adc.44.233.141

Tota, B., Cimini, V., Salvatore, G., & Zummo, G. (1983). Comparative Study of the Arterial and Lacunary Systems of the Ventricular Myocardium of Elasmobranch and Teleost Fishes. *American Journal of Anatomy*, *167*(1), 15–32. https://doi.org/10.1002/aja.1001670103

Tseng, Y.-C., & Hwang, P.-P. (2008). Some Insights Into Energy Metabolism for Osmoregulation in Fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *148*(4), 419–429. https://doi.org/10.1016/j.cbpc.2008.04.009

US EPA. (1996, April). *Ecological Effects Test Guidelines*. https://19january2017snapshot.epa.gov/sites/production/files/2015-07/documents/850-1075.pdf

US National Park Service. (2015). *Rainbow Trout - Shenandoah National Park (U.S. National Park Service)*. https://www.nps.gov/shen/learn/nature/rainbow-trout.htm

Usui, Y., Kimoto, M., Hanashima, A., Hashimoto, K., & Mohri, S. (2022). Cardiac Hemodynamics and Ventricular Stiffness of Sea-Run Cherry Salmon (*Oncorhynchus masou masou*) Differ Critically From Those of Landlocked Masu Salmon. *PLOS ONE*, *17*(11), e0267264. https://doi.org/10.1371/journal.pone.0267264

Varshney, S., Gora, A. H., Siriyappagouder, P., Kiron, V., & Olsvik, P. A. (2022). Toxicological Effects of 6PPD and 6PPD-Quinone in Zebrafish Larvae. *Journal of Hazardous Materials*, *424*(October 2021), 127623. https://doi.org/10.1016/j.jhazmat.2021.127623

Walker, M. K., Spitsbergen, J. M., Olson, J. R., & Peterson, R. E. (1991). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Toxicity during Early Life Stage Development of Lake Trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(5), 875–883. https://doi.org/10.1139/f91-104

Wang, Y., Li, Y., An, R., & Li, K. (2018). Effects of Total Dissolved Gas Supersaturation on the Swimming Performance of Two Endemic Fish Species in the Upper Yangtze River. *Scientific Reports*, *8*(1), 10063. https://doi.org/10.1038/s41598-018-28360-7

Wang, Z., Pu, D., Zheng, J., Li, P., Lü, H., Wei, X., Li, M., Li, D., & Gao, L. (2023). Hypoxia-Induced Physiological Responses in Fish: From Organism to Tissue to Molecular Levels. *Ecotoxicology and Environmental Safety*, *267*, 115609. https://doi.org/10.1016/j.ecoenv.2023.115609

Weber, L. P., Dubé, M. G., Rickwood, C. J., Driedger, K., Portt, C., Brereton, C., & Janz, D. M. (2008). Effects of Multiple Effluents on Resident Fish from Junction Creek, Sudbury, Ontario. *Ecotoxicology and Environmental Safety*, *70*(3), 433–445. https://doi.org/10.1016/j.ecoenv.2007.08.001

Wendelaar Bonga, S. E. (1997). The Stress Response in Fish. *Physiological Reviews*, *77*(3), 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

Werbowski, L. M., Gilbreath, A. N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M. D., Deshpande, A. D., & Rochman, C. M. (2021). Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. *ACS ES&T Water*, *1*(6), 1420–1428. https://doi.org/10.1021/acsestwater.1c00017

Widimsky, P. (2008). Hypokalemia and The Heart. *E-Journal of Cardiology Practice*, *7*, 9–12. https://www.escardio.org/Journals/E-Journal-of-Cardiology-Practice/Volume-7/Hypokalemia-and-the-heart

Wildemann, T. M., Mirhosseini, N., Siciliano, S. D., & Weber, L. P. (2015). Cardiovascular Responses to Lead are Biphasic, While Methylmercury, but Not Inorganic Mercury, Monotonically Increases Blood Pressure in Rats. *Toxicology*, *328*, 1–11. https://doi.org/10.1016/j.tox.2014.11.009

Wilsterman, K., McGuire, N. L., Calisi, R. M., & Bentley, G. E. (2019). Seasonality: Hormones and Behavior. In *Encyclopedia of Animal Behavior* (pp. 599–612). Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.20688-2

Yamauchi, A. (1980). Fine Structure of the Fish Heart. In *Comparative Anatomy and Development* (pp. 119–148). Elsevier. https://doi.org/10.1016/B978-0-12-119401-7.50010-4

Zeng, L., Li, Y., Sun, Y., Liu, L.-Y., Shen, M., & Du, B. (2023). Widespread Occurrence and Transport of *p* -Phenylenediamines and Their Quinones in Sediments across Urban Rivers, Estuaries, Coasts, and Deep-Sea Regions. *Environmental Science & Technology*, *57*(6), 2393–2403. https://doi.org/10.1021/acs.est.2c07652

Zhang, S.-Y., Gan, X., Shen, B., Jiang, J., Shen, H., Lei, Y., Liang, Q., Bai, C., Huang, C., Wu, W., Guo, Y., Song, Y., & Chen, J. (2023). 6PPD and its Metabolite 6PPD-Q Induce Different Developmental Toxicities and Phenotypes in Embryonic Zebrafish. *Journal of Hazardous Materials*, *455*, 131601. https://doi.org/10.1016/j.jhazmat.2023.131601

Zhang, Y., Post, W. S., Blasco-Colmenares, E., Dalal, D., Tomaselli, G. F., & Guallar, E. (2011). Electrocardiographic QT Interval and Mortality. *Epidemiology*, *22*(5), 660–670. https://doi.org/10.1097/EDE.0b013e318225768b

Zhang, Z., Xu, X., Qian, Z., Zhong, Q., Wang, Q., Hylkema, M. N., Snieder, H., & Huo, X. (2024). Association Between 6PPD-Quinone Exposure and BMI, Influenza, and

Diarrhea in Children. *Environmental Research*, *247*, 118201. https://doi.org/10.1016/j.envres.2024.118201

Zhao, Y., Yun, M., Nguyen, S. A., Tran, M., & Nguyen, T. P. (2019). In Vivo *Surface Electrocardiography for Adult Zebrafish*. https://www.jove.com/video/60011/

Zhou, L.-J., Liu, S., Wang, M., Wu, N.-N., Xu, R., Wei, L.-N., Xu, X.-R., Zhao, J.-L., Xing, P., Li, H., Zeng, J., & Wu, Q. L. (2024). Nationwide Occurrence and Prioritization of Tire Additives and their Transformation Products in Lake Sediments of China. *Environment International*, *193*, 109139. https://doi.org/10.1016/j.envint.2024.109139