EFFECTS OF CFT LEGUMINE ROTENONE ON MACROINVERTEBRATES IN FOUR DRAINAGES OF MONTANA AND NEW MEXICO

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Thesis Prepared for the Degree of

Master of Science

University of North Texas

August 2011

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Skorupski, Jr., Joseph, Arthur, Effects of CFT Legumine Rotenone on Macroinvertebrates in Four Drainages of Montana and New Mexico. Master of Science, August 2011, 150 pp., 8 tables, 54 figures, references, 50 titles.

Rotenone is considered essential in the restoration of native fish populations; however, the technique is contentious and criticized, specifically concerning impacts to invertebrates. Knowledge of effects to non-target organisms is important for the management and conservation of fish populations. This thesis has two general objectives: (1) demonstrate the influence CFT Legumine rotenone has on benthic macroinvertebrates for restoration projects in Montana and New Mexico and (2) evaluate the immediate response by means of invertebrate drift.

Chapters 2 and 4 incorporate results from four different restoration projects that examine benthic macroinvertebrate response. Results indicate treatment effects are minimal for Specimen and Cherry Creek projects in Montana. New Mexico projects, Comanche and Costilla Creek suggest a greater influence. Potassium permanganate used to neutralize rotenone, influenced communities in three of the four projects. Regardless, invertebrates in all four projects recovered one-year after treatment. Chapter 3 examines macroinvertebrate drift during rotenone treatment. Results suggest a delayed response compared to previous literature. Rotenone appears to have the greatest immediate influence on the early life stages of Ephemeroptera and Plecoptera. To reduce impacts of rotenone to invertebrates, managers should apply CFT Legumine and use the minimal dosage and duration to complete the projects goal of removing non-indigenous fish species.

ACKNOWLEDGEMENTS

I would like to thank to my advisor Dr. James H. Kennedy, for his support throughout my education at University of North Texas. I would also like to thank Dr. Thomas La Point and Dr. Todd Koel for serving on my graduate committee and providing support in conducting research and writing my thesis. I would like to extend my sincere thanks to Yellowstone Center for Resources, Yellowstone Park Foundation, Turner Endangered Species Fund and Montana State University for funding this research. I would also like to thank Jeff Arnold, Mike Ruhl, Gerald Jacobi, Dan McGuire, Kirk Patten, Pat Clancey and Carter Kruse for sharing valuable information and datasets. Without their help and efforts, this thesis would not have been possible. A special thanks to Charles Randklev and Ben Lundeen for insight and support. Also, my gratitude extends to the Yellowstone fisheries crews who assisted in conducting field collections. Finally, I would like to thank my Fiancé, Erin Fratamico for always supporting my career aspirations.

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CHAPTER 1

INTRODUCTION

The widespread introduction of gamefish continues to contribute to the decline and extirpation of native trout species (Rahel 2000 and 2002). This impact has been most severe for native-trout species with small population sizes and localized distributions. Fishery managers use an assortment of techniques to manage undesirable or non-native fish populations. However, when complete removal of all fish is the goal, as is typical for restoration of a native species, the use of piscicides are required (McClay 2000). Rotenone is one of the most valuable and successful piscicides currently available (McClay 2000 and 2005). The reason for success is due to direct uptake of rotenone across the gills (Ling 2003). Rotenone inhibits mitochondrial respiration by inhibiting Complex I in the electron transport chain, eventually suffocating and killing the organism (Mangum and Madrigal 1999).

Rotenone is derived from the roots of pea plants (Family Leguminosae) including jewel vine (*Derris* spp.) and lacepod (*Lonchocarpus* spp.), that grow in Southeast Asia, South America, Australia and Oceania (Finlayson *et al.* 2000). It has been used for centuries to harvest fish for consumption in its native location by indigenous people, and more recently for greater than 150 years as a commercial insecticide. For more than 70 years, rotenone has become an important tool for fisheries managers in the restoration of native fish species. Although rotenone is a popular and highly effective method in fish management, its use has been criticized and challenged. One significant challenge, and the purpose of this research, is rotenone effects are poorly understood for non-target organisms, specifically aquatic invertebrates (Vinson *et al.* 2010).

Rotenone formulations registered with the United States Environmental Protection Agency (USEPA) as piscicides, are available as powdered extracts or emulsifiable liquids. Differences among rotenone emulsifiable formulations are the inert ingredients, which act as solvents and synergists. Although labeled as inert ingredients most of these chemicals are toxic. Conventional rotenone formulations include petroleum hydrocarbons such as toluene, xylene, benzene, naphthalene, and the synergist piperonyl butoxide. Over the past few years, environmental groups have voiced concerns involving public health, environmental impacts, animal welfare and applicator safety (McClay 2005 and Turner *et al.* 2007). The registration of CFT Legumine addresses several of the issues, which improve the restoration technique. Unlike conventional rotenone formulations, CFT Legumine was designed to reduce or eliminate a number of hydrocarbon compounds and does not include any synergists (McClay 2005; Turner *et al.* 2007; Finlayson *et al.* 2010). Therefore, risks are reduced for applicators, terrestrial species, public health, and the overall environmental impacts. The product is also more difficult for fish to detect, increasing the effectiveness of removal. The disadvantage is that removal of the synergist and hydrocarbons decreases the efficacy of CFT Legumine (as compared to conventional formulations) requiring the active ingredient concentration to be doubled from 25 to 50 ppb to achieve the desired toxicity to fish (Turner *et al.* 2007).

To optimize the application of rotenone, several environmental and biotic factors are taken into account. Rotenone degradation rate in the environment is increased primarily by increasing temperature and sunlight. The calculated half-life of rotenone was 13.9 and 83.9 hours in ponds at temperatures of 24 and 0° C, respectively (Gilderhus *et al.* 1986; Finlayson *et al.* 2000). In reservoirs, the half-life was reported at 41.8 and 84 hours at temperatures of 20–22° C and 10– 20° C (Finlayson *et al.* 2000). Rotenone typically breaks down in flowing water

in less than 24 hours due to dilution and increased hydrolysis and photolysis. Alkalinity and pH are also important factors in the rate of rotenone degradation. Water with high alkalinity (> 170 ppm $CaCO_3$) and pH (> 9.0) degrade rotenone faster than water with lower alkalinity and pH. In addition, the amount of organic matter within the water column will influence rotenone availability, because rotenone binds to organic material including plants. As a result, water with higher suspended organic material requires the application of a higher concentration of rotenone (Finlayson *et al.* 2000). Because of these properties, it is recommended that rotenone be applied to flowing waters only in the summer season. The higher temperatures and higher light penetration during summer helps to ensure toxic effects of the rotenone remain within the project area. The typical lower flows and suspended solids of late summer reduce the amount of rotenone used and improve efficacy to fish (Finlayson *et al.* 2000).

Research statement. – Rotenone is a highly effective tool in the elimination of non-native fish populations; however, there are concerns involving public health, environmental impacts, animal welfare and applicator safety. Multiple courses of action have been taken to address these issues in order to continue the use of rotenone as a fisheries management tool. One of the most recent actions was the registration of CFT Legumine™ Fish Toxicant (U.S. EPA Product Reg No: 75338-2). This formulation has fewer petroleum hydrocarbon solvents, thus reducing concern over the issues mentioned above while maintaining the products efficacy. To date, only one study in the United States has examined the effects of CFT Legumine on benthic macroinvertebrates (BMI). The purpose of my research was to determine the effects of CFT Legumine rotenone on non-target BMI organisms in mountain lakes and streams of the western United States. Field studies were conducted 2009-2010 in East Fork Specimen Creek. The focus was on benthic communities and macroinvertebrate drift; benthic communities were the

only samples collected in 2010. Interpretation of secondary datasets (acquired from other agencies) and data collected in 2009-2010 from East Fork Specimen Creek; the secondary datasets are from three restoration projects in Montana and New Mexico utilizing CFT Legumine.

The specific research objectives were:

Objective I: Determine the effects of CFT Legumine rotenone on benthic macroinvertebrate communities in East Fork Specimen Creek.

Objective II: Determine immediate effects of CFT Legumine rotenone treatment on macroinvertebrate drift.

Objective III: Compare CFT Legumine rotenone effects on benthic macroinvertebrate communities in three additional streams of Montana and New Mexico.

My research hypotheses were:

H_{01}: Benthic macroinvertebrates communities in the East Fork Specimen Creek are not affected (diversity; drift) by the treatment of CFT Legumine rotenone.

H_{a1}: Benthic macroinvertebrates communities in the East Fork Specimen Creek are affected (diversity; drift) by the treatment of CFT Legumine rotenone.

H_{02}: The effects of CFT Legumine rotenone on macroinvertebrate communities does not vary across drainages of the western United States.

H_{a2}: The effects of CFT Legumine rotenone on macroinvertebrates communities does vary across drainages of the western United States.

Laboratory studies. – Laboratory studies have established 50% lethal concentration (LC_{50}) of rotenone for both target and non-target species. Temperature and contact time are the two main variables that affect toxicity. Toxicity measured by 6 and 24 hour LC_{50}'s for 6 species of fish ranged from 3 to 42 and 2.2 to 20 ppb, respectively (Finlayson *et al.* 2010; Marking and Bills 1976; Ling 2003). During a 96 hour LC_{50}, rainbow trout (*Oncorhynchus mykiss)* ranged from 0.84 to 48 ppb of active rotenone (Turner *et al.* 2007). Likewise, after three, six, and 24 hour LC_{50}'s were 8.8, 4.3, and 3.4 ppb of active rotenone, respectively. Results indicate rainbow trout are sensitive to rotenone under a range of conditions (Finlayson *et al.* 2010; Marking and Bills 1976).

In contrast, aquatic invertebrates have a broad range of sensitivity to rotenone, with 6 hour LC_{50}'s ranging from 1.8 to 1,700 ppb of rotenone (Finlayson *et al.* 2010). Specifically, the trichopteran *Hydrosyche* and zooplankton Cladocera had LC_{50}'s of 180 ppb, and 1.8 ppb of active rotenone, respectively. These results suggest broad differences among taxonomic groups (Finlayson *et al.* 2010; Vinson *et al.* 2010; Ling 2003; Marking and Bills 1976). Differences in LC_{50} values have been variously attributed to (1) body surface area to volume ratio, with smaller individuals more susceptible, (2) exoskeleton thickness and frequent molting of the exoskeleton allowing greater permeability, or (3) greater exposure in the pelagic than benthic habitat. While this information is valid, it is important to note that most of the invertebrates evaluated were from lentic habitats. It is also apparent that morphological characteristics to obtain oxygen play a role in toxicity. Aquatic invertebrates that use tracheal gills appear more sensitive than those that acquire oxygen (1) cutaneously, (2) have respiratory pigments, or (3) are aeropnuestic (Vinson *et al.* 2010). Also, mortality for lotic invertebrates was typically near 100% for rotenone concentrations of 50 to 75 ppb and >150 ppb for most lentic taxa depending on exposure time

(Ling 2003; Vinson *et al.* 2010; Engstom-Heg *et al.* 1978). Most of the studies did not specify which formulations were evaluated for toxicity tests. However, none of them would have used CFT Legumine, because it was not available prior to 2003. It is likely that toxicity studies prior to 2003 used Nusyn-Noxfish, Noxfish, or analytical grade rotenone.

Finlayson *et al.* (2010) examined differences in toxicity of synergized Nusyn-Noxfish and non-synergized CFT Legumine rotenone formulations for rainbow trout and lotic aquatic invertebrates. Results using Nusyn-Noxfish on rainbow trout were similar to studies conducted by Marking and Bills (1976) and for the trichopteran larvae *Hydropsyche* (Chandler and Marking 1982). Rainbow trout 4 and 8 hour LC50's were 7.4 and 5.3 ppb for CFT Legumine and 7.7 and 6.2 ppb active rotenone for Nusyn-Noxfish. Macroinvertebrate 4 hour LC50 values ranged from 41 to 274 ppb of CFT Legumine and 18 to 96 ppb active rotenone of Nusyn-Noxfish. Mean 8 hour LC50 values ranged from 34 to 174 ppb of CFT Legumine and 13 to 74 ppb active rotenone of Nusyn- Noxfish. Results suggest the order of sensitivity from least to greatest was Trichoptera, Plecoptera and Ephemeroptera. Finlayson *et al.* (2010) reports an application of 11 ppb would eliminate all fish and less than 50% of insects. More importantly, it would take twice as much Nusyn-Noxfish to produce the similar effects of CFT Legumine on trout. The synergist piperonyl butoxide does not appear to increase the toxicity to trout. However, Nusyn-Noxfish was possibly twice as toxic to aquatic insects because of the synergist or other inert ingredients; therefore, the use of synergized rotenone requires twice as much product, which could likely affect more aquatic insect taxon than CFT Legumine (Finlayson *et al.* 2010).

A majority of the existing laboratory literature lacks important information to assess results accurately and the impacts rotenone has on macroinvertebrates especially in the field. Although the target concentration, temperature and duration were recorded, they typically do not

report the type of formulation applied or differentiate between the rotenone active ingredient and formulation concentrations. Furthermore, active rotenone concentration applied was typically not verified by chemical analysis.

Field studies. – Rotenone effects on invertebrates during restoration projects have been evaluated in both lentic and lotic habitats. Lentic habitat studies began in the 1940s, while lotic studies started in the 1960s. None of these studies used CFT Legumine rotenone. Results have been highly variable, with much of this variation likely related to differences in rotenone dosage (concentration x duration) and intensity of sampling (pre and posttreatment). Pretreatment invertebrate sampling has varied from zero to multiple years. Similarly, posttreatment invertebrate sampling varied from a single posttreatment sample up to four years of posttreatment sampling. Lotic studies rarely exceeded one-year pre or posttreatment sampling. The variety of dosages and sampling designs has created an array of results. In addition, the number of replicates taken and taxonomic resolution was typically not reported, which could equally influence the variability in results.

Recently, Vinson *et al.* (2010) synthesized BMI results of 13 lentic and nine lotic rotenone studies. Generally, lentic studies reported greater rotenone effects on abundance and diversity of zooplankton than benthic organisms. Kiser *et al.* (1963) treated a shallow lake with rotenone powder at 25 ppb of active ingredient. Open-water zooplankton species were completely eliminated, and remained absent for over three months. Zooplankton along the shore were immediately reduced in richness, but took two weeks for all species to be impacted. After two weeks, species began to return, and after five weeks, they were again found in high abundances. Species inhabiting submerged vegetation were minimally affected likely because rotenone binds to the plants before affecting the organisms. Studies that evaluated effects on

lentic benthic organisms reported small differences in total benthic invertebrate abundance, with effects on Chironomidae being greatest (Vinson *et al.* 2010). Blakely *et al.* (2005) reported little change in zooplankton communities or benthic invertebrates after treatment of orchard ponds. Taxa that were affected recovered within six months. However, it was noted that the orchard ponds were previously exposed to contaminants, possibly biasing results. Melaas *et al.* (2001) reported no significant short-term effect was evident in benthic taxa, whereas significant short-term effects were observed for plankton communities, but recovered the following year. Recovery of zooplankton following rotenone treatments were most often reported in terms of organism abundance. Recovery to pretreatment abundances ranged from 1 month to 3 years (Vinson *et al.* 2010).

Three lotic studies had large ranges of pre and posttreatment samples to evaluate rotenone effects on insects (Mangum and Madrigal (1999), Whelan (2002), and Hamilton *et al.* (2009)). The immediate response of aquatic invertebrates to rotenone treatments in streams has been large reductions in abundance and taxa richness. Aquatic insects seem to be more sensitive than non-insects, with the insect groups Ephemeroptera, Plecoptera, and Trichoptera appearing to be the most sensitive. Overall, invertebrate abundances generally returned to pretreatment levels quicker than richness values, likely due to the life history of specific insects. Whelan (2002) reported richness decreased 13% three years after the treatment of 75 ppb active ingredient of Noxfish rotenone. Mangum and Madrigal (1999) reported up to 100% of Ephemeroptera, Plecoptera, and Trichoptera taxa were missing after a second rotenone treatment, but their target concentration was much higher at 150 ppb active ingredient. The treatments were approximately one month apart, and macroinvertebrate samples were taken monthly (7 to 10 days after each treatment). Within one-year 46% of the EP & T taxa recovered, but 21% were still missing after

five years. Hamilton *et al.* (2009) observed total richness reductions of approximately 66% one month after treatment, which rebounded to a 37% difference nine months after treatment. One-year later richness recovered to non-significant ($p< 0.05$) levels prior to treatment. Target application was 250 ppb active ingredient of Prentox PrenFish Toxicant rotenone. Finlayson *et al.* (2010) summarized high concentrations of rotenone (>100 ppb) and treatments exceeding 8 hours, typically resulted in severe impacts to invertebrate richness and abundance. Conversely, lower rotenone concentrations (<50 ppb) and treatments less than 8 hours, resulted in less impact to invertebrate assemblages.

Study areas

Primary data set. – Specimen Creek lies in the northwest corner of YNP in Gallatin and Park Counties of Montana. This waterway is contained within the Absaroka Mountains of the Middle Rockies (level III) and two level IV ecoregions: Absaroka-Gallatin Volcanic Mountains and Mid-Elevation Sedimentary Mountains (Woods 2002). Specimen Creek includes two branches (North Fork and East Fork) forming the Main-Stem of Specimen Creek; eventually flowing into the Gallatin River (Figure 1). The entire Specimen Creek drainage is approximately 76 km^2, containing 62 kilometers of flowing water. The East Fork of Specimen Creek originates at High Lake and flows approximately 27 kilometers until the confluence of the North Fork, which originates 20 kilometers upstream at Crescent Lake. Both branches are second order streams soon after their origin, and increase to third order after their confluence. East Fork Specimen Creek landscape vegetation is predominately coniferous trees with deciduous vegetation in the riparian zones. It meanders through multiple meadows and a recent burn area on the lower reach for approximately two kilometers. The substrate is dominated by rock rubble

except in the low gradient meadows where sand and fine gravel dominate. During study periods, discharge does not exceed approximately one m^3/sec and temperatures are approximately 12°C.

High Lake is located at the uppermost portions of East Fork Specimen Creek at 2,743 meters above sea level. Three inlet tributaries are located along the lakes northern margin and three springs along the lakes western margin. The outlet channel flows approximately 300 meters before reaching a 10 meter natural waterfall, which isolates the High Lake from fish downstream. The surface area of High Lake is approximately three hectares, with the deepest depths reaching 6.5 meters (Koel *et al.* 2007). Historic sampling events recorded temperature ranges from 8 – 17 °C, pH as high as 9.4, and the substrate is dominated by silt and clay (Koel *et al.* 2007).

Historically, the Gallatin River Watershed contained westslope cutthroat trout *(Oncorhynchus clarkii lewisi),* fluvial arctic grayling (*Thymallus arcticus*)*,* mountain whitefish *(Prosopium williamsoni*) *and* mottled sculpin (*Cottus bairdi)*. Stocking events in the early 1900s resulted in the establishment of several non-native game fish species (Behnke 2002 and Varley 1981), including Yellowstone cutthroat trout *(O. c. bouvieri)*, brown trout (*Salmo trutta*), rainbow trout (*O. mykiss*) and brook trout (*Salvelinus fontinalis*). High Lake was fishless until 1937 when it was stocked with Yellowstone cutthroat trout, becoming an upstream source for hybridization with other trout species in the Specimen Creek Drainage (Koel *et al*. 2007). Introduction of these species lead to hybridization and competition with the native, westslope cutthroat trout in the drainage.

Rotenone applications. – Plans were initiated in 2006 to restore westslope cutthroat trout to the East Fork Specimen Creek Drainage after the completion of an Environmental Assessment

(NEPA). This began the process of the removal of non-native species throughout the watershed using rotenone. The first phase of the project started in August 2006 with High Lake, its inlet tributaries, and outlet channel downstream to the natural waterfall barrier (Figure 2). A second treatment followed two weeks later. Both treatments applied 66.2 liters of CFT Legumine (EPA Reg. No. 7538-2) and approximately 0.91 kilograms of Prentox Rotenone Fish Toxicant Powder (EPA Reg. No. 7533-2) over an eight hour periods (C.W.E. Properties Limited, LLC). Total target application concentrations were one part per million**,** which was achieved with the active rotenone concentrations estimated to be 50 parts per billion. Application techniques included two motor operated inflatable boats applying liquid rotenone using Venturi boat bailer pumps and 30 gallon collapsible tanks, backpack sprayers for littoral zones, and Prentox Rotenone Fish Toxicant Powder mixed with gelatin and sand for springs and seeps. A detoxification station to neutralize rotenone with potassium permanganate ($KMnO_4$) was established immediately downstream of the outlet waterfall. A third treatment was deemed unnecessary because no living fish were found after both treatments, in overwintered gillnets (Koel *et. al* 2008). Pure strain westslope cutthroat trout were reintroduced throughout 2007-08 as eyed eggs reared at remote site incubators and various size classes by helicopter (Koel *et. al* 2008).

The second phase of the restoration project began in August 2008 where approximately 25 km of the East Fork Specimen Creek and its tributaries were treated from the waterfall below High Lake to the barrier site (Figure 1). Four application periods occurred in 2008 and 2009. The first two applications were applied two days apart in 2008 and the third and fourth application occurred in August of 2009. A total of 60.6 liters of CFT Legumine and approximately 0.91 kilograms of Prentox powder were applied during the first two treatments. Total target application concentrations were one part per million**,** with the active rotenone

concentrations estimated to be 50 parts per billion. Application techniques included drip stations in streams, backpack sprayers in backwaters and Prentox powder was mixed with gelatin and sand for use in springs and seeps. Drip stations were situated in the uppermost reaches of the watershed and as recharge stations at locations along East Fork Specimen Creek determined by dye tests and bioassays. Rotenone was applied for eight hours. A detoxification station to neutralize rotenone was setup directly below the barrier site where quantities of potassium permanganate ($KMnO_4$) sufficient to achieve approximately three parts per million was added to the stream. Reintroduction of westslope cutthroat trout started in 2010.

Historical macroinvertebrate sampling. – A baseline study of macroinvertebrates found in Specimen Creek Watershed began in 2004. This assessment was initiated due to the potential for a restoration project of pure westslope cutthroat trout. A total of 10 lotic and four lentic sites were sampled at least once prior to treatments for both phases of the restoration project. The 2006 High Lake treatment post samples were collected for all four lentic sites, inlet site (EF7), two outlet channel sites above the water fall (EF5 and EF6), and one site below the treatment zone (EF4) (Figure 2). Lotic sites were collected once after the completion of two treatments, whereas lentic sites were collected after each treatment. All post samples were collected within two week of treatment, except EF4 and EF7 were three weeks after due to gear malfunctions. The 2008 East Fork Specimen Creek treatment post samples were collected at one time period, after both piscicide applications. Collections took place on four of the established Specimen Creek sites (MS1, EF1, EF2, and EF4) (Figure 1). Historical sites followed the same sampling methods as described in Objective 1, except all stream sites and some High Lake samples did not have replicates. All eight Surber, as well as Ekman triplicate grabs were compiled into one sample bottle for their specific site collection.

From the 2006 High Lake treatment, it was determined minimal effects occurred to High Lake benthic macroinvertebrates (Koel *et al.* 2008). At EF4, one kilometer below the treatment area (Figure 2), macroinvertebrate communities were also minimally impacted by the High Lake treatment. The treated inlet and outlet sites demonstrated the most effects from piscicide applications. A total of 68 taxa were found in 2006 prior to treatment and decline to 53 two weeks after. Prior to treatment, 33% and 38% of the invertebrates belonged to EPT taxa found at outlet and inlet stream sites. After treatment, EPT taxa declined to 11% and 10% of all taxa collected in those sites. This indicates that there are negative effects on the inlet and outlet channel macroinvertebrate communities. One-year after treatment, communities recovered in the outlet channel, but little improvement was seen in the inlet site. The outlet channel exhibited an increase in total richness, EPT taxa, and total density (Koel *et al*. 2007).

Secondary datasets. – In the western United States, other state and private entities have been struggling with the protection of westslope cutthroat trout and other cutthroat species. Many techniques are implemented, but for complete removal of competitive or hybridized species, the most effective option is the use of piscicides. While conventional rotenone is still used and just as an effective formulation for removing fish, many had begun to use CFT Legumine rotenone. The option of CFT Legumine addresses many of the controversial issues over the technique. To gain a thorough understanding of how CFT Legumine affects insects three additional datasets were examined. Projects to restore populations of native cutthroat trout in Cherry Creek, Comanche Creek and Costilla Creek drainages were initiated between 1997 and 2008 with an array of sampling designs (Table 1). Cherry Creek is devoted to the restoration of westslope cutthroat trout, whereas the Comanche and Costilla Creek project focus is for indigenous Rio Grande cutthroat trout. Cherry Creek is located in the Absaroka Mountains,

southwest of Bozeman Montana. Selected treatment areas for Cherry Creek are on the Flying D Ranch owned by Turner Enterprises, and the Gallatin National Forest. Costilla and Comanche Creek are located in North Central New Mexico in the Sange de Cristo Mountains, with project waters on boundaries of Turner Enterprises Vermejo Ranch and Carson National Forest. Throughout these projects, invertebrate samples were collected to evaluate the effects of CFT Legumine rotenone on non-target organisms.

Table 1. – Secondary datasets acquired from watersheds where CFT Legumine was applied for the restoration of cutthroat trout throughout different geographic regions of the western United States.

	Cherry Creek	Costilla Creek	Comanche Creek
Sampling Events	5	3	3
Locality	Montana	New Mexico	New Mexico
Number of Sites	6	4	7
Number of Replicates	3	5	5

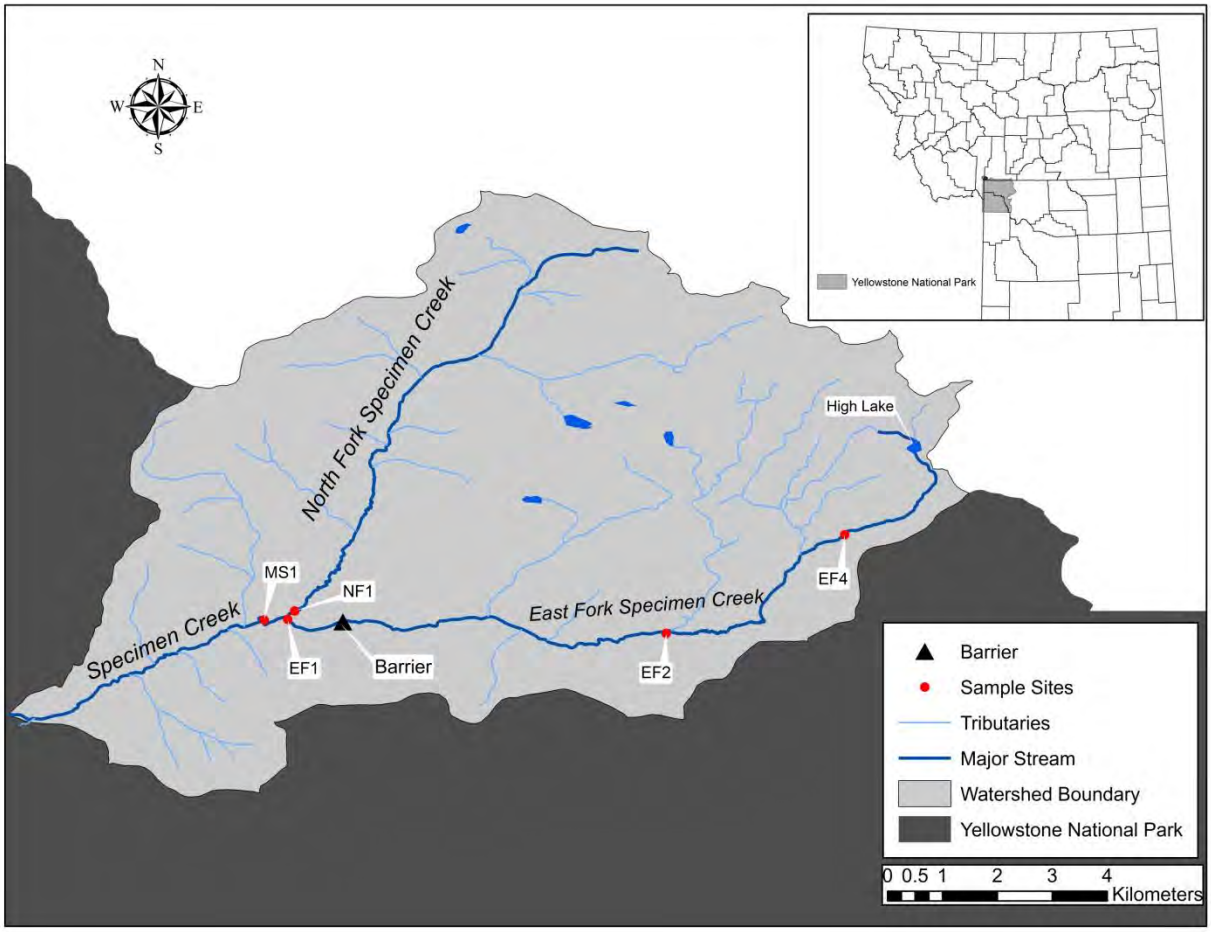

Figure 1. – Map of Specimen Creek, with the light gray boundary designating the watershed. Red dots indicate benthic macroinvertebrate sample locations on the East Fork Specimen Creek (EF1, EF2, and EF4), Main stem Specimen Creek (MS1), and North Fork Specimen Creek (NF1). EF4 and EF2 are within the rotenone treatment area, whereas EF1 and MS1 are below treatment. NF1 is the locality of the reference site. Green dots represent upper and lower treatment drift sites. The triangle indicates the barrier, end of treatment area, and $KMnO_4$ neutralization station.

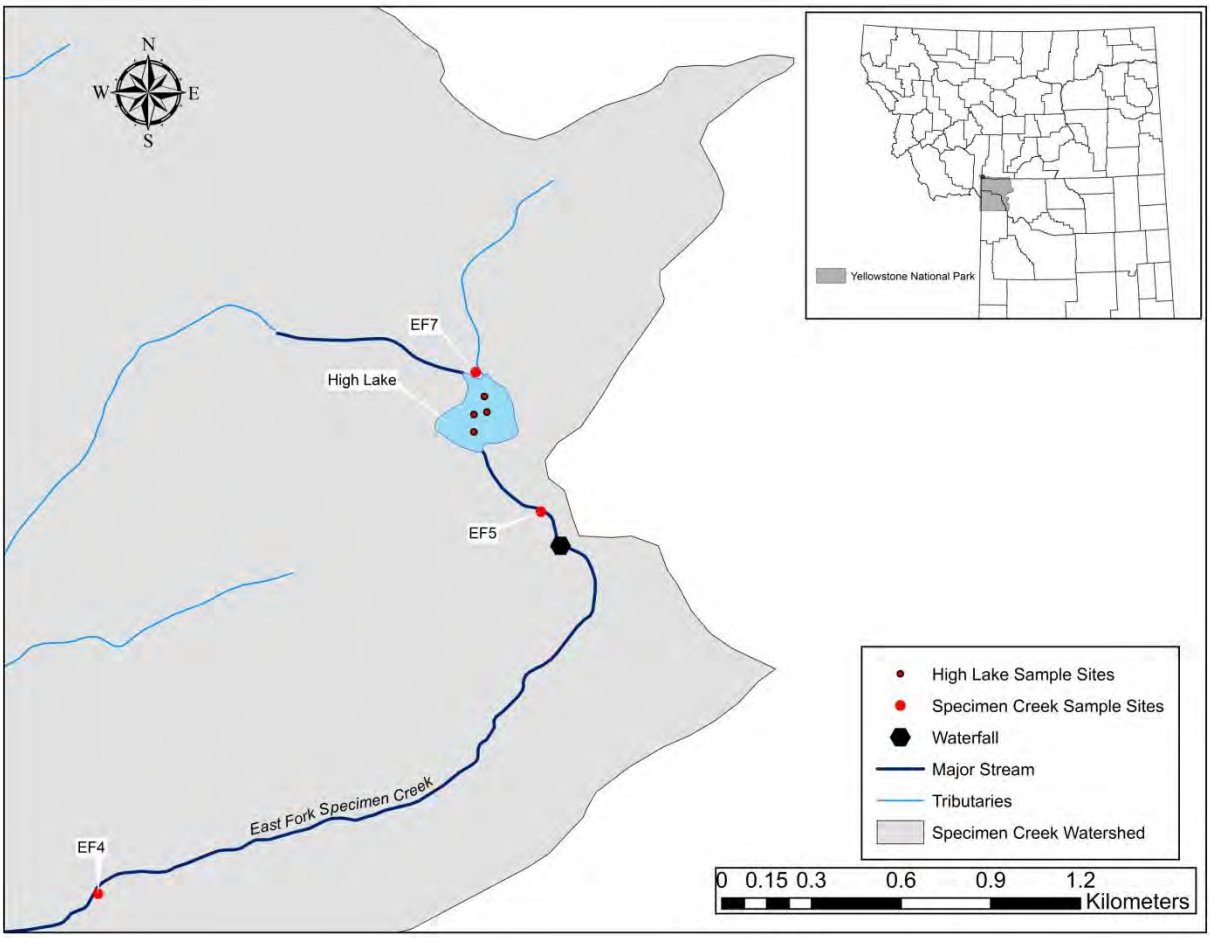

Figure 2. – Map of Specimen Creek, focused on the High Lake sampling area. Large red dots indicate benthic macroinvertebrate sample locations on the East Fork Specimen Creek (EF4, EF5, and EF7), whereas the small red dots represent the four High Lake benthic and Zooplankton sites (HL1-4). All sites are within the rotenone treatment area, except EF4 is downstream. The hexagon indicates the waterfall, end of treatment area, and $KMnO_4$ neutralization station.

CHAPTER 2

EFFECTS OF CFT LEGUMINE ROTENONE ON BENTHIC MACROINVERTEBRATE COMMUNITIES IN EAST FORK SPECIMEN CREEK

Introduction

The widespread introduction of gamefish continues to contribute to the decline and extirpation of native trout species (Rahel 2000 and 2002). This impact has been most severe for native-trout species with small population sizes and localized distributions. This is the case for the westslope cutthroat trout in Yellowstone National Park. Fishery managers use an assortment of techniques to manage undesirable or non-native fish populations. However, when complete removal of all fish is the goal, as is typical for restoration of a native species, the use of piscicides is required (McClay 2000). Rotenone, is one of the most valuable and successful piscicides currently available (McClay 2000 and 2005). For >70 years rotenone has become an important tool for fisheries managers in the restoration of native fish species. Although rotenone is a popular and highly effective method in fish management, its use has been contentious and challenged. One significant challenge and the purpose of this research are rotenone effects are poorly understood for non-target organisms, specifically benthic macroinvertebrates (BMI) (Vinson *et al.* 2010).

Rotenone formulations registered with the United States Environmental Protection Agency (USEPA) as piscicides, are available as powdered extracts or emulsifiable liquids. Differences among rotenone emulsifiable formulations are the inert ingredients, which act as solvents and synergists. Although labeled as inert ingredients most of these chemicals are toxic. Conventional rotenone formulations include petroleum hydrocarbons such as toluene, xylene,

benzene, naphthalene, and the synergist piperonyl butoxide. Over the past few years, environmental groups brought about concerns involving public health, environmental impacts, animal welfare and applicator safety (McClay 2005 and Turner *et al.* 2007). The registration of CFT Legumine addresses several of the issues creating multiple advantages to the product. Unlike conventional rotenone formulations, CFT Legumine was designed to reduce or eliminate a number of hydrocarbon compounds, and does not include the synergist piperonyl butoxide (McClay 2005; Turner *et al.* 2007; Finlayson *et al.* 2010); therefore reducing risks for applicators, terrestrial species, public health, and the overall environmental impacts. The product is also more difficult for fish to detect, increasing the efficiency of the removal. The disadvantage is the removal of the synergist and hydrocarbons decreases the efficacy of CFT Legumine (as compared to conventional formulations) requiring the active ingredient concentration to be doubled from 25 to 50 ppb to achieve the desired toxicity to fish (Turner *et al.* 2007). Given these advantages and disadvantages, it is important to achieve a better understanding of affects to BMI in field applications. The objective of this study was to determine the effects of CFT Legumine rotenone on benthic macroinvertebrate communities before and after treatment of East Fork Specimen Creek. The results of this project will provide information that will aid in the development of management strategies for aquatic systems in Yellowstone National Park (YNP) and elsewhere.

Methods

Study area. – Specimen Creek is located in the northwest corner of YNP in Gallatin and Park Counties of Montana. The entire Specimen Creek drainage is approximately 76 km^2, containing 62 km of flowing water. The East Fork Specimen Creek originates at High Lake and flows approximately 27 km until its confluence with the North Fork. Both branches are second

order streams soon after their origin, and increase to third order after their confluence. East Fork Specimen Creek landscape vegetation is predominately coniferous trees with deciduous vegetation in the riparian zones. It meanders through multiple meadows and a recent burn area on the lower reach for approximately two kilometers. The substrate is dominated by rock rubble except in the low gradient meadows where sand and fine gravel dominate. Collection sites were on the (1) Main-stem Specimen Creek (MS1) approximately 300 meters below the confluence of North and East Fork Specimen Creek, (2) East Fork Specimen Creek (EF1) approximately 100 meters above confluence with the North Fork, (3) North Fork Specimen Creek (NF1) approximately 100 meters above the confluence with the East Fork, (4) East Fork Specimen Creek (EF2) 6.5 km below High Lake, and (5) East Fork Specimen Creek (EF4) 2.5 km below High Lake (Figure 1). The two downstream sites (MS1 and EF1) are below the treatment area, potentially treated with potassium permanganate and rotenone; EF2 and EF4 are within the treatment area, only exposed to rotenone.

Rotenone applications. – In 2008 and 2009, four applications of CFT Legumine rotenone were applied to the East Fork Specimen Creek and its tributaries to remove nonnative rainbow trout *O. mykiss*, brown trout *Salmo trutta*, Yellowstone cutthroat trout *O. clarkii bouvieri,* and hybridized forms of cutthroat/rainbow trout. Approximately 105 liters of CFT Legumine ™ Fish Toxicant (U.S. EPA Product Reg No: 75338-2) (5% rotenone) and approximately 14.5 kilograms of Prentox Fish Toxicant Powder (EPA Reg. No. 7533-2) (7.4% rotenone) were applied to the fish inhabited 20 km section of the East Fork Specimen Creek for eight hours at a target rate of one ppm CFT Legumine (50 ppb rotenone). This concentration was chosen based on the results of travel time estimates, flow calculations, and bioassays. A total of 13 drip stations, spaced 2.5 – 3 hours apart treated the majority of the treatment area. Prentox powder mixed with gelatin

and sand was used to treat springs and seeps and backpack sprayers were used for backwater areas. Potassium permanganate (2-3 ppm) was used to oxidize and neutralize CFT Legumine rotenone below the treatment area.

To examine maximum exposure and persistence of rotenone in the treatment area, water samples were collected from macroinvertebrate sites (MS1, EF1, EF2 and EF4) at three different time periods: before application (pre), estimated peak application (according to travel times), and approximately 48 hours after final treatment(post). Water samples were collected using 5ml amber glass vials with teflon lids to minimize loss of sample material. All samples were placed on ice, and analyzed within 48 hours of collection. Potassium permanganate concentrations were measured directly below the detox station and at EF1.

Aquatic macroinvertebrate collections. – Between 2004 and 2010, benthic macroinvertebrates (BMI) were collected from three sites in East Fork Specimen Creek (EF1, EF2 and EF4), Main-stem Specimen Creek (MS1) and one additional reference site in North Fork Specimen Creek (NF1) (Figure 1). Sampling methodology followed the Wyoming Department of Environmental Quality and Water Quality Division (WDEQ/WQD) (2004) stream benthic macroinvertebrate collection protocols. To evaluate the effects of CFT Legumine on BMI, a minimum of three sampling events were performed at each BMI sampling site around the rotenone treatment sequence: pretreatment, immediate post treatment and one-year post treatment. Study site length was 15, or 30 meters of a riffle, depending on the length of riffle. At each site, discharge (m/s^2), dissolved oxygen (mg/l), pH, conductivity (µS), and water temperature (°C) were measured. Eight random macroinvertebrate samples were collected within the designated riffles. A $0.093m^2$ Surber sampler equipped with 500µm mesh was used to collect macroinvertebrates. Within the Surber sampling area, substrate was disturbed to dislodge

any invertebrates until organisms were washed free (approximately 10cm down). Each of the macroinvertebrate replicates were kept separate and preserved in 85% ethanol for future processing. Samples were processed in total; however, an area sub-sampling method (Elliott 1971) was used if a sample was determined to be too large to finish and/or contained more than 1,000 individuals. In general, insects were identified to genus, whereas non-insects were identified to order or phylum. Even though these levels varied according to each taxonomic group, they were consistent throughout the project. Consistencies in taxonomic resolution ensured differences in response variables were not attributed to variation in taxonomic level.

