LONG-TERM EFFECTS OF ROTENONE ON THE ASSEMBLAGE OF AQUATIC BENTHIC MACROINVERTEBRATES AT A LOCAL LEVEL OF DISTURBANCE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The piscicide rotenone is an important and widely used tool for the conservation of native fishes, but comprehensive evaluations of aquatic benthic macroinvertebrate community response to rotenone exposure are lacking. Therefore, this study investigates the effect of rotenone on aquatic benthic macroinvertebrate (BMI) communities within the context of a watershed-scale restoration effort for westslope cutthroat trout (*Oncorhynchus clarkia lewisi*) conducted between 2007 and 2017. Long term pre- and post-treatment sampling in outlet streams of 13 alpine lakes quantified the response and recovery of BMI exposed to rotenone for periods ranging from weeks to a few months depending on the lake volume and environmental factors that influence the natural neutralization of rotenone. Spatial and temporal quantitative sampling was conducted at the lake outlets for 1 to 6 years before treatment and 2 to 8 years after treatment to capture the variability in density and diversity of the BMI assemblage. Analysis of changes to Ephemeroptera, Plecoptera, Trichoptera (EPT) density and diversity were used as a suitable representative for assessing effects to the entire BMI assemblage. Analysis of variance in EPT density and location shifts in variance in EPT diversity were used to test for significant changes to the EPT assemblage. Significant short-term reductions to EPT density were found 1 year post-treatment at 3 of the 13 lakes, 2 years post-treatment at 1 lake, and no significant reductions found at lakes with 3 or more years of post-treatment monitoring. Significant negative shifts in EPT diversity were found at 2 lakes with only 2 years of post-treatment monitoring, and no significant negative shifts in EPT diversity were found at lakes with 3 or more years of post-treatment monitoring. In all of the study sites, EPT density and diversity returned to baseline conditions within 3 years after treatment, demonstrating resiliency of these taxonomic communities to rotenone exposure.

INTRODUCTION

The piscicide rotenone has been in use as a fisheries management tool in North America since 1934 for quantification of fish populations, control or eradication of unwanted fish species, eradication to control disease, and conservation of native species (McClay 2000). Because rotenone is such an important fisheries management tool and it has the potential for collateral damage to ecologically important nontarget organisms, like aquatic benthic macroinvertebrates (BMI), it is important to gain a better understanding of how much and for how long BMI are affected. This information is critical to build scientific and public support for the continued use of rotenone in fisheries management.

Rotenone is a botanical extract found in many tropical plants in the Leguminosae family (Ling 2003), and is applied to freshwater systems in a variety of manufactured piscicidal products and application methods (Finlayson et al. 2010b). Native people in regions where the plants are indigenous discovered centuries ago how to use the extracts to manipulate fish behavior as a means of harvesting fish for food (Krumholz 1948). Rotenone piscicide products are classified as Restricted Use Pesticides (RUP) due to acute inhalation, acute oral, and aquatic toxicity. Rotenone was approved for reregistration with the U.S. Environmental Protection Agency (EPA) in 2007 for piscicidal use only (USEPA 2007), and is currently undergoing routine reregistration review to incorporate significant research-based technical changes to how rotenone will be used as a tool in fish management. The EPA solicits research-based input from the American Fisheries Society’s Fish Management Chemicals Subcommittee through the Rotenone Stewardship Program, and concerns from various interests including environmental and animal rights groups before issuing a registration decision (AFS

2019). Reviewing new literature related to issues associated with rotenone use is in part the commission of AFS Fish Management Chemicals Subcommittee.

Rotenone affects fish by passing into the blood stream by osmoregulation via the gills and is transported to the cells where it inhibits cellular respiration. An oxygen deficit at the cellular level causes fish to try increasing oxygen absorption through swimming bursts and gulping for oxygen at the water's surface and eventually results in mortality by asphyxiation if the rotenone concentration within the blood is at a lethal level. Rotenone is lethal to salmonid fishes at concentrations as low as 25 parts per billion (ppb) per volume of water (Finlayson et al. 2010a). Unfortunately, rotenone is lethal to BMI at the same concentrations used to eradicate unwanted fish species (Finlayson et al. 2010a). Much like fish, BMI respire through osmoregulatory surfaces such as epithelia, cuticle, chitin, gills, and other structures (Pennak 1989), and undergo the same fate from rotenone toxicity as fish do.

Aquatic invertebrates are an integral component of freshwater ecosystems. They occupy the important role of transferring energy from primary producers to higher trophic levels, and contribute in the release of nutrients back to primary producers (Covich et al. 1999). They are grazers on algae and aquatic macrophytes, predators on smaller invertebrates, and scavengers helping break down decomposing detritus. They are a major food item for many amphibians, reptiles, fish, birds, and mammals. With the understanding of their important roles in ecosystem functions, it is no small concern to question what the potential ecological implications may be from the widespread use of chemicals in fisheries management.

Published laboratory studies have verified the toxicity of rotenone on BMI at the various concentrations used to eradicate fish (Dalu 2015; Engstrom-Heg 1978; Finlayson et al. 2010c). Published field studies have confirmed the laboratory toxicity studies through sampling and comparing BMI taxa richness and abundance before and after fish removal projects using rotenone (Finlayson et al. 2010a; Vinson et al. 2010; Lan Pham et al. 2018). However, published field studies documenting the long-term effect of rotenone on BMI assemblages in natural lotic systems are virtually non-existent. The studies summarized by Vinson et al. (2010) (Table 1) had either very little or no pre-treatment data to compare to the post-treatment results, and none of the studies methods captured the temporal variation in BMI assemblage pre-treatment. Thus, there is an unreliable assessment of the pre-treatment reference condition to compare to the post-treatment results. Post-treatment monitoring was similarly variable, with few studies collecting samples for more than one year. All the field studies report recolonization of common affected taxa, but all the studies stop short at attempting to confirm that BMI assemblages returned to pre-treatment richness and abundance. A relatively recent study in New Zealand streams in 2011 established BMI reference conditions 3 months pre-treatment, and tracked the temporal recovery of BMI densities and taxa richness and community composition changes over a one-year period following rotenone treatment (Lan Pham et al. 2018). Another recent and thorough study in a South African stream monitored the impacts on BMI by conducting a Before-and-After Control-and-Impact (BACI) experimental design for 2 years before treatment and after treatment (Bellingan et al. 2019). This was considered a long-term study, and they found no adverse impacts to the aquatic BMI community. However, this recent long-term study and the studies summarized in Table 1 were from stream treatments where rotenone is typically

Table 1. Summary of published field studies on the effects of rotenone on lotic invertebrates (Vinson et al. 2010:p.65).

Location	Year	Pre-treatment Sampling	Post-treatment Sampling	Observed Changes in Invertebrate Assemblage	Citation
Robinson Creek, CA	1963	None, treated/ untreated comparison.	8 months	10–50% reduction in abundance	Cook and Moore 1969
Green River, UT	1963	2 weeks prior	2 years after	Immediate reduction in abundance of nearly all species. Hydropsychidae (Trichoptera) recovered after 2 yrs., burrowing mayflies extirpated.	Binns 1967
Strawberry River, UT	1990	1 week prior	Annually 5 yrs.	54% decrease in taxa richness after 1 yr., 21% decrease in taxa richness after 5 yrs.	Mangum and Madrigal 1999
Steams in Papau, New Guinea	1990	Immediately prior	Immediately after and then up to 2 yrs.	Significant declines in Dixidae and Hydropsychidae, no change in Leptophlebiidae or in total abundance.	Dudgeon 1999
Silver King Creek, CA	1964-1996	None	Multiple times 1984-2006	Slight reduction in total, Ephemeroptera, Plecoptera, and Trichoptera taxa richness and change in percent dominant taxa.	Trumbo et al. 2000
Manning Creek, UT	1995	1 month prior	1 yr. and 3 yrs.	13% decrease in taxa richness after 3 years.	Whelan 2002
River Ogna, Norway	2001	Just prior	2 mos.	Rapid recolonization of common taxa, a few taxa disappeared.	Kjaerstad and Arnekleiv 2003
Strawberry Creek, Great Basin NP	2000	1 yr. and 1 day prior	1 mo., 9 mo., and 1 yr. after	89% reduction in total taxa richness at 1 month, 22% reduction at 1 year, 4 taxa missing at 1 year, 2 taxa missing at 3 years. 95% reduction in total abundance at 1 month, 47% reduction at 1 year.	Hamilton et al. 2009
Virgin River, UT	2001-2005	None	1 yr.	Little to no change following 2004 and 2005 treatments, study complicated by lack of pre-data and > 20 yrs. of rotenone treatment.	Vinson and Dinger 2006

applied via drip station for as many as 8 hours and then removed. Therefore, the duration of rotenone exposure to the BMI community is short-term, lasting only as long as it takes for the rotenone to clear the stream. Furthermore, treatments were done on stream reaches that are not considered headwater reaches. This is an important factor in that BMI are more likely to rapidly recolonize treated stream reaches from an untreated reach and tributaries upstream.

