EFFECTS OF BROMACIL, DIURON, GLYPHOSATE, AND SULFOMETURON-METHYL ON PERIPHYTON ASSEMBLAGES AND RAINBOW TROUT

Final Report

SPR PROJECT 392

EFFECTS OF BROMACIL, DIURON, GLYPHOSATE, AND SULFOMETURON-METHYL ON PERIPHYTON ASSEMBLAGES AND RAINBOW TROUT

State Planning and Research 392

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16. ABSTRACT

This study documents the testing of several common herbicides used by the Oregon Department of Transportation in vegetation management. The project assessed the short- and long-term effects of Roundup, Krovar and Oust on periphyton and rainbow trout. The active ingredient in Roundup is glyphosate; Krovar uses bromacil and diuron; and Oust uses sulfometuron-methyl. Short-term (96 hour) exposure tests used actual road shoulder runoff collected after herbicide application, using a simulated rain and a natural rain event. Long-term exposure tests assessed effects of a 14-day exposure using lab-mixed solutions of deionized lab water and herbicides, individually and in mixture.

The data showed that the short-term exposure had no statistically significant effects on periphyton. The short-term exposure reduced survivorship of rainbow trout, but the effects were observed both in treated and untreated runoff; thus the toxicity was likely due to other factors.

The long-term exposure tests showed that herbicides, especially Krovar and the mixture of three chemicals, reduced periphyton algal biomass. The declined trend in biomass was more evident in live cell density than in chlorophyll a concentration, suggesting that algal responses to chemicals may vary among groups (green algae vs. diatoms). The long-term exposure had no statistically significant effects on fish mortality and dry weight. Individual herbicide bioassays showed no significant differences between the changes in wet weight, but significant differences in wet weight were found between treatments in the mixture bioassay.

The study showed that periphyton assemblages could be altered by some chemicals. While rainbow trout fish showed no statistical effects for dry weight, the effect on other sublethal endpoints remains a possibility.

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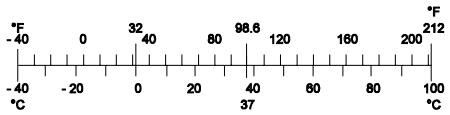
SI* (MODERN METRIC) CONVERSION FACTORS

APPROXIMATE CONVERSIONS TO SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
In	Inches	25.4	Millimeters	Mm
Ft	Feet	0.305	Meters	M
Yd	Yards	0.914	Meters	M
Mi	Miles	1.61	Kilometers	Km
		AREA		
in^2	Square inches	645.2	millimeters	mm^2
ft^2	Square feet	0.093	meters squared	M^2
yd^2	Square yards	0.836	meters squared	M^2
Ac	Acres	0.405	Hectares	Ha
mi^2	Square miles	2.59	kilometers squared	Km^2
		VOLUME		
fl oz	Fluid ounces	29.57	Milliliters	ML
Gal	Gallons	3.785	Liters	L
ft^3	Cubic feet	0.028	meters cubed	m^3
yd^3	Cubic yards	0.765	meters cubed	m^3
NOTE: Volumes greater than 1000 L shall be shown in m^3.				
		MASS		
Oz	Ounces	28.35	Grams	G
Lb	Pounds	0.454	Kilograms	Kg
T	Short tons (2000 lb)	0.907	Megagrams	Mg
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5(F-32)/9	Celsius temperature	°C

APPROXIMATE CONVERSIONS FROM SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
mm	Millimeters	0.039	inches	in
m	Meters	3.28	feet	ft
m	Meters	1.09	yards	yd
km	Kilometers	0.621	miles	mi
		AREA		
mm^2	millimeters squared	0.0016	square inches	in^2
m^2	meters squared	10.764	square feet	ft^2
ha	Hectares	2.47	acres	ac
km^2	kilometers squared	0.386	square miles	mi^2
		VOLUME		
mL	Milliliters	0.034	fluid ounces	fl oz
L	Liters	0.264	gallons	gal
m^3	meters cubed	35.315	cubic feet	ft^3
m^3	meters cubed	1.308	cubic yards	yd^3
		MASS		
g	Grams	0.035	ounces	oz
kg	Kilograms	2.205	pounds	lb
Mg	Megagrams	1.102	short tons (2000 lb)	T
		TEMPERATURE (exact)		
°C	Celsius temperature	1.8C + 32	Fahrenheit	°F



* SI is the symbol for the International System of Measurement

(4-7-94 jbp)

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DISCLAIMER

EFFECTS OF BROMACIL, DIURON, GLYPHOSATE, AND SULFOMETURON-METHYL ON PERIPHYTON ASSEMBLAGES AND RAINBOW TROUT

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EFFECTS OF BROMACIL, DIURON, GLYPHOSATE, AND SULFOMETURON-METHYL ON PERIPHYTON ASSEMBLAGES AND RAINBOW TROUT

EXECUTIVE SUMMARY

Roundup (EPA registration number 524-445), Krovar (EPA registration number 352-352), and Oust (EPA registration number 352-401) are common herbicides that the Oregon Department of Transportation (ODOT) has been using to control roadside vegetation. Water quality criteria do not exist for these compounds. There is limited ecotoxicological data for these herbicides and even less information on the toxicity of these chemicals in complex mixtures.

This project was designed to assess the effects of environmental concentrations of these herbicides on periphyton and rainbow trout. The compounds tested in this project were Roundup with the active ingredient glyphosate; Krovar with the active ingredients bromacil and diuron; and Oust with the active ingredient sulfometuron-methyl. The project consisted of both short-term and long-term exposure tests. The short-term exposure tests assessed the effects on periphyton and rainbow trout of a 96-hour exposure, using actual road shoulder runoff collected after herbicide application. A section of the roadside was sprayed with herbicides using ODOT procedures, and herbicides runoff was collected after a simulated rain and a natural rain event. The long-term exposure tests were designed to assess effects of 14-day exposure using lab-mixed solutions of deionized lab water and selected herbicides, individually and in mixture, based on environmental concentrations on periphyton and rainbow trout.

The data showed that short-term exposure had no statistically significant effects on periphyton assemblages. The short-term exposure did reduce the survivorship of rainbow trout but the effects were observed in runoff from both treated areas and areas untreated with herbicides during the study. Therefore, direct toxicity was probably a result of other factors affecting the quality of runoff.

The long-term exposure tests showed that herbicides, especially Krovar and the mixture of three chemicals, reduced periphyton algal biomass. Declined trend in algal biomass was more evident in live cell density than chlorophyll a concentration, suggesting that algal responses to chemicals may vary among groups (green algae vs. diatoms). The long-term exposure had no statistically significant effects on fish mortality and dry weight. The individual herbicide bioassays showed no significant differences between the changes in wet weight. However, changes in mean rainbow trout wet weight were significant between treatments in the mixture bioassay.

The study showed that periphyton assemblages could be altered by some chemicals. Rainbow trout fish showed no statistical effects for dry weight but the effect on other sublethal endpoints remains a possibility.

1.0 INTRODUCTION

This project was conducted to help the Oregon Department of Transportation (ODOT) determine how roadside herbicide applications affect the ecosystems in nearby streams. The information will provide guidance to their Best Management Practices (BMPs). In 1991 ODOT established the Integrated Vegetation Management Program to control unwanted vegetation growth. An integral part of the program is the use of herbicides. Roundup (EPA registration number 524-445), Krovar (EPA registration number 352-352), and Oust (EPA registration number 352-401) are common herbicides that ODOT has been using to control roadside vegetation. In 1999, ODOT used 5,228 L (1,381 gal) of Roundup, 1,096 kg (2,416 lbs) of Krovar, and 38.5 kg (85 lbs) of Oust.

The U.S. Geological Survey (USGS) in 1996 found a variety of herbicides in Willamette Valley streams *(Anderson et al. 1997)*. In several streams, the same herbicides that were used by ODOT were detected. The detection of these chemicals in Willamette Valley streams, and the recent addition of several salmonid species to the federal threatened and endangered list, raised concerns about the effects of the herbicides on stream ecosystem integrity. Additional information on the aquatic toxicology of these chemicals was necessary to evaluate their effects on aquatic resources.

The compounds tested in this project were Roundup, with the active ingredient glyphosate; Krovar, with the active ingredients bromacil and diuron; and Oust, with the active ingredient sulfometuron-methyl. Water quality criteria do not exist for these compounds. There is limited ecotoxicological data for these herbicides and even less information on the toxicity of these chemicals in complex mixtures. Despite the lack of criteria for these compounds it is important to know the significance of detectable herbicides and their risk to aquatic life. Bioassays are one method for determining the potential effects of these herbicides on aquatic resources.

This project was designed to assess the effects of environmental concentrations of these herbicides on periphyton and rainbow trout. The project consisted of a short-term and a long-term exposure test. The short-term exposure test was designed to assess the effects to periphyton and rainbow trout of a 96-hour exposure, using actual road shoulder runoff collected after herbicide application. A section of the roadside was sprayed with herbicides using ODOT procedures, and herbicide runoff was collected after a simulated rain and a natural rain event. The long-term exposure test was designed to assess effects of 14-day exposure on periphyton and rainbow trout. It used a lab-mixed solution of deionized laboratory water and selected herbicides, individually and in mixture, based on environmental concentrations.

This project is unique because it used environmentally-relevant concentrations of herbicides, individual and complex mixtures of herbicides, a combination of test organisms at different trophic levels (fish vs. algae), and a combination of a single-species (rainbow trout) and multiple-species assemblages (periphyton). Rainbow trout were chosen because of the sensitivity of this

species to contaminants, and the availability of Environmental Protection Agency (USEPA) protocols *(USEPA 1991)*. This species is also important to the Pacific Northwest, and their pollutant sensitivity is more comparable to other salmonid species that have high socioeconomic importance such as coho salmon and cutthroat trout than that of fathead minnows or other species traditionally used in bioassays.

Periphyton are ideal to detect low-dose herbicide concentrations and their cumulative effects in streams. Herbicides, by definition, have adverse effects on photosynthetic organisms. Algae with a short generation time have the highest sensitivity to herbicide contamination *(Solomon et al. 1996)*. They are species-rich and each species may respond to herbicides according to their own physiological characteristics *(see review by Hoagland et al. 1996)*. They are ubiquitous; thus, algal indicators developed from one area may likely be applicable for other regions. Algae form a base for the food web in streams and are tightly coupled to dynamic trophic interactions in streams *(Pan and Lowe 1994)*. Therefore, infererence of herbicide impacts on stream ecosystems can be made from algal responses.

2.0 METHODS

2.1 SHORT-TERM EXPOSURE TESTS: EFFECTS OF HERBICIDE RUNOFF ON PERIPHYTON AND RAINBOW TROUT

2.1.1 Collection of Herbicide Runoff

Researchers with the USGS Oregon Water District designed two studies to collect herbicide runoff. The first study collected runoff after a typical herbicide application and a simulated rain event in the spring of 1999. The study site was the roadside of Highway 211 at Bull Creek near Colton, Oregon. The roadside was divided into two sections and each section was further divided into three plots. One section was sprayed with herbicides while the other section was not sprayed and served as a control. Runoff was collected from each plot in each section at intervals of one day, one week, and two weeks after the herbicide application and the simulated rain event. A total of 8 L (2.1 gal) of runoff were collected and sent to the lab for both fish and algae toxicity tests. In the fall of 1999, a second study collected runoff from treatment and control drainage ditches, and Bull Creek stream samples above and below the road. More detailed information on the study designs, collection methods, and detected herbicide concentrations in the runoff can be found in "Herbicide Use in the Management of Roadside Vegetation, Western Oregon, 1999-2000: Effects on the Water Quality of Nearby Streams." *(Wood 2001)*.

2.1.2 Experimental Designs

A one-way analysis of variance (ANOVA) experimental design was used to test the effects of herbicide runoff on fish and periphyton. Both the fish and periphyton toxicity tests included four treatments for the natural rain event and three treatments for the simulated rain event. For the natural rain event, the treatments were: upper stream water in Bull Creek, downstream water (below the Highway 211), runoff from herbicide plots, and runoff from control plots. For the simulated rain event, the treatments were: control with ambient stream water, runoff from herbicide plots, and runoff from control plots. All treatments had three replicates. Each short-term exposure test lasted for 96 hours.