Data analysis. – To evaluate the impacts of CFT Legumine on benthic macroinvertebrates, a combination of univariate and multivariate statistical techniques were used. Changes in BMI community structure were graphically presented using Nonmetric multidimensional scaling (NMDS) and agglomerative cluster analysis. For both, dissimilarity was measured using Morisita-Horn index for insect and Ephemeroptera, Plecoptera, Trichoptera (EPT) abundance. This index was chosen because it is unaffected by differences in species richness and sample size (Krebs 1989). Pretreatment vs. posttreament differences in BMI community composition were tested using a one-way analysis of variance (ANOVA). Tukey's multiple range tests separated differences by site over sampling events and sites vs. the reference site (NF1) at the same sampling event. Insect abundance, insect richness, EPT abundance and EPT richness were the response variables used in the ANOVA. Because BMI data prior to 2009 was composited, we analyzed these data using only NMDS and cluster analysis. However, statistics do not report lost or gained taxa, which is an important component regarding piscicide treatments. Taxa present before treatment that were not found after one-year of treatment, and taxa that were not present prior to treatment but were collected once treatments began were

reported. All statistics were performed and figures generated using R version 2.6.2 (R Development Core Team, http://www.R-project.org) and Statistical Analysis System (SAS) version 9.2 (SAS Institute 2008).

Physio-chemical measurements. – When quantifying differences in response variables and communities, it was important to incorporate variables that can cause natural variation over space and time. For example, statistical differences produced by variation among sample sites or within a site over the years, could produce false results. To ensure that differences observed in the study are from treatment effects of CFT Legumine and not random background variation were evaluated using Principal Component Analysis (PCA) on physio-chemical variables. Variables are displayed in Table 4, for the five sites.

Results

Residue rotenone measurements. – There was no detection of rotenone at MS1 and EF1 in any samples. Therefore, the measured application of (2 ppm) potassium permanganate was sufficient in neutralizing rotenone. The detection of rotenone at EF2 in the second lower treatment indicates trace amounts of rotenone after the upper treatment. EF2 is located directly below the intermediate barrier and end of the upper treatment area. Even though there was detection of rotenone at this time period, sentinel fish located at EF2 did not die throughout the duration of the upper treatment. EF2 was exposed to the highest concentration of rotenone (20 ppb). There was no detection (< 1.0 ppb) of rotenone at any macroinvertebrate sites approximately 48 hours after final treatment. Measurements of potassium permanganate directly below the application and at EF1 indicated concentrations of 2.2 and 1.1 ppm, respectively.

Benthic community. – A total of 57 insect taxa were collected at the five sample sites, dominated by the family Chironomidae and EPT genera (Appendix A and B). Five taxa present during pretreatment sampling were not present after treatments. Six taxa that were not present before treatment were collected after treatment. Most individuals gained or lost were in low abundance and not consistently sampled throughout the years. The ephemeropteran, *Diphetor* was present after treatment higher in the drainage (Table 3).

Physio-chemical measurements. – The PCA biplot of environmental variables indicates there were differences in habitat between EF4 and the four other sites (Figure 3). The first two components accounted for 70.1% of the total variation (Table 5). Water temperature and dissolved oxygen were correlated with PC_1, whereas discharge and conductivity were correlated with PC_2. The first component (PC_1) represents a gradient moving from right to left (Figure 3) in which sites with a high water temperature and low dissolved oxygen are located on the left hand side of the biplot. The second component (PC_2) represents a gradient moving from bottom to top in which sites with high discharge and conductivity are near the top of the biplot. Considering both components, the biplot indicates that EF4 sampling events were different from the other four sites due to high water temperature and low discharge. Because of this, differences in BMI at EF4 were likely attributed to environmental conditions rather than the application of CFT Legumine.

Pretreatment temporal and spatial comparisons. – BMI community patterns were consistent in NMDS and cluster analysis. Over time, no apparent pattern was seen within sites, indicating low community variability among years (Figures 4-7). However, all sampling events of EF4 showed dissimilarity from the other sites in both analyses (Figures 4-7). This suggests that BMI community structure was different at this site, probably the result of differences in

habitat identified in the PCA. EF4 was the most upstream site, and in close proximity to High Lake. This site was influenced by a lentic system that has higher water temperatures and lower discharge than other sites.

2008 treatment analysis. –Dissimilarity in BMI community composition was greatest following treatment with CFT Legumine (Figure 8-11). Sites (MS1 and EF1) below the $KMnO_4$ detox station were the most dissimilar immediately after treatment for insect and EPT abundance. Sites (EF2 and EF4) within the treatment area were the most similar to their pretreatment communities for insect abundance(Figure 8 and 9); however, when comparing sites using EPT abundance both grouped with the two downstream immediate post treatment sites (Figure 10 and 11). One-year post treatment communities are the most similar to their pretreatment sites for insect and EPT abundance (Figure 8-11). Results indicate $KMnO_4$ has a greater effect on insect and EPT communities than sites only exposed to rotenone. In addition, one-year after treatment, all sites regardless of being exposed to rotenone or $KMnO_4$ were similar to the pretreatment sampling event, demonstrating a recovery of community structure.

2009 treatment analysis. – NMDS and cluster analysis were similar to the 2008 treatment results (Figures 12-15). The greatest dissimilarity in BMI community composition was within the immediate post sampling event for insect and EPT abundance. Sites (MS1 and EF1) below $KMnO_4$ detox station were the most dissimilar immediately after treatment for insect and EPT abundance. Sites (EF2 and EF4) within the treatment area shared a similarity to immediate post detox sites, but were still grouped to their pretreatment and/or one-year post treatment communities for insect and EPT abundance (Figures 12-15). One-year post treatment communities are the most similar to their pretreatment sites for insect and EPT abundance. Results indicate that the 2009 treatment had impacts on EPT and insect communities at the four

sites (Figures 12-15). The difference was the detox influenced sites were the most dissimilar to other sites, whereas treatment sites still showed some similarity to their pretreatment and/or one-year post samples. In addition, one-year after treatment, all sites regardless of being exposed to rotenone or $KMnO_4$ were the most similar to the pretreatment sampling event, demonstrating a recovery of community structure.

Insect richness, EPT richness, insect abundance and EPT abundance were significantly different (One way ANOVA, $p < 0.05$) (Figures 16-19). Tukey's multiple range test determined immediate post insect richness samples below the detox station (MS1 and EF1) to be significantly different from the reference site (NF1) (Figure 16). Despite their pre and post treatment samples not being statistically different, abundance did decline. Minimal changes were seen at the treatment sites (EF2 and EF4) through time, indicating rotenone treatment did not significantly affect insect richness.

EPT richness declined the greatest immediately after treatment at sites (EF1 and MS1) below the detox station (Figure 17). MS1 immediate post was significantly different from its pretreatment level and the reference site. EF1 immediate post treatment was only significantly different from the reference site. All three EF2 sampling events were significantly different from their coinciding reference site. These differences are likely due to consistent differences in EPT richness when comparing EF2 to the reference site. Results do not indicate significant affects from the application of rotenone, because EF2 immediate post EPT richness was higher than pretreatment levels.

Insect abundance at MS1 immediate post treatment was significantly different from its pretreatment levels and the reference site (Figure 18). The pretreatment EF2 sampling event was

significantly different from the reference site; however, the value was significantly greater. The EF2 one-year post treatment sampling event was significantly different from its pretreatment level. At the one-year post sampling event, insect abundance at many of the sites (including NF1) was less than pre and post treatment levels. This pattern could be related to environmental variables rather than rotenone applications. If this reduction was due to rotenone it would likely result in a greater pattern of significant decrease when comparing pre and post sample events.

EPT abundance at MS1, EF1 and EF2 immediate post treatment were significantly different from their pretreatment level and the post treatment reference site (Figure 19). The EF2 one-year post treatment sampling event was significantly different from its pretreatment level. EF4 immediate and one-year post treatment sampling events were significantly different from their corresponding reference site. Results indicate EPT abundance was the most influenced response variable.

Discussion

Samples were collected at the same seasonal period throughout the project to minimize natural variation (particularly temperature regimes) that can influence macroinvertebrate communities. Water temperature and discharge are known to influence BMI community structures. For example, EF4 in the PCA biplot indicated habitat differences (Figure 3); regardless, EF4 was consistently grouped together indicating within site differences are minimal. It is important not to disregard these differences and interpret the data carefully. Vannote and Sweeney (1980) describe that different temperature regimes will influence insect communities. Streams with a similar total annual accumulation of degree days, can have seasonal variation in thermal regimes, affecting life history components and the distribution of aquatic insects. Continuous monitoring of water temperature could provide a better understanding of how

variability in temporal and spatial thermal regimes in the study area impacts community structure. Regardless of these differences, this study is an important step in fisheries management piscicide techniques. The use of rotenone is an ever-growing contentious issue and information demonstrating progression is vital. This study is the first to evaluate CFT Legumine effects on BMI in field applications.

Traditionally the impacts of piscicides on BMI have been assessed using only univariate statistical techniques. However, in this chapter both a univariate and multivariate approach was used to assess the ecological impact of CFT Legumine application. The results of this study suggest this approach was more appropriate because differences in abundance and richness are difficult to interpret using only one technique, such as an ANOVA. The 2008 and 2009 NMDS and cluster analysis results mirror each other. In general, communities were the most dissimilar in the four immediate post treatment sites, with the greatest dissimilarity at sites below the detox station (Figure 8-15). Even though sites within the treatment area (EF2 and EF4) grouped with EF1 and MS1 immediately after treatment, they were still most similar to their pre and one-year post treatment samples. This indicates there was an impact to BMI communities, but these differences were not enough to disassociate EF2 and EF4 from their pre and one-year post levels. Despite a compelling pattern, there are differences when comparing insect and EPT communities. Following treatment, EPT community affects were more prominent than insect communities were immediately; EF4 and EF2 were more dissimilar related to their pre and one-year post samples. It was clear that EPT communities experience the greatest impact from the application of rotenone.

The four response variables suggest CFT Legumine does not impact insect and EPT richness and insect abundance; however, it does reduce EPT abundance. EF2 was significantly

different (ANOVA, $p < 0.05$) immediately after treatment from its pretreatment level and reference site, whereas, EF4 was only significantly different from its reference site. This indicates impacts were not as significant at EF4. The locality of EF2 exposed insects to residue rotenone from the upper treatment and a higher concentration (20 ppb) during treatment (Table 2). Sites influenced the greatest were below the detox station. EPT and insect abundance and EPT and insect richness at MS1 and EF1 decreased, with the greatest impacts on EPT abundance. The results of this study indicate that BMI recovered rapidly following the application of CFT Legumine. EF2 one-year post treatment was the exception, but this difference can perhaps be attributed to differences in environmental conditions and not an impact from rotenone applications. The 2010 water temperature regime could have been different, resulted in the decrease of abundance seen at most sites (including the reference) in the 2010 sampling year (Figure 18 and 19); the decrease of abundance in most sites was speculation and not evidence for dismissal. However, analysis of the dataset using NMDS and cluster analysis indicates that although there were differences for the response variable, community composition before and after treatment was essentially the same (Figure 15).

Previous literature (Binns 1967; Cook and Moore 1969; Mangum and Madrigal 1999; Trumbo *et al.* 2000; Whelan 2002; Vinson and Dinger 2006; Hamilton et al. 2009; Finlayson 2010) has reported a range of different impacts to macroinvertebrate assemblages. Most report immediate reductions of assemblages in some capacity. Recovery for common taxa was rapid however, it may take and several years for rare taxa to recover (Vinson *et al.* 2010). This study compared to others using conventional rotenone formulations only shares resemblance in decline and rapid recovery at sites below the detox station. Chemical analysis did not detect rotenone below the detox station. Thus, CFT Legumine treatment minimally affected BMI, and short-

term affects were observed from detoxification using potassium permanganate. However, the 2006 treatment of High Lake showed minimal influence from potassium permanganate applications (Koel *et al.* 2008). The causes of these differences are unclear, but could be attributed to distance and travel times of the detox station to the sample site. There is limited information of how potassium permanganate responds under different environmental conditions. Evaluation of potassium permanganate response in the environment and its affects to macroinvertebrates would help managers understand its impacts to BMI. Sites within the treatment area illustrate minimal affects immediately after treatment. As suggested by Finlayson *et al.* (2010), the use of CFT Legumine, which does not contain the synergist piperonyl butoxide, reduced the impacts to aquatic invertebrate communities in comparison to other studies implementing conventional formulation. It is probable that the treatment concentration (50 ppb) and design reduced the impacts to invertebrates. Headwater reaches deemed fishless were not treated, rotenone drip stations were operated for 8 hours, drainage treatment was split into different phases (High Lake 2006; East Fork Specimen Creek 2008 and 2009), different stages of East Fork Specimen Creek treatment (upper and lower) minimizing the background demand of rotenone and use of less potassium permanganate, and chemical measurements of rotenone and potassium permanganate were taken.

Management implications

Based on the results presented in this chapter, the following are recommendations to minimize impacts and maximize recolonization of BMI during native trout restoration: (1) apply the minimum dosage to eliminate fish; (2) operate rotenone drip stations eight hours or less per treatment; (3) apply unsynergized formulations (CFT Legumine); (4) partition the drainage into multiple treatments with intermediate barriers and allow time between treatments for dispersal

and recolonization of invertebrates; (5) do not treat headwater areas that are fishless, which leaves a source for recolonization of downstream treated reaches; (6) place caged sentinel fish throughout the treatment area to monitor treatment effectiveness; (7) and collect water samples to monitor potassium permanganate and rotenone concentrations throughout the treatment area.

Table 2. – 2009 rotenone concentrations (ppb) at macroinvertebrate sites. NA = not sampled and ND = no detected rotenone concentrations. The symbol in parenthesis indicate treatment event (UT1 = upper treatment one, UT2 = upper treatment two, LT2 = lower treatment two).

	Pretreatment	Peak exposure	48 hours after treatment
MS1	NA	ND	ND
EF1	ND	ND	ND
EF2	ND (UT1), 1.9 (LT2)	1.0 (UT1), 20.0 (LT2)	ND
EF4	ND (UT1, UT2)	7.8 (UT1), 8.9 (UT2)	ND

Table 3. – Macroinvertebrate taxa lost and gained at sites in the Specimen Creek drainage. Taxonomic groups are identified in parenthesis (E = Ephemeroptera, T = Trichoptera, and C = Coleoptera). Site and sampling event code with example is as follows: Site (EF4), sampling time relative to treatment (PRE), and sampling year (06), and combined is EF4PRE06.

Status	Taxa	Site and sampling event	Abundance
Lost	*Diphetor* (E)	EF4PRE06	5
		EF4PRE06	5
		EF4PRE07	13
	Arctopsyche (T)	MS1PRE07	9
	Homophylax (T)	MS1PRE08	3
		EF2PRE08	16
	Desmona (T)	EF4POST06	5
Gained	*Allomyia* (T)	EF1ONEYR10	1
		EF2POST09	1
	Amphicosmoecus (T)	EF2PRE09	1
	Cryptochia (T)	EF4POST09	1
	Goereilla (T)	EF2POST09	1
	Ametor (C)	MS1PRE08	1
	Dytiscidae (C)	MS1PRE09	1
		EF1PRE09	1
		EF2ONEYR10	1
		EF4PRE09	7

Table 4. – Mean and sample size of physio-chemical measurements at sample sites collected between 2004 and 2010.

Site	Water Temp (°c).	DO(mg/L)	pH	Conductivity(µS)	Discharge(m/s^2)
MS1	8.8 (6)	9.0 (6)	7.4 (6)	53 (6)	0.798 (6)
NF1	8.5 (4)	9.4 (4)	7.1 (4)	57 (4)	0.300 (4)
EF1	7.6 (8)	9.4 (8)	7.6 (8)	50 (8)	0.513 (8)
EF2	5.8 (8)	9.3 (8)	7.7 (8)	48 (8)	0.289 (8)
EF4	10.3 (7)	8.5 (7)	7.2 (7)	35 (7)	0.017 (7)

Table 5. – Principal component vectors for PCA on environmental variables in the comparison of sites analysis.

	PC_1	PC_2	PC_3	PC_4	PC_5
Water Temperature	-0.553	0.382	-0.238	0.167	0.681
DO	0.589	-0.306	0.207	0.319	0.644
PH	0.167	-0.350	-0.899	-0.192	0.065
Conductivity	0.425	0.559	0.022	-0.681	0.206
Discharge	0.372	0.572	-0.303	0.608	-0.272
Proportion of variance	0.392	0.309	0.183	0.066	0.049
Cumulative percent of variance	39.2	70.1	88.5	95.1	100

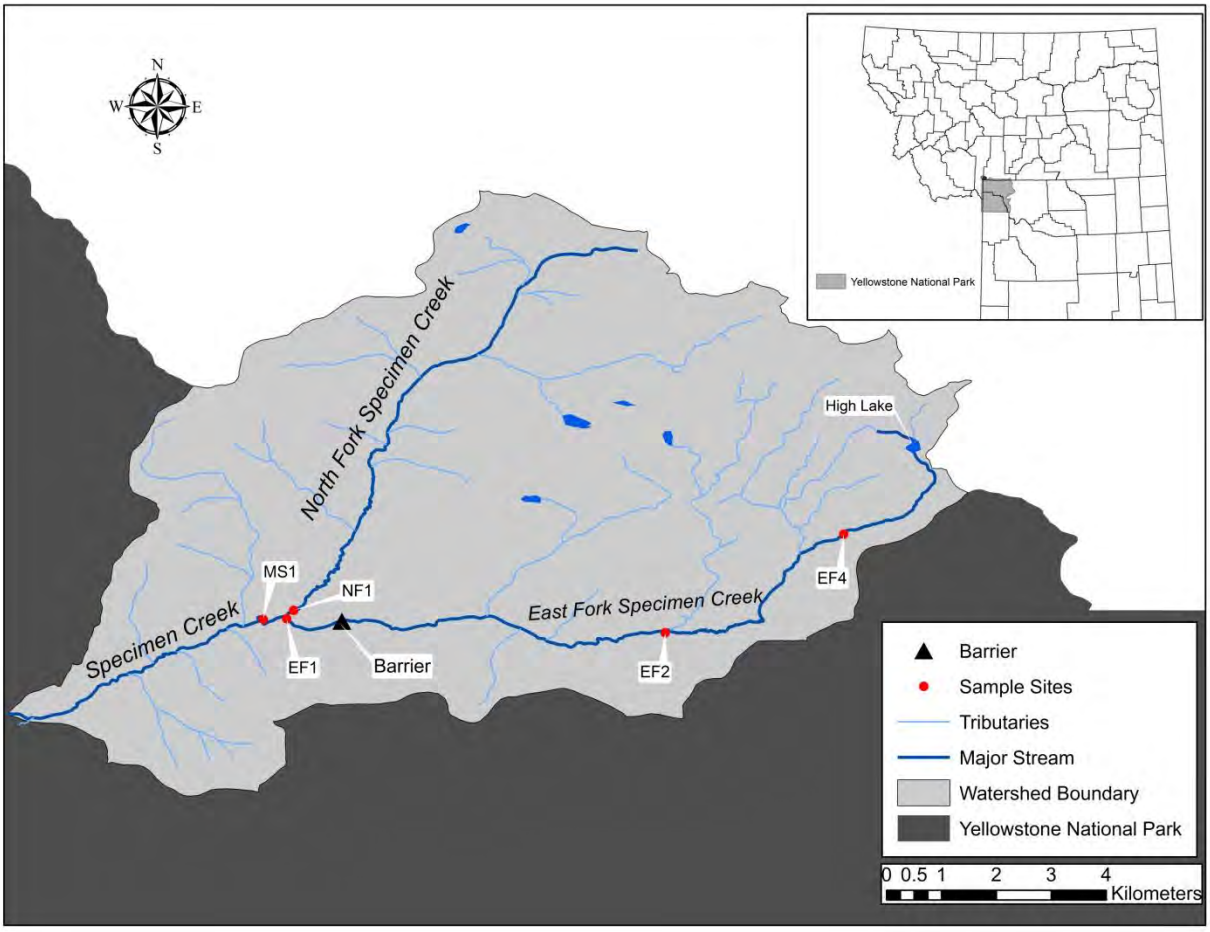

Figure 1. – Map of Specimen Creek, with the light gray boundary designating the watershed. Red dots indicate benthic macroinvertebrate sample locations on the East Fork Specimen Creek (EF1, EF2, and EF4), Main stem Specimen Creek (MS1), and North Fork Specimen Creek (NF1). EF4 and EF2 are within the rotenone treatment area, whereas EF1 and MS1 are below treatment. NF1 is the locality of the reference site. The triangle indicates the barrier, end of treatment area, and KMnO4 neutralization station.

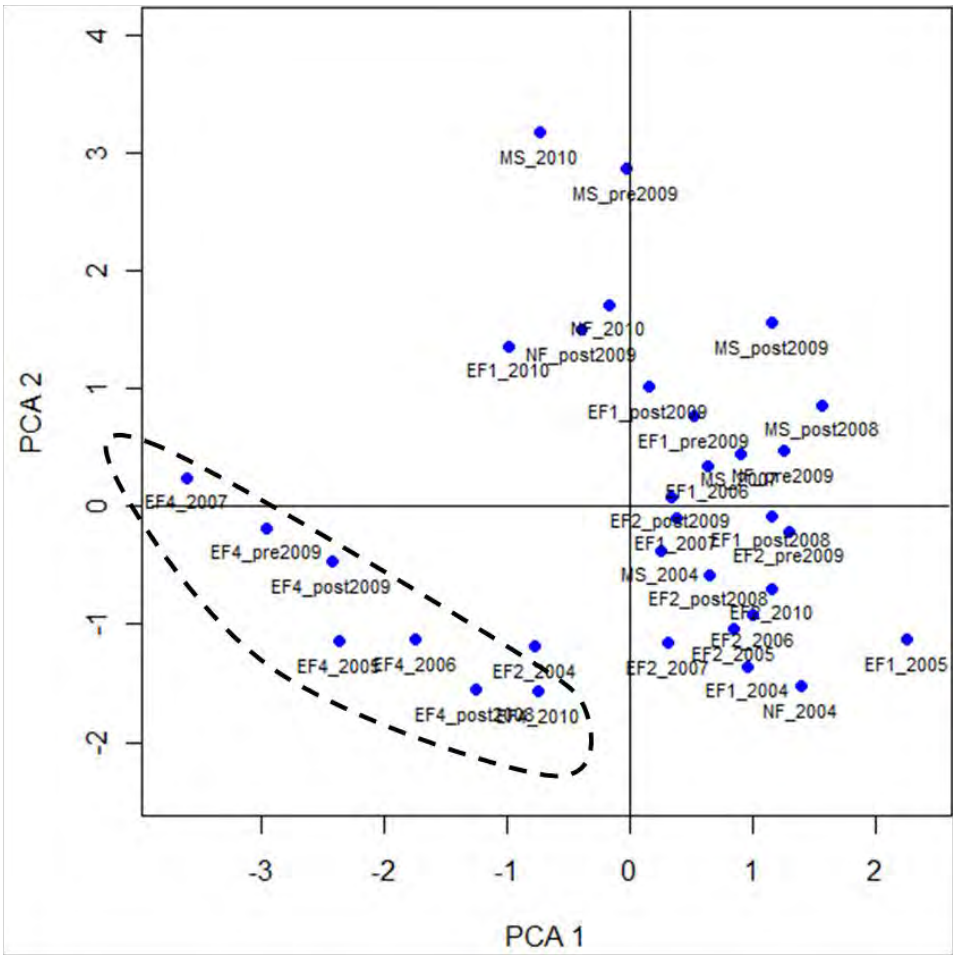

Figure 3. – Principal component analysis of environmental variables at sites from 2004 to 2010. The encircled sites are EF4 at all sampling events.

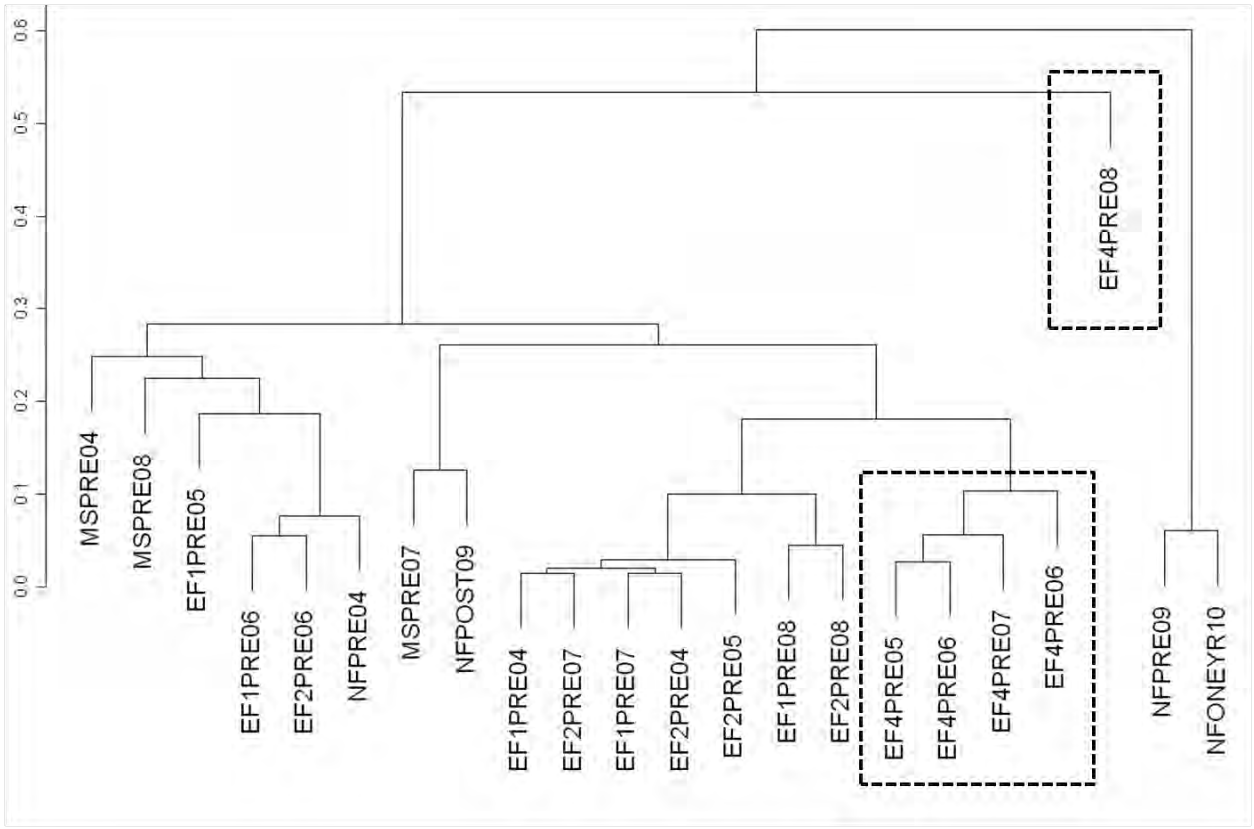

Figure 4. – Cluster analysis using Morisita-Horn similarity index for pretreatment (2004-2009) insect abundance. Sites within dashed lines are EF4 samples.

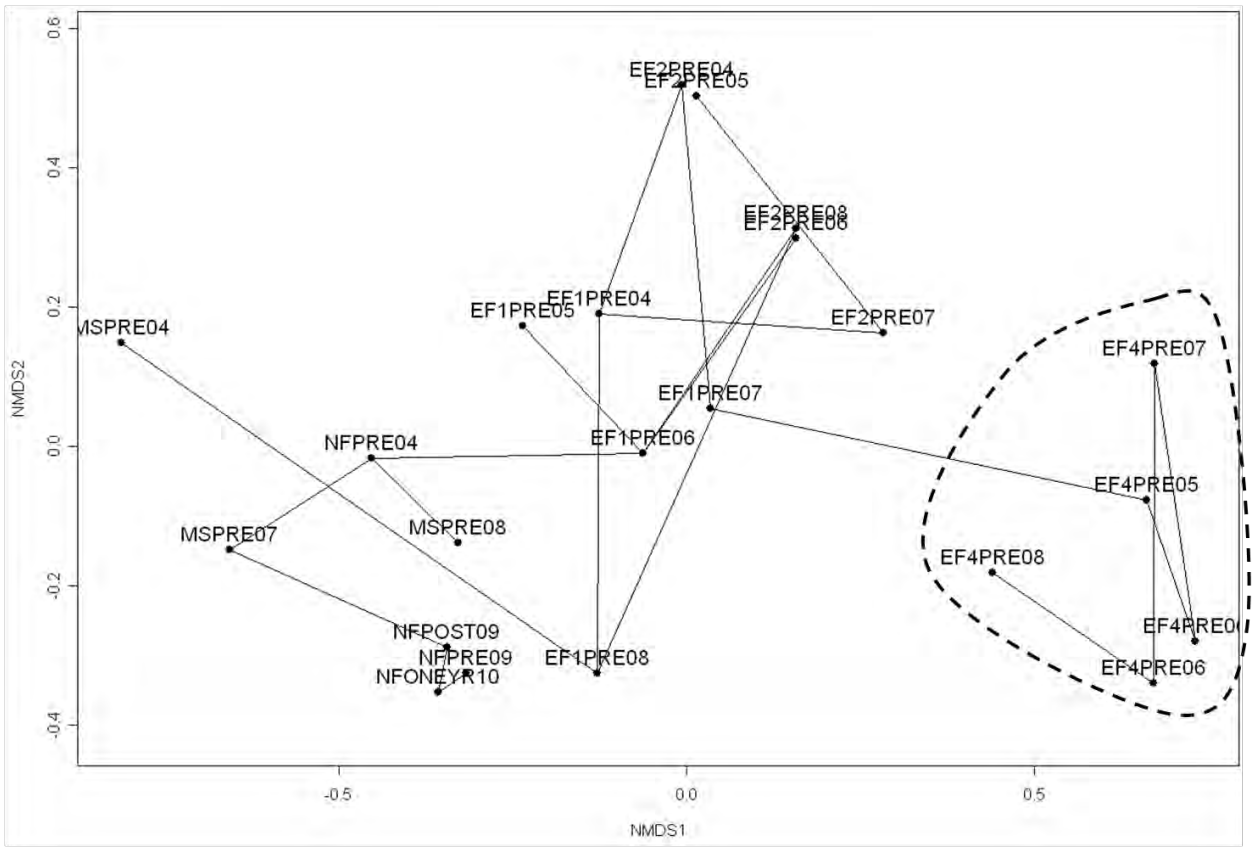

Figure 5. – NMDS spanning tree using Morisita-Horn similarity index for pretreatment (2004-2009) insect abundance. Sites within dashed lines are EF4 samples.

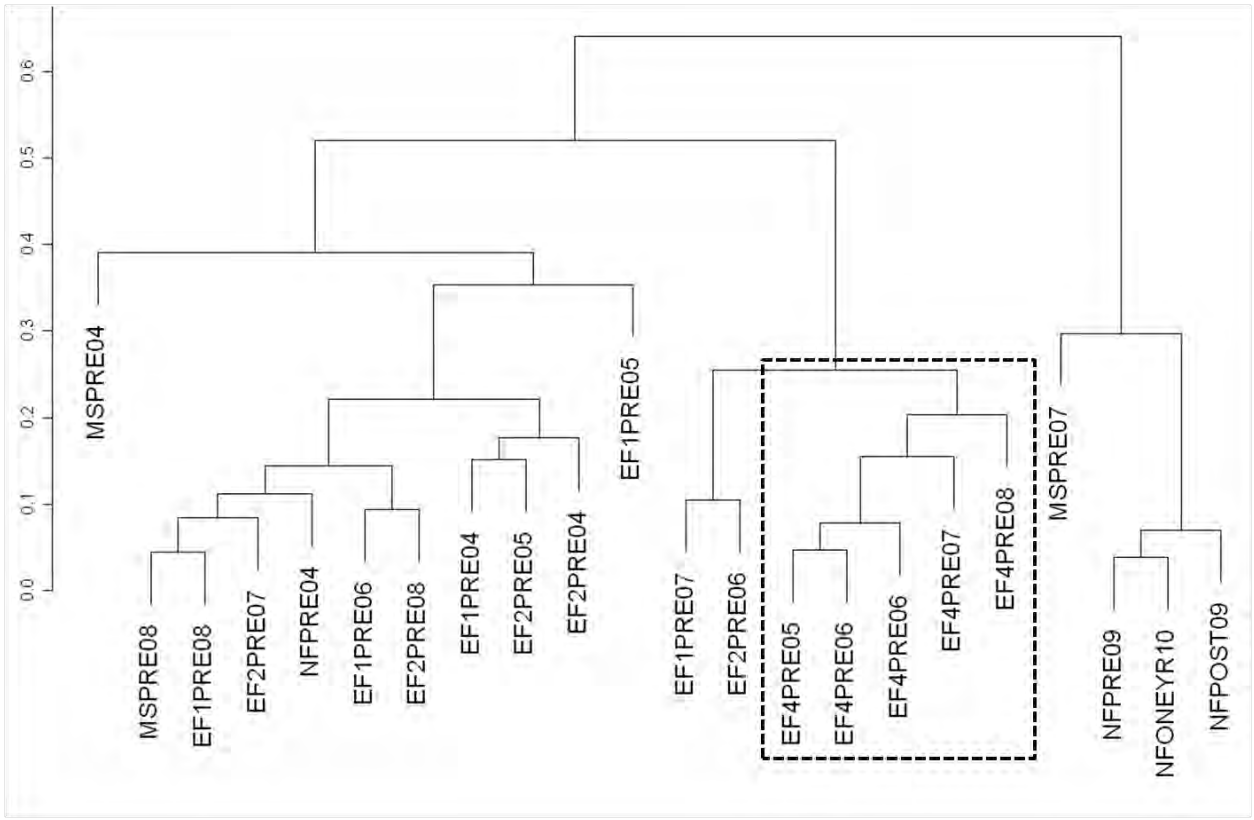

Figure 6. – Cluster analysis using Morisita-Horn similarity index for pretreatment (2004-2009) EPT abundance. Sites within dashed lines are EF4 samples.

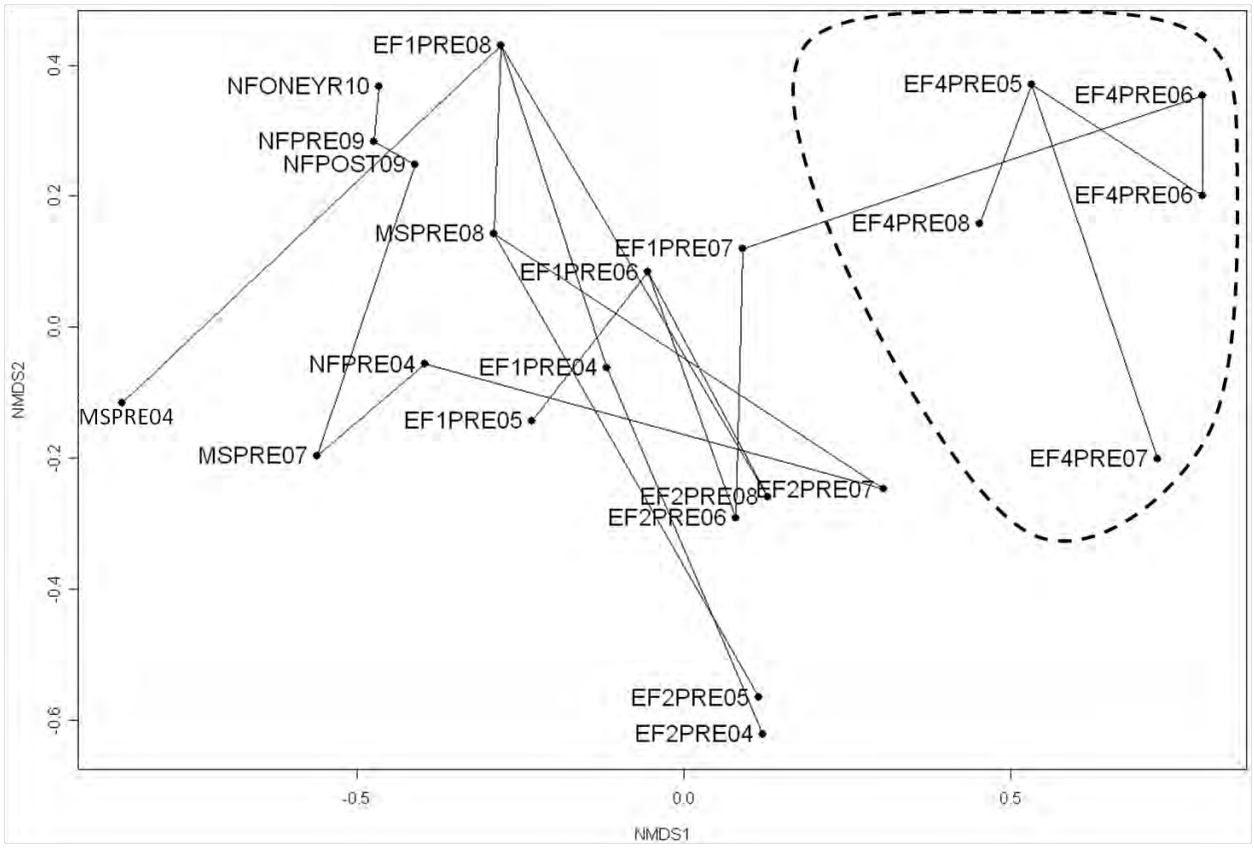

Figure 7. – NMDS spanning tree using Morisita-Horn similarity index for pretreatment (2004-2009) EPT abundance. Sites within dashed lines are EF4 samples.

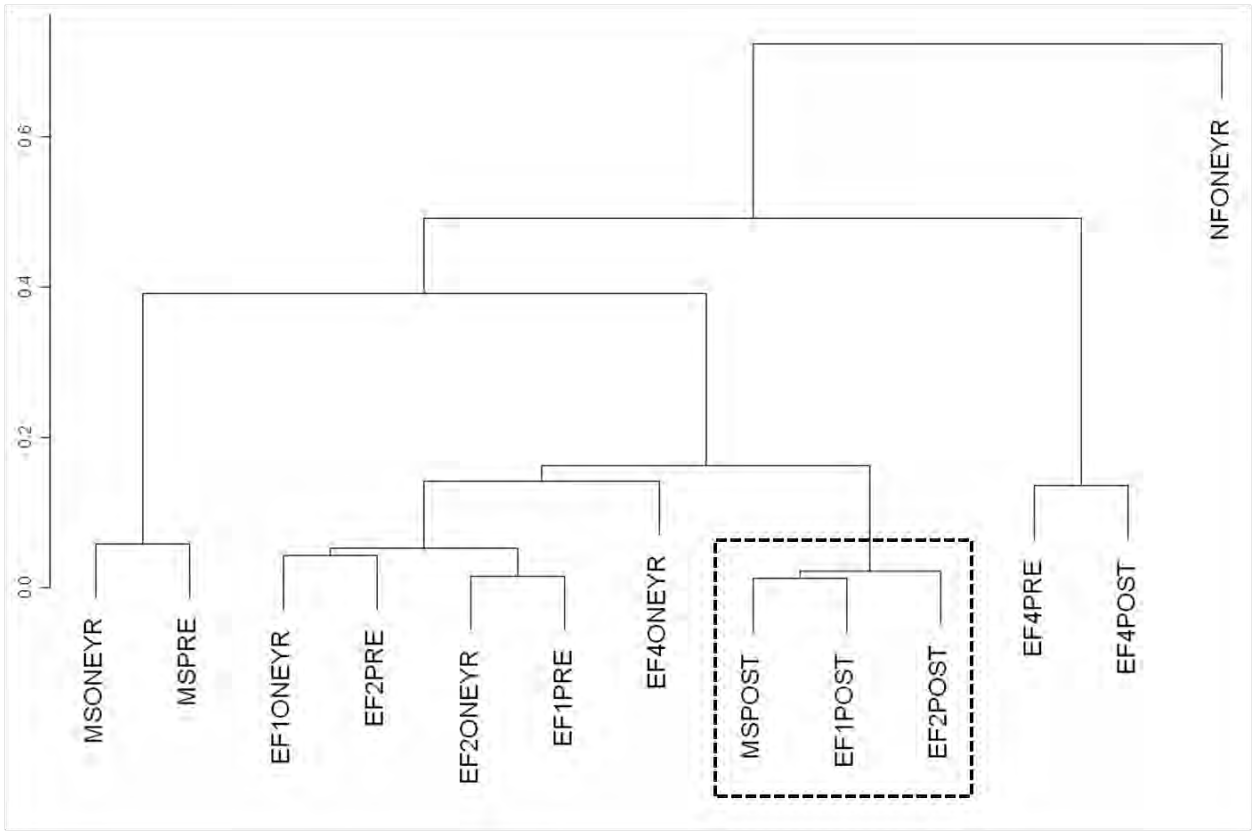

Figure 8. – Cluster analysis using Morisita-Horn similarity index for 2008 pre, immediate post and one-year posttreatment insect abundances. Sites within dashed lines are immediate post samples most dissimilar to other samples.