Unlike all the other studies, this study monitors the response and recovery of the BMI community in 13 lake outlet streams that, in all but 1 of the 13 cases, are considered to be the headwaters of the drainage. In addition, the entire lake volume was treated and exposure time to the stream invertebrates was much longer; i.e. several days, weeks, or even months depending on the lake volume and factors that influence the natural neutralization of rotenone such as dilution and environmental degradation. Furthermore, it is typical in fish removal projects to purposely treat the outlet stream long distances downstream to accomplish the objective of removing most if not all the target fish that have dispersed downstream from the lake population. In most cases, the prolonged exposure to rotenone treated water exiting a lake is considered a benefit in accomplishing the objective of successful complete eradication of unwanted fish species. Depending on the physical and environmental characteristics of the aquatic system treated, rotenone treated water has the potential to travel at lethal concentrations for very long distances downstream. Therefore, a study is warranted that broadens the temporal assessment of the pre-treatment BMI assemblage and tracks the long-term recovery of BMI after a fish removal project using rotenone in a headwater lake with a year-round effluent. We hypothesize that there may be long-term adverse effects to the BMI assemblage beyond 2 years after treatment in a headwater stream that had a prolonged exposure to rotenone-treated lake water.

METHODS

Study Area

This study investigates 15 years of BMI monitoring data collected during a westslope cutthroat trout (*Oncorhynchus clarkia lewisi*) conservation project that treated 13 high mountain lakes with rotenone in Northwest Montana. The lakes and associated stream network were treated to remove mixed and hybridized stocks of cutthroat and rainbow trout populations, and restocked the next year with a pure strain of westslope cutthroat. All the lakes are located in the Swan Mountain Range on the Flathead National Forest; 6 lakes are within the Bob Marshall Wilderness, 5 within the Jewel Basin Hiking Area, 2 outside special designated management, and all lakes drain to the South Fork Flathead River drainage (Figure 1). The lakes were treated from 2007 to 2017 and the data collection period began in 2005 through 2019 (Table 2). All the lakes were treated at the normal use concentration of 1 ppm using a liquid formulation of rotenone under trade names Prenfish or CFT Legumine. Both formulations contain 5% of the active ingredient rotenone.

Monitoring Sites

Samples were collected from the lakes tail-out/riffle habitats at the lake outlets. Seasonal runoff in these habitats is buffered by the lake which produces gradual changes to discharge. Thus, these flowing water habitats are relatively stable, contain coble and gravel substrates typical of riffle habitats, and store large quantities of allochthonous material. Flowing water habitats frequently possess more habitat heterogeneity and increased taxa (Thorp & Covich 1991). Therefore, theoretically the outlet habitats would

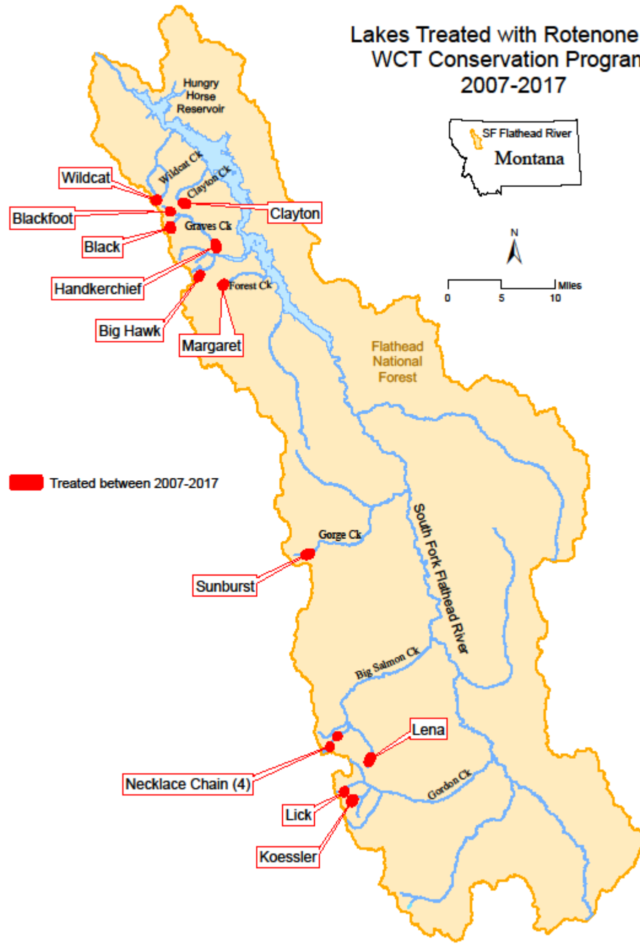

Figure 1. South Fork Flathead Lakes treated with rotenone from 2007 to 2017

contain a diverse host of common lotic and lentic taxa and would receive the longest duration of chronic exposure to rotenone from the treated lake water.

Sampling Method

Quantitative samples were collected from randomly selected areas in the lake outlet habitat using a 0.09 m^2 (1 ft^2) Surber stream bottom sampler with 500-micron mesh. Sample area per sample is 0.18 m^2 (2 ft^2). Vinson (1996) suggests collecting a series of 0.1 m^2 or larger samples per habitat to capture the high spatial heterogeneity of stream BMI. The number of samples collected per sampling date ranged from 1 to 3

Table 2. South Fork Flathead lakes treated with rotenone from 2007 to 2017.

Year Treated	Lake	Land Management	Size (Acres)	Max Depth (ft)	Volume (Acre Feet)
2007	Blackfoot	JBHA	16	22	205
	Black	JBHA	49	157	4,493
2008	Bighawk	JBHA	27	41	612
2009	Margaret	FNF	46	79	1,962
	Clayton	JBHA	62	193	6,948
2010	Wildcat	JBHA	40	83	2,066
2011	Necklace #5	BMW	14	21	54
	Necklace #8	BMW	14	30	153
2012	Lick	BMW	19	26	141
2013	Lena	BMW	74	80	2,682
2014	Koessler	BMW	86	173	5,731
2016	Handkerchief	FNF	51	24	517
2017	Sunburst	BMW	148	220	12,407

FNF (Flathead National Forest Non-wilderness), JBHA (Jewel Basin Hiking Area), BMW (Bob Marshall Wilderness).

samples (sample area 2 ft^2 to 6 ft^2). Samples were collected from a nearby control site for 2 of the 13 lake treatments (Handkerchief and Sunburst). Replicate samples were collected monthly during June, July, August and September for one to six years before rotenone application. Post-treatment samples were collected the following year after treatment and replicated seasonally and annually for 2 to 5 years to monitor the response, recolonization, and recovery of the BMI community (Table 3). A total of 86 pre-treatment samples and 187 post-treatment samples were collected. Note: The pre- and post-treatment sampling was not necessarily conducted over consecutive years. For example, at Blackfoot Lake pre-treatment samples were collected in 2005 and 2007, and post-treatment samples collected 2008, 2009, 2010, 2011, 2015. The seasonal and annual temporal sampling captures the variability in BMI assemblage (Underwood 1994). An exception to seasonal sampling was made for the 6 lakes located in the wilderness where remoteness and scheduling limited sampling to one visit per year usually in August.

Additionally, the 2019 sample data for Handkerchief Lake and Aeneas Creek control site are unavailable at this time. Thus, the analyses for both Handkerchief and Sunburst Lake have only 2 years of post-treatment monitoring data. Annual post-treatment monitoring is ongoing for both sites and for at least for 1 additional year at Lena and Koessler Lake. Samples were fixed with 95% ethyl alcohol in Nalgene bottles and transported back to a lab for sorting and identification. Sample specimens were identified primarily to genus and, when possible, to species thus using the lowest level of identification for each taxon and treating each taxon (family, genus, or species) as equivalent. Texts used for identification were Brown (1976), Merritt et al. (2008), Pennak (1989), Stewart & Stark (1993), Thorp & Covich (1991), and Wiggins (1996).

Table 3. Number of years and samples collected for each lake pre- and post-rotenone treatment.

Lake	No. Samples Pre	*No. Years Sampled Pre	No. Samples Post	*No. Years Sampled Post
Black	3	1	13	5
Blackfoot	6	2	15	5
Big Hawk	3	1	14	5
Margaret	6	1	10	5
Clayton	2	2	13	5
Wildcat	5	2	16	5
Necklace #5	4	4	11	5
Necklace #8	4	4	7	3
Lick	3	3	8	4
Lena	4	4	7	3
Koessler	5	5	7	3
Handkerchief	17	6	27	2
Aeneas creek (Control)	9	1	27	2
Sunburst	9	5	6	2
Inspiration creek (Control)	6	2	6	2
Totals	86		187	

* not necessarily consecutive.