2.1.3 Fish Bioassay Experimental Procedure

The methods used for the rainbow trout bioassay were according to the USEPA protocol *(1991)*. The bioassay was conducted as follows: approximately 4 L (1 gal) of control, stream, or plot water were placed for 96 hours in a 5 L (1.3 gal) glass aquarium with juvenile rainbow trout between the age of 15 to 30 days. There were a minimum of ten individuals per replicate, with each treatment performed in triplicate. Basic water chemistry such as dissolved oxygen, pH, temperature, conductivity, and hardness were monitored daily. The number of dead fish was recorded every 24 hours. Test temperatures were approximately 12^{o}C (53.6°F).

2.1.4 Periphyton Bioassay Experimental Procedure

All experiments were conducted in cylindrical recirculating Plexiglas laboratory stream chambers with a diameter of 25 cm (9.85 in); holding a volume of 3 L (0.8 gal). The chambers contained 2 L (0.53 gal) of water collected from the field plots. Each stream chamber was placed on a magnetic plate and the water was circulated continuously by a magnetic stir bar in the chamber to simulate the flow in natural streams. Water temperature, light intensity, and photoperiod were adjusted to simulate the conditions in the natural stream.

Prior to the experiment, clay tiles (5.29 cm^2 or 0.82 in^2) were placed in Eagle Creek in the Clackamas River Basin and colonized by periphyton for three weeks to establish natural periphyton communities *(Pan and Lowe 1994)*. The tiles were retrieved from the creek and a minimum of nine randomly selected tiles were placed in each chamber. Periphyton samples were assayed for chlorophyll a (chl a)[1] and species composition according to standard methods *(APHA 1992)*. Chl a concentrations were analyzed fluorometrically following disruption of cells (by grinding) and extraction with acetone *(APHA 1992)*. At least 500 diatom valves were counted and identified at 1000X magnification.

2.1.5 Data Analysis

Analysis of variance (ANOVA) was used to assess if herbicide runoff had significant effects on fish mortality and algal assemblages ($p=0.05$) *(Zar 1999)*. If the null hypothesis was rejected ($p<0.05$), a multiple comparison test was conducted to test which treatment significantly differed from the others.

2.2 LONG-TERM EXPOSURE TESTS: EFFECTS OF ROUNDUP, KROVAR, AND OUST INDIVIDUALLY AND IN MIXTURE ON PERIPHYTON AND RAINBOW TROUT

2.2.1 Experimental Designs

Four rainbow trout bioassays and four periphyton bioassays were run separately, one for each individual herbicide (n=3) and one for a mixture of the three herbicides. Each rainbow trout bioassay had five treatments. The individual herbicide assays had concentrations of: 0.0, 0.1, 1.0, 10.0, and 100.0 μg/L (parts per billion). The mixture-of-herbicides assays had concentrations that were: 0.0, 0.3, 2.6, 25.5, and 255.0 μg/L. The mixture of herbicides used in the bioassays was the same as the ratios used during ODOT application; which were 1.5:1.8:0.2 for Roundup, Krovar, and Oust, respectively. The concentrations chosen for these assays encompass the environmental concentrations found in Wood's study *(Table 13, 2001)*. Wood *(2001)* estimated that theoretical concentrations of herbicides in Bull Creek, resulting from a hard rainfall of 7.6 mm/hr (0.3 in/hr) between the first day and 2 weeks after herbicide application to the road shoulder, may vary from

[1] Chlorophyll a (chl a) is the green steroid pigment of autotrophic algae and plant cells, which is the receptor of light energy in photosynthesis.

0.06 to 1.5 µg/L for diuron, 0.01 to 0.6 µg/L for sulfometuron-methyl, and 0.02 to 1.8 µg/L for glyphosate, respectively.

Each periphyton bioassay had four treatments (due to limited numbers of the experimental apparatus). The concentrations the individual herbicide assays were: 0.0, 1.0, 10.0, and 100.0 µg/L. The concentrations for the mixture of herbicides assay were: 0.0, 2.6, 25.5, and 255.0 µg/L. All of the treatments in both fish and periphyton assays were performed in triplicate (Figure 2.1).

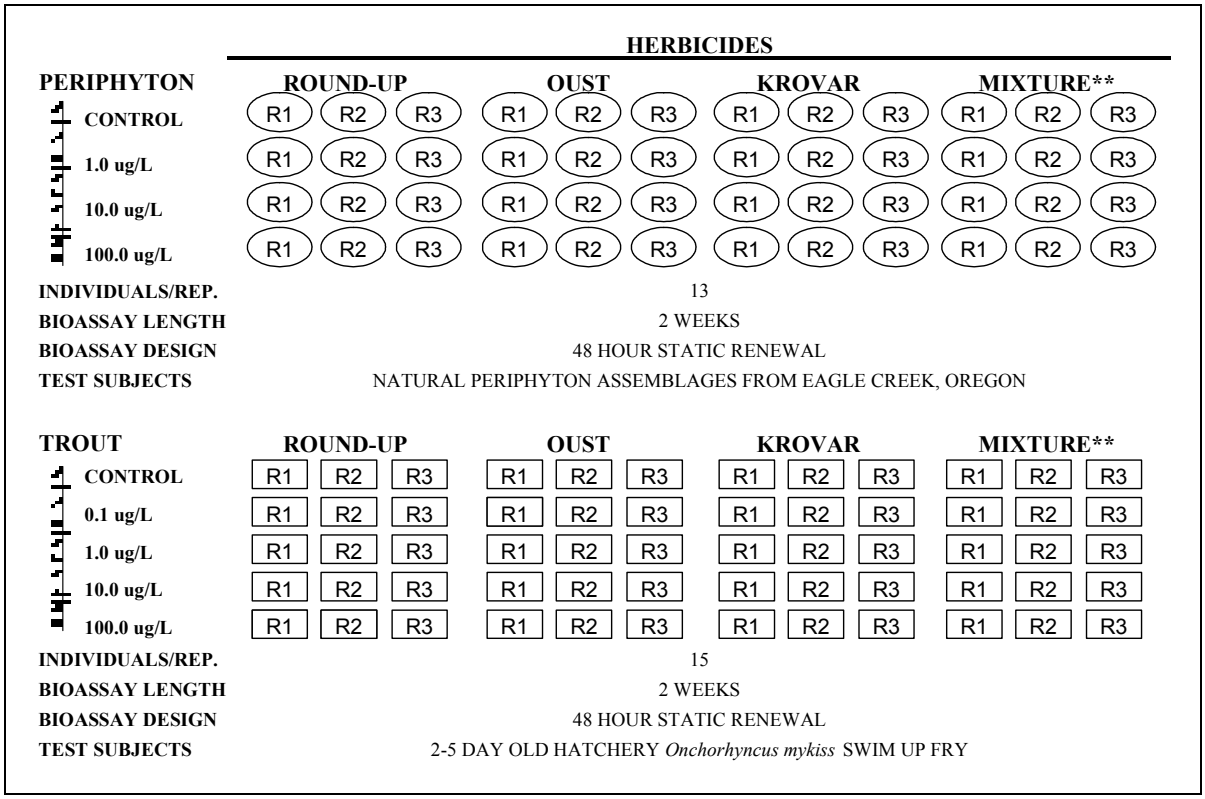

Figure 2.1: Experimental design of periphyton and rainbow trout assays depicting concentrations, replicates, experimental parameters, and sources of test subjects
(** Mixture assays had concentrations 2.55 times the concentrations of the individual herbicide assays.)

2.2.2 Fish Assay Experimental Procedure

These procedures were a modified format of the USEPA *Oncorhynchus mykiss* and *Salvelnus fontinalis* "7-day Survival and Growth Test Method" *(Lazorchak 2002)*. Rainbow trout 2-5 day post-swim up fry were obtained from Leaburg and Roaring River hatcheries. These fish represent a sensitive life stage, which has a low natural mortality rate. The fish were collected 48 hours prior to each assay and allowed to acclimate. There were no mortalities during the acclimation process. Temperature, pH, dissolved oxygen, conductivity, and hardness were measured at the hatcheries during each pickup for comparison to laboratory conditions. Fish for each replicate were weighed as a group at the beginning of each assay (Mettler PB300).

Reconstituted water was made in 946 L (250 gal) batches. Moderately hard water was prepared by adding $NaHCO_3$, $CaSO_42H_2O$, $MgSO_4$, and KCl to deionized water according to EPA guidelines *(Marking and Dawson 1975)*. Submerged pumps were used to keep the solution mixed and aerated.

Commercial grade herbicides were acquired from ODOT. Roundup (glyphosate), Krovar (diuron and bromacil), and Oust (sulfometuron-methyl) were used in their commercial form (active ingredient with surfactants and inert ingredients). Concentrations used in the bioassays were based on the commercial form. Stock solutions of each individual herbicide were made by dissolving the herbicide into deionized water just before the beginning of each assay. These stock solutions were added directly to the treatments to give the appropriate concentration at each renewal.

Herbicide concentrations were verified for the selected treatments to assure target concentrations were within range. Composite samples of the three replicates were sent to Pacific Agricultural Laboratories. All compounds were tested according to EPA or Monsanto methodologies.

The bioassays were conducted in a temperature controlled cold room at DEQ. Glass aquaria holding 18.9 L (5 gal) were arranged on shelves in close approximation to each other. The treatments and fish were randomly assigned to aquaria. Black plastic was attached to the outside of the aquaria to prevent interaction between the fish in separate aquaria.

The bioassays were 14-day, 48-hour static renewal. The two-week duration was used based on the persistence reported by Wood *(2001)*. The 48-hour renewal process was performed to maintain an adequate physical chemical water quality and to replenish the herbicide lost due to uptake, absorption, or volatilization. For each renewal, 13.5 L (3.56 gal) of the test solution was removed from the aquaria with a peristaltic pump. Reconstituted water was then pumped into the tanks, and the appropriate amount of herbicide stock solution was added. The tanks did not require aeration, which decreased the volatilization of the herbicides.

The aquaria were monitored for temperature (YSI 550), dissolved oxygen (YSI 550), and pH (Beckman 250) daily to ensure consistent conditions. Conductivity (YSI 30) and hardness (LaMotte Kit) were measured at the beginning of each assay, and when a new batch of reconstituted water was prepared to assure consistent conditions. The cold room temperature was variable between assays, but was generally consistent for each assay. The temperature was 15-18°C (59-65°F) for the assay using Oust. The temperature ranged 5-17°C (41-63°F) for the assay using Krovar, due to a failure in the air conditioning unit. The temperature was 9-11°C (48-52°F) for the Roundup assay and the mixture assay. The photoperiod was 12 light:12 dark for all of the assays.

The fish were fed 0.3 g (0.01 oz) of feed (Bio-diet fish feed) approximately 30 minutes following the renewal. This was based on a 3% body-weight feeding regime that is often used by hatchery programs. Any mortality during the assays were noted, then removed from the aquaria and frozen in a -20°C (-4°F) freezer.

Prior to termination of an assay, the fish were held off feed for 24 hours to reduce stress. The fish were weighed as a group while alive (Mettler PB300) to determine their growth over the two-week bioassay. A lethal dose of MS-222 was then used to kill the fish, which were frozen immediately, stored at -20°C (-4°F) for approximately three months and then held at -80°C (112°F) until analysis.

Rainbow trout assay endpoints include:

1. Mortality: As individuals died during the bioassays, they were removed, aquaria and treatment were noted, and they were frozen at -20°C (-4°F). Cumulative mortality for each aquarium was used to represent the mortality for each replicate.
2. Wet weights: The fish were weighed (Mettler PB300) as a group for each replicate before the first dosing, and then weighed at the termination of the assay. The difference was calculated as the wet weight change. The wet weight change is referred to as "wet weight" in this document.
3. Dry weights: The fish were removed from the freezer and weighed as a group on pre-combusted tins using a precision scale (Mettler Toledo DeltaRange AT261). They were then dried in a 60°C (140°F) oven for 24 hours and weighed again. The dried remains were scraped off the tins, weighed and stored at -20°C (-4°F).

