IMPACTS OF CONTAMINATED SEDIMENT REMEDIATION ON EARLY LIFE STAGES OF RAINBOW TROUT

By

JOHN RICHARD FOLTZ

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To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of JOHN FOLTZ find it satisfactory and recommend that it be accepted.

_____
Jeffery L. Ullman, Ph.D., Chair

_____
Marvin J. Pitts, Ph.D.

_____
Jeremy A. Rentz, Ph.D.

_____
Gary H. Thorgaard, Ph.D.

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Abstract

By John Richard Foltz, M.S.
Washington State University
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Chair: Jeffrey L. Ullman

Fish in the salmonid family are of particular importance economically, socially, and ecologically. Consequently, a great deal of interest has been given to researching environmental impacts on salmonid health to better protect these valued species. Sediment contamination is vast and poses a potential ecological risk in watersheds across the United States. Contaminated sediments may directly or indirectly affect fish populations. Current sediment remediation practices primarily involve capping, dredging or monitored natural attenuation approaches – each has its own drawbacks in relation to the aquatic ecosystem. An innovative remedial approach, using solid peroxide within the sediment to increase available oxygen for microbial degradation of organic contaminants, was examined in this study. Sediments are low oxygen environments; increasing the oxygen available to be used as the terminal electron acceptor during microbial degradation may facilitate this process.

The effects of this unique remediation method on rainbow trout at two different life stages were evaluated in relation to environmental parameters and fish condition. Remediated and non-remediated contaminated sediment impacts on yolk-sac fry and juvenile trout were

evaluated following 29 and 30 day exposures, respectively. Contaminant uptake, environmental condition and fish condition were evaluated. Results suggest that dissolved oxygen within the water was significantly increased in aquaria undergoing the remediation treatment. The contaminant used in this study, phenanthrene, was degraded in both remediated and un-remediated treatments and was not significantly increased by the remediation treatment. This may be a result of the experimental set-up, and given more time significant differences might be observed. Fish condition factor, a relationship between length and weight, varied across treatment types. However, all fish condition factors were relatively close to 1.0, a value suggesting good fish condition. Fish were observed to uptake phenanthrene during Phase I, but not Phase II – likely a result of lower sediment phenanthrene concentrations during Phase II. This suggests the remediation treatment resulted in an observable impact on environmental parameters, specifically dissolved oxygen, and fish condition.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..... iii

ABSTRACT..... iv

LIST OF FIGURES vii

1.0 Introduction and Literature Review 1

1.1 Background 2

1.2 Fish response to contaminant uptake and accumulation 3

1.3 Effects of organic contaminants at critical life stages 6

1.4 Methods of contaminant exposure 8

1.5 Traditional remediation of contaminated sediments and impacts on fish 11

1.6 Oxygen and biodegradation of PAHs in sediment 12

1.7 Summary 13

1.8 Objectives 14

1.9 Significance 14

2.0 Impacts of contaminated sediment remediation on early life stages of rainbow trout 16

2.1 Introduction 16

2.2 Materials and Methods 17

2.3 Results 24

2.3.1 Environmental Conditions 24

2.3.2 Fish Condition 30

2.4 Discussion 38

3.0 Conclusions and Recommendations for Future Research 47

4.0 Literature Cited 50

5.0 Appendix A 56

6.0 Appendix B 57

LIST OF FIGURES

Figure 1. Rainbow trout at embryonic, yolk-sac, and juvenile life stages…….. 2

Figure 2. Dissolved Oxygen vs Temperature between replicates…….. 27

Figure 3. Water pH in aquaria with sediment…….. 28

Figure 4. Phenanthrene degradation in sediment…… 29

Figure 5. Phase I Fish Condition Factors…….. 32

Figure 6. Phase II Fish Condition Factors…….. 33

Figure 7. Phenanthrene concentration in fish tissue…… 35

Figure 8. Phenanthrene HPLC Standard Curve…….. 56

Figure 9. Sediment Phenanthrene concentration HPLC Chromatogram…….. 56

Figure 10. Phenanthrene GC-MS Standard Curve…… 57

Figure 11. Fish Tissue Phenanthrene concentration GC-MS Chromatogram…… 57

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Fish in the salmonid family are of particular importance economically, socially, and ecologically. Consequently, a great deal of interest has been given to researching environmental impacts on salmonid health to better protect these valued species. Fish are exposed to any pollutants that enter aquatic ecosystems, including organic legacy contaminants from anthropogenic origins. Incorporating salmonids in toxicological research helps elucidate contaminant impacts on fish considering their relative sensitivity and unique life history.

Many scientific studies have examined effects of organic contaminants, often through waterborne exposure, on different life stages of aquatic organisms and fish (Chen and White, 2004; Honkanen et al., 2001; Koponen et al., 1998 and 2000; Milston et al., 2003; Petersen and Kristensen, 1998; Walker et al., 1996). The embryonic stage may be the most sensitive and important of all the salmonid life stages, and represents an important period for evaluating the effects of contaminants (Hendricks et al., 1980; von Westernhagen, 1988). In addition, the embryonic and post-hatch periods maintain a close association with the substrate and sediment, because these are benthic life stages prior to absorption of the yolk-sac and swim-up (Figure 1). Although contaminant uptake may be sub-lethal, other detrimental impacts may arise later in life, including increased disease susceptibility and decreased reproductive success (Cachot et al., 2007; Milston et al., 2003; Talmage et al., 1999). Due to the hydrophobic nature of organic contaminants and issues surrounding bioavailability, examining early fish life stages exposed to sediment-borne contaminants presents an ecologically relevant approach for evaluating detrimental effects.

Figure 1. Photograph of the embryonic (left), yolk-sac (middle) and juvenile (right) stages of rainbow trout.

1.1 Background

Anthropogenic impacts on the natural environment are vast and often detrimental. Sediments contaminated as a result of human activity may have a direct negative affect on aquatic biota or present a source of contaminants that can bioaccumulate in the food chain. Contaminated sediments pose a potential ecological risk in watersheds across the United States, including water bodies where chemical water quality criteria are at or below acceptable levels. According to the United States Environmental Protection Agency (USEPA), there are 2,800 fish advisories in the United States for the types of contaminants often found in sediment (USEPA, 2004). These advisories affect more than 544,000 river miles, 71% of the National coast and more than 95,000 lakes including all 5 of the Great Lakes (USEPA, 2004). Contaminated sediment impacts may directly or indirectly affect aquatic ecosystems, human health and economics. Past studies have examined numerous contaminant impacts to different aquatic species (Cachot et al., 2007; Hankanen et al., 2001; Koponen et al., 1998, 2000; Oliver and Niimi, 1988; Petersen and Kristensen, 1998; Pruell et al., 1993; Sundberg et al., 2006; Walker et al., 1996). Still, there is much left to understand concerning contaminated sediment impacts on aquatic life.

Sediments in aquatic ecosystems provide a contaminant sink, which adversely affect both benthic and pelagic organisms (Chen and White, 2004). Many organic chemicals, such as chlorinated compounds, have high partitioning coefficients, which suggests that the distribution of these compounds are likely to associate with the sediment and organic matter rather than with the water. Low concentrations may be found in the water column while the sediment may have orders of magnitude higher concentrations.

The Clean Water Act has established 126 priority pollutants based upon toxicity, persistence and degradation potential (FWPCA, 2008). The list includes heavy metals which tend to be highly toxic and bioaccumulate, pesticides and herbicides that encompass many diverse compounds, polychlorinated biphenyls (PCBs) which are extremely persistent and bioconcentrate, and polycyclic aromatic hydrocarbons (PAHs). Of the 16 PAHs listed as priority pollutants, phenanthrene was used as the contaminant of interest in this study.

1.2 Fish Response to Contaminant Uptake and Accumulation

In order to understand the potential impact of a particular compound on an organism, one must recognize the primary exposure routes, which are typically dictated by where the organism resides. The potential for the compound to be transferred from the environment to the organism depends not only on the morphological, physiological and biochemical attributes of the organism (Giulio and Hinton, 2008), but also on the bioavailability of the chemical. Since many chemicals tend to partition into the sediments, pollutant uptake is typically limited in the water column and relatively high in non-aqueous parts of the ecosystem (i.e. the substrate and sediment). For an organism to be greatly impacted by a hydrophobic contaminant, it typically

must have some association with the substrate and sediment – most fish species receive contaminant loads through the food chain. If an organism has a direct association with contaminated sediment, an additional opportunity for pollutant uptake is presented.

Contaminant uptake can occur via several exposure routes depending on organism characteristics. These pathways can include absorption through membranes and ingestion. Generally, bioconcentration refers to chemical accumulation by non-dietary means. The term bioaccumulation refers to chemical accumulation within an organism by all potential pathways, including dietary intake. Biomagnification is the result of elevated contaminant concentrations in organisms with an increase in trophic level. Once the contaminant has entered the organism it can be eliminated, transformed into another compound or concentrated within the organism (Giulio and Hinton, 2008).

The bioconcentration factor (BCF), which is the relation of the chemical concentration in the environment to that in the fish under steady state conditions, is used to quantify the magnitude of accumulation in aquatic systems. This term has the units of water volume/tissue weight. Variations in BCF between species or life stages are thought to be accounted for by differences in lipid content (Giulio and Hinton, 2008). Simlarly to the BCF, an organism has an elimination rate. A compound can be excreted, metabolized or otherwise eliminated, in addition to being concentrated within an organism. These possible mechanisms can cause a response by the organism to a specific compound. Responses in fish can possibly be observed in the form of stress, a change in osmoregulation, changes in liver enzyme production, behavior changes, effects on the nervous, endocrine or immune systems or cancer development (Giulio and Hinton, 2008).

Contaminant uptake can vary based upon intra-species differences and relative association with the sediment (Pruell et al., 1993). For instance, Gewurtz et al. (2000) observed differences in accumulation and maximum contaminant uptake of PAH and PCB in benthic invertebrates. Variations were attributed to habitat preference and diet. Species that had a greater association with the sediment had significantly higher levels of contaminant uptake (Gewurtz et al., 2000).

Biomagnification of organic contaminants is an issue of concern. Food chain biogmagnification of PCBs and other chlorinated hydrocarbons (e.g., DDT) in Lake Ontario resulted in salmonid contaminant concentrations almost two orders of magnitude greater than pelagic primary and secondary producers (Oliver and Niimi, 1988).

Fish and other organisms can also metabolize organic compounds once they have entered the body. For a contaminant to be metabolized or biotransformed, specific enzymes must be present. In fish, the induction of liver cytochrome P450 (CYP1A) enzymes result from metabolism of organic contaminants (Fragoso et al., 2006). The CYP1A1 specifically catalyses ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH). CYP1A1, and as a consequence EROD activity, can be induced in fish by polychlorinated dibenzo-*p*-dioxins (PCDDs) and related poly aromatic compounds (Stegeman and Kloepper-Sams, 1987).

Additional responses to contaminants can be observed on a larger scale. Mortalities resulting from contaminant uptake have been observed during the larval stage (Sundberg et al., 2006). Mortality was also observed during the yolk-sac stage of lake trout (*Salvelinus namaycush*) and rainbow trout (*Oncorhynchus mykiss*) and was dose dependent (Walker et al., 1996). Walker et al. (1996) observed that rainbow trout exhibited a higher resistance to

contaminants than lake trout. Hollert et al. (2003) observed mortality of zebrafish (*Danio rerio*) embryos as a result of contact with native sediment with unknown contaminants. Differences in hatching time and sublethal development abnormalities also arose (Hollert et al., 2003). Cachot et al. (2007) found similar results when medaka (*Oryzias latipes*) exposed to PAHs and PCBs extracted from field sediment showed higher mortality in the embryo-larval stages and an increase in spinal deformities. Additionally, mutations in the liver, transverting Guanine:Cytosine and Thymine:Adenine, were observed (Cachot et al., 2007).