Analyses

The sample data were initially analyzed with stacked bar charts which showed immediate changes to post-treatment abundance and taxa richness of all observed taxa at all of the lakes. There were unquestionably immediate adverse changes to Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) (EPT) abundance and taxa richness. However, apart from EPT, adverse changes in abundance and richness were not observed to BMI taxa that are relatively tolerant to environmental disturbance, such as Diptera (i.e. Chironomidae, Empedidae, Simuliidae, Tipulidae) and other common taxa (i.e. Coleoptera, Hemiptera, Odonata, Gastropoda, Ostracoda, Pelecypoda, Oligochaeta, Nematoda, Tubellaria). In fact, in many cases the dipterans increased in abundance the first year after rotenone treatment and progressively decreased to pre-treatment proportional abundance as EPT abundance and richness progressively increased through the monitoring period. Therefore, the analysis for this study focused on the impacts to the relative abundance (density) and taxa richness (diversity) of the EPT assemblage at each lake and control site. The EPT community is considered the most sensitive to changes in water quality (Mitchell & Stapp 1997), thereby analyzing EPT data to detect shifts in density and diversity are reasonable measures for assessing the long-term adverse effects to the BMI assemblage from rotenone. Density was calculated as the total number of EPT specimens per 2 ft^2 sample, and a diversity metric calculated as the total number of EPT taxa per 2 ft^2 sample (Hayslip 2007). All the pre- and post-treatment EPT density and diversity data are listed in Appendix A. Statistical analyses and plots were produced in R (R Core Team 2018).

The EPT pre-treatment density data were initially analyzed for normality at each lake and control site. Pre-treatment density data were grouped and analyzed using a Shapiro-Wilk Normality Test, $p > 0.05$, to accept the null hypothesis that no significant departure from normality in density data is found. Next, boxplots comparing the distributions of the grouped pre-treatment and grouped annual post-treatment samples were produced for visual analysis. A Kruskal-Wallis non-parametric ANOVA model was employed to compare variance in distributions between the grouped pre-treatment samples and the grouped annual post-treatment samples, followed by a Dunn pairwise test for significance between all sample groups, $p > 0.05$, to accept the null hypothesis that no difference in variance is found. The Kruskal-Wallis model is a rank-sum test method for coping with serious outlying observations (Ramsey & Schafer 2013), which is a common observation in the EPT density data, and for comparing multiple groups of independent random samples.

The EPT diversity data for each lake and control site were initially analyzed with boxplots for visual analysis of the distributions in diversity data between the individually grouped pre-treatment and grouped post-treatment samples. Next, a Levene's test was employed to test for homogeneity of variance between the grouped pre- and post-treatment samples. The grouped pre-treatment and grouped post-treatment sample data were compared for equality of variance, $p > 0.05$, to accept the null hypothesis that no difference in variance is found between pre- and post-treatment sample groups. The Levene's (median) test for equality of two variances is based on deviations from the median and is a robust test of equality of variances of two populations that depart from normality (Ramsey & Schafer 2013). Lastly, a Wilcoxon test was employed to analyze for significant location shifts, $p \leq 0.05$, in distribution of diversity data between the pre- and post-treatment sample groups. The Wilcoxon test is a distribution-free signed-ranked test for comparing two independent random groups of samples. The test uses the ranks of the magnitude of the differences in addition to their signs and is resistant to outliers (Ramsey & Schafer 2013).

RESULTS

EPT Density

Results of the Shapiro-Wilk Normality Test for sites where pre-treatment sample sizes are small (n=3-9), no significant departure from normality, $p > 0.05$, was found in EPT density. Where the pre-treatment sample size is relatively more robust, e.g. Handkerchief lake (n=18), with 95% confidence the EPT density data do not fit a normal distribution, $p \leq 0.05$ (Table 4).

Results from the ANOVA suggest with 95% confidence that there were significant differences, $p \leq 0.05$, in EPT density between pre- and post-treatment samples at 5 of the 15 sites (Table 4). The significant result is either from a significant decrease in EPT density 1 to 2 years post treatment, or a significant long-term increase in density post treatment. More specifically, 3 of the 5 lakes (Black, Handkerchief, Sunburst) had a significant decrease in EPT density between pre- treatment and 1 year post treatment, and Sunburst 2 years post treatment. The other 2 lakes with a significant difference (Blackfoot and Margaret) had a significant increase in EPT density between pre-treatment and one or more post-treatment groups. The Dunn pairwise test sorted out significance between the paired pre-treatment post-treatment sample groups. The density boxplots in Figures 2 through 8 illustrate the results of the Kruskal-Wallis test.

Based on the analysis, we can infer that there were short-term adverse effects to the EPT density for 1 to 2 years post treatment at 3 of the 13 lakes monitored. However, EPT density returned to pre-treatment levels or higher 3 years post treatment for those 11 lakes that have 3 or more years of post-treatment monitoring, and 12 lakes with 2 or more years post-treatment monitoring — Sunburst Lake being the only exception with only 2 years of post-treatment monitoring.

EPT Diversity

With the exceptions at Necklace #5 and Lick Lake, the results from Levene's test for homogeneity of variance between the grouped pre-treatment samples and grouped post-treatment samples suggest that no significant difference in variance, $p > 0.05$, was found in EPT diversity between pre- and post-treatment groups (Table 4). Significant differences at both Necklace #5 and Lick are a result of variance being greater pre-treatment. The results from the Wilcoxon test suggest with 95% confidence that there was a significant location shift, $p \leq 0.05$, in diversity post-treatment at 3 of the 13 lakes (Wildcat, Handkerchief, Sunburst). There was a significant negative shift at Handkerchief and Sunburst, and a significant positive shift at Wildcat. The diversity boxplots in Figures 2 through 8 illustrate the results from the Levene's test for equal variance and the Wilcoxon test for location shifts in diversity.

Based on the analysis, we can infer that there were short-term adverse effects to EPT diversity at 2 of the 13 lakes (Handkerchief and Sunburst). Again, both lakes have only 2 years of post-treatment monitoring, so no assessment of long-term effects to EPT diversity can be made at this time. We can also infer that no adverse effects to EPT diversity were found at the other 11 lakes where there is 3 or more years of post-treatment monitoring.

Control Sites

Results from the control locations were mixed. Results from the Dunn pairwise test suggest with 95% confidence that a significant change was found, $p \leq 0.05$, in EPT density between pre-treatment and 1 year post-treatment in Inspiration creek, and no significant changes to EPT density post-treatment in Aeneas creek. Additionally, results from the pairwise test for Aeneas creek suggest with 95% confidence there was a significant increase, $p \leq 0.05$, in EPT density between pre-treatment and 2 years post-treatment. Results from the Wilcoxon test suggest with 95% confidence that there was a significant positive location shift, $p \leq 0.05$, in diversity post-treatment in Aeneas creek, and no significant shifts found in EPT diversity in Inspiration creek. The density and diversity boxplots in Figures 8 and 9 illustrate the results from the Kruskal-Wallis test and Wilcoxon test.

The fact that no significant changes to EPT density and diversity at the Handkerchief Lake control site (Aeneas Creek) were found suggests that other environmental factors apart from rotenone exposure likely do not account for the significant changes to EPT density and diversity post treatment at Handkerchief Lake. However, significant changes to EPT density at the Sunburst Lake control site (Inspiration Creek) suggest that some environmental factor significantly changed EPT density 1 year post treatment at the control site. We believe this is a false positive that other environmental factors apart from rotenone could account for the change to EPT density and diversity post treatment at Sunburst Lake. The false positive is likely the result of the sampling location at Inspiration Creek being relatively close to the nearest point of rotenone application. The stream distance from the closest application point was approximately 80-m downstream of the sampling location. In contrast, the stream distance from closest application point to the Aeneas Creek control site during the Handkerchief Lake treatment was approximately 650 m. We believe that the significant changes to EPT density 1 to 2 years post treatment at the Inspiration Creek control site are likely due to poor judgement in selecting a sampling site in close proximity to rotenone application. We conclude that selecting a sampling site to control for environmental factors apart from treatment is very important to prevent the potential for false positives.

Table 4. P-values for EPT density and diversity statistical analysis.