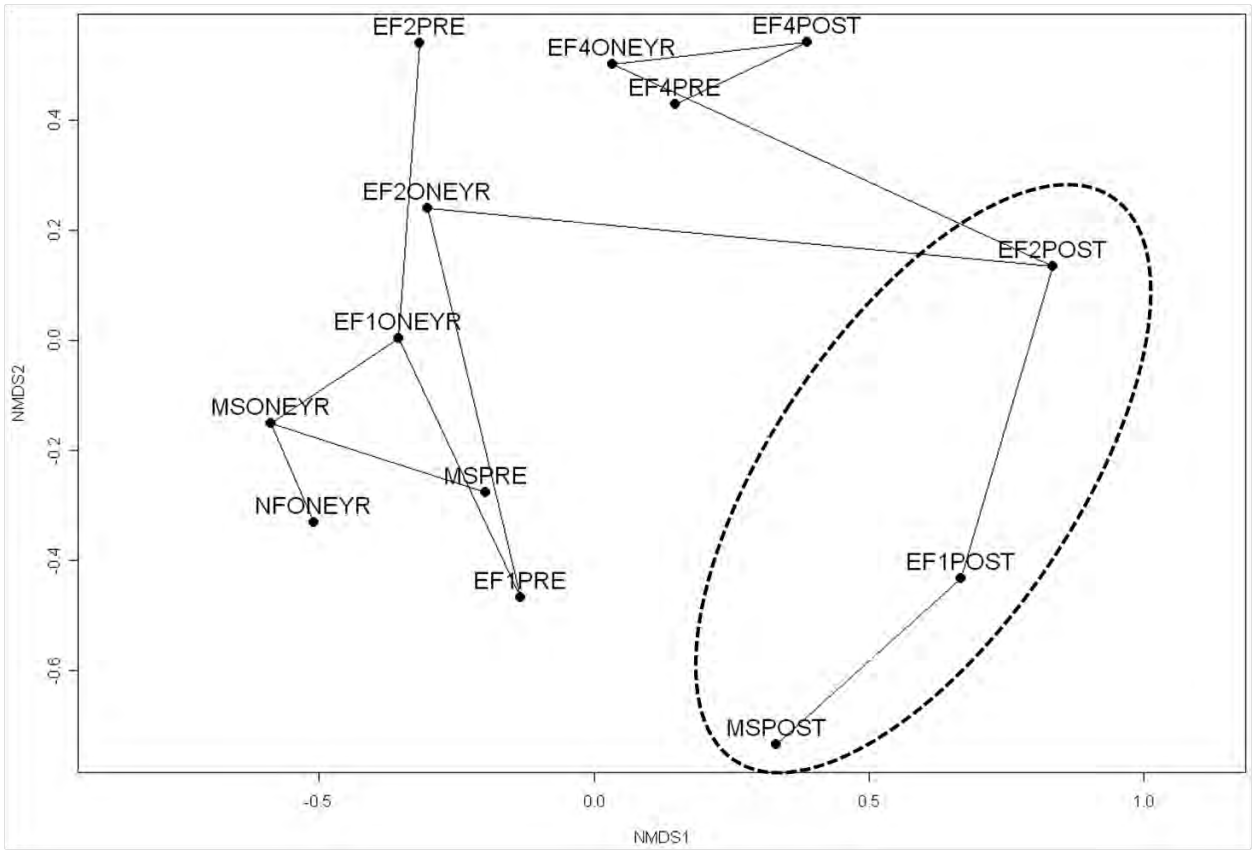

Figure 9. – NMDS spanning tree using Morisita-Horn similarity index for 2008 pre, immediate post and one-year posttreatment insect abundances. Sites within dashed lines are immediate post samples most dissimilar to other samples.

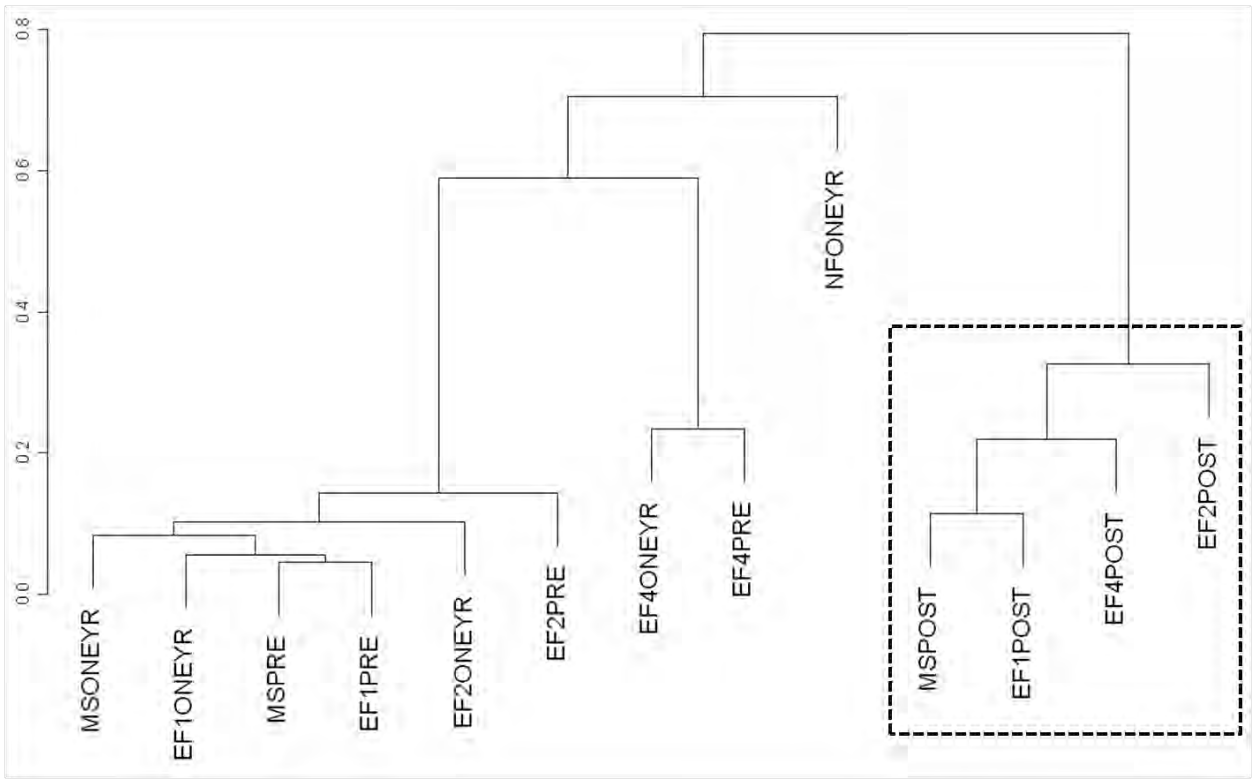

Figure 10. – Cluster analysis using Morisita-Horn similarity index for 2008 pre, immediate post and one-year posttreatment EPT abundances. Sites within dashed lines are immediate post samples.

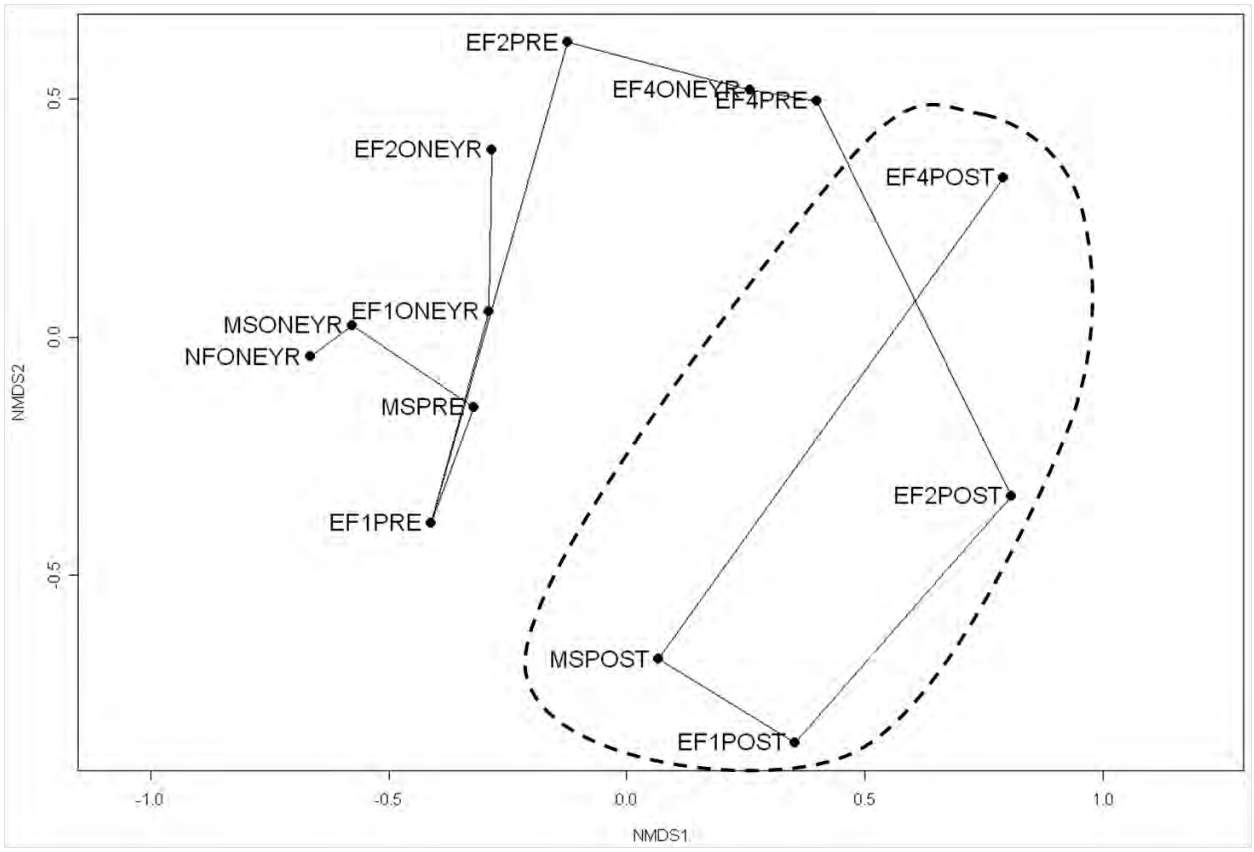

Figure 11. – NMDS spanning tree using Morisita-Horn similarity index for 2008 pre, immediate post and one-year posttreatment EPT abundances. Sites within dashed lines are immediate post samples.

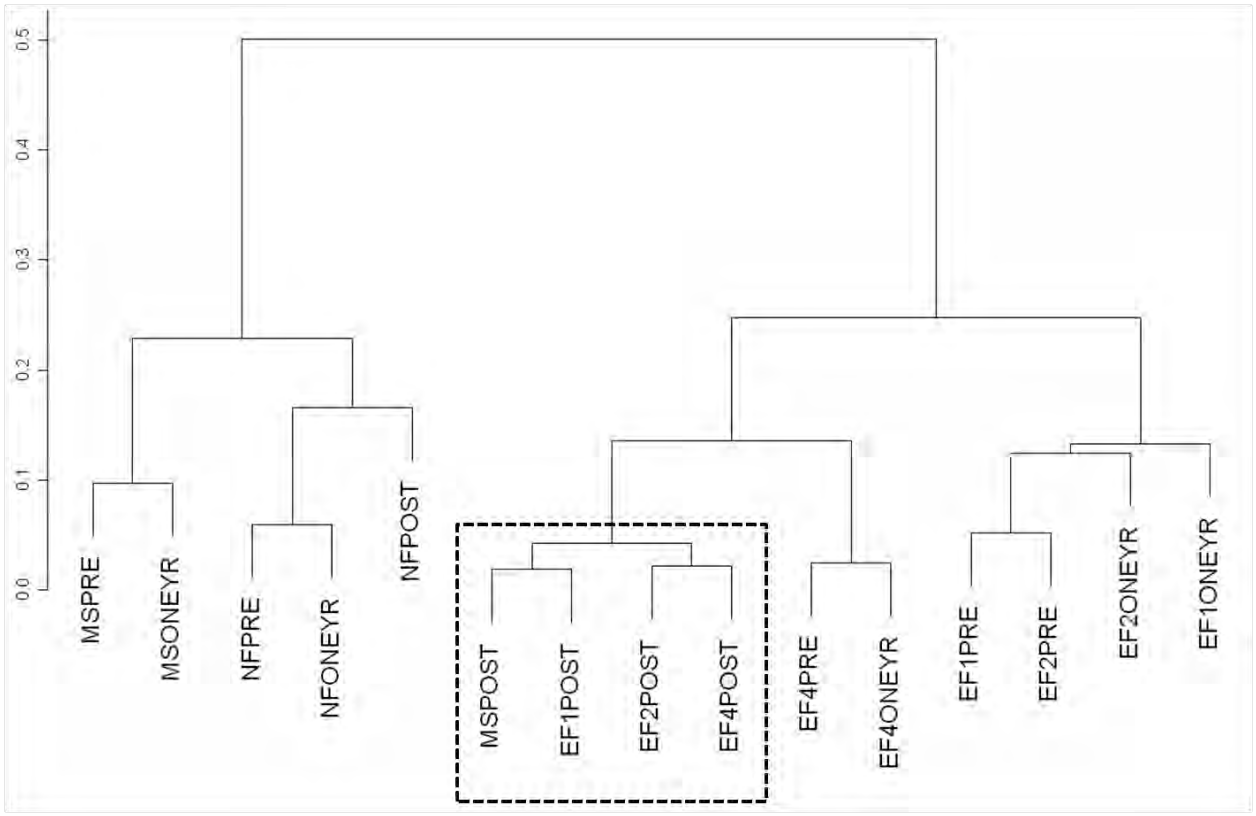

Figure 12. – Cluster analysis using Morisita-Horn similarity index for 2009 pre, immediate post and one-year posttreatment insect abundances. Sites within dashed lines are immediate post samples.

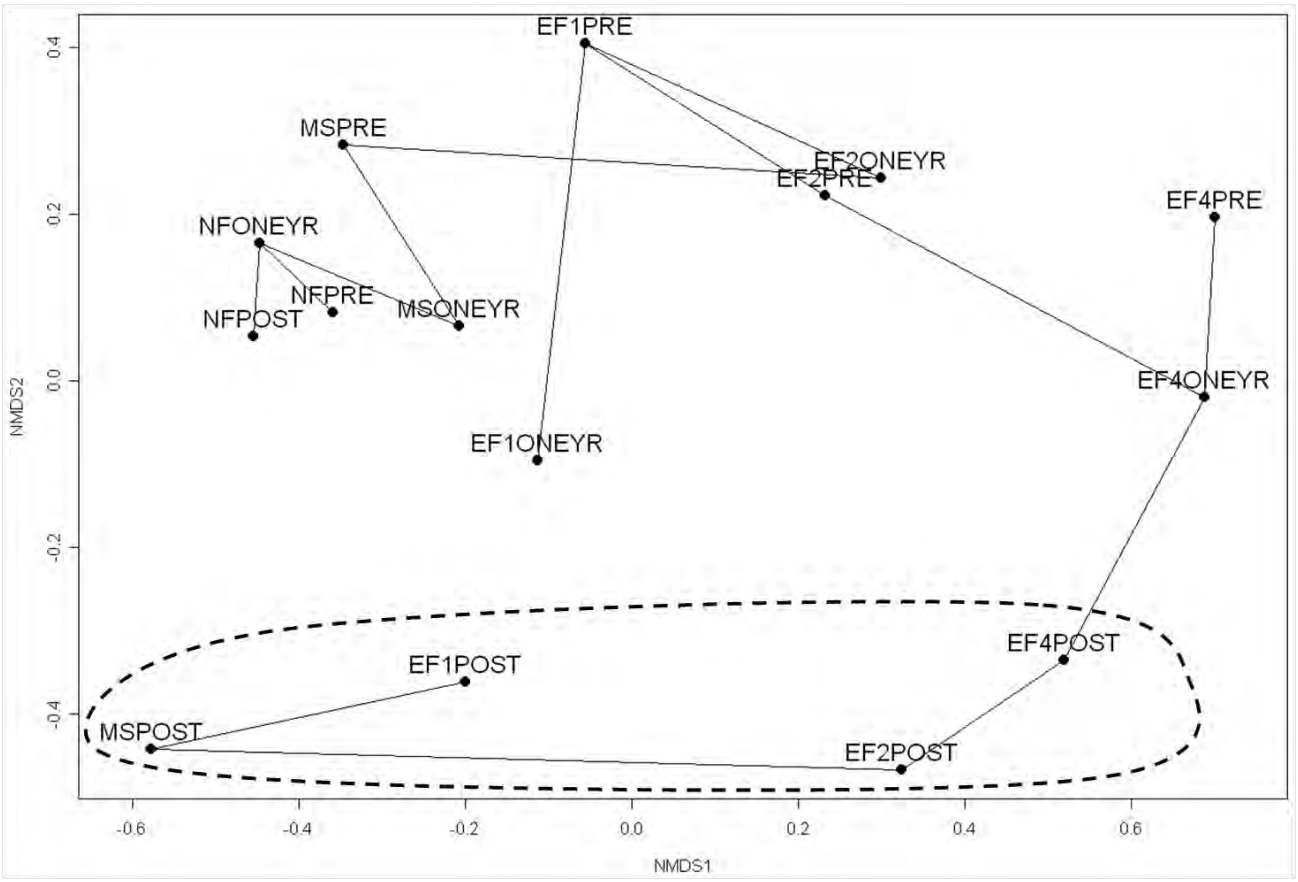

Figure 13. – NMDS spanning tree using Morisita-Horn similarity index for 2009 pre, immediate post and one-year posttreatment insect abundances. Sites within dashed lines are immediate post samples.

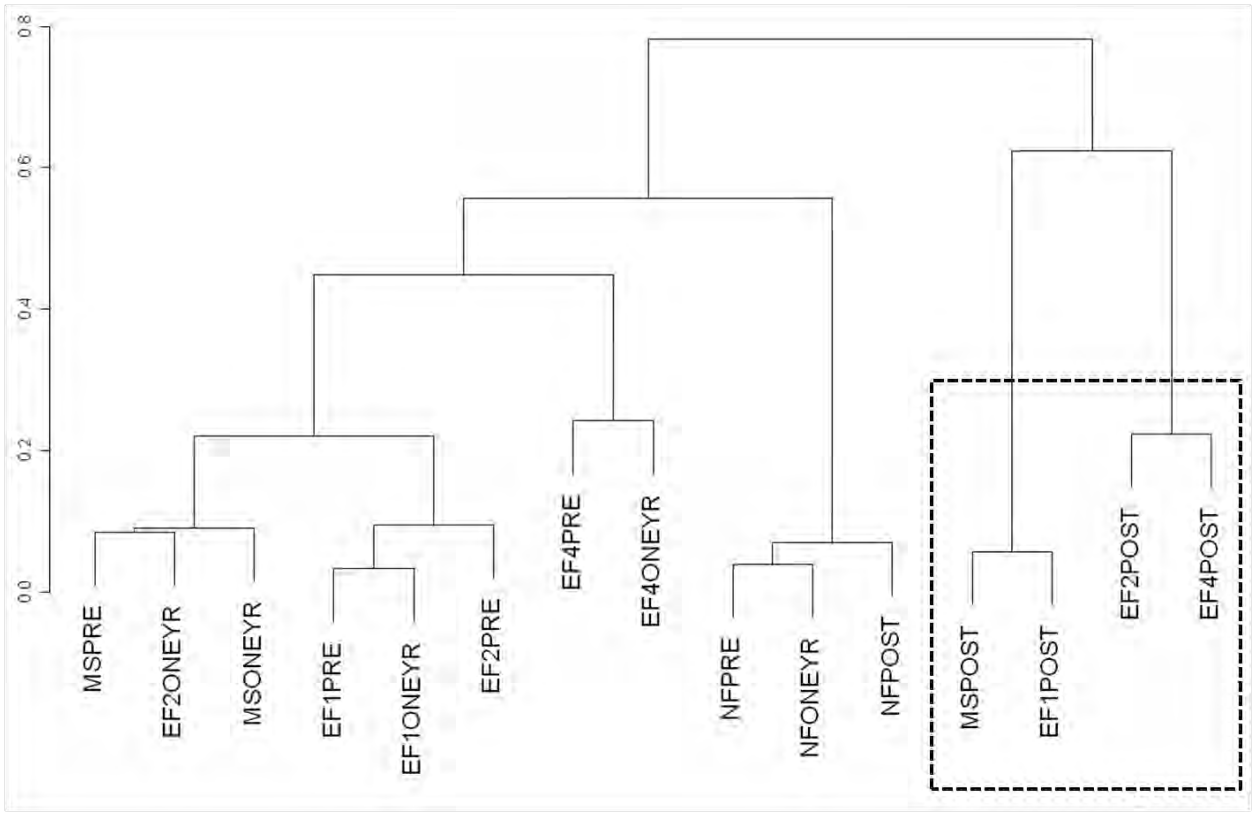

Figure 14. – Cluster analysis using Morisita-Horn similarity index for 2009 pre, immediate post and one-year posttreatment EPT abundances. Sites within dashed lines are immediate post samples.

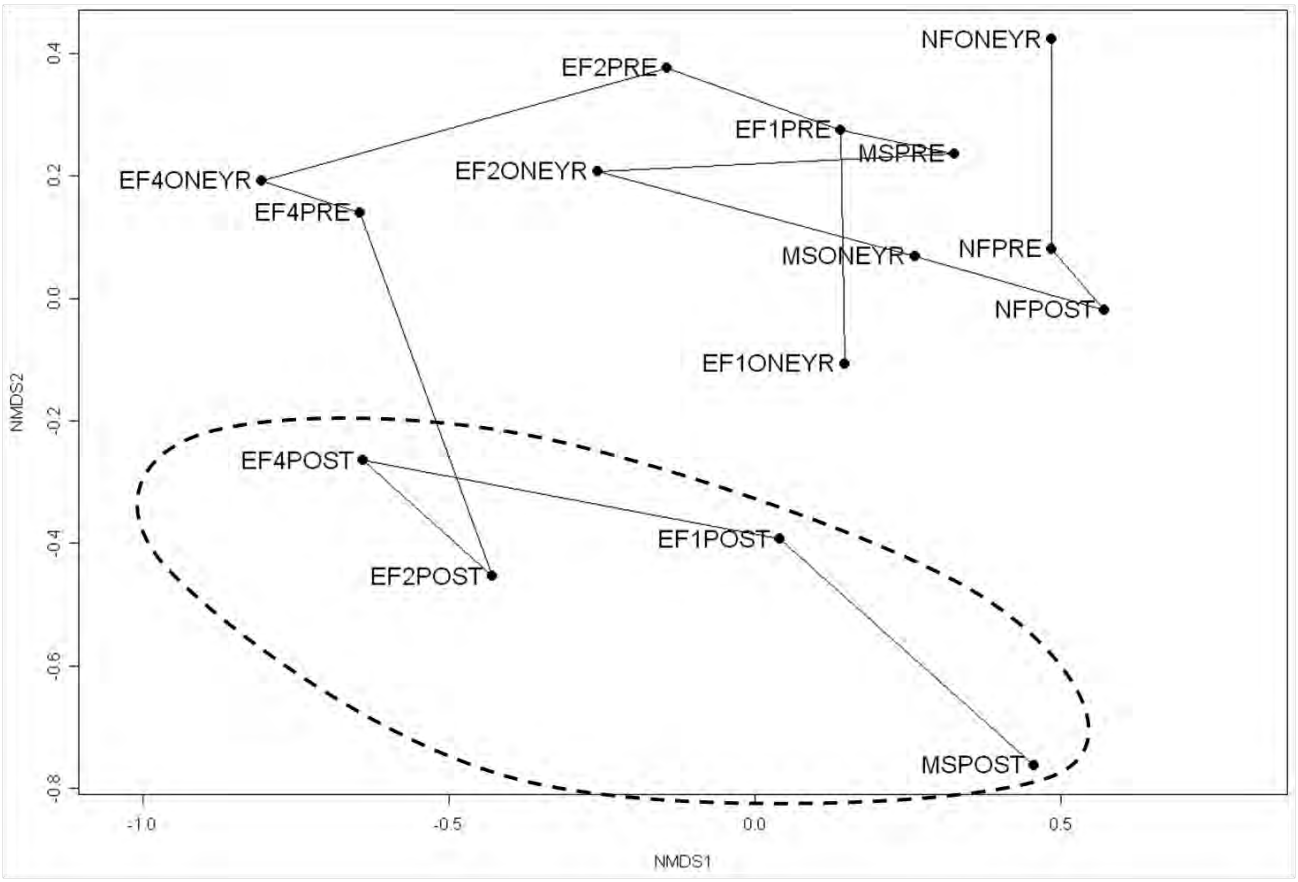

Figure 15. – NMDS spanning tree using Morisita-Horn similarity index for 2009 pre, immediate post and one-year posttreatment EPT abundances. Sites within dashed lines are immediate post samples.

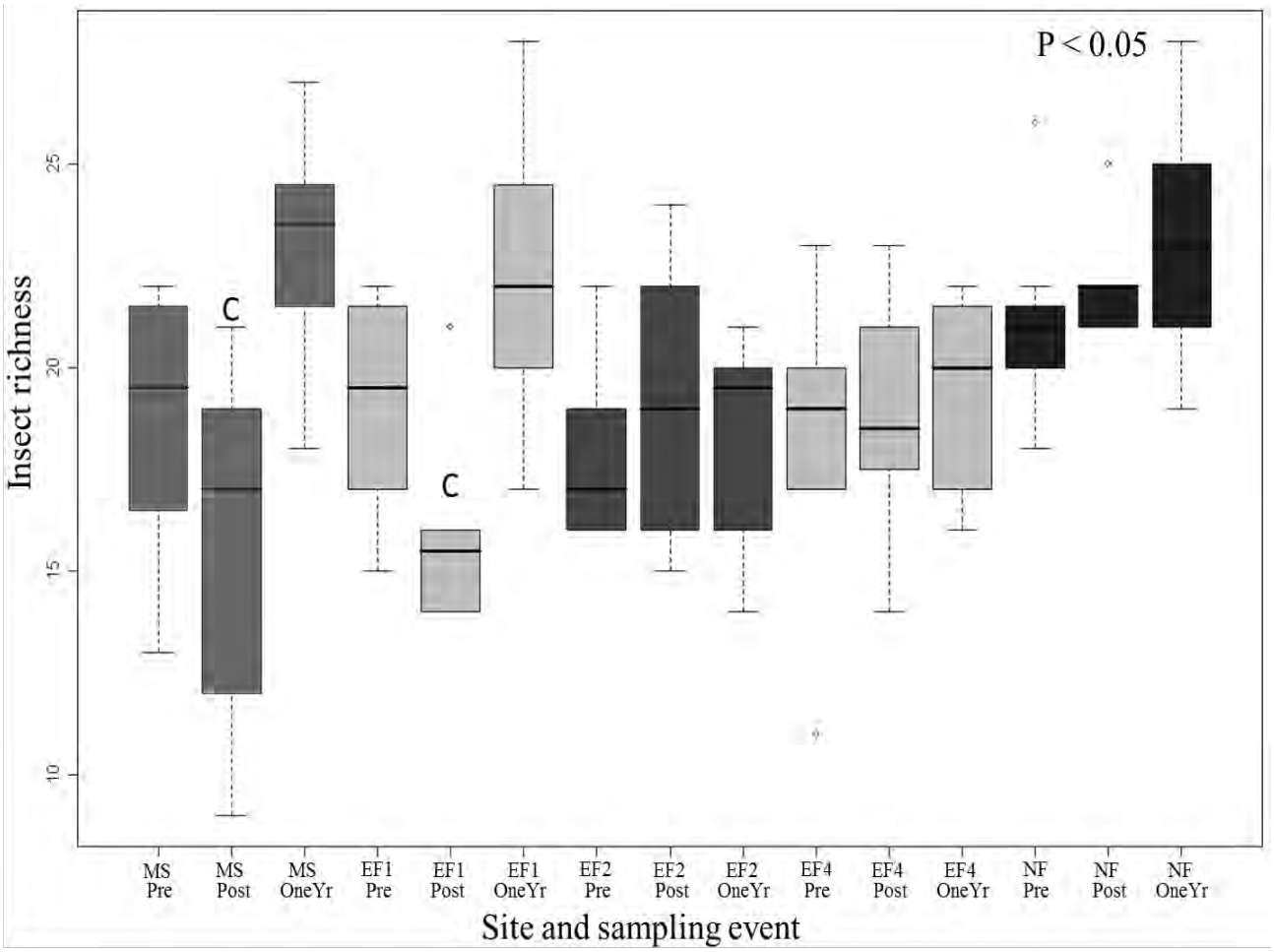

Figure 16. – Boxplot of insect richness for pre, immediate post and one-year posttreatment samples. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the reference site during the same sampling event.

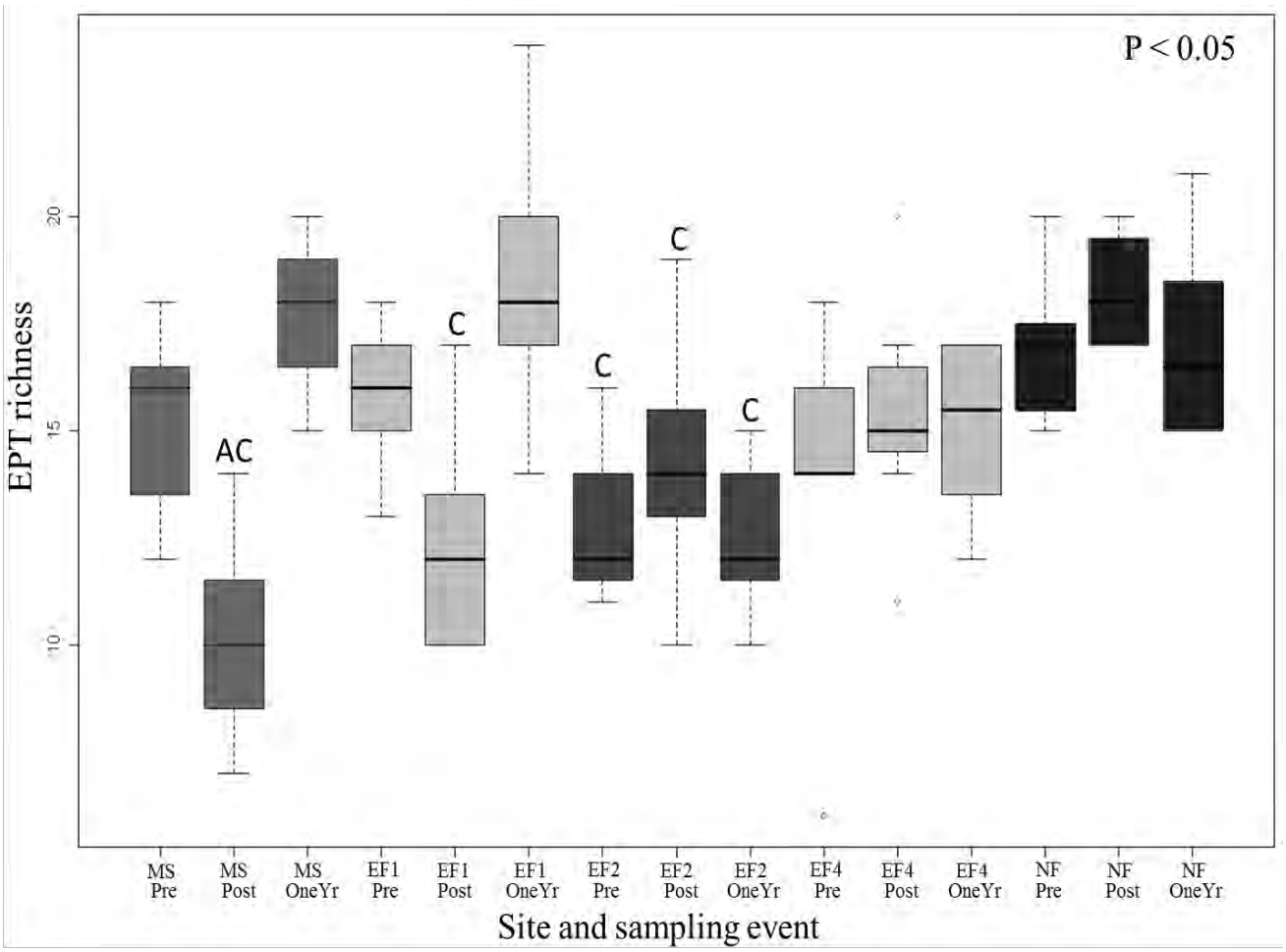

Figure 17. – Boxplot of EPT richness for pre, immediate post and one-year posttreatment samples. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the reference site during the same sampling event.

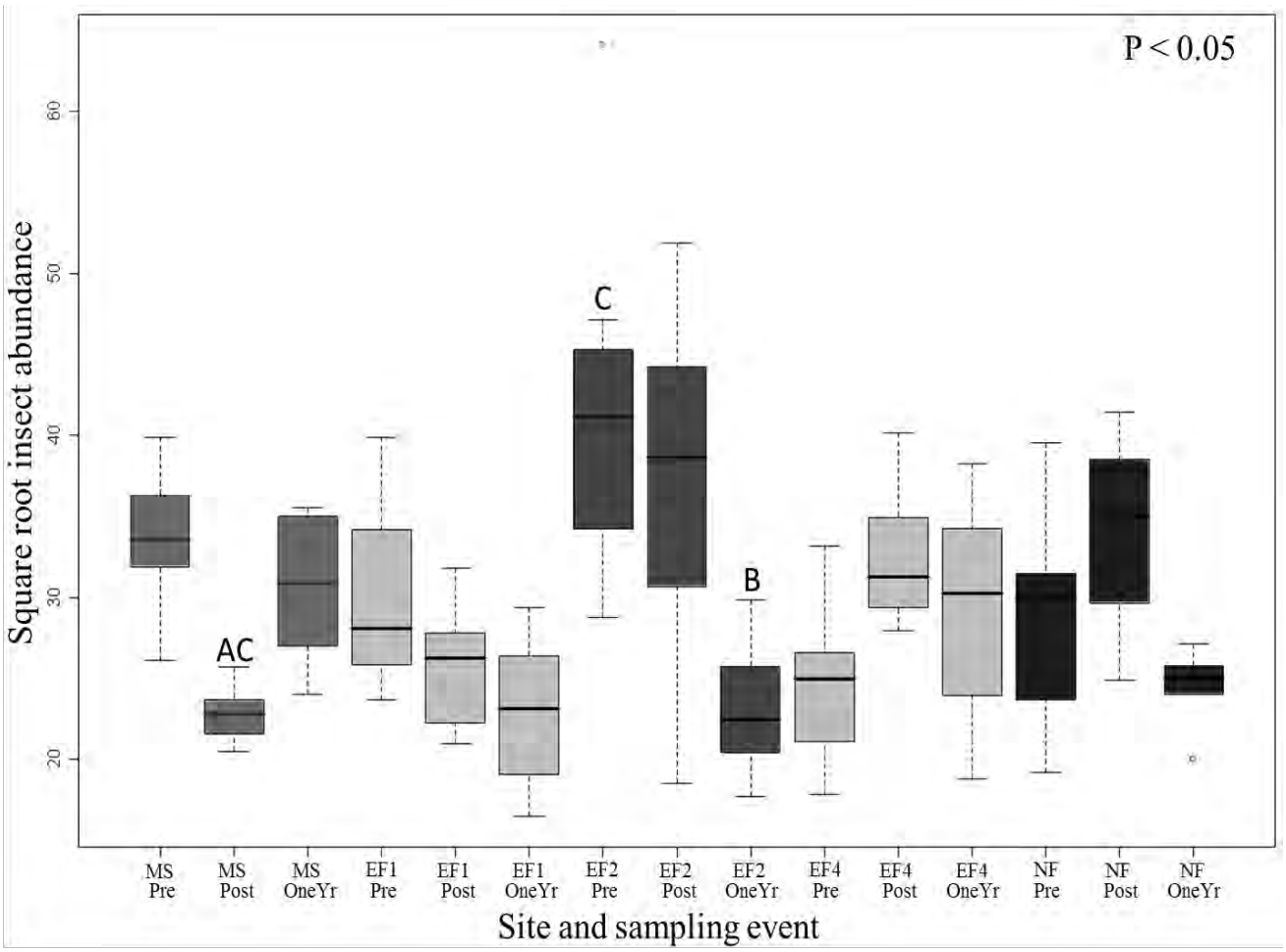

Figure 18. – Boxplot of square root transformed insect abundances for pre, immediate post and one-year posttreatment samples. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the reference site during the same sampling event.

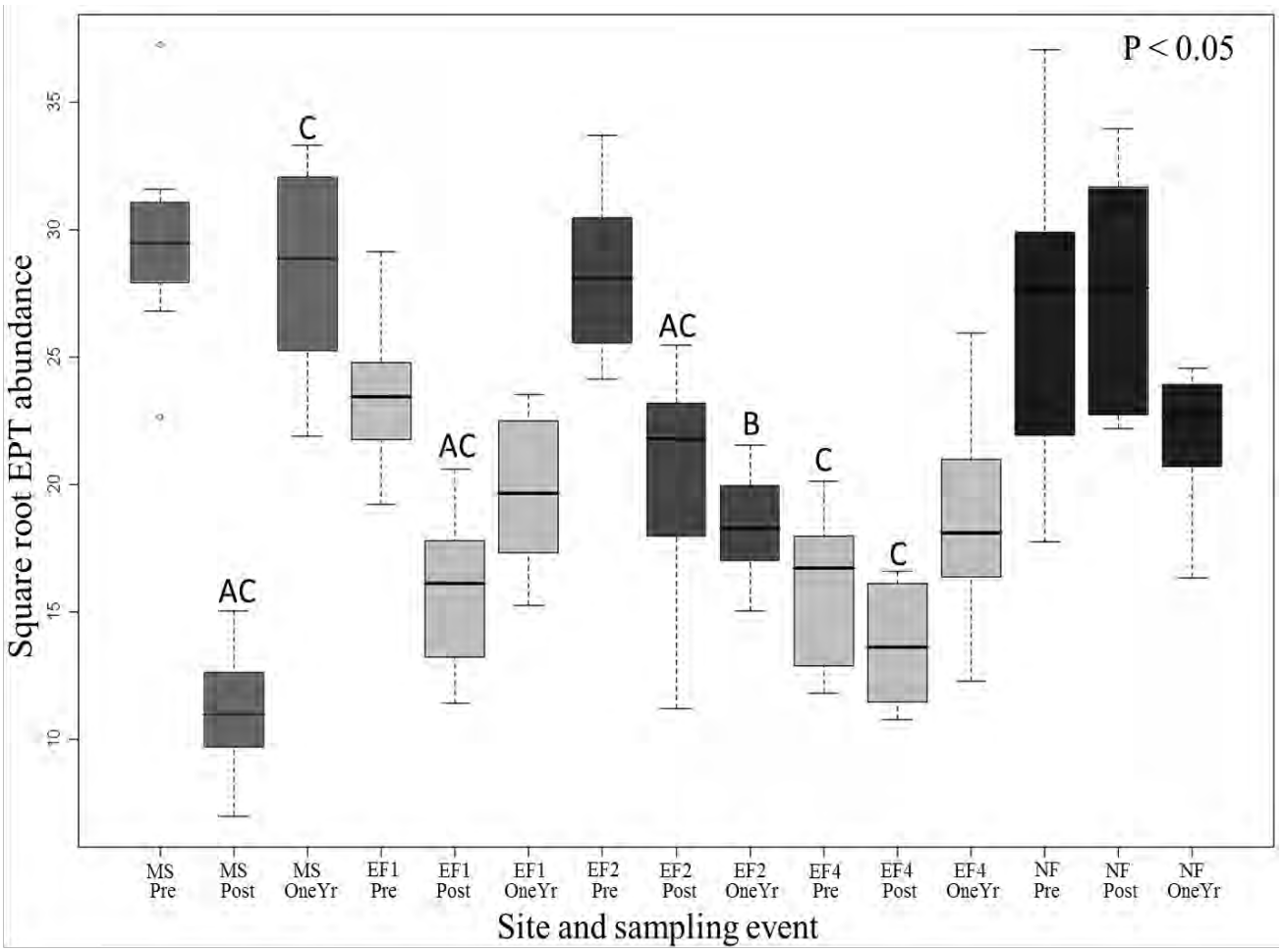

Figure 19. – Boxplot of square root transformed EPT abundances for pre, immediate post and one-year posttreatment samples. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the reference site during the same sampling event.