2.2.3 Periphyton Assay Experimental Procedure

Unglazed ceramic tiles (5.29 cm^2 or 0.82 in^2) were attached to 5.1 x 20.3 x 61.0 cm [H x W x L] (2 x 8 x 24 in) concrete blocks with clear silicone rubber sealant. The tiles were used as a known area that provided periphyton with a uniform surface to colonize. The blocks were placed in Eagle Creek and were left to colonize for 4-6 weeks. They were all placed within 50 m (164 ft) of each other in the same riffle habitat unit, in 20-30 cm (8-12 in) of water.

Upon transport, two blocks were removed from the stream and placed in coolers covered by 2.5-5.0 cm (1-2 in) of water. Temperature, pH, dissolved oxygen, conductivity, and hardness of the Eagle Creek water were measured during each collection to ensure that laboratory conditions were comparable to the stream conditions to which the algae were accustomed.

The assays began within an hour of arrival at the DEQ cold room. The outer rim of tiles was discarded to avoid any possible edge effects on the block. Two-liter (0.53 gal), plastic, circular stream chambers were filled to 1.5 L (0.40 gal) with reconstituted water. Tiles were then randomly distributed to each stream chamber until each chamber contained 13 tiles. Each tile was inspected for chironomids (Order: *Diptera,* Family: *Chironomidae*) using both the naked eye and the dissecting scope. Chironomids were removed, counted, and preserved in 100% ethanol to reduce the grazing of periphyton.

The reconstituted water was made and maintained in the same manner as the water used for the rainbow trout bioassays. The same methods were used to mix, store, and administer the herbicides as was previously done with the rainbow trout bioassays. Herbicide concentrations were not verified in the test solutions for the periphyton bioassays due to limited resources.

Nutrients were added to the stream chambers after every dosing. This was done to create an environment for the periphyton to continue growth. $NaNO_3$ and $NaH_2PO_4H_2O$ were dissolved in deionized water at the beginning of every assay in a ratio of 8:1 (N:P). After each static renewal the stream chambers were dosed with nutrients.

The stir bars were set to spin on a medium velocity, dosed with the appropriate herbicide(s), and spiked with $NaNO_3$ and $NaH_2PO_4H_2O$. Fluorescent grow lights were put on automatic timers with a photoperiod of 12 light:12 dark and placed 8 cm (3.1 in) above the surface of the water in the chambers. The temperature of the cold room was consistently 10-12°C (50-53.6°F) for all four of the assays.

Temperature, pH, and dissolved oxygen were measured regularly to ensure consistency from day to day and tank to tank. Conductivity and hardness were measured at the beginning of each assay and at the initial use of a new batch of reconstituted water.

Each bioassay was a two-week long, 48-hour static renewal. The 48-hour renewal process was performed to supply fresh aerated water and account for any degradation or uptake in the tanks. For each renewal, the fluorescent lights were removed and water parameters measured. Chironomids were removed, and 1.35 L (0.36 gal) of water was removed by a peristaltic pump. The tanks were filled to 1.50 L (0.40 gal) with reconstituted water. Herbicide stock solution was added to each chamber in the appropriate amount, and nutrient stock solutions were added to all chambers.

Prior to the termination of the periphyton assays, the stir bars were turned off and the chambers were allowed to settle for five minutes. The tiles were removed from the chambers, and were immediately frozen in the dark to prevent photodegradation. Within two weeks of the assay termination, the tiles were scraped with a toothbrush in the dark and periphyton assemblages put into suspension with 135 mL (4.56 fl oz) of deionized water. This solution was divided into three partitions for chl a, assemblage composition analysis, and a reserve sample, and then frozen in a -20°C (-4°F) freezer.

Samples were preserved in a 10% formalin solution at a ratio of 1:1, and stored in the refrigerator. The samples were stained by the addition of Fast Green dye to create a distinction between live and dead cells. Wet mounts were produced and sealed using clear nail polish. The slides were analyzed within four days of production using a Lecia DM microscope at 1,000x magnification. A total of 250 live cells were counted on each slide, and identified to the genus level. Krammer and Lange-Bertalot *(1986, 1988, 1991)* and Patrick and Reimer *(1966, 1975)* were referenced for diatom taxonomy.

2.2.4 Data Analysis

Analysis of variance (ANOVA) was used to assess if herbicides had significant effects on fish and periphyton assemblages ($p=0.05$) *(Zar 1999)*. If the null hypothesis was rejected ($p<0.05$), a multiple comparison test was conducted to test which treatment significantly differed from another.

3.0 RESULTS

3.1 SHORT-TERM EXPOSURE TESTS: EFFECTS OF HERBICIDE RUNOFF ON PERIPHYTON AND RAINBOW TROUT

3.1.1 Simulated Rain Event

The runoff collected one day, one week, and two weeks after herbicide application had no significant effects on algal biomass, measured as chl a ($p>0.05$), as Figure 3.1 shows. The assay with exposure to the runoff collected one day after herbicide application showed a chl a concentration in the control plots approximately nine times higher than those in both the sprayed plots and stream water. However, variation among the control plots was high. The only significant difference in chl a was detected between the stream water and sprayed plots with the runoff collected two weeks after herbicide application ($p<0.05$). However, chl a concentration was not significantly different between the control and sprayed plots ($p>0.05$).

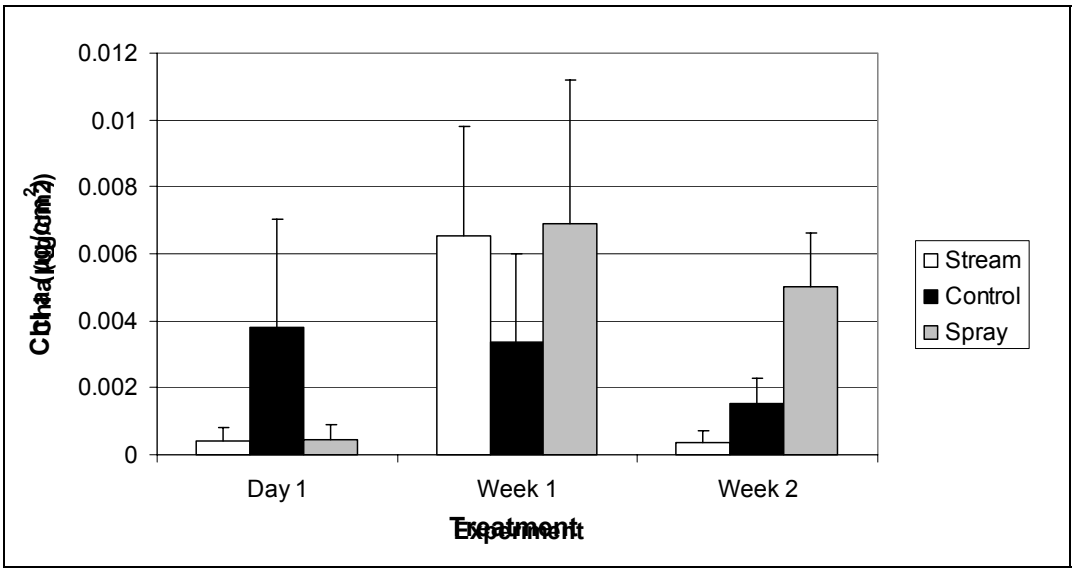

Figure 3.1: Comparison of chlorophyll a concentrations (mean + standard error) among the three treatments (Runoff was collected 1 day, 7 days, and 14 days after herbicide spray and a simulated rain event.)

The runoff that was collected one day, one week, and two weeks after herbicide application had no significant effects on diatom species richness ($p>0.05$), as Figure 3.2 shows. Diatom species richness was only significantly different between the stream water and control/sprayed plots. However, diatom species richness was not significantly different between the control and sprayed plots ($p>0.05$).

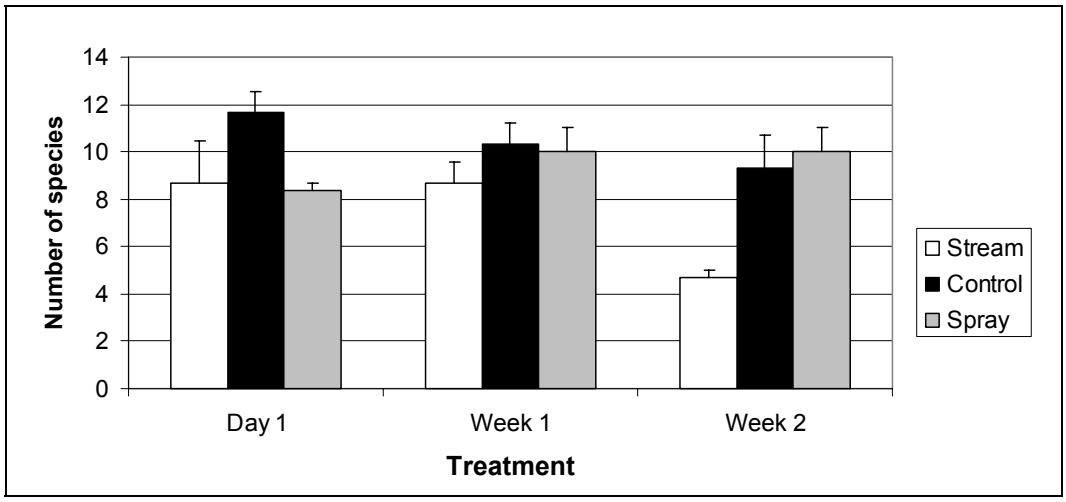

Figure 3.2: Comparison of diatom species richness (mean + standard error) among the three treatments (Runoff was collected 1 day, 7 days, and 14 days after herbicide spray and a simulated rain event.)

The runoff collected one day after herbicide application had no significant effects on relative abundance of *Cocconeis placentula*, a dominant diatom species. However, the runoff collected one week and two weeks after herbicide application had significant effects this taxon ($p=0.01$, $p<0.001$), as can be seen in Figure 3.3. Relative abundance of *C. placentula* was significantly lower in the control plots than the other two treatments with the runoff collected one week after the herbicide application. Relative abundance of *C. placentula* was lowest in the control plots, and highest in the stream water.

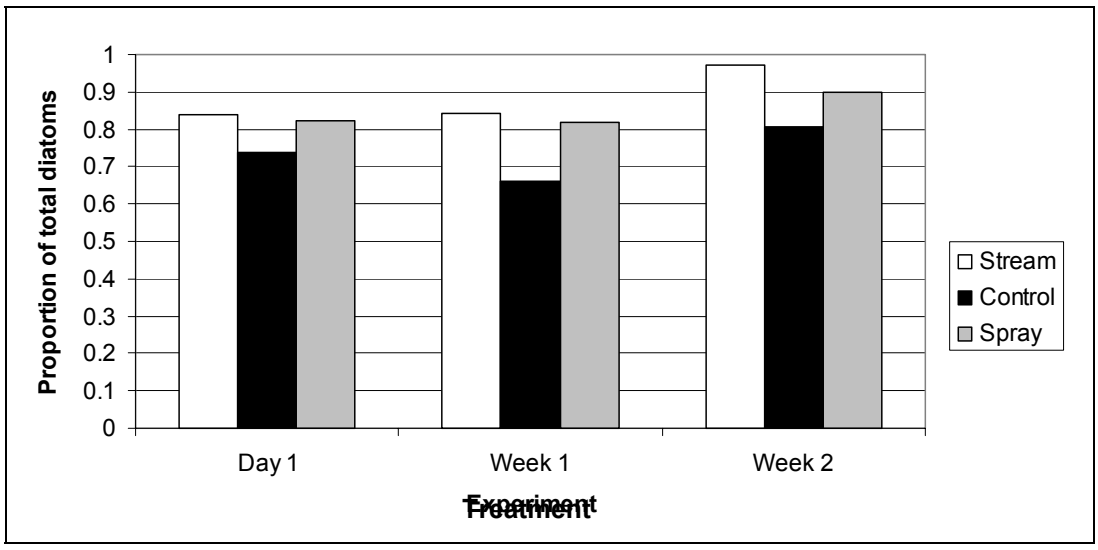

Figure 3.3: Comparison of proportion of *Cocconeis placentula* among the three treatments (Runoff was collected 1 day, 7 days, and 14 days after herbicide spray and a simulated rain event.)