	EPT Density			EPT Diversity	
Lake	Sample Size	Normality*	K-W ANOVA	Homogeneity of Variance	Wilcoxon
Black	3	0.10	0.03	0.15	0.64
Blackfoot	6	0.47	0.02	0.26	0.35
Big Hawk	3	0.87	0.11	0.45	0.37
Margaret	6	0.62	0.02	0.12	0.26
Clayton	2	N/A**	0.25	0.20	0.23
Wildcat	5	0.90	0.09	0.60	0.04
Necklace #5	4	0.30	0.10	0.02	0.32
Necklace #8	4	1.00	0.17	0.80	0.09
Lick	3	0.67	0.10	0.05	0.47
Lena	4	0.24	0.06	0.45	0.44
Koessler	5	0.88	0.26	0.61	0.80
Handkerchief	18	0.001	0.02	0.39	0.04
Aeneas creek (Control)	9	0.76	0.07	0.78	0.006
Sunburst	9	0.92	0.006	0.26	0.002
Inspiration creek (Control)	6	0.17	0.07	0.50	0.93

* Pre-treatment samples only. Minimum sample size = 3
** Sample size too small for normality test.

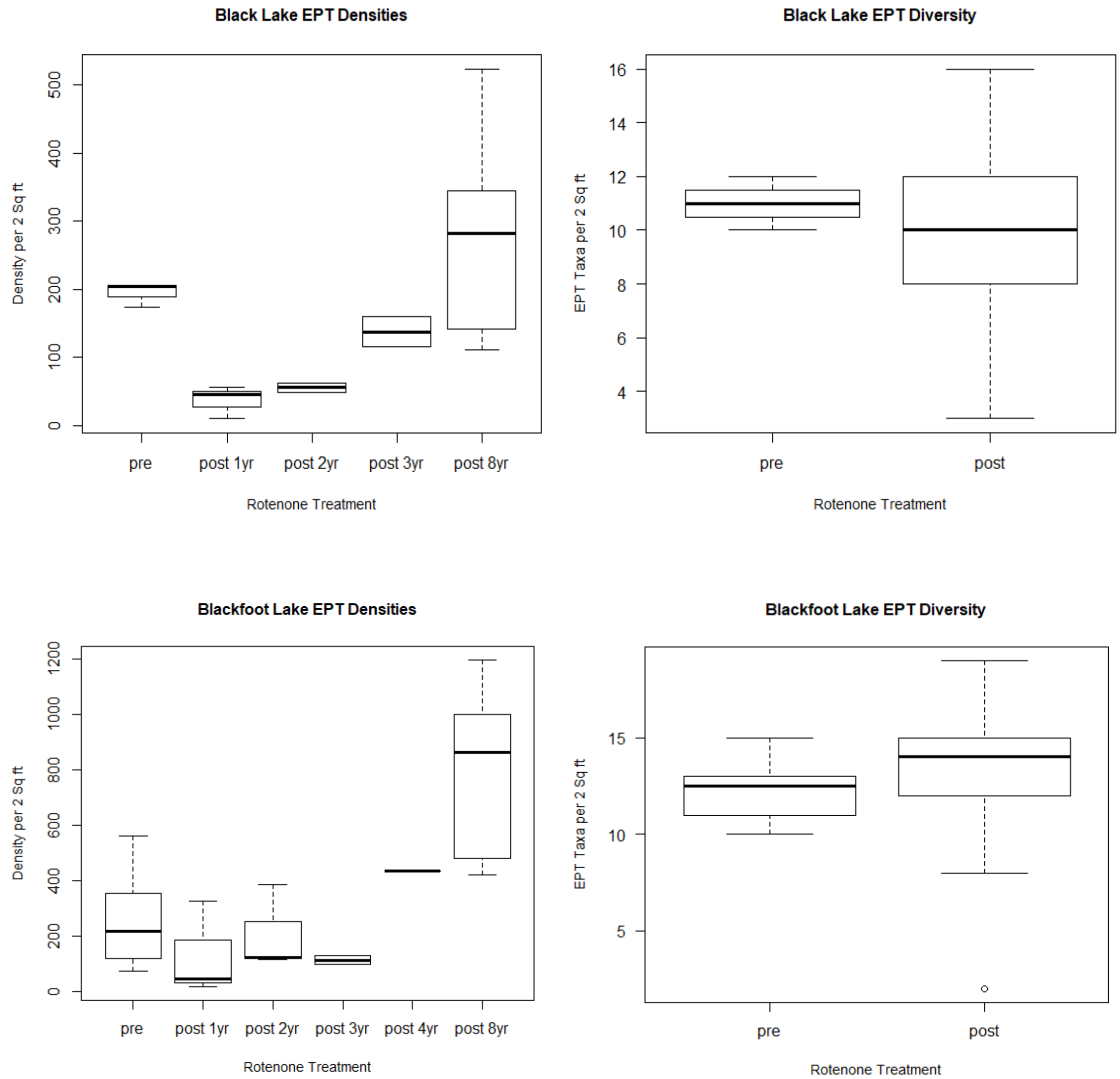

Figure 2. Boxplots of EPT densities and diversity pre- and post-treatment for Black and Blackfoot Lake.

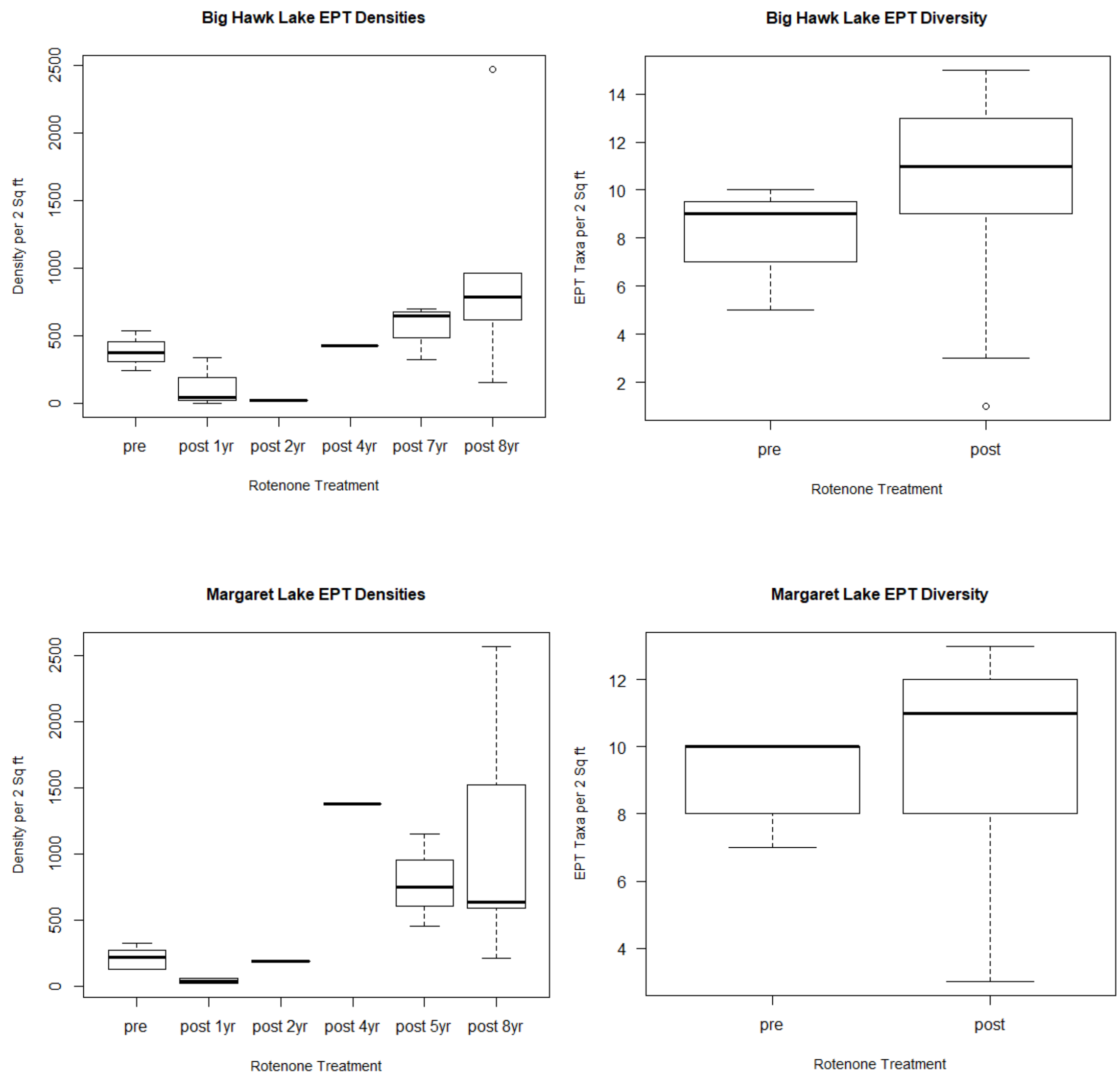

Figure 3: Boxplots of EPT densities and diversity pre- and post-treatment for Big Hawk and Margaret Lake.