CHAPTER 3

IMMEDIATE EFFECTS OF CFT LEGUMINE ROTENONE TREATMENT ON MACROINVERTEBRATE DRIFT

Introduction

The loss and decline of native fish species is a result of non-native species introductions that compete and hybridize with the native populations (Rahel 2000 and 2002). Piscicides, specifically rotenone, is one of the few management techniques that can remove non-native fishes and restore native fish habitat (McClay 2000 and 2005). Rotenone has been used for centuries to harvest fish for consumption in its native location by indigenous people, and more recently for greater than 150 years as a commercial insecticide. For greater than 70 years, rotenone has become an important tool for fisheries managers in the restoration of native fish species. Although rotenone is a popular and valuable method in fisheries management (McClay 2000), its use has been criticized and challenged.

Over the past few years, environmental groups have brought about concerns involving public health, environmental impacts, animal welfare and applicator safety (McClay 2005 and Turner *et al.* 2007). Rotenone formulations registered with the United States Environmental Protection Agency (USEPA) as piscicides, are available as powdered extracts or emulsifiable liquids. Differences among rotenone emulsifiable formulations are the inert ingredients, which act as solvents and synergists. Although labeled as inert ingredients most of these chemicals are toxic. Conventional rotenone formulations include petroleum hydrocarbons such as toluene, xylene, benzene, naphthalene, and the synergist piperonyl butoxide. The registration of CFT Legumine addresses several of the issues creating multiple advantages to the product. Unlike

conventional rotenone formulations, CFT Legumine was designed to reduce or eliminate a number of hydrocarbon compounds, and does not include any synergists (McClay 2005; Turner *et al.* 2007; Finlayson *et al.* 2010); therefore reducing risks for applicators, terrestrial species, public health, and the overall environmental impacts. However, CFT Legumine effects in field applications are poorly understood for non-target organisms, specifically aquatic invertebrates.

A need for studies evaluating the advanced rotenone formulation (CFT Legumine) and methods to minimize rotenone effects on invertebrates is crucial. The objective of this paper is to provide specific recommendations to advance piscicide treatments by utilizing information collected on benthic macroinvertebrate drift during the application of CFT Legumine in East Fork Specimen Creek. The sampling of insects before, immediately after and one-year after treatment provides a snapshot of the benthic macroinvertebrate communities; whereas sampling drift throughout the treatment conveys how invertebrates respond to the application of rotenone. Drift is a macroinvertebrate response that can be behavioral or caused by a disturbance, defined as catastrophic drift (Waters 1965 and 1972). Drift response is directly related to insect tolerance of rotenone (Dudgeon 1990; Gladso *et al.* 2002; Arnekleiv 1997; Cerreto 2004; Arnekleiv *et al*. 2001). As suggested by Finlayson *et al.* (2010), CFT Legumine is not as detrimental to selected macroinvertebrates compared to conventional formulations. Therefore, it is suspected that using CFT Legumine will minimize impacts and change invertebrate drift response. In this study, we examine the drift pattern of insects at two localities and differences in life stages of insect larvae, specifically Ephemeroptera and Plecoptera. Using this information, we predict how improved management techniques would influence macroinvertebrate drift.

Methods

Study area. – Specimen Creek is located in the northwest corner of Yellowstone National Park (YNP) in Gallatin and Park Counties of Montana. The entire Specimen Creek drainage is approximately 76 km^2, containing 62 km of flowing water. The East Fork of Specimen Creek originates at High Lake and flows approximately 27 km until the confluence of the North Fork, which originates 20 km upstream at Crescent Lake (Figure 20). Both branches are second order streams soon after their origin, and increase to third order after their confluence. East Fork Specimen Creek landscape vegetation is predominately coniferous trees with deciduous vegetation in the riparian zones. It meanders through multiple meadows and a recent burn area on the lower reach for approximately two kilometers. The substrate is dominated by rock rubble except in the low gradient meadows where sand and fine gravel dominate.

Rotenone applications. – In 2008 and 2009, four applications of CFT Legumine rotenone were applied to the East Fork Specimen Creek and its tributaries to remove nonnative rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, Yellowstone cutthroat trout *O. clarkii bouvieri,* and hybridized forms of cutthroat/rainbow trout. Approximately 105 liters of CFT Legumine ™ Fish Toxicant (U.S. EPA Product Reg No: 75338-2) (5% rotenone) and approximately 14.5 kilograms of Prentox Fish Toxicant Powder (EPA Reg. No. 7533-2) (7.4% rotenone) were applied to the fish inhabited 20 km section of the East Fork Specimen Creek for eight hours at a target rate of one ppm CFT Legumine (50 ppb rotenone). The application was based on the results of travel time estimates, flow calculations, and bioassays. A total of 13 drip stations, spaced 2.5 – 3 hours apart treated the majority of the treatment area. Prentox powder mixed with gelatin and sand was used to treat springs and seeps and backpack sprayers were

used for backwater areas. Quantities of potassium permanganate were applied to achieve 2-3 ppm to neutralize CFT Legumine rotenone downstream of the treatment area.

Macroinvertebrate drift sampling. – Aquatic macroinvertebrate drift samples were collected from two locations (upper and lower) in the treatment area on the East Fork Specimen Creek during the third piscicide treatment. The upper drift site was collected with one net due to a narrow stream channel. The lower drift site consists of three replicate nets, all located in a line perpendicular to the thalweg. Nets were positioned at 30 minutes of travel time below a drip station to sample insects consistently at both sites. All of the nets were in a similar riffle or run habitat. Each net was 30cm wide and 1 meter long with a mesh size of 200µm. Velocity (m/s) and depth (cm) measurements were taken at the net opening to estimate water volume filtered at the end of the eight hour treatment; drifting insect densities are expressed as the number/m^3. A total of four separate 30 minute drift samples were taken throughout the 8 hour treatment period: (1) 30 minutes before treatment to start of treatment, (2) 30 to 60 minutes, (3) 180 to 210 minutes, and (4) 330 to 360 minutes. Samples were taken during the daytime, therefore were not influenced by behavioral drift (Waters 1965). Each of the macroinvertebrate replicates were kept separate and preserved in 85% ethanol until processed. Samples were processed in total; however, an area sub-sampling method (Elliott 1971) was used if a sample was determined to be too large to finish and/or contained more than 1,000 individuals. In general, insects were identified to genus, whereas non-insects were identified to order or phylum. Even though these levels varied according to each taxonomic group, they were consistent throughout the project. Consistencies in taxonomic resolution ensured differences in response variables were not attributed to variation in taxonomic level.

To address management strategies the data was interpreted to provide information on possible ways to improve restoration plans. Studies indicated aquatic invertebrates that have gills (Ephemeroptera, Plecoptera, and Trichoptera), smaller invertebrates with larger surface area to volume ratio and less sclerotized exoskeletons appear more sensitive to rotenone (Engstrom-Heg *et al.* 1978; Ling 2003; Vinson *et al.* 2010). We predict that these results are applicable, but believe that the life stage of these individuals was the underlying cause of higher sensitivity rather than just size. Earlier life stages, have larger surface area to volume ratio, less sclerotized exoskeletons, a higher metabolic rate, and molt their exoskeleton more often (Kiffney and Clements 1994 and 1996), which collectively contribute to a higher susceptibility to rotenone. The hemimetabolous Ephemeroptera and Plecoptera nymphs were classified into life stage groups based on wing pad development (Heise *et al*. 1987). Ephemeroptera life stage groups are as follows: (I) no wingpads present, (II) wingpads are starting to develop, but not past abdominal segment one, (III) wingpads developed past abdominal segment one, (IV) wingpads fully developed, has become darkened and swollen. Plecoptera life stage groups are as follows: (I) no wingpads present, (II) wingpads was starting to develop, but not fully developed, (III) wingpad fully developed, has becoming darkened and swollen.

Data analysis. – The lower treatment drift samples were analyzed for differences in density of macroinvertebrates drifting by Analysis of Variance (ANOVA) using Statistical Analysis System (SAS). Dunnett's Multiple Range test was used to determine which samples are significantly different from the pretreatment drift sample. The upper treatment samples were not statistically analyzed because there were not replicates.

Results

Rotenone treatment effected macroinvertebrate drift at both sample sites compared to the sampling event prior to treatment (Figure 21 and 22). At the lower site, total drift, insect and EPT densities were significantly different from pretreatment levels for all three sampling periods (One-way ANOVA, $p < 0.05$). Throughout the treatment, the upper and lower sites were similar to each other in their pattern of response to rotenone application. In general, drift increases at 30-60 minutes, but was greatest during the 180-210 (lower) and 330-360 (upper) minute sampling periods (Figure 21 and 22). No divergent drift pattern was observed when comparing total drift, insect, and EPT density (Figure 21 and 22); however, some taxonomic groups appear to be disproportionately affected by rotenone treatment.

In general, Plecoptera and Ephemeroptera are the dominant drifters, but timing of the drift response suggests differences in sensitivity to rotenone. The 30-60 minute sampling period had the lowest density during the treatment, but had the greatest density of Plecoptera than any other sample (Table 6). Within this sampling period, Plecoptera nymphs accounted for greater than 80% of the drifting insects (Table 7). This indicates plecopterans peaked within the first 30 minutes of treatment and immediately responded to rotenone than any other taxonomic group. The 180-210 minute sample had the highest density of Ephemeroptera at the lower site, and the second greatest for the upper site (Table 6). Regardless, Ephemeroptera nymphs dominated approximately 60% of the drifting insects (Table 7). This demonstrates that Ephemeroptera peaked at the lower site, and increased in large quantities at the upper site; however, while they are dominant at this sampling period, other insects also began to drift in low proportions (Table 7). The last sampling period (330-360 minutes) had differences in total drift density at sites, which was likely due to the upper site not having replicates and an increased number of

Ephemeroptera (Table 6). However, approximately 50% and 40% Ephemeroptera taxa dominated the upper and lower sites, respectively (Table 7). This indicates Ephemeroptera were still drifting in high quantities at the lower site, and their densities had peaked at the upper site. Although, their dominance had decreased from the prior sampling period, indicating other taxa had again increased in density, suggesting they are becoming intolerant to rotenone.

The separation of Ephemeroptera and Plecoptera into life stage groups shows a distinct difference in tolerance to rotenone application. Group I had the greatest density in all three sampling periods during treatment; however, the later life stages increase in proportion as treatment continued (Figure 23 - 26). This indicates early life stages had the highest sensitivity to rotenone, and as exposure continued, later life stages become more vulnerable causing them to drift.

In recent restoration projects, the treatment time was reduced from 8 to 4 hours (M. Ruhl, Yell-NPS and C. Kruse, Turner Enterprises, personal communication). If this design were affective, it would potentially reduce the impacts to invertebrates. Using the drift values, I determined how this could have influenced the Specimen Creek insect drift. In the 480 minutes of treatment, I sampled 90 minutes. Assuming the three 30 minute drift samples are representative of the 480 minutes of treatment, I estimate the percentage of invertebrates that would not have been drifting. I recorded a value of zero for the last 30 minutes of treatment to not over estimate drifting invertebrates. These estimates indicate a 4 hour application would decrease total drift, insect and EPT density by 53, 52 and 47%, respectively (Figures 27-32). These results suggest rotenone projects could further reduce the impacts to invertebrates, but should be interpreted carefully. A thorough understanding of how a drifting individual responds

through time was limited. However, concentration and duration of exposure are the limiting factors that influence the impacts to invertebrates.

Discussion

Rotenone application resulted in an increase in macroinvertebrate drift in East Fork Specimen Creek. Catastrophic drift, defined as the physical disturbance of the bottom fauna, usually by drought, high temperatures, anchor ice, pollution, and insecticides (Waters 1972), is numerously reported during the application of rotenone (Dudgeon 1990; Gladso *et al.* 2002; Arnekleiv 1997; Cerreto 2004). However, in rotenone studies catastrophic drift was interpreted as an immediate drift response and not reported consistently. For example, the interpretation of immediate has ranged from 30 to 120 minutes of rotenone exposure (Dudgeon 1990; Arnekleiv *et al.* 2001; Erikson *et al.* 2009). What is clear is all studies demonstrate that density rapidly increased then slowly decreased over time or remains high throughout the treatment. Dudgeon (1990) and Arnekleiv *et al.* (2001) used a drift sampling design comparable to this study and observed a peak in drift in the first 30 minutes after rotenone application. Unfortunately, Dudgeon (1990) did not report rotenone concentrations and Arnekleiv *et al.* (2001) reports a 0.5-1.0 ppm formulation was applied, but was likely much higher in concentration at the locality of drift sites. In contrary to other studies, a peak in drift did not occur within the first 30 minutes of treatment. Peak drift occurred 180 – 210 minutes after exposure to rotenone. The delay of drift response reported with CFT Legumine suggests rotenone impacts are reduced in some proportion compared to other studies using different formulations (Dudgeon 1990; Arnekleiv *et al.* 2001). The reason was likely due to the use of CFT Legumine formulation and the concentration applied.

Although total drift, insect, and EPT density did not peak in the first 30 minutes of application, some plecopterans were collected during this time period. This was the peak drift period for this order, which were predominantly nymphs belonging to the family Perlodidae (Appendix C and D). Morphotypes encountered indicate that multiple genera of early instars were present. All other plecopterans had a delayed response, (including some perlodids) increasing in density later in the treatment. Engstrom-Heg *et al.* (1978) reported that perlodids were very sensitive to rotenone, linking the immediate drift response to low rotenone tolerance. Previous literature also reported the immediate response of perlodids to rotenone applications (Dudgeon 1990; Gladso and Raddum 2002; Arnekleiv 1997; Cerreto 2004; Kjarstad and Arnekleiv 2011). It is important to note that perlodids were the only insect identified as sensitive in the study by Engstrom-Heg *et al.* (1978) and demonstrate their peak drift in the first 30 minutes after rotenone application in the Specimen Creek study. Other sensitive taxa were present in the Specimen Creek BMI population study and were present later in the drift study. Thus, demonstrating taxa were disproportionately influenced and drift was not a proportional reflection of the benthic community. Other studies report that most baetid mayflies immediately respond to rotenone applications (Arnekleiv *et al.* 2001). No ephemeropterans or specifically baetids peaked in drift density until later in the treatment. A difference of response was observed among Ephemeroptera genera, and the same genera at the two sites. Regardless of the site, it is evident that Ephemeroptera drift was dominated by only a few taxa (Appendix C and D). The difference in response of ephemeropterans could be due to multiple factors: (1) difference in site community composition, (2) sensitivity to rotenone and (3) developmental stage of the nymph. Reporting the dominant taxon drifting at the sampling event and their peak in drift will account for differences in taxon abundances in the community. Comparison of Ephemeroptera genera

drifting between sites provides important information, because it demonstrates that different taxa and communities will respond differently, which has been reported frequently (Dudgeon 1990; Gladso and Raddum 2002; Arnekleiv 1997; Arnekleiv *et al.* 2001; Cerreto 2004; Kjarstad and Arnekleiv 2011). The developmental stage of individuals during treatment influences how insects respond to rotenone application.

Differences of drifting Ephemeroptera and Plecoptera based on wing pad development were noted in Specimen Creek. As discussed earlier, perlodids were the most abundant insect drifting in the first 30 minutes of treatment. At the upper and lower sites, Group I perlodids accounted for 97% and 88% (respectfully) of the drifting Ephemeroptera and Plecoptera (Figure 24 and 26). In the two later sampling events, Group I also dominated, but later developmental groups became more prevalent. Differential responses at different developmental stages had been noted by other studies. Gladso and Raddum (2002) reported that early instars responded immediately and had higher sensitivity to rotenone than later instars, and the later instars of the Trichoptera: *Rhyacohila nubila* had higher survival rates. Likewise, Erikson *et al.* (2009) found that early instars of drifting Trichoptera: *Rhyacohila nubila* had 100% mortality in the first three hours of the treatment, while instars 4 and 5 were generally alive. Drifting Trichoptera: *Polycentropus flavomaculatus* larvae had 80-90% mortality in instars 4 and 5, while earlier instars had 100% mortality. Later in the treatment, all larval instars collected were dead. In contrary to the two studies, Arnekleiv (1997) reports that greater than 95% of drifting larvae were dead, which could be a result of a different formulation and concentration. Erikson *et al.* (2009) applied CFT Legumine with concentrations at a maximum of 45 ppb active ingredient, and Arnekleiv (1997) applied Gull-Viks rotenone with concentrations potentially reaching 5 ppm formulation. Determination of live and dead individuals in Specimen Creek could not be made

for early instars, which dominated the drift samples. Regardless, the response of early life stages in Specimen Creek and other studies are consistent. Early life stages are the more sensitive to rotenone applications and as exposure time continues, later life stages are also affected.

Considering the findings of the Specimen Creek treatment regime and other studies, it is clear rotenone affects to benthic macroinvertebrates can be reduced; however, results are not absolute and projects should be considered individually. A reduction of concentration and duration of treatment that still removed non-native fish populations would intuitively reduce impacts. Finlayson *et al.* (2010) determined that mean 4 hour LC50 values ranged between from 4.8 - 11.0 ppb for rainbow trout, which was below the application rate of Specimen Creek (Figure 33). As implied by Figures 27-32 and previous literature, a reduction of treatment time could potentially reduce the mortality of insects. Knowing the position of the drift sites implies we captured invertebrates drifting due to the highest concentration along the degradation curve of rotenone. Samples collected during treatment suggest these individuals were exposed to approximately 30 ppb of rotenone (Figure 33). Therefore, it would be expected that further downstream, where insects are exposed to a lower concentration would not be as impacted. These results should be interpreted carefully. Attempts had been made to quantify live and dead drifting individuals throughout a treatment, but results varied (Arnekleiv 1997; Gladso and Raddum 2002; Erikson *et al.* 2009). In addition, quantifying how long a live individual drifts and if they continue to survive through treatment and after treatment has not been attempted. It is unclear if the macroinvertebrates immediately drifting that are alive recover and become part of the benthic community downstream. However, what is understood is prolonged exposure and higher concentrations cause more macroinvertebrates to drift, increases the impacts to later life

stages, and an increase in mortality. Therefore, applying CFT Legumine, reducing exposure time and lower effective rotenone concentrations could reduce impacts.

Recommendations

To fully understand and minimize rotenone effects on BMI, I recommend the following: (1) laboratory studies to determine survivorship of different insects (size dependent) and survivorship of different life stages within insects, specifically the most sensitive orders Ephemeroptera, Plecoptera and Trichoptera; (2) take adequate pretreatment samples classifying size classes and life stage groups within the project area, to determine potential effects to BMI community; (3) dynamic study to determine spatial and temporal differences of insect drift from rotenone treatment; (4) and apply unsynergized formulations (CFT Legumine).

Table 6. – Drift densities of taxonomic groups from the rotenone treated sites. The lower site densities are mean ± standard deviation. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

Upper Site	0	30-60	180-210	330-360
Total	15.61	154.28	860.88	1105.57
Non-insects	3.63	7.16	313.15	371.15
Ephemeroptera	2.39	2.82	330.10	379.74
Plecoptera	0.05	119.63	43.92	24.63
Trichoptera	0.14	0.53	3.72	29.07
Coleoptera	0.05	0.05	0.10	2.96
Diptera	9.36	24.11	169.89	298.02

Lower Site	0	30-60	180-210	330-360
Total	15.69 ± 4.76	187.49 ± 77.23	848.49 ± 158.11	569.69 ± 98.57
Non-insects	6.00 ± 2.30	12.49 ± 2.16	225.68 ± 20.19	170.121 ± 20.86
Ephemeroptera	2.91 ± 0.95	10.47 ± 3.44	358.77 ± 79.93	153.24 ± 33.02
Plecoptera	1.12 ± 0.42	144.67 ± 68.54	87.32 ± 22.13	80.54 ± 11.88
Trichoptera	1.12 ± 0.15	3.066 ± 0.65	10.71 ± 1.65	14.61 ± 1.86
Coleoptera	0.01 ± 0.02	0.05 ± 0.08	0.017 ± 0.03	0.34 ± 0.22
Diptera	4.53 ± 1.06	16.74 ± 2.95	166 ± 43.77	150.84 ± 33.51

Table 7. – Drift proportional abundance of taxonomic groups from the rotenone treated sites. The lower site densities are mean ± standard deviation. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

Upper Site	0	30-60	180-210	330-360
Ephemeroptera	19.92	1.91	60.27	51.71
Plecoptera	0.40	81.31	8.02	3.35
Trichoptera	1.20	0.36	0.68	3.96
Coleoptera	0.40	0.03	0.02	0.40
Diptera	78.09	16.39	31.02	40.58

Lower Site	0	30-60	180-210	330-360
Ephemeroptera	29.82 ± 5.18	6.05 ± 1.01	57.55 ± 3.077	38.29 ± 6.82
Plecoptera	11.33 ± 2.46	82.27 ± 27.77	14.01 ± 1.72	20.14 ± 2.01
Trichoptera	11.66 ± 0.73	1.81 ± 0.33	1.73 ± 0.09	3.65 ± 0.14
Coleoptera	0.15 ± 0.26	0.04 ± 0.05	0.0 ± 0.0	0.08 ± 0.04
Diptera	47.07 ± 7.45	9.84 ± 0.34	26.7 ± 4.56	37.86 ± 8.59

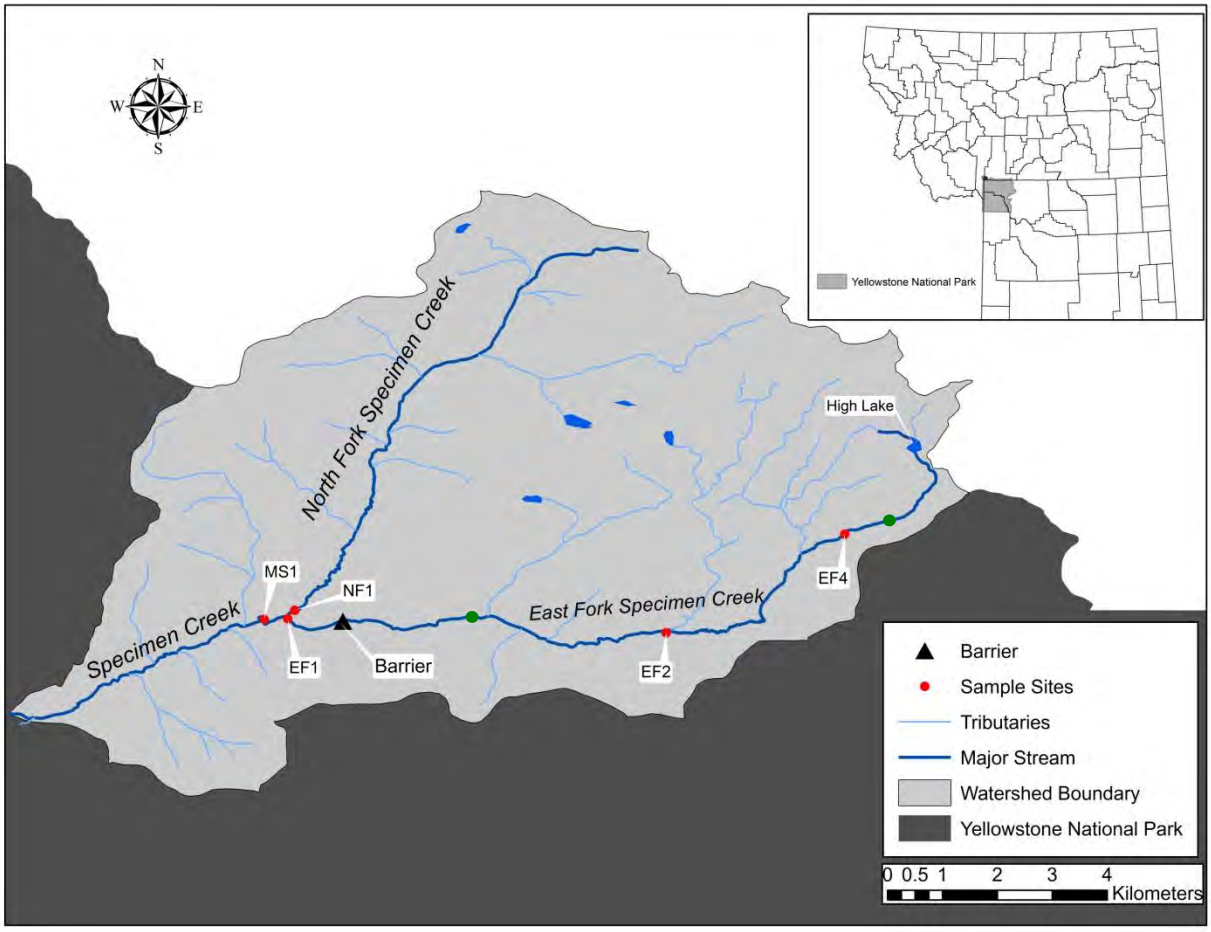

Figure 20. – Map of Specimen Creek, with the light gray boundary designating the watershed. Red dots indicate benthic macroinvertebrate sample locations on the East Fork Specimen Creek (EF1, EF2, and EF4), Main stem Specimen Creek (MS1), and North Fork Specimen Creek (NF1). EF4 and EF2 are within the rotenone treatment area, whereas EF1 and MS1 are below treatment. NF1 is the locality of the reference site. Green dots represent upper and lower treatment drift sites. The triangle indicates the barrier, end of treatment area, and $KMnO_4$ neutralization station.

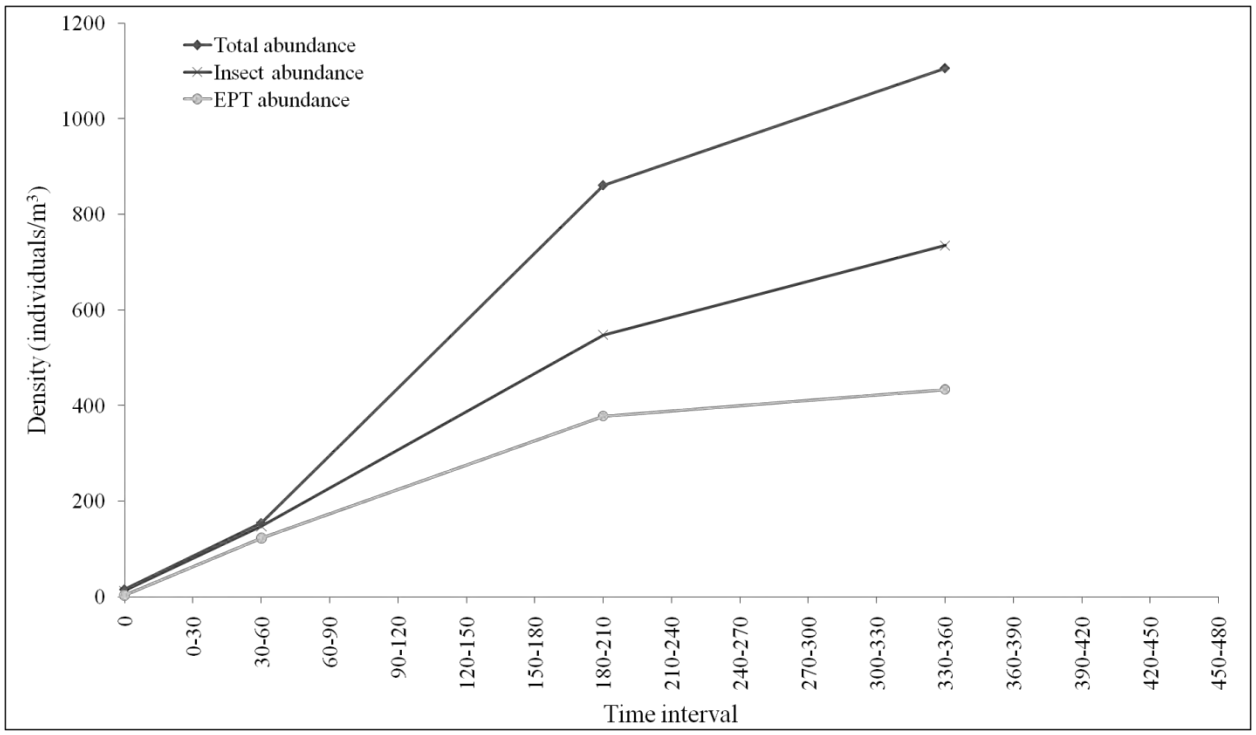

Figure 21. – Drift densities of total, insect and EPT from the upper rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

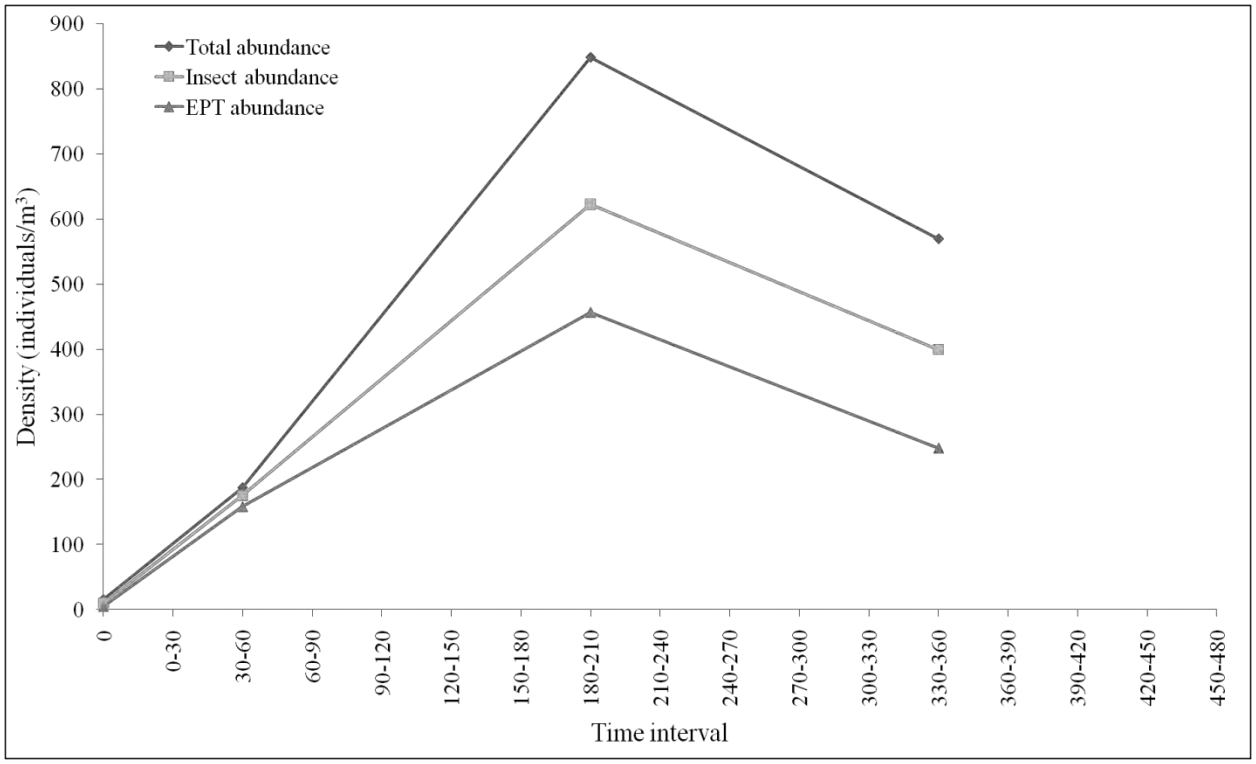

Figure 22. – Drift density of total, insect and EPT abundance from the lower rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

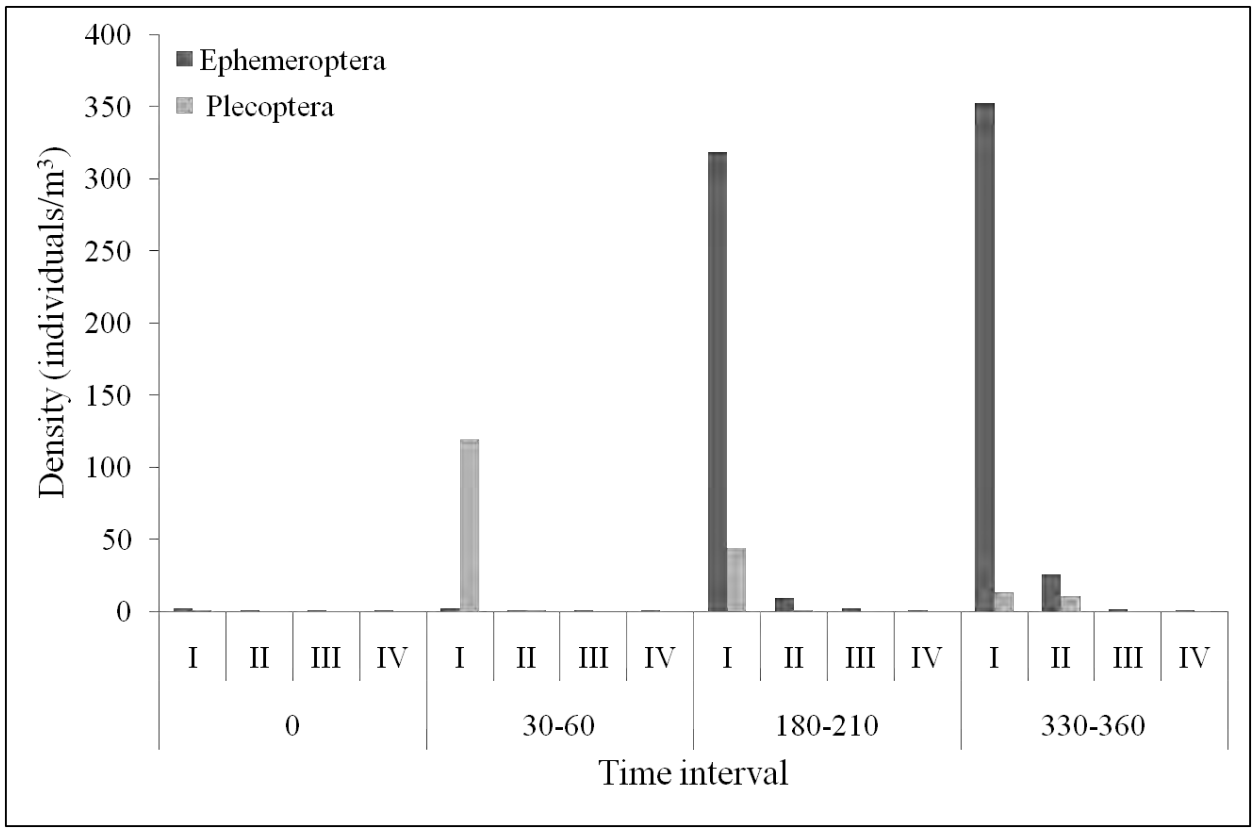

Figure 23. – Drift densities for wingpad development groups of Ephemeroptera and Plecoptera from the upper rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

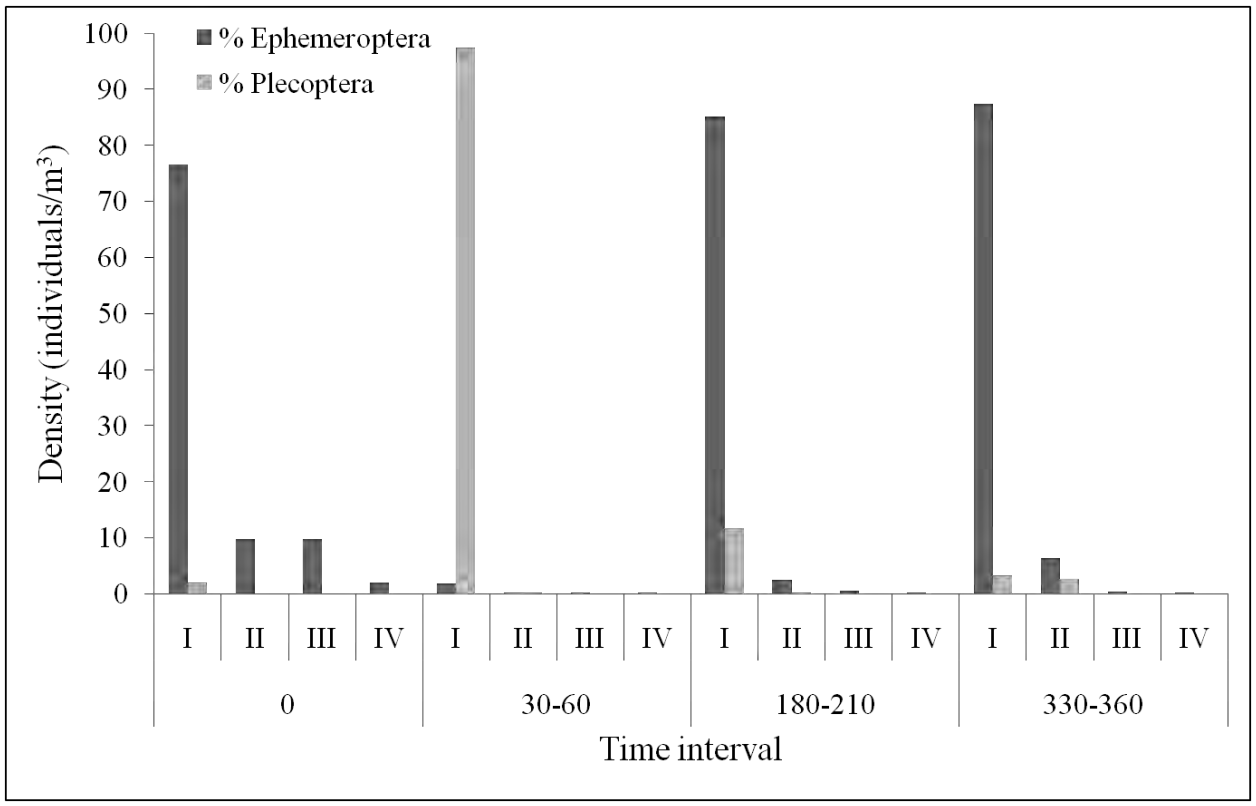

Figure 24. – Drift proportional abundances for wingpad development groups of Ephemeroptera and Plecoptera from the upper rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

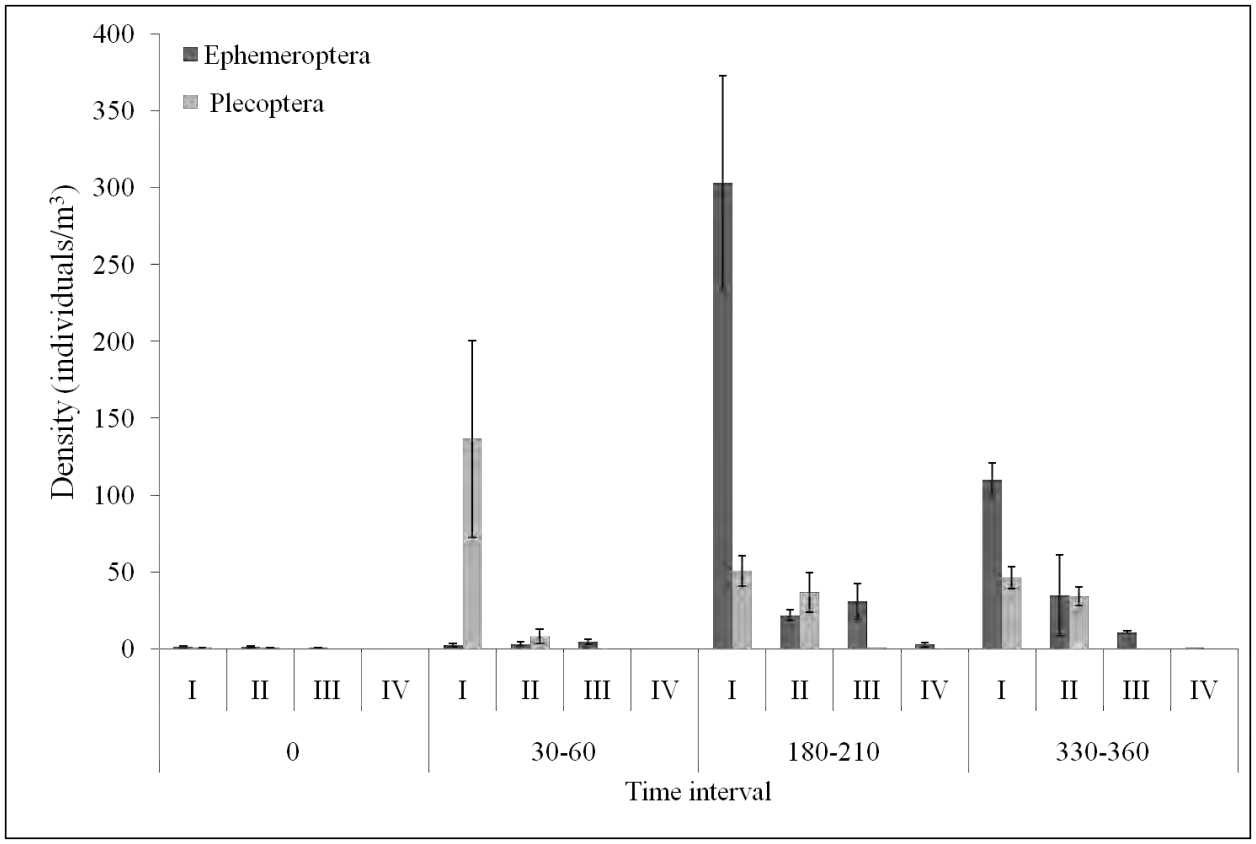

Figure 25. – Drift densities (mean ± standard deviation) for wingpad development groups of Ephemeroptera and Plecoptera from the lower rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