There were significant decreases in rainbow trout survivorship for the untreated control and the treated plots as compared to the assays conducted with stream water at 24 and 48 hours ($p<0.05$), shown in Figure 3.4. The data suggests that the roadside runoff from both the control and treated plots had an effect on rainbow trout survivorship. The contribution from the applied herbicides on decreased survivorship was unknown because the runoff would contain a mixture of other chemicals, such as metals.

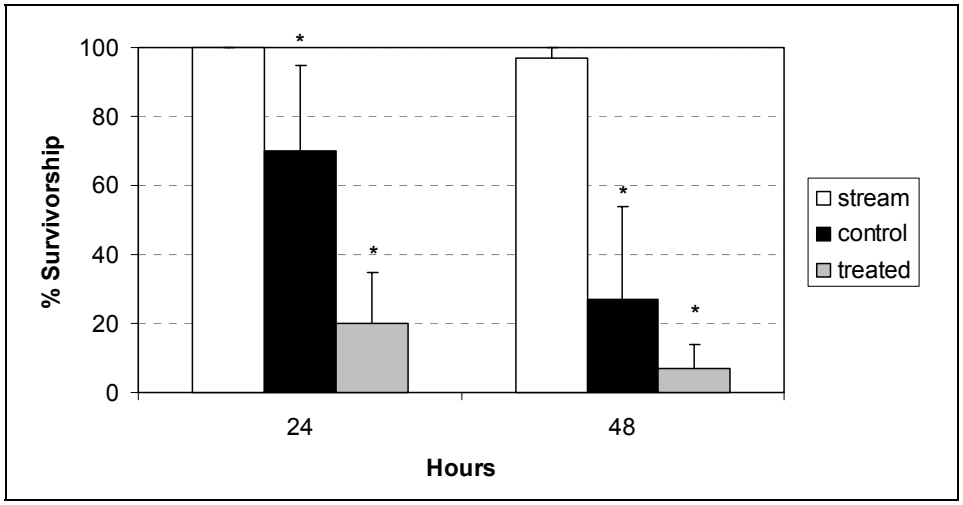

Figure 3.4: Means and standard errors of the rainbow trout mortality within 24 and 48 hours (* = significant difference from stream ANOVA $p<0.05$ MLR LSD $p<0.05$)

3.1.2 Natural rain event

Figure 3.5 shows that chl a concentrations were significantly different among the four treatments ($p=0.03$). A multiple comparison test showed that the chl a concentration in the control plots was significantly higher than that in the upstream water. Diatom species richness was lowest in the sprayed plots (Figure 3.6). However, the difference among the four treatments was not statistically significant ($p=0.46$).

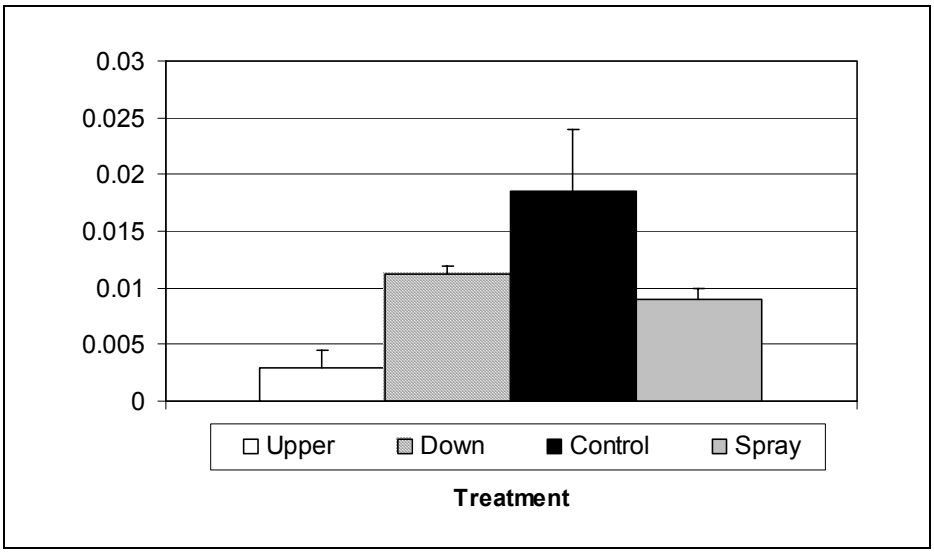

Figure 3.5: Comparison of chlorophyll a concentrations (mean + standard error) among the four treatments

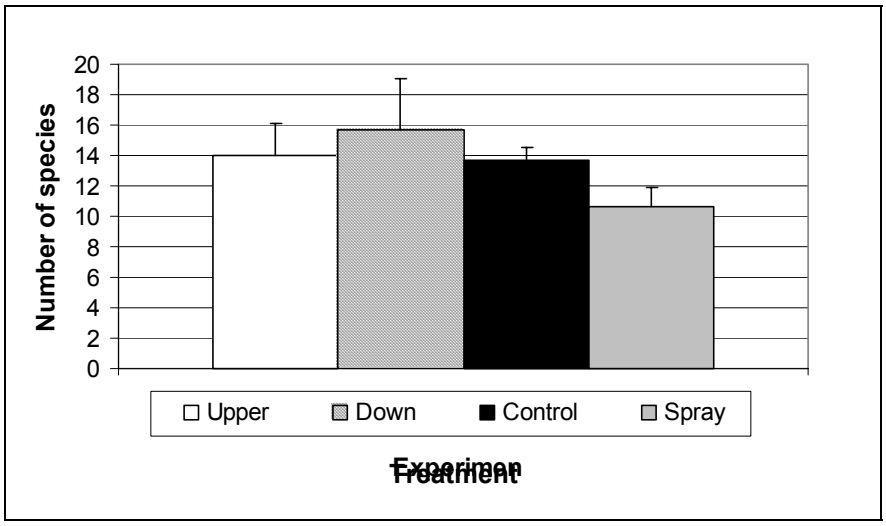

Figure 3.6: Comparison of diatom species richness (mean + standard error) among the four treatments

The relative abundance of *Achnanthes minutissima* was significantly different among the treatments ($p<0.001$), shown in Figure 3.7. This taxon was significantly more abundant in the controls than both the downstream water and sprayed plots. The relative abundance of this taxon in the sprayed plots was also significantly lower than the upstream water. Other two common species, *A. deflexa* and *C. placentula*, were not significantly different among the treatments ($p>0.05$).

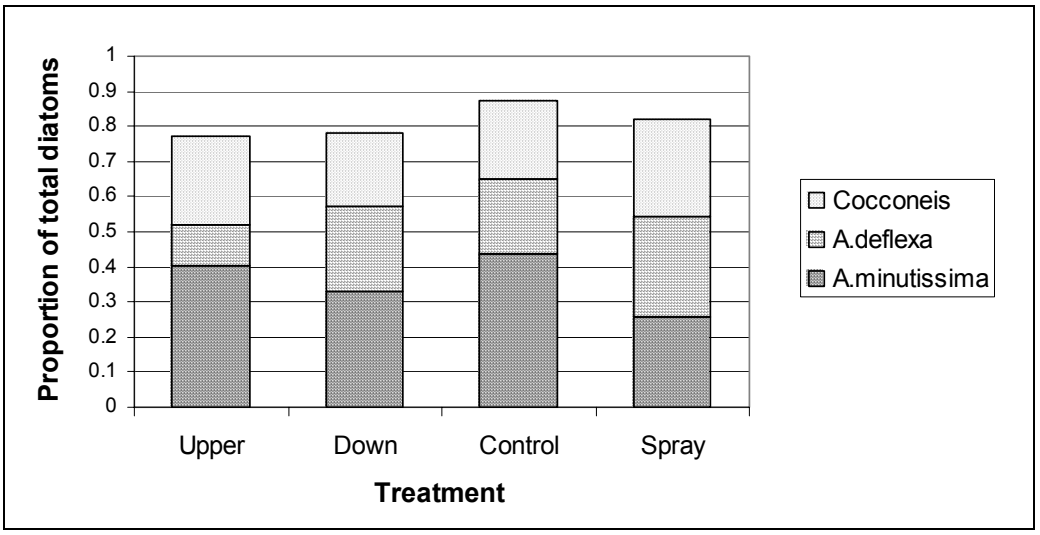

Figure 3.7: Comparison of proportion of three dominant diatom species among the four treatments

Figure 3.8 shows that the runoff collected during the natural rain event had no significant effects on rainbow trout survivorship at 24, 48, 72, or 96 hours of exposure ($p<0.05$). The bioassay with water from the upstream location had a lower survivorship than other locations but was not significantly different.

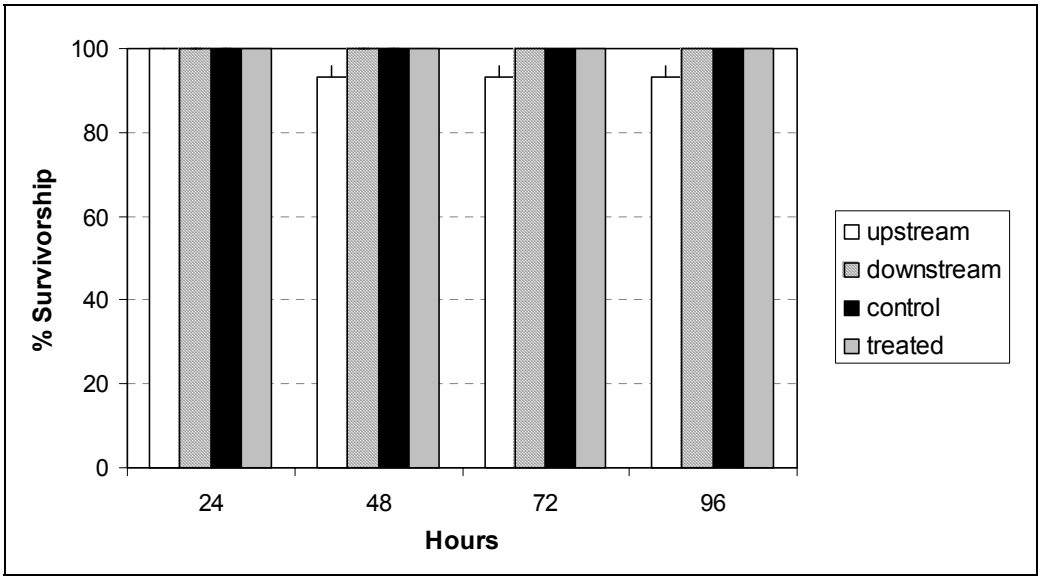

Figure 3.8: Means and standard errors of the rainbow trout survivorship during the four-day exposures

3.2 LONG-TERM EXPOSURE TESTS: EFFECTS OF ROUNDUP, KROVAR, AND OUST INDIVIDUALLY AND IN MIXTURE ON PERIPHYTON AND RAINBOW TROUT

3.2.1 Herbicide Concentrations

The concentration of glyphosate for the assay treated with Roundup was within 5-73% of the expected values, erring towards a lower concentration in every instance. The diuron concentrations in the Krovar bioassay were within 3-8% of the expected values. The bromacil concentrations in the Krovar bioassay were within 3-7% of the expected values with one value that was four times higher than the expected concentration of 1 μg/L which was probably due to analytical error. The concentration of sulfometuron-methyl for the assay treated with Oust was within 33-73% of the expected values. The concentrations for the bioassay using the mixture of chemicals were within 0-48% of the expected values for all four compounds.

3.2.2 Physico-chemical Conditions

The physico-chemical conditions of the test solutions were within the ranges described by EPA bioassays *(USEPA 1985)*, and provided stable conditions for the test subjects (Table 3.1). The periphyton assays had dissolved oxygen levels that ranged from 7.8 to 9.4 mg/L (parts per million), pH was between 7.6 and 9.2, the water hardness was 16.0 mg/L for all the assays, and the conductivity was 53.0 to 61.0 μS/cm (135 to 155 μS/in). Temperatures during the periphyton assays ranged between 10.7 and 17.5°C (51.3 to 63.5°F). With the exception of temperature, these conditions were similar to Eagle Creek.