1.3 Effects of Organic Contaminants at Critical Life Stages

Fish toxicity and bioaccumulation experiments are usually performed on juveniles (Connell, 1990). Although this does not represent a particularly sensitive life state, it provides feasibility and practicability in the laboratory. On the other hand, early fish life stages are commonly considered the most sensitive periods to environmental stress, particularly the yolk-sac stage and the embryonic stage prior to completion of gastrulation (Hendricks et al., 1980; von Westernhagen, 1988). There are limited possible routes through which a developing fish embryo or a post-hatch fish can accumulate a contaminant. Due to limited mobility and no exogenous feeding, these two life stages must passively uptake a contaminant. Therefore, the contaminant must be passed from the parent fish or acquired upon being deposited in the substrate.

Salmonids in particular may be impacted at early life stages by contaminated sediment. Petersen and Kristensen (1998) proclaimed that for fish species having a long embryonic stage, such as salmon, it is important to include this period when examining lipophilic chemicals.

Salmonids have large lipid-rich yolk reserves that make the egg/embryonic stage a good candidate to accumulate large amounts of lipophilic organic chemicals. This, in addition to other salmonid life history characteristics of relatively large eggs and long developmental time, suggests that salmonid embryos may accumulate chemicals readily (Honkanen et al., 2001).

Differences in uptake and response to contaminants are linked to specific life stages. Peterson and Kristensen (1998) observed that the capability of embryonic fish to eliminate or metabolize a contaminant is reduced as compared to a fish at a more developed life stage. PAHs in particular are metabolized at a slower rate in embryonic cod (*Gadus morhua*), herring (*Clupea harengus*) and zebrafish (Peterson and Kristensen, 1998). Van der Oost et al. (2003) observed that limited biotransformation and elimination of contaminants takes place during life stages prior to the eyed-stage in rainbow trout. Kopenen et al. (2000) found that the no observable affect concentration of PCB-77 was lower for yolk-sac fry than for eyed embryos (Kopenen et al., 2000). Kopenen et al. (1998) showed rapid uptake (3 days to steady state) of PCB-77 at three concentrations in rainbow trout at the eyed stage. The fish microsomal CYP1A monooxygenase system was shown to be sensitive at the lowest concentration (1µg L^{-1} PCB-77 per 10ng $embryo^{-1}$)(Kopenen et al., 1998).

Observable differences in mortality and toxic effects have also been shown between life stages. Sundberg et al. (2006) observed a difference in mortality and toxic effects between rainbow trout at the larval and the embryonic stages. Walker et al. (1996) observed that mortality did not significantly increase for either rainbow or lake trout eggs when exposed to contaminants (2,3,7,8-tetrachlorodibenzo-p-dioxins (TCDDs), polychlorinated dibenzo-p-dioxins

and dibenzofurans (PCDDs and PCDFs) and PCBs); rather mortality increased in a dose-response pattern during the yolk-sac stage (Walker et al., 1996).

Contaminant exposure at the embryonic or yolk-sac stages may not always cause mortality, but can cause problems later in life. Even a brief exposure to pollutants can cause fish health concerns. Exposure to low waterborne concentrations of DDE (10 ppm) for one hour at fertilization and two hours at hatch caused immune-suppression in Chinook salmon (*Oncorhynchus tshawytscha*) one year post-hatch even though no differences in length, weight, sex ratio, gonadal development and mortality were observed (Milston et al., 2003). This suggests that exposure to contaminants at ecologically relevant levels may decrease fish fitness by increasing the likelihood of health problems during its life. Hollert et al. (2003) observed differences in hatching time and sub-lethal development abnormalities in zebrafish exposed to contaminated sediment (Hollert et al., 2003). Fish that develop abnormally may not die as a result of contamination, but are less fit to survive to reproduction. Cachot et al. (2007) also observed spinal mutations and mortality in medaka exposed to PAH and PCB contaminated sediment extracts. Fish that did survive were placed in a contaminant free environment for 35 weeks, after which detrimental effects were observed in the form of liver and gonadal tumors (Cachot et al., 2007).

1.4 Methods of Contaminant Exposure

There are four basic ways to expose fish and aquatic species to contaminants in a laboratory setting: waterborne, injection, feed, and sediment-borne. Each method has

advantages and disadvantages. Method selection depends on what is trying to be tested and observed.

A waterborne exposure method is a quick method that eliminates complex interactions with the sediment and likely results in quick uptake in fish as a result of the hydrophobic nature of organic contaminants. Petersen and Kristensen (1998) evaluated PAH metabolism at different life stages in zebrafish, cod, herring and turbot (*Scophthalmus maximus*) using a waterborne exposure. Koponen et al. (1998 and 2000) utilized a waterborne exposure method when comparing PCB-77 effects on two different life stages of rainbow trout, sub-lethal stress and the induction of liver cytochrome P450. Milston et al. (2003) employed a waterborne exposure of TCDD, in this case to expose Chinook salmon embryos for a quick period, and observed immune-suppression one year post hatch. Walker et al. (1996) utilized a waterborne exposure when using lake trout embryos, instead of other contaminant exposure methods, citing the sensitivity of the species life stage. Waterborne exposure is relevant when evaluating contaminants with that are highly soluble, such as bisphenol A (BPA). Honkanen et al. (2001) exposed salmonids to BPA (120-300 mg/L) using a waterborne exposure to evaluate biotransformation rates with variation in water temperature. Despite its widespread use, the waterborne exposure method is not ecologically relevant as water concentrations are usually low relative to sediment concentrations. Solubility of a contaminant may also pose a problem as high aqueous concentration of contaminants may not be achieved.

The contaminant injection method is an exposure technique that ensures that the contaminant of interest enters the fish body. This method has been shown to claim less than 1% mortality of fish embryos due to the injection itself (Åkerman and Balk, 1995). Sundberg et

al. (2006) first extracted contaminants from natural sediment then injected them into rainbow trout embryos post-fertilization. Walker et al. (1996) utilized the injection method when evaluating intra-species response differences when comparing lake and rainbow trout. This method is useful in guaranteeing the contaminants will be in the fish, but it is not a natural pathway. This method may cause additional stress due to handling and the physical injection, which may confound observed effects. This method also does not reveal any information regarding uptake or bioaccumulation.

Exposing fish to contaminants through feed is a method that is ecologically relevant, as it mimics a natural contaminant exposure pathway. However, this method is irrelevant for fish not feeding, such as during the yolk-sac stage. When trying to evaluate effects of fish at life stages before full digestive tract development, other methods must be used. This method may also present complications, as the desired level of pollutant uptake may not be achieved or occur at all due to palatability issues with contaminated feed.

The final method reviewed here is a sediment-borne exposure. This method is ecologically relevant, as sediments are where contaminants are generally found at higher levels. This method is also acceptable for exposure at early fish life stages due to their close association with the substrate. Hollert et al. (2003) exposed embryonic zebrafish to contaminated field sediments to evaluate the potential of a sediment contact assay for toxicological evaluation. Contaminants were observed to be bioavailable and accumulated in the embryos, causing mortality and sub-lethal impacts (Hollert et al., 2003). Embryonic medaka were evaluated by Cachot et al. (2007) using the same sediment-borne contaminant exposure method to evaluate if the contaminants were bioavailable and were taken up. Mortality and

spinal deformities were observed in addition to liver mutations and liver and gonadal tumor formation 35 weeks after exposure (Cachot et al., 2007). Sediment-borne exposure of contaminants is ecologically relevant, although it does not guarantee contaminant uptake. Depending on what specific parameter is being evaluated in a study, a method should be selected based upon that methods advantages and drawbacks. In this study, the sediment-borne method is relevant, because the research examined the impacts of a sediment remediation technique on fish.

1.5 Traditional Remediation of Contaminated Sediments and Impacts on Fish

Traditional PAH-contaminated sediment remediation generally involves removal with offsite treatment, capping, or monitored natural recovery. Contaminated sediment removal is accomplished by dredging, which is expensive and controversial. Dredging results in sediment resuspension in the aquatic ecosystem, which is a major aquatic health risk on its own and also increases potential PAH exposure (Palermo, 2001). In-situ remediation is possible by capping or monitored natural recovery. Capping employs a clean isolating material placed over the contaminated sediment which can be simple or complex. The goal of the cap is to physically isolate the contaminated area from aquatic organisms and limit contaminated sediment resuspension. This method is similar in cost to dredging, but leaves the pollutant on site. Re-exposure of the contaminated sediment due to damage to the cap would render the remediation technique invalid (Palermo, 2001). Monitored natural recovery (MNR) is a remediation option that lets natural processes reduce PAH concentrations. This remediation approach implements a program to monitor contamination levels to ensure that degradation is

occurring. Monitored natural recovery is relatively less expensive, although the contaminants stay in the environment and recovery takes time (Palermo, 2001).

Remediation of contaminated sediments by dredging, capping, and abatement of contaminant sources has been shown to increase aquatic ecosystem health over time (Adams et al., 2005; Myers et al., 2008; Yang and Baumann, 2006). However, in the short term, Blom et al. (1998) conducted a study suggesting that the contaminated sediment removal will decrease fish health due to increased pollutant bioavailability due to dredging. On the other hand, over a period of two years, fish heath increased and stress and mortality decreased post-dredging as a result of contaminant removal and downstream transport of the resuspended contaminants (Blom et al., 1998). Thus, remediation of contaminated sediments yields mixed results depending on the temporal scale. However, the overall general theme is that a reduction in contaminant availability increases fish health. Other remediation strategies work well in terrestrial systems, but these methods have not been shown to adapt well to the aquatic environment where aquatic species cannot escape their environment and are subjected to anything that enters the water. However, evaluating alternative methods for remediating contaminated sediment in the aquatic system may help to increase the health of the aquatic environment.

1.6 Oxygen and Biodegradation of PAHs in Sediment

Microbial degradation is the major PAH-removal mechanism in sediments and occurs most quickly and efficiently when oxygen is utilized as the terminal electron acceptor (Lei et al., 2005). Aerobic phenanthrene degradation was reported to be two times as efficient as

anaerobic degradation (Tang et al., 2006). Additionally, PAHs are reduced compounds that generally degrade via oxidation (Cerniglia, 1992). Generally sediments, except for at the water interface, are depleted of oxygen and anaerobic degradation occurs at a relatively slower rate as compared to aerobic degradation.

The methods developed and used in this study utilize the knowledge that sediments are depleted of oxygen. If oxygen, the terminal electron acceptor used in PAH microbial degradation, is artificially added to sediment, bioremediation may be enhanced. Oxygen additions to contaminated sediment have been observed to increase microbial degradation in laboratory mesocosms (Schaffnit, 2009). However, the impact of this remediation technique on aquatic life has not been evaluated.

1.7 Summary

Organic contaminants in the aquatic ecosystem are a significant issue of concern. Many scientific studies have addressed the plethora of organic contaminants, often through waterborne exposure, on different life stages of aquatic organisms and fish. The embryonic stage may be the most sensitive of all the life stages and represents an important period for evaluation of the effects of contaminants. Due to the hydrophobic nature of organic contaminants and issues surrounding bioavailability, sediment-borne contaminant exposure to embryonic fish may be the most ecologically relevant method to determine detrimental effects. Furthermore, the incorporation of salmonids in toxicological research can contribute to the understanding of contaminant uptake and impacts, as their sensitivity and life history are unique and important.

1.8 Objectives

The primary objective of this research was to investigate the toxicological effects of sediment-bound contaminants and remediation on the early life stages of fish, specifically to:

1. Design an experiment that evaluates the impact of contaminated sediment on rainbow trout at different life stages; and
2. Evaluate the impact of contaminated sediment remediation upon rainbow trout.