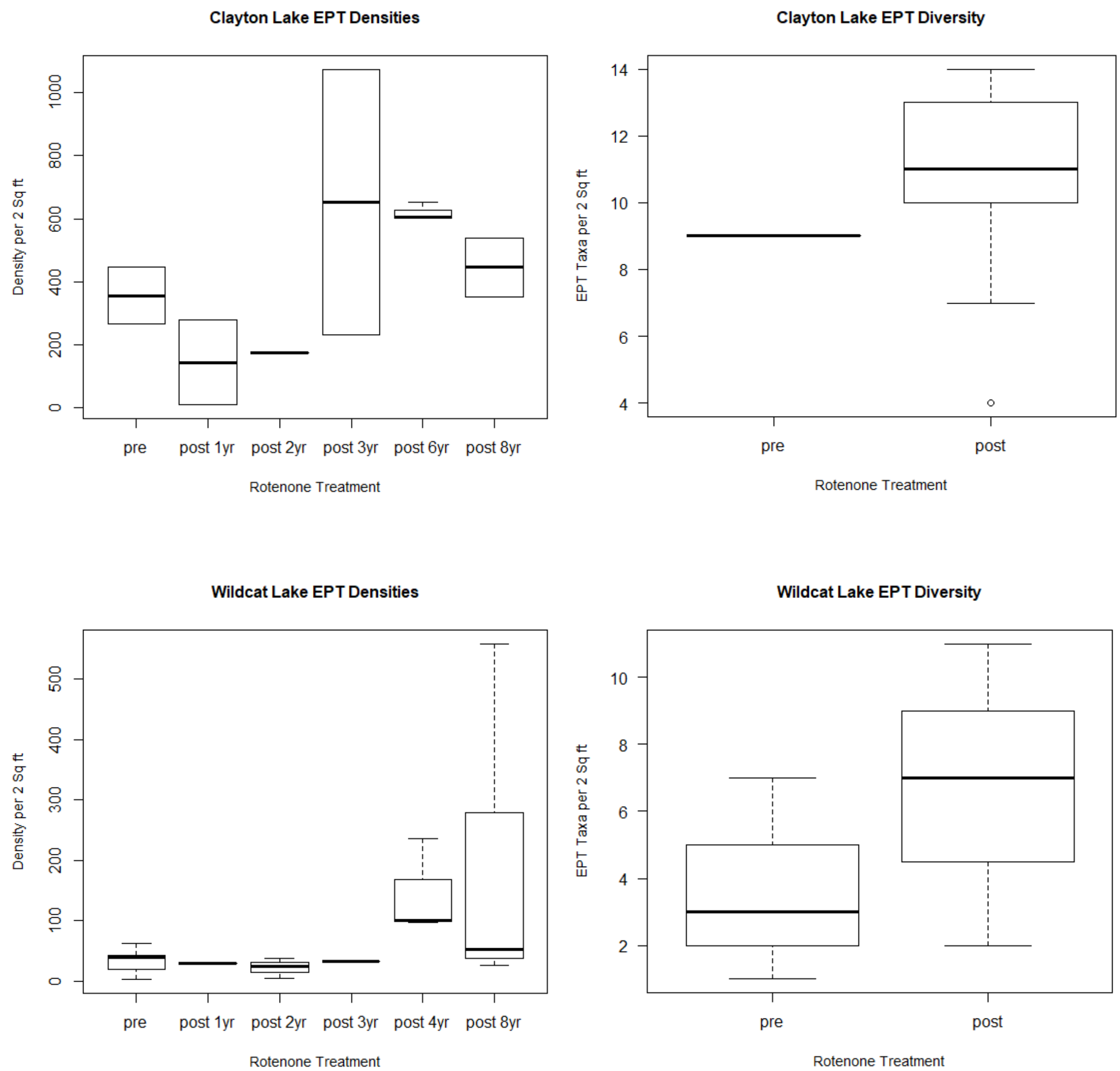

Figure 4: Boxplots of EPT densities and diversity pre- and post-treatment for Clayton and Wildcat Lake.

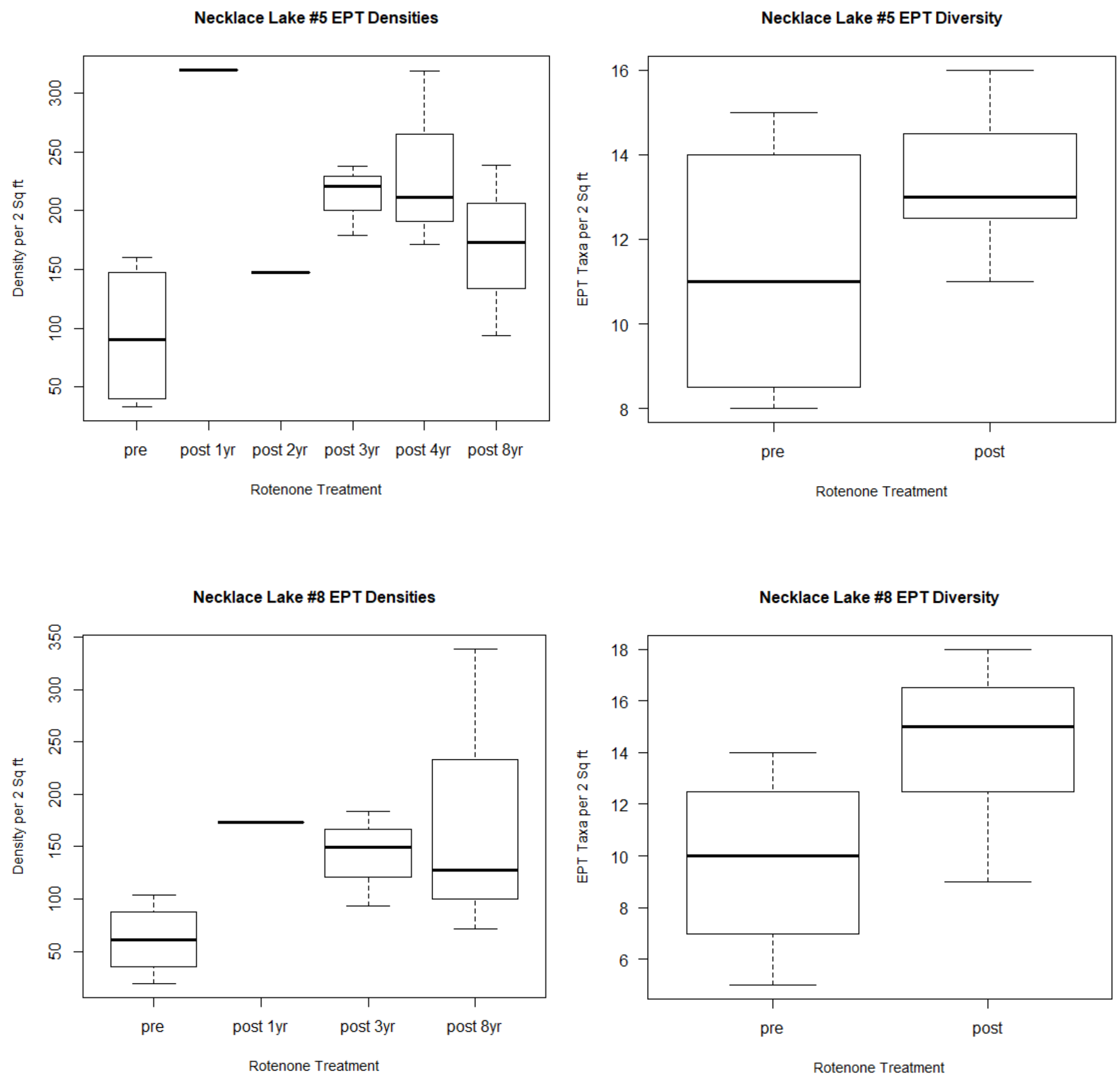

Figure 5: Boxplots of EPT densities and diversity pre- and post-treatment for Necklace #5 and Necklace #8 Lake.