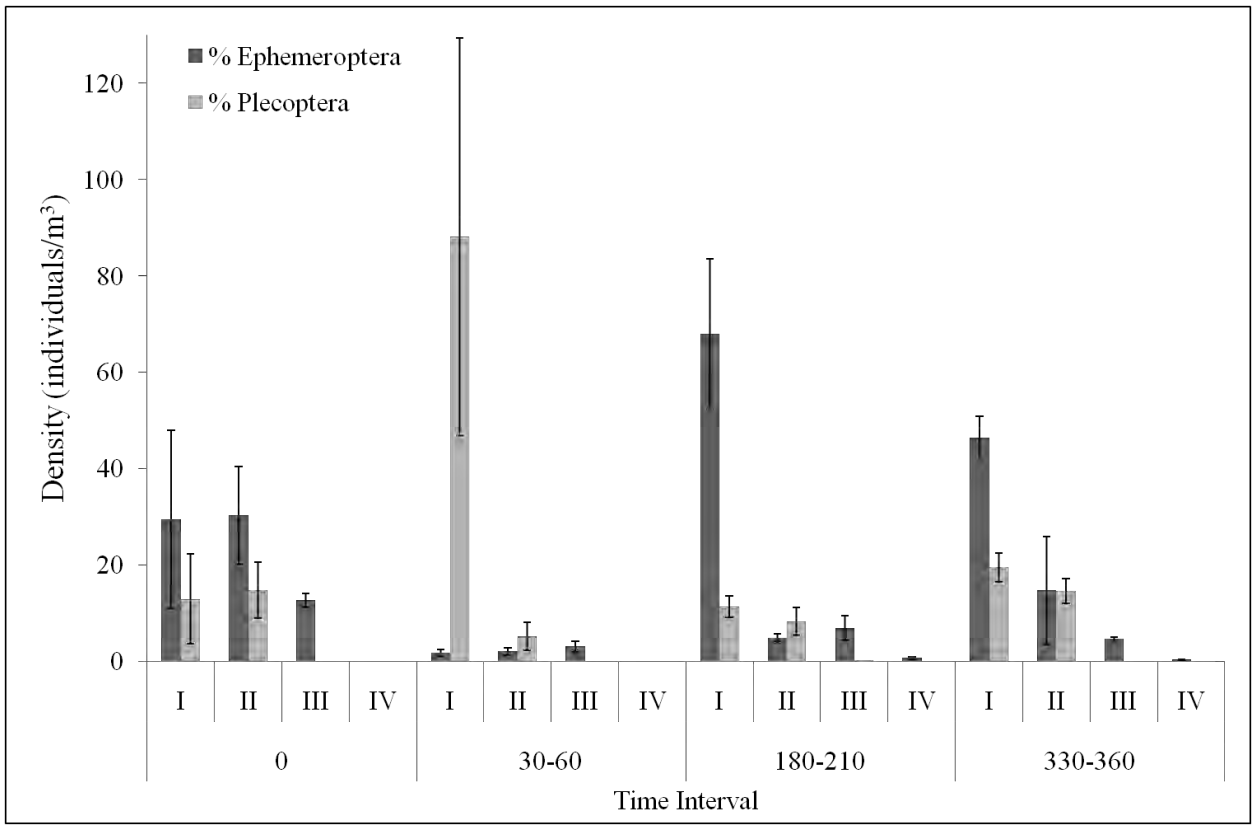

Figure 26. – Drift proportional abundances (mean ± standard deviation) for wingpad development groups of Ephemeroptera and Plecoptera from the lower rotenone treated site. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

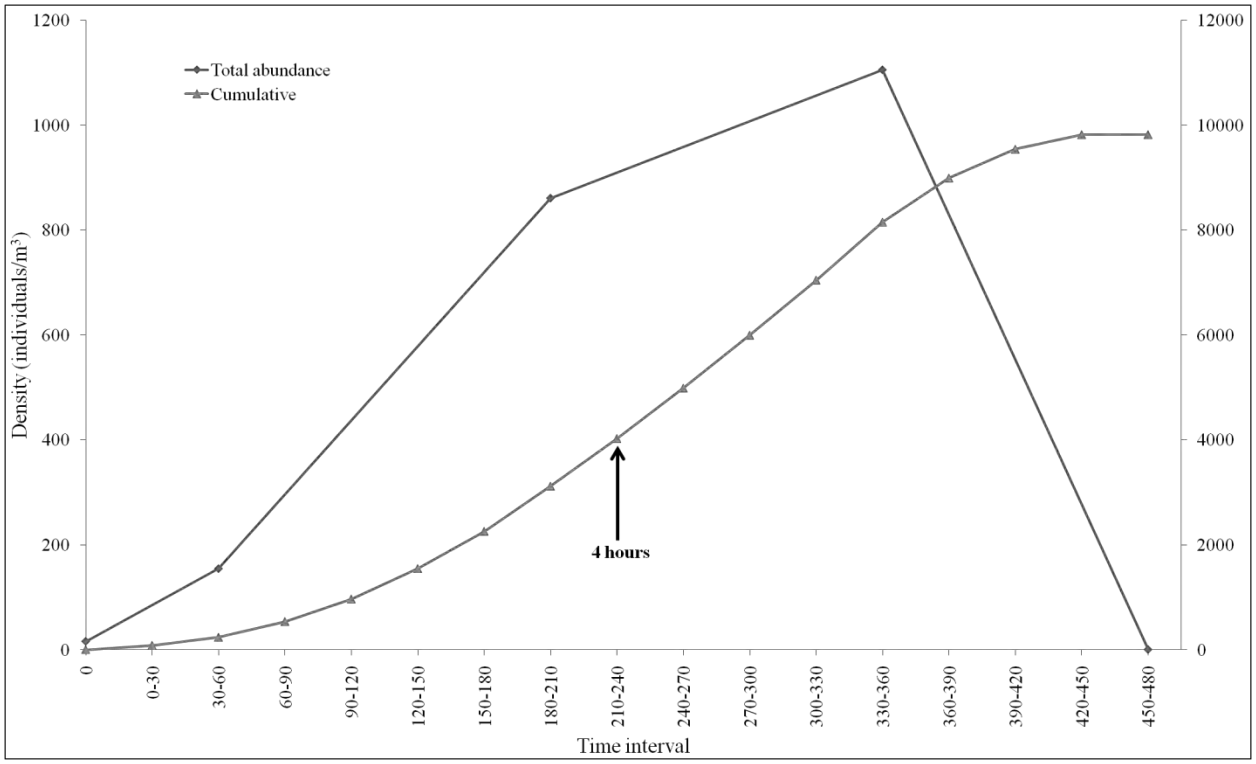

Figure 27. – Drift densities of total invertebrates from the lower rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

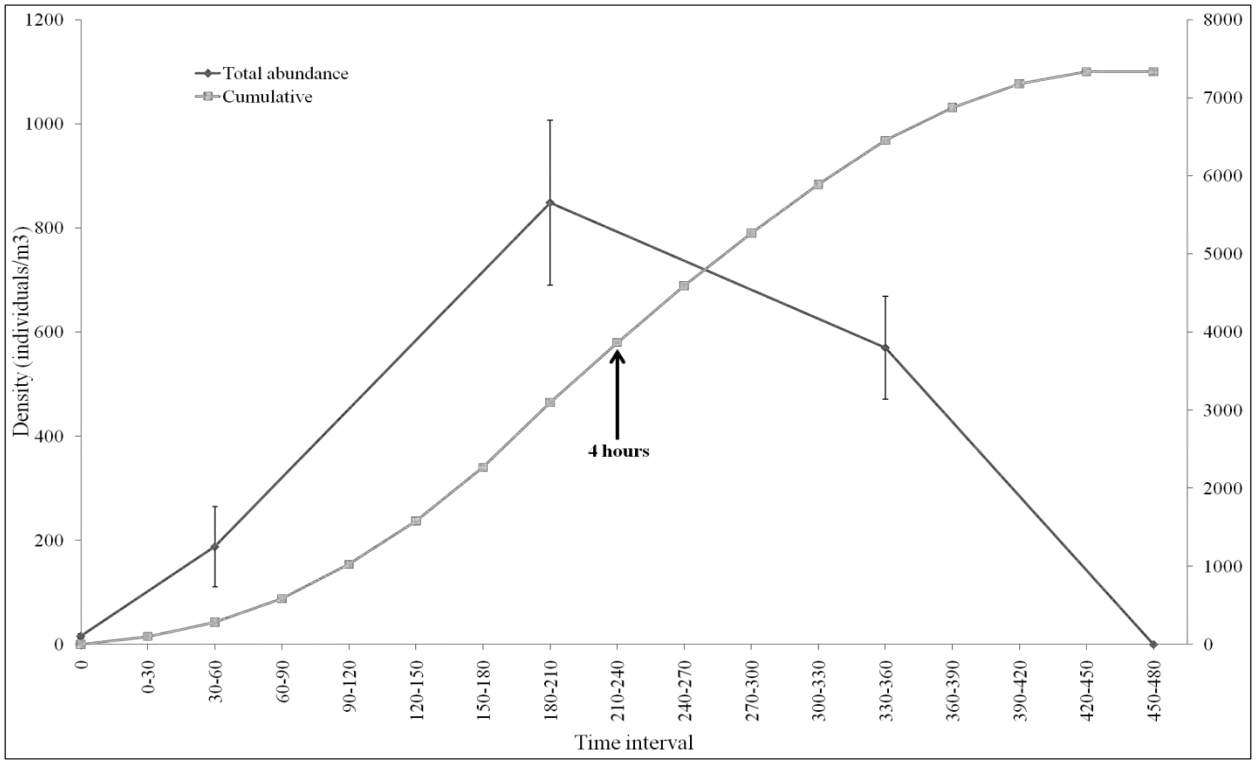

Figure 28. – Drift densities (mean ± standard deviation) of total invertebrates from the lower rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

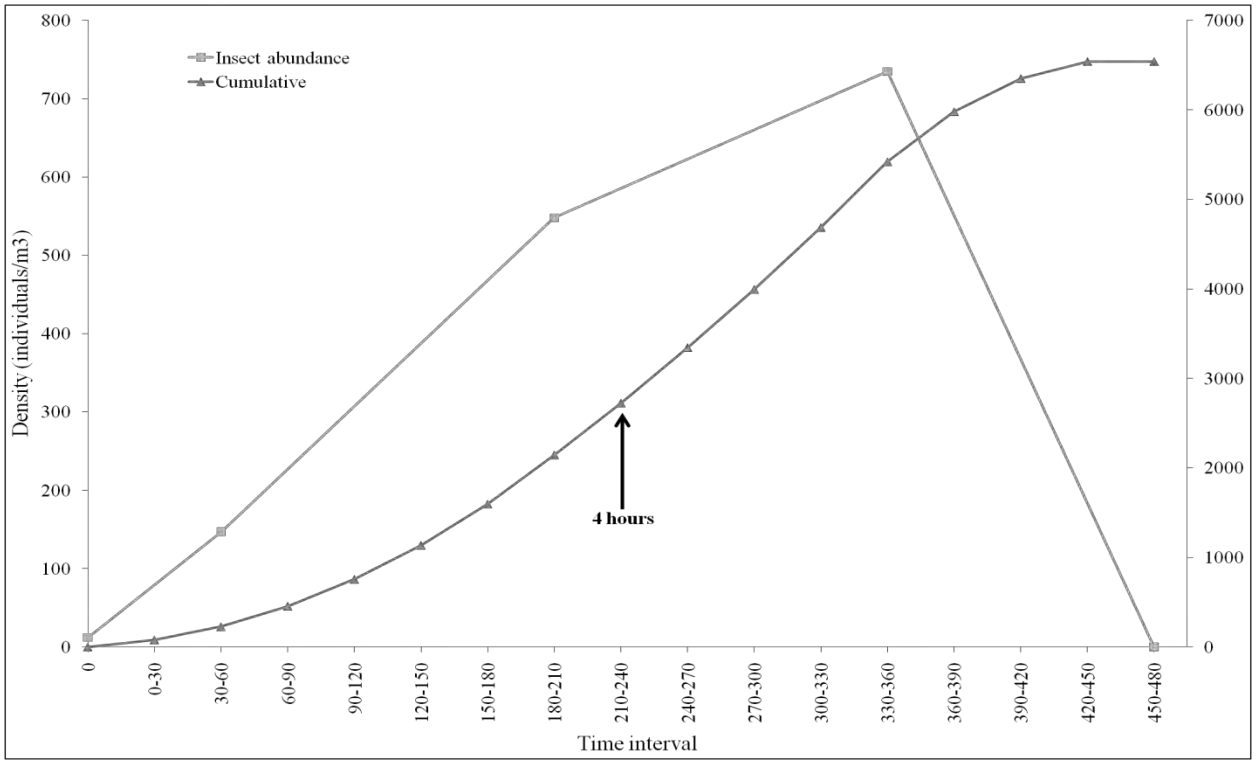

Figure 29. – Drift densities of insects from the upper rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

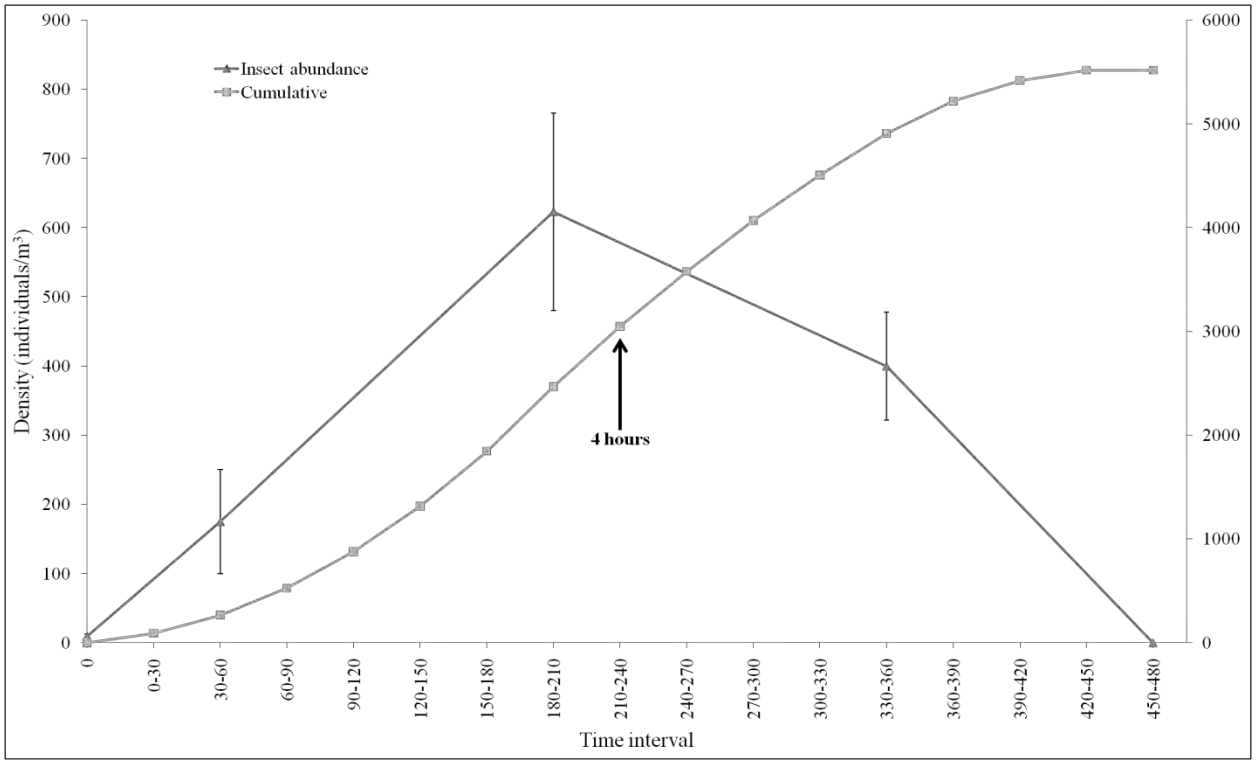

Figure 30. – Drift densities (mean ± standard deviation) of insects from the lower rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

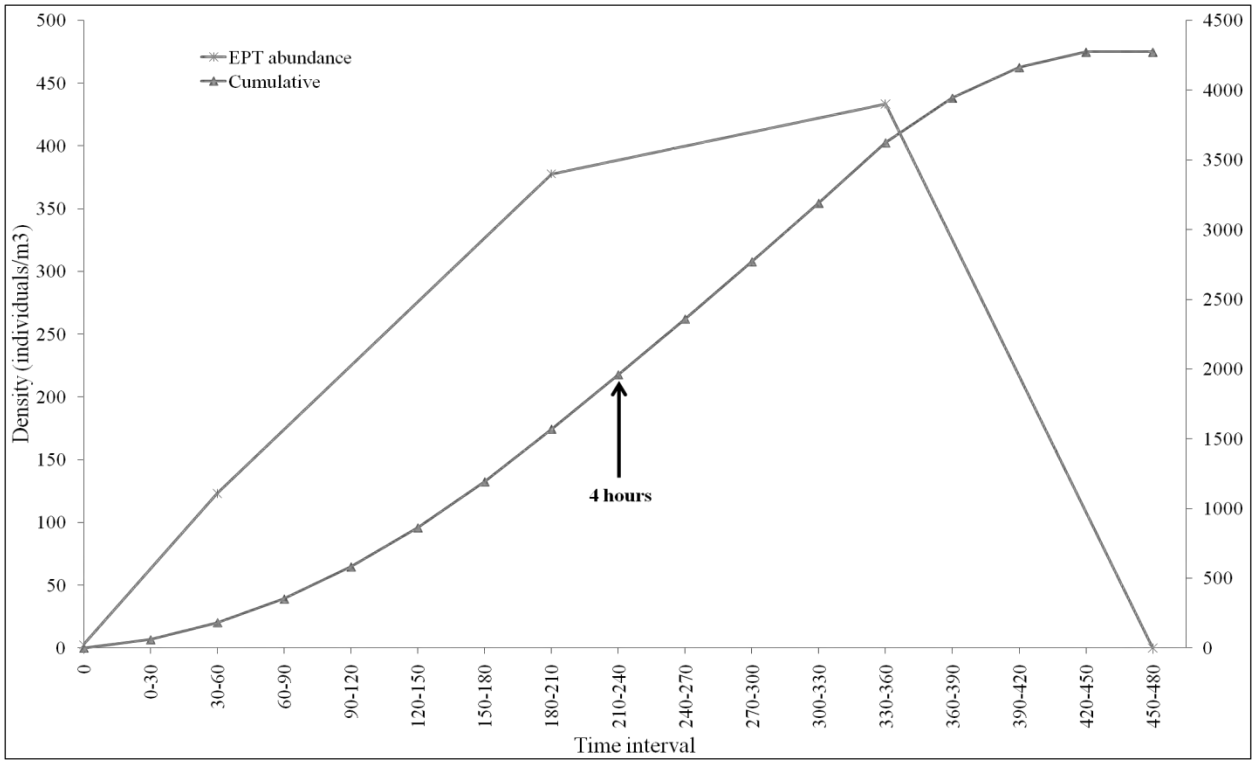

Figure 31. – Drift densities of EPT from the upper rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

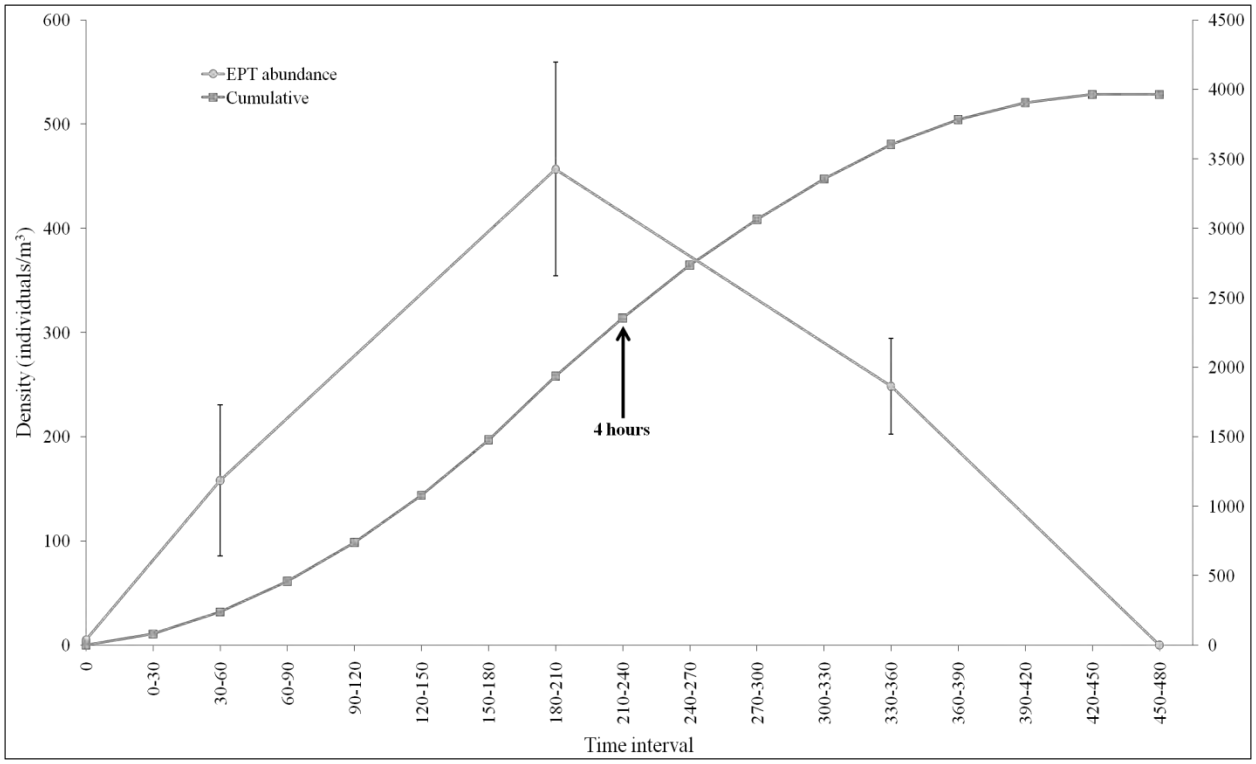

Figure 32. – Drift densities (mean ± standard deviation) of EPT from the lower rotenone treated site with a generated cumulative curve based on a predicted drift pattern. Rotenone application was initiated at 0 minutes and continued for 480 minutes. The 0 sample period represents pretreatment conditions.

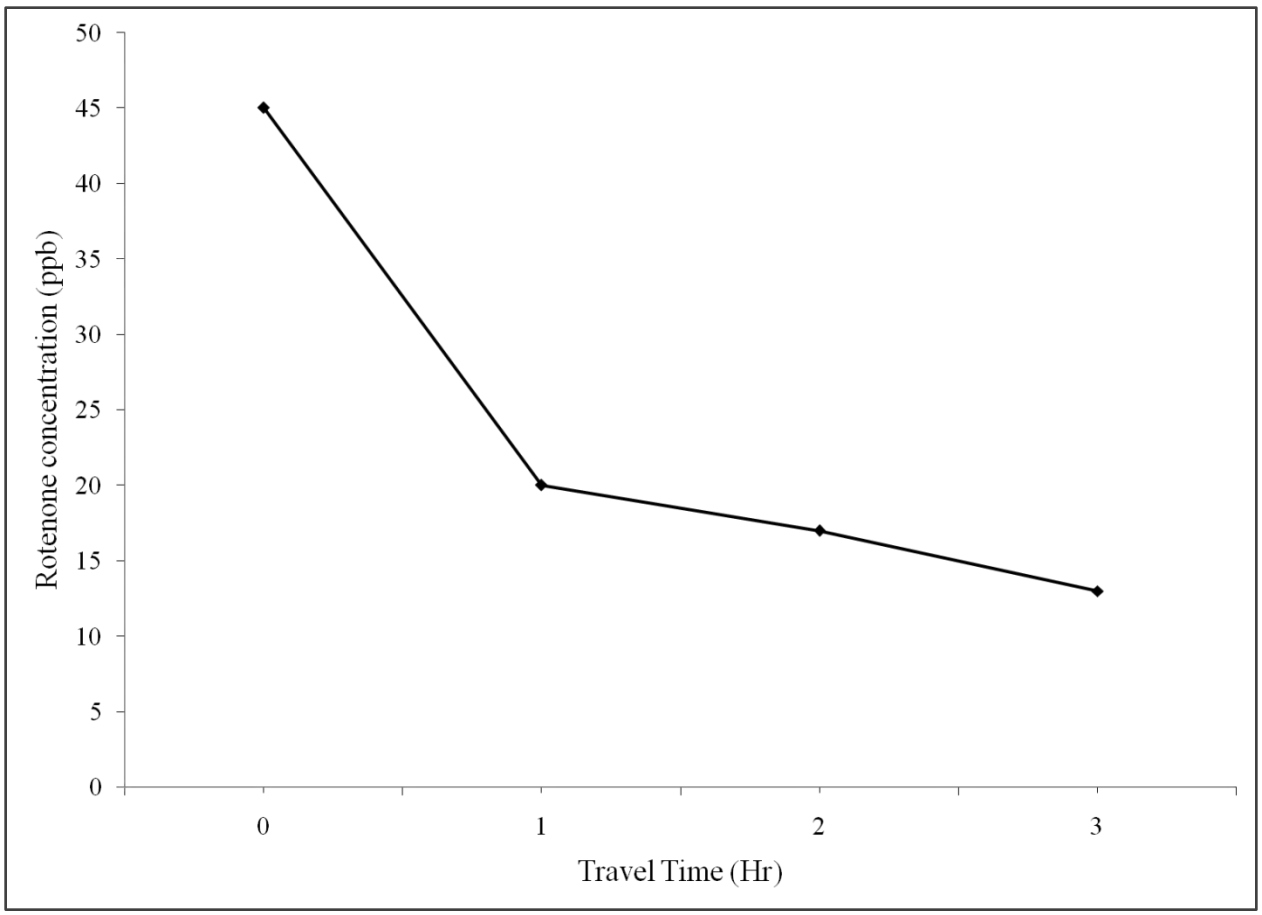

Figure 33. – Water samples collected during rotenone treatment on Specimen Creek demonstrated the rapid breakdown of rotenone in the natural environment. (Data collected by D. Skaar, Montana Fish, Wildlife and Parks.)

CHAPTER 4

COMPARISON OF CFT LEGUMINE ROTENONE AFFECTS ON BENTHIC MACROINVERTEBRATE COMMUNITIES IN FOUR STREAMS OF MONTANA AND NEW MEXICO

Introduction

Fishery managers use an assortment of techniques to manage undesirable or non-native fish populations. However, when complete removal of all fish is the goal (typically done for restoration of a native species) the use of piscicides is required (McClay 2000). Rotenone is one of the most valuable and successful piscicide currently available (McClay 2000 and 2005). For >70 years rotenone has become an important tool for fisheries managers in the restoration of native fish species. Although rotenone is a popular and highly effective method in fisheries management, its use has been contentious and challenged. One significant challenge are rotenone effects are poorly understood for non-target organisms, specifically benthic macroinvertebrates (BMI) (Vinson *et al.* 2010).

Rotenone formulations registered with the United States Environmental Protection Agency (USEPA) as piscicides, are available as powdered extracts or emulsifiable liquids. Differences among rotenone emulsifiable formulations are the inert ingredients, which act as solvents and synergists. Although labeled as inert ingredients most of these chemicals are toxic. Conventional rotenone formulations include petroleum hydrocarbons such as toluene, xylene, benzene, naphthalene, and piperonyl butoxide. Over the past few years, environmental groups have highlighted concerns involving public health, environmental impacts, animal welfare and applicator safety (McClay 2005 and Turner *et al.* 2007). The newly licensed rotenone (CFT

Legumine) was developed in response to these concerns. Unlike conventional rotenone formulations, CFT Legumine was designed to reduce or eliminate a number of hydrocarbon compounds, and does not include the synergist piperonyl butoxide (McClay 2005; Turner *et al.* 2007; Finlayson *et al.* 2010); therefore reducing risks for applicators, terrestrial species, public health, and the overall environmental impacts.

CFT Legumine has increased in use, but with limited evaluation on its effects to macroinvertebrates. In addition, large scale comparisons have been lacking and suggested by previous studies to gain a thorough understanding of impacts (Vinson *et al.* 2010). Organizations, including but not limited to Yellowstone National Park, Turner Enterprises Inc., Montana Fish Wildlife and Parks and New Mexico Game and Fish successfully applied CFT Legumine to manage fish populations in four drainages within Montana and New Mexico. The willingness of these organizations to share data sets provides the opportunity to have a comprehensive study of the effects CFT Legumine has on macroinvertebrate communities in different streams and geographic regions. The objective of this study was to compare the effects of CFT Legumine rotenone on benthic macroinvertebrate of the four projects. The results of this project will provide information to multiple organizations that will aid in the development of management strategies for aquatic systems.

Methods

Primary dataset. – Specimen Creek is located in the northwest corner of YNP in Gallatin and Park Counties of Montana (Figure 34). The entire Specimen Creek drainage is approximately 76 km^2, containing 62 km of flowing water. The East Fork of Specimen Creek originates at High Lake and flows approximately 27 km until the confluence of the North Fork. Five Collection sites were sampled to monitor treatment effects. The downstream sites MS1 and

EF1 (Detox) are below the treatment area, and only treated with potassium permanganate; EF2 and EF4 (Treatment) occur within the treatment area, are only exposed to rotenone; and NF1 is the control site. Further treatment and sample design detail is located in Table 8.

Secondary datasets. – Projects to restore populations of native cutthroat trout in Cherry Creek, Comanche Creek and Costilla Creek drainages were initiated between 1997 and 2008 with an array of rotenone treatment and sampling designs (Table 8). Cherry Creek is devoted to the restoration of westslope cutthroat trout, whereas the Comanche and Costilla Creek project focus is for indigenous Rio Grande cutthroat trout. Cherry Creek is located in the Absaroka Mountains, southwest of Bozeman Montana in Gallatin County (Figure 34). Selected treatment areas for Cherry Creek are on the Flying D Ranch owned by Turner Enterprises, and Gallatin National Forest. Five sites were sampled to monitor treatment effects of two treatment phases of the project. From downstream to upstream site localities are as follows: A and B (phase IV), C and D (phase III) and site E control site upstream of phase III treatment. The downstream sites A and B (Detox) are below the treatment area, and treated in combination of rotenone and potassium permanganate in 2009; however in 2010 phase IV sites, A and B (Treat (D)) are within the treatment area. Sites C and D (Treatment) were always within the treatment area, only exposed to rotenone.

Comanche and Costilla Creek are located in Taos County of North Central New Mexico in the Sange de Cristo Mountains, with project waters on boundaries of Turner Enterprises Vermejo Ranch and Carson National Forest (Figure 34). In Comanche Creek, Five sites were sampled to monitor treatment effects to macroinvertebrates. From downstream to upstream, site localities are as follows: Site 5 (Control) on Costilla Creek, just upstream of confluence with Comanche Creek, Site 4 (Detox) was 1.6 miles below the detoxification station and sites 1, 2 and

3 (Treatment) are within the treatment area. In Costilla Creek, four sites were sampled to monitor treatment effects to macroinvertebrates. In a downstream to upstream sequence, site localities are as follows: Site 4 (Detox), located 0.3 miles downstream of the detoxification station, sites 2 and 3 (Treatment) are within the treatment area, and site 1 was above the treatment area designated as a control site.

Rotenone applications. – Projects applied CFT Legumine ™ Fish Toxicant (U.S. EPA Product Reg No: 75338-2) (5% rotenone) and Prentox Fish Toxicant Powder (EPA Reg. No. 7533-2) (7.4% rotenone) to the selected study area at a target rate of one ppm CFT Legumine (50 ppb a.i.). This concentration was chosen based on the results of travel time estimates, flow calculations, and bioassays. Prentox powder mixed with gelatin and sand was used to treat springs and seeps and backpack sprayers were used for backwater areas. Potassium permanganate oxidized and neutralized CFT Legumine rotenone below the treatment area.

Aquatic macroinvertebrate collections. – To evaluate the effects of CFT Legumine on BMI in the four projects, a minimum of three sampling events were performed at each site around the rotenone treatment sequence: pretreatment, immediate posttreatment and one-year posttreatment. From 2008-2010, BMI were collected during five sampling events at five macroinvertebrate sites in the Specimen Creek drainage. Sampling methodology follows the Wyoming Department of Environmental Quality and Water Quality Division (WDEQ/WQD) (2004) stream benthic macroinvertebrate collection protocols. Eight random macroinvertebrate samples were collected within the designated riffles. A 0.093 m^2 Surber sampler equipped with 500µm mesh was used to collect macroinvertebrates. Within the Surber sampling area, substrate was disturbed to dislodge (approximately 10cm down) invertebrates until suspected organisms are washed free. Each macroinvertebrate replicate was kept separate and preserved in 85%

ethanol for future processing. Samples were processed in total; however, an area sub-sampling method (Elliott 1971) was used if a sample was determined to be too large to finish and/or contained more than 1,000 individuals. In Cherry Creek, BMI were collected during four sampling events at five sites from 2009-2010. Three replicate traveling kick samples were collected at each site using a D-frame kick net equipped with 900 µm mesh. Riffle habitats were sampled by disturbing the substrate and dislodging invertebrates starting from one bank and working towards the other. Equal time and area of replicates were used to standardize sampling efforts at each site. Replicates were kept separate and preserved in 85% ethanol until processed. In the laboratory, Rapid Bioassessment Protocol III sorting methodology (Plafkin et al. 1989) was employed to obtain approximately 300 organism subsample from each kick-net collection. The Comanche Creek project performed five sampling events from 2007-2009 at the five BMI sites. Five quantitative samples in riffle habitat were collected at each site using a modified 0.059 m^2 Hess type circular sampler (Jacobi 1978) with 500 µm mesh at each of the four Comanche Creek and one Costilla Creek locations. Samples were stored separately and preserved in 90% ethyl alcohol for future processing. Samples were processed in total. In Costilla Creek, benthic samples were taken during three sampling events at four sites. Five samples in riffle habitat were collected at each site using a modified 0.059 m^2 Hess circular sampler (Jacobi 1978) equipped with 500 µm mesh. Samples were stored separately and preserved in 90% ethyl alcohol for future processing. Samples were processed in total.

In general, insects were identified to genus, whereas non-insects were identified to order or phylum. Even though these levels of identification varied according to each taxonomic group, they were consistent across projects. Consistencies in taxonomic resolution ensured differences in response variables noted between projects were not attributed to variation in taxonomic level.

Data analysis. – To evaluate the impacts of CFT Legumine on BMI, a combination of univariate and multivariate statistical techniques were used. Changes in BMI community structure were graphically presented using Nonmetric multidimensional scaling (NMDS) and verified with agglomerative cluster analysis. Dissimilarity was measured using Morisita-Horn index for Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundance. This index was chosen because it is unaffected by differences in species richness and sample size (Krebs 1989). Pretreatment vs. posttreament differences in BMI community composition was tested using a one-way analysis of variance (ANOVA). Tukey's multiple range tests was used within each dataset to separate differences by site (Treatment, Detox and Control) over sampling events and site vs. the control site at the same sampling event. Insect abundance, insect richness, EPT abundance and EPT richness were the response variables used in the ANOVA. All statistics were performed and figures generated using R version 2.6.2 (R Development Core Team, http://www.R-project.org) and Statistical Analysis System (SAS) version 9.2 (SAS Institute 2008).

Results

Insect abundance, insect richness, EPT abundance and EPT richness were significantly different (One way ANOVA, $p < 0.05$) for all four datasets (Figures 35-50). In general, Tukey's multiple range tests determined (1) Specimen and Cherry Creek had greater significant differences at detox sites compared to treatment sites, (2) Comanche Creek had greater significant differences at treatment sites compared to detox sites and (3) Costilla Creek had significant differences at both treatment and detox sites, but results were not congruent for each response variable. Greater differences were observed in EPT abundance and richness response variables. Regardless of significant differences in response variables and site (treatment vs.

detox) differences within each dataset, recovery was indicated in one-year post samples for all four datasets (Figures 35-50).

Insect abundance. – Minimal changes in insect abundance were seen at Specimen and Cherry Creek treatment and detox sites through time, indicating minimal impacts (Figure 35 and 36). However, Specimen Creek immediate post detox sample was statistically significantly different from the control site, indicating a slight impact (Figure 35). In addition, Cherry Creek one-year post detox sample was statistically significantly different from its pretreatment level, but was likely due to natural variability (Figure 36). Comanche Creek immediate post treatment samples were reduced, but only the 2007 sample was statistically significantly different from its pretreatment level. Minimal changes were observed at the detox site (Figure 37). Costilla Creek immediate post sample levels decreased, but were not statistically significant for either treatment or detox sites (Figure 38).

Insect richness. – The Specimen Creek immediate post detox sample was significantly different from its pre and control site levels. Changes were observed at the treatment site, but were not statistically significant. Although the one-year post treatment sample was significantly different from the corresponding control site, it could be attributed to the increase in the control site level (Figure 39). Cherry Creek demonstrated treatment and detox sites were not statistically significantly different. However, one-year post detox sample was significantly different from its pretreatment level, but was possibly due to natural variability (Figure 40). Comanche Creek immediate post treatment samples were reduced significantly from there pretreatment and control site levels. Immediate post detox samples were slightly reduced, but were not significant (Figure 41). Costilla Creek immediate post samples were significantly different from pretreatment levels for both treatment and detox sites (Figure 42).

EPT abundance. – Specimen Creek immediate post detox sample was significantly different from its pre and control site levels. The immediate post treatment sample was only significantly different from the control site and was not greatly reduced (Figure 43). Cherry Creek immediate post detox samples were significantly different from pretreatment levels, but the 2009 sample was reduced more than 2010. The 2010 immediate post (Treat(D)) detox site was treated with rotenone and not potassium permanganate. Thus, differences are due to rotenone treatment. The 2010 immediate post treatment sample was significantly different from its pretreatment level. The control site also showed the same pattern, but was not significant (Figure 44). It was likely that a portion of the decrease in the 2010 treatment samples was due to natural variability. Comanche Creek immediate post treatment samples were reduced significantly from their pretreatment levels. A decrease in the immediate post detox sample was not observed (Figure 45). Costilla Creek immediate post treatment sample was significantly different from pretreatment levels. A decrease in the immediate post detox sample was not observed (Figure 46).

EPT richness. – Specimen Creek immediate post detox sample was significantly different from its pre and control site levels. Although the pretreatment sample was significantly different from the control site, minimal changes were observed through time (Figure 47). Cherry Creek immediate post detox sample was significantly different from the pretreatment level. The 2010 immediate post treatment sample was significantly different from the corresponding control level, but was only slightly reduced from the pre sample. The 2009 immediate post treatment samples were slightly reduced (Figure 48). Comanche Creek immediate post treatment samples were reduced significantly from their pre and control site levels. Immediate post detox samples were reduced, but not significant; they follow the same pattern as the control site through time

(Figure 49). Costilla Creek immediate post treatment and detox samples were significantly different from pre and control site levels (Figure 50).

NMDS analysis. – The greatest dissimilarity in BMI community composition was within the immediate post sampling event for EPT abundances. One-year post treatment communities are the most similar to their pretreatment sites (Figures 51-54). Specimen and Cherry Creek sites below the $KMnO_4$ detox station were the most dissimilar immediately after treatment (Figures 51 and 52). Specimen Creek immediate post treatment samples were also distanced from pre and one-year post samples, but were most similar to their pretreatment communities (Figure 51). Cherry Creek 2009 immediate post treatment samples were also dissimilar, but the 2010 immediate post samples were grouped with pre and one-year post samples. This indicates the 2009 treatment had a greater impact compared to 2010 (Figure 52). Comanche Creek immediate post treatment samples were the most dissimilar, with the 2007 treatment being the greatest. Sites influenced by detox were linked to the immediate post treatment samples, but were not grouped with them. This indicates detox sites were slightly impacted, but not as great as treatment sites (Figure 53). Costilla Creek immediate post treatment and detox samples were dissimilar from pre and one-year post samples. However, the detox site was still linked to the pre and one-year post samples, indicating a slightly greater impact to the treatment sites (Figure 54).