Our hypothesis was that there will be a negative impact of contaminated sediment upon the fish as compared to the non-contaminated sediment. Additionally, we hypothesized that the remediation will not significantly change environmental parameters or negatively impact fish as compared to those not remediated.

1.9 Significance

Innovative research was proposed and funded to evaluate a novel remedial method, specifically on creosote (composed of PAHs) contaminated sediment in an attempt to improve fish and wildlife habitat. The remedial method presented provides an alternative to the established approaches of dredging, capping and monitored natural recovery that applies reactive solids comprised of chemical oxidants to improve biological degradation. This method will attempt to degrade contaminants in situ to eliminate the potential of increasing bioavailability and sedimentation resuspension by dredging and increase degradation rates compared to capping or monitored natural recovery. In laboratory experiments, Schaffnit (2009) observed enhanced degradation of phenanthrene (a PAH component of creosote) using these reactive solids compared to degradation rates in control groups.

Wildlife and ecosystem health implications associated with the proposed remediation technique are a potential concern. While the initial study has shown that the proposed reactive solid treatment can degrade phenanthrene, the potential effects on fish are unknown. The surrounding concern lies within degradation byproducts. The aim of this research was to assess whether sediments undergoing treatment with reactive solids impact biota. To accomplish this, comparisons were made using rainbow trout reared in the presence of untreated toxic sediment, the same sediment undergoing treatment and uncontaminated sediment undergoing treatment. Two life stages were evaluated, yolk-sac and juvenile, to evaluate the direct and indirect impacts of contaminated sediment exposure. In this manner, the benthic yolk-sac stage served as a surrogate for bottom dwelling biota while the juvenile life stage represented free swimming pelagic organisms.

The significance of this work will be to better understand the impact of contaminated sediments on salmonids by providing general toxicological data on contact exposure to sediments. Additionally, this study aims to evaluate the effects of the potential remedial practices presented in this study on rainbow trout. Furthermore, this study aims to improve toxicology studies by adding a method for examining toxicity and potentially establishing a quick toxicological examination method for early fish life stage exposure. We also expect this sediment-borne contaminant exposure method to be useful in other toxicological tests, as it may provide a more ecologically relevant method of examination compared to waterborne experiments.

CHAPTER 2

2.0 IMPACTS OF CONTAMINATED SEDIMENT REMEDIATION ON EARLY LIFE STAGES OF RAINBOW TROUT

2.1 Introduction

Fish in the salmonid family are of particular importance economically, socially, and ecologically. Consequently, a great deal of interest has been given to researching environmental impacts on salmonid health to better protect these valued species. Fish are exposed to any pollutants that enter aquatic ecosystems, including organic legacy contaminants from anthropogenic origins. Incorporating salmonids in toxicological research helps elucidate contaminant impacts on fish considering their relative sensitivity and unique life history.

Many scientific studies have examined effects of the plethora of organic contaminants, often through waterborne exposure, on different life stages of aquatic organisms and fish (Chen and White, 2004; Honkanen et al., 2001; Koponen et al., 1998 and 2000; Milston et al., 2003; Petersen and Kristensen, 1998; Walker et al., 1996). The early life stages may be the most sensitive and important of all the salmonid life stages for evaluating the effects of contaminants (Hendricks et al., 1980; von Westernhagen, 1988). In addition, the embryonic and post-hatch periods maintain a close association with the substrate and sediment, because these are benthic life stages prior to absorption of the yolk-sac and swim-up. Although contaminant uptake may be sub-lethal, other detrimental impacts may arise later in life, including increased disease susceptibility and decreased reproductive success (Cachot et al., 2007; Milston et al., 2003; Talmage et al., 1999).

Due to the hydrophobic nature of organic contaminants and issues surrounding bioavailability, examining early fish life stages exposed to sediment-borne contaminants presents an ecologically relevant approach for evaluating detrimental effects. Additionally, the remediation of these sediments is an area of interest. This study examined the impact of sediment-borne contamination and a novel remediation method upon the post-hatch and juvenile stages of rainbow trout, using the polyaromatic hydrocarbon (PAH) phenanthrene as a surrogate contaminant. The proposed remediation method utilizes reactive solids comprised of chemical oxidants to enhance biological degradation. Sediments are low oxygen environments, and increasing the oxygen available to be used as the terminal electron acceptor during microbial degradation may facilitate this process. This technique will potentially allow for degradation to occur in situ at a quicker rate than methods such as capping or monitored natural attenuation and decrease the amount of sediment perturbation associated with dredging. The impact of the proposed sediment remediation technique upon environmental parameters and fish condition were evaluated.

2.2 Materials and Methods

Fish

Rainbow trout gametes were obtained from Troutlodge®, Sumner, Washington. Eggs were fertilized in the presence of an activation fluid (8.75 g Sodium Chloride, 1.2 g TrisBase, 1.5 g Glycine/1 L water), and were subsequently washed with reverse osmosis (RO) purified water amended with RO Right Salts® to yield a total dissolved solids concentration of 200mg/L, pH 8.0. This water formulation was used during incubation in a heath tray incubator at 15°C±2°.

Post-hatch fish were kept in re-circulating holding tanks (15°C±2°) and fed approximately 1.5% of their body weight daily upon the start of exogenous feeding prior to the experiments. During the first phase of the experiment, juvenile fish were used. During the second phase of the experiment, rainbow trout two days post-hatch were used.

Sediment Substrate and Chemicals

Natural stream sediment (3.4% organic matter) was collected from Paradise Creek, Pullman, Washington. This sediment was used as collected or artificially contaminated. Phenanthrene ($C_{14}H_{10}$) was used as a model contaminant to examine the effectiveness of the chemical oxidant as a remediation method and its potential subsequent impacts on rainbow trout. Contaminated sediments were spiked with phenanthrene (95% purity), acquired from Sigma-Aldrich (St. Louis, MO), using a shell-coating method modified from Ke et al. (2003). Three grams of phenanthrene were dissolved in 200 mL of acetone in a 500-mL glass bottle. The phenanthrene/acetone mixture was poured over 1 L clean, dry sand and mixed in an aluminum pan and left under a hood for two hours to allow for acetone evaporation. The mixture was then mixed again and poured into a 4-L amber glass bottle and left uncapped under a hood to ensure volatilization of all the acetone. Sand was then transferred to a 1-L amber plastic Nalgene® bottle and stored in the freezer until the time of use. The dry sand was then poured into a 5-gallon bucket containing 24 kg of Paradise Creek sediment and homogenized for 20 min with an electric paint mixer. Homogenized sediment was left in the bucket for 24 hours after mixing to allow phenanthrene adsorption to sediment organic matter. The estimated phenanthrene concentration was 117 mg/kg wet weight. Sediment,

contaminated or uncontaminated, was then added in one of three treatment types to aquaria at a depth of approximately 40 mm; a fourth treatment utilized water only to serve as a control.

Solid magnesium peroxide cubes were made and utilized as the potential remediation method tested in this study. Solid cubes consisting of magnesium peroxide ($Mg0_2$), magnesium oxide (MgO) and magnesium hydroxide ($Mg(OH)_2$) marketed as Oxygen Release Compound (ORC® Regenesis Bioremediation Products, San Clemente, CA) were created by mixing 150 g ORC® powder with 81 mL of deionized water in a glass beaker. The resulting slurry was approximately 65% ORC®, within the range recommended by the manufacturer. The slurry was poured into plastic ice cube trays (90 cubes, 0.5 inch per side, per tray) and set out overnight to dry at room temperature. Once dry, the cubes were removed and sorted to ensure cubes were similar in size.

Experimental Design

Contaminated sediment and sediment remediation impacts upon rainbow trout were determined using two fish age classes under four experimental treatments replicated thrice. Phase I utilized juvenile fish (mean fork length, nose to the fork in tail, 46.5mm, sd=4.2). Phase II utilized rainbow trout two days post-hatch. Fish were exposed to one of three treatments: 1) phenanthrene contaminated sediment, 2) phenanthrene contaminated sediment with remediation, 3) non-contaminated sediment with remediation, and 4) a control of water without sediment. During Phase II, pebbles were added to the surface of the sediment to minimize sediment related mortality of the newly hatched rainbow trout.

The experiment was conducted in an environmental chamber where the temperature remained constant within each replicate. During Phase I the photoperiod was kept on a 12 hour light/12 hour dark cycle. During Phase II, the newly hatched fish were reared under continuous dark conditions until exogenous feeding began, at which point the photoperiod was changed to the same conditions as Phase I. The water solution used was as described previously and aerated via an air stone in 5-gallon aquaria. Aquaria were non-filtered static systems with the exception of a daily water change during Phase I and every other day during Phase II. The water change consisted of removing approximately 50% of the water (2.5 gallons). This was accomplished by drawing the water down using a siphon to the top of a 100-mL beaker placed inside the aquaria. Water was then added back in the aquarium by allowing the water to flow into the 100-mL beaker, which acted as a water flow barrier to minimize sediment re-suspension. Water removed from each aquarium was run through a 1-L column of granulated activated carbon (GAC). Each aquarium was populated with 10 juvenile fish and approximately 40 yolk-sac fish for Phase I and II, respectively.

Aquaria were maintained for a total of 68 days. The same aquaria and sediment were used in both Phase I and Phase II. Fish were present for 30 and 29 days for Phase I and II, respectively. A six day hiatus occurred between phase one and two as a result of waiting for rainbow trout embryonic development. During each phase, a daily record of each aquarium was made to document fish mortality, dissolved oxygen, pH, and temperature prior to the water change. A YSI Professional Plus® (Yellow Springs, OH) instrument with dissolved oxygen, pH, and temperature probes, calibrated to manufacturer specifications, was used to monitor the aquaria daily.

Sediment Sampling, Extraction, and Analysis

Sediment grab samples were taken from each tank at three time intervals, the start of the experiment, at the end of Phase I and at the end of Phase II. Sediment samples were frozen until analysis. Phenanthrene was extracted from the sediment using a 48-h solvent extraction. Prior to extraction, samples were dried overnight (105°C) and powdered using an acetone-rinsed mortar and pestle. Approximately 20 g was transferred to a clean 60-mL vial to which 50 mL methlyene chloride was added. The extraction vials were vortexed every 12 h. After 48 h, 25 mL of the supernatant was removed from each sample and transferred to a clean 30-mL vial. A solvent exchange was then conducted, evaporating the methylene chloride via nitrogen gas while heated in a water bath at 50°C and reconstituting the extracted phenanthrene in 20 mL methanol.

Phenanthrene concentrations in the extracts were determined using high pressure liquid chromatography (HPLC) on an Agilent HP 1100 HPLC equipped with an Agilent 1100 diode array detector. Compound separation was achieved using a Supelco Supelcosil LC-PAH 5 µm column (15 cm x 4.6 mm; Sigma-Aldrich, St. Louis, MO), operated at a 26°C column temperature, a 1 mL/min flow rate, and a 50 µL injection volume. A phenanthrene standard curve was created using the following concentrations: 100, 50, 10, 5, and 1 mg/L in methanol (8, Appendix A).

Water Sampling, Extraction, and Analysis

The water removed daily from each aquarium was passed through a 1-L column of GAC to capture any phenanthrene in the water. GAC samples for each aquarium were then frozen

and saved until extraction. Phenanthrene was extracted from the GAC in 1-gallon glass jars using a 400:200 mL solvent ratio of acetone:methlyene chloride. The jars were shaken by hand every 12 h for 48 h. A 50-mL subsample was then extracted into a 65-mL vial. A solvent exchange was then conducted, evaporating the methylene chloride via nitrogen gas while heated in a water bath at 50°C and reconstituting the extracted phenanthrene in 20 mL methanol.

A positive control GAC sample spiked with 0.3 mg phenanthrene was prepared to evaluate the efficiency of the extraction method. This sample was extracted in the same manner as the aquaria GAC samples. Phenanthrene concentrations in all extracts were determined using HPLC with the same parameters as the sediment sample extracts.