Table 3.1: Means and ranges (in parentheses when multiple measurements) of the physico-chemical parameters of test solution

Bioassay	Location	Temperature (°C)	Dissolved oxygen (mg/L)	pH	$CaCO_3$ (mg/L)	Conductivity (μS/cm)
ROUND-UP	Hatchery	6.30	9.30	7.10	18.00	46.30
	Fish tanks	10.3 (8.6-12.2)	8.5 (7.7-9.4)	7.2 (7.1-7.4)	16.00	60.00
	Eagle Creek	7.00	10.20	6.50	16.00	35.00
	Algae chambers	14.4 (12.4-16.2)	8.6 (7.9-9.2)	7.9 (7.8-8.0)	16.00	53.00
KROVAR	Hatchery	6.70	9.60	7.80	28.00	44.80
	Fish tanks	11.6 (4.5-17.0)	8.0 (7.0-10.4)	7.1 (6.9-7.2)	20.00	62.00
	Eagle Creek	7.10	10.20	6.50	16.00	40.00
	Algae chambers	15.7 (14.0-17.5)	8.5 (8.1-9.0)	7.9 (7.8-8.1)	16.00	56.00

Bioassay	Location	Temperature (°C)	Dissolved oxygen (mg/L)	pH	$CaCO_3$ (mg/L)	Conductivity (µS/cm)
OUST	Hatchery	8.80	10.50	7.60	32.00	52.90
	Fish tanks	16.6 (14.9-17.9)	8.2 (7.1-8.9)	7.6 (7.4-7.8)	20.00	69.00
	Eagle Creek	7.10	10.20	6.40	16.00	38.00
	Algae chambers	15.7 (14.0-17.1)	8.4 (7.8-9.4)	8.7 (8.3-9.2)	16.00	61.00
MIXTURE	Hatchery	8.60	9.70	7.40	20.00	44.90
	Fish tanks	10.5 (8.0-12.7)	7.9 (6.6-9.1)	7.6 (7.5-7.6)	16.00	56.00
	Eagle Creek	7.10	10.20	6.10	16.00	42.00
	Algae chambers	13.4 (10.7-16.5)	8.5 (7.8-9.4)	7.8 (7.6-7.8)	16.00	55.00

The rainbow trout bioassays had dissolved oxygen values that ranged between 6.6 and 10.4 mg/L, the pH ranged from 6.9 to 7.8, the hardness ranged between 16 and 20 mg/L, and the conductivity ranged from 56.0 to 69.0 µS/cm (142 to 175 µS/in). The temperatures were variable for the rainbow trout bioassays, and fluctuated from 4.5 to 17.9°C (40.1 to 64.2°F), as shown in Table 3.1. The dissolved oxygen, pH, hardness, and conductivity were very similar to the water at the hatchery where the rainbow trout eggs had been raised.

3.2.3 Periphyton

Effects on chl a concentrations varied among the chemicals (Table 3.2). In the Krovar and mixture assays, chl a concentrations in the highest concentration treatments were significantly lower than that in the controls (ANOVA and LSD, $p<0.05$), shown in Figure 3.9. Chl a concentrations were, however, not significantly different among the treatments in the Roundup and Oust assays (ANOVA, $p>0.05$), also shown in Figure 3.9.

The density of live algal cells significantly decreased along each chemical concentration gradient assay ($p<0.05$), as shown in Figure 3.10. The two higher treatments of Roundup had significantly fewer cells per cm^2 than the control and lowest treatment ($p<0.01$). The Oust assay had similar results with decreasing cell density as the concentration increased ($p<0.01$). The Krovar and mixture assays showed a significant decrease in live cell density between every treatment ($p<0.01$), as seen in Table 3.2.

A total of 13 genera of periphyton were identified in the assemblages from these experiments. Out of 13 genera, 10 genera are diatoms. *Cocconeis placentula* was the most abundant taxon, comprising approximately 35% of the algal assemblages in every assay.

Table 3.2: ANOVA summary table for the results of the periphyton assays

ENDPOINT	HERBICIDE	ANOVA p-values
Chlorophyll a	Roundup	0.95
	Oust	0.13
	Krovar	0.03**
	Mixture	<0.01**
Density	Roundup	<0.01**
	Oust	<0.01**
	Krovar	<0.01**
	Mixture	<0.01**
Relative abundance: Chlorophyta	Roundup	0.07
	Oust	<0.01**
	Krovar	<0.01**
	Mixture	0.04**
Relative abundance: Cyanophyta	Roundup	<0.01**
	Oust	0.14
	Krovar	0.09
	Mixture	0.04**
Relative abundance: *Cocconeis* spp.	Roundup	<0.01**
	Oust	0.76
	Krovar	<0.01**
	Mixture	<0.01**
Relative abundance: diatoms other than *Cocconeis* spp.	Roundup	0.67
	Oust	0.17
	Krovar	0.09
	Mixture	0.06
Proportion of live *Cocconeis* cells to all *Cocconeis* cells	Roundup	<0.01**
	Oust	<0.01**
	Krovar	<0.01**
	Mixture	<0.01**

** denotes a significant difference between treatments ($p<0.05$).

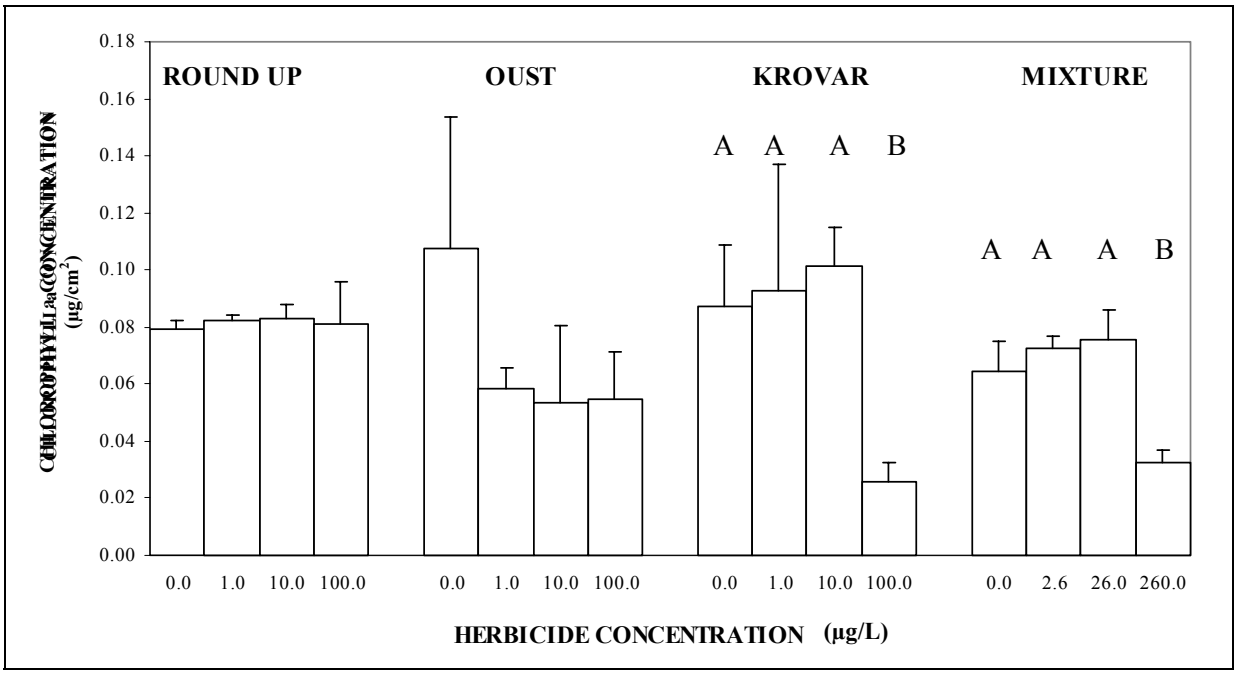

Figure 3.9: Means (n=3) and standard errors of the chlorophyll a concentrations (µg/cm^2) for the periphyton assays across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures
(Dissimilar letters indicate significant differences between treatments (ANOVA and LSD, $p<0.05$))

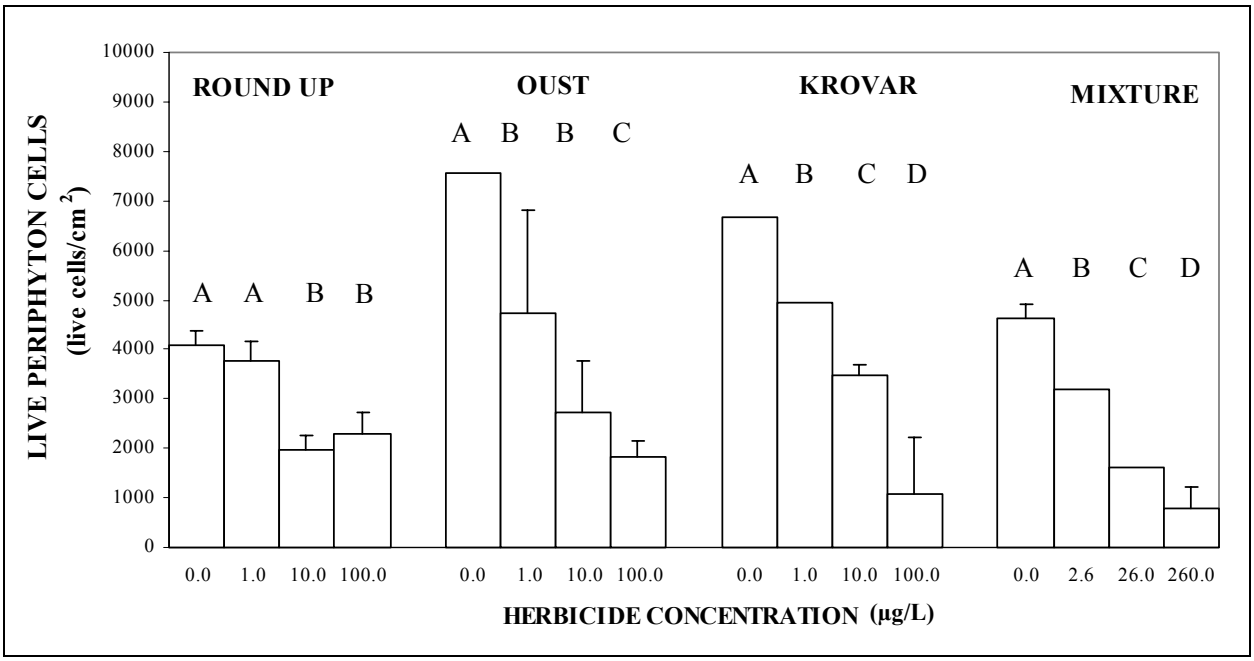

Figure 3.10: Means (n=3) and standard errors of the live cell density (live cells/cm^2) for the periphyton assays across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures
(Dissimilar letters indicate significant differences between treatments (ANOVA and LSD, $p<0.05$)).

Figure 3.11 shows that the relative abundances of *C. placentula* cells were significantly different for the Roundup, Krovar, and mixture assays ($p<0.05$). The Roundup assay did not show a consistent trend. The Krovar and mixture assays generally showed the relative abundances of this taxon were highest in the 100 μg/L concentration.