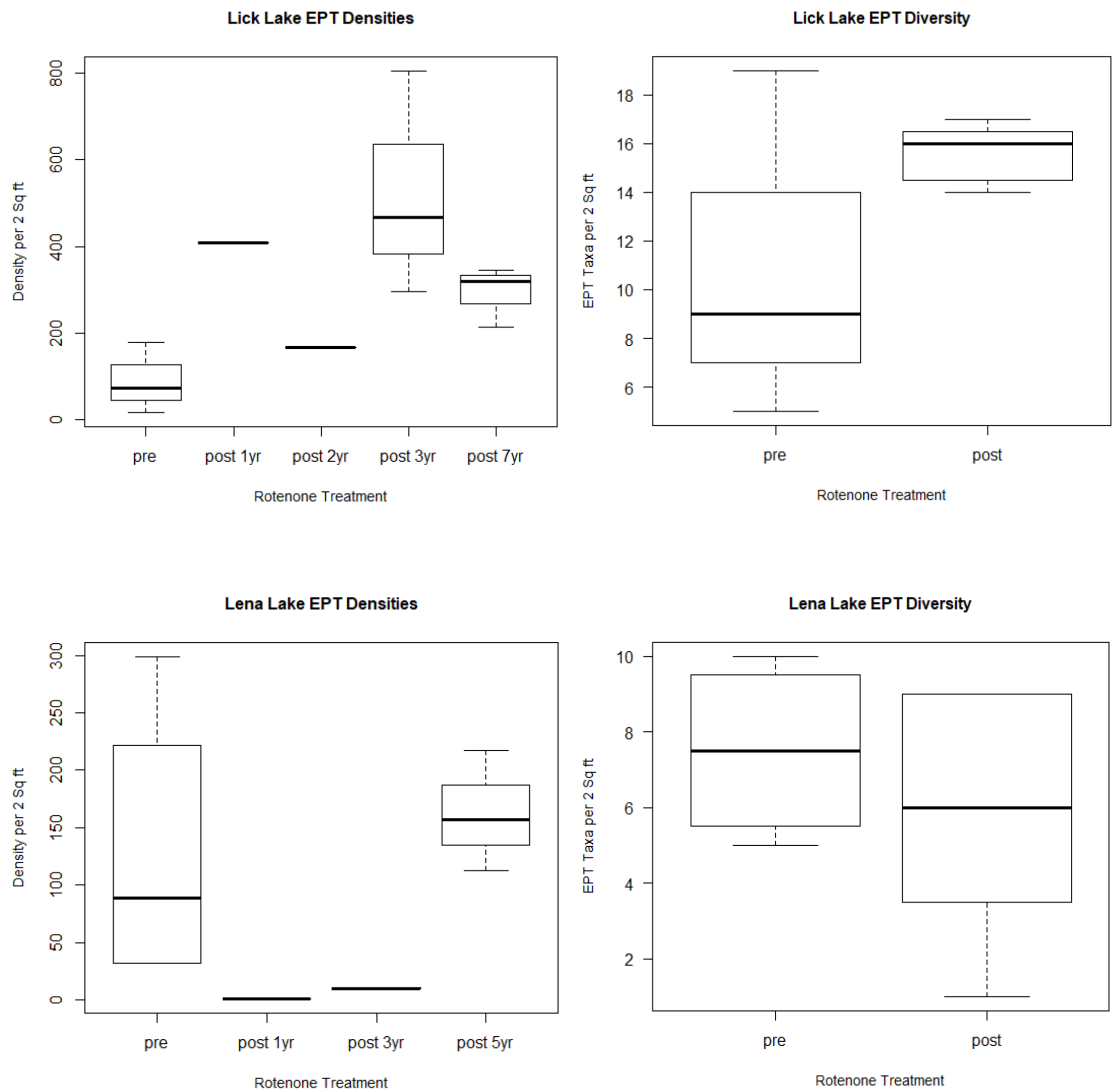

Figure 6: Boxplots of EPT densities and diversity pre- and post-treatment for Lick and Lena Lake.

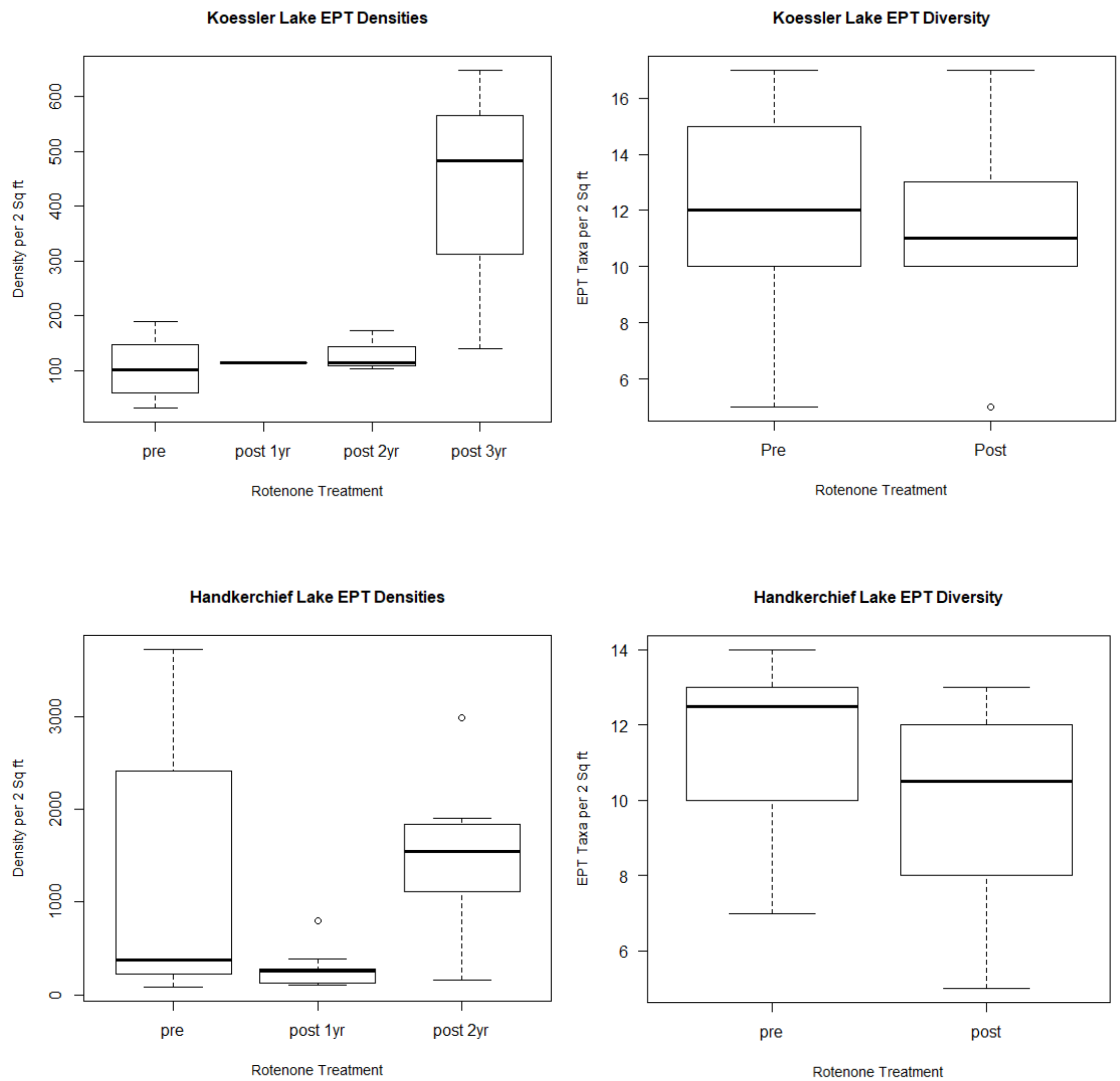

Figure 7: Boxplots of EPT densities and diversity pre- and post-treatment for Koessler and Handkerchief Lake.