Fish Tissue Sampling, Extraction, and Analysis

At the end of each experimental phase, fish were weighed and measured after being euthanized by a lethal dose of tricaine methanesulfonate (MS-222). The livers were removed from five fish in each replicate from Phase I, weighed and frozen. Whole fish were freeze dried in a Multi-Dry® dryer (FTS Systems Inc., Stone Ridge, NY) for five days and frozen at -80°C prior to extraction; liver samples were not freeze dried.

Phenanthrene present in the fish tissue samples was extracted using a Dionex ASE 200 Accelerated Solvent Extractor (ASE). Prior to extraction, samples were dried overnight (105°C) and ground using an acetone-rinsed mortar and pestle. The sample was transferred to clean 22-mL stainless steel extraction cells (Dionex Corporation, Sunnyvale, CA) and topped off with clean sand to minimize solvent waste. Extraction was performed according to the

manufacturer's instructions at the recommended system conditions, using a system pressure of 1,500 psi, oven temperature of 100°C, oven heat-up and static time of 5 min, with a 1:1 solvent ratio of dichloromethane and acetone. The extraction cell was flushed with 60% of the cell volume and purged with nitrogen at 150 psi for 1 min. Extract was collected in clean 60-mL vials. A solvent exchange was then conducted, evaporating the methylene chloride/acetone via nitrogen gas while heated in a water bath at 50°C and reconstituting the extracted phenanthrene in 20 mL methlyene chloride prior to gas chromatography-mass spectrometry (GC-MS) analysis

A positive control to examine the efficiency of the ASE extraction of phenanthrene from fish tissue was performed. Eight rainbow trout not previously exposed to phenanthrene were sacrificed by applying a lethal dose of MS-222. Four fish were injected with 20 µg of phenanthrene and split into two replicates and all eight were dried overnight (105°C). These fish were prepared and extracted in the same method described previously.

For these fish extracts, an Agilent 7890A GC and Agilent 5975C MS was used. Compound separation was achieved using a J & W Scientific DB5-MS column (30 cm x 250 µm x 0.5 µm; Agilent Tech., Santa Clara, CA) with a sample injection of 1 µL. The inlet temperature was 280°C and the oven temperature was set at 80°C for 2 min, 12°C for 1 min, and then 280°C for 1 min with constant helium flow at 1.5 mL/min. MS parameters included a 320°C transfer temperature, a 230°C source temperature and a 150°C quadrupole temperature. Acquisition was made using the selective ion monitoring (SIM) mode with the primary mass 178 and confirming masses of 152 and 76 for phenanthrene. A phenanthrene standard curve was

created using the following concentrations: 100, 50, 10, 5, and 1 mg/L in methanol (Figure 10, Appendix B).

Statistical Analysis

T-tests, ANOVA and Tukeys tests (significance $t<0.05$) were used to evaluated statistical significance. Statistical comparisons, using a t-test, were made between contaminated sediment treatments with and without remediation in water dissolved oxygen levels. ANOVA and Tukeys tests were utilized to make statistical comparisons of fish condition factors as a result of treatment type and the environmental parameters pH, dissolved oxygen, and temperature, as well as to compare phenanthrene concentration in the fish. Statistical comparisons, using a t-test, were also made between fish condition factor for each of the treatment means for treatments with sediment.

2.3 Results

2.3.1 Environmental Conditions

Temperature during Phases 1 and 2

The temperature within each aquarium was similar throughout each phase of the experiment (Figure 2). Variation occurred between replicates within the treatments based upon their location in the environmental chamber. Aquaria located near the bottom of the environmental chamber were colder than those located at the top; each treatment had a replicate at each level in the environmental chamber. Replicates located on the top shelf of the environmental chambers had the highest temperatures with a range of 13.5—15.3°C for Phase I

and 14.7—15.3°C for Phase II. Replicates located at the bottom of the environmental chambers had the lowest relative temperatures with a temperature range of 2.0—9.9°C for Phase I and 3.3—13.3°C for Phase II. Replicates located in the middle had a temperature range of 12.4—14.4°C for Phase I and 12.9—15.4°C for Phase II. Treatments 1 (contaminated sediment) and 2 (contaminated sediment with remediation) were in one environmental chamber and treatments 3 (non-contaminated sediment with remediation) and 4 (water only) in another.

Dissolved Oxygen during Phases 1 and 2

Dissolved oxygen was also constant within a given aquarium (Figure 2). As dissolved oxygen saturation is a function of temperature, the observed dissolved oxygen level varied between replicates based on the location each treatment replicate with in the environmental chamber. Aquaria with lower temperatures had higher dissolved oxygen levels. Again, replicates located in the upper part of the environmental chamber had the lowest relative dissolved oxygen concentration. Water dissolved oxygen was greater than 7.66 mg/L in all replicates. Replicates located in the lower part of the environmental chamber had the highest relative dissolved oxygen concentration and lowest relative temperatures.

Although temperatures were comparable between replicates located in similar sections of the environmental chamber, there was a statistical difference observed between dissolved oxygen levels in the contaminated sediments with and without remediation. During Phase I, replicates of treatment 1 and 2 in the top of the environmental chamber had statistically different dissolved oxygen levels ($t<0.0001$), as did replicates of treatment 1 and 2 in the middle of the environmental chamber ($t=0.0022$) and replicates of treatment 1 and 2 located in the

bottom of the environmental chamber (t=0.0242). During Phase II, replicates of treatment 1 and 2 located at the top of the environmental chamber were statistically different (t<0.0001), as were replicates of treatment 1 and 2 located in the bottom of the environmental chamber (t<0.0001).

pH during Phases 1 and 2

The pH of the water was consistent in replicates over time (Figure 3). Phase II had a higher pH in the corresponding aquaria for each replicate. Contaminated sediment replicates had a similar mean pH as the contaminated sediment with remediation replicates; mean pH ranged from 8.29 to 8.68 in all aquaria. The greatest error (standard deviation/mean) for pH was less than 1.5% in all aquaria with sediment (treatments 1, 2 and 3) for both phases.

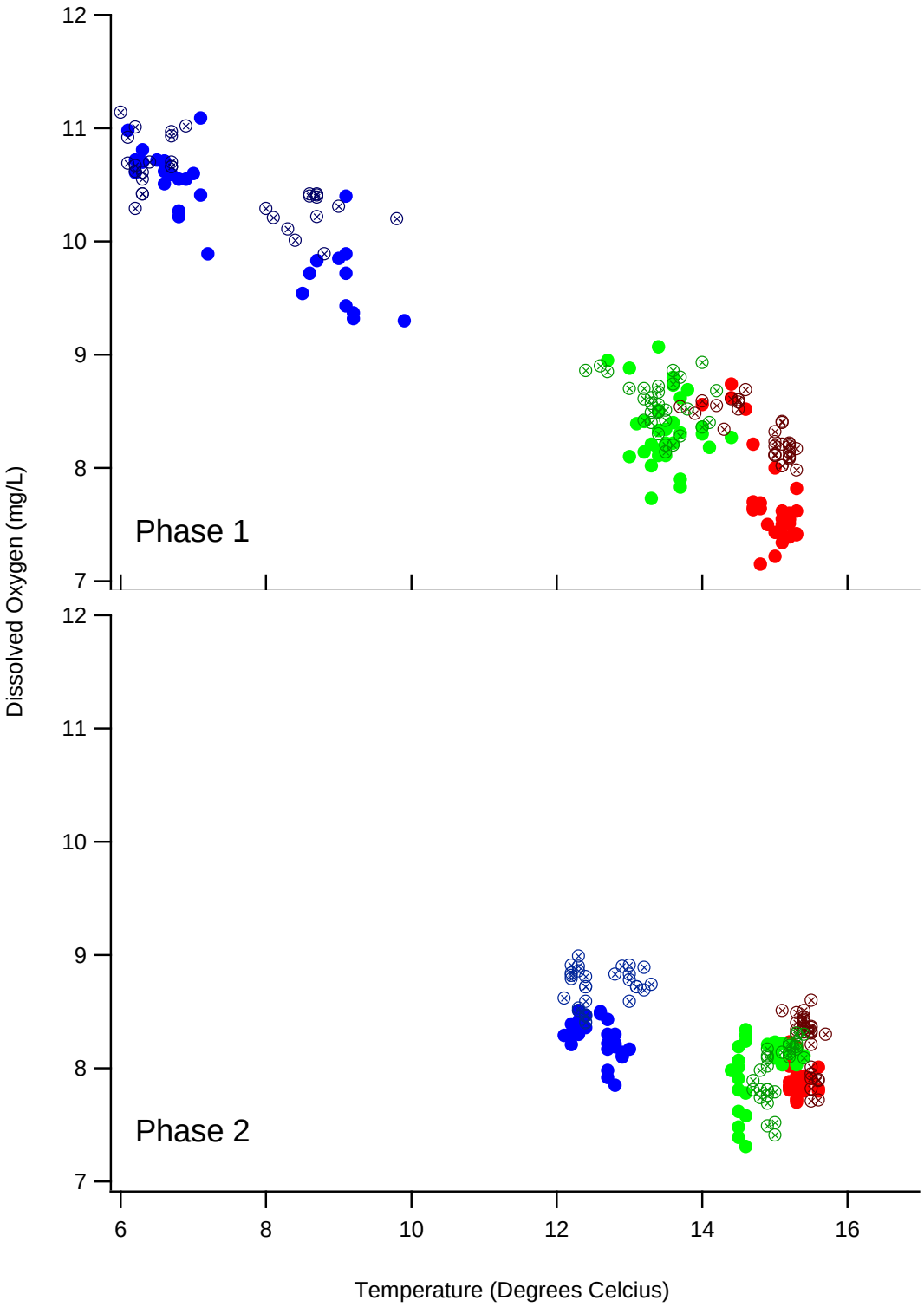

Figure 2. Temperature (°C) plotted against dissolved oxygen (mg/L) for contaminated sediment replicates (closed circles) and contaminated sediment with remediation replicates (open circles with x's). The differences in color represent different replicates, warm (red), mid (green) and blue (cool).