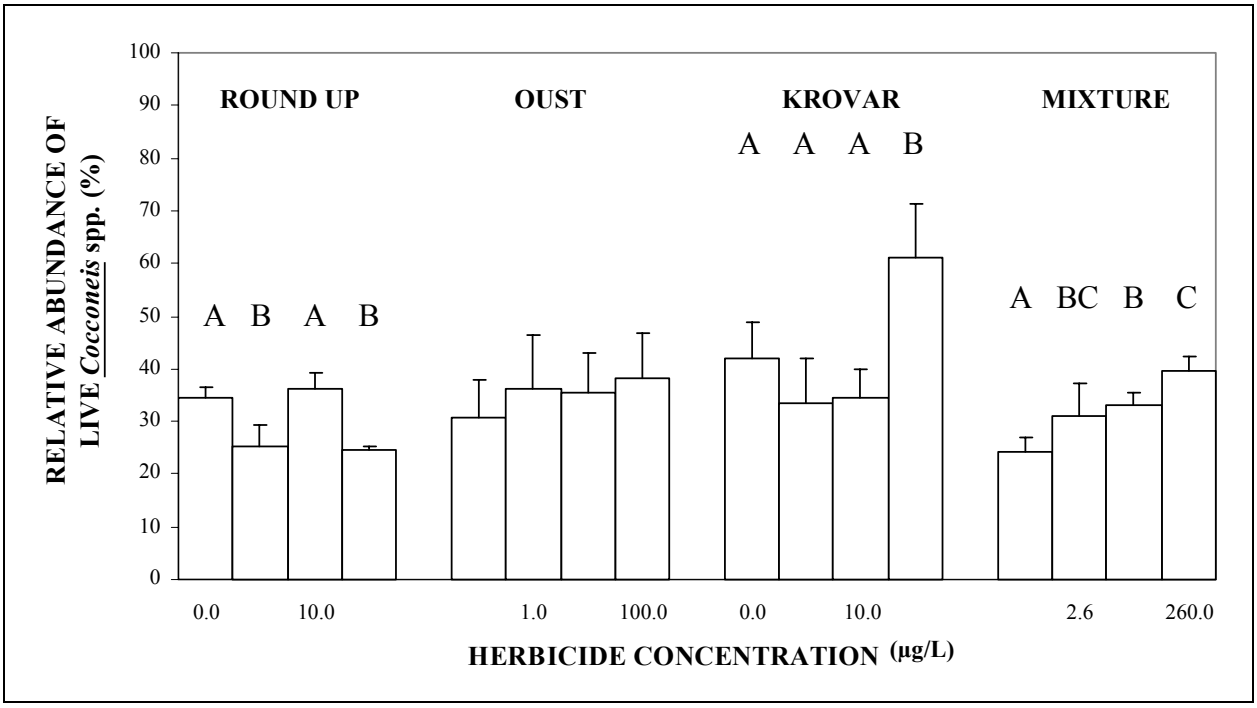

Figure 3.11: Mean (n=3) and standard error of the relative abundances of live *Cocconeis* spp. cells across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures (Dissimilar letters indicate significant differences between treatments (ANOVA and LSD, $p<0.05$))

The Cyanophyta relative abundances were significantly different in the Roundup and mixture assays ($p<0.05$), shown in Figure 3.12. The Roundup assay did not show a consistent trend with increasing concentration in the relative abundance of Cyanophyta. The mixture assay showed a decrease in Cyanophyta relative abundance with increasing herbicide concentration.

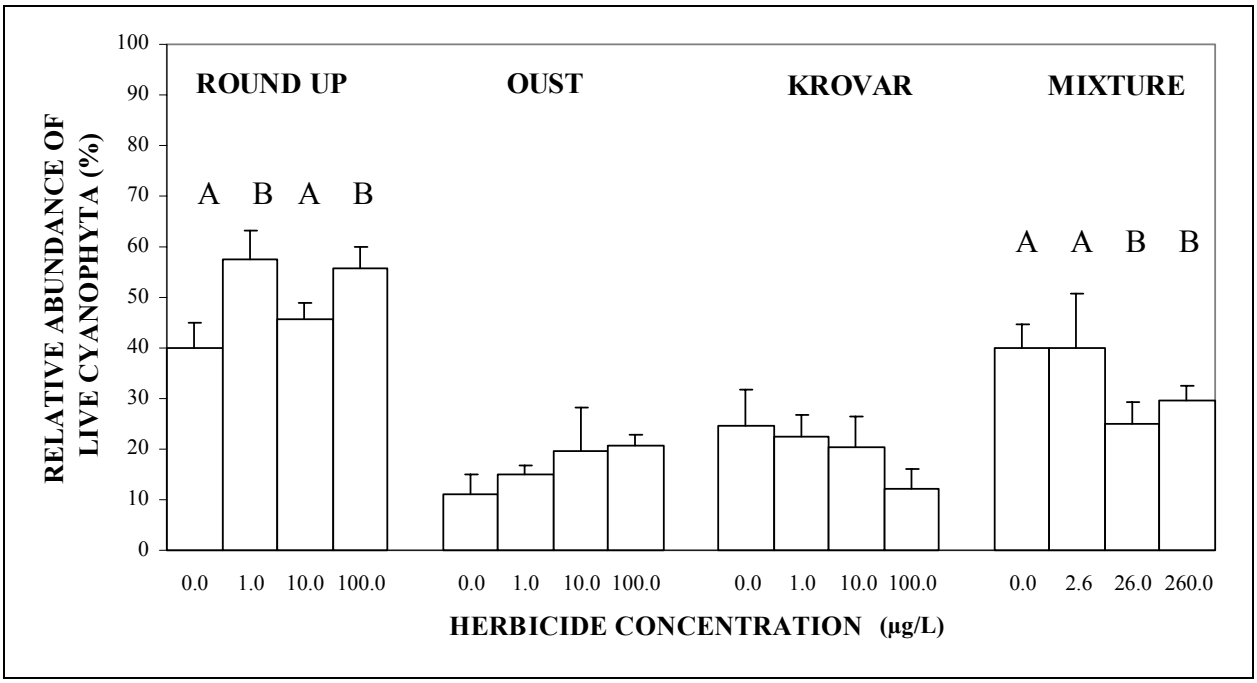

Figure 3.12: Mean (n=3) and standard error of the relative abundances of live Cyanophyta cells across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures (Dissimilar letters indicate significant differences between treatments (ANOVA and LSD, $p<0.05$))

The Chlorophyta relative abundances had significant differences in all of the assays except Roundup ($p<0.05$), as Figure 3.13 shows. The Oust and mixture assays show a consistent decrease in Chlorophyta relative abundance with increasing concentration. The Krovar assay is not as consistent, and shows an initial increase and then a dramatic decrease at the highest concentration.

The relative abundances of the fourth group, diatoms excluding *C. placentula*, showed no significant differences between the treatments in any of the assays ($p>0.05$), as shown in Figure 3.14.

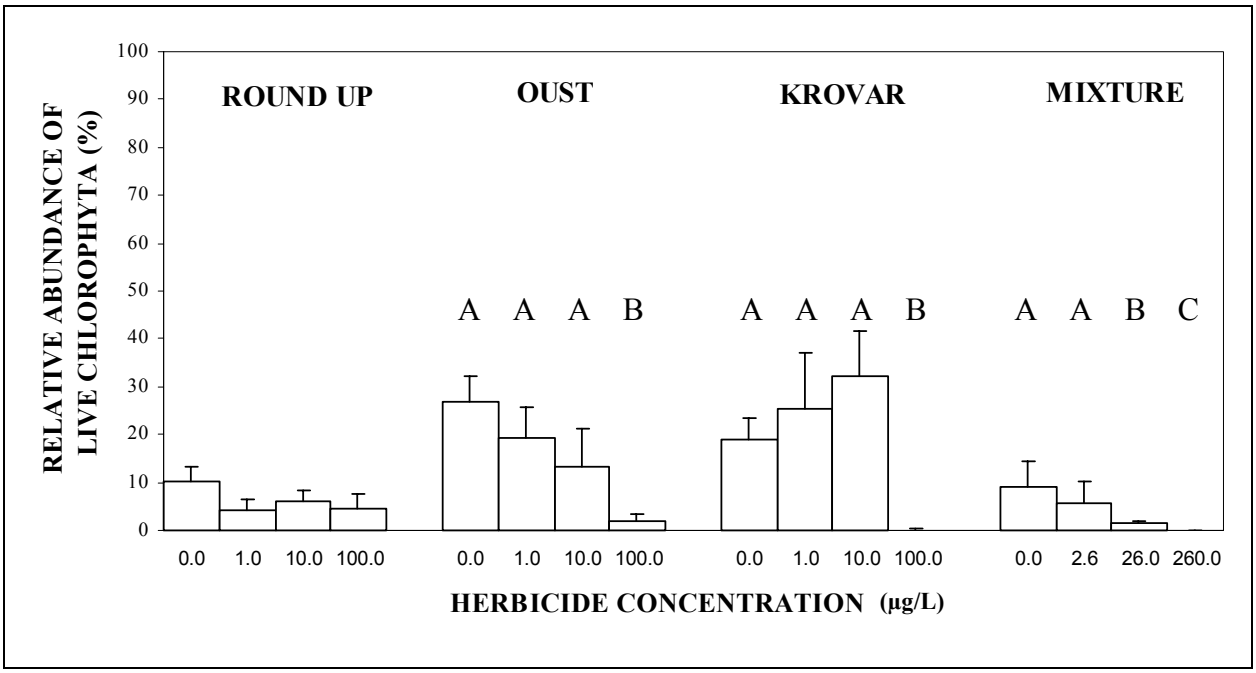

Figure 3.13: Mean (n=3) and standard error of the relative abundances of live Chlorophyta cells across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures (Dissimilar letters indicate significant differences between treatments (ANOVA and LSD, $p<0.05$))

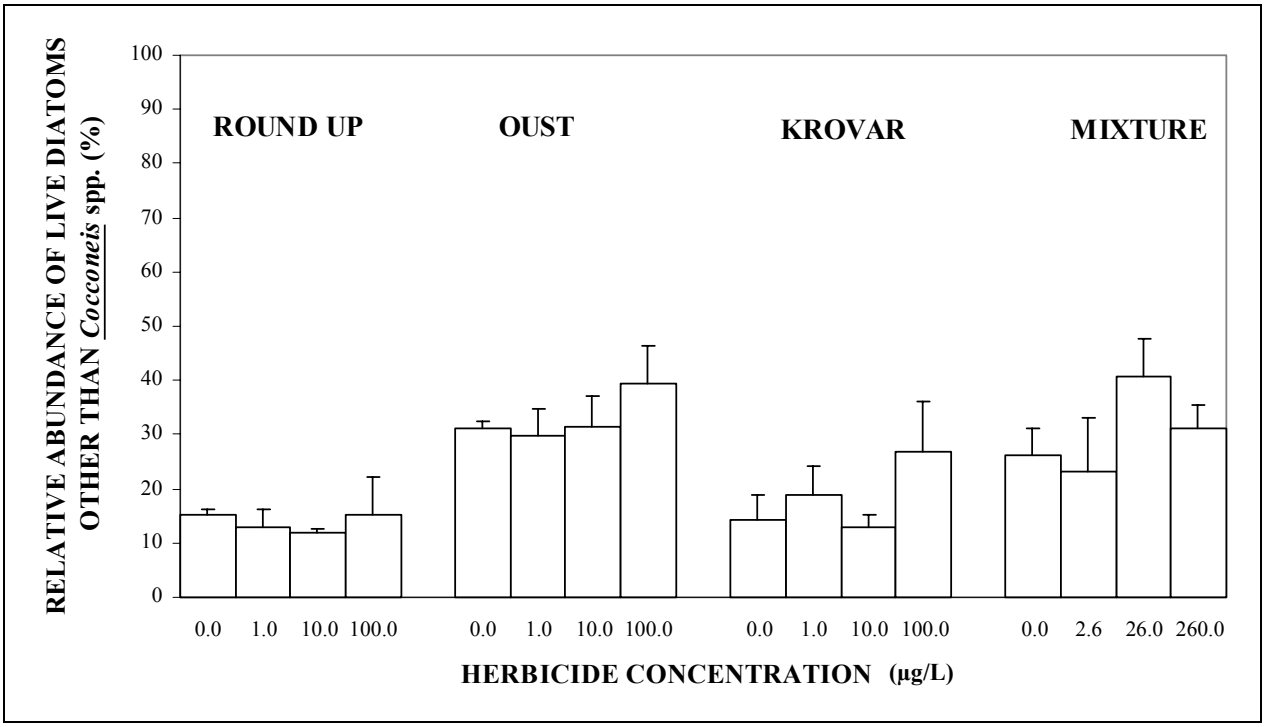

Figure 3.14: Mean (n=3) and standard error of the relative abundances of live diatoms other than *Cocconeis* spp. cells across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients after two-week exposures (There were no significant differences between treatments (ANOVA $p>0.05$))

3.2.4 Rainbow Trout

3.2.4.1 Mortality

Rainbow trout mortality was not significantly different between treatments in any of the bioassays (Figure 3.15). Mortality at the Roaring River hatchery for this cohort of fish