Discussion

Influences of rotenone on BMI were similar within geographic region whereas effects from potassium permanganate differed among the datasets. Specimen and Cherry Creek demonstrated minimal impacts from rotenone and significant influences from potassium permanganate. Comanche and Costilla Creek demonstrated influences from rotenone, but only

Costilla Creek had statistically significant differences from potassium permanganate. Analysis of the datasets using NMDS exhibited a similar pattern, which strengthens our interpretation. Traditionally the impacts of piscicides on BMI have been assessed using only univariate statistical techniques. However, in this chapter both a univariate and multivariate approach was used to assess the ecological impact of CFT Legumine application. The results of this study suggest this approach was more appropriate because consistent differences using multiple statistical techniques evaluating effects by means (abundance and richness) and the community structure provide a powerful interpretation (Green 1979).

Vinson *et al.* (2010) indicated that differences in BMI effects were due to treatment design, BMI study objectives and sampling intensity and natural variation in toxicity among species. In the four studies, treatment design was similar except for duration of application. Each study applied 50 ppb (a.i.) of CFT Legumine, broke the treatment area into multiple phases and only applied to fish inhabited areas. Specimen Creek treatment duration was eight hours, whereas the others were four. Differences in BMI responses to treatment across the studies were attributed to, variation of toxicity to macroinvertebrate taxa, BMI community structures and BMI sampling design. Specimen and Cherry Creek had less impact to their BMI communities from rotenone treatment compared to Comanche and Costilla Creek. The streams in similar geographic regions (Montana vs. New Mexico) exhibited similar patterns of impact due to rotenone application. It was likely those different taxa that were found in benthic communities in different regions have a different sensitivity to rotenone caused these differences. Engstrom-Heg *et al.* (1978), points out that different taxa, even within a genera, can have large differences in sensitivity. Differences within the community structure could also influence the effects as demonstrated in Chapter 3. For instance, if the community were comprised of only early life

stages during treatment, you would expect for impacts to be greater. Regardless, communities recovered to pretreatment levels one-year later. Another factor that needs to be considered is the proximity of the BMI sample sites to the drip stations. Rotenone exposure changes because of the time and distance from the treatment source, thus the proximity of a sample site will determine the extent of exposure to the BMI community. At BMI sites, water samples should be collected to monitor rotenone levels to aid in understanding effects. Based on study results, distance from the detox station was the factor for differences in detox effects observed among datasets. Three of the datasets demonstrate effects from potassium permanganate, but Comanche Creek influences were negligible. The impacted detox sites in Specimen, Cherry and Costilla were approximately 1.0, 0.5 and 0.3 miles (respectively) below the detox station and all were observed to be exposed to potassium permanganate. Comanche Creek BMI detox site was 1.6 miles below the detox station and was not observed to be exposed to potassium permanganate. In addition, mortality of sentinel fish did not occur from detox in proximity of the BMI sites. Therefore, potassium permanganate has a greater impact on BMI compared to cutthroat trout. Even though there were significant impacts from potassium permanganate application, the affected area seems to be within a short distance downstream of the detox station. Currently there is limited information of how potassium permanganate responds in the environment or its effects on BMI. This study demonstrates that within a certain distance of the detox station invertebrates were significantly influenced. This information is vital to understand its impacts and further minimize impacts to BMI communities in the future use of rotenone.

Previous literature (Binns 1967; Cook and Moore 1969; Mangum and Madrigal 1999; Trumbo *et al.* 2000; Whelan 2002; Vinson and Dinger 2006; Hamilton et al. 2009; Finlayson 2010) has reported a range of impacts to macroinvertebrate populations. Most report minor

reductions of populations, but some report substantial impacts. Recovery for common taxa was rapid; however, it may take and several years for rare taxa to recover (Vinson *et al.* 2010). This study compared to others using conventional rotenone formulations demonstrate less impacts to the macroinvertebrate communities. For example Hamilton *et al.* (2009), reported dramatic reductions in BMI population response variables. As observed by Finlayson *et al.* (2010), the use of CFT Legumine, which does not contain the synergist piperonyl butoxide, reduced the impacts to aquatic invertebrate communities in comparison to other studies implementing conventional formulation. It is probable that the formulation and treatment design reduced impacts to invertebrates.

This study is an important step in fisheries management piscicide techniques. The use of rotenone is an ever-growing contentious issue and information demonstrating progression in practices is vital. This study is the first to evaluate CFT Legumine effects on BMI in field applications in multiple drainages. The interpretation of multiple datasets provides a powerful and robust comprehensive understanding of CFT Legumine effects to BMI communities. Natural variation in the environment spatially and temporally can influence results. Green (1979) discusses that a control site is important for spatial and temporal control. He states the best way to demonstrate effects is by comparison with a control. The power of utilizing multiple datasets is exhibited when trying to partition variability in Comanche Creek. Comanche Creek had the most thorough sampling of a control site; utilizing the control site enhanced our interpretation. If this was not present you would assume that the same variability seen in the detox site was not natural and indeed from the detox station. The information in this study provides a robust insight into the influence of CFT Legumine to invertebrate communities and provides information to help minimize impacts to BMI while still achieving the projects goal.

Management implications

Based on the results presented in this chapter, the following are recommendations to minimize impacts and maximize recolonization of BMI during native trout restoration: (1) apply the minimum dosage to eliminate fish; (2) operate rotenone drip stations eight hours or less per treatment; (3) apply unsynergized formulations (CFT Legumine); (4) partition the drainage into multiple treatments with intermediate barriers and allow time between treatments for dispersal and recolonization of invertebrates; (5) do not treat headwater areas that are fishless, which leaves a source for recolonization of downstream treated reaches; (6) place caged sentinel fish throughout the treatment area to monitor treatment effectiveness; (7) and collect water samples to monitor potassium permanganate and rotenone concentrations throughout the treatment area, specifically at BMI sample sites.

Table 8. – Datasets that implemented the application of CFT Legumine for the restoration of cutthroat trout throughout different geographic regions of the western United States.

	Cherry Creek	Costilla Creek	Comanche Creek	Specimen Creek
Treatment Year(s)	2007- 2010	2008	2007 and 2008	2008 and 2009
Number of Treatments	5 (1/yr); 2009 (2/yr) Phase III: 5 Phase IV: 1	2	2 (1/yr)	4 (2/yr)
Target Concentration (a.i.)	50 ppb	50 ppb	50 ppb	50 ppb
Sampling Events	5	3	5	5
Locality	Montana	New Mexico	New Mexico	Montana
Number of Sites	5	4	5	5
Number of Replicates	3	5	5	8

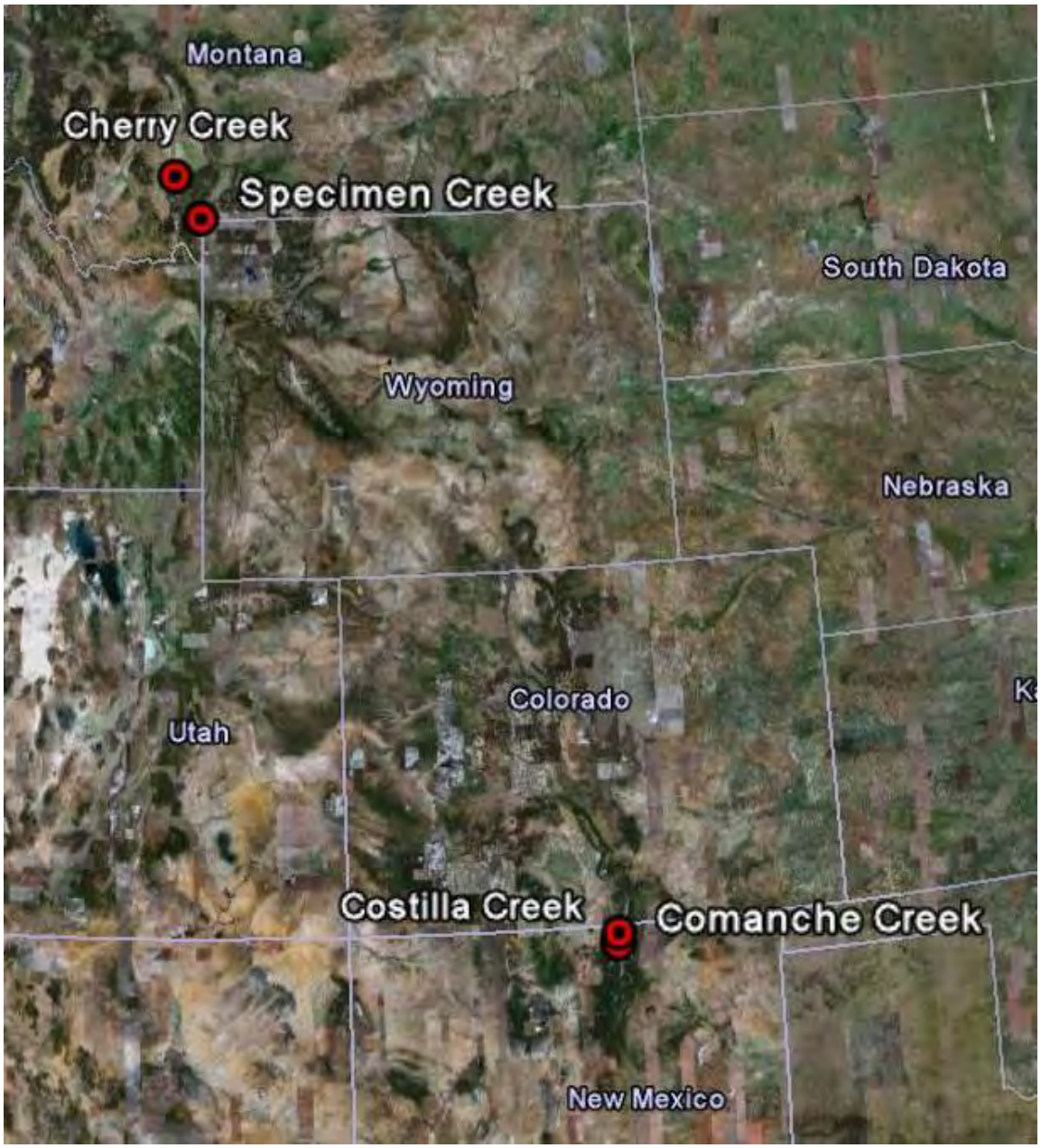

Figure 34. – Localities of four datasets examined in Montana and New Mexico.

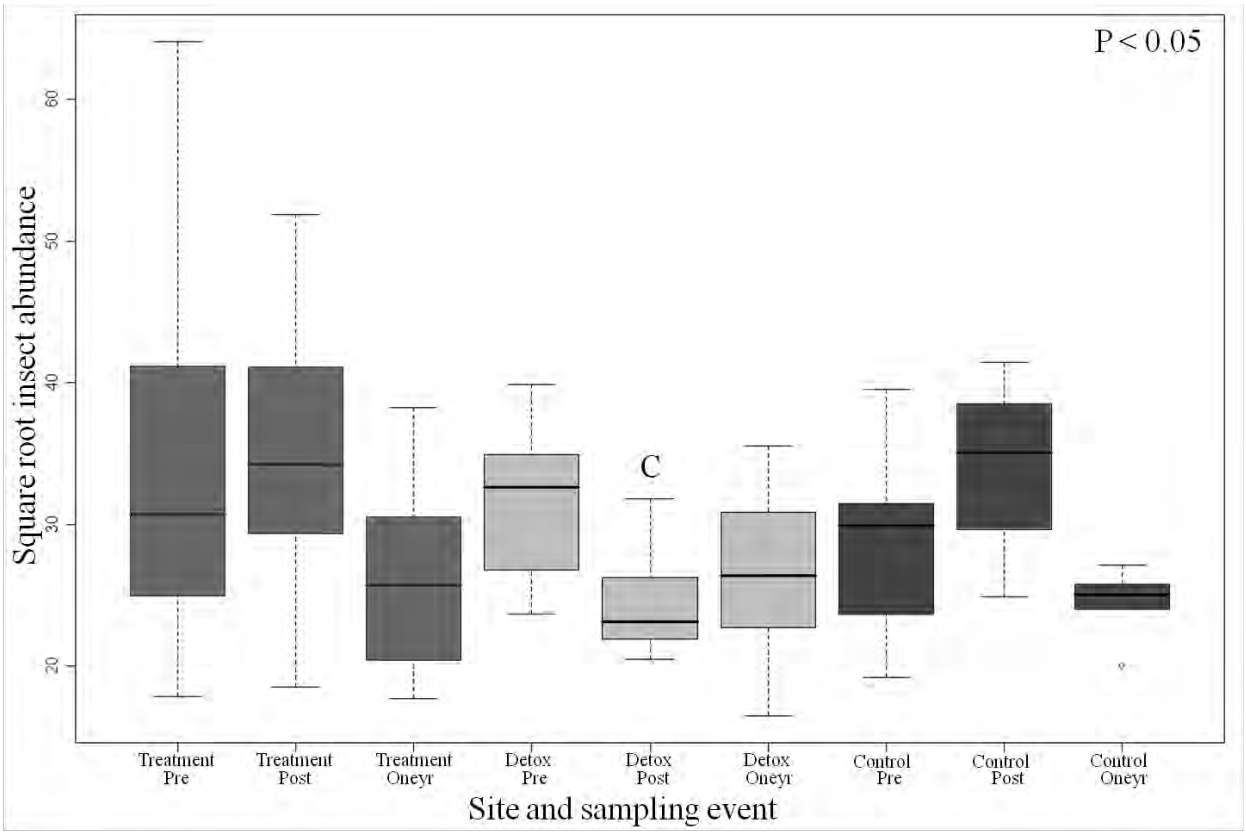

Figure 35. – Boxplot of square root transformed insect abundances for pre, immediate post and one-year posttreatment samples in the Specimen Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

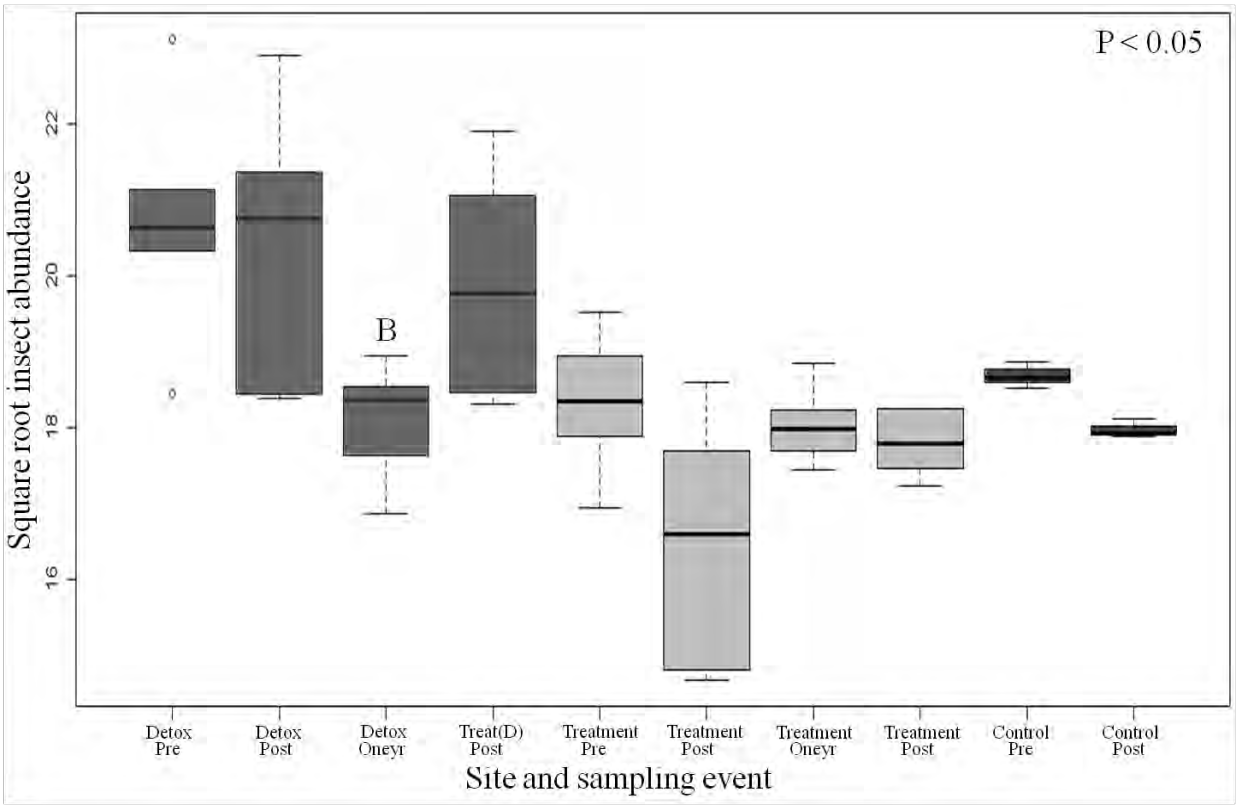

Figure 36. – Boxplot of square root transformed insect abundances for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Cherry Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

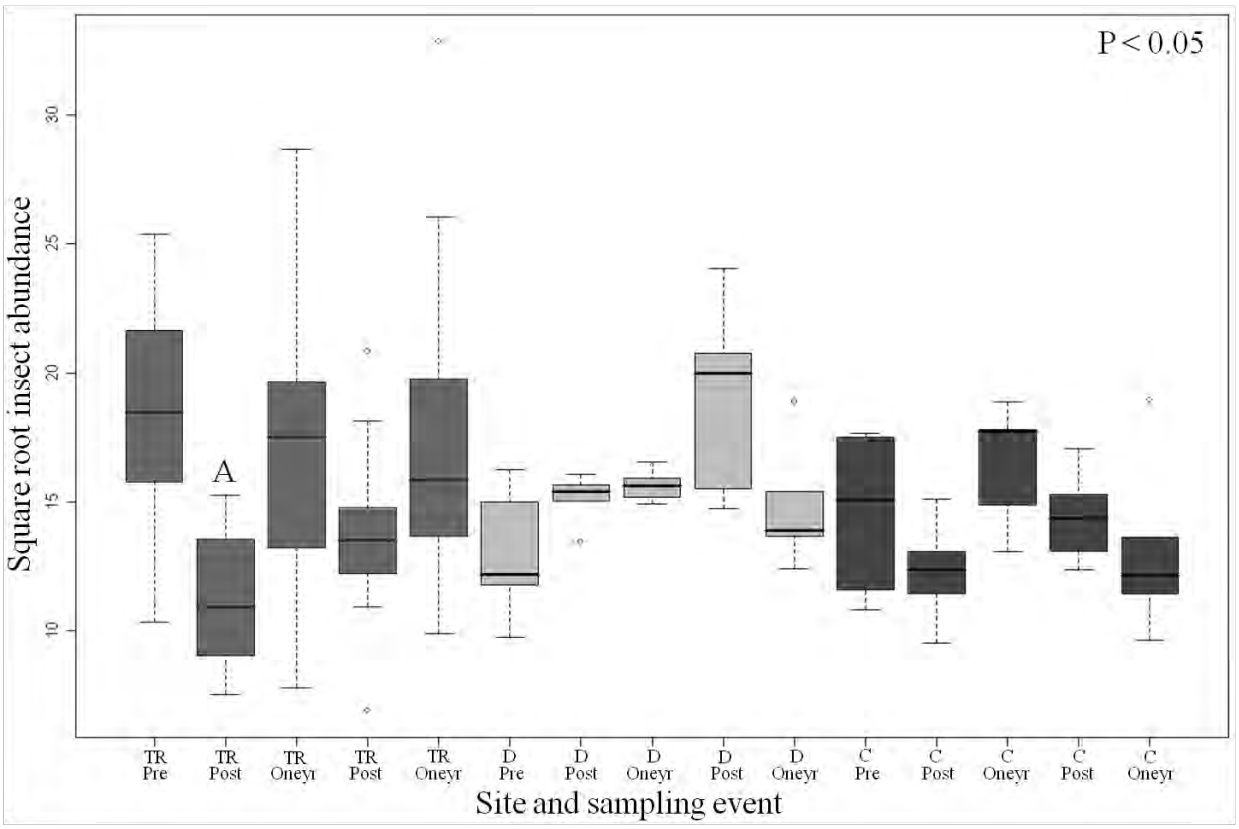

Figure 37. – Boxplot of square root transformed insect abundances for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Comanche Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

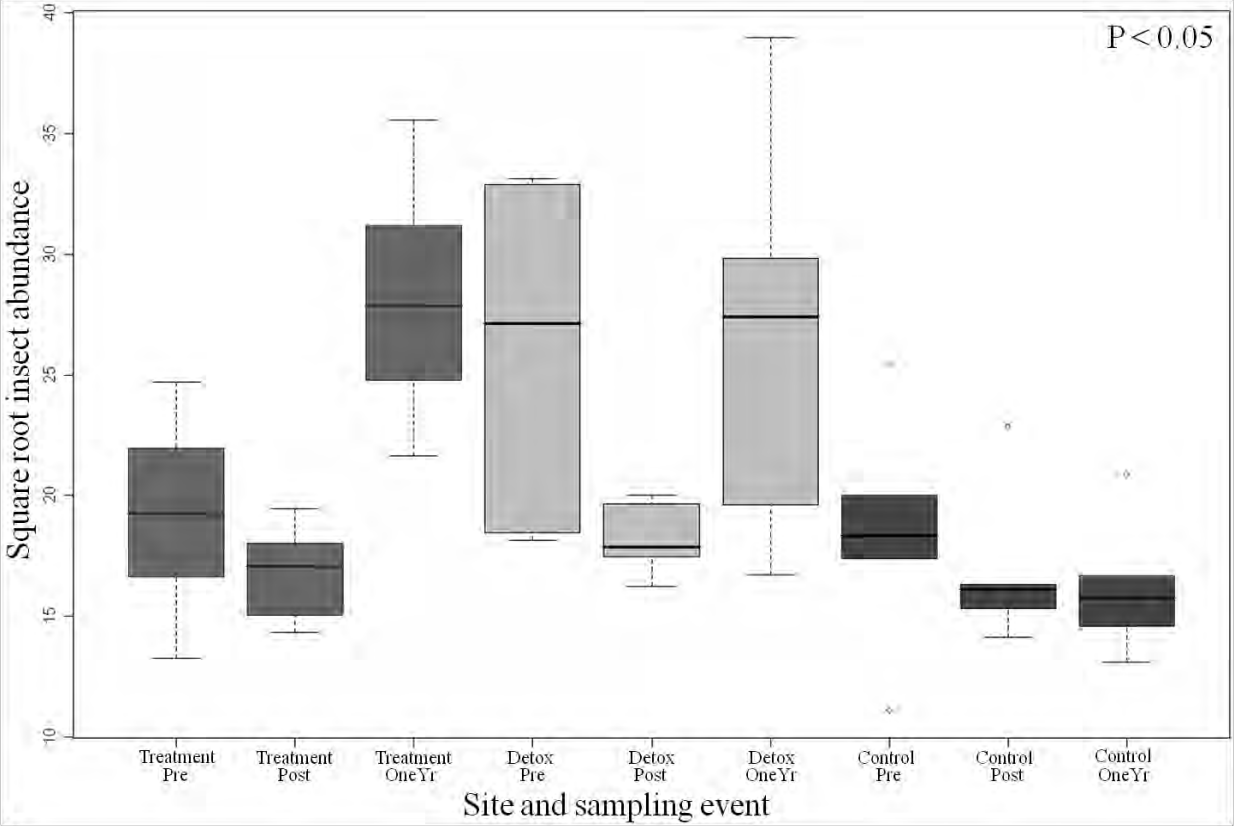

Figure 38. – Boxplot of square root transformed insect abundances for pre, immediate post and one-year posttreatment samples in the Costilla Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

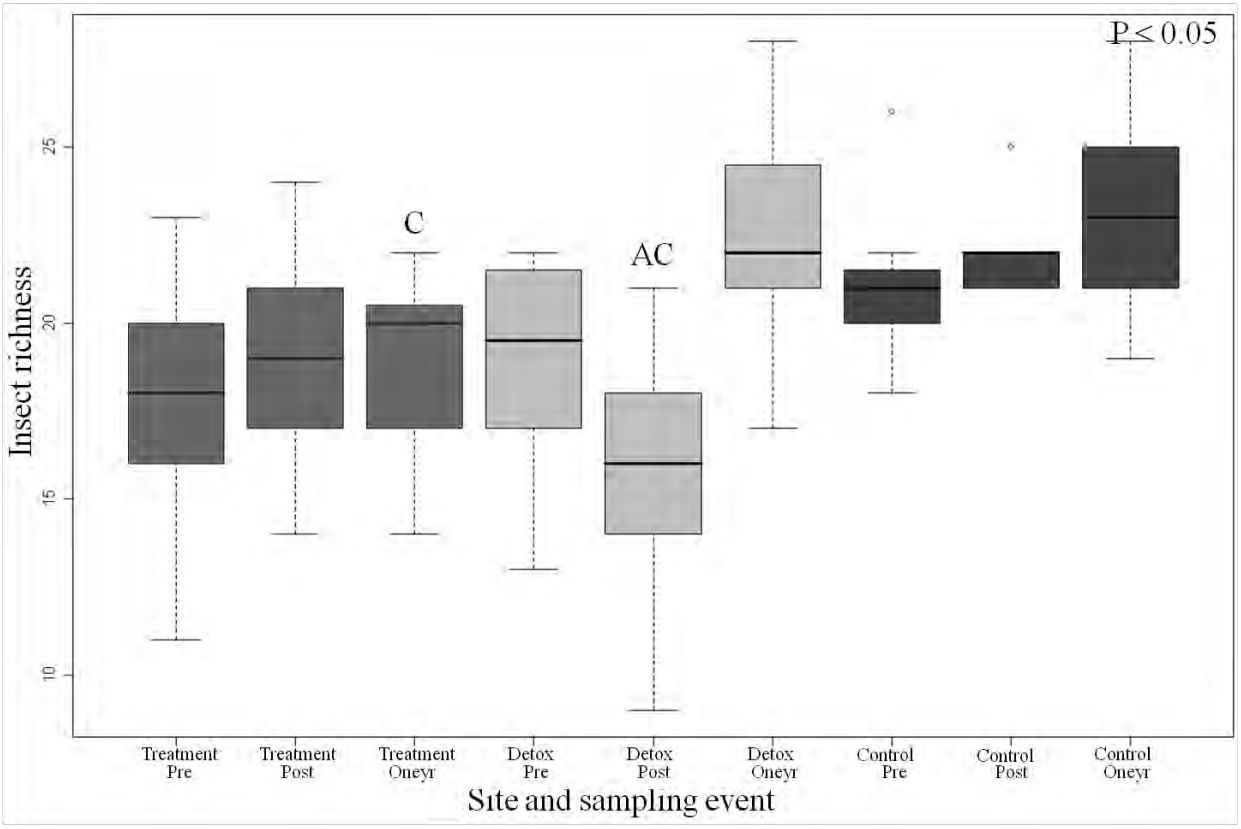

Figure 39. – Boxplot of insect richness for pre, immediate post and one-year posttreatment samples in the Specimen Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

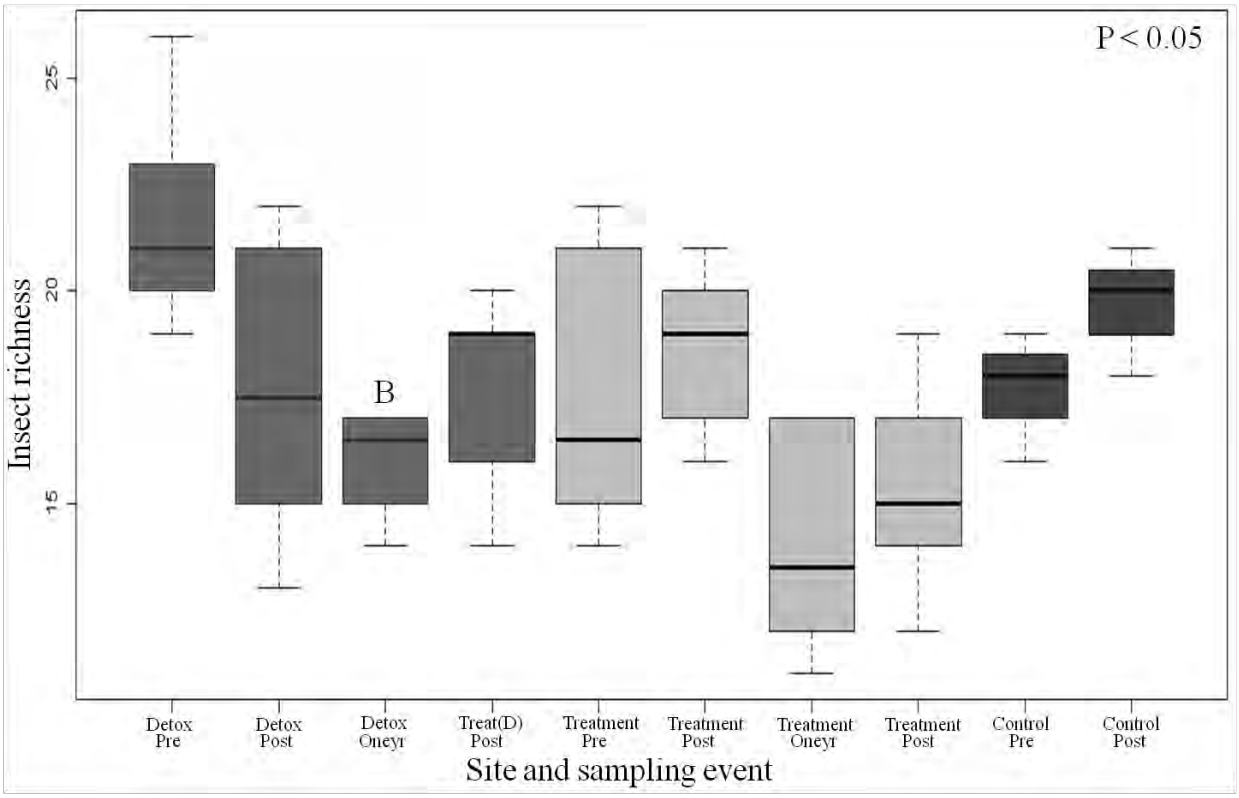

Figure 40. – Boxplot of insect richness for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Cherry Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

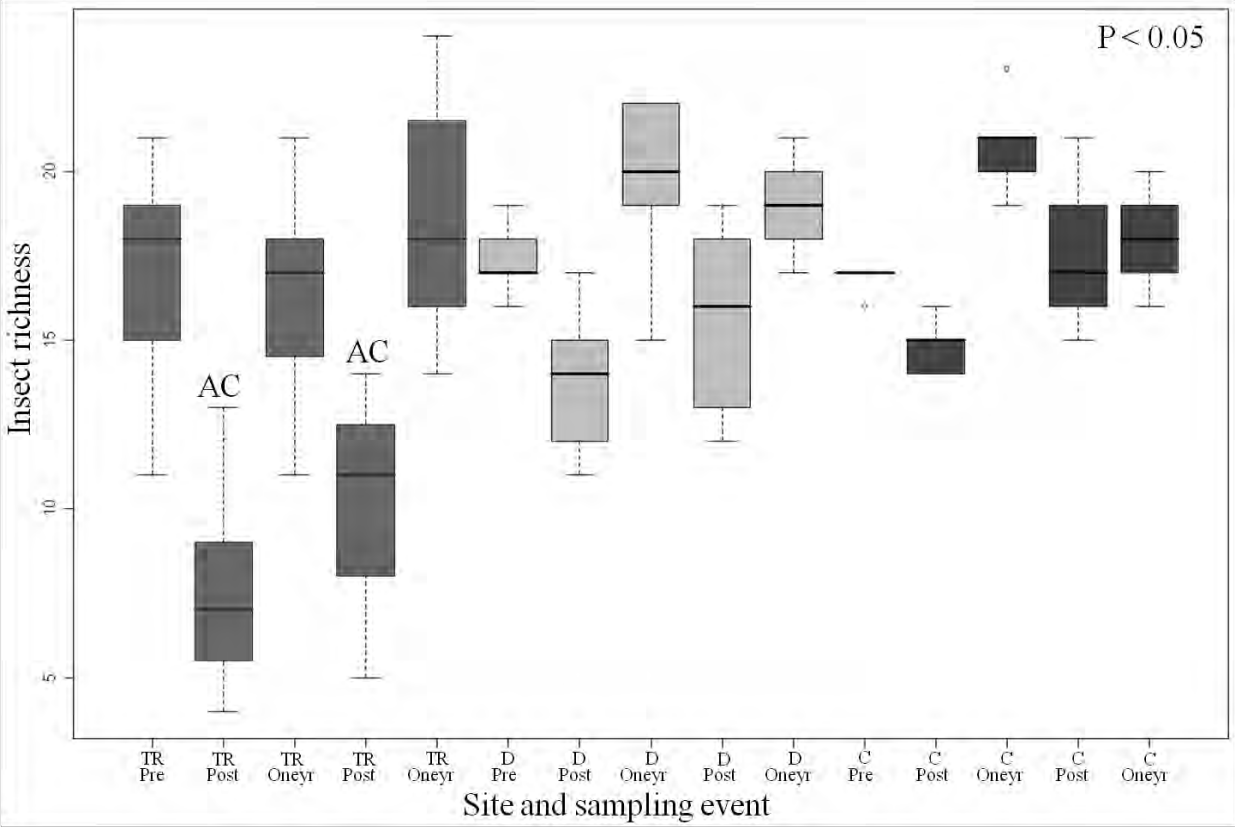

Figure 41. – Boxplot of insect richness for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Comanche Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

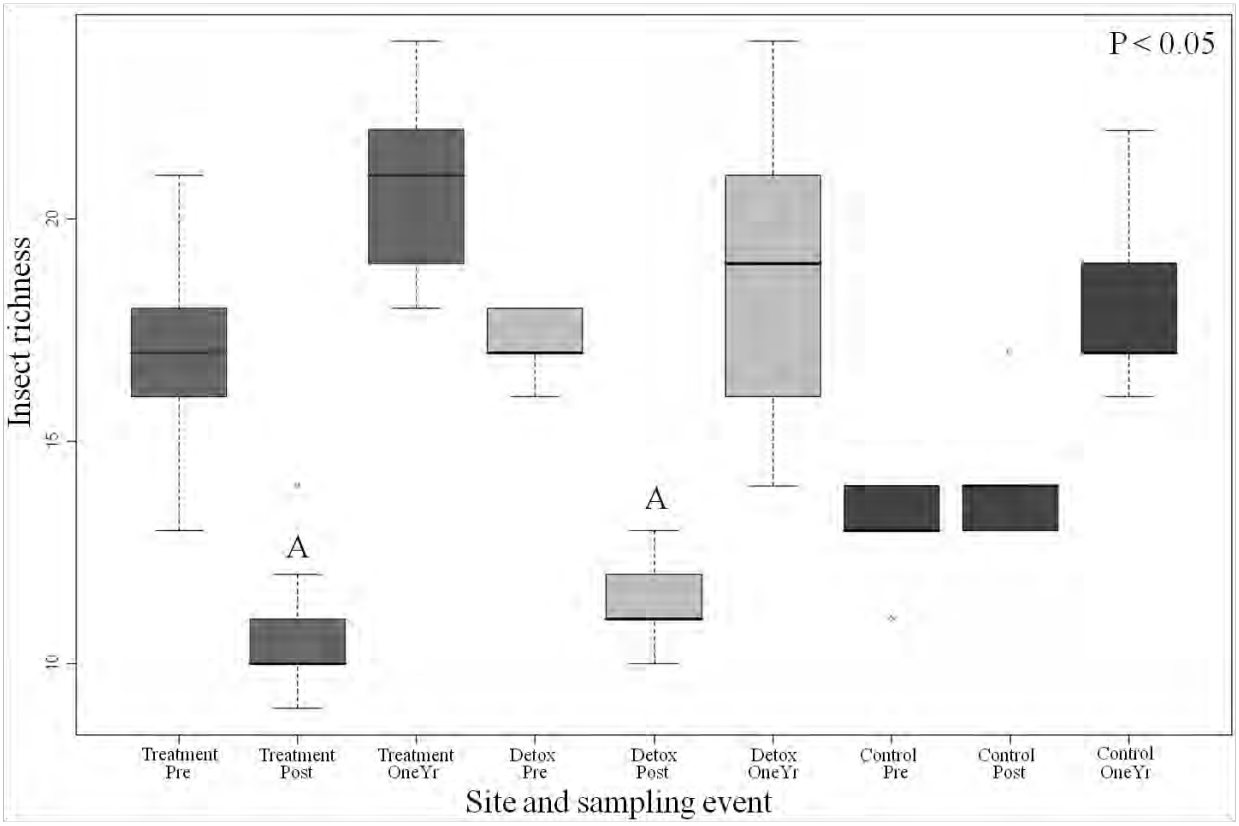

Figure 42. – Boxplot of insect richness for pre, immediate post and one-year posttreatment samples in the Costilla Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

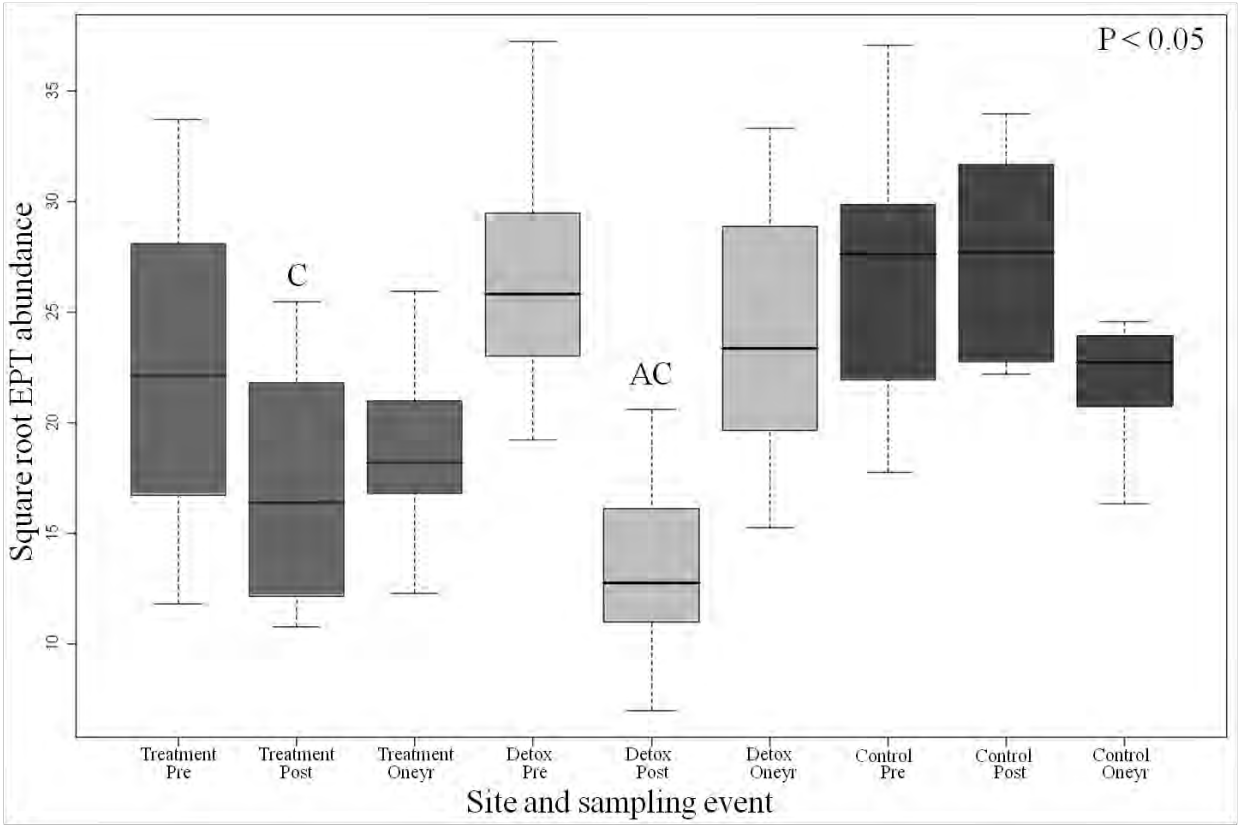

Figure 43. – Boxplot of square root transformed EPT abundances for pre, immediate post and one-year posttreatment samples in the Specimen Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