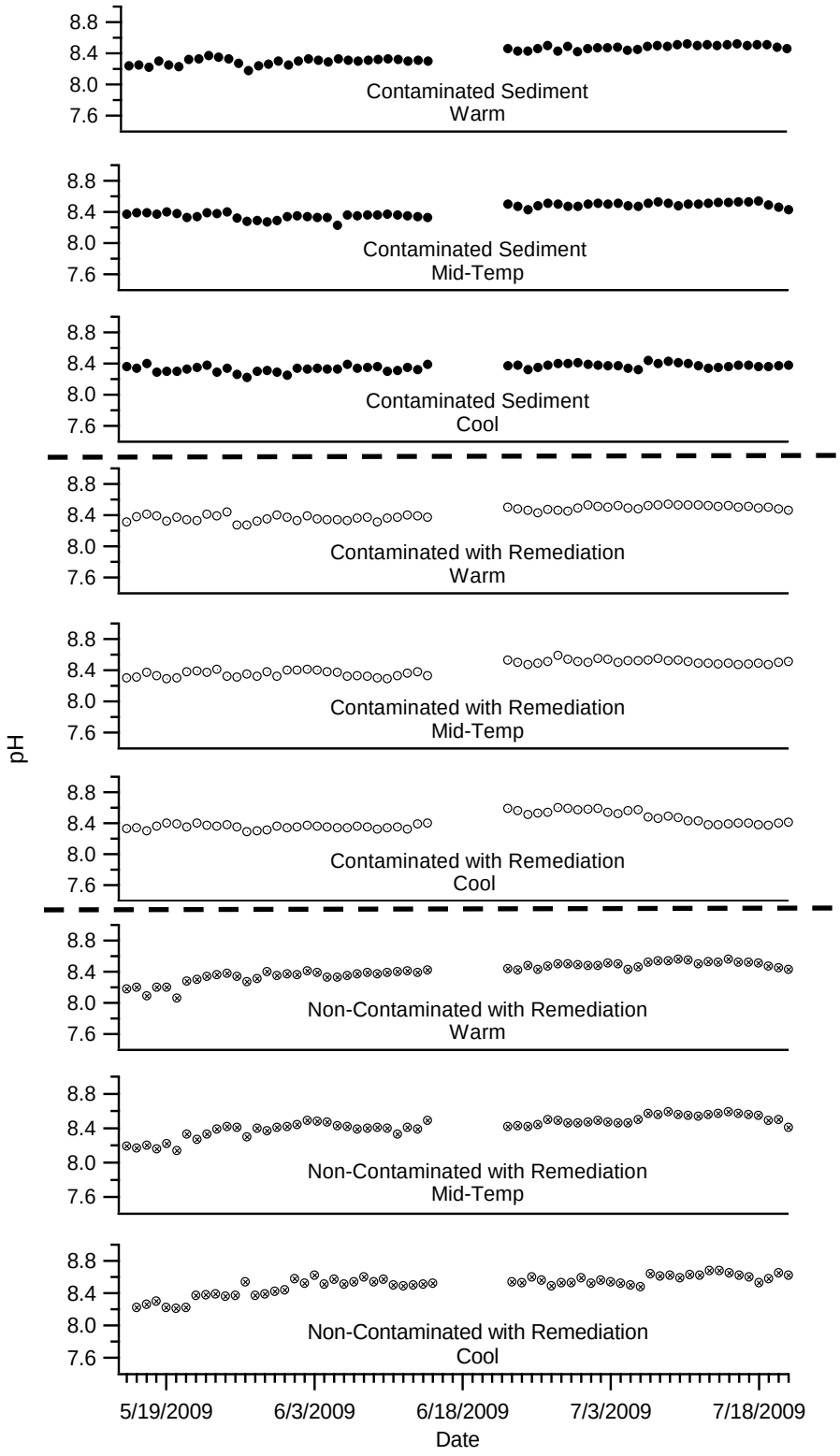

Figure 3. Daily water pH values for aquaria with the three sediment treatments.

Sediment Phenanthrene Degradation

Sediment phenanthrene concentrations decreased significantly from initial levels under both the remediation and non-remediation treatments (Figure 4). The highest decrease in phenanthrene concentration during Phase I was observed in the contaminated sediment treatments with a total decline of 92%. During Phase II, the highest level of degradation was observed in the contaminated sediments without remediation; however, the aquarium with the greatest decrease in phenanthrene concentration was observed in a sediment replicate undergoing the remediation treatment. Phenanthrene degradation byproducts were seen at relatively low levels in relationship to phenanthrene (Figure 9, Appendix A).

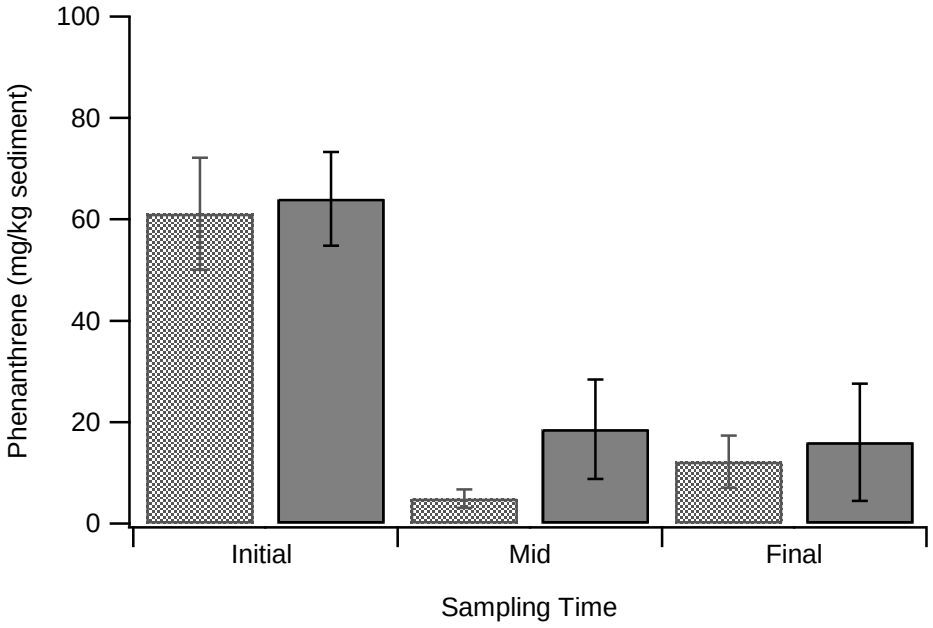

Figure 4. Phenanthrene concentration (mg/kg sediment) during the start of Phase I (Initial), the end of Phase I (Mid), and the end of Phase II (Final). Lighter colored bars on the left represent the mean value of the contaminated sediment treatment and the darker bars on the right represent the mean values of the contaminated sediment with remediation treatment. Error bars represent standard deviation.

2.3.2 Fish Condition

Length-Weight Relationships

The condition factor, "k", was used in this study to normalize the length and weight of fish between replicates due to possible differences in growth as a result of temperature. The condition factor is a relative measurement of the robustness or well being of a fish; a healthy fish should have a condition factor close to 1.0. The condition factor was determined by the equation:

$$k = (100{,}000 \times W)/L^3$$

where k = condition factor, W = weight in grams, and L= length in millimeters. During Phase I, condition factors were highest in the contaminated sediment undergoing the remediation treatment (mean k=0.99, sd=0.05), followed by the non-remediated contaminated sediment treatment (mean k=0.96, sd=0.06), and finally the non-contaminated sediment with remediation and the water only treatments (mean k=0.94, sd=0.06 and 0.07 respectively) (Figure 5). Condition factors were relatively close to 1.0 for all treatments. Statistical differences in fish condition factor were observed between fish in the contaminated sediment with remediation treatment and the non-contaminated sediment with remediation treatment (t=0.0013) as well as between fish in the contaminated sediment with remediation treatment and the water only treatment (t=0.0034). The survival in all aquaria in Phase I was 100%.

In Phase II condition factors were highest in the non-contaminated sediment with remediation (k=1.06, sd=0.25), followed by the non-remediation contaminated sediment

treatment (k=0.99, sd=0.19), then the water only treatment (k=0.97, sd=0.20) and finally the contaminated sediment undergoing remediation (k=0.94, sd=0.19) (Figure 6). All condition factors were relatively close to 1.0 as in Phase I. Statistical differences in fish condition factor were observed between fish in the contaminated sediment treatment and the contaminated sediment with remediation (t=0.0280), the contaminated sediment undergoing remediation treatment and the non-contaminated sediment undergoing remediation treatment (t=0.0003), and the non-contaminated sediment undergoing remediation treatment and the water only treatment (t=0.0117). However, the contaminated sediment undergoing remediation treatment had the highest survival (90%) followed by the contaminated sediment (89%), and then the non-contaminated sediment undergoing remediation (88%) with the water only treatment having the lowest survival (81%). The only significant difference, using a t-test to compare treatment survival means, was observed between treatment 2 and 4 (t=0.0496).

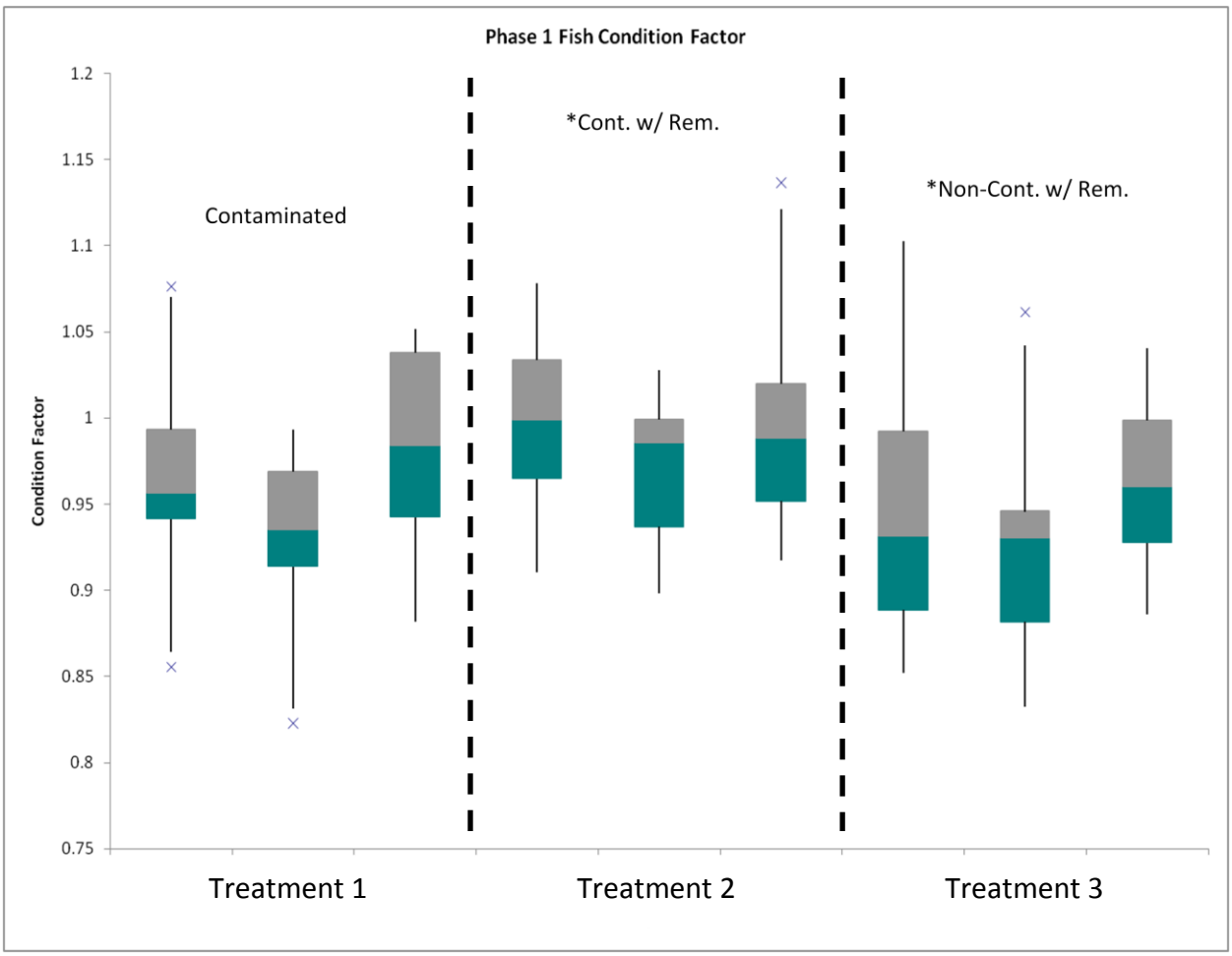

Figure 5. Fish condition factor (k) for Phase I (juvenile). Each treatment has three replicates. The change in color within the box represents the median fish k, the lower half of the box represents the 1st quartile, the top the 3rd quartile. The bottom whisker represents the lower of the minimum k value or 1.5 X * (3rd quartile – 1st quartile). "x" represents an outlier as determined by a k value less than or greater than 1.5 * (3rd quartile – 1st quartile). Mean k values were significantly different between treatments 2 and 3 (denoted by *).