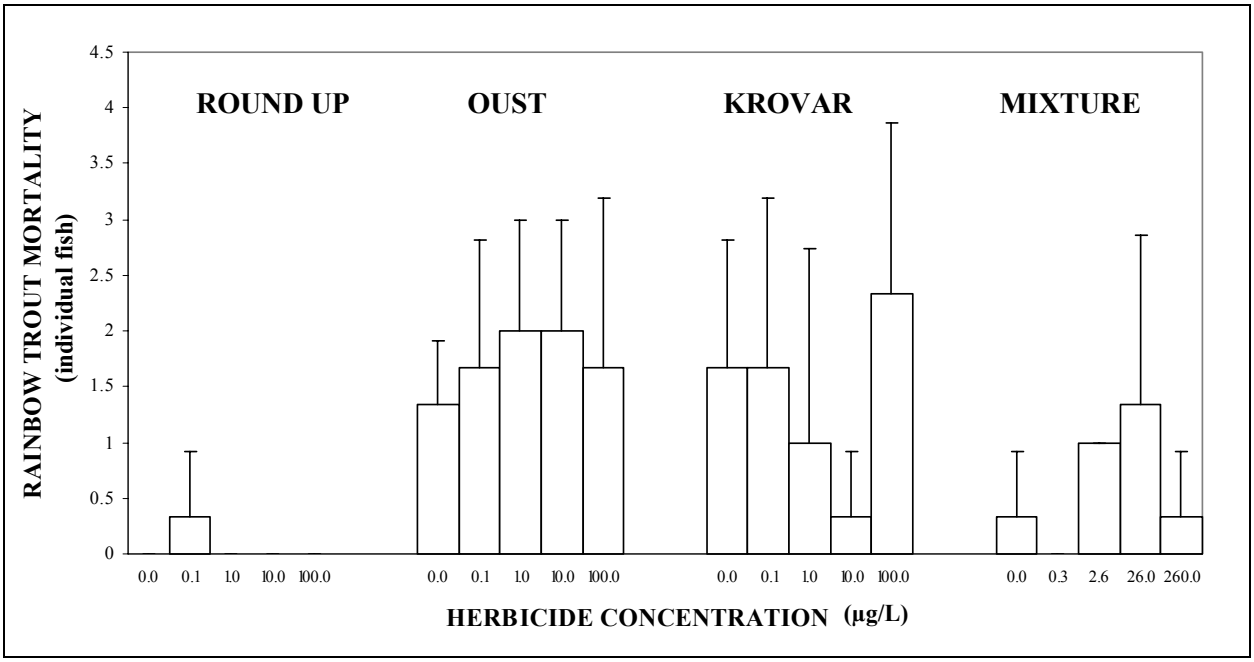

Figure 3.15: Means (n=3) and standard errors of the rainbow trout mortality during the two-week exposures across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients (There were no significant differences between treatments (ANOVA $p>0.05$))

3.2.4.2 Wet weight

The individual herbicide bioassays showed no significant differences between the changes in wet weights (Figure 3.16). However, changes in mean rainbow trout wet weight were significant between treatments in the mixture bioassay. There was a decreasing trend for wet weight with increasing herbicide concentration in the mixture bioassay with significant differences from the control in the 2.6 and the 255.0 µg/L treatments ($p<0.05$) while the 0.3 and the 25.5 µg/L treatments were not different from the control (Table 3.3). There was a trend of reduced growth with concentration in the Roundup and Krovar bioassays and a trend of increased wet weight in the Oust bioassay but the differences were not significant between controls and treatments.

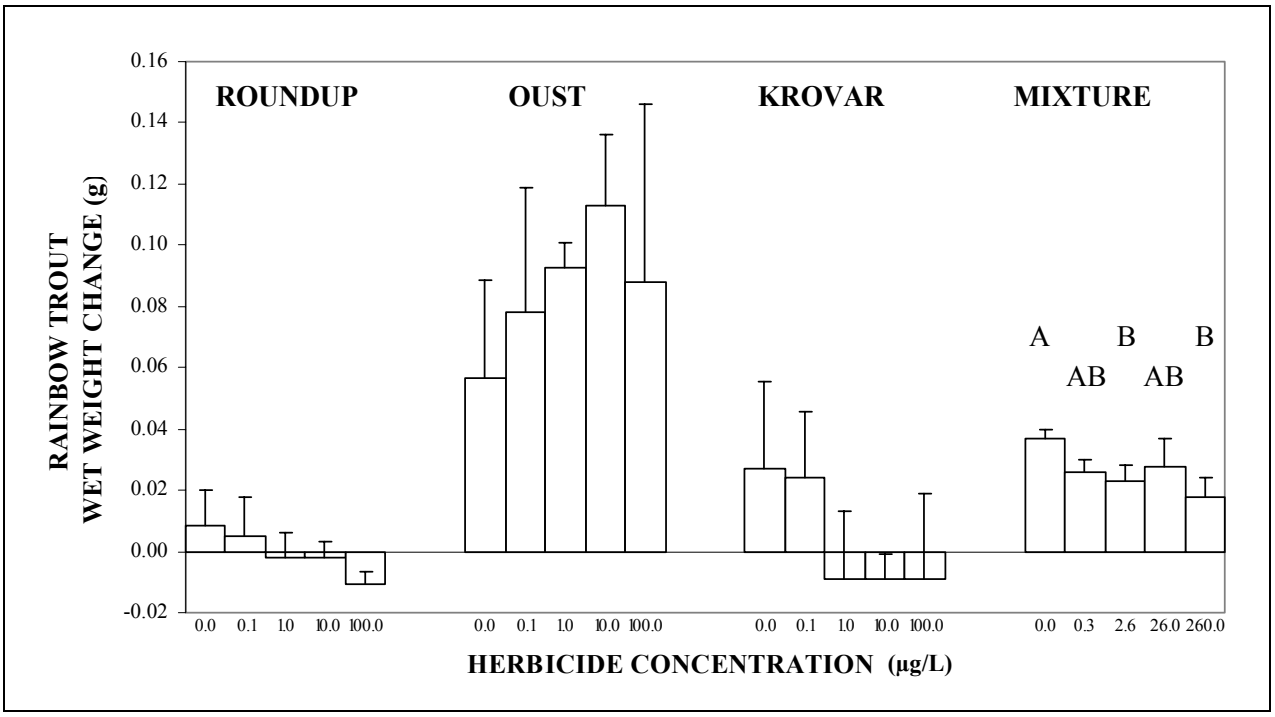

Figure 3.16: Means (n=3) and standard errors of the rainbow trout wet weight gain/loss after two-week exposures across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients
(Dissimilar letters indicate significant differences between treatments (ANOVA and LSD $p<0.05$))

Table 3.3: ANOVA summary table for the results of the rainbow trout assays

ENDPOINT	HERBICIDE	ANOVA p-values
Mortality	Roundup	0.45
	Oust	0.94
	Krovar	0.49
	Mixture	0.27
Wet weight	Roundup	0.16
	Oust	0.48
	Krovar	0.16
	Mixture	0.04**
Dry weight	Roundup	0.8
	Oust	0.75
	Krovar	0.64

** denotes a significant difference between treatments ($p<0.05$).

3.2.4.3 Dry weight

There were no significant differences between treatments for the rainbow trout dry weights at the end of the two-week bioassays for any of the bioassays (see Table 3.3). Variability was low in the Roundup bioassay while the Oust, Krovar, and mixture bioassays were more variable (Figure 3.17).

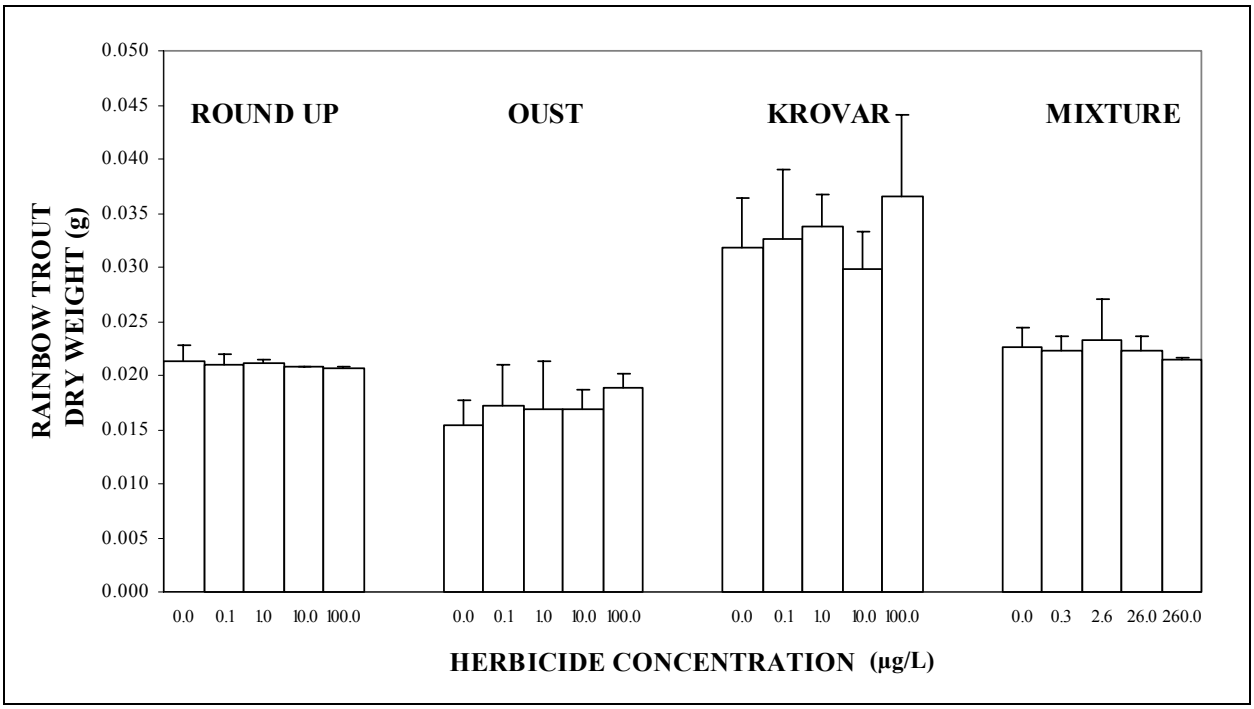

Figure 3.17: Means (n=3) and standard errors of rainbow trout dry weights at the end of each two-week exposure across the Roundup, Oust, Krovar, and mixture of herbicides concentration gradients (There were no significant differences between treatments (ANOVA $p>0.05$))

4.0 DISCUSSION

4.1 SHORT-TERM EXPOSURE TESTS: EFFECTS OF HERBICIDES RUNOFF ON PERIPHYTON AND RAINBOW TROUT

It was expected that herbicide runoff would have adverse effects on chl a concentrations *(Hoagland et al. 1996)*. Statistically, chl a concentrations were, however, not significantly different between the control and sprayed plots for both simulated and natural rain event assays. Lack of significant difference between the two treatments may be partially due to relatively low herbicide concentrations in the runoff. Wood *(2000)* reported that the simulated rain event one day after the herbicide application generated <1 mg/L glyphosate and diuron and even lower concentration of sulfometuron-methyl in the roadside runoff. Ma et al. *(2001)* reported that the EC_{50} (median effect concentration) for a green alga, *Chlorella pyenoidosa,* with a 96-hour exposure, was 3.5 mg/L for glyphosate and 14.2 mg/L for sulfometuron-methyl, respectively. However, the same study showed that the EC_{50} was 0.001 mg/L for diuron, which was much lower than the concentration detected in the runoff one day after the application.

Variability in environmental conditions among the replicates (individual plots) within the control and sprayed treatments may also confound the assessment. Average chl a concentrations in the control plots were higher than the spray plots in both the simulated rain day-one assay and the natural rain assay. Variability among the control plots in the chl a concentrations was high, however, which may partially contribute to the lack of statistical difference between the two treatments. Higher chl a concentration in the control plots may be due to a combination of nutrient enrichment from soil runoff and low or no herbicide residues in the runoff. The runoff collected from both the control plots and sprayed plots, in this case, was a "black box" in terms of their chemical properties, except for targeted herbicide compounds. Environmental conditions for each plot, such as soils and flow, may be different. Also, the replicates for both the control and sprayed treatments may not be homogenous in their chemical properties (e.g., nutrients, metals, etc.).