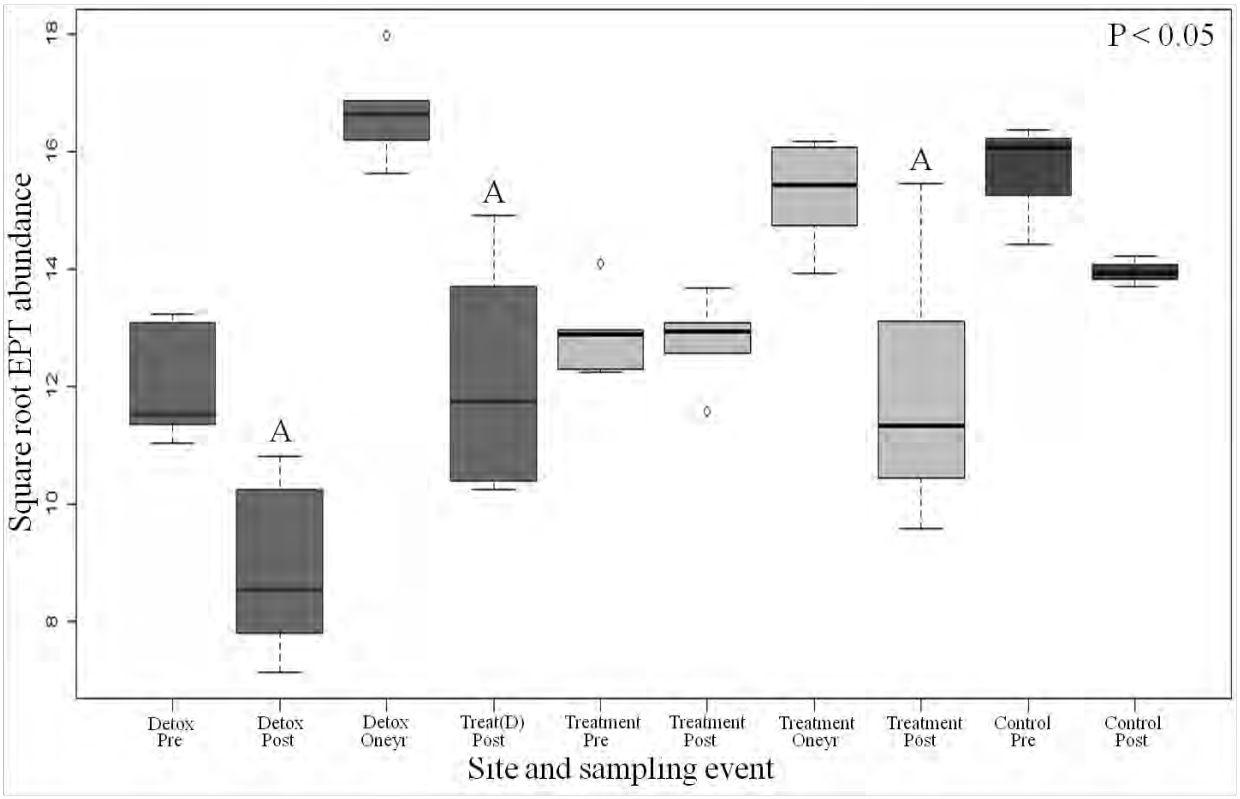

Figure 44. – Boxplot of square root transformed EPT abundances for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Cherry Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

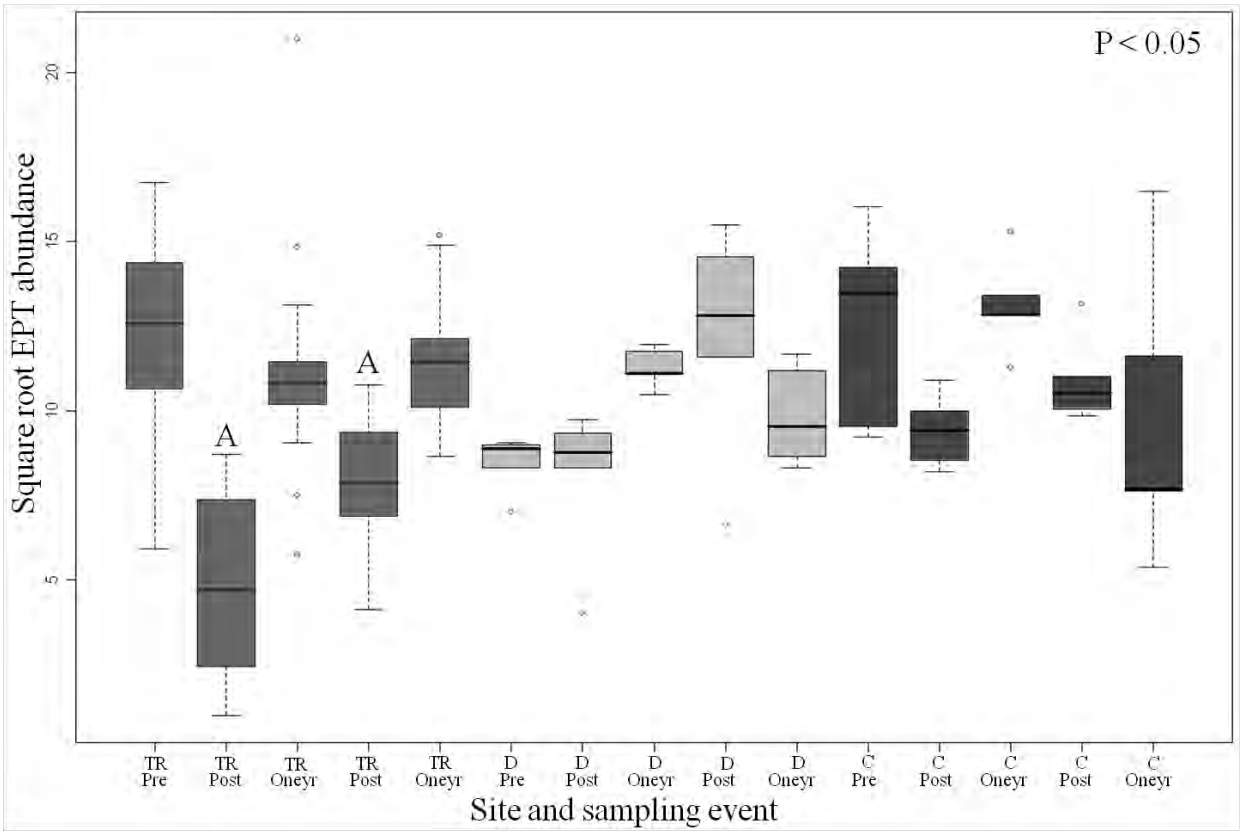

Figure 45. – Boxplot of square root transformed EPT abundances for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Comanche Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

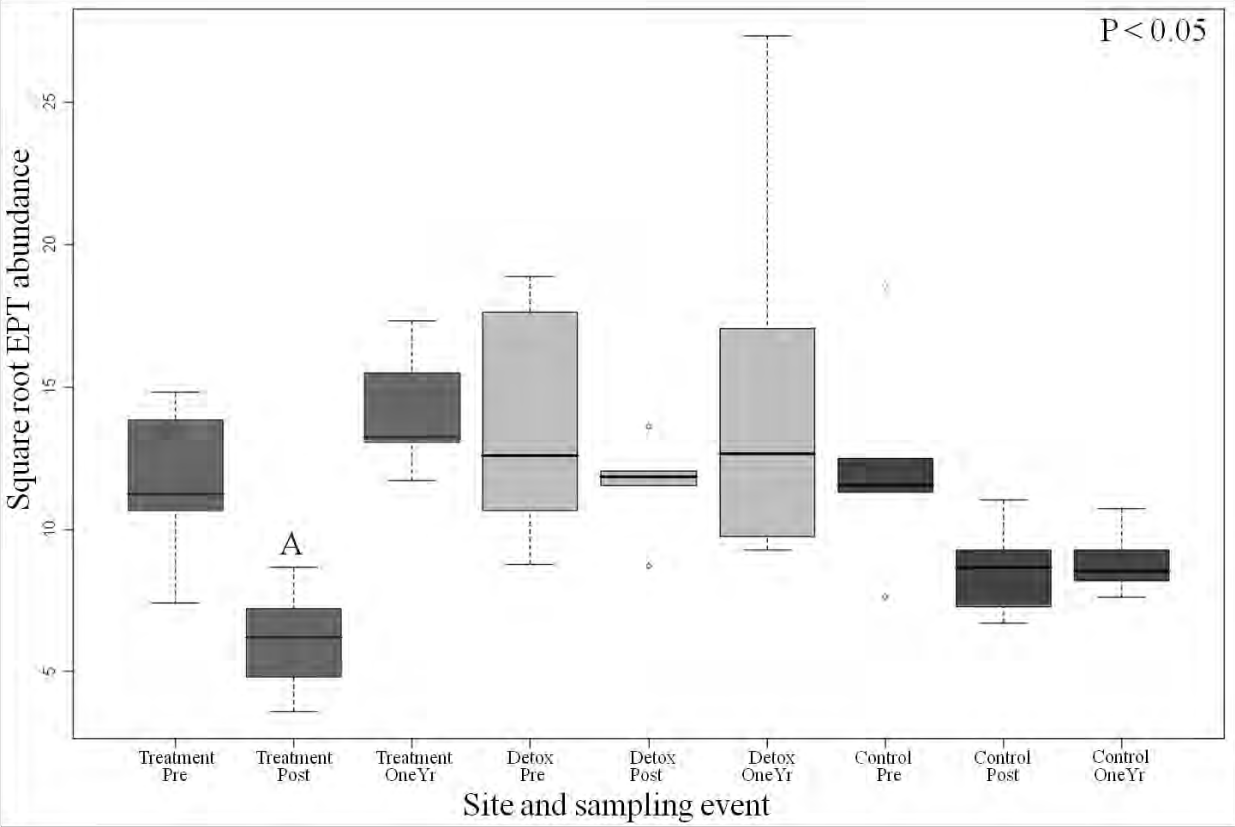

Figure 46. – Boxplot of square root transformed EPT abundances for pre, immediate post and one-year posttreatment samples in the Costilla Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

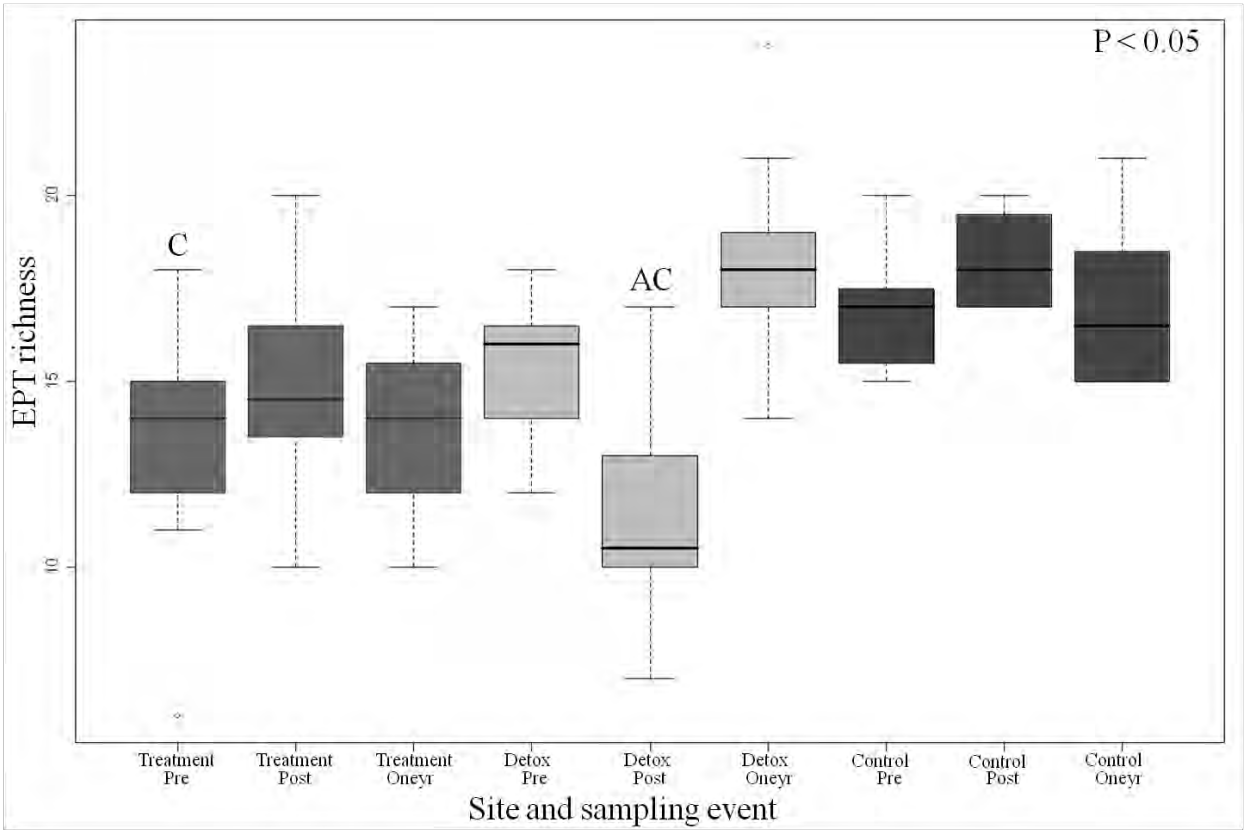

Figure 47. – Boxplot of EPT richness for pre, immediate post and one-year posttreatment samples in the Specimen Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

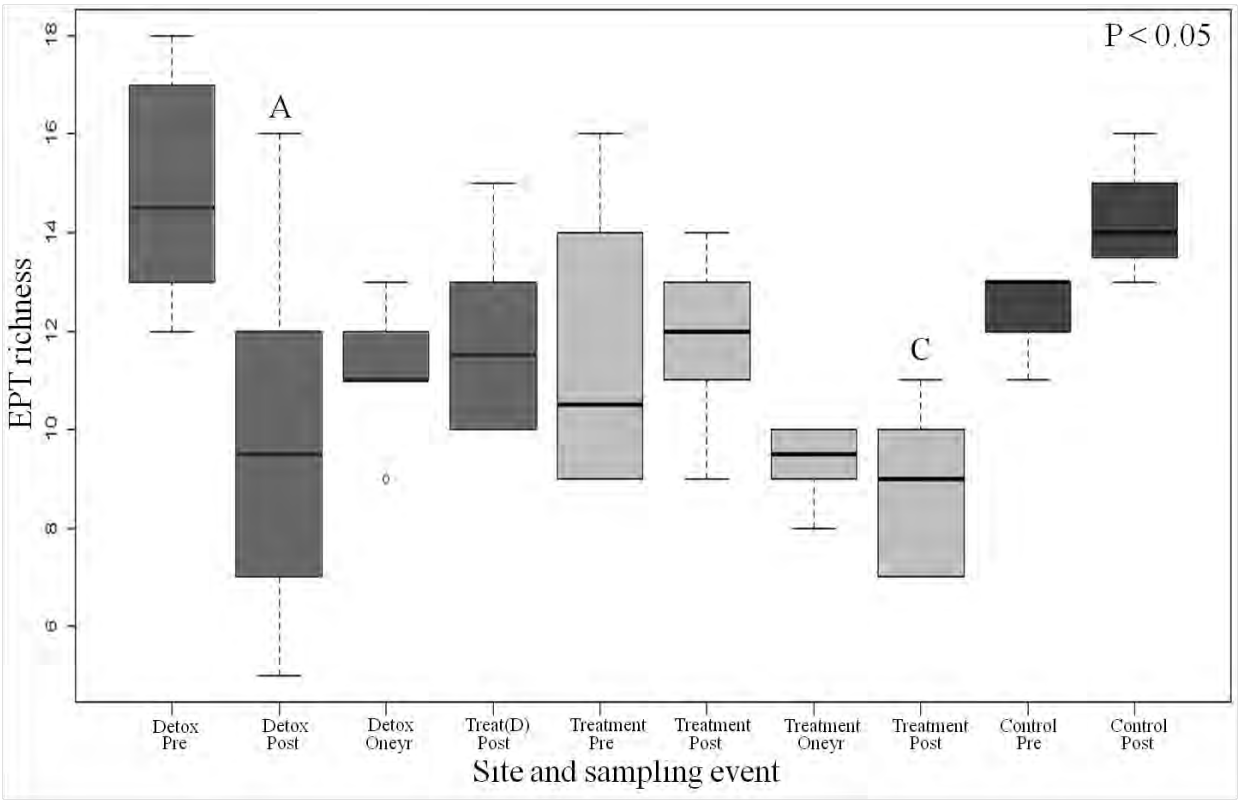

Figure 48. – Boxplot of EPT richness for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Cherry Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

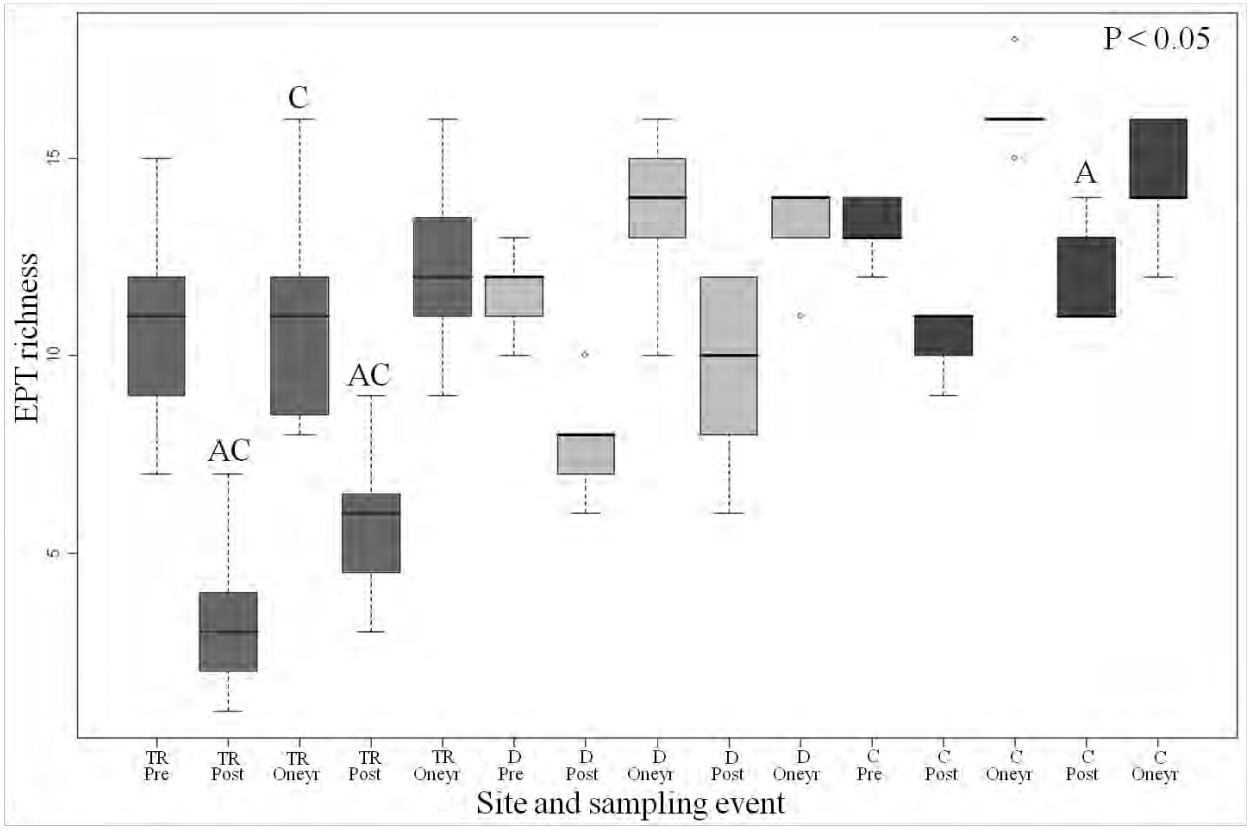

Figure 49. – Boxplot of EPT richness for pre, immediate post, one-year post (also second treatment year pre) and post (second treatment year) treatment samples in the Comanche Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

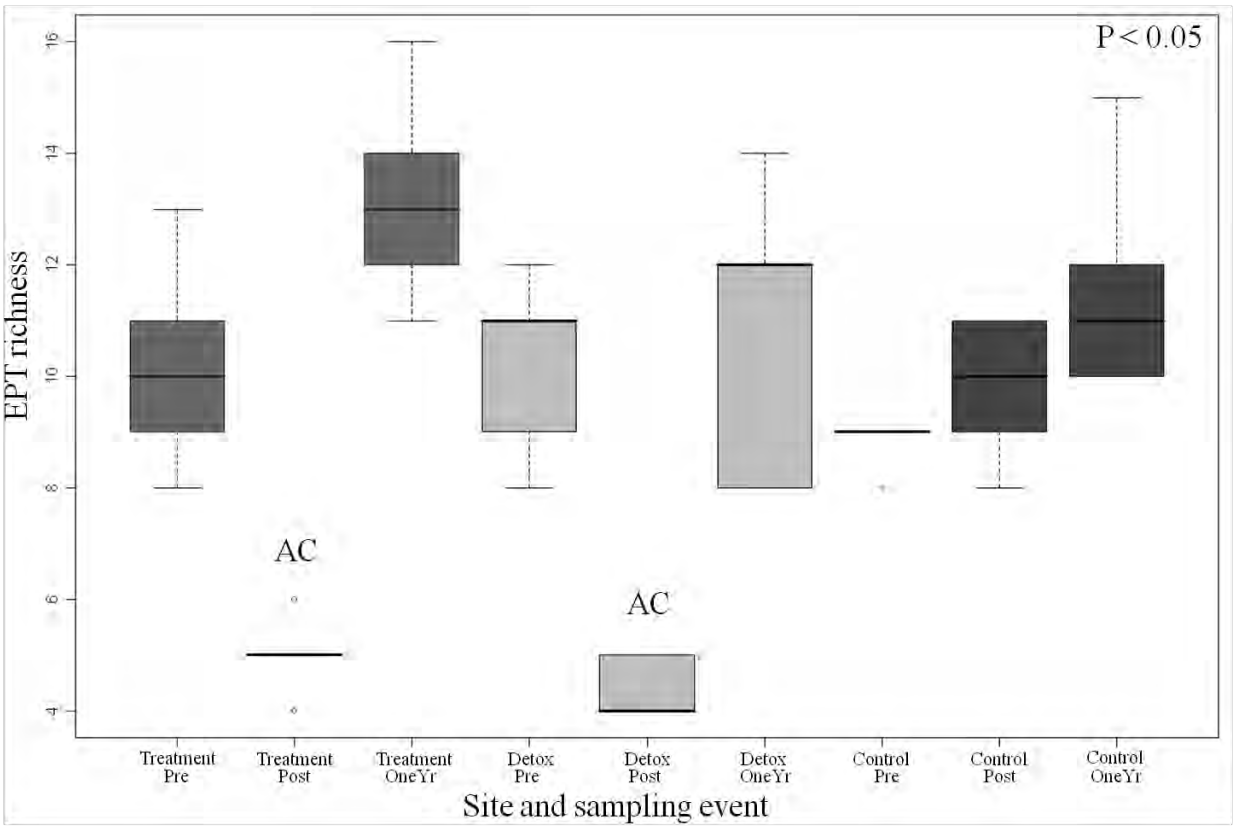

Figure 50. – Boxplot of EPT richness for pre, immediate post and one-year posttreatment samples in the Costilla Creek drainage. The letter "A" indicates pre and post differences by site; "B" indicates pre and one-year post differences by site; "C" indicates a site different from the control site during the same sampling event.

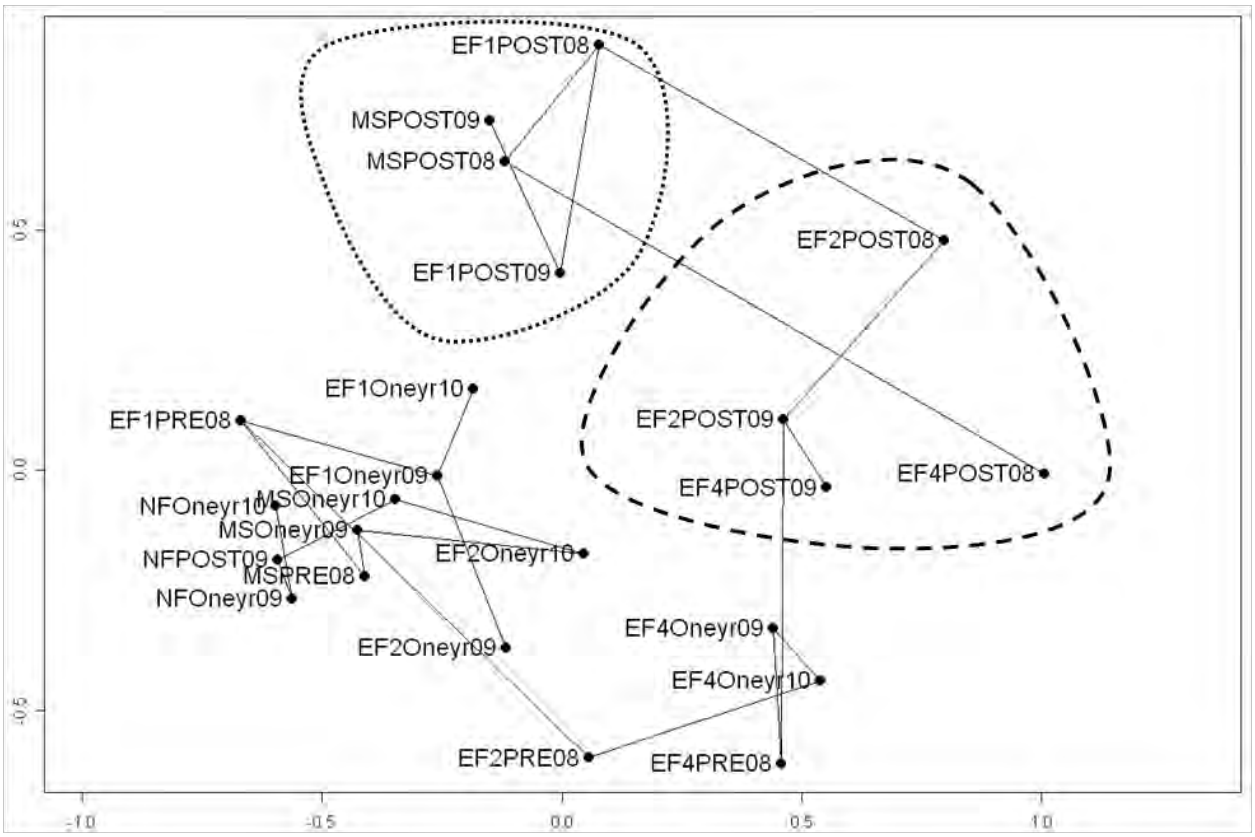

Figure 51. – NMDS spanning tree using Morisita-Horn similarity index for pre (2008), immediate post (2008 and 2009) and one-year post (2009 and 2010) treatment EPT abundances in the Specimen Creek drainage. Circled samples are post treatment (dashes) and detox (diamonds) sites.

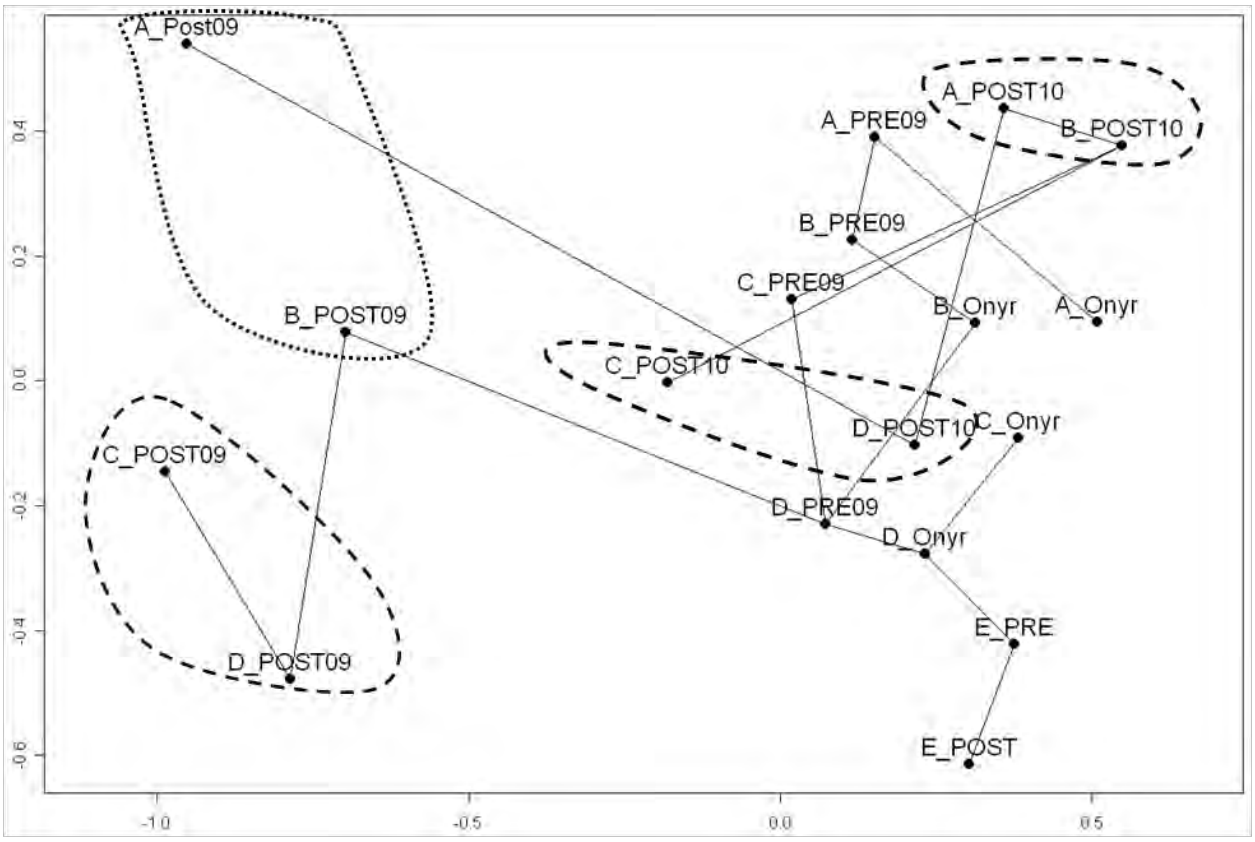

Figure 52. – NMDS spanning tree using Morisita-Horn similarity index for pre (2009), immediate post (2009 and 2010) and one-year post (2009) treatment EPT abundances in the Cherry Creek drainage. Circled samples are post treatment (dashes) and detox (diamonds) sites.

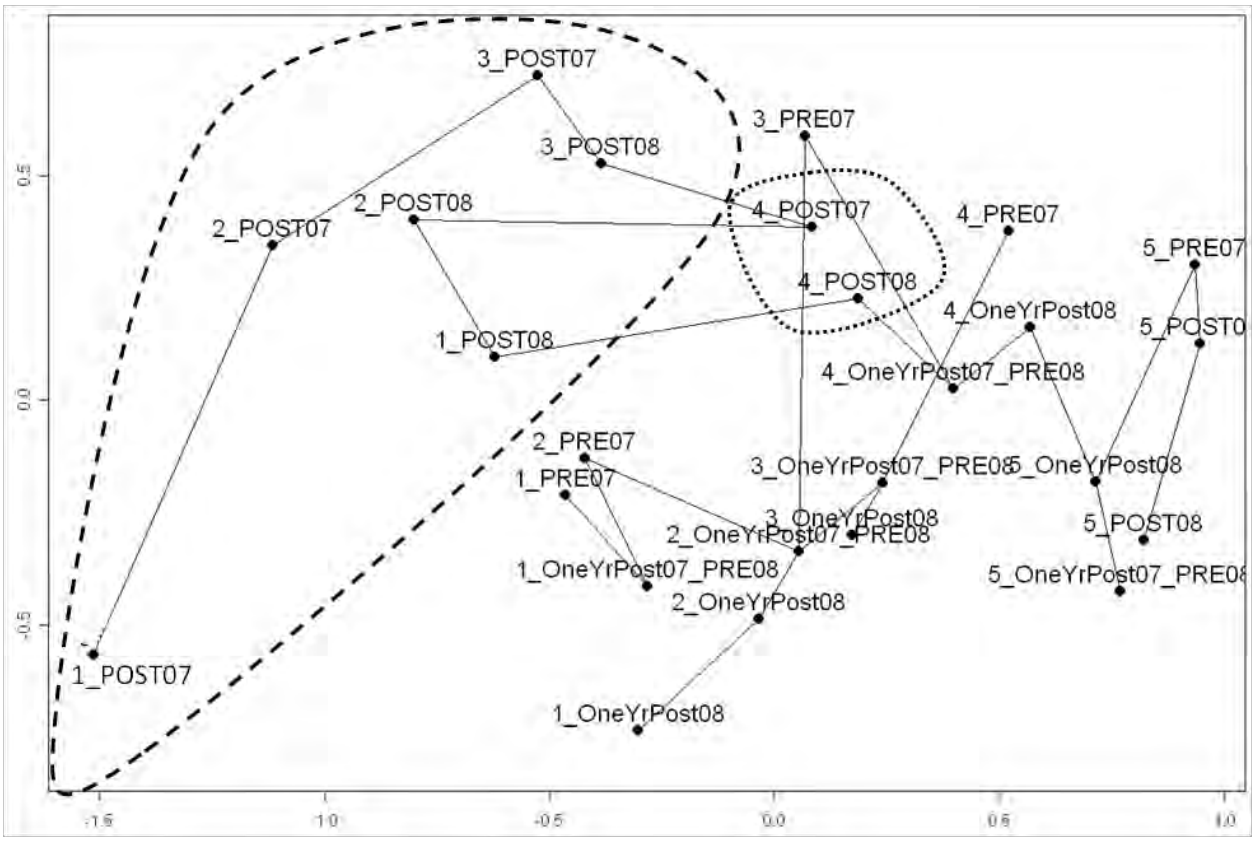

Figure 53. – NMDS spanning tree using Morisita-Horn similarity index for pre (2007), immediate post (2007 and 2008) and one-year post (2008 and 2009) treatment EPT abundances in the Comanche Creek drainage. Circled samples are post treatment (dashes) and detox (diamonds) sites.

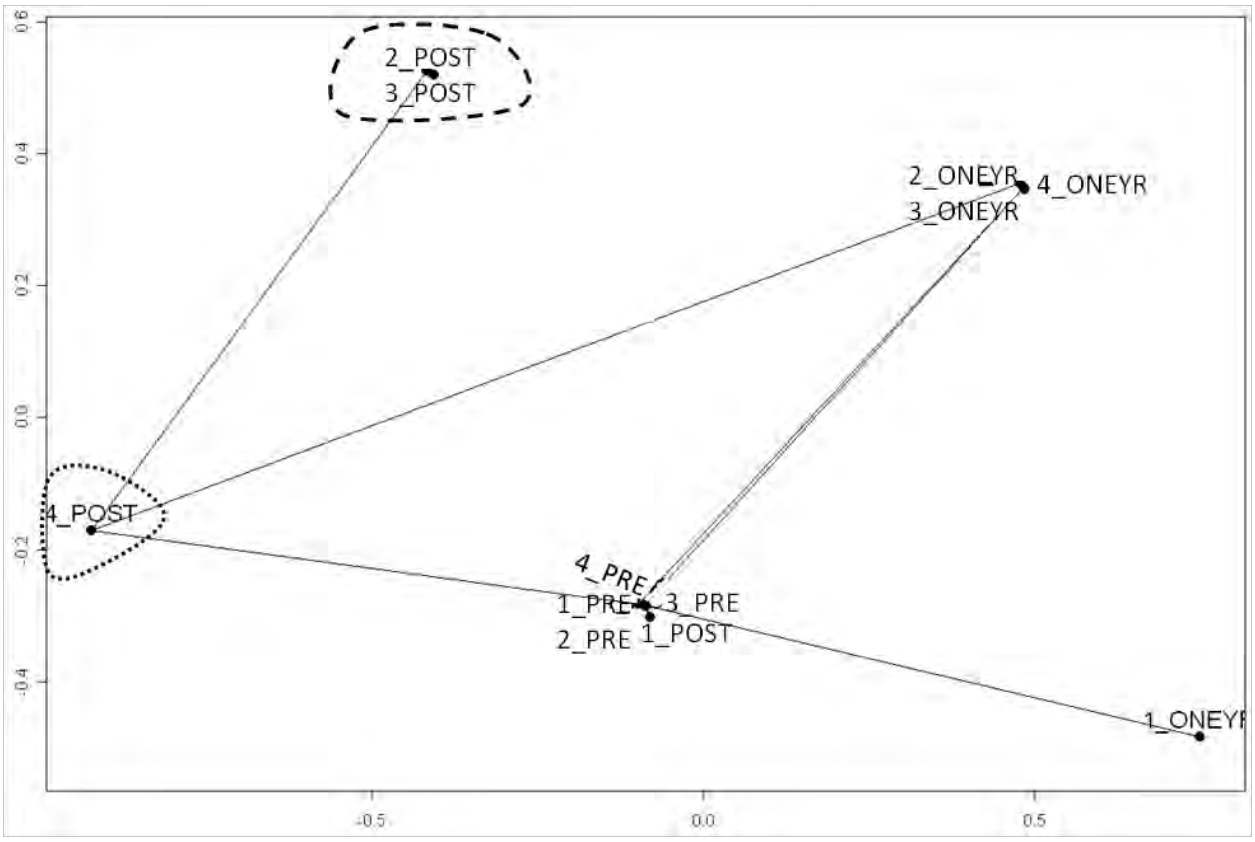

Figure 54. – NMDS spanning tree using Morisita-Horn similarity index for pre, immediate post and one-year posttreatment EPT abundances in the Costilla Creek drainage. Circled samples are post treatment (dashes) and detox (diamonds) sites.

CHAPTER 5

CONCLUSIONS

The homogenization of fishes is caused by the replacement of native species with non-indigenous species (Rahel 2000 and 2002). Piscicides, particularly rotenone is considered essential in the restoration of native fish populations; however, their use is contentious and criticized, specifically concerning impacts to invertebrates. Knowledge of effects to non-target organisms is important for the management and conservation of fish populations. This thesis has two general objectives: (1) demonstrate the influence CFT Legumine rotenone has on benthic macroinvertebrates for restoration projects in Montana and New Mexico and (2) evaluate the immediate response by means of invertebrate drift from the application of CFT Legumine rotenone. Both objectives are important to understanding of the influence CFT Legumine has on benthic macroinvertebrates and in improving fisheries restoration efforts.

Chapter 2 examines benthic macroinvertebrate response during the Specimen Creek restoration project. This project is one of the first to utilize CFT Legumine and demonstrate its effects to the invertebrate community. A recent laboratory study has suggested that CFT Legumine has fewer impacts to invertebrates compared to conventional formulations (Finlayson *et al.* 2010); this chapter evaluates the concept in a field application. Results indicate CFT Legumine treatment effects are minimal; however, detoxification of rotenone using potassium permanganate influenced the BMI community. This chapter demonstrates that CFT Legumine did not influence BMI communities compared to previous studies. However, potassium permanganate studies should be performed to understand how it reacts in the environment and is toxic to invertebrates.

Chapter 3 examines macroinvertebrate drift during rotenone treatment. Previous studies have observed peak drift in the first 30 minutes of application that sustained for the duration of treatment (Dudgeon 1990 and Arnekleiv *et al.* 2001). Results demonstrate peak drift does not occur in the first 30 minutes. However, some plecopterans do peak in the first 30 minutes, demonstrating their sensitivity to rotenone. In all sampling events, early life stages of Ephemeroptera and Plecoptera are dominant; however, as treatment continues later life stages begin to drift. Thus, early life stages are the most susceptible to rotenone, but as exposure time continues later life stages become intolerant and drift. Reducing treatment duration to four hours could potentially reduce to impacts to invertebrates and protect later life stages.

Chapter 4 follows a similar approach to Chapter 2, but incorporates results from Specimen Creek and three other restoration projects that utilized CFT Legumine. Recent literature has indicated that comparisons of studies with similar study designs are limited and needed to understand treatment affects (Vinson *et al.* 2010). This study provides a comprehensive perspective of CFT Legumine effects to benthic macroinvertebrates in two geographic regions. Projects in the same geographic region had similar treatment results. However, potassium permanganate effects were observed in three of the four projects. It is likely that exposure to BMI is the cause for differences in the projects. The projects demonstrating effects were all within close proximity to the detoxification station. Regardless, invertebrates recovered one-year after treatment. To reduce impacts of rotenone managers should apply CFT Legumine and use the minimal dosage and duration to complete the projects goal.