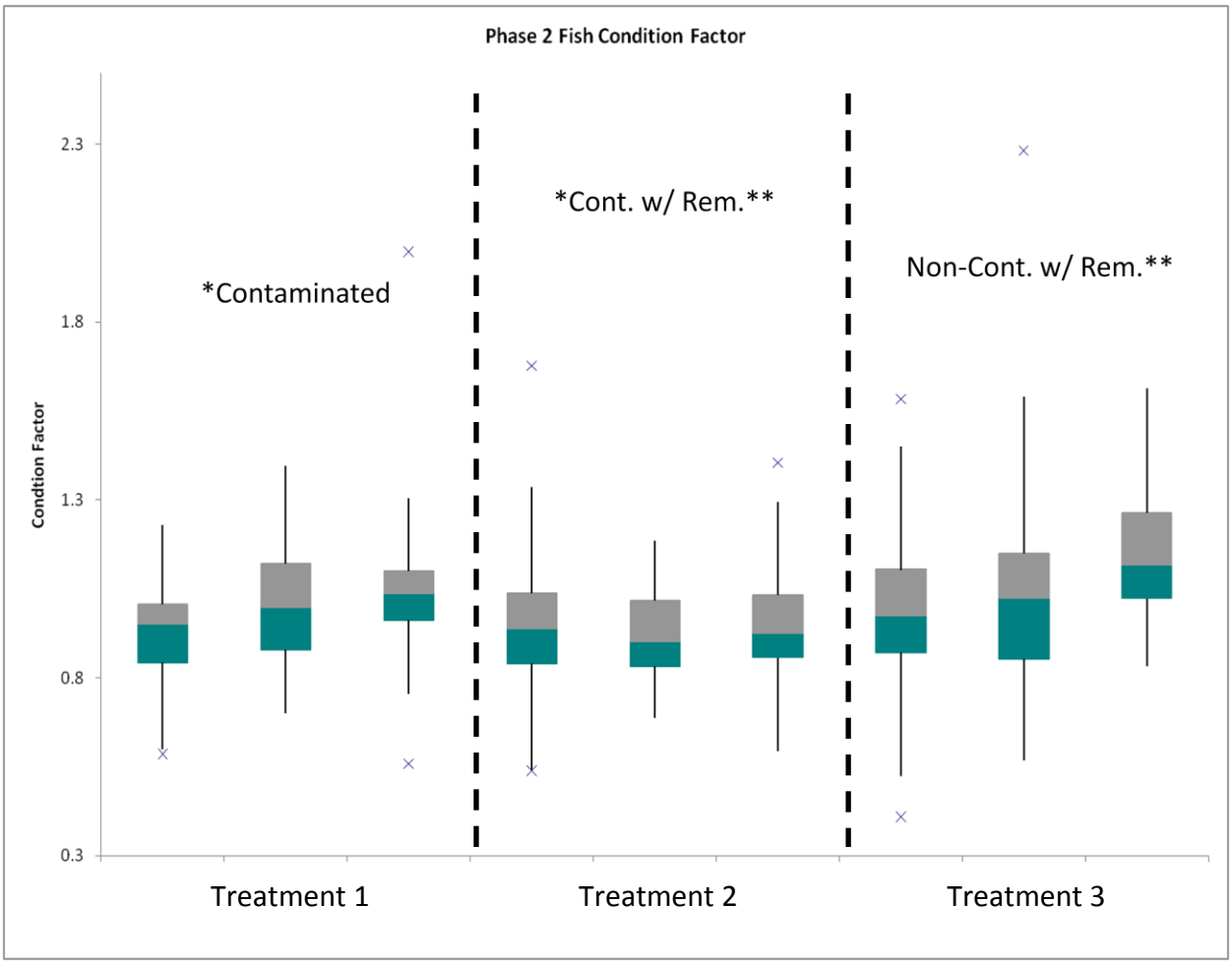

Figure 6. Fish condition factor (k) for Phase II (yolk-sac stage). The change in color within the box represents the median fish k, the lower half of the box represents the 1st quartile, the top the 3rd quartile. The bottom whisker represents the lower of the minimum k value or 1.5 X * (3rd quartile – 1st quartile). "x" represents an outlier as determined by a k value less than or greater than 1.5 (3rd quartile – 1st quartile). Mean k values were significantly different between treatments 1 and 2 (denoted by *) and treatments 2 and 3 (denoted by **).

The hepatosomatic index (HSI), the ratio of liver weight to body weight, was measured and varied across treatments. Increases in HSI have been linked to fish exposure to PAH contamination (Pinkney et al., 2001). The lowest mean HSI value was observed in the contaminated sediment undergoing remediation treatment (mean HSI=1.08, sd=0.25). The highest mean HSI was observed in the water only treatment (mean HSI=1.38, sd=0.50), with the contaminated sediment (mean HSI=1.21, sd=0.40) and non-contaminated sediment undergoing remediation (mean HSI=1.14, sd=0.33) yielding intermediate HSI values. Variation in HSI values was large as indicated by the standard deviation. Only treatments 2 and 4 were statistically different (t=0.0464).

Fish Accumulation of Phenanthrene

Phase I of the experiment showed that phenanthrene accumulated in the rainbow trout tissue. In the contaminated sediment treatment, phenanthrene values ranged from 26.0 to 34.7 mg per kg of fish tissue, with a mean value of 31.8 mg per kg fish tissue (sd=5.1 mg/kg) (Figure 7). In the contaminated sediment with remediation, phenanthrene ranged from 4.4 to 114.0 mg per kg of fish tissue, with a mean value of 67.7 mg per kg of fish tissue (SD=56.7 mg/kg). No statistical difference in fish uptake of phenanthrene was observed between treatment 1 and 2 (ANOVA, F=0.337). Phenanthrene degradation byproducts were not seen in large amounts relative to phenanthrene in the fish tissue (Figure 11, Appendix B).

Phase II of the experiment showed that phenanthrene accumulation in the yolk-sac rainbow trout was not detected. The two positive control samples of fish injected with phenanthrene yielded phenanthrene recoveries from fish tissue of 79% and 84%.

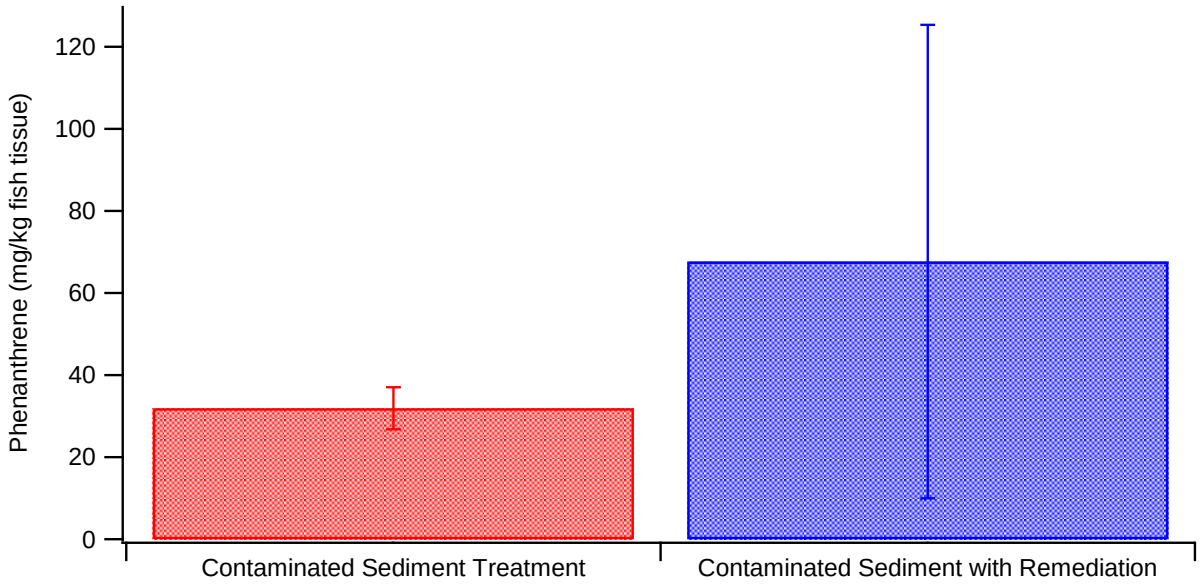

Figure 7. Mean phenanthrene concentrations in fish tissue for Phase 1 (mg phenanthrene/kg fish tissue). Error bars represent the standard deviation.

Impacts of Treatment and Environmental Conditions upon Fish Condition Factors

Phase 1

Results of ANOVA analysis comparing the mean fish condition factor between treatments 1, 2 and 3 (treatments with sediment present) in Phase I suggests that there are differences when comparing the treatments together, although it is not significant at the 0.05 level (F=0.087). However, this suggests that there are differences between fish condition factors between individual treatments. Using the Tukey method to compare individual treatments showed that treatments 2 (contaminated sediment undergoing remediation,) and 3 (non-contaminated sediment undergoing remediation) exhibited a statistical difference (P=0.077). No statistical differences were observed in fish condition factors between treatments 1 and 2, or 1 and 3 (P=0.282 and 0.589, respectively).

When comparing fish condition factors between treatment types after adjusting these values for temperature, dissolved oxygen and pH variation, no significant impacts was observed. ANOVA analysis when taking temperature into account yielded F=0.064 as compared to F=0.087 before adjusting for temperature. ANOVA analysis when accounting for dissolved oxygen and pH yielded F=0.072 and F=0.119, respectively, as compared to the F=0.087 value when comparing fish condition factor with just the treatment type. Tukey analysis between each individual treatment, suggests that temperature, dissolved oxygen and pH do not have an effect on condition factor by treatment type, as similar P values are found when accounting for the three variables as when just comparing fish condition factor with treatment type. All statistical results were checked for normality using the Shapiro-Wilk test and variability of

normal distribution. Variability in normal distribution was adjusted as needed using the Kendal Roger method.

Phase II

Results of ANOVA analysis comparing the mean fish condition factor between treatments 1, 2 and 3 (treatments with sediment present) in Phase II suggests that there are differences when comparing the treatments together, although it is not significant at the 0.05 level (F=0.139). However, this suggests that there are differences between fish condition factors between individual treatments. Using the Tukey method to compare individual treatments showed that treatments 2 (contaminated sediment undergoing remediation) and 3 (non-contaminated sediment undergoing remediation) exhibited a statistical difference (P=0.122). No statistical differences were observed in fish condition factor between treatments 1 and 2, or 1 and 3 (P=0.503 and 0.509 respectively).

When comparing fish condition factors between treatment types after adjusting these values for temperature, dissolved oxygen and pH variation, no significant impacts was observed. ANOVA analysis taking temperature into account yielded F=0.184 as compared to F=0.139 before adjusting for temperature. ANOVA values when accounting for dissolved oxygen and pH are F=0.221 and F=0.111 respectively as compared to the F=0.139 value when comparing fish condition factor with just the treatment type. Tukey analysis between each individual treatment suggests that temperature, dissolved oxygen and pH do not have an effect on condition factor by treatment type. P values are lower when comparing just the treatment and the fish condition factor than when accounting for the three variables. All statistical results

were checked for normality using the Shapiro-Wilk test and variability of normal distribution. Variability in normal distribution was adjusted as needed using the Kendal Roger method.

2.4 Discussion

Environmental Condition

Within the non-static aquaria, which received daily or every other day water changes, the sediment remediation did not have an impact on temperature. The temperature, regulated by the environmental chamber, was considerably stable within each aquarium. Differences within treatments were observed based upon the location within the environmental chamber – those closer to the refrigeration output were relatively cooler than those located further away. A remedial impact upon temperature was not observed.