Herbicide runoff may have significant effects on diatom species composition. More than 80% of the diatom assemblages were *Cocconeis placentula*. The relative abundance of this taxon was lower in the control plots than in the sprayed plots. The difference persisted for all three simulated rain assays and the difference was statistically significant for two out of three simulated rain assays. For the natural rain event assay, diatom assemblages were more diverse than those used for the simulated rain assay in spring.

Relative abundance of *Achnanthes minutissima* was significantly higher in the control plots than in the sprayed plots, while relative abundance of *C. placentula* and *A. deflexa*, two other common species, were not statistically different between the two treatments. Effects of herbicides on algal species composition have been reported *(see review by Hoagland et al. 1996)*. For example, DeLorenzo et al. *(1999)* found that atrazine (herbicide) altered algal composition and reduced

most taxa's abundance in a mesocosm study. Alteration of algal species composition may be due to inhibitory effects of herbicides on autotrophic organisms and increases of bacteria which compete for limited nutrients with algae.

The rainbow trout mortalities observed in the assay for the simulated rain event may be due to a variety of reasons. The chemistry and quality of roadside runoff can be highly variable and could contain heavy metals, PAHs (polynuclear aromatic hydrocarbons), and other chemicals which could cause toxicity. Only herbicides were measured in this study and not the other chemicals that could occur in roadside runoff.

4.2 LONG-TERM EXPOSURE TESTS: EFFECTS OF ROUNDUP, KROVAR, AND OUST INDIVIDUALLY AND IN MIXTURE ON PERIPHYTON AND RAINBOW TROUT

4.2.1 Herbicide Toxicity to Periphyton

Chlorophyll a concentrations. Effects on chl a concentrations varied among the chemicals. The chl a concentrations decreased in both Krovar and mixture assays while this measure showed no statistical differences among the treatments in both Roundup and Oust assays. The herbicide diuron has a high affinity to the Q_B binding site of the photosystem II photosynthetic complex.[2] By excluding Q_B from binding at this location, electron transfer from Q_A to Q_B is limited *(Sandmann and Bolger 1986)*. Diuron in this study was persistent within a 48-hour period. The diuron concentrations in the Krovar bioassay were within 3-8% of the expected values. The literature on effects of these chemicals on algae is limited. Haynes et al. *(2000)* reported that chl a concentrations of three sea grass species declined when exposed to 10.0 μg/L of diuron.

The chl a results for the Roundup and Oust assays did not show a significant difference in any of the treatments. Lack of the significant responses to these chemicals may be partially due to relatively low concentrations and low persistence of these chemicals during the assays. Glyphosate is an EPSP (Excitatory Post-Synaptic Potentials) synthase inhibitor. Acute toxicity of glyphosate to autotrophs causes an elevation in glyoxylate concentration that inhibits an enzyme involved in carbon fixation *(Ma et al. 2001)*. This further inhibits photosynthesis, to cause premature cellular death *(Lydon and Duke 1988)*. Sáenz et al. *(1997)* investigated the effects of glyphosate on *S. quadricauda* chl a concentrations and found the 96-hour NOEC (No Observable Effect Concentration)[3] to be 0.77 mg/L. This value is approximately an order of magnitude higher than the nominal concentrations used in this study. Ma et al. *(2001)* examined the acute toxicity of metasulfuron-methyl (a related sulfonylurea herbicide) to *Chlorella pyrenoidosa* chl a and found the EC_{50} to be 14.22 mg/L. This concentration is more than an order of magnitude higher than the concentrations used in the present study. In addition, the glyphosate concentrations may not be stable during the testing period. The concentration of glyphosate for the assay treated with Roundup was within 5-73% of the expected values. The

[2] Q_A and Q_B are forms of plastoquinone, electron carriers in the light phase of photosynthesis.
[3] NOEC is the highest concentration of toxicant in which the values for the observed parameters are not statistically different from the controls

concentration of sulfometuron-methyl for the assay treated with Oust was within 33-73% of the expected values.

Density of Live Algal Cells. Current literature regarding the density of live cells in exposures to the herbicides used was extremely sparse. Gausch et al. *(1997)* investigated the effects of atrazine on periphyton and used chl a, cell density (live and dead), and community composition as endpoints to determine the conditions that affect the toxicity of atrazine to periphyton.

Live cell density decreased along the chemical concentration gradients in the Oust, Krovar, and mixture assays. The live cell density results for these assays were consistent with chl a results. However, it was interesting that in the Roundup and Oust assays there is a difference in the results between the chl a and live cell density analyses. Changes in live cell density but not chl a concentrations may be due to changes in community structure. Blanck et al. *(1984)* found that algal species sensitivity to herbicides varied by three orders of magnitude. Sensitivities of algal species may stem from differences in: physiological sensitivities, growth forms, or uptake rates. Contributions of each algal cell to total biomass may depend on their cell sizes and amount of chl a pigments per cell. Chl a concentrations may remain unchanged while species composition may change in response to slightly increased stress intensity *(Schindler 1987)*. Sensitivity of species composition to low-intensity stresses may be due to their ecological redundancy in their multi-species assemblages. The apparent decrease in live cell density of all the assays indicates that although chl a analysis showed some changes in the algal biomass, there is more change occurring at the community level of these ecological concentrations than the chl a analysis indicates.

Periphytic community assemblages may shift as a result of chronic exposures to herbicides *(Kosinski & Merkle 1984; Hoagland et al. 1993; Gausch et al. 1997)*. The decreases in live cell density suggest that the herbicides used in this study may show a higher sensitivity in the analysis than the chl a analysis. In contrast Hoagland et al. *(1993)* found significant decreases in the chl a concentration in lentic algae when exposed to atrazine and bifenthrin while cell densities were not different. Although this study appears to present opposing conclusions, Hoagland et al. *(1993)* did not differentiate between live and dead cells. As a result, the differentiation between viable and non-viable cells is crucial in interpreting impacts to the community structure. Only cells that actively convert light energy to chemical energy continue to contribute to the primary production of an aquatic system.

Relating the density of cells to changes in the relative abundance of guilds, there is a pattern of a significant decrease in chlorophyta amongst the higher treatments except for the Roundup assay. The Roundup assay had decreased cell densities in the two highest treatments but the relative abundance of cells was moderately constant. The Oust, Krovar, and mixture assays had defined decreases in the density, and the relative abundance showed decreases in chlorophyta. The differential effects on specific species in algal communities have shown chlorophyta to be more sensitive than diatoms to exposures of simazine and terabutryn *(Gurney & Robinson 1989)* and atrazine *(Hoagland et al. 1993; Gausch et al. 1998)*. These results are similar to those found in this study regarding the sensitivity of species, with the exception of the Roundup assay.

4.2.2 Herbicide Toxicity to Rainbow Trout

There is limited information on ecologically relevant concentrations of individual or complex mixtures of herbicides and their effects on fish. Lethality was usually the endpoint used in the few studies that have been reported. The concentrations used in these studies were generally orders of magnitude higher than the stream concentrations measured in Oregon *(Anderson et al. 1997)*. Acute bioassays for registered pesticides are required but extrapolations to ecological effects from chronic low-level exposures are difficult. The acute toxicity for the individual herbicides used in this study was as follows: The 96-hour LC_{50} (median lethal concentration) for rainbow trout in Roundup bioassays ranged from 2.0-50.0 mg/L *(Folmar et al. 1979; Hildenbrand et al. 1982; Wan et al. 1989)* and was 148.0 mg/L for Oust *(Dupont 1998)*. The LC_{50} for the Krovar herbicide was not available, but the 96-hour LC_{50} for rainbow trout using diuron was 3.5 mg/L and the bromacil 48-hour LC_{50} for rainbow trout was 56.0 mg/L *(U.S. National Library of Medicine 1995)*. These concentrations were greater than the concentrations used in this study.

Glyphosate is an organophosphate compound but does not inhibit acetylcholine esterase (AchE) activity like many other organophosphates because glyphosate is missing an ester which makes organophosphate insecticides AchE inhibitors. Although glyphosate is thought to not affect the nervous system in the same way as organophosphate insecticides, glyphosate adversely affected the immune response of Bolti fish by decreasing lymphocytes, inhibiting protein synthesis, and affecting regulatory mechanisms *(El-Gendy et al. 1998)*. Although the endpoints used for this study showed no effect there may be more sensitive endpoints that could be affected.

Sulfometuron-methyl targets the enzyme activity of acetolactate synthase, which then inhibits meristematic growth *(Cox 1993)*. Animal cells do not contain acetolactate synthase. There is evidence that these chemicals could cause toxicity through mechanisms separate from their designed mechanisms of toxicity. Some sufonylurea herbicides affect insulin secretion *(Melander et al. 1989)* and blood pressure *(Walden 1991)* and sulfometuron-methyl were teratogenic in frogs at concentrations of 5.0 mg/L *(Fort et al. 1999)*.

Toxicity data on the formulation Krovar was not available but there was toxicity data available for the active ingredient diuron. Diuron decreased egg hatch and survival of fathead minnows at concentrations of 8.3 mg/L *(Nebeker & Schuytema 1998)*. In other studies, growth and survival of fathead minnow eggs and newly hatched fry were significantly reduced at 1.0 mg/L *(Call et al. 1987)*. Although this herbicide has been used for many years, there is relatively little information on its toxicity in animals.

This study provided important information that the sublethal endpoint growth, as measured by dry weight, was unaffected by environmentally relevant concentrations of Roundup, Krovar, Oust. Dry weight can be more accurately measured than wet weight. In addition, dry weight can be used as a measure of the body's ability to convert food into muscle and bone. However, dry weight would not capture the effects of weight gain due to retention of water that could be a result of factors affecting water balance.

The effects observed in wet weight may be an artifact of methodology but disruption of osmoregulation and edema cannot be ruled out at this time. Other sublethal endpoints such as osomoregulation, DNA synthesis, or suppressed immune function are generally more sensitive endpoints that could be adversely affected by chronic exposures. These physiological, biochemical, and molecular responses to herbicides could be used as early warning signals of adverse effects at higher levels of biological organization.

5.0 CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

The data showed that short-term exposure to the herbicides had no statistically significant effects on periphyton. The short-term exposure did reduce the survivorship of rainbow trout but the effects were observed in runoff from areas treated and untreated with herbicides during the study. Therefore, direct toxicity was probably a result of other factors affecting the quality of runoff. Long-term exposure tests showed that herbicides, especially Krovar and the mixture of three chemicals, reduced algal biomass. Declined trend in algal biomass was more evident in live cell density than chl a concentration, suggesting that algal responses to chemicals may vary among groups (green algae vs. diatom). The long-term exposure had no statistically significant effects on fish mortality and dry weight. The individual herbicide bioassays showed no significant differences between the changes in wet weights. However, changes in mean rainbow trout wet weight were significant between treatments in the mixture bioassay.

The study was constrained in several aspects. First, only the targeted herbicide compounds in roadside runoff were quantified. It remains difficult to interpret the difference or lack of difference between the control and sprayed plots, due to incomplete information on chemical properties of the runoff such as nutrients and metals. Second, due to limited funding, the degradation of each chemical during the assays was assessed sporadically. It is very important to know the fate of each chemical consistently throughout the testing period.

The study showed that periphyton assemblages could be altered by some chemicals. While the rainbow trout fish showed no statistical effects for dry weight, the effect on other sublethal endpoints remain a possibility. Studies on the effects of these chemicals on endocrine function and osmoregulation in fish may be necessary to assess the potential sublethal effects of these chemicals. It is unclear what the ecological implications may be. Changes in periphyton biomass and species composition are tightly coupled with macroinvertebrate grazers in streams *(Steinman 1996)*. A study on effects of different algal growth forms (green algae vs. diatom) on macroinvertebrate grazers may be necessary to assess the potential effects of these herbicides on stream ecosystems.

Biota such as periphyton assemblages should be used as indicators to assess ODOT's herbicide Best Management Practices.

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