APPENDIX A

SUMMARY OF SPECIMEN CREEK RESPONSE VARIABLES (MEAN ± SD) AND INSECT TAXA (MEAN ABUNDANCE) FOR THE FIVE SAMPLE SITES FOR 2009-2010 PRE, IMMEDIATE POST, AND ONE-YEAR POST SAMPLING EVENTS

	MS1		
Response variable	Pre	Post	One-yr. post
Insect abundance	1149 ± 284.1	521.6 ± 75.80	958.0 ± 271.4
Insect richness	18.8 ± 3.37	15.9 ± 4.42	23.1 ± 2.64
EPT abundance	891.6 ± 249.3	128.4 ± 54.52	824.9 ± 234.3
EPT richness	15.2 ± 2.05	10.2 ± 2.49	17.9 ± 1.64
Taxa			
Coleoptera			
Ametor	0.125	0	0
Cleptelmis	0	0	0
Dytiscidae	0.125	0	0
Heterlimnius	3.125	4.125	4.625
Diptera			
Antocha	0	0	0
Ceratopogoninae	0.75	3.625	3.25
Chironomidae	250.8	333.8	121.5
Clinocera	1.5	1.875	0.75
Dicranota	0	0	0.125
Dixella	0	0	0
Glutops	0.125	1.625	1.875
Hexatoma	0.875	0.625	0.375
Limnophila	0	0.25	0.125
Pericoma	0	47.25	0
Prosimulium	0.125	0	0.5
Simulium	0	0.125	0
Ephemeroptera			
Ameletus	0.125	0	0.5
Baetis	342.9	0.25	198.1
Caudatella	0	0	0.875
Cinygma	0	0	0
Cinygmula	29.75	0.125	55.25
Diphetor	0	0	0
Drunella	235.1	0.75	314.5
Epeorus	136.1	0	66
Ephemerella	3.625	1.5	6.625
Paraleptophlebia	0	0	0
Rhithrogena	40	0.125	58
Serratella	8.75	0	7.5

Plecoptera			
Capniidae	6.875	0.25	8.75
Doroneuria	0	0	0
Hesperoperla	0	0	0
Isoperla	0	0	0.125
Kogotus	0.125	0	0
Leuctridae	1.125	2.5	1.625
Megarcys	5.75	0	2.75
Paraperla	0.5	0	0.5
Sweltsa	3.875	3.625	15.5
Taeniopterygidae	0	0.25	0
Visoka	0	0.375	0.875
Yoraperla	0.125	0	0
Zapada	6.5	1.25	4.625
Trichoptera			
Allomyia	0	0	0
Amphicosmoecus	0	0	0
Anagapetus	0	0	0
Apatania	0	0.875	0.125
Arctopsyche	0	0	0
Cryptochia	0	0	0
Desmona	0	0	0
Dicosmoecus	1.75	0.125	1
Ecclisomyia	0	2.125	0.125
Glossosoma	1.375	27.38	17.75
Goereilla	0	0	0
Homophylax	0	0	0
Lepidostoma	0	0	0
Micrasema	0	0	0
Neophylax	0	0	0
Neothremma	41.12	42.12	36.38
Oligophlebodes	1.25	0	0.75
Parapsyche	4.125	13.25	5.375
Psychoglypha	0	0	0
Rhyacophila	20.75	31.5	21.25

	EF1		
Response variable	Pre	Post	One-yr. post
Insect abundance	925.4 ± 358.1	671.2 ± 191.8	542.1 ± 209.0
Insect richness	19.1 ± 2.64	15.9 ± 2.23	22.4 ± 3.50
EPT abundance	561.6 ± 141.2	258.0 ± 98.50	397.0 ± 118.8
EPT richness	15.9 ± 1.73	12.4 ± 2.38	18.6 ± 2.82
Taxa			
Coleoptera			
Ametor	0	0	0
Cleptelmis	0	0	0
Dytiscidae	0.125	0	0
Heterlimnius	4.25	5.125	10.25
Diptera			
Antocha	0	0	0
Ceratopogoninae	0	0	0.375
Chironomidae	346.5	394	130.3
Clinocera	0	0	0
Dicranota	0	0.625	0.125
Dixella	0	0	0
Glutops	5.75	8.25	1.75
Hexatoma	0	0.75	0.125
Limnophila	0	0.625	0
Pericoma	0	0.625	0
Prosimulium	6.5	3.25	2.125
Simulium	0.625	0	0
Ephemeroptera			
Ameletus	1.25	0.375	4.125
Baetis	205.2	5.75	145.6
Caudatella	0	0	0.25
Cinygma	0	0	0
Cinygmula	5.125	0	4
Diphetor	0	0	0
Drunella	56.87	3.25	24.88
Epeorus	75	3.25	38.12
Ephemerella	4	0.5	5.875
Paraleptophlebia	0	0	0
Rhithrogena	22.5	0	13.5
Serratella	10.25	0	3.5

Plecoptera			
Capniidae	0	1.25	0.375
Doroneuria	0	0	0
Hesperoperla	0	0	0
Isoperla	0	1.25	0.25
Kogotus	0	0	0.25
Leuctridae	2.625	4.875	2.125
Megarcys	30.12	9.25	3.5
Paraperla	1.125	0.875	1.75
Sweltsa	34	33.38	23.5
Taeniopterygidae	0	0	0
Visoka	3	3.375	3.125
Yoraperla	0	0	0.125
Zapada	5.375	1.875	0.5
Trichoptera			
Allomyia	0	0	0.125
Amphicosmoecus	0	0	0
Anagapetus	1.75	0	0
Apatania	0	0	0
Arctopsyche	0	0	0
Cryptochia	0	0	0
Desmona	0	0	0
Dicosmoecus	1.375	0.125	0.375
Ecclisomyia	0	1.75	0.25
Glossosoma	6	54.88	22.75
Goereilla	0	0	0
Homophylax	0	0	0
Lepidostoma	0	0.125	0
Micrasema	0.625	0.625	0.625
Neophylax	0.25	0	0
Neothremma	40.12	67.25	49.62
Oligophlebodes	0	0	10.5
Parapsyche	4.125	8.125	10
Psychoglypha	0	0	0
Rhyacophila	50.88	55.875	27.38

	EF2		
Response variable	Pre	Post	One-yr. post
Insect abundance	1847 ± 1017	1483 ± 758.8	547.0 ± 192.2
Insect richness	17.8 ± 2.25	19.6 ± 4.00	18.4 ± 2.39
EPT abundance	808.6 ± 190.3	432.8 ± 169.1	342.6 ± 82.56
EPT richness	12.8 ± 1.83	14.8 ± 3.37	12.6 ± 1.51
Taxa			
Coleoptera			
Ametor	0	0	0
Cleptelmis	4.125	9.625	1.125
Dytiscidae	0	0	0.125
Heterlimnius	54.38	32.62	30.75
Diptera			
Antocha	0.75	0.125	0.25
Ceratopogoninae	0	0	0.125
Chironomidae	955.4	987.8	159.5
Clinocera	0.625	0.875	0.125
Dicranota	0	0.125	0
Dixella	0	0	0
Glutops	8.875	14	5.125
Hexatoma	0	0	0
Limnophila	0	0	0
Pericoma	1.125	1.25	0.25
Prosimulium	12.88	4	6.5
Simulium	0.125	0	0.5
Ephemeroptera			
Ameletus	3.125	1.625	1.875
Baetis	428.1	0.25	92.75
Caudatella	0	2.625	0
Cinygma	0	0	0
Cinygmula	46.75	33.25	12.5
Diphetor	0	0	0
Drunella	43.38	19.38	94.5
Epeorus	73	0.125	39
Ephemerella	18.88	74.62	14.38
Paraleptophlebia	0	0	0
Rhithrogena	0.125	0	0.625
Serratella	4	0.125	0.125

Plecoptera			
Capniidae	0	0.375	0
Doroneuria	0	0.25	0.75
Hesperoperla	0	1.375	0
Isoperla	11.25	15.75	0.125
Kogotus	0	0	0
Leuctridae	0.25	2.625	0.125
Megarcys	28.62	0.625	1.125
Paraperla	0.125	1.375	0
Sweltsa	44.12	89.5	24.62
Taeniopterygidae	0	0	0
Visoka	0.75	1.5	2.5
Yoraperla	0	3.625	0
Zapada	35.12	112.1	10
Trichoptera			
Allomyia	0	0.125	0
Amphicosmoecus	0.125	0	0
Anagapetus	0	0	0.125
Apatania	0	0.125	0
Arctopsyche	0	0	0
Cryptochia	0	0	0
Desmona	0	0	0
Dicosmoecus	0.625	0	0
Ecclisomyia	0	4.25	1.875
Glossosoma	0.75	1.375	1
Goereilla	0	0.625	0
Homophylax	0	0	0
Lepidostoma	0	0.125	0
Micrasema	0.125	1.625	0
Neophylax	0	0	0
Neothremma	6	3.625	0
Oligophlebodes	0	0.25	0.125
Parapsyche	0.125	0	0
Psychoglypha	0	0	0
Rhyacophila	63.25	59.5	44.5

	EF4		
Response variable	Pre	Post	One-yr. post
Insect abundance	622.4 ± 243.2	1065 ± 276.2	900.5 ± 402.5
Insect richness	18.2 ± 3.54	18.9 ± 2.80	19.4 ± 2.39
EPT abundance	260.8 ± 94.76	193.6 ± 67.81	362.8 ± 158.5
EPT richness	14.0 ± 3.58	15.4 ± 2.56	15.1 ± 1.96
Taxa			
Coleoptera			
Ametor	0	0	0
Cleptelmis	0	0	0
Dytiscidae	0.875	0	0
Heterlimnius	97.75	149.75	134.5
Diptera			
Antocha	1.75	0.25	0.75
Ceratopogoninae	1.125	0.75	2.5
Chironomidae	257.9	715.5	395.4
Clinocera	0	0	0.625
Dicranota	0	1.25	0
Dixella	0	0	0
Glutops	1.625	2.75	3.875
Hexatoma	0	0	0
Limnophila	0	0.625	0.125
Pericoma	0	0.125	0
Prosimulium	0	0	0
Simulium	0.625	0	0
Ephemeroptera			
Ameletus	23.38	10.75	22.62
Baetis	31.25	0.625	97.62
Caudatella	0	0	0
Cinygma	0.75	2.625	7.75
Cinygmula	57.75	2.875	55.38
Diphetor	0	0	0
Drunella	17.62	10.25	2.875
Epeorus	0.5	0.625	6.75
Ephemerella	3	20.25	41.25
Paraleptophlebia	6	3.625	5.25
Rhithrogena	0	0	0
Serratella	0	0	0

Plecoptera			
Capniidae	0.25	9.75	0
Doroneuria	0.125	0	8.5
Hesperoperla	0	0.125	0
Isoperla	1	9.125	0.75
Kogotus	0	0	0
Leuctridae	0.75	15	3.25
Megarcys	6.25	0.5	0.625
Paraperla	0.125	1.25	0
Sweltsa	15	25.38	16.12
Taeniopterygidae	0	0	0
Visoka	16.38	10.38	40.62
Yoraperla	0	0	0
Zapada	46	19.5	18.38
Trichoptera			
Allomyia	0	0	0
Amphicosmoecus	0	0	0
Anagapetus	0	0	0
Apatania	0	0	0
Arctopsyche	0	0	0
Cryptochia	0	0.125	0
Desmona	0	0	0
Dicosmoecus	0.125	0.125	0
Ecclisomyia	0	8.375	1
Glossosoma	0	0	0
Goereilla	0	0	0
Homophylax	0	0	0
Lepidostoma	0	0	0
Micrasema	5	2.375	0.625
Neophylax	0	0	0
Neothremma	8.875	10.62	2
Oligophlebodes	0	0	0
Parapsyche	0.75	0.125	1.125
Psychoglypha	0.5	2	0
Rhyacophila	19.38	27.25	30.25

	NF1		
Response variable	Pre	Post	One-yr. post
Insect abundance	858.6 ± 379.2	1193 ± 388.6	609.1 ± 98.5
Insect richness	21.8 ± 2.71	22.0 ± 1.31	23.1 ± 2.90
EPT abundance	750.8 ± 341.4	778.2 ± 257.3	488.4 ± 111.9
EPT richness	17.5 ± 2.07	18.2 ± 1.28	17.0 ± 2.33
Taxa			
Coleoptera			
Ametor	0	0	0
Cleptelmis	0	0	0
Dytiscidae	0	0	0
Heterlimnius	2.375	2.5	4
Diptera			
Antocha	0	0	0
Ceratopogoninae	0.125	0.625	0.75
Chironomidae	97.62	396.2	108.4
Clinocera	0.375	0.625	1.625
Dicranota	1.375	6	0.875
Dixella	0	0	0
Glutops	4.375	3	3
Hexatoma	0.25	0.75	0.5
Limnophila	0.125	0.625	0.625
Pericoma	0	4	0
Prosimulium	1.25	0	0.875
Simulium	0	0.625	0.125
Ephemeroptera			
Ameletus	0.75	2.625	0.375
Baetis	21.38	84.12	20.88
Caudatella	0	0	0
Cinygma	0	0	0
Cinygmula	38	19.62	32.5
Diphetor	0	0	0
Drunella	494.5	377	255.5
Epeorus	60.12	35.88	86.12
Ephemerella	3	19	3.875
Paraleptophlebia	0	0	0
Rhithrogena	46.25	125.9	16.38
Serratella	2.625	3.5	4.75

Plecoptera			
Capniidae	26.25	27.12	12.12
Doroneuria	0	0	0
Hesperoperla	0	0	0
Isoperla	0.625	0	0
Kogotus	1	0.5	0.125
Leuctridae	0.75	3	0.875
Megarcys	2	3.375	0.75
Paraperla	1.125	0.25	0
Sweltsa	6.5	4.75	4.625
Taeniopterygidae	0	9	0
Visoka	0.125	0.125	0.25
Yoraperla	0.375	0.25	0
Zapada	14.25	15	7.625
Trichoptera			
Allomyia	0	0	0
Amphicosmoecus	0	0	0
Anagapetus	0	0	0
Apatania	0.375	0.75	0
Arctopsyche	0	0	0
Cryptochia	0	0	0
Desmona	0	0	0
Dicosmoecus	0.75	0.375	1.875
Ecclisomyia	0	0	0.125
Glossosoma	3.875	30.38	14
Goereilla	0	0	0
Homophylax	0	0	0
Lepidostoma	0.75	0	0
Micrasema	0	0	0
Neophylax	0	0	0.375
Neothremma	6.625	2.75	1.125
Oligophlebodes	1.625	0	8.75
Parapsyche	2.5	2.5	2.625
Psychoglypha	0	0	0
Rhyacophila	14.62	10.5	12.75

APPENDIX B

SUMMARY OF SPECIMEN CREEK INSECT TAXA (TOTAL ABUNDANCE) FOR THE FIVE SAMPLE SITES FOR 2004-2008 SAMPLING EVENTS

	MS1			
	2004	2007	Pre 2008	Post 2008
Taxa				
Coleoptera				
Ametor	0	0	0	0
Cleptelmis	0	0	0	0
Dytiscidae	0	0	0	0
Heterlimnius	0	45	16	29
Diptera				
Antocha	0	0	0	0
Ceratopogoninae	0	45	19	31
Chironomidae	471	1427	263	770
Clinocera	3	0	6	0
Dicranota	0	0	0	2
Dixella	0	0	0	0
Glutops	0	9	6	4
Hexatoma	3	18	3	7
Limnophila	0	0	0	4
Pericoma	0	108	0	83
Prosimulium	3	18	9	2
Simulium	0	9	0	0
Ephemeroptera				
Ameletus	0	0	0	0
Baetis	309	243	496	0
Caudatella	3	63	0	0
Cinygma	0	0	0	0
Cinygmula	5	601	126	0
Diphetor	0	0	0	0
Drunella	13	745	196	2
Epeorus	113	207	142	0
Ephemerella	3	54	13	0
Paraleptophlebia	0	0	0	0
Rhithrogena	34	215	79	0
Serratella	3	0	32	0
Plecoptera				
Capniidae	11	9	25	2
Doroneuria	0	0	0	0
Hesperoperla	0	0	0	0
Isoperla	0	0	0	0
Kogotus	0	0	3	0

Leuctridae	0	9	0	0
Megarcys	0	9	6	0
Paraperla	0	9	3	2
Sweltsa	8	27	22	31
Taeniopterygidae	0	81	0	0
Visoka	0	0	6	2
Yoraperla	3	0	3	0
Zapada	41	216	57	0
Trichoptera				
Allomyia	0	0	0	0
Amphicosmoecus	0	0	0	0
Anagapetus	0	0	0	0
Apatania	40	27	9	2
Arctopsyche	0	9	0	0
Cryptochia	0	0	0	0
Desmona	0	0	0	0
Dicosmoecus	0	0	3	2
Ecclisomyia	0	0	3	7
Glossosoma	332	215	9	20
Goereilla	0	0	0	0
Homophylax	0	0	3	0
Lepidostoma	3	0	0	0
Micrasema	0	0	0	0
Neophylax	0	0	3	2
Neothremma	0	27	0	7
Oligophlebodes	0	18	0	0
Parapsyche	5	45	9	27
Psychoglypha	0	0	0	0
Rhyacophila	106	242	110	168

	EF1					
	2004	2005	2006	2007	Pre 2008	Post 2008
Taxa						
Coleoptera						
Ametor	0	0	0	0	0	0
Cleptelmis	0	0	0	0	0	0
Dytiscidae	0	0	0	0	0	0
Heterlimnius	12	60	16	20	40	18
Diptera						
Antocha	0	0	0	0	8	0
Ceratopogoninae	0	0	0	0	0	0
Chironomidae	4762	3219	1083	3023	1816	761
Clinocera	0	20	0	0	0	0
Dicranota	0	0	0	0	0	0
Dixella	0	0	0	0	0	0
Glutops	23	40	11	39	8	25
Hexatoma	0	0	0	0	0	0
Limnophila	0	0	0	0	0	0
Pericoma	0	0	0	0	0	2
Prosimulium	196	40	27	10	65	0
Simulium	12	80	32	0	0	0
Ephemeroptera						
Ameletus	12	0	16	29	0	0
Baetis	1263	1610	559	274	694	0
Caudatella	0	0	0	20	0	0
Cinygma	0	0	0	0	0	0
Cinygmula	23	181	323	127	105	0
Diphetor	0	0	0	0	0	0
Drunella	104	282	205	40	193	0
Epeorus	161	724	479	137	282	0
Ephemerella	104	20	0	30	56	0
Paraleptophlebia	0	0	0	0	0	0
Rhithrogena	23	80	32	78	16	0
Serratella	58	20	11	0	8	0
Plecoptera						
Capniidae	0	20	5	10	16	2
Doroneuria	0	0	0	0	0	0
Hesperoperla	0	0	0	0	0	0
Isoperla	0	0	0	20	0	0
Kogotus	0	0	0	0	8	0

Leuctridae	0	0	0	0	0	0
Megarcys	81	20	145	78	16	0
Paraperla	0	0	11	20	0	0
Sweltsa	115	181	135	186	73	38
Taeniopterygidae	0	0	0	10	8	0
Visoka	35	40	5	88	8	7
Yoraperla	46	60	16	20	16	0
Zapada	600	885	398	509	105	0
Trichoptera						
Allomyia	0	0	0	0	0	0
Amphicosmoecus	0	0	0	0	0	0
Anagapetus	0	0	0	20	0	0
Apatania	46	80	11	20	0	0
Arctopsyche	0	0	0	0	0	0
Cryptochia	0	0	0	0	0	0
Desmona	0	0	0	0	0	0
Dicosmoecus	12	0	5	0	8	
Ecclisomyia	0	0	0	0	8	22
Glossosoma	12	121	32	117	153	74
Goereilla	0	0	0	0	0	0
Homophylax	0	0	0	0	0	0
Lepidostoma	0	0	0	0	0	0
Micrasema	0	0	0	0	0	0
Neophylax	0	0	0	20	32	0
Neothremma	23	2354	242	59	16	65
Oligophlebodes	0	0	0	0	0	0
Parapsyche	0	20	11	10	16	18
Psychoglypha	0	0	0	0	8	0
Rhyacophila	219	482	101	157	80	163

	EF2					
	2004	2005	2006	2007	Pre 2008	Post 2008
Taxa						
Coleoptera						
Ametor	0	0	0	0	0	0
Cleptelmis	15	1	5	0	97	38
Dytiscidae	0	0	0	0	0	0
Heterlimnius	10	4	24	172	113	66
Diptera						
Antocha	0	0	3	0	32	0
Ceratopogoninae	0	0	0	0	0	0
Chironomidae	2160	323	317	3218	3130	1600
Clinocera	5	1	0	0	0	0
Dicranota	0	0	0	0	0	0
Dixella	0	0	0	0	0	0
Glutops	15	8	19	54	97	47
Hexatoma	0	0	0	0	0	0
Limnophila	0	0	3	0	0	0
Pericoma	15	0	0	0	0	0
Prosimulium	60	9	13	0	242	0
Simulium	5	0	16	11	0	0
Ephemeroptera						
Ameletus	0	0	0	11	16	19
Baetis	393	36	132	581	1404	0
Caudatella	20	0	8	0	0	19
Cinygma	0	0	0	0	0	0
Cinygmula	45	8	91	215	452	0
Diphetor	0	0	0	0	0	0
Drunella	50	11	109	366	323	46
Epeorus	21	0	102	97	710	0
Ephemerella	207	5	19	86	258	114
Paraleptophlebia	0	0	0	0	0	9
Rhithrogena	0	0	0	0	0	0
Serratella	5	1	3	0	32	0
Plecoptera						
Capniidae	0	0	0	0	0	0
Doroneuria	0	0	5	11	16	0
Hesperoperla	0	0	3	0	16	0
Isoperla	101	7	11	54	81	0
Kogotus	0	0	0	0	0	0

Leuctridae	0	0	0	0	0	0
Megarcys	0	1	35	11	81	0
Paraperla	5	0	0	0	0	0
Sweltsa	25	5	56	118	500	76
Taeniopterygidae	0	0	0	0	0	0
Visoka	0	0	3	0	16	0
Yoraperla	30	1	22	43	323	9
Zapada	428	9	194	161	500	208
Trichoptera						
Allomyia	0	0	0	0	0	0
Amphicosmoecus	0	0	0	0	0	0
Anagapetus	0	0	0	0	0	0
Apatania	0	0	0	22	0	0
Arctopsyche	0	0	0	0	0	0
Cryptochia	0	0	0	0	0	0
Desmona	0	0	0	0	0	0
Dicosmoecus	0	0	0	0	0	0
Ecclisomyia	0	0	0	32	0	19
Glossosoma	0	0	0	0	16	19
Goereilla	0	0	0	0	0	0
Homophylax	0	0	0	0	16	9
Lepidostoma	0	0	0	0	0	0
Micrasema	10	0	0	22	48	47
Neophylax	0	0	0	0	0	0
Neothremma	146	8	5	43	97	104
Oligophlebodes	0	0	0	0	0	0
Parapsyche	0	0	3	0	0	0
Psychoglypha	0	0	0	0	0	0
Rhyacophila	131	22	86	75	145	255

	EF4					
	2005	2006	2006	2007	Pre 2008	Post 2008
Taxa						
Coleoptera						
Ametor	0	0	0	0	0	0
Cleptelmis	0	0	0	0	0	0
Dytiscidae	0	0	0	0	0	0
Heterlimnius	404	132	202	457	627	633
Diptera						
Antocha	111	8	62	27	4	25
Ceratopogoninae	0	0	0	27	4	31
Chironomidae	2058	739	552	2338	315	564
Clinocera	0	0	3	0	4	0
Dicranota	0	0	3	0	0	0
Dixella	0	0	0	0	0	0
Glutops	10	22	8	94	9	31
Hexatoma	0	0	0	0	0	0
Limnophila	0	3	0	13	0	0
Pericoma	0	0	0	0	0	0
Prosimulium	0	0	0	0	0	0
Simulium	0	0	0	0	0	0
Ephemeroptera						
Ameletus	40	67	46	13	27	50
Baetis	10	16	19	13	31	0
Caudatella	0	0	0	0	0	0
Cinygma	0	5	5	0	0	0
Cinygmula	262	94	264	686	116	0
Diphetor	0	5	5	13	0	0
Drunella	131	16	40	376	49	6
Epeorus	10	5	0	0	4	0
Ephemerella	61	8	19	269	18	0
Paraleptophlebia	71	8	78	175	27	62
Rhithrogena	0	0	0	0	0	0
Serratella	0	0	0	0	0	0
Plecoptera						
Capniidae	0	11	8	0	4	0
Doroneuria	61	27	27	13	76	0
Hesperoperla	0	0	0	13	0	0
Isoperla	0	0	8	13	4	0
Kogotus	0	0	0	0	4	0

Leuctridae	0	3	22	0	0	0
Megarcys	61	0	30	0	54	0
Paraperla	0	3	0	13	4	19
Sweltsa	151	137	159	67	175	37
Taeniopterygidae	0	0	0	0	0	0
Visoka	131	113	94	673	72	50
Yoraperla	0	0	0	0	4	0
Zapada	474	280	339	821	152	12
Trichoptera						
Allomyia	0	0	0	0	0	0
Amphicosmoecus	0	0	0	0	0	0
Anagapetus	0	0	0	0	0	0
Apatania	0	16	8	0	54	0
Arctopsyche	0	0	0	0	0	0
Cryptochia	0	0	0	0	0	0
Desmona	0	0	5	0	0	0
Dicosmoecus	10	0	0	0	0	0
Ecclisomyia	0	16	5	0	0	0
Glossosoma	0	0	0	0	0	0
Goereilla	0	0	0	0	0	0
Homophylax	0	0	0	0	0	0
Lepidostoma	0	0	0	0	0	
Micrasema	91	0	3	282	76	37
Neophylax	0	0	0	0	0	0
Neothremma	20	0	0	27	63	12
Oligophlebodes	0	0	0	0	0	0
Parapsyche	0	5	8	40	4	6
Psychoglypha	30	11	0	0	0	0
Rhyacophila	232	78	116	188	93	174

	NF1
	2004
Taxa	
Coleoptera	
Ametor	0
Cleptelmis	0
Dytiscidae	0
Heterlimnius	22
Diptera	
Antocha	0
Ceratopogoninae	16
Chironomidae	732
Clinocera	5
Dicranota	0
Dixella	0
Glutops	5
Hexatoma	11
Limnophila	0
Pericoma	0
Prosimulium	0
Simulium	5
Ephemeroptera	
Ameletus	0
Baetis	503
Caudatella	0
Cinygma	0
Cinygmula	145
Diphetor	0
Drunella	398
Epeorus	247
Ephemerella	11
Paraleptophlebia	0
Rhithrogena	113
Serratella	22
Plecoptera	
Capniidae	11
Doroneuria	0
Hesperoperla	0
Isoperla	0
Kogotus	0

Leuctridae	0
Megarcys	27
Paraperla	0
Sweltsa	43
Taeniopterygidae	5
Visoka	0
Yoraperla	0
Zapada	291
Trichoptera	
Allomyia	0
Amphicosmoecus	0
Anagapetus	0
Apatania	5
Arctopsyche	0
Cryptochia	0
Desmona	0
Dicosmoecus	0
Ecclisomyia	0
Glossosoma	151
Goereilla	0
Homophylax	0
Lepidostoma	0
Micrasema	0
Neophylax	0
Neothremma	5
Oligophlebodes	0
Parapsyche	124
Psychoglypha	0
Rhyacophila	81

APPENDIX C

DRIFT DENSITIES OF TAXON IDENTIFIED FROM THE UPPER TREATMENT SITE IN EAST FORK SPECIMEN CREEK. SAMPLES IN BLUE BOXES REPRESENT WHEN EPT TAXON PEAKED IN DRIFT

	0	30-60	180-210	330-360
Ameletus	0.67	0.67	29.84	182.69
Baetis	0.19	0.14	2.43	0.67
Cinygma	0.05	0.00	42.77	7.16
Cinygmula	0.05	0.00	41.63	94.90
Diphetor	0.00	0.05	204.26	63.73
Drunella	0.00	0.00	0.00	0.29
Ephemerella	1.19	1.67	8.26	27.16
Paraleptophlebia	0.24	0.29	0.91	3.15
EPHEMEROPTERA	2.39	2.82	330.10	379.74
Doroneuria	0.00	0.00	0.86	0.24
Malenka	0.00	0.05	0.43	3.44
Perlodidae	0.00	118.20	7.35	2.39
Sweltsa	0.00	1.34	4.96	5.63
Visoka	0.00	0.00	1.10	0.86
Zapada	0.05	0.05	29.21	12.08
PLECOPTERA	0.05	119.63	43.92	24.63
Dicosmoecus	0.00	0.00	0.05	0.05
Ecclisomyia	0.10	0.00	0.00	0.00
Micrasema	0.00	0.24	0.86	1.29
Neothremma	0.00	0.00	0.00	0.24
Parapsyche	0.00	0.24	0.67	0.43
Psychoglypha	0.00	0.00	0.00	0.14
Rhyacophila	0.05	0.05	2.15	26.92
TRICHOPTERA	0.14	0.53	3.72	29.07
Dytiscidae	0.00	0.00	0.05	0.05
Heterlimnius	0.05	0.00	0.00	2.91
Hydrophilidae	0.00	0.05	0.05	0.00
COLEOPTERA	0.05	0.05	0.10	2.96
Antocha	0.00	0.00	0.00	0.24
Ceratopogoninae	0.05	0.00	0.00	0.00
Clinocera	0.00	0.00	0.67	0.86
Dicranota	0.00	0.00	0.24	0.00
Dixa	0.00	0.24	0.00	0.24

Pidicia	0.00	0.00	0.00	0.14
Pericoma	0.05	0.00	0.43	0.43
Prosimulium	0.00	0.00	0.00	0.24
Simulium	0.00	0.24	0.24	0.00
Chironomidae	9.26	23.63	168.32	295.87
DIPTERA	9.36	24.11	169.89	298.02
TOTAL	15.61	154.28	860.88	1105.57

APPENDIX D

DRIFT DENSITIES OF TAXON IDENTIFIED FROM THE LOWER TREATMENT SITE IN EAST FORK SPECIMEN CREEK. SAMPLES IN BLUE BOXES REPRESENT WHEN EPT TAXON PEAKED IN DRIFT

	0	30-60	180-210	330-360
Ameletus	0.14	0.46	48.48	45.10
Baetis	0.80	5.09	24.41	11.49
Caudatella	0.00	0.00	4.52	3.29
Cinygma	0.01	0.00	0.00	0.00
Cinygmula	0.70	2.35	270.33	85.93
Drunella	0.01	0.00	0.69	1.46
Epeorus	0.13	1.93	7.55	3.01
Ephemerella	0.06	0.10	1.33	2.56
Rhithrogena	1.04	0.54	1.46	0.31
Serratella	0.02	0.00	0.00	0.09
EPHEMEROPTERA	2.91	10.47	358.77	153.24
Capniidae	0.09	0.09	14.49	3.58
Leuctridae	0.00	0.00	0.00	0.12
Malenka	0.00	0.00	0.05	0.00
Perlodidae	0.21	140.36	18.44	17.4
Sweltsa	0.04	2.62	11.84	32.28
Visoka	0.00	0.05	0.72	0.29
Yoraperla	0.34	0.58	0.36	0.23
Zapada	0.32	0.97	41.40	26.65
PLECOPTERA	1.12	144.67	87.32	80.54
Dicosmoecus	0.00	0.01	0.01	0.01
Ecclisomyia	0.41	0.66	1.62	2.71
Glossosoma	0.40	0.30	1.36	1.83
Neothremma	0.26	1.97	3.32	2.44
Parapsyche	0.00	0.00	0.67	1.60
Trichoptera	0.01	0.00	0.16	0.27
Psychoglypha	0.00	0.01	0.03	0.06
Cryptochia	0.00	0.06	0.00	0.00
Rhyacophila	0.03	0.06	3.53	5.69
TRICHOPTERA	1.12	3.07	10.71	14.61
Heterlimnius	0.01	0.05	0.01	0.34
Hydrophilidae	0.00	0.00	0.01	0.00
COLEOPTERA	0.01	0.05	0.02	0.34

Antocha	0.00	0.05	0.00	0.00
Dicranota	0.00	0.05	0.05	0.01
Dixa	0.00	0.00	0.05	0.00
Pericoma	1.07	0.99	1.04	1.15
Prosimulium	0.27	1.87	1.35	0.67
Tipula	0.00	0.00	0.00	0.05
Chironomidae	3.20	13.78	163.50	148.96
DIPTERA	4.53	16.74	166.00	150.84
TOTAL	15.69	187.49	848.49	569.69

LITERATURE CITED

Arnekleiv, J. V., D. Dolmen, K. Aagaard, T. Bongard and O. Hanssen. 1997. Effects of rotenone treatment on the bottom-fauna of the Rauma and Henselva watercourses, More og Romsdal County. Part 1: Qualitative investigations. Vitenskapsmus. Rapp. Zool. Ser. 1997–8. (in Norwegian).

Arnekleiv, J. V., D. Dolmen and L. Ronning. 2001. Effects of rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. In: E. DOMINGUEZ (ed.), Trends in Research in Ephemeroptera and Plecoptera. Kluwer Academic/Plenum Publishers: 77–88.

Benke, R.J. 2002. Trout and salmon of North America. The Free Press. New York, NY.

Binns, N. A. 1967. Effects of rotenone treatment on the fauna of the Green River, Wyoming. Fisheries Research Bulletin 1, Wyoming Fish and Game Commission. Cheyenne.

Blakely, T. J., W. L. Chadderton, and J. S. Harding. 2005. The effect of rotenone on orchard pond invertebrate communities in the Moteueka, South Island, New Zealand. DOC Research and Development Series 220, Department of Conservation, Wellington, New Zealand.

Chandler, J. H. Jr., and L. L. Marking. 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. Progressive Fish-Culturist 44:78–80.

Cook, S. F. Jr. and R. L. Moore. 1969. The Effects of Rotenone Treatment on the Insect Fauna of a California Stream. Trans. Amer. Fish. Soc., 3: 539-544.

Cumming, K. B. 1975. History of fish toxicants in the United States. Pages 5-21 in P. H. Eschmeyer, editor. Rehabilitation of fish populations with toxicants: a symposium. American Fisheries Society, North Central Division, Special Publication 4, Bethesda, Maryland.

Dudgeon, D. 1990. Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks on two Papau New Guinea streams. Archives Hydrobiologia 199:35-53.

Elliott, J. M. 1971 Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association. Scientific Publication 25.

Engstrom-Heg, R., R. T. Colesante, and E. Silco. 1978. Rotenone tolerances of stream bottom insects. New York Fish and Game Journal 25:31-41.

Eriksen, T. E., J. V. Arnekleiv and G. Kjaerstad. 2009. Short-term effects on riverine Ephemeroptera, Plecoptera and Trichoptera of rotenone and aluminum sulfate to eradicate Gyrodactylus salaris. Journal of Freshwater Ecology 24: 597–607.

Finlayson, B. J., R. A. Schnick, R. L. Cailteux, L. DeMong, W. D. Horton, W. McClay, C. W. Thompson, and G. J. Tichacek. 2000. Rotenone use in fisheries management: administrative and technical guidelines manual. American Fisheries Society, Bethesda, Maryland.

Finlayson, B., W. Somer, and M. R. Vinson. 2010. Rotenone toxicity to rainbow trout and several mountain stream insects. North American Journal of Fisheries Management 30:102-111.

Gilderhus, P. A., J. L. Allen, and V. K. Dawson. 1986. Persistence of rotenone in ponds at different temperatures. North American Journal of Fisheries Management 6:126-130.

Gladso, J. A. and G. G. Raddum. 2002. Rotenone treatment of a west Norwegian River: effects on drift of invertebrates. Verh. Int. Ver. Theor. Angew. Limnol. 28: 764–769.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley & Sons, New York.

Hamilton, B. T., S. E. Moore, T. B. Williams, N. Darby, and M. R. Vinson. 2009. Comparative effects of rotenone and antimycin on benthic macroinvertebrate diversity in two streams in Great Basin National Park. North American Journal of Fisheries Management 29:1620-1635.

Hauer, F. R., G. A. Lamberti. 2007. Methods in Stream Ecology. Elservier Inc. Oxford, UK.

Heise, B.A., Flannagan, J.F. and Galloway, T.D. 1987. Life histories of Hexagenia limbata and Ephemera simulans (Ephemeroptera) in Dauphin Lake, Manitoba. Journal of North American Benthological Society 6: 230-240.

Jacobi, G. Z. 1978. An inexpensive circular sampler for collecting benthic macroinvertebrates in streams. Archive. Fur Hydrobiologia. Pp. 83:126-131.

Kiffney, P.M. and W.H. Clements. 1994. Effects of metals on a Rocky Mountain stream macroinvertebrate assemblage in experimental microcosms. Journal of North American Benthological Society 13:511–523.

Kiffney, P. M. and Clements, W. H. 1996. Size-dependent response of macroinvertebrates to metals in experimental streams. Environmental Toxicology and Chemistry 15: 1352–1356

Kjaerstad G. and J. V. Arnekleiv. 2011. Effects of Rotenone Treatment on Lotic Invertebrates. International Review Hydrobiologia 96: (1) 58–71.

Kiser, R. W., J. R. Donaldson, and P. R. Olson. 1963. The effect of rotenone on zooplankton populations in freshwater lakes. Transactions of the American Fisheries Society 92:17-24.

Koel, T. M., J. L. Arnold, P. E. Bigelow, P. D. Doepke, B. D. Ertel, and M. E. Ruhl. 2007. Yellowstone Fisheries & Aquatic Sciences: Annual Report, 2006. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2007-04.

Koel, T. M., J. L. Arnold, P. E. Bigelow, P. D. Doepke, B. D. Ertel, and M. E. Ruhl. 2008. Yellowstone Fisheries & Aquatic Sciences: Annual Report, 2007. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2008-02.

Krebs, C.J. 1989. Ecological Methodology. Harper Collins Inc., New York.

Ling, N. 2003. Rotenone. a review of its toxicity and use for fisheries management. Science for Conservation 211, Wellington.

Mangum, F. A. & J. L. Madrigal. 1999. Rotenone effects on aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. J. Freshwater Ecology 14: 125–135.

Marking, L., and T. Bills. 1976. Toxicity of rotenone to fish in standardized laboratory tests. U.S. Fish and Wildlife Service, Investigations in Fish Control 72, Washington, D.C.

McClay, W. 2000. Rotenone use in North America. Fisheries 25(4):15-21.

McClay, W. 2005. Rotenone use in North America (1988–2002). Fisheries 30(4):29–31.

Merritt R.W., K. W. Cummins, M. B. Berg. 2008. Aquatic Insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Melaas, C. L., K. D. Zimmer, M. G. Butler, and M. A. Hanson. 2001. Effects of rotenone on aquatic invertebrate communities in prairie wetlands. Hydrobiologia 459:177-186.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89/001.

Rahel, F.J. 2000. Homogenization of fish faunas across the United States. 2000. Science 288:854-856.

Rahel, F.J. 2002. Homogenization of freshwater faunas. Annual Review of Ecology and Systematics. Volume 33:291-315. Turner L., S. Jacobson, L. Shoemaker. 2007. Risk Assessment for Piscicidal Formulations of Rotenone. Compliance Services International.

SAS Institute Inc. 2008. Configuration Guide for SAS® 9.2 Foundation for Microsoft® Windows®, Cary, NC: SAS Institute Inc.

Trumbo, J., S. Siepmann, and B. Finlayson. 2000. Impacts of rotenone on benthic macroinvertebrate populations in Silver King Creek, 1990-1996. California Department of Fish and Game Pesticide Investigation Unit, Rancho Cordova.

Turner L., S. Jacobson, L. Shoemaker. 2007. Risk Assessment for Piscicidal Formulations of Rotenone. Compliance Services International.

Varley, J. D. 1981. A history of fish stocking activities in Yellowstone National Park between1881 and 1980. Information paper No. 35. Yellowstone National Park. 94 pp.

Vannote, R. L. and B. W. Sweeney. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. The American Naturalist 115(5): 667-695.

Vinson, M.R. and E.C. Dinger. 2006. Effects of rotenone on aquatic invertebrate assemblages in the Virgin River in the vicinity of St. George, Utah. Washington County Water Conservation District, St. George, Utah. 137 pp.

Vinson, M. R., E. C. Dinger, and D. K. Vinson. 2010. Piscicides and invertebrates: after 70 years, does anyone really know? Fisheries 45(2):61-71.

Woods, A. J., J. M. Omernik, J. A. Nesser, J. Shelden, J.A. Comstock, S. H. Azevedo. 2002. Ecoregions of Montana, 2nd edition (color poster with map, descriptive text, summary tables, and photographs). Map scale 1:1,500,000.

Waters T. F. 1965. Interpretation of Invertebrate Drift in Streams. Ecology. 46 (3): 327-334.

Waters T. F. 1972. The drift of stream insects. Annual Review Entomology. 17:253-272.

WDEQ/WQD. 2004. Manual of Standard Operating Procedures for Sample Collection and Analysis. Wyoming Department of Environmental Quality, Water Quality Division, Watershed Program, Cheyenne, WY.

Whelan, J. 2002. Aquatic macroinvertebrate monitoring results of the 1995 and 1996 rotenone treatments of Manning Creek, Utah. Utah Department of Natural Resources, Publication 02-02, Salt Lake City.