Dissolved oxygen saturation in water is highly correlated with temperature, but was significantly higher in replicates undergoing remediation. Within the range of temperatures observed during this experiment, a higher dissolved oxygen concentration would be expected in aquaria with lower temperatures. This trend was observed, however, there was a significant difference between dissolved oxygen levels between aquaria with contaminated sediment not undergoing remediation and those undergoing remediation. The goal of the remediation method utilized in this experiment was to add oxygen to the sediment to increase microbial degradation of phenanthrene. It seems that additional oxygen was added to the water as well. Even with the constant water changes, dissolved oxygen was significantly greater in the remediated replicates, suggesting that oxygen release was rapid within the aquaria. Water

changes did remove the top portion of the water column, but aeration via pump and air stone resulted in constant water mixing within aquaria.

The statistical increase in dissolved oxygen in the water as a result of remediation could potentially be useful in scenarios other than contaminant remediation. During this experiment, daily water dissolved oxygen concentrations were all above 7 mg/L, most likely a result of constant aeration. At or above this level, no detrimental impacts should be observed to aquatic life due to oxygen limitations. However, in a situation where aquatic dissolved oxygen levels are at concentrations that may be detrimental to aquatic life, a similar remedial strategy could be beneficial. Highly polluted or anthropogenically influenced eutrophic bodies of water can result in high biological oxygen demand that create "dead zones." Utilizing the novel remediation proposed in this experiment could increase dissolved oxygen levels in the sediment and/or closely associated bottom water strata.

Water pH levels were also stable during the experiment, most likely a result of water changes. This would suggest that the remediation proposed in this experiment would not significantly alter the pH in a dynamic system. In a different complementary study investigating the application of ORC® as a sediment remediation method that used static systems without fish, pH significantly increased in the remediation treatments (Schaffnit, 2009). Thus in a lentic system (e.g. a lake) without flow, pH may be of concern.

Phenanthrene degradation occurred in both the remediated and non-remediated treatments with contaminated sediment, but no statistical difference was observed. The large decrease in phenanthrene in these two treatments could have resulted from the prevalence of the newly added, bioavailable phenanthrene used as an energy source by microorganisms. The

method in which the sediment was prepared and mixed prior to the study may also help explain the similar phenathrene degradation rates, even in the absence of the remediation treatment. Oxygen was likely added into the sediment during sediment phenanthrene mixing, such that it was not initially anoxic. In addition, other phenanthrene degradation mechanisms were likely occurring within the sediment enhancing degradation, even without oxygen additions. These mechanisms are physical, chemical and biological, even though this study aimed to enhance microbial biodegradation using reactive solids to increase oxygen levels.

An unexpected increase in the sediment phenanthrene concentration was observed from the middle sampling point to the end sampling point in the remediated sediment. Grab samples were used to evaluate phenanthrene concentrations in the sediments, and a non-uniform spatial distribution of this compound may have created an experimental error that accounts for this phenomenon. Degradation is likely not occurring in a uniform fashion within the sediment and a degree of spatial variation is expected.

Given additional time, differences in the sediment phenanthrene concentrations may have been observed such that the remediated sediment had a lower level than the un-remediated sediment. This is because the readily bioavailable phenanthrene, nutrients and oxygen would be used relatively quickly by microbial degraders. As nutrients and oxygen are used in both sediments, oxygen would continue to be supplied via the solid peroxide blocks in the remediated sediment. Given time to reach a steady state, the sediment without remediation would reach low dissolved oxygen concentrations.

Thresholds for sediment PAH concentrations proposed by Johnson et al. (2002) are less than 1 ppm dry weight of sediment. This level is based upon a significant increase in liver

lesions and diminished indicators of reproductive function in fish above this level (Johnson et al., 2002). In this study, although the sediment was artificially spiked, phenanthrene levels were much higher than the 1 ppm threshold in both the remediated and un-remediated treatments. This suggests that the sediment phenanthrene levels observed during this experiment were at a level that may impart liver lesions and affect reproductive function, especially if given adequate exposure time.

The release of degradation byproducts or an increase in bioavailability is possible during contaminant degradation. Byproducts are also an issue of concern in addition to the initial contamination. Degradation byproducts should be considered when conducting future remediation experiments of this type.

Fish Condition

During Phase I the juvenile fish condition factors were highest in the contaminated sediment undergoing remediation, followed by the contaminated sediment and then the non-contaminated sediment with remediation. This is the opposite of what was expected, as the contaminated sediment would be expected to have a negative impact upon fish condition. However, during this phase no mortality was observed and condition factors were close to 1 and all were above 0.8. This suggests that neither the contamination in the sediment, nor the remediation, nor the degradation byproducts had a large impact upon fish condition. It is possible that because the juvenile fish had a relatively low association with the sediment, little impact would be observed and therefore minimal differences would be observed in fish condition factors. This might suggest that phenanthrene and degradation byproduct levels in

the water were insignificant in affecting fish condition factor. Fish at this point in their lives are heartier than during earlier life stages. Further examination into sediment byproducts and fish metabolism of phenanthrene is needed.

During Phase II, fish condition factors for the yolk-sac stage trout were the opposite of those observed in Phase I. The non-contaminated sediment undergoing the remediation treatment had the highest fish condition factors, followed by the contaminated sediment treatment and then the contaminated sediment with remediation treatment. This was expected as a result of both the initial contamination and potential degradation byproducts from the contaminated sediment undergoing remediation having an impact upon fish condition. The non-contaminated sediment with remediation was expected to have the highest fish condition factor as a result of no contamination. The association between the yolk-sac fry and the sediment is large, as physical contact occurs between the fish and the sediment during this stage. Physical contact with the contaminant or degradation byproducts could have resulted in the observed differences in fish condition factors between treatments. The treatment undergoing remediation could potentially be making the contaminant and degradation byproducts more bioavailabe.

Mortality was observed during the second phase of the experiment. Even though observed fish condition factors were lowest in the contaminated sediment with remediation treatment of the three sediment treatments, survival was the highest in this treatment (90%). Fish condition factor did not account for mortalities, thus this specific treatment could have been lowered by fish that were in poor condition but did not die as they may have in other treatments. Overall, all the sediment treatments provided a higher survivorship (90%, 89%,

88%) of fish than the water only treatment (81%). Fish mortality specifically due to PAH contamination has been observed in previous studies (Fragoso et al., 2006; Jonsson et al., 2004). Although this experiment utilized just phenanthrene instead of a group of PAHs that would be found in naturally contaminated sediment, exposure to the single contaminant could help explain mortality in contaminated treatments. Differences in mortality between treatments in this experiment could be a result of the sediment acting as a sink and buffer to the fish waste or that the remedial practice had a positive impact upon fish survivorship.

Differences in condition factors could be attributed to unmeasured environmental differences between replicates. Potential differences within aquaria could have resulted from the preparation method or variations in sediment composition even after homogenization. Other factors could include fish density in aquaria and resulting fish waste products. Fish behavior, as a result of density and relative differences in fish size, could also impact both condition factor and survivorship. Other un-realized environmental condition impacts may also be influencing fish condition.

The hepatosomatic index, which was only evaluated during the first phase of the experiment, revealed convoluted results. The water only treatment provided the highest HSI, followed by the contaminated sediment treatment, non-contaminated sediment with remediation, and finally the contaminated sediment with remediation. Expected results would have been to see both contaminated sediment treatments to have the highest HSI as this value has been shown to positively correlate with a contaminated environment, specifically in regards to elevated PAH metabolite levels in fish (Pinkney et al., 2003). Although this study did not examine PAH metabolites, this premise suggests that these metabolites were either limited in

the contaminated sediment treatments or did not affect HSI. However, Yang and Baumann (2006) observed little correlation between HSI and PAH contamination when examining brown bullhead (*Ameiurus nebulosus*), but concluded this was due to low PAH exposure concentrations. In this study, HSI correlation with contaminated sediment may not have been high as a result of human error while dissecting the fish to remove the liver or other environment effects within the aquaria, in addition to insufficient exposure to phenanthrene and its metabolites.

Accumulation of phenanthrene in fish tissue was observed in Phase I of this study. There was a greater mean phenanthrene accumulation in fish tissue within the contaminated sediment undergoing remediation than in the contaminated sediment treatment only. This may indicate that the remediation method tested increased the phenanthrene bioavailability to the fish. When comparing the extracted phenanthrene concentration (mg/kg) in the sediment with the extracted phenanthrene concentration in the fish tissue, there was approximately a 1:1 ratio in the contaminated sediment with remediation treatment versus a 2:1 ratio in the contaminated sediment treatment. However, the lowest amount of accumulation detected was observed in one of the treated sediment replicates. The standard deviation was higher in the contaminated sediment with remediation compared to the contaminated sediment treatment (sd=5.1 vs 56.7 mg/kg). The large phenanthrene concentration difference observed in the sediment undergoing remediation could have non-uniform distribution of phenanthrene in the sediment due to degradation rates being higher near the reactive solids, and the random selection of sediment samples for analysis.

In comparison to other sediment remediation techniques, the novel method tested in this study may have a similar or lesser impact upon fish than the established methods. Cohen et al. (2001) observed higher stress levels, as measured by oxygen consumption, in fish exposed to remediated crude oil as compared to control groups. Two remediation techniques were used: a water accommodated fraction and a chemically dispersed crude oil water accommodated fraction (Cohen et al., 2001). When dredging contaminated sediment, Blom et al. (1998) observed an increase in histopathological damage of rainbow trout immediately after and up to two years after remediation (Blom et al., 1998). Pending minimal disturbance of the sediment when implementing the novel remediation tested here, the solid peroxide method could potentially increase contaminant degradation while limiting fish health impacts.

Phenanthrene levels accumulated in fish during Phase II were below detection levels. The relative phenanthrene abundance in the sediment was less during Phase II than during Phase I, which may account for in the negligible uptake by the fish even though there was a closer physical association with the sediment compared to Phase I. Fragoso et al. (2006) showed that direct physical contact of fish with contaminated sediment results in less contaminant uptake by fish, but not significantly so, than indirect fish to sediment contact based upon EROD activity associated with contaminant breakdown in fish (Fragoso et al., 2006).

In summary, the remediation proposed in this study did not appear to negatively affect the environmental parameters measured or fish condition, even though contaminant uptake was observed during Phase I. Over the time period of this study, no significant difference was observed between the amount of phenanthrene degradation in the non-remediated and remediated treatments. However, treatments undergoing remediation increased water

dissolved oxygen significantly, but did not greatly affect pH. There were significant differences observed in fish condition factors during Phase I between treatments 2 (contaminated sediment undergoing remediation) and 1 (contaminated sediment). Significant differences were also observed in condition factors between treatments 3 (contaminated sediment undergoing remediation) and 2 and treatments 2 and 3 in Phase II. However, fish condition factors were still close to 1 in all treatments.

CHAPTER 3

3.0 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Practical Remediation Implementation

In this experiment the proposed remediation method did not significantly improve phenanthrene degradation. However, in a complementary study examining the same remediation method under different conditions did yield a significant reduction in sediment phenanthrene levels (Schaffnit, 2009). There were several variations between the two experimental designs, as the other study utilized a static system without bioturbation from fish, water changes, and water circulation. Additionally, the sizes of the mesocosms used were much smaller in scale and the entire sediment undergoing treatment was analyzed rather than smaller sub-samples. It may be the case that the extent of degradation was underestimated in the sediment undergoing remediation due to the selection of sub-samples from a non-uniform sediment. Finally, the temperature under which this study was conducted was lower than that maintained in Schaffnit (2009) in order to provide adequate conditions for the trout, although the durations over which the studies were conducted were the same. This decrease in temperature could have slowed biological activity, and thus decreased degradation rates.

Evaluating the effectiveness of this remediation method using different sediment types is something that should be evaluated. Sediment characteristics, such as particle size and organic matter content, may help dictate the remediation potential. Experimentation on other contaminant types may also prove to be advantageous. Additionally, a field or pilot study may be beneficial in determining the remediation effectiveness under real-world conditions. In this scenario, a method must be determined to get the reactive solids into the sediment. Injection

of a peroxide slurry, solid spikes, or another method must be utilized to minimize sediment re-suspension and an increase in turbidity.