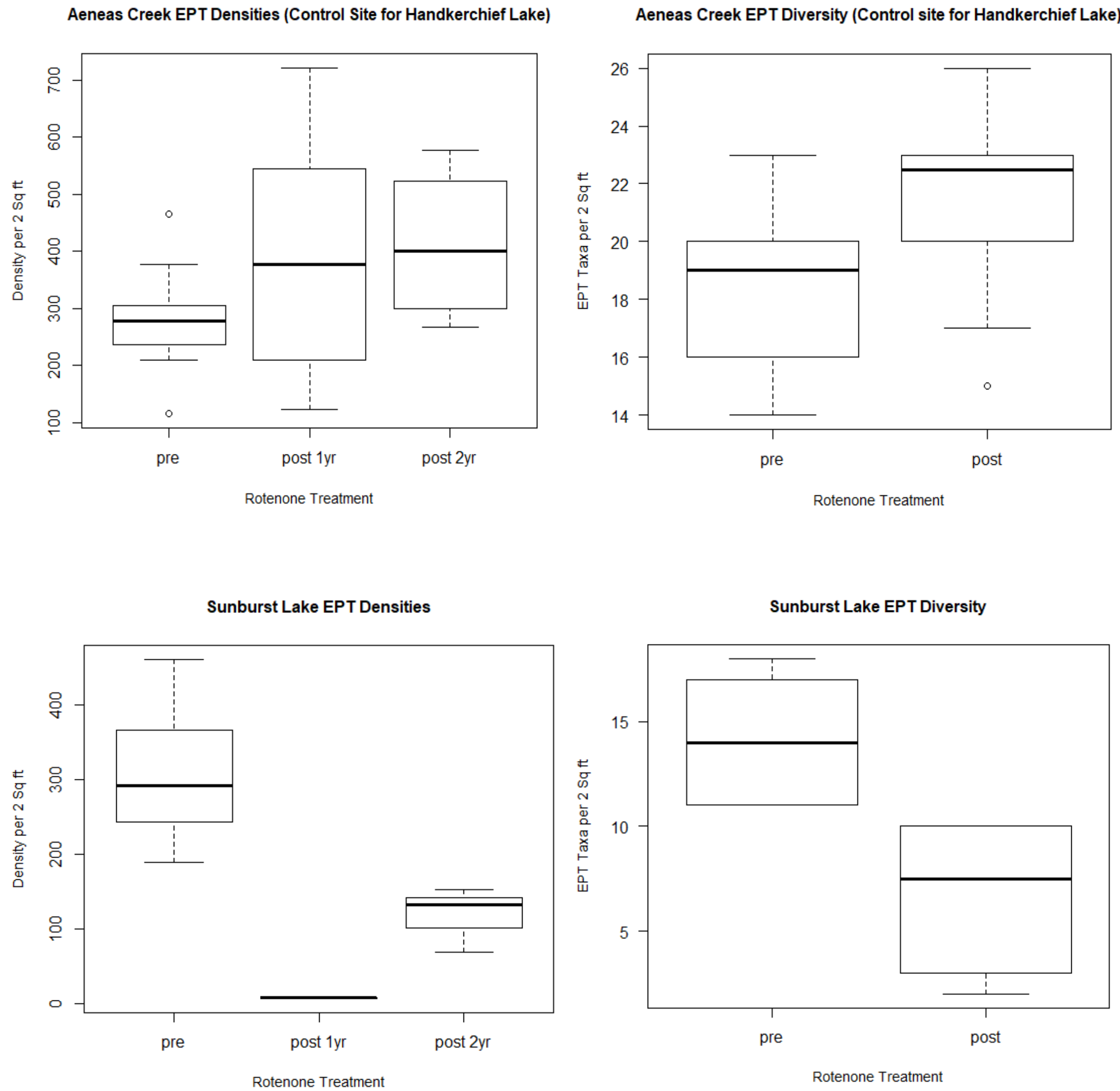

Figure 8: Boxplots of EPT densities and diversity pre- and post-treatment for Aeneas Creek and Sunburst Lake.

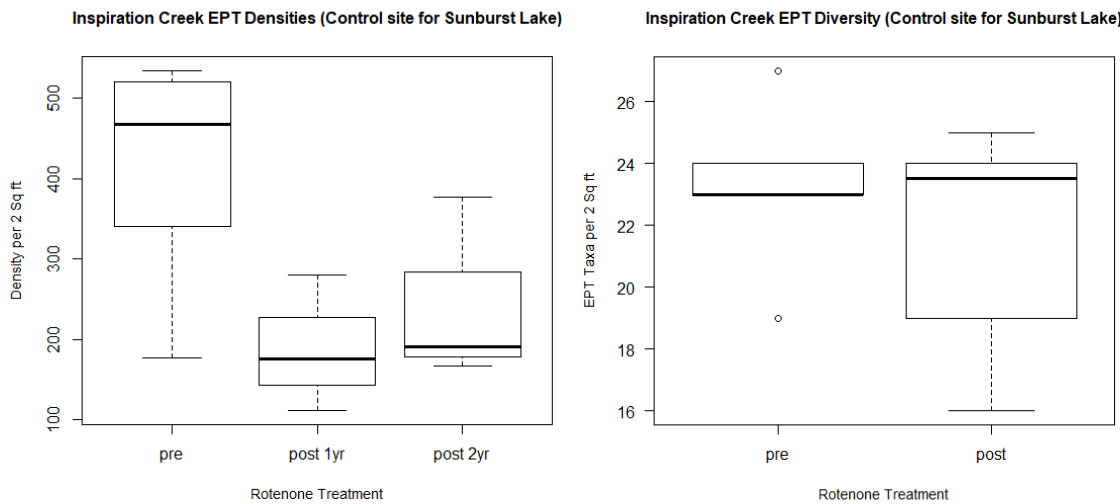

Figure 9: Boxplots of EPT densities and diversity pre- and post-treatment for Inspiration Creek.

The data analysis provides strong evidence that there are likely short-term adverse effects to EPT density and diversity after rotenone treatment. However, the analysis also provides strong evidence that there are likely no long-term adverse effects to EPT density and diversity after treatment. EPT seem to be very resilient in recolonizing headwater habitats within 1 year after a prolonged disturbance to water quality from rotenone application; and BMI assemblages recover to similar density and diversity within 2 to 3 years after rotenone treatment. Thus, the BMI assemblages recovered with no adverse effects to density and diversity at all the lakes with 3 or more years of post-treatment monitoring. Therefore, based on the sampling and analysis methods used in this study we conclude that no long-term effects to EPT density and diversity were found in the lake outlet streams. Thus, it can be inferred that the BMI assemblages recovered with no long-term adverse impacts from the normal use of rotenone to remove unwanted fish species from headwater lakes in Northwest Montana.

DISCUSSION

It is apparent that prolonged, localized rotenone exposure to BMI in headwater streams is not sufficient to have long-term adverse effects on the BMI assemblage at the local level of disturbance in Northwest Montana. Recovery of EPT assemblages in headwater streams is likely due to early recolonization from winged adults dispersing from untreated waters in nearby drainages and/or from lower untreated reaches and tributaries within the drainage. The linear distances between treated mountain lake headwaters and the closest untreated waters are easily overcome by the dispersal mechanisms and reproduction strategies possessed by EPT.

BMI life history attributes of dispersal and reproduction aid in rapid recolonization and recovering assemblages back to pre-disturbance condition. This resilience serves to rapidly restore the ecological processes for which aquatic BMI contribute. Therefore, fishery managers can be confident that the aquatic systems will recover in reasonable time and provide sufficient and stable sources of food to sustain the restocked fish populations 1 year after rotenone treatment. Furthermore, other natural resource managers and citizen interest groups can be confident that aquatic systems that have been treated with rotenone will recover and overall ecosystem health will recover to pre-treatment conditions within 1 to 2 years.

Based on the sampling methods used in this study and the results they produced, our study confirms what other studies have reported, that there are no long-term adverse effects to the aquatic BMI assemblages from the normal use of rotenone to remove unwanted fish populations. However, this study has limitations in its methods of sample collection. We recognize that sampling effort across the data collection period from 2005 to 2019 was inconsistent in the number of samples collected, sample area, and number of years of pre-treatment sampling. Pre-treatment sampling effort was recognized as minimal at best for the first 5 years of the collection period, so sampling effort was increased to strengthen the confidence in the results. The decision to include control site sampling and choosing an appropriate location should have been made earlier in the collection period to give consistency and strength in the results from those locations as well.

A broader application from our results could be used to make assumptions about the short and long-term effects to aquatic BMI from other prolonged chemical-related disturbances to water quality from point and non-point contamination sources such as: residential, commercial, industrial and agricultural land-use practices, or human-caused catastrophic spills.

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APPENDICES

APPENDIX A

EPT DENSITY AND DIVERSITY DATA PRE- AND POST-TREATMENT FOR ALL LAKES AND CONTROL SITES.

Lake	Treatment	Date Sampled	EPT Density (2 ft^2)	EPT Diversity (2 ft^2)
Black	pre	Jun-07	204	12
	pre	Jul-07	206	10
	pre	Aug-07	173	11
	post 1yr	Jul-08	10	3
	post 1yr	Aug-08	45	6
	post 1yr	Sep-08	56	7
	post 2yr	Jul-09	62	10
	post 2yr	Aug-09	49	8
	post 3yr	Aug-10	115	9
	post 3yr	Sep-11	160	11
	post 8yr	Jun-15	345	13
	post 8yr	Jun-15	220	16
	post 8yr	Jun-15	524	15
	post 8yr	Aug-15	142	11
	post 8yr	Aug-15	111	10
	post 8yr	Aug-15	344	12
Blackfoot	pre	Jun-05	73	10
	pre	Jul-05	144	12
	pre	Aug-05	562	11
	pre	Jun-07	120	15
	pre	Jul-07	355	13
	pre	Aug-07	292	13
	post 1yr	Jun-08	17	2
	post 1yr	Aug-08	326	12
	post 1yr	Sep-08	46	8
	post 2yr	Jul-09	121	13
	post 2yr	Aug-09	384	14
	post 2yr	Sep-09	114	12
	post 3yr	Jun-10	99	15
	post 3yr	Aug-10	128	9
	post 4yr	Sep-11	434	14
	post 8yr	Jul-15	998	17
	post 8yr	Jul-15	788	15