Fish Related Aspects

If additional laboratory studies are to be performed based upon the experimental design used in this study, additional parameters could be evaluated and modifications could be made in the design. Contaminant metabolite analysis in the sediment, water and fish tissue would provide greater insight into the degree of degradation in the sediment, what the metabolite pathways are, and if metabolites are a concern in regards to fish health. This additional analysis would also help to evaluate how much degradation is occurring and if it is indeed the result of microbial activity. Furthermore, determining the contaminant metabolites in fish would be beneficial to assess how much of the contaminant is actually affecting the fish. Fish liver enzyme activity could similarly be evaluated to determine if fish are metabolizing contaminants.

If conducting this study again, evaluating more fish parameters would be beneficial. Within the aquaria, measurements of ammonia, nitrate and nitrite should be measured to evaluate if fish waste products affect fish condition factor values under the conditions examined. With more funding, fish behavior could also be evaluated, via video camera, examining the interaction between fish and the sediments.

Changing the feeding regime from a surface feed to one that includes food grown in the sediment, such as invertebrates, may also give a better estimate of contaminant uptake and accumulation within fish. Contaminant accumulation also occurs via the food chain (Oliver and

Niimi, 1988) where salmonids have significantly higher pollutant concentrations than species at lower trophic levels. Either incorporating a food source that lives within the sediment or providing feed with the chemical in question could help mimic natural contaminant pathways. Muirhead et al. (2006) utilized such a method by feeding brine shrimp bio-encapsulated with PBDE-47 to medaka.

A comparison of this study and one conducted in the field examining all fish species present could provide practical information on how this remediation method effects on the local biota. Including other fish species would be constructive, as each has its own niche and association with sediment. This would also provide a more robust dataset, depending on how long the contamination has been in the environment.

Other Remediation Uses

The novel remediation method proposed in this study may also be beneficial outside the realm of contaminant remediation. Dissolved oxygen levels observed in the contaminated sediment with remediation were significantly higher than those in the non-remediated sediment. Implementing this remediation in a system with a high biological oxygen demand could possibly improve environmental quality and ecosystem function.

4.0 LITERATURE CITED

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5.0 APPENDIX A: HPLC STANDARD CURVES AND SAMPLE CHROMATOGRAM

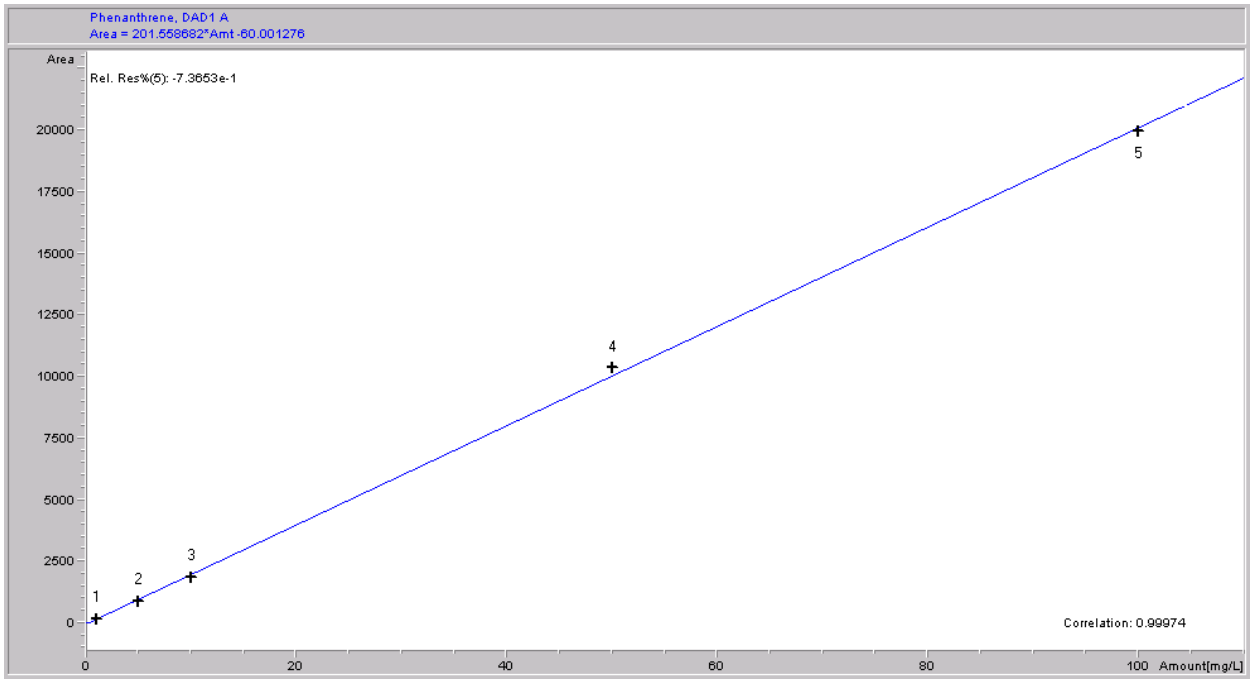

Figure 8. Phenanthrene standard curve using HPLC. Values were 1, 5, 10, 50, 100-mg/L phenanthrene in methanol, calculated R^2=0.999.

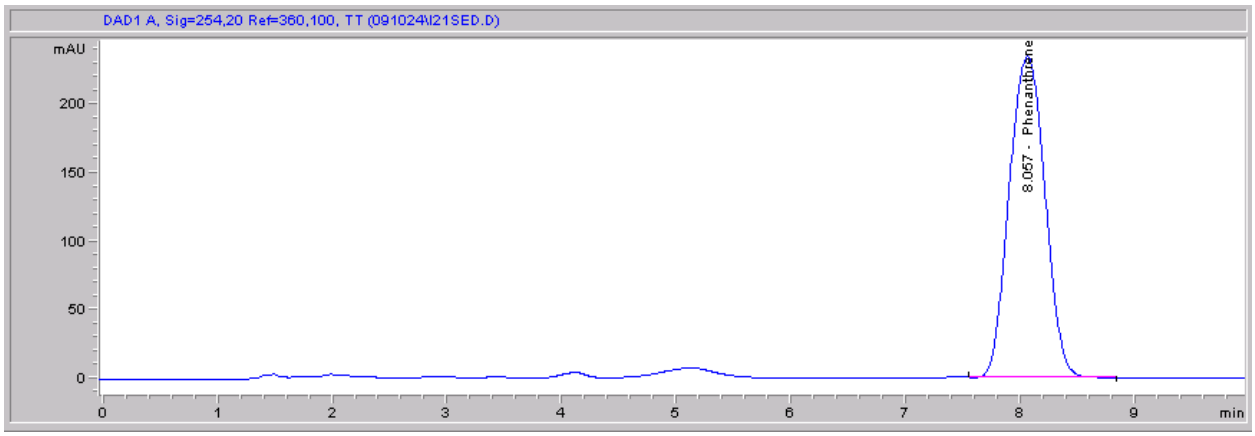

Figure 9. HPLC chromatogram output of phenanthrene extracted from a sediment sample.

6.0 APPENDIX B: GC-MS STANDARD CURVE AND POSITIVE CONTROL CHROMATOGRAM

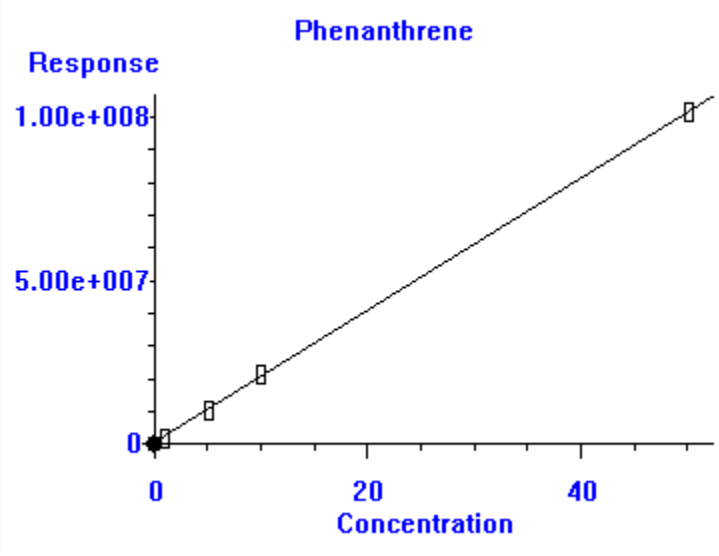

Figure 10. Phenanthrene standard curve using GC-MS. Values were 1, 5, 10, 50, 100-mg/L phenanthrene in methanol, calculated R^2=0.999.

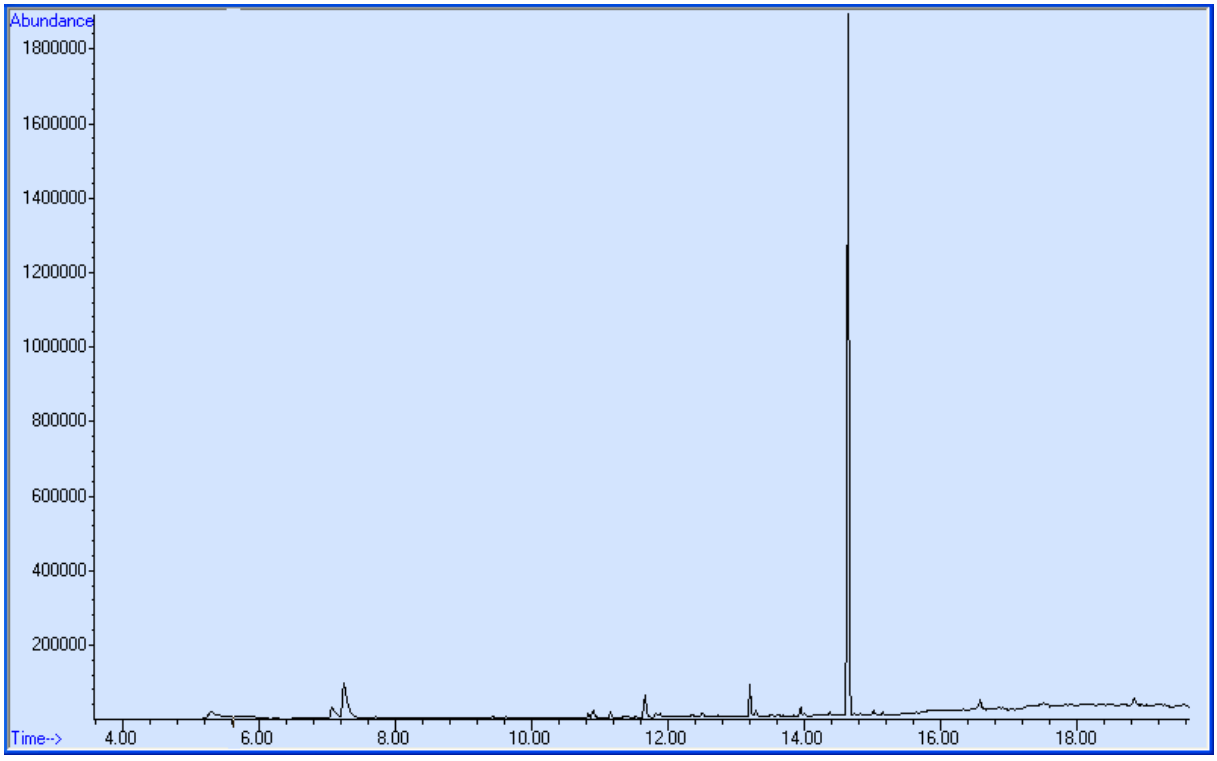

Figure 11. GC-MS chromatogram output of phenanthrene extracted from a fish tissue sample.