	post 8yr	Jul-15	933	19
	post 8yr	Aug-15	422	17
	post 8yr	Aug-15	1196	15
	post 8yr	Aug-15	481	13
Big Hawk	pre	Jul-08	374	5
	pre	Aug-08	245	9
	pre	Sep-08	537	10
	post 1yr	Jul-09	1	1
	post 1yr	Aug-09	46	3
	post 1yr	Sep-09	338	9
	post 2yr	Jul-10	23	4
	post 4yr	Aug-12	430	12
	post 7yr	Aug-15	323	11
	post 7yr	Aug-15	652	14
	post 7yr	Aug-15	701	11
	post 8yr	Jul-16	155	9
	post 8yr	Jul-16	673	11
	post 8yr	Jul-16	619	9
	post 8yr	Aug-16	963	13
	post 8yr	Aug-16	909	15
	post 8yr	Aug-16	2476	14
Margaret	pre	Jun-05	276	8
	pre	Jul-05	128	7
	pre	Aug-05	233	10
	pre	Jul-09	130	10
	pre	Aug-09	328	10
	pre	Sep-09	203	10
	post 1yr	Jul-10	21	3
	post 1yr	Aug-10	60	8
	post 2yr	Jul-11	187	8
	post 4yr	Jul-13	1381	10
	post 5yr	Jul-14	454	13
	post 5yr	Jul-14	751	8
	post 5yr	Jul-14	1156	8
	post 8yr	Jul-17	594	12
	post 8yr	Jul-17	664	11
	post 8yr	Jul-17	212	13
	post 8yr	Aug-17	606	12
	post 8yr	Aug-17	1526	13
	post 8yr	Aug-17	2574	12
Clayton	pre	Jul-09	265	9
	pre	Aug-09	446	9

	post 1yr	Jul-10	10	4
	post 1yr	Aug-10	278	7
	post 2yr	Jul-11	175	10
	post 3yr	Jul-12	232	11
	post 3yr	Aug-12	1074	14
	post 6yr	Jul-15	604	11
	post 6yr	Jul-15	651	11
	post 6yr	Jul-15	601	13
	post 8yr	Jul-17	353	13
	post 8yr	Jul-17	538	14
Wildcat	pre	Jul-09	40	2
	pre	Aug-09	19	3
	pre	Jul-10	3	1
	pre	Aug-10	63	7
	pre	Sep-10	43	5
	post 1yr	Sep-11	29	3
	post 2yr	Jul-12	25	5
	post 2yr	Aug-12	38	4
	post 2yr	Sep-12	5	5
	post 3yr	Aug-13	33	7
	post 4yr	Sep-14	100	8
	post 4yr	Sep-14	98	11
	post 4yr	Sep-14	237	8
	post 8yr	Jul-18	40	4
	post 8yr	Jul-18	54	7
	post 8yr	Jul-18	37	2
	post 8yr	Aug-18	26	9
	post 8yr	Aug-18	104	9
	post 8yr	Aug-18	53	6
	post 8yr	Sep-18	559	9
	post 8yr	Sep-18	454	10
Necklace #5	pre	Aug-08	33	9
	pre	Aug-09	134	8
	pre	Aug-10	47	13
	pre	Aug-11	160	15
	post 1yr	Aug-12	320	15
	post 2yr	Aug-13	147	12
	post 3yr	Aug-14	179	15
	post 3yr	Aug-14	221	13
	post 3yr	Aug-14	238	14
	post 4yr	Aug-15	171	13
	post 4yr	Aug-15	211	11

	post 4yr	Aug-15	319	12
	post 8yr	Aug-19	173	16
	post 8yr	Aug-19	239	13
	post 8yr	Aug-19	94	13
Necklace #8	pre	Aug-08	19	5
	pre	Aug-09	71	11
	pre	Aug-10	51	9
	pre	Aug-11	104	14
	post 1yr	Aug-12	173	17
	post 3yr	Aug-14	149	12
	post 3yr	Aug-14	93	13
	post 3yr	Aug-14	184	15
	post 8yr	Aug-19	71	9
	post 8yr	Aug-19	339	16
	post 8yr	Aug-19	128	18
Lick	pre	Aug-07	179	9
	pre	Aug-11	16	5
	pre	Aug-12	73	19
	post 1yr	Aug-13	408	17
	post 2yr	Aug-14	166	16
	post 3yr	Aug-15	467	16
	post 3yr	Aug-15	296	14
	post 3yr	Aug-15	806	17
	post 7yr	Aug-19	346	16
	post 7yr	Aug-19	214	14
	post 7yr	Aug-19	320	15
Lena	pre	Aug-08	32	6
	pre	Aug-09	33	5
	pre	Aug-12	299	9
	pre	Aug-13	145	10
	post 1yr	Aug-14	1	1
	post 3yr	Aug-16	11	2
	post 3yr	Aug-16	10	5
	post 3yr	Aug-16	10	6
	post 5yr	Aug-18	217	9
	post 5yr	Aug-18	157	9
	post 5yr	Aug-18	113	9
Koessler	pre	Aug-07	59	5
	pre	Aug-11	31	10
	pre	Aug-12	147	15
	pre	Aug-13	190	17
	pre	Aug-14	101	12

	post 1yr	Jul-15	114	10
	post 2yr	Jul-16	172	11
	post 2yr	Jul-16	113	10
	post 2yr	Jul-16	103	5
	post 3yr	Aug-17	649	17
	post 3yr	Aug-17	483	15
	post 3yr	Aug-17	140	11
Handkerchief	pre	Jul-10	81	9
	pre	Aug-10	191	10
	pre	Jul-11	327	7
	pre	Jul-12	125	11
	pre	Aug-12	371	14
	pre	Jul-13	379	14
	pre	Aug-13	897	12
	pre	Jul-14	222	11
	pre	Jul-14	701	13
	pre	Jul-16	380	13
	pre	Jul-16	83	10
	pre	Jul-16	332	10
	pre	Aug-16	2298	14
	pre	Aug-16	2594	13
	pre	Aug-16	2932	9
	pre	Sep-16	2416	14
	pre	Sep-16	3730	13
	pre	Sep-16	2470	13
	post 1yr	Jul-17	391	9
	post 1yr	Jul-17	255	9
	post 1yr	Jul-17	108	8
	post 1yr	Aug-17	800	12
	post 1yr	Aug-17	279	13
	post 1yr	Aug-17	102	12
	post 1yr	Sep-17	128	7
	post 1yr	Sep-17	279	5
	post 1yr	Sep-17	180	7
	post 2yr	Jul-18	155	11
	post 2yr	Jul-18	711	13
	post 2yr	Jul-18	1545	13
	post 2yr	Aug-18	1840	10
	post 2yr	Aug-18	1686	8
	post 2yr	Aug-18	2986	11
	post 2yr	Sep-18	1330	13
	post 2yr	Sep-18	1902	7

	post 2yr	Sep-18	1118	11
Aeneas creek (Control for Handkerchief)	pre	Jul-05	209	15
	pre	Aug-05	278	17
	pre	Jul-16	221	15
	pre	Jul-16	116	14
	pre	Jul-16	280	20
	pre	Aug-16	324	19
	pre	Aug-16	254	17
	pre	Aug-16	255	20
	pre	Sep-16	465	23
	pre	Sep-16	288	20
	pre	Sep-16	377	21
	post 1yr	Jul-17	209	19
	post 1yr	Jul-17	157	15
	post 1yr	Jul-17	123	17
	post 1yr	Aug-17	305	20
	post 1yr	Aug-17	545	24
	post 1yr	Aug-17	413	24
	post 1yr	Sep-17	568	22
	post 1yr	Sep-17	722	23
	post 1yr	Sep-17	377	20
	post 2yr	Jul-18	310	21
	post 2yr	Jul-18	300	23
	post 2yr	Jul-18	400	23
	post 2yr	Aug-18	267	20
	post 2yr	Aug-18	292	21
	post 2yr	Aug-18	523	26
	post 2yr	Sep-18	471	23
	post 2yr	Sep-18	566	23
	post 2yr	Sep-18	577	25
Sunburst	pre	Aug-13	189	18
	pre	Aug-15	287	18
	pre	Aug-16	347	16
	pre	Aug-16	385	11
	pre	Aug-16	296	11
	pre	Aug-17	221	14
	pre	Aug-17	266	14
	pre	Aug-17	461	11
	post 1yr	Aug-18	9	2
	post 1yr	Aug-18	6	3
	post 1yr	Aug-18	7	5
	post 2yr	Aug-19	152	10

	post 2yr	Aug-19	132	10
	post 2yr	Aug-19	69	10
Inspiration creek (Control for Sunburst)	pre	Aug-16	457	23
	pre	Aug-16	341	23
	pre	Aug-16	521	24
	pre	Aug-17	177	19
	pre	Aug-17	479	23
	pre	Aug-17	535	27
	post 1yr	Aug-18	175	19
	post 1yr	Aug-18	111	16
	post 1yr	Aug-18	280	25
	post 2yr	Aug-19	166	24
	post 2yr	Aug-19	190	23
	post 2yr	Aug-19	377	24